ANNUAL MEETING OF THE INTERNATIONAL CELL SENESCENCE ASSOCIATION

ICSA
International Cell Senescence Association

ATHENS
9-12.9.2019

www.icsa2019-athens.gr

Cellular Senescence: the bright & dark side

Program & Conference information

Organizers
Vassilis G. Gorgoulis
Valery Krizhanovsky
Dimitris Kletsas
Konstantinos Evangelou

BIOMEDICAL RESEARCH FOUNDATION OF THE ACADEMY OF ATHENS (BRFAA)

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Keynote Speakers

Curtis C. Harris , USA Judith Campisi , USA Yossi Shiloh , Israel

Invited Speakers

Adams Peter, USA
Aidinis Vassilis, Greece
Baker Darren J., USA
Benetos Athanasios, France
Ben-Porath Ittai, Israel
Bischof Oliver, France
Blow Julian, UK
Burma Sandeep, USA
Chondrogianni Niki, Greece
Collado Manuel, Spain
Demaria Marco, Netherland
Di Micco Raffaella, Italy
Eliopoulos Aristidis, Greece
Ferbeyre Gerardo, Canada
Försch Sebastian, Germany
Fousteri Maria, Greece
Frisan Teresa, Sweden
Gagos Sarantis, Greece
Garinis Georgios, Greece
Giatromanolaki Alexandra, Greece
Gonos Efstathios S., Greece
Gorgoulis Vassilis G. , Greece/UK
Hara Eiji, Japan
Horvath Steve, USA
Kletsas Dimitris, Greece
Kostourou Vasso, Greece
Krizhanovsky Valery, Israel
Lewis D. John, USA
Liborio Vetrano Davide, Sweden
Logothetis Christopher, USA
Lygerou Zoi, Greece
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Welcome Letter

Dear Colleagues and Friends,

It is with great pleasure to welcome you at the 2019 Conference of the International Cell Senescence Association (ICSA) focusing on “The bright & dark side” of cellular senescence. The Conference will be held on September 9-12, 2019 in Athens, a city of great importance since ancient times for culture, philosophy and scientific thinking. The historic centre of Athens is an open-air museum, yet the city’s cultural and social life takes place amid these ancient landmarks, merging past and present.

Yesterday’s fact was that telomere attrition, activated oncogenes and other stimuli induce senescence. Today, we aim at the underlying mechanisms while tomorrow we aspire to manipulate this cellular state as to prevent associated diseases. We welcome you to discuss the latest evidence in the field of cellular senescence at the Biomedical Research Foundation of the Academy of Athens. The conference aims to cover a diverse range of interdisciplinary topics focused on cellular senescence with worldwide attendance. We have received a large number of high quality abstracts and it is a great pleasure that many of them have been submitted by young investigators. In several sessions, prominent, as well as younger scientists will present their recent findings on various topics.

For further information you can visit our website www.icsa2019-athens.gr which will constantly be kept updated with information regarding the “The bright & dark side” conference.

On behalf of the International Cell Senescence Association and the Local Organizing Committee we would like to thank all speakers and participants for their scientific contribution, as well as sponsors and exhibitors for their support.

We look forward to hosting you in Athens.

The Organisers,

Vassilis G. Gorgoulis
Valery Krizhanovsky
Dimitris Kletsas
Konstantinos Evangelou

Biomedical Research Foundation of the Academy of Athens (BRFAA)

The Biomedical Research Foundation of the Academy of Athens (BRFAA) is the most recent addition to the Life Sciences Research organizations in Greece, which begun its activities in 2004. It is located at a distance of 3 km from downtown Athens, and is housed in a modern 32,000 m^2 building. The main goal of BRFAA is to achieve excellence in the Biomedical Sciences by recruiting high quality investigators carrying out cutting-edge basic and translational research and by training young researchers in a state-of-the-art facilities, which provide a particularly stimulating scientific environment and strong research infrastructures. At present, BRFAA consists of four Research Centers (Institutes) specialized in different aspects of biomedical research focusing on the following research areas: gene regulation, stem cells, neurobiology, developmental biology, aging, cancer, inflammation, stress and metabolic syndromes, cardiovascular pathophysiology and environmental health issues. BRFAA research activities are supported by an impressive infrastructure of state-of-the-art core facilities, in addition to basic molecular biology equipment.

How to get there

• From Katechaki Metro Station (blue point 1), find the exit (there is only one) and turn right to Mesogeion Avenue (Image 1-arrows).
• Walk on Mesogeion Avenue for about 600 meters and once you see on your right hand the entrance of Sotiria Hospital (white point 2) turn right (Images 2-4). Show your identity card or badge at the security control spot and enter the hospital (arrows).
• Follow the yellow line on the road (Image 5-inset) until you reach the Hospital's Cafeteria (yellow point 3 and Image 5). Turn right behind the large building that is exactly opposite of the cafeteria's entrance (Image 6-arrows) and walk around it until you reach a traffic islet (Images 7&8-arrows). In about 15 meters turn left (Image 9-arrows) and immediately right (Image 10-arrows) (follow the signs "ICSA Conference").
• You will see a grey steel door (red point 4) in front of you (Images 11&12). Pass through and turn right (Image 12-arrows). Show your identity card or badge at the security control spot and enter the Biomedical Research Foundation of the Academy of Athens (BRFAA) (green point 5) (Image 12).
• Straight ahead and on the left you will find the main entrance that leads to the auditorium.

Mini-Shuttle Bus Transportation

Available from the Sotiria Hospital entrance (Starting point-thick arrow in Image 4) every 20-30 min, from 08:00 to 14:00. Request from the driver debarkation after the Hospital's cafeteria (Traffic islet-Images 7&8).

Map

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General information

Welcome desk - opening hours

The registration desk opens at 15.00 on September 9th, 2019. To receive your registration package at any other time, please head to the welcome desk during coffee breaks or contact: Contact@icsa2019-athens.gr.

Transportation to the conference

Shuttle bus will be available for the invited speakers to access the BRFAA from their hotel. 09/09 from hotel 16.00, from BRFAA 21.30 | 10/9 & 11/09 from hotel 08.15, from BRFAA 17.00 | 12/09 from hotel 08.15, from BRFAA 16.30
Metro tickets will be provided to all participants at registration for transport during the whole days of the conference.

WiFi

A free public Wi-Fi connection is available at the conference area of the Biomedical Research Foundation of the Academy of Athens (Wi-Fi : BRFAA_Guests)

Oral sessions

Scientific sessions are taking place in the auditorium on ground floor of the Biomedical Research Foundation of the Academy of Athens. Information for presenters: Please give your presentation before your session in a USB key to Kostas Evangelou: cnevagel@med.uoa.gr.

Poster Sessions

Two poster sessions will take place at the auditorium of the BRFAA, as follow:
Poster session I will start on Tuesday, September 10th at 17:05 PM (No 1-50)
Poster session II will start on Wednesday, September 11th at 18:00 PM (No 50-93)
IMPORTANT: All posters should be removed at the end of each session.Kindly note that poster size must not exceed 100 cm (width) x 120 cm (height) (portrait orientation).

Coffee breaks & lunches

Coffee breaks and lunch buffets will be served in the Hall on the ground floor of the BRFAA just outside of the conference theater. Access is strictly limited to registered participants while seating will be available indoors as well as outdoors in designated areas.

Medical support

Medical support will be available during the whole conference. For emergency call at: +30 6977865680 or +30 6973980566

Social events

Welcome Dinner & Cocktail

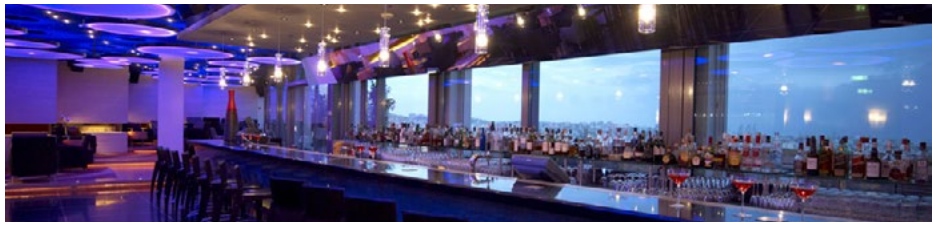
A reception and dinner cocktail will be served in the Hall on the ground floor of the BRFAA just outside of the conference theater on Monday 8th at 20:35 pm.

Address:
(BRFAA) Biomedical Research Foundation
4, Soranou Ephessiou Street, Athens 115 27
or scan the QR code to open location on
your phone's Google Maps.

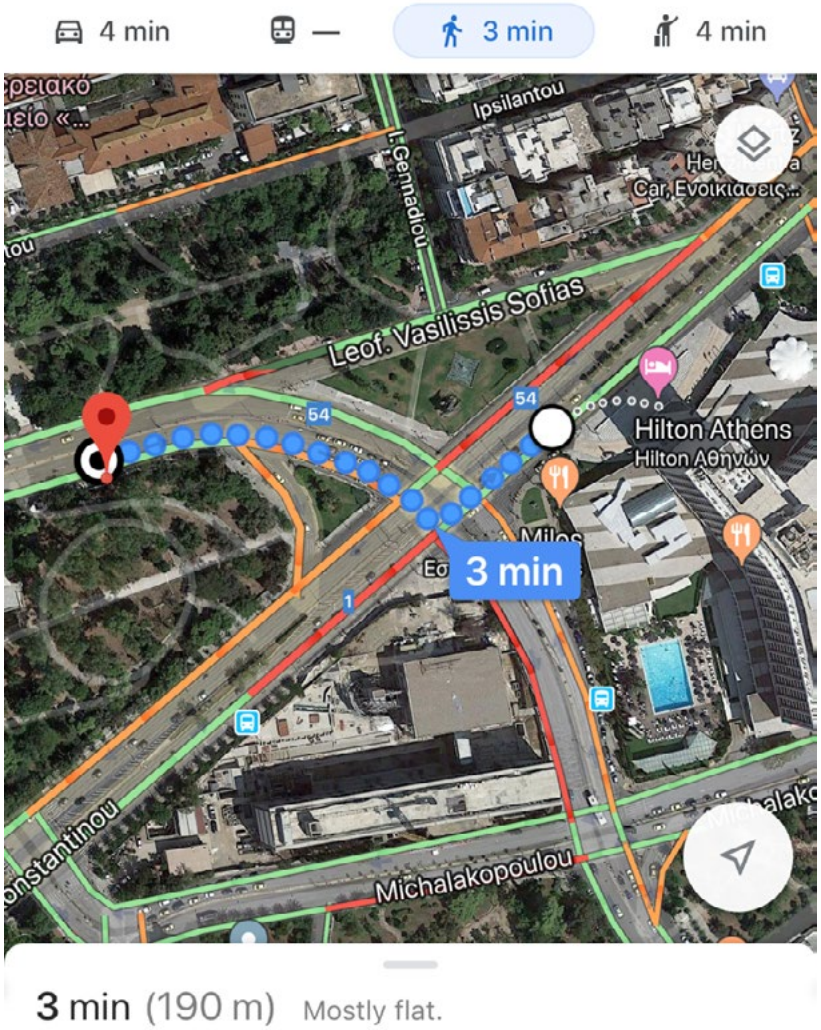
Gala Dinner

The Gala Dinner of the ICSA 2019 conference – "The bright & dark side" will take place September 11th 2019 at the Galaxy Hall of the Hilton Athens Hotel. The Gala will feature the lecture: "Human Evolution at the Crossroads: new discoveries and insights from the paleoanthropology of Greece", by Prof. Katerina Harvati-Papatheodorou from the Senckenberg Center for Human Evolution and Paleoenvironments, University of Tübingen, Germany.
The chic and stylish rooftop Galaxy Venue at the Hilton Athens hotel offers superb views of Athens and the Acropolis.

Address:
46, Vassilissis Sofias Avenue, Athens, 11528
or scan the QR code to open location on
your phone's Google Maps.



How to get to Athens Hilton



Directions to Athens Hilton from closest metro station

Exit the Evangelismos metro station

Turn right onto Leof. Vasilissis Sofias avenue E054/E083

Head southwest on Leof. Vasilissis Sofias E01/E054/E083 toward Leof. Vasileos Alexandrou avenue

Prof. Dr. Harvati-Papatheodorou

Professor Harvati is a paleoanthropologist specializing in Neanderthal evolution, modern human origins and the application of 3-D geometric morphometric and virtual anthropology methods to paleoanthropology. She conducted her doctoral studies (2001) at the City University of New York and the New York Consortium on Evolutionary Primatology (NYCEP). Before becoming Full Professor at the University of Tübingen in 2009, she was Assistant Professor at New York University (2001-2004) and Senior Researcher at the Max Planck Institute for Evolutionary Anthropology (2004-2009). Since 2009 Harvati also holds an adjunct Professorship of Anthropology at the City University of New York Graduate Center and is resource faculty for the New York Consortium of Evolutionary Primatology.

Harvati is the recipient of two ERC grants (ERC Starting Grant 'Paleoanthropology at the Gates of Europe', 2011; ERC Consolidator Grant 'Human Evolution at the Crossroads', 2016). She currently directs the Cross-Faculty DFG Centre for Advanced Studies 'Words, Bones, Genes, Tools: Tracking linguistic, cultural and biological trajectories of the human past' at the University of Tübingen. Her work has been published in Nature, Science, PNAS, Nature Ecology & Evolution and other specialist as well as general science journals. Her research was named one of the top 10 scientific discoveries of the year 2007 by TIME magazine for demonstrating the African origin of all modern humans. She was elected Fellow of the American Association for the Advancement of Science in 2010 and in 2014 she was awarded the Research Award of the state of Baden-Württemberg in 2014 (basic research). She has conducted fieldwork in Europe and Africa, most recently in Greece.

About the Gala Dinner presentation:

Greece lies at the crossroads between three continents, and represents a logical gateway through which early human populations might have repeatedly passed on the way to and from Europe. It also represents one of the three European Mediterranean peninsulas which acted as refugia for fauna, flora and possibly human populations during glacial times. Evidence from this region is therefore essential in order to test hypotheses about the course of human evolution in Europe. Despite its geographic importance, however, paleoanthropological research has until recently been relatively neglected. This presentation will review new discoveries from the fossil and paleolithic record of Greece in the context of broader questions in European paleoanthropology.

Scientific Program

Day 1 **September 9**

Opening Session

15.00-17.00 Registration

17.00-17.15 Welcome and introduction
(Gorgoulis, Serrano, Young ICSA)

17.15-18.00 **Yossi Shiloh (EMBO Key Note Lecture)**
The DNA damage response:
Where genome stability meets human morbidity

18.00-18.15 Coffee Break

Session - Chairperson: Vassilis Gorgoulis Senescence: Genome - Epigenome (I)

18.15-18.35 Vassilis Gorgoulis
Genomic instability and "escape" from oncogene-induced senescence

18.35-18.55 Julian Blow
How cells ensure complete genome duplication and what happens when this fails

18.55-19.15 Zoi Lygerou
DNA replication licensing aberrations as drivers of genomic instability

19.15-19.35 Steve Horvath (young ICSA invited speaker)
Epigenetic clock analysis of cellular senescence

19.35-19.55 Dafni Pefani
Molecular insights into the rDNA damage response

19.55-20.15 Wojciech Niedzwiedz
A novel synthetic lethal interaction with the BRCA tumour suppressor pathway

Scientific Program

10 minutes speed talks

20.15-20.25 Jean-Marc Lemaitre
Manipulating senescence through reprogramming to improve tissue homeostasis in aging

20.25-20.35 Alena Shen
Epigenetically targeting senescent cells prevents the deleterious effects of glucocorticoids on growing skeleton

20.35 **Welcome Reception-Dinner & Cocktail**

Scientific Program

Day 2 **September 10**

Session - Chairperson: Peter D. Adams — Senescence: Genome - Epigenome (II)

09.00-09.45 **Curtis Harris (Key Note)**
Aging and cancer: p53 isoforms

09.45-10.05 Peter D. Adams
A novel mito-nuclear retrograde signaling pathway is a target for suppression of cytoplasmic chromatin fragments (CCF) and SASP

10.05-10.25 John M. Sedivy
Somatic retrotransposition in cellular senescence and aging

10.25-10.45 Aristides Eliopoulos
A role for the CD40 pathway in cellular senescence

Session — 10 minutes speed talks

10.45-10.55 Frédérick A. Mallette
KDM4A inhibition induces a senescence-like phenotype and increases sensitivity to Bcl2 inhibitors in MLL-AF9 acute myeloid leukemia

10.55-11.05 Tamir Chandra
Global changes in regulatory networks in senescence

11.05-11.35 Coffee Break

Session - Chairperson: Oliver Bischof — Senescence: Genome - Epigenome -Telomeres

11.35-11.55 Oliver Bischof
Breaking the S-ENesence CODE: Towards senescence reversal and therapeutic opportunities

Scientific Program

11.55-12.15 Sarantis Gagos
Alternative Lengthening of Telomeres in neoplasia: a complex immortalization pathway with features of senescence

12.15-12.35 Athanasios Benetos
Telomeres dynamics: From the cellular senescence to the human aging

Session - Chairperson: Manuel Serrano — Senescence: Metabolism (I)

12.35-12.55 Manuel Serrano
Iron deposition associated to hemolysis causes senescence and fibrosis

12.55-13.15 Nektarios Tavernarakis
Autophagic pathways regulating mitochondrial homeostasis in ageing and neurodegeneration

10 minutes speed talks

13.15-13.25 Andrew Young
Autophagy-mediated reduction in tolerogenicity in facilitating senescent cell clearance from mouse liver

13.25-13.35 Nicholas Rettko
Probing the surface of senescent cells for p16 MHC-peptide complexes

13.35-14.35 Lunch Break

Session - Chairperson: Angel Nebreda — Senescence: Metabolism (II)

14.35-14.55 Angel Nebreda
Autophagy-induced senescence is regulated by p38α signaling: implications for chemotherapy

Scientific Program

14.55-15.15 Isabel Varela-Nieto
Cell senescence, autophagy and apoptosis cooperate in tissue remodeling during early inner ear morphogenesis

15.15-15.35 Gerardo Ferbeyre
A cytosolic NAD+ regenerating complex bypasses senescence and contributes to transformation

15.35-15.55 Ewa Sikora
Interplay between cell senescence and autophagy; the lesson from post-mitotic neural and highly mitotic cancer cells

15.55-16.15 Ioannis P. Trougakos
Proteome instability as a main hallmark of ageing and cancer

16.15-16.35 Niki Chondrogianni
Eat, pray, degrade: Potential anti-ageing strategies offered by Mother Nature (and not only)

10 minutes speed talks

16.35-16.45 Raquel Buj
Suppression of p16 bypasses senescence through a mTORC1-mediated nucleotide metabolic reprogramming

16.45-16.55 Haoran Zhu
Harnessing senescence to control oncogene-driven cancer through the metabolic intervention

2 minutes speed talks

16.55-17.10 Ayush Srivastava, Covadonga Huidobro, Koji Itahana, Eva González-Suarez, Amr Omer, Nafsika Chala

17.05 **Poster Session I (posters 1-50)**

Scientific Program

Day 3 **September 11**

Session - Chairperson: Marco Demaria — Drug discovery - Senolysis -Therapeutics

09.00-09.20 Marco Demaria
CDK4/6 inhibitors induce and modulate senescence responses

09.20-09.40 Laura Niederhofer
Identifying key senescent cell types that drive aging

09.40-10.00 John D. Lewis (Oisin Biotechnologies)
Systemic senolysis in naturally aged mice using a fusogenix gene therapy approach

10.00-10.20 Paul D. Robbins
Progress towards development of clinically-relevant senotherapeutics for extending healthspan

10.20-10.40 Manuel Collado
Identification and characterization of cardiac glycosides as a novel class of broad spectrum senolytic compounds

10.40-11.00 Evangelia Pavlatou
Nanomaterials and cellular senescence

11.00-11.30 Coffee Break

Session - Chairperson: Valery Krizhanovsky — Senescence - Inflammation - Immunosenescence

11.30-11.50 Valery Krizhanovsky
Immune system controls dynamics of accumulation of senescent cells during ageing

11.50-12.10 Teresa Frisan
Bacterial genotoxins and senescence in vivo: is there a role of the tissue microenvironment?

12.10-12.30 Argyris Papantonis
Autophagy-induced and SASP-free senescence in human cells and its anti-cancer implications

Scientific Program

12.30-12.50 Raffaella Di Micco
Dissecting the impact of cell senescence in the hematopoietic compartment

12.50-13.10 George Garinis
DNA damage and innate immune signaling in development and disease

13.10-13.30 Ourania Tsitsilonis
Immune signatures in age-related diseases

13.35-14.35 Lunch Break

Session - Chairperson: Christopher Logothetis Senescence in Human Diseases

14.30-14.50 Christopher Logothetis
Transitioning from a prognostic to a predictive paradigm of prostate cancer classification

14.50-15.10 Richard Nicholas
Accelerated ageing of the brain in multiple sclerosis

15.10-15.30 Clemens A. Schmitt
Senescence in oncology: linking cell biology to clinical care

15.30-15.50 Alexandra Giatromanolaki
Markers of senescence and cancer prognosis

15.50-16.10 Sandeep Burma
Elimination of senescent astrocytes in the brain tumor microenvironment attenuates glioblastoma recurrence after radiotherapy

16.10-16.30 Sebastian Försch
Cellular senescence - from biological phenomenon to valuable clinical tool

Scientific Program

10 minutes speed talks

16.30-16.40 Utz Herbig
Humans increasingly develop senescent immune cells with advancing age

16.40-16.50 Deborah Milligan
Development of a 3D living skin equivalent to explore the influence of senescence on the skin ageing phenotype

2 minutes speed talks

16.50-17.00 Takehiro Yamanaka, Saba Manshaei, Satotaka Omori, Marc-Alexandre Olivier, Wioleta Grabowska

17.00-18.00 Panel Discussion - General Assembly

18.00 **Poster Session II (posters 50-93)**

20.30 Gala Dinner
Galaxy Hall of the Hilton Athens Hotel
46, Vassilissis Sofias Avenue, Athens, 11528
Katerina Harvati-Papatheodorou
Human Evolution at the Crossroads: new discoveries and insights from the paleoanthropology of Greece

Scientific Program

Day 4 **September 12**

Session - Chairperson: Andrea Maier **Age related diseases -Aging - Geroscience**

09.00-09.20 Davide Liborio Vetrano
Multimorbidity phenotypes as a potential target for intervention

09.20-09.40 Andrea Maier
The future of Clinical Biogerontology

09.40-10.00 Darren Baker
Incriminating senescent cells to neurodegenerative pathologies

10.00-10.20 Efstathios Gonos
Proteasome activation delays aging and progression of age-related diseases

10.20-10.40 Rebecca Matsas
Exploring p.A53T-alpha synuclein pathology in a human induced pluripotent stem cell-based model

10.40-11.00 Maria Fousteri
Molecular control of genome surveillance mechanisms and their implication in cancer and human pathology

11.00-11.30 Coffee Break

Session - Chairperson: Dimitris Kletsas **The Senescence Microenvironment**

11.30-11.50 Eiji Hara
Cellular senescence and cancer: a gut microbial connection

11.50-12.10 Dimitris Kletsas
Senescent cells as modulators of the tumor microenvironment

12.10-12.30 Ittai Ben-Porath
Tumor promoting activities of senescent cells in the skin and pancreas

Scientific Program

12.30-12.50 Vasso Kostourou
Endothelial cell adhesion is a central regulator of blood vessel homeostasis and pathology

12.50-13.10 Daniel Muñoz-Espín
Effects of chemotherapy-induced SASP in lung cancer progression

13.10-13.30 Vassilis Aidinis
Autotaxin (ATX), a novel player in chronic inflammation and fibrosis

13.30-14.30 Lunch Break

10 minutes speed talks

14.30-14.40 Ana O'Loghlen
Novel regulators of senescence: in disease and health

14.40-14.50 Ryan J Wallis
Investigating the Interplay between Senescence, Exosomes and Inflammation

14.50-15.50 **Judith Campisi (Key Note)**
The many lives of senescent cells

The DNA damage response: where genome stability meets human morbidity

Author: Yossi Shiloh

The David and Inez Myers Laboratory for Cancer Genetics, Department of Human Molecular Genetics, and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.

Maintenance of genome stability is critical for cellular homeostasis and prevention of undue cell death, cancer and premature aging. A central axis in maintaining genome stability is the DNA damage response (DDR), which copes continuously with numerous DNA lesions inflicted mainly by endogenous reactive oxygen species. It is not surprising, therefore, that the DDR has evolved into a massive but fine-tuned signaling network that involves many proteins. The DDR is robustly activated by DNA double-strand breaks (DSBs). The primary transducer of the DSB response is the homeostatic serine-threonine kinase, ATM, which mobilizes the network by initiating a phosphorylation cascade. Loss of ATM due to null mutations at the ATM gene leads to a prototypic genome instability syndrome – ataxia-telangiectasia (A-T), a focal point of interest in our lab. A-T includes progressive cerebellar degeneration, chronic lung disease, endocrine abnormalities, premature aging, cancer predisposition, chromosomal fragility and extreme sensitivity to DSB-inducing agents. We found that primary fibroblasts from A-T patients exhibit premature senescence in culture, under either ambient or 3% oxygen pressure; and that fibroblasts from A-T carriers exhibit senescence rates intermediate between those of A-T cells and control cells. These findings are additional evidence of the segmental premature aging observed in A-T patients, further substantiating the link between genome instability, cellular senescence and aging.

Extending these studies on this link to a combination of two DDR genes, we generated mouse models combining mutations in the Atm and Wrn genes. The human WRN gene is mutated in Werner syndrome (WS), a genome instability disorder characterized primarily by segmental premature aging. The helicase-nuclease WRN – a member of the RecQ helicase family – is involved in numerous DNA transactions including DNA repair pathways. Wrn-/- mice have no discernible phenotype, while Atm-/- mice exhibit sterility, cancer predisposition and radiation sensitivity. Importantly, we find that the Atm-/-//Wrn-/- double knockout (dKO) genotype is embryonic lethal and dKO cells do not grow due to early, acute premature senescence. Other Atm/Wrn genotypes are alive but show various degrees of segmental premature aging and associated morbidity, including the double-heterozygous genotype, Atm+/-//Wrn+/-, which exists in human populations.

Generalizing from these studies, it is clear that sequence alterations in the multitude of DDR genes affect DDR effectiveness, creating a broad continuum of genome stability among humans. At one extreme are the genome instability syndromes, but further along this spectrum presumably lie chronic conditions that affect morbidity and aging. Most of these sequence alterations exist in heterozygous genotypes. For example, A-T carriers have long been suspected to be cancer-prone. When we performed carrier detection in two communities and followed their epidemiology over two decades, we found an excess of cardiovascular diseases and predisposition to many cancer types among the carriers, suggesting a slight premature aging in these individuals. This correlates with the intermediate acceleration of senescence in cultured primary fibroblasts from these individuals.

The combinations of sequence variations in DDR genes are expected to be highly individual and to determine a personal “genome stability index”. This index will correlate with aging pace and associated morbidity on the one hand and be a guide in precision medicine on the other.

Genomic instability and "escape" from oncogene induced senescence

Author: Vassilis G Gorgoulis

1. Molecular Carcinogenesis Group, Department of Histology and Embryology, School of Medicine, National and Kapodistrian University of Athens, 75 Mikras Asias Str, Athens, GR-11527, Greece;
2. Biomedical Research Foundation of the Academy of Athens, 4 Soranou Ephessiou Str., Athens, GR-11527, Greece;
3. Faculty Institute of Cancer Sciences, University of Manchester, Manchester Academic Health Science Centre, Wilmslow Road, Manchester, M20 4QL, UK;
4. Center for New Biotechnologies and Precision Medicine, Medical School, National and Kapodistrian University of Athens, 75 Mikras Asias Str, Athens, GR-11527, Greece

Oncogene-induced senescence is considered an important tumor suppressor mechanism. Yet, if not removed timely by the immune surveillance system it will present a detrimental side. Though the latter is attributed to the so called senescence-associated-secretory-phenotype (SASP), recent reports demonstrate that "escape" from senescence may represent another unfavorable outcome. Herein, we present evidence that oncogene-induced DNA damage, occurring from the early phases of senescence, forms a driving force participating in the "escape" phenotype. Within this frame we also present an in silico pipeline that discriminates driver from passenger genetic events linked with drug responsiveness.

Oral presentations

Session 09/09 Senescence: Genome - Epigenome (I)

How cells ensure complete genome duplication and what happens when this fails

Author: Julian Blow

Federico Tinarelli1, Ananya Kar1, Patricia Heyn2, Andrew Jackson2 and J. Julian Blow1
1. School of Life Sciences, University of Dundee, Scotland, UK
2. MRC Human Genetics Unit, University of Edinburgh, Scotland, UK

Prior to S phase entry, replication origins must be licensed for use by binding double hexamers of MCM2-7. If two converging replication forks irreversibly stall with no dormant origin between them – a 'double fork stall' – the DNA in between cannot be replicated by conventional means because no new origins can be licensed once S phase has started. Mathematical and computer modelling suggests that in organisms with gigabase genome sizes, such as humans, there is a high probability of DNA replication not being complete at the end of S phase due to the occurrence of double fork stalls. We have tentatively mapped out a pathway of 'Post-S Phase DNA Synthesis' or PoSDAS, by which replication is completed despite the occurrence of double fork stalls. This involves: recognition of stalled replication forks by the Fanconi Anaemia system in S phase; additional DNA synthesis occurring during G2 and early mitosis; segregation of unreplicated DNA via Ultrafine Anaphase Bridges; protection of unreplicated DNA by 53BP1 during G1; final resolution during the next S phase. This heterodox view suggests that it takes more than one cell cycle (S, G2, M, G1 and the second S phase) to complete DNA replication. In order to investigate the consequences of under-replication, we have been investigating the molecular defects in Meier-Gorlin disease. This human genetic disease is caused by mutations in the proteins involved in origin licensing/MCM2-7 loading. We have engineered human and mouse cells lines with one of the most common Meier-Gorlin mutations in the ORC1 subunit. These cell lines typically show a defect in the rate and quantity of MCM2-7 loaded onto DNA. One consequence of this is an increase in the number of spontaneous 53BP1 Nuclear Bodies, consistent with the PoSDAS pathway being over-active. The ORC1 mutation also increases the proportion of primary cells that are in 'unlicensed G1' - potentially able to proliferate but not actively loading MCM2-7 onto DNA. This may represent a 'pre-senescent' state that is a consequence of under-replication exceeding the capacity of the PoSDAS pathway.

Moreno, A. et al (2016). Unreplicated DNA remaining from unperturbed S phases passes through mitosis for resolution in daughter cells. Proc Natl Acad Sci USA 113, E5757-64. Al Mamum, M. et al (2016). Inevitability and containment of replication errors for eukaryotic genome lengths spanning Megabase to Gigabase. Proc Natl Acad Sci USA 113, E5765–E5774. Caroll, et al (2018). Lgr5+ intestinal stem cells reside in an unlicensed G1 phase. J Cell Biol 217, 1667-1685.

DNA replication licensing aberrations as drivers of genomic instability

Author: Zoi Lygerou

Department of Biology, School of Medicine, University of Patras, Patras 26504, Greece.

To maintain genome stability, dividing cells must ensure that every part of their genome is replicated once and only once per cell cycle. Chromatin is licensed for a new round of replication in the G1 phase, through the loading of the hexameric MCM replicative helicase onto multiple origins along the genome. Accurate spatial and temporal control of DNA replication licensing is essential for genome stability. We show that DNA replication licensing takes place in waves in the long G1 phase of human cells. Minimal licensing at the end of mitosis is followed by a wave of licensing close to the G1 to S phase transition, which is responsible for loading the majority of MCM proteins onto chromatin. Cdc6 is the rate limiting factor which governs this second licensing wave. Cells entering S-phase in the absence of the second MCM-loading wave show signs of replication stress, fail to fire dormant origins when challenged with hydroxyurea and exhibit DNA damage. Over-licensing also leads to replication stress. Ectopic expression of Cdc6 and / or Cdt1 leads to rereplication, double-strand breaks and the activation of multiple DDR pathways which act in competition. Silencing of key DDR factors shows that homology-mediated pathways lead to enhanced drug resistance through gene copy number increases in rereplicating cells, and are counter-acted by non-homologous-end-joining pathways. Both under and over-licensing are therefore linked to genomic instability and genome plasticity, which drive genome evolution.

Oral presentations

Session 09/09 Senescence: Genome - Epigenome (I)

Epigenetic clock analysis of cellular senescence

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DNA methylation based biomarkers of aging known as “epigenetic clocks” can be used to measure the age of any human tissue, cell type, or fluid that contains DNA. DNA methylation age captures aspects of biological age, e.g. it predicts lifespan and healthspan in large scale epidemiological studies. In general, epigenetic clocks are not simply markers of cellular senescence. Using primary cells, telomerase-expressing cells and oncogene-expressing cells of the same genetic background, we have shown that induction of replicative senescence (RS) and oncogene-induced senescence (OIS) are accompanied by epigenetic aging. However, senescence induced by DNA damage (radiation) is not, even though RS and OIS activate the cellular DNA damage response pathway, highlighting the independence of senescence from epigenetic aging. Consistent with this, we observed that telomerase-immortalised cells aged in culture without having been treated with any senescence inducers or DNA-damaging agents, re-affirming the independence of the process of aging from telomeres and senescence.

Molecular insights into the rDNA damage response

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The rDNA repeats that give rise to the ribosomal DNA (rDNA) are organised in the most prominent nuclear structure, the nucleolus. Instability within the rDNA repeats has been connected with decreased lifespan and malignant phenotypes. Breaks within the rDNA repeats result in persistent lesions that can lead to alteration of the rDNA copy number and genomic instability. Due to challenging replication, excessive transcription and repetitive nature, regulation of the rDNA damage response bears unique features including re-organisation of the nucleolus, translocation of the broken ends into the nucleolar periphery for homology mediated repair and establishment of histone modification marks to regulate Polymerase I transcriptional activity.
Our studies aim to understand how the nucleolus is re-organised in the presence of rDNA breaks and identify novel regulators of the rDNA damage response. We found the tumour suppressor RASSF1A at the sites of rDNA breaks and propose that RASSF1A recruitment is necessary for regulation of Polymerase I transcription, repair of rDNA breaks and survival upon rDNA damage.

Oral presentations

Session 09/09 Senescence: Genome - Epigenome (I)

A novel synthetic lethal interaction with the BRCA tumour suppressor pathway

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Accurate replication of DNA is essential to preserve genomic integrity and failure to do so results in a variety of chromosomal instability-associated diseases including cancer. A key to this is the ability of cells to stabilise and restart stalled replication forks. Here, I will provide evidence that the EXD2 nuclease is essential to this process. Our data show that EXD2 is recruited to stalled replication forks and cells lacking EXD2 or expressing a nuclease-dead version of the protein display high levels of replication-associated genome instability. Mechanistically, we show that EXD2 acts to counteract fork reversal in the same pathway as RECQ1, and this activity is critical for suppression of uncontrolled degradation of newly synthesised DNA. Consistent with this, purified EXD2 can process substrates mimicking regressed forks in vitro, and in vivo its nuclease activity is required to supress the collapse of terminally regressed forks and subsequent mitotic catastrophe. Unexpectedly, we also discover that depletion of EXD2 confers a synthetic lethal interaction with BRCA1/2, suggesting a non-redundant function in genome stability between these repair factors. In summary, our work identifies a novel mechanism of fork reactivation, underpinned by EXD2's nuclease activity, by which cells balance fork regression with fork restoration to maintain genome stability. In addition, this work offers a potential new therapeutic target for tumours with BRCA1/2-deficiency.

Aging and cancer: p53 isoforms

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The p53 network is an intrinsic monitoring and responsive pathway of telomeric attrition and chronic stress involved in cellular aging and senescence (1). Cellular senescence, in cancer cells, can be a tumor suppressive mechanism that can be activated by p53. Cellular senescence of tumor stromal cells can enhance carcinogenesis and tumor progression. We and others are currently studying the molecular mechanisms of cellular senescence in normal human cells and the role of p53 and its isoforms in aging and cancer (2-4). Our research focuses on the functional role of p53 isoforms. e.g., "dominant negative" Δ133p53 and "co-transactivator" of wild-type p53, p53β, both as natural regulators of cellular senescence. DNA repair and stem cell biology are dysregulated in cancer, including damaging side effects of radiation and chemotherapy, and aging diseases, such as Hutchinson-Gilford Progeria Syndrome and Alzheimer's Disease (A-C). Because of molecular evolution, Δ133p53 is specifically present in humans and primates, but not in other organisms, including mice, because of lack of an initiating methionine codon corresponding to the human codon 133 at exon 5 of TP53 (D)

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A novel mito-nuclear retrograde signaling pathway is a target for suppression of cytoplasmic chromatin fragments (CCF) and SASP.

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Cellular senescence is a potent tumor suppressor mechanism and also a cause of aging and aging related diseases. Senescence is characterized by a stable cell cycle arrest and a complex pro-inflammatory secretome termed the senescence associated secretory phenotype (SASP). We recently discovered that cytoplasmic chromatin fragments (CCF), extruded from the nucleus of senescent cells, are triggers of SASP through activation of the innate immunity cytosolic DNA sensing cGAS-STING pathway. However, the upstream initiating signal of CCF formation remains unknown. Here, we show that dysfunctional mitochondria, linked to downregulation of nuclear-encoded mitochondrial oxidative phosphorylation genes, trigger a ROS-JNK retrograde signalling pathway that drives CCF formation and hence SASP. In the nucleus, formation of CCF is controlled by 53BP1. Importantly, we show that low doses of HDAC inhibitors restore expression of mitochondrial genes, improve mitochondrial function and suppress CCF and SASP in senescent cells, and suppress oxidative stress, inflammation and tissue damage in mouse models. Overall, our findings delineate an extended mitochondria-to-nucleus retrograde signalling pathway that initiates formation of CCF during senescence and is a potential target for drug-based interventions to inhibit the pro-aging SASP.

Somatic retrotransposition in cellular senescence and aging

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All organisms have evolved diverse mechanisms to repress the activity of their endogenous transposable elements (TEs). As a result of these defenses the activity of TEs in somatic cells was believed to be very low. Recent evidence however suggests that retrotransposable elements (RTEs) can be derepressed in some contexts in somatic mammalian tissues, for example during periods of embryonic development, or in a variety of cancers. We have shown that cellular senescence, an important component of mammalian aging, is accompanied by an increase in RTE transcription. This activation occurs late in the senescence process and is a hallmark of "deep" senescence. More recently we have found that during natural aging of mouse tissues, and in particular in advanced age, several families of RTEs become active. We will discuss the molecular processes that may lead to the progressive derepression of RTEs with age, as well as the consequences of their activation on the host. We suggest that somatic retrotransposition is a hitherto unappreciated aging process, that activation of RTEs is likely to be an important contributor to the progressive dysfunction of aging cells, and that these processes could be ameliorated therapeutically.

Oral presentations

Session 10/09 Senescence: Genome - Epigenome (II)

A role for the CD40 pathway in cellular senescence

Author: Aristides G. Eliopoulos

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CD40, a tumour necrosis factor (TNF) receptor family member, is expressed in a variety of cell types, including B lymphocytes, macrophages, fibroblasts, endothelial and epithelial cells, and this widespread expression is likely to account for its central, yet complex role in normal physiology, inflammatory and anti-tumour properties. Herein, we provide evidence to support a role for constitutive CD40 signalling in cell senescence. We show that co-expression of CD40 and CD40L induces senescence in primary epithelial cells and fibroblasts. Gene expression profiling identifies a plethora of chemokines/cytokines as targets of CD40 signaling that include CXCR2 and TGF-β family ligands. The knock-down of CXCR2 largely alleviates the CD40-mediated induction of senescence and p21 expression. Using mutational analysis of the CD40 cytoplasmic tail, we demonstrate that the specific binding of the TRAF2 adaptor and ubiquitin ligase to CD40 is mostly responsible for CXCR2 ligand expression through NF-kappaB signaling. Taken together, our results suggest a role for the constitutive engagement of the CD40L/CD40/NF-kappaB activation pathway in orchestrating a self-amplifying secretory network in which CXCR2-binding chemokines convey growth arrest.

Breaking the S-ENesence CODE: Towards senescence reversal and therapeutic opportunities

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Cellular senescence limits the proliferation of dysfunctional cells in response to diverse forms of cellular stress, playing essential roles in tumor suppression and tissue regeneration, while also contributing to age-related pathologies. Here, we will discuss our latest results using multidimensional profiling to define the temporal organization of the senescence transcriptional program, the transcription factor network topology that controls it and its underlying epigenome dynamics. Together, our findings identify dynamically regulated chromatin configurations critical for senescence competence, thus hinting at promising targets for manipulating the senescence program for therapeutic benefit.

Oral presentations

Session 10/09 Senescence: Genome - Epigenome -Telomeres

Alternative Lengthening of Telomeres in neoplasia: a complex immortalization pathway with features of senescence

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Human malignancies overcome replicative senescence either by activating the reverse transcriptase telomerase or by utilizing alternative lengthening of telomeres (ALT). From yeast to humans, the ALT pathway is operated by break-induced replication (BIR), a repair pathway for one-ended DNA double-strand breaks (DSBs) that requires DNA polymerase delta, depends on RAD52 and leads to conservative DNA neo-synthesis. ALT is characterized by extreme deviation in telomere length, frequent telomere dysfunction and highly increased chromosomal instability. ALT tumors or immortalized cells display increased rates of telomeric sister chromatid exchanges, produce high abundances of extrachromosomal telomeric DNA and frequently form promyelocytic leukemia (PML) protein nuclear bodies that bring together telomeres, telomere specific proteins and DNA repair factors. Beyond cancer, the activation of ALT-like pathways appears important for mammalian reproduction and stem cell pluripotency. There is increasing evidence that as in yeast, in mammals may operate more than one pathway capable to elongate telomeres in absence of telomerase. Further understanding of Alternative Lengthening of Telomeres will significantly impact the development of efficient oncotherapeutic strategies and will play a very important role in regenerative medicine.

Telomeres dynamics: From the cellular senescence to the human aging

Author: Prof. Athanasios Benetos MD, PhD

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Telomeres, the non-coding repetitive DNA sequences (TTAGGG) located at the ends of chromosomes, play a crucial role in cellular replicative capacity and in chromosome stability. Telomere length (TL) is genetically determined at birth, decreasing thereafter with age. Individuals with shorter TL have increased risk for earlier appearance of age-related degenerative diseases and a shorter life expectancy. The prevailing view in telomere epidemiology is that TL is associated with accelerated aging since it serves as a biomarker of the cumulative burden of inflammation and oxidative stress during adult life. Our recent findings in the field of arterial aging and atherosclerotic cardiovascular disease, support the tenet that a comparatively short leukocyte TL exist prior to the clinical manifestations of atherosclerosis, reflecting mainly higher LT attrition rates during the first years of life. Since short telomeres precede atherosclerosis, we hypothesize that TL is not just a simple marker, but a real determinant of arterial aging. It is therefore important to assess the telomere regulation in the younger and its consequences in older individuals.

Iron deposition associated to hemolysis causes senescence and fibrosis

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It is estimated that up to 30% of western world death cases originate from fibrotic diseases. Fibrosis is the formation of excessive connective tissue in a reparative process. The rigidity of the fibrotic tissue and the associated inflammation interfere with normal organ function manifesting in deteriorating health conditions. To date there is no cure for fibrotic diseases. Analyzing mice with kidney, lung or cardiac fibrosis, we found that tissue iron deposition is a common hallmark of fibrosis. We found elevated levels of heme in the sera of fibrotic mice and hemorrhagic red blood cell (RBCs) accumulation in their fibrotic tissues, suggesting that iron accumulation originates from hemolytic RBCs. In vitro, lysed RBCs or iron alone were sufficient to induce fibroblast activation, expression of inflammatory cytokines and senescence, hallmarks of fibrosis. In vivo, local administration of iron or lysed RBCs were sufficient to induce lung or kidney fibrosis depending on the administration route. Iron chelators were effective in preventing folic acid induced kidney fibrosis. Gene expression analysis of patients with idiopathic pulmonary fibrosis (IPF) versus controls identified altered iron homeostasis gene expression as a defining feature of human IPF. We suggest that fibrotic diseases, at least in some cases, are vascular diseases in which chronic microvascular injuries result in tissue deposition of iron and heme to toxic levels, which on its own is sufficient to initiate the fibrotic cascade.

Autophagic pathways regulating mitochondrial homeostasis in ageing and neurodegeneration

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Mitochondria, the main energy hub of the cell, are highly dynamic organelles, playing essential roles in fundamental cellular processes. Mitochondrial function impinges on several signalling pathways modulating cellular metabolism, cell survival and healthspan. Maintenance of mitochondrial function and energy homeostasis requires both generation of newly synthesized and elimination of dysfunctional mitochondria. Impaired mitochondrial function and excessive mitochondrial content are major characteristics of ageing and several human pathophysiological conditions, highlighting the pivotal role of the coordination between mitochondrial biogenesis and mitophagy. However, the cellular and molecular underpinnings of mitochondrial mass homeostasis remain obscure. We found that DCT-1, the Caenorhabditis elegans homolog of mammalian BNIP3 and BNIP3L/NIX, is a key mediator of mitophagy promoting longevity under stress. DCT-1 acts downstream of the PINK-1-PDR-1/Parkin pathway and is ubiquitinated upon mitophagy-inducing conditions to mediate the removal of damaged mitochondria. Accumulation of damaged mitochondria triggers SKN-1 activation, which initiates a bipartite retrograde signaling pathway stimulating the coordinated induction of both mitochondrial biogenesis and mitophagy genes. Taken together, our results unravel a homeostatic feedback loop that allows cells to adjust their mitochondrial population in response to environmental and intracellular cues. Age-dependent decline of mitophagy both inhibits removal of dysfunctional or superfluous mitochondria and impairs mitochondrial biogenesis resulting in progressive mitochondrial accretion and consequently, deterioration of cell function.

Autophagy-induced senescence is regulated by p38α signaling: implications for chemotherapy

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Apoptosis and senescence are two mutually exclusive cell fate programs that can be activated by stress. The factors that instruct cells to enter into senescence or apoptosis are not fully understood, but both programs can be regulated by the stress kinase p38α. Using an inducible system that specifically activates this pathway, we show that sustained p38α activation suffices to trigger massive autophagosome formation and to enhance the basal autophagic flux. This requires the concurrent effect of increased mitochondrial reactive oxygen species production and the phosphorylation of ULK1. Moreover, we demonstrate that macroautophagy induction by p38α signaling determines that cancer cells preferentially enter senescence instead of undergoing apoptosis. In agreement with these results, we present evidence that the induction of autophagy by p38α protects cancer cells from chemotherapy-induced apoptosis by promoting senescence. Our results identify a new mechanism of p38α-regulated basal autophagy that controls the fate of cancer cells in response to drug treatments.

Cell senescence, autophagy and apoptosis cooperate in tissue remodeling during early inner ear morphogenesis

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The embryonic development of the organs requires tissue reshaping that is highly regulated at the genetic level. During the earliest stages of otic development, the otocyst undergoes rounds of cell proliferation to grow a spherical structure formed by a pseudostratified epithelium, which contains most of the molecular cues to form the adult inner ear. Programmed cell death has been proposed as the singular cellular process that sculpts the shape of the embryonic inner ear. However, recent evidences suggest that it is coordinated with other tissue remodeling events that ensure the outcome of morphogenesis in an increasing number of developing tissues. Cellular senescence participates in tissue repair, cancer, aging, and, more recently, it has been associated with embryonic morphogenesis. In this regard, senescent cells show a highly regulated temporal pattern in the developing vertebrate inner ear. Cell senescence is transiently associated with dorsal areas of the otocysts that show increased apoptosis and reduced proliferation, indeed the induction of senescence occurs at the time at which the endolymphatic duct is being formed. Furthermore, modulation of senescence by Navitoclax and Palbociclib disrupts otic vesicle morphology. Cell autophagy provides the elimination of damaged organelles and the energy required to facilitate remodeling. At the molecular level, transforming growth factor β2, its receptors and downstream targets are expressed in the developing otocysts. Transforming growth factor β2 activation drives the senescent response in the otocyst leading to morphogenesis and cell differentiation. We propose here that senescence and apoptotic cell death coordinately contribute to tissue remodeling along the development of the vertebrate inner ear.

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Oral presentations

Session 10/09 Senescence: Metabolism (II)

A cytosolic NAD+ regenerating complex bypasses senescence and contributes to transformation

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Senescence is a barrier against tumor formation maintained by the tumor suppressors p53 and RB, whose actions on the cell cycle are well understood. However, their role on the metabolic rewiring that underpins senescence is largely unknown. Here we show that a previously unappreciated cytosolic NAD+ regenerating complex (CNARC) bypasses senescence and promotes cellular transformation. CNARC is formed by Malate Dehydrogenase (MDH1), the Malic Enzyme 1 (ME1) and a cytosolic fraction of Pyruvate Carboxylase (PC). These enzymes are repressed in senescent cells, but their levels are restored by inactivation of p53 or RB. Exogenous expression of the three CNARC enzymes bypassed senescence in response to onocogenic RAS or STAT3 depletion, whereas their inactivation restored senescence in cells with disabled p53. CNARC enzymes were found highly expressed in a mouse model of prostate cancer driven by PTEN/STAT3 loss and in samples from human prostate cancer patients. Collectively, we provide evidence for a hitherto elusive NAD+ regenerating pathway which overcomes senescence and contributes to tumorigenesis.

Interplay between cell senescence and autophagy; the lesson from post-mitotic neural and highly mitotic cancer cells

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According to established markers of cell senescence, such as SA-β-gal activity, ROS production and secretory phenotype, both non-dividing neural and indefinitely proliferating cancer cells can achieve a senescence-like state. A considerable amount of literature has been published on the intertwining of cell senescence and autophagy, however, the reports are not consistent as to whether functional or disturbed autophagy is indispensable for cell senescence. That is why we wanted to contribute to this debate. Using rat cortical neurons and human ATM-deficient neural cells (A-T cells) we have documented a DNA-damage-independent senescence-like state in cells grown in vitro. Senescing A-T cells had impaired autophagic flux and showed disturbances in mitophagy, which indicates that neural cell senescence is associated with autophagy disturbance. In MCF-7 breast cancer cells, which have a quite high autophagic index (functional basal autophagy), drug-induced lysosomal membrane permeabilization (LMP) led to both induction of autophagy (lysophagy) and inhibition of the autophagic flux. Prolonged LMP resulted in rapid caspase-independent death of all cells, but if the drug was withdrawn after short treatment and the autophagic flux were restored, the cells survived and part of them underwent senescence. In MDA-MB-231 breast cancer cells, which are characterized by a very low autophagic index (halted autophagic flux), therapy-induced senescence did not influence it, proving that these cells can proliferate and cease to proliferate (undergo senescence) independently from autophagy disturbances. However, senescence escapers had increased autophagy index, which means that autophagy was restored in these cells. In HCT 116 colon cancer cells (with high autophagic index), therapy-induced senescence was strictly connected with autophagic flux inhibition. The lesson from the cellular models used seems to be that disturbances in autophagy are in some cases linked to the features of senescence (neural cells), but neither proper functioning of autophagy nor autophagy disturbances can be considered as a cause or consequence of cancer cell senescence.

Oral presentations

Session 10/09 Senescence: Metabolism (II)

Proteome instability as a main hallmark of ageing and cancer

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Viability of metazoans largely depends on their ability to regulate metabolic processes for producing energetic molecules, as well as on their capacity to mount anti-stress responses for retaining the integrity and functionality of biomolecules (e.g. genome and proteome). At the whole organism level, these pathways and responses require complicated, largely unknown in its details, co-regulation and wiring of cell autonomous and non-autonomous mechanisms. Proteome stability in cells is assured by the proteostasis network (PN), a modular integrated system which ensures proteome quality control at both basal conditions and under conditions of proteotoxic stress. Key components of the PN are the molecular chaperones and the two main degradation machineries, namely the Autophagy Lysosome- and the Ubiquitin Proteasome- pathways. The PN branches are functionally coordinated by stress sensors, which respond to deregulated proteostasis and/or to increased amounts of stressors. These sensors are mostly short-lived transcription factors, e.g. forkhead box O (Foxo), heat shock factor-1 (Hsf1) and nuclear factor erythroid 2-related factor (Nrf2), that mobilize cytoprotective genomic responses against various types of stress. Our analyses in normal human cells and at the model organism Drosophila melanogaster indicate that the functionality of proteostatic modules and anti-stress responses decline during aging and these events fuel the appearance of aging and/or age-related diseases. Notably, by analyzing several cellular models of carcinogenesis in human cells and also biopsies from patients we found that tumor progression is characterized by aberrant reactivation of proteostatic and anti-stress modules in advanced metastatic tumors.

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Eat, pray, degrade: Potential anti-ageing strategies offered by Mother Nature (and not only)

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Ageing is a complex process affected by both genetic and environmental factors, characterized by a gradual failure of functionality, reduced stress response and resistance, leading to enhanced probability for age-related diseases and mortality. During the last decades, natural compounds have attracted the attention of researchers in the quest of bioactive phytochemicals with anti-ageing properties. For few of those compounds an extra advantage appears; many of them have been shown to decelerate the progression of age-related diseases with emphasis on aggregation-related diseases. Using the nematode Caenorhabditis elegans along with the replicative senescence model of human primary fibroblasts, we have identified compounds that are part of our diet with anti-oxidation, anti-ageing and anti-aggregation activities. Some of the identified compounds promote their anti-ageing activity through activation of the proteasome, others through the activation of Nrf2 transcription factor, while others through inhibition of glucose transporters (GLUTs). Our work identifies new bioactive compounds with anti-ageing and/or anti-aggregation properties or reveals additional beneficial properties on already known bioactive compounds.

CDK4/6 inhibitors induce and modulate senescence responses

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Most used anti-cancer therapeutic approaches are based on impairing mitosis and targeting highly proliferative cells. The non-specificity of these interventions often leads to short- and long-term side effects, and a general accelerated aging phenotype. Chemotherapy agents induce premature cellular senescence in both mice and humans. Chemotherapy-induced senescent cells develop a strong secretory phenotype (SASP) and contribute to chemotoxicity. Accordingly, genetic or pharmacological senolysis delays or prevents the onset of several therapy-induced side effects. Here, we show that a novel class of oncological drugs -- the inhibitors of Cyclin-Dependent Kinases (CDK)-4/6 – also induces a permanent state of growth arrest and premature senescence in culture and in vivo. The growth arrest is dependent on p53 transcriptional activity, and cellular models lacking functional p53 restore proliferation upon drug withdrawal. In contrast to chemotherapy, CDK4/6i-induced senescent cells do not develop a pro-inflammatory SASP as shown by RNAseq, cytokine arrays and in-tissue analyses. CDK4/6i-induced senescent cells do not exert any paracrine detrimental effects, and are well tolerated in mice with minimal side effects. Interestingly, treatment of chemotherapy-induced senescent cells with CDK4/6i is sufficient to reduce the SASP and promote health benefits. Together, our data suggest that CDK4/6i can generate de novo cellular senescence without detrimental features, but also reduce the SASP of pre-existing pro-inflammatory senescent cells. Unraveling mechanisms regulated by CDK4/6 inhibition might provide insights into the relationship between senescence-associated growth arrest and secretion, and can be exploited for the design of anti-cancer therapies with reduced side effects.

Identifying key senescent cell types that drive aging

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Senescent cells are well-established to play a causal role in aging and age-related disease. They appear to do so largely via their secretory phenotype, which disrupts tissue homeostasis and drives chronic sterile inflammation. We found that endogenous DNA damage, which occurs spontaneously as a consequence of normal cellular metabolism, is sufficient to drive cellular senescence in vivo. Ercc1$^{-/\Delta}$ mice, which express 5% of the normal complement of the DNA repair endonuclease ERCC1-XPF, at 5 months of age, have an equivalent amount of oxidative cyclopurine adducts as 30 month-old wild-type mice. The young mutant mice also have the same burden of senescent cells as aged WT mice as measured by a p16-luciferase reporter transgene or qRT-PCR measurement of p16, p21 and SASP factor mRNA. Notably, the location and level of senescence marker expression is nearly identical between Ercc1$^{-/\Delta}$ and old WT mice across a panel of 14 tissues. Administration of senolytics drugs to Ercc1$^{-/\Delta}$ mice to eradicate senescent cells improves age-related symptoms, histopathology and functional decline as occurs in aged WT mice. To begin to define which senescent cells must be targeted in vivo in order to achieve the maximum benefit from senolytics, we created a panel of tissue-specific Ercc1 mutant mice. In most cases, deletion of Ercc1 in a single organ or cell type resulted in premature accumulation of senescent cells in that tissue and premature onset of age-related disease. This approach enables us to identify which senescent cells are most potent at driving senescence and aging in trans, and therefore must be targeted therapeutically.

Oral presentations

Session 11/09 Drug discovery - Senolysis -Therapeutics

Systemic senolysis in naturally aged mice using a fusogenix gene therapy approach

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Therapeutic approaches to eliminate senescent cells (SCs) in vivo using transgenic mouse models have demonstrated significant improvements in lifespan, reduction of cancer and amelioration of age-related degeneration. Unfortunately, this approach requires that the organism be genetically engineered from the embryo and thus cannot be implemented in humans. We describe a clinically viable gene therapy consisting of a suicide gene under a senescent cell promoter delivered in vivo with fusogenic lipid nanoparticles (LNPs). These LNPs employ fusion-associated small transmembrane (FAST) proteins that can efficiently transduce a wide range of cells in vivo without observed toxicity or off-target effects. Selective ablation of target cells is then achieved through the expression of a potent pro-apoptotic transgene driven by a senescence-associated promoter such as p16Ink4A or p53. Here, we describe targeting of p16Ink4A-positive or p53-positive cells in vitro and in vivo using this method and the in vivo clearance of SCs in a dose-dependent manner. Results of repeated dosing using constructs targeting various promoters are presented, including biodistribution and toxicity of LNPs in non-human primates. We will also present results of a lifespan/healthspan study in a cohort of naturally aged C57BL/6 mice (105 week-old at start of trial), where we observe significant improvements in median lifespan, as well as improvements in associated health indicators such as bone density. In summary, this approach represents a first-in-class therapeutic that targets cells based on transcriptional activity, rather than surface markers or metabolism.

Progress towards development of clinically-relevant senotherapeutics for extending healthspan

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Cellular senescence is caused by the accumulation of DNA damage and/or other cellular stressors that drives proliferating or terminally differentiated non-dividing cells to a state characterized by replicative arrest, profound chromatin changes, increased expression of the cell cycle inhibitor p16Ink4a and resistance to apoptosis. Some senescent cells can develop a senescence-associated secretory phenotype (SASP), consisting of pro-inflammatory cytokines, chemokines, and extracellular matrix-degrading proteins, which have deleterious paracrine and systemic effect. Cell senescence is one of the hallmarks of aging known to negatively influence a healthy lifespan and thus represents a target for drug development. Drugs able to kill senescent cells specifically in cell culture, termed senolytics, or suppress markers of senescence, termed senomorphics, can reduce the senescent cell burden in vivo and extend healthspan. We have developed a senescent cell culture, C12FDG-based SA-ß-gal assay for screening for agents able to function as senotherapeutics. Using this assay, we identified a number of senotherapeutics including HSP90 inhibitors, Bcl-2 family inhibitors, fisetin and the combination of dasatinib/quercetin as senolytics and IKK/NF-kB inhibitors, mitochondrial targeted free radical scavengers and young stem cell-derived extracellular vesicles as senomorphics. The activity of these senotherapeutics to reduce senescence and extend healthspan has been confirmed in the Ercc1-/Δ mouse model of accelerated aging carrying a p16INK4a-Luciferase reporter and, in some cases, in naturally aged mice. In addition, several of these senotherapeutic compounds currently are in Phase IIb clinical trials for assessing their ability to reduce the senescent cell burden. Progress towards optimizing the activity of these identified senotherapeutics as well as the identification of novel senotherapeutics will be presented.

Oral presentations

Session 11/09 Drug discovery - Senolysis -Therapeutics

Identification and characterization of cardiac glycosides as a novel class of broad spectrum senolytic compounds

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The development of compounds with specific cytotoxic activity in senescent cells, known as senolytics, offers an extraordinary support for the idea that senescence is causally involved in age-related diseases and holds the promise of new tools to treat them. Using a cell-based phenotypic screening, we identified Cardiac Glycosides (CGs) as a family of compounds with novel senolytic activity. CGs, by targeting the Na+/K+ ATPase pump, cause a disbalanced electrochemical gradient in which Na+/K+ and H+ are altered within the cell causing depolarization and acidification. Senescent cells present a slightly depolarized plasma membrane and higher concentrations of H+, making them more susceptible to the action of CGs. These vulnerabilities can be exploited for therapeutic purposes as evidenced by the in vivo eradication of tumors xenografted in mice after treatment with the combination of a senogenic and a senolytic drug. The senolytic effect of CGs was also effective in the elimination of senescence-induced lung fibrosis. The use of this experimental approach is a promising tool to identify new compounds with senolytic activity that could potentially be used to develop novel effective treatments against age-related diseases.

Nanomaterials and cellular senescence

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Senescent cells are found to be aberrantly accumulated to a variety of ageing and multiple chronic diseases in humans. It is reasonable that the elimination of senescent cells could ameliorate and perhaps revert the progression of senescence. The development of innovative therapeutic and diagnostic applications or drug delivery systems based on nanotechnology could allow the specific targeting of senescent cells [1]. It is now well established that nanomaterials, characterized by high specific surface area that correlates with high interfacial chemical and physical reactivity can in turn, be biologically reactive in various ways either by inducing cell death or by binding and stimulating a cellular response. Magnetic nanoparticles (NPs), for MRI, or nanomaterials for PET, SPECT etc. have been developed in order to optimize the detection of senescent cells for diagnostic purposes. Coated mesoporous silica nanoparticles, zinc oxides are among the most famous NPs which can interact with senescent cells, focusing on clearance and tissue repair and regeneration as a monotherapy or in combination with chemotherapy or radiotherapy [2].
Our approach is to use NPs, such as titanium dioxide, in order to induce controlled ROS-mediated cellular senescence on cancer cells, as it is well known that photoactivated TiO2 can generate ROS which are implicated in cellular senescence, as an alternative “chemotherapeutic” treatment avoiding the side effects [3]. In the next level, the develop or the use of another type pf NPs in order to trigger the clearance mechanism could be the transformation of the phenotype of these cells, allowing cell repair.

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Immune system controls dynamics of accumulation of senescent cells during ageing

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Cellular senescence is a stress response that imposes stable cell-cycle arrest in damaged cells, preventing their propagation in tissues. However, senescent cells accumulate in tissues in advanced age, where they might promote tissue degeneration and malignant transformation. The dynamics of this accumulation, its basic rules and regulating mechanisms are still mysterious. Immune system controls presence of senescent cells in different pathologies, but the extent of immune-system involvement in regulating age-related accumulation of senescent cells, and its consequences, are unknown. We show that Prf1-/- mice with impaired cell cytotoxicity exhibit both higher senescent-cell tissue burden and chronic inflammation. They suffer from multiple age-related disorders and lower survival. Strikingly, pharmacological elimination of senescent-cells by a Bcl-2 family inhibitor ABT-737 partially alleviates accelerated aging phenotype in these mice. In progeroid mice reduced survival is also concomitant with accumulation of senescent cells. Impaired cell cytotoxicity further promotes senescent-cell accumulation and shortens lifespan of these mice. ABT-737 administration during the second half of life of these progeroid mice abrogates senescence expression signature in multiple tissues and increases median survival. Our findings show for the first time that accumulation of senescent cells during aging is regulated by the immune system. We also demonstrate that even transient elimination of senescent cells can lead to reduction of inflammatory load, improved structure and function of multiple tissues, and extend median survival. In order to understand the rules of senescent cell dynamics we performed longitudinal measurements of senescent cells following senescence induction in mice. We show that senescent cells are in rapid turnover in young mice. However, they slow their own removal rate in old mice, leading to persistent fluctuations in amount of senescent cells. Our findings shed new light on mechanisms governing senescent-cell accumulation in aging and possibilities of its therapeutic modulation.

Bacterial genotoxins and senescence: is there a role of the tissue microenvironment?

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Bacterial genotoxins are unusual effectors that cause DNA damage in the eukaryotic cells, inducing activation of the DNA damage response, which often results in induction of senescence. These toxins are produced by Gram negative commensal and pathogenic bacteria, and are enriched in the microbiota of Inflammatory Bowel Disease (IBD) and colorectal cancer (CRC) patients. However, their role in the modulation of the host-microbial interaction in health and disease remains unclear. Using isogenic strains of Salmonella enterica expressing or non-expressing a functional genotoxin, known as typhoid toxin, we showed that the presence of this effector suppresses the intestinal inflammatory response to the infection. This effect was associated with reduced recruitment of leukocytes and macrophages at 5 days post-infection, and decreased levels of T lymphocytes at 10 days post-infection. The macrophages present in the mucosa of mice infected with the genotoxic strain expressed predominantly the alternatively activated phenotype (M2). Activation of the NF-kB, assessed by nuclear translocation of the p65 subunit, was similar in mice infected with the control or the genotoxic Salmonella strain indicating that a functional typhoid toxin induced a qualitatively different immune response, which was confirmed by the transcriptomic analysis showing reduced mRNA levels for T helper (Th) 1 cytokines (IFN-γ and IL12), and increased mRNA levels for Th2 cytokines (IL10, IL13 and IL9). We detected presence of senescence cells both in the epithelial and stroma compartment

Oral presentations

Session 11/09 Senescence - Inflammation - Immunosenescence

Autophagy-induced and SASP-free senescence in human cells and its anti-cancer implications

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Autophagy is intimately involved in cellular senescence and directly implicated in the induction of the SASP. Here, we apply a small molecule inhibitor designed to interfere with the mode-of-action of HMGB family proteins and obtain a fast, homogeneous, and SASP-free induction of replicative senescence across populations of human primary cells. We then use a range of whole-genome approaches to follow changes in gene expression (RNA-seq), protein levels (quantitative proteomics), and translation rates (Ribo-seq). Integrative data analysis uncovers a link between chromatin reorganization, autophagy induction, and SASP regulation with HMGBs at the center of this network. Finally, our discovery offers a potential anti-cancer intervention path as exemplified by application of this small molecule inhibitor to lymphoma cancer samples from a cohort of patients.

Dissecting the impact of cell senescence in the hematopoietic compartment

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Cellular senescence is a physiologic stress response program elicited, by DNA damage accumulation and the expression of activated oncogenes. Senescence is characterized by proliferation arrest and by the activation of a senescence-associated secretory phenotype characterized by factors linked to inflammation, proliferation, and modulation of the extracellular matrix, collectively named as SASP. Senescence contributes to tumor suppression and tissue repair. However, accumulation of senescent cells leads to aging and chronic inflammatory pathologies. Despite the well-characterized role of senescence programs in differentiated fibroblasts or epithelial cells, many fundamental aspects of senescence in stem cell physiology remain poorly elucidated. Hematopoetic Stem Cells (HSCs) serve as a lifelong reservoir for mature blood cells. Importantly, the capacity of HSCs to constantly replenish the hematopoietic compartment upon stressors requires active mechanisms that ensure a careful balance between HSC self-renewal potential and differentiation outputs. We dissected the molecular determinants of HSCs response to several senescence-inducing stimuli, including DNA Double Strand Breaks (DSBs) and activated oncogenes. We studied the transcriptional impact of senescence stressors in an unbiased manner and interrogated individual cells within the heterogeneous hematopoietic stem/progenitor population. We also deciphered the complex network of inflammatory SASP by protein arrays and identified potential cytokines/chemokines responsible to transmit senescence signals to neighboring cells in a paracrine fashion. Finally, our mechanistic studies led to the identification of novel therapeutic targets that, when inhibited, counteracted potential detrimental effects of cellular senescence on HSCs functionality and during hematopoietic reconstitution.

Oral presentations

Session 11/09 Senescence - Inflammation - Immunosenescence

DNA damage and innate immune signaling in development and disease

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Recent advances suggest that DNA damage and innate immune responses are intimately linked in mammals triggering chronic inflammation, cellular malfunction and tissue degeneration with advancing age. Using a unique series of animal models with engineered defects in nucleotide excision repair, we will discuss the functional role of persistent DNA damage signaling in immune activation and the role of DNA damage-driven chronic inflammation in metabolic disorders and age-related diseases.

Immune signatures in age-related diseases

Author: Ourania Tsitsilonis

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Immunosenescence, i.e., age-related changes that affect the immune system both at functional and structural level, is a crucial component of ageing. It is manifested, among others, by the increased vulnerability of aged individuals to infectious diseases, their decreased ability to respond to vaccination, the increased frequency of autoimmune disorders, the higher prevalence of cancer, and the systemic and chronic low-grade inflammation, else termed inflammaging. The increase of the later with age is now considered a substantial driver of the higher cancer incidence and progression in old people. Immunosenescence affects the function of innate and adaptive immune cells, leading to reduced numbers of naïve B and T cell populations and increased numbers of terminally differentiated T cells in the periphery, dysfunctional chemotaxis and phagocytosis, dysregulated cytokine production and impaired responsiveness of immune cells to TLR and cytokine signaling. In an effort to identify biomarkers of "healthy" ageing, we studied the frequency and competence of immune cell populations in the peripheral blood of older adults who received an adjuvanted influenza vaccine, as well as in the blood and bone marrow of aged patients with multiple myeloma, and propose immune signatures that can eventually be used to predict response to vaccination and drug treatment, respectively. We also present preliminary results of a novel flow cytometry-based method, with which senescent cells stained with SenTraGor® can be easily detected and quantified.

Transitioning from a prognostic to a predictive paradigm of prostate cancer classification

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Advances in the treatment of prostatic carcinoma have not paralleled advances in other common tumor types. This has in part been overcome by the recent understanding of the underlying biology that accounts for the clinical phenotype. It is becoming clear that prostate cancer is driven by clinically relevant and heterogeneous disease biology that is often, but not always, manifest at presentation. The evidence that, with time, a low-grade adenocarcinoma with a combined Gleason score of 6 can become a potentially lethal cancer still does not exist. This has created a therapeutic dilemma because early diagnosis with unclear penetrance exposes patients to the risk of overtreatment, whereas patients with more advanced disease are exposed to the risk of ineffective or delayed intervention. To overcome this, we have shifted to a biological classification of the disease. It is now recognized that 60% of patients can be grouped into therapeutically relevant categories that predict androgen indifference, responsiveness to AR signaling inhibition, and may also anticipate bone metastases. Our understanding of the molecular drivers of androgen indifference or castrate progression is now being applied to develop a marker-driven predictive strategy. Future studies will examine the interactions between these "domains of progression" and "functionalize" these observations using corresponding animal models inform discovery. The results will be used to link biology to clinical decision making. However, none of these account for the dimension of time and the evolution of cancer under the influence of therapy. Liquid biopsy strategies enable longitudinal monitoring of patients. We can now monitor the evolution of the epithelial compartment of the cancer over time and assess the homotypic and heterotypic cell–cell interaction central to the development of prostate cancer bone metastases. This is preliminarily achieved by serial characterization of the transcriptional profiles of exosomes in circulation, plasma DNA, and cytokine. Taken together, these data allow us to intervene and make informed course adjustments based on markers that promise to transform the care of patients with prostate cancer, optimizing treatment in a disease state–specific manner.

Accelerated ageing of the brain in multiple sclerosis

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Background: Brain atrophy occurs in both normal ageing and in multiple sclerosis (MS), but it occurs at a faster rate in MS, where it is the major driver of disability progression. Here, we employed a neuroimaging biomarker of structural brain ageing to explore how MS influences the brain ageing process.
Methods: In a longitudinal, multi-centre sample of 3,565 MRI scans in 1,204 MS/clinically isolated syndrome (CIS) patients and 150 healthy controls (HCs) (mean follow-up time: patients 3·41 years, HCs 1·97 years) we measured 'brain-predicted age' using T1-weighted MRI. Brain-predicted age difference (brain-PAD) was calculated as the difference between the brain-predicted age and chronological age. Positive brain-PAD indicates a brain appears older than its chronological age. We compared brain-PAD between MS/CIS patients and HCs, and between disease subtypes. In patients, the relationship between brain-PAD and Expanded Disability Status Scale (EDSS) at study entry and over time was explored.
Results: Adjusted for age, sex, intracranial volume, cohort and scanner effects MS/CIS patients had markedly older-appearing brains than HCs (mean brain-PAD 11·8 years [95% CI 9·1-14·5] versus -0·01 [-3·0-3·0], p<0·0001). All MS subtypes had greater brain-PAD scores than HCs, with the oldest-appearing brains in secondary-progressive MS (mean brain-PAD 18·0 years [15·4-20·5], p<0·05). At baseline, higher brain-PAD was associated with a higher EDSS, longer time since diagnosis and a younger age at diagnosis. Brain-PAD at study entry significantly predicted time-to-EDSS progression (hazard ratio 1·02 [1·01-1·03], p<0·0001): for every 5 years of additional brain-PAD, the risk of progression increased by 14·2%.
Conclusion: MS increases brain ageing across all MS subtypes. An older-appearing brain at baseline was associated with more rapid disability progression, suggesting 'brain-age' could be an individualised prognostic biomarker from a single, cross-sectional assessment.

Oral presentations

Session 11/09 Senescence in Human Diseases

Senescence in oncology: linking cell biology to clinical care

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Oncogene-induced senescence (OIS) is widely accepted as a pivotal tumor-suppressive barrier against imminent malignant transformation. Likewise, there is ample evidence that therapy-induced senescence (TIS) – upon chemotherapy, but probably other modern cancer therapeutics as well – operates as a tumor-controlling response program, especially if apoptosis as the primary drug effector mechanism is no longer available. Fueled by elegant work from transgenic mouse models in which p16INK4a-high senescence-like cells may be continuously removed during aging, leading to reduced age-related organ pathologies and extended lifespan, chronic cancer cell senescence is increasingly viewed as a detrimental component of long-term outcome – irrespective of pre-clinical and clinical data underscoring a lasting benefit of an intact as compared to an impaired senescence capacity in the first place. How senescent cancer cells may promote tumor progression and relapse is an issue of debate, but chronic inflammation via the senescence-associated secretory phenotype (SASP), occasional cell-cycle re-entry out of senescence, and the execution of novel, senescence-acquired capabilities such as senescence-associated stemness reflect some of the less desirable features senescent cells may present with. On the contrary, senescent cells not only prompt their own removal by the host's innate immune system, but might evoke a somewhat more specific anti-tumor immune control that may require ongoing re-stimulation by senescent cancer cells. With all this in mind, comprehensive time-course analyses of genetically senescence-capable vs. -incapable tumors in their natural, immune-competent environments are needed to judge the actual net contribution of tumor cell senescence on treatment outcome. Simultaneously, pre-clinical studies in appropriate model settings are critical to systematically evaluate the impact of senolytic treatment strategies, i.e. co-therapies that aim at selectively eliminating senescent cells, or feature-focused strategies, e.g. co-therapies that seek to ablate the SASP, on long-term outcome. Examples of our approaches to this central problem in clinical oncology will be presented at the conference.

Markers of senescence and cancer prognosis

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The accumulation of senescent cells that have entered a permanent cell cycle arrest occurs gradually in all normal and malignant tissues. Such cells, being far from being inactive, affect bi-directionally the tissue function, as they induce both deleterious and beneficial effects. On the one hand, senescent cells contribute to the creation of a proinflammatory milieu, promoting tissue degeneration and carcinogenesis. On the other, the entrance of damaged cells in senescence protects against cancer development and is essential in tissue repair and remodeling. The role of senescent cells in the growth, invasion, and metastasis of cancer and how they affect prognosis or response to antineoplastic therapy remains obscure. A significant problem in the study of senescence in malignancy is the lack of reliable methods to identify senescent cells and, more importantly, specific subtypes of senescence (e.g., secretory phenotypes). Senescent cells exhibit specific morphological and functional changes that are used to develop methods for their identification. Accumulation of lysosomes is a central feature detectable by the overactivity of beta-galactosidase (SA-bgal), the Sudan black and SenTraGor histo/immunohistochemical detection of lipofuscins, or even the detection of LAMPs and TFEB. Accumulation of mitochondria with decreased membrane potential is another hallmark, eventually a result of reduced mitophagy, that can be detected by measurement of the increased content of endonuclease G (EndoG) and high ROS production. Loss of Lamin B1 and nuclear accumulation of EndoG are markers of nuclear changes developed in senescent cells. Up-regulation of caveolin-1, detection of DEP1 and B2MG or oxidized forms of Vimentin are useful markers to detect senescence-related changes of plasma membrane composition. Chronic DNA damage detectable by γH2AX phosphorylated histone accumulation in nuclei, up-regulation of cyclin-dependent kinase inhibitory molecules like p16INK4a or secretion of pro-inflammatory and tissue remodeling factors like CXCL1/CXCL2, IL-10, and IL-13 are also important markers of specific types of senescence. Our knowledge of all the above senescence-related pathways in the prognosis of cancer patients and the results of therapy is limited. Overexpression of p16, for example, has been related to early relapse of prostate cancer, advanced stage and poor prognosis in lung and breast cancer. High expression levels of lamin B1 predicts disease recurrence in prostate and pancreatic cancer. Accumulation of γH2Ax in the nuclei is associated with poor response of breast cancer to chemotherapy, ovarian, and gastric cancer. The prognostic and predictive role of senescence should be thoroughly examined in the future, also given the importance of targeting such cells for the development of therapeutic anti-cancer approaches.

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Oral presentations

Session 11/09 Senescence in Human Diseases

Elimination of senescent astrocytes in the brain tumor microenvironment attenuates glioblastoma recurrence after radiotherapy

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Glioblastomas (GBM) are lethal brain tumors for which ionizing radiation (IR) remains the mainstay of therapy. However, these tumors inevitably recur, and the recurrent tumors are highly therapy resistant. During GBM therapy, both the tumor and the surrounding brain tissue are irradiated with 50-60 Gy of IR. IR induces senescence in multiple cell types, and senescent stromal cells are known to promote the growth of neighboring tumor cells by secreting a panoply of pro-tumorigenic cytokines and chemokines which create a senescence-associated secretory phenotype (SASP). We hypothesize that IR-induced senescence of normal brain cells in the tumor microenvironment is a powerful driver of tumor recurrence and therapy resistance. To examine if IR triggers senescence of normal brain cells, we intra-cranially irradiated C57BL/6J mice, and found that irradiated brains exhibited widespread senescence by 30 days post-IR, with the astrocytic population being highly susceptible. Genomic analyses of irradiated brains revealed an altered transcriptomic profile which included upregulation of CDKN1A (p21), a key enforcer of senescence, and increased expression of a number of bona fide SASP proteins including HGF, the ligand for the RTK Met. We orthotopically implanted mock-irradiated or irradiated mice, at 30 days post-IR, with a limiting number of syngeneic GL261 or CT2A glioma cells. Pre-irradiation of mouse brains resulted in a striking increase in tumor growth rates, with the resulting tumors showing a more aggressive phenotype including increased infiltration, angiogenesis and necrosis, and activation of Met. Importantly, irradiated p21-/- mouse brains did not exhibit SASP and failed to promote tumor growth, thereby substantiating the link between IR-induced senescence and GBM development. Irradiated primary astrocytes underwent senescence in vitro and secreted a number of SASP factors including HGF. Senescent astrocytes could promote the migration and invasion of glioma cells in vitro, and this could be attenuated with HGF-neutralizing antibodies or by the Met inhibitor Crizotinib. These findings indicate that SASP factors (like HGF) in the irradiated GBM microenvironment could drive GBM recurrence after radiotherapy via the activation of RTKs (like MET) in the tumor cells. Significantly, we found that senolytic drugs can selectively kill senescent astrocytes both in vitro and in vivo resulting in decreased HGF levels and attenuated growth of glioma cells. These results are of great translational significance as they indicate that adjuvant therapy with senolytic drugs might attenuate GBM recurrence after radiotherapy.

Cellular senescence - from biological phenomenon to valuable clinical tool

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Although our knowledge of the mechanisms underlying cellular senescence increases constantly, its' clinical implications are somewhat less clear. This holds true especially in the field of clinical oncology and pathology. This can at least partly be attributed to the fact, that (a) there are a plethora of different stimuli leading to a number of different "types" of cellular senescence. And (II) until recently, there is no valid marker, to reliably identify senescent cells in particular on archived patient material. In various projects we are thus establishing a marker profile to reliably detect cellular senescence on FFPE material of large cohorts of cancer patients. We hope to gain a further insight into the prognostic and predictive implications of senescent cells and their interactions with various other tissue components, i. e .immune cells. This is achieved by using state-of-the-art digital image analysis and comprehensive tissue morphometry on advanced tissue microarrays. Results of these multi-staining experiments are then correlated with detailed clinical and survival data of hundreds of patients with various types of cancers. They are then further explored in an in vitro co-culture model of proliferating or senescent tumor cells and various immune cell subtypes.

Multimorbidity phenotypes as a potential target for intervention

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Aging is featured by the progressive accumulation of chronic diseases, which leads to multimorbidity. Diseases start piling up since the early adult life, and their burden in old age is cause of disability, poor quality of life and shorter survival. However, multimorbidity does not progress at the same pace across different individuals, and evidence showed that a relevant share of the older population could live free from disability in spite of multimorbidity. During this presentation, the speaker will support the idea that the study of the longitudinal development of multimorbidity clusters, the knowledge of the mechanisms underlying a faster development of multimorbidity, and the investigation of the relationship existing between multimorbidity, functional decline and frailty, might help to identify new phenotypic targets for intervention to slow down the aging process.

The future of Clinical Biogerontology

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The aging process occurs gradually with a high degree of inter and intra-individual differences. As such, within an aging population there is significant variation in the prevalence and severity of age related diseases and functional impairment. This variability between individuals is thought to be reflected by their biological age. Currently, the clinical (geriatric) assessment is a multidimensional, interdisciplinary diagnostic process used to determine an individual's medical, psychological and functional capability at older age. While the clinical assessment utilizes well-established markers of physical and functional parameters, it does not include any molecular measures that indicate underlying pathophysiological mechanisms. Combining functional measures with molecular markers could improve the current clinical assessment by identifying individuals undergoing a rapid aging process. Cellular senescence is one of the prominent markers of ageing and it has been shown that the number of senescent cells is higher at advanced chronological and biological age. Although no biomarkers indicative of biological age are currently being utilized in the clinical setting, promising research advancements would suggest their application in intervention studies aiming to slow down the ageing process.

Oral presentations

Session 12/09 Age related diseases -Aging - Geroscience

Incriminating senescent cells to neurodegenerative pathologies

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Perhaps the single greatest risk factor for the development of most chronic degenerative diseases in people is advancing age. A potential molecular mechanistic explanation of this relationship is that fundamental processes of aging actively promote tissue dysfunction and age-associated diseases. One plausible culprit may be the accumulation of senescent cells, as recent studies have found cellular senescence is a fundamental process of aging that often associates with disease states. These cells are thought to disrupt normal tissue functions through both cell intrinsic and extrinsic mechanisms.
Importantly, cells with features reminiscent of senescence have been found in a variety of neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, suggesting that senescent cells may contribute to neurodegenerative pathology. Using mice prone to neurodegeneration due to neurofibrillary tangle deposition, we find that senescent cells actively promote the initiation, progression, and severity of symptoms. Through elimination of these cells, by either genetic or pharmacological means, we were able to dramatically attenuate disease pathology. Importantly, treatment of mice with established pathology with senescent-cell eliminating strategies can reverse histological features of AD pathology. These data suggest that these cells may be potential therapeutic targets for intervention in debilitating neuropathologies. Additional studies related to senescent cells in other neurological conditions will also be highlighted.

Proteasome activation delays aging and progression of age-related diseases

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Aging and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have studied proteasome function in replicative senescence and cell survival (Mol Aspects Med 35, 1-71; Ageing Res Rev 23, 37-55). We have observed reduced levels of proteasome content and activities in senescent cells due to the down-regulation of the catalytic subunits of the 20S complex (J Biol Chem 278, 28026-28037). In support, partial inhibition of proteasomes in young cells by specific inhibitors induces premature senescence which is p53 dependent (Aging Cell 7, 717-732). Stable over-expression of catalytic subunits or POMP resulted in enhanced proteasome assembly and activities and increased cell survival following treatments with various oxidants. Importantly, the developed "proteasome activated" human fibroblasts cell lines exhibit a delay of senescence by approximately 20% (J Biol Chem 280, 11840-11850; J Biol Chem 284, 30076-30086). Similar proteasome activation in human mesenchymal stem cells not only increases their lifespan, but also enhances stemness significantly (Free Rad Biol Med 103, 226-235). Moreover, additional findings indicate that the recorded proteasome activation by many inducers is Nrf2-dependent (J Biol Chem 285, 8171-8184). Finally, we provide evidence that proteasome activation is an evolutionary conserved mechanism, as it can delay aging in vivo and, importantly, it also confers deceleration of aggregation-related pathologies, such as Alzheimer's or Huntington's diseases (FASEB J 29, 611-622). Given these findings, recent work has identified a proteasome activator that decelerates aging and Alzheimer's disease progression (Antiox Redox Signal 25, 855-869).

There are no financial interests to disclose

Oral presentations

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Exploring p.A53T-alpha synuclein pathology in a human induced pluripotent stem cell-based model

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Parkinson's disease (PD) remains an incurable neurodegenerative disorder with variable clinical characteristics, age of onset and course of progression. The hallmark of PD, whether sporadic or familial, is the deposition of protein aggregates, which are composed mainly of alpha-synuclein (αSyn). αSyn is a pre-synaptic protein with N-terminal binding to acidic lipids that can sense and generate changes in membrane curvature, suggesting its participation in presynaptic events, including endocytosis and exocytosis whilst its involvement in presynaptic organization has been postulated in mice. However, the mechanisms through which mutant αSyn affects synaptic organization in a human setting remain unknown. αSyn is the major gene linked to sporadic Parkinson's disease, while the G209A (p.A53T) αSyn mutation causes a familial form characterized by early onset and a generally severe phenotype, including non-motor manifestations (Polymeropoulos, M.H., et al., Science, 1997). In this study, using cell reprogramming technologies, we have developed a robust induced pluripotent stem cell (iPSC)-based model of PD from patients harboring the p.A53T-αSyn mutation that faithfully simulates disease pathogenesis and uncovers novel disease-relevant phenotypes at basal conditions, including protein aggregation, compromised neuritic outgrowth and contorted axons with swollen varicosities containing αSyn and tau, as well as reduced synaptic connectivity (Kouroupi et al, PNAS, 2017). Patch-clamp electrophysiology and global transcriptome analysis suggested defects in synapse formation and function. Electron microscopy (EM) of p.A53T neurons indicated impaired organization of synaptic vesicle pools, microtubule disorganization and a striking accumulation of autophagic vacuoles. In agreement, impaired autophagic activity and lysosomal protein degradation was shown by immunofluorescence and biochemical analysis. Finally, artificial synapse formation (ASF) assay was used to study synaptogenesis of p. A53T neurons and monosynaptic rabies virus (RBV) tracing to assess p.A53T neuronal circuitry. Our model provides mechanistic insight of αSyn-associated neuropathology and could be useful to develop strategies to treat PD and other synucleinopathies.

Molecular control of genome surveillance mechanisms and their implication in cancer and human pathologies

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It has become increasingly apparent that, upon genotoxic stress, a major transcription-driven cellular response is activated in human cells. As recently reported1, this response involves a well-orchestrated transient alteration of the regular transcription firing program leading to fast and widespread release of RNA Polymerase II (Pol II) from promoter-proximal pausing (PPP) sites into elongation. This mechanism maximizes sensing of DNA lesions as more Pol II molecules encounter damages and thus guarantees uniform repair of actively transcribed genes regardless of damage location and transcript stability or expression level. In turn, this mechanism limits mutagenesis as demonstrated by surprisingly low and homogenous level of C>T substitutions detected across expressed regions in genotoxic-agents exposed cancer tissues such as melanoma and lung adenocarcinoma. We now have addressed the molecular details powering such transcription-driven response. By establishing precise maps of chromatin state, we addressed in highly resolutive fashion the molecular details driving the unforeseen synergy between increased chromatin accessibility and post-UVC damage transcription dynamics. We revealed that continuation of a functional transcription initiation process, driven by both sense and antisense transcription at active TSSs, throughout the damage-recovery period was required for the extended and uniform repair of transcription-blocking damages2. In addition to the above, employing high throughput NGS methodologies, we found that, the rapid senescent response that is elicited upon induction of the replication licensing factor CDC6 and is characterised by a major alteration in RNA synthesis profile3, is accompanied by substantial chromatin reorganization of proximal and distal transcription regulatory regions. Taken together, our systems biology approaches reveal important insights on how faulty transcription regulation might provoke genomic instability and disclose a new avenue for drugging DNA repair, since perturbation of Pol II initiation and transition into productive elongation in tumour cells could augment chemo-therapeutics efficacy.

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Cellular senescence and cancer: a gut microbial connection

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Over the last few decades, it has become apparent that oncogenic proliferative signals are coupled to a variety of growth inhibitory responses, such as the induction of apoptotic cell death or irreversible cell cycle arrest known as "cellular senescence". Thus, both apoptosis and cellular senescence are thought to act as important tumor suppression mechanisms. Unlike apoptotic cells, however, senescent cells remain viable for long periods of time and accumulate with age in various organs and tissues in vivo. Moreover, recent studies have revealed that although cellular senescence initially functions as a tumor suppressive process through induction of stable cell cycle arrest, it may eventually promote chronic inflammation through secretion of various pro-inflammatory factors called "senescence associated secretory phenotype (SASP)". It is therefore quite possible that accumulation of senescent cells in vivo may contribute to inflammatory disorders, such as cancer. However, it remains unclear how cellular senescence is actually induced in vivo.

The human commensal microbiome provides a variety of benefits that contribute to proper functional activity in the host through the modulation of functional processes such as signal transduction, immunity and metabolism. The unbalance of this microbial profile, or dysbiosis, has been correlated with the development of several diseases such as cancers. Here, we introduce our recent work on identification and characterization of gut bacteria which have potential to cause senescence-like phenotypes in various human cells, focusing on positive and negative roles of cellular senescence in cancer development. We believe that a better understanding of the molecular mechanisms involved will lead to new strategies for the prevention of cancer.

Senescent cells as modulators of the tumor microenvironment

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Stromal fibroblasts are key modulators of tumor microenvironment. In addition, in response to various genotoxic anticancer treatments they can become senescent, thus affecting local tissue homeostasis due to their catabolic and inflammatory phenotype. In this vein, we have previously shown that senescent cells enhance significantly the growth of cancer cells in co-cultures in vitro and in immunocompromised mice in vivo. Here, we present evidence that curative doses of ionizing radiation can provoke premature senescent of breast stromal fibroblasts, both in vitro and in vivo. These cells are characterized by an enhanced collagenolytic activity and they also have an altered expression of several proteoglycans. In particular, they overexpress syndecan-1 (SDC1), a poor prognostic factor in breast cancer development via a TGF-β-mediated autocrine loop leading to SDC1 overexpression due to the activation of the Smad pathway and of the transcription factor sp1. In addition, senescent cells down-regulate the expression of decorin, the latter having an anti-tumorigenic effect. This phenomenon is regulated by the paracrine action of a multitude of growth factors and of signaling pathways. Interestingly, these phenomena are enhanced by cancer cells in a paracrine manner. These data indicate that ionizing radiation-mediated premature senescent human breast stromal fibroblasts have an altered proteoglycan expression towards breast cancer development and these changes are enhanced by tumor cells via paracrine mechanisms.

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Tumor promoting activities of senescent cells in the skin and pancreas

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Cellular senescence is a coordinated program activated by cells in response to stress or damage. p16-expressing senescent cells accumulate in tissues during normal aging, and recent work has demonstrated their contribution to various diseases, yet their functions are generally poorly understood. In the context of cancer, senescence primarily suppresses tumor development by inducing cell cycle arrest; however, senescent cells that remain in lesions can have diverse effects, some of which promote tumorigenesis. It is currently unclear whether senolytic drugs, that preferentially eliminate senescent cells, can be beneficial for cancer treatment. To understand whether the continued presence of p16-expressing cells within tissues influences tissue structure and propensity to cancer, we activated p16 in the mouse epidermis. We found that after 6 months of activation, p16 expression led to epidermal hyperplasia and dysplasia, reminiscent of human premalignant conditions such as actinic keratosis. Furthermore, p16-expressing mice formed more papillomas upon carcinogen treatment than control mice. We found that the Wnt pathway is activated in p16-expressing epidermis, and drives hyperplasia. Thus, p16-expressing cells can promote tissue hyperpriliferation, suggesting that premalignant lesion formation can be promoted by the presence of such cells in damaged tissue regions. Senescent cells form within premalignant PanIN lesions in the pancreas formed by Kras activity. We found that these cells express high levels of the Cox2 pro-inflammatory enzyme, and that this activity promotes PanIN growth. Together our findings indicate that p16 expression and senescence can stimulate early premalignancies, and suggest that the pharmacologic elimination of these cells can be preventive of disease formation and progression.

Endothelial cell adhesion is a central regulator of blood vessel homeostasis and pathology

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Vascular homeostasis requires the translation of signals received from the extracellular microenvironment in the form of circulating factors and mechanical stimuli inside the cells, in order to elicit a cell response and regulate cell behaviour. The sites where this integration occurs are endothelial cell-matrix adhesions, that are formed by a dynamic network of signaling and adaptor proteins, organized around integrin transmembrane receptors, called adhesome. Although the role of adhesome members in orchestrating cancerous behavior is well documented, their function on the vascular system is largely unexplored. This presentation will address the role of key adhesome regulators in blood vessel morphogenesis and function during homeostasis and cancer.

Oral presentations

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Effects of chemotherapy-induced SASP in lung cancer progression

Authors: Estela González-Gualda[1], David Macías-Gutierrez[1], José-Ezequiel Martín[2], Guy Slater[4], Christian Frezza[3], Masashi Narita[4], Daniel Muñoz-Espín[1]

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Lung cancer is the leading cause of cancer-related deaths in our society. In the case of non-small cell lung cancer (NSCLC) the treatment of choice is frequently platinum-based chemotherapy in combination with other drugs. However, it often results in treatment failure, which in turn leads to only about 10% of the patients surviving within a 5-year window from the first diagnosis. Chemotherapeutic drugs, such as cisplatin, can induce cellular senescence, a process that can implement a strong paracrine secretion (SASP), reported in other models to increase the inflammatory milieu and promote malignant phenotypes. However, the impact of chemotherapy-induced senescence in the lung still remains to be elucidated. In this work, we analysed the paracrine effects of different chemotherapy-induced senescence types in human and mouse lung cancer cells. Co-cultures of senescent and lung cancer cells were performed, as well as senescent conditioned media was collected to analyse the effect on a variety of functional assays, including proliferation, migration, stemness and mitochondrial metabolism. Our results show that platinum-induced SASP leads to an increased rate of division, migration and gain of colony and sphere-forming traits of recipient lung cancer cells, in addition to promoting a metabolic rewiring. High-throughput gene expression profiling and proteomics analyses allowed us to gain insight into the molecular mechanisms driving these effects. Of note, our results have been validated in vivo with tumour xenografts and orthotopic models of lung carcinogenesis. Together, our data indicate that the paracrine effects of chemotherapy- induced senescence depend on the type of drug used, and suggest that platinum-based treatment of lung cancer cells promote malignant phenotypes in a cell non- autonomous manner, which might contribute to tumour relapse and an aggressive growth potential.

Autotaxin (ATX), a novel player in chronic inflammation and fibrosis

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Autotaxin (ATX, ENPP2) is a secreted lysophospholipase D, widely present in biological fluids. ATX catalyzes the hydrolysis of lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA), a growth factor-like, signaling phospholipid. The pleiotropic LPA effects are mediated through its G-protein-coupled receptors (LPARs) that are widely expressed, exhibiting overlapping specificities. LPA affects multiple cell types, including epithelial cells, endothelial cells, fibroblasts, macrophages and other leucocytes in various ways. Therefore, LPA participates in the regulation of many processes such as vascular homeostasis and skeletal remodeling, lymphocyte trafficking and immune regulation, demyelination and chronic pain, that are intricately involved in the pathogenesis of different chronic inflammatory diseases. Increased ATX expression and LPA levels have been detected in patients with different chronic inflammatory diseases, most notably idiopathic pulmonary fibrosis (IPF), chronic liver and cardiovascular diseases, rheumatoid arthritis (RA) and multiple sclerosis (MS). Genetic and pharmacologic studies in mice have confirmed the pathogenetic role of ATX/LPA in chronic inflammation, and provided the proof of principle for therapeutic interventions, as exemplified by the ongoing clinical trials for IPF.

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The many lives of senescent cells

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Since it was first formally described in the 1960s, cellular senescence has undergone a myriad of transformations in both characteristics and function. This transformation includes the recognition that senescent cells can have either beneficial and deleterious effects, depending on the biological context, arguing that the senescence response is an example of evolutionary antagonistic pleiotropy. Among the beneficial effects, the senescence response protects organisms from malignant tumorigenesis, likely promotes the initiation of parturition, and significantly contributes to wound healing, tissue repair and tissue regeneration. Among the deleterious effects, senescent cells are now known to promote many aging phenotypes and most, if not all, the major age-related pathologies. At the heart of both effects of senescent cells is the senescence-associated secretory phenotype (SASP) -- the propensity of senescent cells to secrete numerous biologically active molecules. The SASP includes not only the initially-described cytokines, chemokines, growth factors and other proteins, but now also includes many bio-active lipids and small molecular weight damage-associated molecular patterns (DAMPs). The SASP is known or strongly suspected to confer some of the beneficial effects of senescent cells. In addition, because many of these SASP factors are pro-inflammatory, the SASP is now known or strongly suspected to drive a plethora of age-related pathologies, ranging from neurodegeneration to cancer, which are caused or exacerbated by chronic inflammation. The SASP has now expanded to >500 molecules, some as purely secreted factors and some in exosomes. I will discuss this newly expanded SASP and how it is helping our understanding, and translation, of the many aspects of the senescence response.

Manipulating senescence through reprogramming to improve tissue homeostasis in aging

Quentin Alle, Nelly Béchir, Mélissa Gabanou, Camille Lemey, Enora Le Borgne, Laure Lapasset, Alexandre Prieur, Franck Pellestor, De Vos J, Ollivier Milhavet and Jean-Marc Lemaitre

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Aging is a complex process modulated by genetic and epigenetic factors. It is marked by progressive appearance of age-related pathologies and a decrease of cell and tissue regenerative capacity. Among the many molecular and cellular mechanisms underlying aging, cellular senescence acts as a powerful tumour-suppressor mechanism, which also contributes to tissue aging. Removal of senescent cells from certain tissues can prevent or delay tissue dysfunction, suggesting that strategies able to decrease or delay the senescence effect might be beneficial in aging. Although, the iPSCs reprogramming process, using the 4 Yamanaka factors, OCT4, SOX2, KLF4, C-MYC (OSKM)1, has been described to favour senescence, establishing senescence as a barrier to reprogramming, we were able to derive iPSCs efficiently from senescent cells and from centenarian donors cells, using an optimized reprogramming strategy based on 6 factors (OCT4, SOX2, KLF4, C-MYC, NANOG, LIN28)2. This strategy allowed to overcome the senescent state and both gene expression patterns, telomere length and metabolism were rejuvenated after reprogramming into iPSCs and re-differentiation into fibroblasts. We further investigated for a different reprogramming regimen using OSKM factors to avoid senescence promotion. We showed that in vitro experiments inducing transiently OSKM factors in fibroblasts induced FOXO3a, an actor of the stress response, and that DNA damage and senescence was reduced. In addition, autophagy was activated in parallel. To reproduce this activity in vivo, we derived

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a mouse transgenic murine model, allowing both a controlled expression of OSKM by doxycycline and recapitulating the human phenotype of the accelerated aging syndrome of Hutchinson-Gilford Progeria (HGPS). We firstly established a specific induction protocol that significantly extend the life expectancy of this accelerating aging mice confirming our hypothesis and previous results. Then, we investigated for health and tissue integrity improvement. Unexpectedly, we observed that a single transient reprogramming induction for a short period of time, in the early life was able to increase lifespan, and delayed age-related pathologies like osteoporosis, and idiopathic pulmonary fibrosis of HGPS mice. An improvement of tissue integrity was also observed in spleen, kidneys and skin, suggesting that the protective effect of this transient reprogramming might persist in aging. Altogether, our results demonstrate that this new reprogramming strategy is a pertinent approach to explore potential rejuvenation mechanisms and its propagation in aging.

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Epigenetically targeting senescent cells prevents the deleterious effects of glucocorticoids on growing skeleton

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Glucocorticoids (GCs) are among the most effective treatments for many chronic inflammatory and autoimmune conditions in children. However, GC treatment has adverse effects on the growing skeleton, including osteoporosis and growth retardation. The mechanisms underlying these adverse effects are unclear. In this study, we report that daily injection of methylprednisolone (MPS), a synthetic GC, in growing young mice induces cellular senescence in metaphysis of long bone as indicated by increased number of SA-βGal+ and p16INK4a+ cells. In situ immunofluorescence staining of bone tissue sections and single-cell imaging flow cytometry analysis of single bone/bone marrow cells revealed that the senescent cells are primarily CD144+ vascular endothelial cells. These vascular cells lost proliferative capacity and gradually diminished in metaphyseal region of the mice with MPS treatment. The abnormal bone phenotype caused by MPS treatment, including compromised angiogenesis and osteogenesis as well as growth retardation and bone loss, were largely improved in Cdh5-CreERT2; p16flox/flox mice, in which p16INK4a is deleted selectively in vascular endothelial cells in a tamoxifen-inducible manner. We also investigated the molecular mechanisms underlying GC-induced bone vascular cell senescence. Vascular endothelial cells isolated from the metaphyseal region of MPS-treated mice have greatly decreased expression of polycomb histone methyltransferase enhancer of zeste homolog 2 (Ezh2), a key epigenetic regulator of cellular senescence by increasing the H3K27me3 mark at the promoter regions of the cellular senescence inducing genes. ChIP-qPCR assays demonstrated that H3K27me3 was enriched at the promoter regions of p16INK4a, p15INK4b, and p21CIP1 in bone vascular cells from Vehicle-treated mice, but this repressive marker was lost at these regions in the cells isolated from MPS-treated mice. GSK-J4, a small molecule that selectively inhibits H3K27 demethylases, elevated H3K27me3 mark in the key senescence genes in vascular endothelial cells, attenuated blood vessel senescence, and rescued MPS-impaired bone growth and bone loss. Therefore, GC-induced cellular senescence in bone is epigenetically regulated by Ezh2 and its H3K27me3 mark. These findings show the importance of bone blood vessel senescence in mediating glucocorticoid action on growing bone and present new opportunities for manipulating epigenetic factors to treat growth retardation and pediatric osteoporosis.

KDM4A inhibition induces a senescence-like phenotype and increases sensitivity to Bcl2 inhibitors in MLL-AF9 acute myeloid leukemia

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Epigenetic modifications regulate gene expression, genome stability, and nuclear architecture. A better understanding of the implications of epigenetic differences in oncogenic cells is a promising strategy in the development of new therapeutics and clinical care. We have determined that KDM4A, an epigenetic regulator of the JUMONJI lysine demethylase family, is overexpressed in tissues from MLL-AF9 pediatric acute myeloid leukemia (AML) patients. We aim to characterize the precise epigenetic function of KDM4A in pediatric MLL-AF9 AML and its role in modulating gene expression and chromatin architecture. We are also investigating the potential of pharmacological inhibition of KDM4A as a novel therapeutic strategy in AML treatment. The loss of KDM4A resulted in decreased cell proliferation, increased expression of markers of cell differentiation, and repressed transcription of genes involved in leukemic maintenance, including leukemic-required c-Myc. In the MLL-AF9 leukemia genome, a lineage-specific super-enhancer element is required for the robust expression of the Myc oncogene. We observed an increase in chromatin repressive histone modifications at the Myc super-enhancer in KDM4A-depleted cells, as well as a decrease in the recruitment of BRD4, an epigenetic remodeling factor required for robust super-enhancer activity. Our results also indicate that KDM4A-depleted or inhibited cells express markers of cellular senescence, including increased levels of anti-apoptotic Bcl-2 family proteins. Notably, the inhibition of KDM4 proteins followed by treatment with Bcl-2 inhibiting compounds enhanced apoptosis in MLL-AF9 leukemic cells. In AML, malignant leukemic stem cells have an unlimited potential for self-renewal and are blocked in an undifferentiated state. Our results demonstrate that KDM4A inhibition and depletion leads to growth arrest and a senescence-like phenotype in MLL-AF9 leukemic cells in vitro. Our study will decipher a precise molecular role of KDM4A in the maintenance of leukemic proliferation and describe a potential novel therapeutic approach for treatment of MLL-AF9 AML, a disease still plagued with dismal survival rates.

Global changes in regulatory networks in senescence

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Senescent cells have a distinct gene expression profile, which is often accompanied by the spatial redistribution of heterochromatin into senescence-associated heterochromatic foci (SAHF). Our previous work identified global changes in the nuclear architecture in oncogene-induced senescence using Hi-C, however the link between higher order structure and gene expression remains unknown. Combining single-cell ATAC-seq and promoter capture Hi-C, we find a link between global changes in nuclear organisation and transcriptional output, suggesting senescence activation underlies a distinct gene regulatory rewiring, including the use of senescence-specific regulatory elements. Extending our analysis to co-culture experiments, we also find distinct regulatory elements separating primary and secondary senescent cells.

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Autophagy-mediated reduction in tolerogenicity in facilitating senescent cell clearance from mouse liver

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To study the immune-mediated elimination of senescent cells, we have used a mouse liver model wherein transduced oncogenic NRAS induces hepatocyte senescence[1]. The current state of knowledge for this model is that the senescent hepatocytes secret inflammatory factors (e.g. CCL2), which recruit monocyte derived macrophages that in turn depend on mutant NRAS-specific CD4 T-helper cells [1,2]. However, our data suggests that organ 'tolerogenicity' provides an additional layer of regulation. The liver has a unique immune environment: even in normal physiological situations it is constantly exposed to microbial or other exogenous products derived from the gut, hence the liver is tolerogenic by nature [3]. One major player is the tissue resident macrophage (the Kupffer cell, KC), which are self- renewing and act as sentinels. The tolerogenic function of KCs is critical for liver homeostasis (without it the liver would be always be inflamed), although an excess of exogenous products can switch KCs to a more 'inflammatory' state. Consistent with this idea, immunophenotyping of the mouse livers in these different settings using mass cytometry (CyToF), flow cytometry, and immunohistochemistry suggests that, upon the induction of senescence in the hepatocytes, tolerogenic KC numbers decline, potentially facilitating senescent cell clearance by monocyte derived macrophages. Previously, we and others have shown that autophagy modulates the SASP in culture, however the functional relevance of autophagy in senescence in vivo remains unclear. Interestingly, when we block autophagy within our senescent hepatocytes, the proportion of tolerogenic KCs returns to normal and senescent cell elimination is delayed. Importantly, those autophagy defective senescent hepatocytes that persist develop cancer after several months. These data suggest a non-autonomous tumour suppressive role for autophagy through inhibiting tolerogenic KCs. Tissue resident macrophages may also have similar roles in other tissues such as in lung, intestine, and the peritoneum (where they are commonly exposed to external antigens, and thus also have tolerogenic features). A better understanding of how senescent cell elimination is influenced by organ tolerogenicity may reveal new targets for early intervention in tumour immunity.

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Probing the surface of senescent cells for p16 MHC-peptide complexes

Nicholas Rettko

Nicholas J. Rettko and James A. Wells - University of California, San Francisco

Recent transgenic mouse models have shown removing p16-expressing senescent cells provide benefits in lifespan and delaying the onset of aging phenotypes[1,2]. However, eliminating native human cells expressing p16 has remained a challenge. One potential approach to target senescent cells is by generating biologics that bind to MHC-peptide complexes (pMHC's). We hypothesize senescent cells display peptides derived from p16 on the cell surface that could serve as senescent specific antigens and targetable biomarkers. Utilizing our Fab-phage display technology, we have generated antibodies that bind to p16 pMHC's across multiple HLA alleles, both for previously identified and computationally predicted peptides. Using biolayer interferometry and flow cytometry, we demonstrate our antibodies bind specifically to these complexes in a peptide-dependent manner. These antibodies serve as the foundation in designing multiple biological derivatives for validation and application, including IgG's, bi-specific T cell engagers (BiTEs), and chimeric antigen receptor (CAR) T cells. We will use these different avenues to probe the surface of senescent cells and explore therapeutic potential.

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Suppression of p16 bypasses senescence through a mTORC1-mediated nucleotide metabolic reprogramming

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Oncogene-induced senescence (OIS) is a bona-fide tumor suppressor mechanism characterized by increased expression of the cell-cycle regulator p16 and decreased levels of deoxyribonucleotides. The absence of p16 predisposes cells to tumorigenesis, and its expression is low or null in many human cancers. There is currently no approved targeted therapy for p16 low tumors. Hence, delineating the molecular mechanisms downstream of p16 suppression is critical for identifying new therapeutics for these patients. Transformed and tumorigenic cells require increased dNTP synthesis to fuel the genome replication that sustains their unregulated cell cycle and proliferation. Interestingly, both suppression of p16 and addition of exogenous nucleosides bypasses OIS to allow for transformation and tumorigenesis. Therefore, it is likely that the cell cycle and nucleotide metabolism are linked. Here we used senescence as a model to study the link between p16 and nucleotide metabolism. We found that depletion of p16 increases nucleotide synthesis to bypass senescence induced by multiple stimuli, including endogenous dNTP depletion and BRAFV600E expression. Mechanistically, we found that suppression of p16 increases mTORC1-mediated translation of the pentose phosphate pathway enzyme ribose-5-phosphate isomerase (RPIA) to upregulate production of ribose-5-phosphate and nucleotides. Underscoring the importance of this pathway in human cancers, cancer cells with low p16 expression are more sensitive to both mTORC1 inhibitors and rely upon RPIA protein expression for proliferation both in vitro and in vivo. In summary our data demonstrate that loss of p16 increases nucleotide synthesis to bypasses senescence through upregulation of the mTORC1-RPIA axis. These mechanistic insights have broad implications for understanding pro-tumorigenic metabolism. Moreover, this study provides a new metabolic vulnerability for p16 low cancer cells, which may be exploited for therapy.

Harnessing senescence to control oncogene-driven cancer through the metabolic intervention

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PI3K/AKT/mTORC1 signaling network regulation is essential for homeostatic control of cell growth, proliferation, and survival. Aberrant activation of this signaling network is an early driver of many sporadic human cancers. Paradoxically, PI3K/AKT/mTORC1 hyperactivation causes a senescence-like phenotype in nontransformed cells, which acts as a protective brake against tumour transformation1. Using a whole protein-coding genome RNAi screen, we identified a number of essential regulators of AKT-induced senescence (AIS). We have also shown AKT-induced senescent cells display a metabolic phenotype distinct from the Warburg effect to maximize energy production2. These metabolic alterations contribute to AIS establishment and maintenance, and interfering metabolic reprogramming may cause senescence-bypass or escape and promote tumorigenesis. Here, we show that Cystathionine β-Synthase (CBS), identified from a whole protein-coding genome RNAi screen, plays a critical role in AIS maintenance. CBS is a key enzyme catalyzing the conversion of homocysteine and serine to cystathionine and involving in H2S and glutathione formation3. Integrating transcriptome and metabolic profiling data, we explore the molecular mechanisms underpinning AIS escape induced by loss of CBS. CBS depletion causes intracellular L-homocysteine accumulation and promotes global DNA hypomethylation, such effects lead to transcriptome rewiring and contribute to cell cycle re-entry of AIS cells. Our findings thus reveal a connection between metabolic rewiring and epigenetic regulation by CBS in AIS maintenance. The effect of CBS depletion on AIS escape implicates a potential tumor suppressor role of CBS. Here, we show that CBS depletion promotes the transformation of normal gastric epithelial cells in synergy with PTEN knockout. Furthermore, restoration of CBS expression in gastric cancer cells induces senescent-like phenotype, and inhibited cell proliferation and tumor growth both in vitro and in vivo. CBS restoration in gastric cancer cells elevates intracellular H2S production and re-engages senescence brake, providing a new option for targeting gastric cancer cells. Overall, our study identifies a novel metabolic regulator of AIS and uncovers new potentially targetable metabolic vulnerabilities of PI3K/AKT/mTORC1-driven cancers.

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Humans increasingly develop senescent immune cells with advancing age

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In mammals, senescent cells (SCs) accumulate with aging in multiple tissues, particularly at sites of pathogenesis of age-related diseases and conditions. This aging-associated accumulation is surprising, given that SCs can be cleared efficiently during embryonic development as well as from various pathological conditions by innate and adaptive immune responses. One hypothesis that would explain why SCs accumulate with advancing age in mammalian tissue is that immune cells themselves increasingly undergo senescence as we age, thereby progressively weakening immune responses that would otherwise clear SCs from aged tissue. We will present evidence that supports this hypothesis.In order to analyze the abundance of senescent immune cells in humans, we developed a protocol that can accurately and efficiently quantify and isolate SCs from mixed cell populations. The technique relies on flow cytometry and measures high activity of senescence associated β-galactosidase (SA-β-gal) using a cell permeable and fluorogenic β-gal substrate. It thus labels all senescent cells, regardless of their senescence-inducing signal. We demonstrate that this protocol allows us efficiently quantify and isolate SA-β-gal expressing cells from human cell cultures, mixed cell populations, tissue, and from peripheral blood. Sorted and isolated cells can be further characterized by immunofluorescence analysis, lysed to harvest intact RNA and chromatin, analyzed for gene expression, and cultured for cell proliferation assays. Analysis of peripheral blood mononuclear cells (PBMCs) from a cohort of healthy human donors (n=18 to date) revealed that some PBMC subsets increasingly display high SA-β-gal activity with advancing age, while others do not. The most striking increase was observed for CD8+ T cell populations, in which the percentages of cells with high SA-β-gal activity increased from 23 ± 6 % to 60 ± 14 % in donors in their 20's and 60's, respectively ($p = 8x10-6$). Data for four other PBMC subsets will also be presented. CD8+ T cells with high SA-β-gal activity, but not cells with low activity, displayed other hallmarks of cellular senescence, including lack of proliferation in culture, high gene expression levels of p16INK4a, p21, and GLB1, telomere dysfunction-induced DNA damage foci (TIF), as well as high nuclear p16 protein levels. Although the nature of effector immune cells that interact with SCs in the context of aging is still unclear, our study supports the model that immune cell senescence, triggered by telomere dysfunction and other stresses, is a contributing factor to the decline of immune responses and senescent cell accumulation in aging humans.

Development of a 3D living skin equivalent to explore the influence of senescence on the skin ageing phenotype

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The accumulation of senescent cells with age in vivo causes loss of tissue function and reduced regenerative capacity. Changes associated with senescence disrupt tissue homeostasis by acting on surrounding cells and matrices, and the senescent-associated secretory phenotype (SASP) is thought to trigger senescence in neighbouring cells. However, the paracrine effect and subsequent influence on the ageing phenotype in tissues remains unclear. Previously within the group, a protocol was developed to revert deeply senescent (DS) primary human epithelial cells (Lowe et al., Genome Biology) and fibroblasts (unpublished) to an early proliferating (EP) phenotype. Comparing EP, DS and reversal cells has allowed us to more deeply understand the molecular mechanisms of senescence. To further investigate these mechanisms within the context of ageing, a 3D organotypic skin equivalent (LSE) to model either young or aged skin was developed. Firstly, a 2D model of senescence was established by serially passaging human dermal fibroblasts (HDFs) to DS. These DS HDFs displayed a panel of senescence markers, including SA-β-Gal, p16 and p21. To create a 3D LSE, EP HDFs were suspended in a dermal matrix and proliferating human epidermal keratinocytes (HEKs) were seeded on the surface to create an epidermis. DS HDFs were added in increasing proportions to the dermal matrix and the subsequent LSE phenotype was analysed. LSEs containing only EP HDFs show clear stratification of the epidermis, whereas LSEs containing a mixed population of EP/DS HDFs showed altered stratification and changes in the expression of senescence markers in the epidermis. The epidermal phenotype in LSEs constructed with only DS HDFs in the dermis was distinct from LSEs constructed with an EP/DS HDF mixed population. To understand the interaction between EP and DS HDFs, single cell-RNA sequencing was run on EP, mixed and DS populations in 2D and 3D. Transcriptomic data showed that each population was distinct and may help us understand why the epidermal phenotype was altered in DS LSEs.

Novel regulators of senescence: in disease and health

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The activation of the cellular phenotype- senescence- is considered a key hallmark and inducer of ageing. One of the most prominent characteristics of senescence is the release of chemokines, cytokines and extracellular matrix remodelling protein, named SASP. However, the contribution of other non-soluble mediators and the role of lipid metabolism is less well characterised. Here, I will show how extracellular vesicles act as mediators of intercellular communication during senescence1. Furthermore, I will unveil novel regulators of senescence: from enzymes regulating lipid metabolism2 to novel previously uncharacterised SASP factors.

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Investigating the interplay between senescence, exosomes and inflammation

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Extracellular vesicles (EVs) are increasingly becoming appreciated as major players in cellular communication, with roles in cancer and the immune system. Exosomes represent the most widely investigated subset and have been demonstrated to carry a variety of functional cargos including proteins, miRNAs and mRNAs. This work investigates the contribution of exosomes to the paracrine signalling of the senescence-associated secretory phenotype (SASP).

Here, we demonstrate that exosome production increases in HRas:ER oncogene-induced senescent IMR90 fibroblasts, as well as replicatively senescent adult Human Mammary Fibroblasts (HMFs). Proteomic data, comparing the composition of exosomes to the soluble fraction of the SASP, indicated that many canonical SASP factors are associated with the vesicular fraction of the secretome. Furthermore, membrane and intracellular proteins identified in exosomes from OIS cells may represent a potential mechanism for non-cell autonomous paracrine signalling beyond the traditional SASP.

Given their small size, the technical challenges of studying exosomes from senescent cells will also be discussed. One particular obstacle is dissecting the composition of vesicles from the wider SASP, due to the issue of co-isolated soluble proteins. We will present a high purity isolation procedure combing traditional differential centrifugation with an additional size exclusion chromatography step.

How senescence is driving the development of brain

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Multiciliated ependymal cells are specialized glial cells which line the inner walls of the mammalian brain cavities, called ventricles. They are essential for continuous cerebrospinal fluid flow in the ventricles and to maintain brain homeostasis throughout life. Any defect in these cells leads to major neurological diseases, among which early born lethal hydrocephalus is the most severe. It is crucial to decipher how these cells are formed during development. We previously demonstrated that ependymal cells derive from radial glia during embryonic stages. While studying the largely unknown molecular mechanisms behind ependymal cell development, we found that mTOR, a key metabolic pathway is active during the process of ependymal cell differentiation. By blocking the mTOR pathway, the differentiation of ependymal cell was arrested, confirming the essential role of mTOR pathway in ependymal cell differentiation. Interestingly, we also found that differentiating ependymal cells express markers of cellular senescence, showing the link between mTOR and senescence in these differentiating cells. Additional studies on the transcriptome of differentiating ependymal cells revealed replicative stress pathways to be active during the differentiation process, pointing towards possible inducers of the observed senescence-like state. This transient senescence-like state, which appears only during the process of ependymal cell differentiation and disappearing later, is enigmatic when compared with the conventional permanent state of senescence. By using various genetic models of mice, and pharmacological techniques, we are studying aspects of this peculiar, transiently expressed senescence-like state. It is remarkable to observe the pathways, which are usually detrimental to the tissue health and associated with ageing, are also actively responsible for development of brain.

Lung mechanical stretch triggers a senescence-like response

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Introduction: Senescence is currently understood not only as a long-term consequence of cell stress, but also as part of the acute response to injury. Abnormalities in the interaction between DNA and nuclear envelope may trigger senescence. As the nuclear envelope has recently been involved in mechanotransduction, we hypothesized that mechanical stretch could activate senescence-related cell pathways, aimed to minimize tissue damage.

Objectives: Our objectives were to study the activation of senescence-related molecular pathways after lung stretch caused by mechanical ventilation, and to clarify the short-term consequences of this activation.

Methods: Lung injury was induced by intratracheal HCl instillation and mechanical ventilation in C57Bl/6 mice. After 135 minutes, animals were sacrificed and lungs harvested. Lung damage was assessed in histological sections, and neutrophilic infiltration and Il6 expression were quantified as markers of inflammation. Senescence was characterized by appearance of macroH2A-positive heterochromatin foci and expression of p53 (Tp53), p16 (Cdkn2a) and p21 (Cdkn1a). DNA damage and heterochromatin abundance were quantified by western blots against γ-H2AX and HP1-α respectively. Senescence-associated heterochromatin foci were also assessed in lung sections from patients who died after mechanical ventilation and non-ventilated controls. To clarify the role of senescence in lung damage, mice treated with lopinavirritonavir (that ameliorates mechanical stretch within the nuclear envelope) or vehicle were compared after lung damage and mechanical ventilation. Similarly, animals lacking Cdkn1a (p21) and their wildtype counterparts were studied to clarify the role of this factor.

Results: Acid instillation and mechanical ventilation caused lung injury, with an increase in histological scores and apoptotic cell counts. Interestingly, both hits, but not acid instillation alone was related to the appearance of senescence-associated heterochromatin foci. Among the canonical markers of senescence, only Cdkn1a (p21) increased with injury. Samples from mechanically ventilated patients showed also heterochromatin foci. The mechanical stretch caused alterations in the nuclear envelope, namely an increase in Lamin-A/Lamin-B ratio, an increase in the DNA damage marker γ-H2AX and in HP1-α. Treatment with lopinavir-ritonavir decreased lung injury and apoptosis in spite of no changes in DNA damage. However, this treatment dampened all the observed changes in chromatin structure (senescenceassociated heterochromatin foci and abundance of HP1-α) and markedly increased the expression of Cdkn1a (p21). Finally, lung injury and apoptosis were markedly increased after acid instillation and mechanical ventilation in Cdkn1a-/- mice, compared to their wildtype counterparts.

Conclusions: Mechanical stretch triggers senescence in injured lungs. This response is aimed to limit epithelial apoptosis and minimize injury.

The potential role of multi-drug resistance protein ABCB1 in tumor suppression and longevity in bat

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One of the factors that contribute to longevity is strong tumor suppression. Most cancer research utilizes cancer-prone, short-lived models such as mouse and rat. However, emerging evidence suggests that long-lived mammals such as whale and elephant have unique anticancer strategies that are not conserved in human and could potentially inform new treatments for human cancers. It has been known that longevity is positively correlated with body size. Among the long-lived mammals, bats are unique since bats live much longer than other mammals of equivalent size. For example, some bats live up to 40 years although the body size is similar to mice which only live around two years. Bats are also known to have extremely low cancer incidence. However, underlying anti-cancer mechanisms are unclear. We discovered that bat-derived cells exhibit enhanced DNA damage resistance to toxic chemical exposure compared to human and mouse cells. We found that bat cells accumulate less chemical than human and mouse cells and that efficient drug efflux mediated by the ABC transporter ABCB1 underlies this improved response to genotoxic reagents. We showed that the high ABCB1 expression protected bat cells from DNA damage and cell death induced by a chemotherapeutic drug. In humans, ABCB1 is expressed only in organs of detoxification, excretion, and protective barriers, such as kidney, intestine, liver, and brain. On the other hand, ABCB1 is much more highly and ubiquitously expressed in multiple cell lines and tissues derived from bats compared to humans. Furthermore, increased drug efflux and high expression of ABCB1 are conserved across multiple bat species (Koh et al., 2019). Accumulation of DNA damage is known to induce cancer development and cellular senescence. We propose that genotoxic efflux as a potential tumor-suppressive mechanism that contributes to the low incidence of cancer and longevity in bats. We also would like to share our ongoing studies of using bat ABCB1 as a potential tool to develop novel treatment strategies in human.

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A dual role for RANK signaling pathway in mammary tumorigenesis

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RANK signaling regulates mammary epithelial differentiation and mediates mammary tumorigenesis driven by progesterone and carcinogens. RANK overexpression in mouse and human mammary cell lines induces stemness and promotes tumorigenesis and metastasis. Loss of function genetic and pharmacologic approaches demonstrates that RANK promotes mammary tumorigenesis in tumor-prone MMTV-NEU and MMTV-PYMT oncogene driven models. Unexpectedly, we found a significantly longer latency to tumor formation and reduced tumor incidence in transgenic mice overexpressing both RANK and NEU or RANK and PYMT as compared to the single mutants. Analyses of preneoplasic lesions in double mutants suggested that the progression from hyperplasias to mammary intraepithelial neoplasia (MIN) was impaired. No differences in proliferation or apoptosis were observed but an increase in the number of senescent cells was found in the hyperplastic lesions of double mutant mice. Senescent cells were observed in MMTV-RANK mammary ducts and hyperplasias and in mammary epithelial cells growing in vitro, in the absence of other oncogenic stimuli. Moreover, infection of WT mammary epithelial cells (mecs) and mouse embryonic fibroblast (MEFs) with RANK overexpressing vectors (but not PyMT or Neu) led to DNA damage and senescence. Similar to RAS, RANK-induced senescence is driven by p16/p19 but not p53. Importantly, RANK-induced senescence is essential for RANK driven stemness, as the increased mammosphere forming ability of RANK overexpressing cells is not observed after senolytic treatments. We uncovered an unexpected dual role for RANK in the mammary epithelia able to induce stemness but also oncogene-induced senescence and preventing mammary tumorigenesis.

G3BP1 controls cGAS-dependent senescence-associated secretory phenotype to promote cancer progression

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The mechanisms and factors through which we can control senescent cells to prevent their deleterious impacts on age-related diseases, without bearing the consequences of their elimination are still elusive. Here we show that the ras-GAP-SH3-Binding Protein 1 (G3BP1) is essential for the activation of the senescence-associated secretory phenotype (SASP) and their effects on cancer progression. G3BP1 depletion or pharmacological inhibition impairs the activation of the cGAS pathway preventing the expression and secretion of SASPs. Despite this, these cells are still able to commit to senescence. SASPless senescent fibroblasts depleted of G3BP1 are unable to enhance the proliferation of cancer cells in vitro and tumor progression in vivo. Together, our data demonstrate that G3BP1 is a viable target to prevent senescence-induced age-related diseases, such as cancer, while potentially preserving beneficial function of senescent cells.

Altered mechanobiology of senescent endothelial cells

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Background: Endothelia are connected monolayers of endothelial cells lining human vessels. The healthy endothelium is a dynamic tissue that integrates mechanical and biological stimuli generated by the flow and by circulating molecular factors. It is naturally developed to control vascular homeostasis acting as selective barrier between the vessel lumen and the surrounding tissues, preventing thrombosis, modulating the response to infective agents and inflammatory insults, controlling the interaction with circulating immune cells, and promoting vascular repair. Endothelial function decays progressively with age, in a process of tissue senescence that typically accompanies cardiovascular disease [1]. Senescent endothelial cells are associated with complex structural and functional alterations in the vasculature.
Methods: In order to gain fundamental knowledge on the mechanobiology of endothelia in health and senescence, we artificially induced senescence in vitro. In our model we use a chronic treatment with the tumor necrosis factor alpha (TNF-α), as is in line with the chronic inflammation condition of cardiovascular patients [2]. We verify the senescence phenotype measuring the expression of SA-β-GAL and other biochemical markers of senescence. Finally, we study the mechanical properties of senescent endothelial cells and their behavior under physiological hemodynamic conditions [3].
Results: Our results provide novel insights on the mechanical alterations in senescent endothelial cells.

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Identification of an essential metabolic pathway to maintain senescent cell viability

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Senescent cells have been reported to accumulate in various tissues with the advance of organismal age, contributing to various age-related phenotypes and disorders. Therefore, elimination of senescent cells from aged tissues has been proposed to ameliorate these deleterious consequences. For this purpose, elucidation of the mechanism by which the viability of senescent cells is maintained would provide a cue to develop innovative technologies and chemical compounds that selectively induce lethality in senescent cells in vivo. In this study, we found that senescent cells showed a dysregulated intracellular environment. In order to compensate for the intracellular environment dysregulation, senescent cells adopted an altered metabolic pathway that improved intracellular environment and increased the survival. On the basis of these observations, we found that treatment with a chemical compound which targeted the altered metabolic pathway induced lethality in senescent cells both in vitro and in vivo. These results suggest that senescent cells require the metabolic pathway to maintain their viability and its inhibition offers a promising strategy for eliminating senescent cells in vivo.

2 min. presentations
Day Three 11/09

Stem cell senescence drives age-attenuated induction of pituitary tumours in mouse models of paediatric craniopharyngioma

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Senescent cells can promote tumour progression through the activation of a senescence-associated secretory phenotype (SASP), but it remains unknown whether these cells can also initiate tumourigenesis in vivo. Adamantinomatous craniopharyngioma (ACP) is a paediatric pituitary tumour associated with high morbidity and premature mortality. Activating mutations in CTNNB1 (encoding β-catenin) are present in most ACPs. Previous research on ACP mouse models (Hesx1Cre/+; Ctnnb1lox(ex3)/+ and Sox2CreERT2/+; Ctnnb1lox(ex3)/+) has shown that tumours are induced in a paracrine manner, whereby SOX2+ve pituitary stem cells expressing oncogenic beta-catenin have tumour- inducing potential but are not the tumour-initiating cells (Andoniadou et al., 2013). Specifically, targeting expression of oncogenic beta-catenin to HESX1 embryonic precursors or SOX2 adult pituitary stem cells leads to formation of clusters of stem cells, and induction of tumours resembling ACP derived from non-stem cells. To characterise further this paracrine model of tumourigenesis we have combined mouse genetics with molecular and histological studies in our mouse models and human ACP tumours. Here, we show that clusters contain senescent cells with activated SASP and the paracrine tumours harbour somatic mutations suggesting cell transformation. Transcriptomics of purified populations, by flow cytometry or laser-capture micro-dissection, corroborate that cluster cells are analogous and share a common signature of senescence and SASP in mouse and human ACP. Expression of oncogenic β-catenin in Sox2 pituitary stem cells from aged mice (>6 months) results in formation of clusters with attenuated senescence/SASP and reduced tumour-inducing potential. Likewise, genetic deletion of the WNT repressor adenomatous polyposis coli (Apc) generates clusters with ameliorated senescence/SASP response and failure to form tumours (Gonzalez-Meljem et al., 2017). Finally, we show that expression of a mutant form of BRF1 (ZFP36L1), a negative regulator of SASP-mediated inflammation (Herranz et al., 2015), in the context of the ACP mouse models results in a significant decrease in tumour burden, implicating SASP as the driver of tumour induction in ACP. Together, these data provide evidence that tumour-associated senescent cells can transform surrounding cells in vivo and initiate tumourigenesis in a non-cell autonomously manner through SASP signalling.

Characterization of cellular senescence in vivo at a single cell resolution

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Cellular senescence is triggered by diverse genotoxic stimuli, including telomere erosion, activated oncogenes, reactive oxygen species, and DNA damage. An important hallmark of senescent cells is its inability to proliferate in response to physiological mitotic stimuli, which can limit the ability of stem cells in tissue and organ renewal. Another is the appearance of senescence-associated secretory phenotypes (SASP), such as robust secretion of numerous growth factors, cytokines, proteases, and other proteins, which may cause deleterious effects on tissue microenvironment. Recent studies using genetically engineered mouse models or senolytic drugs indicate that the accumulation of senescent cells (p16ink4a-positive cells) in the body accelerates age-related alterations such as arteriosclerosis and kidney injury, thus limiting the healthy lifespan. However, cell origin, spatial-temporal dynamics, and characterization of senescent cells in vivo are largely unknown. Here, we develop a new mouse model whose senescent cells are detected and isolated at a single cell resolution. Characterization of fibroblasts from mouse tails revealed that the mouse model can be useful to specifically detect and isolate senescent cells. Analysis of tissues/organs section showed that senescent cells are detected in various tissues/organs even in young age, suggesting clearance of senescent cells as a defense mechanism against the accumulation. Moreover, single-cell RNA-seq analysis of mouse kidney indicated that some cell types are preferentially origins of senescent cells and have cell-type specific senescence gene expression signatures. Further analyses are in progression and will be discussed in this presentation.

2 min. presentations
Day Three 11/09

Telomeric sister chromatid fusions trigger irreversible genome damage for telomere-initiated senescence

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Replicative senescence is the permanent proliferative arrest caused by gradual telomere attrition at each round of genome replication. This DNA end loss leads to telomere uncapping (TU), which occurs when critically shortened telomeres lose their protective shelterin complex, revealing free chromosome ends recognized as DNA double-strand breaks. TU is proposed to directly trigger a p53-dependent DNA damage response (DDR) that actively sustains a stable senescence-associated (SA) proliferative arrest (SAPA)1. Since telomeres are heterogeneous in length within single cells, the number of short telomeres necessary for senescence onset remains poorly defined. Furthermore, accumulating evidence suggests that normal cells can tolerate a certain level of TU before entering SAPA, which lead us to hypothesize that TU cannot, in and of itself, trigger stable SAPA. We use controlled shelterin inactivation to trigger TU and subsequent SAPA in normal human fibroblasts2, and validate our observations during natural replicative senescence. We show that telomere dysfunction alone cannot trigger senescence. While continued TU generates stable DDR activation at telomeric ends, this overall weak DDR allows the rapid bypass of a primary proliferative arrest and thereby, a re-entry into the cell cycle3. The subsequent return into S-phase, and more precisely DNA synthesis, allows sister chromatid fusions at telomeres mediated by homologous recombination. During the ensuing mitosis, fused telomeres lead to additional DNA breaks and to genomic instability (GI) including chromosome bridges or micronuclei, which then sustains a definitive p53-mediated secondary SAPA. The loss of p53 in the presence of TU prevents both primary and secondary proliferative arrests, leading to amplified genomic instability. During naturally occurring replicative senescence, interphase cells that have already undergone SAPA display GI, while cells captured in mitosis with TU have not yet undergone telomere fusions explaining their continued, albeit slowed, proliferation. Our results support a new multistep model defining entry into telomere-mediated replicative senescence in normal cells, which is not directly induced by telomere uncapping, but rather by an amplification of DNA lesions caused by critically short telomere fusions that lead to permanent irreparable genome damages.

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The putative role of IQGAP1 in intercellular protein transfer and senescence.

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It is well established that senescent cells can communicate with neighboring cells using secreted factors such as cytokines and chemokines. This way of communication is commonly known as senescence associated secretory phenotype (SASP). Several years ago it was shown that cells can also communicate by intercellular protein transport (IPT), which involves direct exchange of proteins between two cells via cellular bridges (CBs). Recent studies show that in senescent cells intensity of IPT increases. The research also revealed that Cdc42 and actin polymerization are indispensable for this process to occur in human fibroblasts. Here we evaluate the hypothesis that other protein – IQGAP1, a scaffold protein with binding sites for both, actin and Cdc42, is involved in IPT. To investigate our hypothesis we used human vascular smooth muscle cells (VSMCs) as we observed that these cells are characterized by increased formation of protrusions when undergoing replicative senescence (RS) or stress induced premature senescence (SIPS). It was shown that senescent VSMCs are involved in pathogenesis of some cardiovascular diseases, including atherosclerosis. We showed that in VSMCs endogenous IQGAP1 is uniformly distributed within cytoplasm and is also present in CBs and other protrusions formed by the cells. The level of endogenous IQGAP1 decreased in senescent VSMCs. We also observed gradual decrease in the level of endogenous Cdc42 in cells undergoing SIPS (contrary to the findings described for fibroblasts). We showed that IPT occurs in VSMCs based on observation of migration of dye between differentially stained cells using flow cytometry. To evaluate the role of IQGAP1 in protrusion formation and IPT, we created two mutated forms of the protein: one lacking CHD domain responsible for actin binding, and the other lacking GRD domain, which has the ability to bind Cdc42. We did not observe any significant differences in dye exchange between control and variants were either of mutated forms of IQGAP1 was expressed. However, to our surprise, cells harboring mutated IQGAP1 had changed morphology, were characterized by decreased proliferation, increased time of division and appearance of some senescence markers (increased activity of senescence associated β-galactosidase and induction of SASP). These findings suggest that IQGAP1 dysfunction can induce senescence, however more research is needed to confirm this. We are yet to find if introduction of mutated IQGAP1 influences protrusion formation.

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Poster presentations

Nod-like receptor pyrin domain-containing protein (NLRP6) interferes with DNA single-strand break repair (SSBR) pathway and induces senescence of colorectal cancer cells

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Cellular senescence is a stress-induced state characterized by a robust cell cycle arrest, an apoptosis resistance, a pro-inflammatory cytokines secretion, and a persistence of unrepaired DNA damages. During aging, senescent cells accumulate in most tissues and organs and contribute to most age-associated disorders and pathologies. In the gastro-intestinal tract,the tissue capacity to repair injuries declines with age, potentially leading to chronic inflammatory diseases and to colorectal cancer. However, nothing is known about the contribution of epithelial cell senescence in the wound healing of the intestinal epithelium. We havepreviously established that the inflammasome component NLRP6 contributes to proper gut wound healing and protects mice from colitis and colorectal cancer[1]. Our results indicate that NLRP6 interacts with CSNK2, a serine-threonine kinase which is involved in the DNA single-strand break repair (SSBR) pathway. We have previously highlighted that senescence in epithelial cells results from the accumulation of unrepaired SSBs, because of a default of phosphorylation of the scaffold protein XRCC1 by the serine-threonine kinase CSNK2, a primordial step in the SSB repair pathway[2]. In this context, we aim in this project to investigatea potential role of NLRP6 inintestinal epithelial cell senescence and how this interferes with the efficacy of gut wound healing. We first showedthat NLRP6 overexpressed induces cell deathin colorectal cancer cell lines. This overexpression also increases the sensitivity of colorectal cancer cells to oxidative stress induced by H2O2 treatment.Indeed NLRP6 overexpression accelerates premature senescence prompted byhydrogen peroxide-induced DNA damagein colorectal cancer cells and this seems to involve the alteration the SSBs repair pathway. Indeed, cells overexpressing NLRP6 show a decrease in XRCC1 phosphorylation and the activation of the p53/p21(TP53/CDKN1A) pathway following H2O2 treatment. These results suggest that NLRP6 could play a role in age-associated colitis and colorectal cancer by interfering with the repair of DNA damage of oxidative origin, thereby favoring intestinal epithelial cell senescence.

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Resumption of autophagic flux in MDA-MB-231 breast cancer cells, that escaped senescence-like state

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Triple negative MDA-MB-231 (mt TP53) breast cancer cells after doxorubicin treatment underwent cellular senescence, but they were still capable of recovering the clonogenic growth. On day 4 after treatment most of the cells expressed hallmarks of senescence, such as: arrest in the G2/M phase of the cell cycle, increased level of the cell cycle inhibitor, p21, enlarged and flattened morphology, increased SA-β-gal activity, lipofuscin and lipids droplets accumulation. Moreover, they secreted more proinflammatory cytokines, thus displaying features of senescence-associated secretory phenotype (SASP). On day 9 after treatment DNA image cytometry revealed a protracted duration of the S phase of the cell cycle, premature mitosis, and eventual mitotic slippage into a cycling polyploid with a chromosome number varying from 8 to 16n. Additionally, electron microscopy analysis revealed accumulation of dense, non-degraded material in these cells. After day 19 most of the cells were both Ki-67- and BrdU-positive, which proves that they were cycling. Moreover, these senescent-like cells were characterised by DNA damage, as revealed by accumulation of γH2AX foci and activation of DNA damage response. We found that proteins involved in DNA repair were preferentially localized in the main nucleus, which probably results in its repair. Interestingly, small, unrepairable DNA fragments, highly positive for γH2AX, colocalized with polyubiquitinated conjugates and autophagy adaptor protein, SQSTM1/p62, but did not colocalize with lysosomes. These results suggest the existing of the process of removal of damaged DNA, however independent from autophagy. Moreover, we collected cells, which escaped senescence and established 'escapers' cell lines. The escapers were bigger, more granular, less invasive and more sensitive to doxorubicin than parental (non-induced to senescence) cells. The most surprising difference among these two cell lines was activation of the autophagic flux in escapers cells, whereas parental cells were characterised by a complete autophagic flux blockade. Autophagy resumption in descendants was accompanied by down regulation of the Rubicon protein, an endogenous autophagy inhibitor, as well as translocation of the Transcription Factor EB into nucleus, indicative of lysosome biogenesis. Escapers underwent doxorubicin induced senescence, however they were significantly more resistant to the induced DNA damage estimated as the number of γH2AX foci. Therefore we conclude that MDA-MB-231 cells treated with doxorubicin can go through a life cycle, that includes senescence/polyploidization followed by depolypoidization, which in turn gives rise to a progeny characterized by autophagy capacity completely different from that of parental pre-senescent cells.

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Poster presentations

Investigating the chromatin structure and the role of HP1α in prematurely and replicatively senescent vascular smooth muscle cells

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Histone modifications play a crucial role in the proper maintenance of chromatin architecture and thus control gene expression level throughout the cell's lifespan. Senescent cells, characterized by permanent cell cycle arrest, exhibit global histone hypomethylation resulting in reduction of inactive heterochromatin in favour of loosely packed euchromatin. Our preliminary results demonstrated, however, that the level of histone methylation varies between different types of senescence. We analysed the compaction of chromatin and nucleus structure during replicative (RS) and stress induced premature senescence (PS) in vascular smooth muscle cells (VSMC). In prematurely senescent cells, where senescence was induced by doxorubicin (DNA-damage dependent) or curcumin (DNA-damage independent), and in replicatively (RS) senescent cells, marks of euchromatin and heterochromatin, and protein HP1α were visualized. This had revealed a drastic loss of all histone marks in RS in contrast to PS, where the level of decrease depended on the type of modification. The phenomenon was further confirmed by Western blot technique. Chromatin condensation analysis confirmed gradual loss of heterochromatin in PS and RS. This loss is also linked with higher roughness of nucleus, resulting in higher surface roughness (as analyzed with AFM). Moreover, microarray analysis revealed vast changes in gene expression between RS and PS. All of these changes implicate distinct differences in nuclear organization depending on the type of senescence. Further investigation of chromatin architecture might serve as a promising diagnostic tool recognizing source of senescence and therefore help in reducing the risk of age-related diseases, for instance atherosclerosis.

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The linkage between endometrial cell senescence and decidualization in female fertility

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Cell senescence is considered as a common reaction of proliferating cells on the DNA damage. Senescent cells stop dividing and harbor a lot of impairments in various intracellular systems. Presence of senescent cells within tissues may mediate their malfunctioning. Here we focused on the investigation of the entangled and complex relationship between senescence and tissue-specific decidual differentiation of endometrial stromal cells (ESCs). ESCs are a critical component of stromal compartment of endometrium lining uterus, and their normal functioning is crucial in terms of female fertility. ESCs are capable for proliferation and tissue-specific differentiation, mediating both cyclic restoration of the functional layer of endometrium and hormone-induced decidualization of endometrial stroma required for embryo implantation. Previously we have shown that premature senescence is the primary stress reaction of ESCs. Within the present study we tried to elucidate possible outcomes of the existence of senescent ESCs within endometrium.
Initially, we tested the ability of senescent ESCs towards decidual differentiation. To do so, we estimated the most common features accompanying decidual differentiation: mesenchymal-to-epithelial morphological switch, expression of the key transcription factor FOXO1 and expression of decidual marker genes prolactine and IGFBP-1. According to our results, senescent ESCs are unable to decidualize compared to control cells. When unraveling the molecular cause of such reduced differentiation ability, we revealed that nuclear translocation of progesterone receptor is impaired in senescent ESCs. Other supposed effects of senescent cells existence within tissues are associated with their paracrine activity. Therefore, we next assessed the effects of senescent ESCs on their young counterparts by applying 2D and 3D co-culturing models, as well as by using SASP. Firstly, we observed that co-culturing with senescent ESCs induced paracrine senescence in the neighboring young cells. Secondly, senescent cells disturbed decidual differentiation in the adjacent ESCs. Notably, negative effects of direct co-culturing with senescent ESCs were replicated when using SASP alone. Together, these data indicate several undesirable consequences of senescent ESCs appearance within endometrial tissue: differentiation ability of such cells is reduced, by secreting SASP they transmit senescence and perturb decidualization of their healthy surroundings. Unraveled outcomes may be further considered in terms of altered endometrial plasticity and sensitivity to invading embryo, thus contributing to the female infertility curing.

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Cell surface changes associated with chemotherapy-induced senescence in ovarian clear cell carcinoma

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Ovarian cancer is the third deadliest gynecological cancer in the world, partially due to drug resistance. Indeed, ovarian cancer cells treated with chemotherapies can stop proliferating and show a senescence-like phenotype, instead of dying. Furthermore, it has been demonstrated that some chemotherapy-induced senescent (CIS) cells can re-enter the cell cycle and proliferate, which could lead to the relapse of the patient. Despite this knowledge, in 25 years, the overall 5- year survival rate only increased by 3%, highlighting the need for new treatments. To develop new treatments or propose sequential pipelines to target CIS cells, it is necessary to better characterize them. Therefore, we decided to perform transcriptomic and cell-surface proteomic analyses on an ovarian clear cell carcinoma cell line that undergoes prolonged growth arrest with many characteristics of senescence upon chemotherapy treatment. Both techniques showed many changes at the mRNA level and cell-surface of the CIS cells compared to control cells. We notably found that 246 and 125 cell surface proteins were significantly upregulated and downregulated, respectively, in senescent cells. Supporting our model, we found a signature of proteins already known to be involved in senescence, such as Cavin-1, CD47, and major vault protein, among others. We are currently using a CRISPR/Cas9 genome wide screen and a shRNA screen targeting up-regulated cell surface proteins to identify genes encoding cell-surface proteins that could be used to distinguish CIS cells and then target them. Overall, our results demonstrate that CIS alters the cell surface distribution of many proteins involved in diverse biological functions. Careful analysis of these changes will improve our understanding of CIS, but also help in the design of improved chemotherapy for ovarian cancer.

Paracrine effects of the SASP on human cells

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Cellular senescence is a proliferative arrest induced by different stimuli, such as telomere dysfunction, oncogene activation, and other stresses. Cells undergoing senescence remain metabolically active and secrete numerous cytokines and other molecules, collectively called the Senescence-Associated Secretory Phenotype (SASP). Molecules secreted from senescent cells exert various effects and perform multiple functions as they can reinforce the senescence growth arrest in an autocrine manner, induce paracrine senescence in surrounding cells, stimulate transdifferentiation, as well as promote reprogramming and cellular plasticity of mouse cells. Collectively, recent studies suggest that the functions of the SASP are diverse and complex, cell type specific, and temporally regulated. Here we investigate the plasticity- inducing effects of the SASP in human cells.

Using a tamoxifen-inducible H-RasV12 (Ras) expression system to rapidly and synchronously induce senescence of human fibroblasts, we show that normal somatic human fibroblasts surrounding Ras-senescent cells develop features of stem cells, such as high expression levels of Sox2, Oct4 and Nanog, in a time dependent manner. These effects were independent of cell-cell contacts, as conditioned medium collected at certain times after Ras-induction similarly stimulated expression of stemness genes in somatic human cells. Genome-wide gene expression analysis revealed several genes, including cytokines, that are temporally upregulated following Ras-induction and we are currently investigating the potential role of these gene products on promoting plasticity in neighboring cells. Our work suggests that a temporally evolving SASP induces cellular plasticity of human cells in a paracrine manner, potentially in order to promote tissue regeneration at sites of tissue damage.

Poster presentations

ASB14 : Discovery of a new E3 ubiquitin ligase implicated in cellular senescence and tumor suppression

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Cellular senescence is a tumour suppressor mechanism that is inactivated by genetic or epigenetic changes during carcinogenesis. Senescent cells are enriched in aging organisms and benign tumours but are noticeably absent in malignant tumours. Consistent with the role of senescence as an anticancer response, several tumour suppressors regulate senescence whereas their inactivation allows cells to bypass the process. Interestingly, chemotherapy, radiation or certain microRNAs can block tumour progression by restoring the senescent response in tumour cells. Gerardo Ferbeyre's group found that the senescence response to oncogenes or DNA damage involves aberrant activation of MAP kinases signalling pathways leading to protein degradation, a process that we named senescence-associated protein degradation or SAPD. We discovered that SAPD occurs secondary to aberrant protein phosphorylation through an ubiquitin-mediated protein degradation pathway, in which E3 ubiquitin ligases constitute the key recognition elements. Targets of SAPD include proteins required for cell cycle, cell migration, ribosome biogenesis and nuclear functions, which explains how tumour progression is aborted in senescent cells. Based on our results, we proposed that E3 ubiquitin ligases play a major role in senescence by promoting protein degradation. To identify E3 ligases required for protein degradation during oncogene-induced senescence, we performed a screening of shRNAs covering all E3 ligases of the mouse genome, in which we identified ASB14 as an important gene for senescence establishment. ASB14 belong to a family of E3 ubiquitin ligases (ASB1-18) that contain multiple ankyrin repeats whose functions have not been identified. We have found promising evidence that linksASB14 with senescence and tumour suppression, as ASB14 levels strongly increase in senescence and benign lesions, whereas it is undetectable in a pancreatic ductal adenocarcinoma cell line array. The goal of this project is to characterize the molecular mechanisms of ASB14in pancreatic cancer and senescence.

PAI-1 is a master-regulator of senescence transmission within the population of endometrial stromal cells

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The capacity of endometrial stromal cells (ESCs) to proliferate and differentiate in tissue-specific decidual direction is critical for endometrial plasticity and receptivity to embryos, what highlights their role in female fertility. Previously, we established that ESCs may undergo premature senescence, accompanied by proliferation loss and different intracellular alterations, in response to various stresses. This fact implies possible negative effects for female reproduction. The present study aimed to elucidate whether and how senescence may be transmitted within the ESCs population.
Firstly, we examined the effects of co-culturing of young ESCs with senescent cells in 2D and 3D models. We revealed that senescent ESCs may induce paracrine senescence in young counterparts. Secondly, we checked whether this phenomenon might be reproduced by factors secreted by senescent ESCs, termed senescence associated secretory phenotype (SASP). Indeed, conditioned medium from senescent ESCs could promote senescence in neighboring cells. Raising a question about what fraction of SASP has more adverse effects on the young ESCs, we compared the influence of soluble factors and extracellular vesicles. Although the negative effect of soluble factors was more significant, both fractions were responsible for paracrine senescence propagation in the population of ESCs. Therefore, we focused on the precise analysis of the overall SASP protein content. According to mass-spectrometry data, confirmed by ELISA and Western blotting, we identified plasminogen activator inhibitor-1 (PAI-1) to be the most prominent protein secreted by senescent ESCs. Of note, PAI-1 contained in both soluble fraction and extracellular vesicles. This finding pushed us to investigate the possible PAI-1 role in triggering SASP-mediated paracrine senescence in ESCs. By applying CRISPR/Cas9 techniques we disclosed that PAI-1 secreted by senescent ESCs may serve as the master-regulator of paracrine senescence transduction within the ESCs population. The results of this study allowed establishing the fact of senescence transmission in the population of ESCs as well as expand understanding of the mechanisms of this phenomenon. Based on this knowledge, we can consider PAI-1 as a target for preventing senescence propagation within the ESCs population, what can make a contribution to the treatment of endometrium dysfunction.

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Poster presentations

Major traits of the senescent phenotype of nucleus pulposus intervertebral disc cells persist under the specific microenvironmental conditions of the tissue.

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Intervertebral discs (IVDs) are the joints of the spine, mainly consisting of extracellular matrix (ECM) with a low number of cells embedded therein. Low cellularity stems from nutrient deprivation due to the lack of blood supply, as well as from the hypoxic and hyperosmotic conditions prevailing in the tissue. Intervertebral disc degeneration (IDD) has been firmly connected with low back pain, a major age-related disease, whereas degenerated discs have been characterized by increased proteolytic activity and accumulation of senescent cells. While the catabolic phenotype of senescent IVD cells has been documented, whether this phenotype is preserved under the harsh conditions met in the IVD milieu has never been investigated. Here we showed that a combination of low glucose, hypoxia, high osmolality and absence of serum is anti-proliferative for young disc cells. In addition, we demonstrated for the first time that classical senescence markers, namely p16INK4a, p21WAF1 and ICAM-1, remain up-regulated in senescent cells under these conditions. Finally, up-regulation of the main senescence-associated ECM degrading enzymes, i.e. MMP-1, -2 and -3 was maintained in this strict environment. Conservation of IVD cells’ senescent phenotype under the actual conditions these cells are confronted with in vivo further supports their possible implication in IDD.

Kouroumalis et al. Mech Ageing Dev. 177 (2019);177:118-127.

Genome wide screens for senescence inducers: using a suicide switch to deplete unwanted populations

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Our suicide switch approach can streamline screens for growth arrested populations and has been applied successfully to identify new senescence inducers in cancer cells.

Cellular senescence is a state of stable cell cycle arrest which is important for wound healing and is thought to suppress the growth of premalignant cells. However, the accumulation of long-lasting senescent cells can drive age-related pathology through a senescence-associated secretory phenotype. We have recently demonstrated that senescence can be exploited in a two-step approach to kill cancer cells. To find new vulnerabilities that can induce senescence in RAS mutant cancer cells, we set out to do a CRISPR-Cas9 mediated knockout screen comparing proliferating and growth arrested cells with and without a senescence-associated secretory phenotype. Screening for senescence inducers at a genome-wide scale is challenging as the cells become growth arrested and the senescent phenotype takes 1-2 weeks to develop; and billions of negative cells will keep proliferating which makes the workflow unfeasible for a regular lab. Therefore, previous efforts were either screens for activation of a senescence reporter using a targeted library or genome wide dropout screens.

In order to distinguish senescent cells with a secretory phenotype from other growth arrested cells in a genome wide screen, we developed a suicide switch to eliminate proliferating cells during the screen allowing a subset of growth arrested cells to adapt a secretory phenotype. Using our system, we have identified genes that can trigger growth arrest in RAS mutant cancer cells and genes associated with the secretory phenotype of senescent cells. Validated hits contain several components of the autophagy pathway, and small molecule inhibition of these targets gave robust senescence induction. In addition, consecutive treatment with ABT-263 quickly eliminated the cells, which offers possibilities for potential cancer treatment.

Poster presentations

Spatial coordination between a miRNA locus and its target gene during the transition to senescence

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miRNAs are potent modulators of gene expression known for the post-transcriptional control they exert on mRNAs in the cytoplasm. Over the last decade, accumulating evidence show that microRNAs may also localize in the nucleus and form complexes with proteins of the RNAi machinery. Still, while the cytoplasmic mode-of-action of microRNAs is well described, the mechanisms by which nuclear miRNAs find and regulate their targets remain elusive. It has been proposed that nuclear miRNAs may invoke both transcriptional gene silencing or activation. Furthermore, we previously showed that spatial miRNA-networks do form, and it is thus attractive to assume that these networks might facilitate the targeting and regulatory action of nuclear miRNAs. Here, we present evidence of the miR-3145 locus engaging in spatial regulatory interactions with the PERP gene, which encodes a p53-associated effector critical for cellular senescence entry by human primary lung fibroblasts. First, we used profiling of chromatin- and "transcription factory"-enriched miRNAs to find that miR-3145 is specific to nuclei of proliferating cells, and becomes suppressed upon senescence, when PERP is strongly induced. Next, we applied native i4C-seq and intronic RNA FISH in proliferating and senescent cells from different donors to show that the two loci interact spatially in proliferating cells, and that abolishing this interaction promotes PERP activation and senescence entry. Then, a combination of "factory" RNA-IP and ChIP allowed us to identify the Drosha protein in complex with the pre-miR-3145 as the key effectors in this coordinated interaction, and the PERP promoter as they main target region. Last, we use a CRISPRa approach to show how PERP activation can lead to a senescent phenotype. As a result, we propose a novel regulatory axis that exploits the three-dimensional folding of human chromosomes to coordinate targeting of gene loci by nuclear miRNAs via Drosha in a co-transcriptional manner.

Poster presentations

DNA replication dynamics during senescence progression

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Organismal aging entails a gradual decline of normal physiological functions and a major contributor to this decline is failure of the cell cycle, known as senescence. Senescence can result from telomere diminution leading to a finite number of population doublings, known as replicative senescence, or from oncogene overexpression (OIS), as a protective mechanism against cancer. Senescence is associated with large-scale chromatin re-organization, changes in gene expression and in the spatiotemporal program of DNA replication. Replication stress is a complex phenomenon, defined as the slowing or stalling of replication fork progression and/or DNA synthesis, which has serious implications for genome stability, and consequently in human diseases. Aberrant replication fork structures activate the replication stress response leading to the activation of so-called dormant origins, thought to be a safeguard mechanism to complete DNA replication on place and time. However the relationship between replicative stress and the changes in the spatiotemporal program of DNA replication in senescence progression was unclear.

To further analyse DNA replication dynamics and the relationship between replicative stress and chromatin reorganization, we followed firstly the reorganization of chromatin dynamics features associated to OIS induction, as well as changes of the DNA replication program, and we identified that the 3D architecture changes associated to SAHF formation are related to late replication domains. Then, we generated genome-wide Replication timing (RT) profiles of proliferative and pre-senescent cells from distinct age donors, as well as from a patient with Hutchinson-Gilford progeria syndrome (HGPS). We found that, despite substantial alterations in replication origin activity associated to replication stress, the RT program is almost stable during senescence progression (Rivera-Mulia et al., 2018). However, we were able to identify specific genomic regions that replicate at distinct times in ageing cells, as DNA replication timing signature of aging (Rivera Mulia et al. 2017).

We demonstrated previously the existence of a cell type specific program of DNA replication origins activities (Besnard et al., 2012). To further investigate for the dynamics DNA replication program during senescence progression, we combined single DNA fiber combing of replicated DNA, origin mapping by sequencing of short nascent strands at the origins. We firstly demonstrated that, progression into senescence leads to reduced replication fork rates and activation of dormant origins in different senescence induction situations. Then, we analysed SNS by a new bioinformatic method to descriminate between closed origin peaks and to follow the activation of dormant origins. We identifed an increased number of origin peaks almost conserved in proliferative and pre-senescent cells and a specific signature of origin usage associated to senescence progression.

Altogether, our results demonstrate that during senescence progression, replication stress triggers an increased activity of pre-existing replication origins, called dormant origins, but that the replication timing is largely resistant to this replication stress. It alos suggests that changes observed in RT associated to physiological and accelerated aging might be a consequence of repetitive or chronic stress, giving new insights into the interplay between senescence, aging and DNA replication programs.

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Identifying novel pro-senescence compounds for p16-positive basal-like breast cancer

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Arresting uncontrolled proliferation of cancer cells by targeting pro-senescence pathways is a promising therapeutic strategy for a variety of cancer subtypes. The signatures targeted by pro-senescent therapies are unique to each cancer type, which presents a challenging and dynamic area of research (1). Palbociclib is a CDK4/6 inhibitor which has been approved as a senostatic drug for HR-positive and HER2-negative breast cancer (2). However, current senostatic therapies, such as palbociclib, do not target p16-positive basal like breast cancer (BLBC) cells. Therefore, this project aims to find novel pro-senescence compounds that potentially resensitise BLBC cells to endogenous p16, thereby inducing cell cycle arrest and providing a novel treatment route for tumours with a current unmet need. Using genome-wide siRNA screening, 25 gene targets have been identified that induce senescence when knocked down in BLBC cells. These include ribosomal proteins RPS3A and RPS7, which have been extensively characterised in the lab. Importantly, the knockdown of these proteins is well tolerated in normal mammary epithelial cells. Senescence induction in p16-positive BLBC cells appears to be driven by p16 nuclear translocation. As such, these findings highlight the possibility of driving p16-positive BLBC cells into a senescent state. Subsequently, a large-scale compound screen was performed to identify drugs that induce senescence in p16-positive BLBC cells. A 10,000-diversity compound library was initially investigated in a high-throughput screen at two concentrations. From this, we have identified 447 compounds that induced senescence in p16-positive BLBC cells using multi-parameter phenotypic analysis. Currently, these compounds are being validated in a high-throughput secondary 10-point dose response assay with the goal of identifying lead compounds for the drug discovery pipeline. These compounds will be analysed to discover their mode of action, with the potential that some of them may work through ribosomal stress-induced senescence pathways previously identified by the lab. Ultimately, small molecular compounds identified in the screen could be used as a targeted therapy for both BLBC and a variety of other p16-positive cancer types.

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Poster presentations

Elimination of age-related senescent cells improve healthspan and tissue homeostasis

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Cellular senescence is a state of irreversible growth arrest, which relies on the activity of the cyclin-dependent kinase (CDK) inhibitor p16. Senescent cells activate the senescence associated secretory phenotype (SASP), a complex secretory program that promotes many physiological processes and helps to maintain tissue homeostasis. However, senescent cells are reported to aberrantly accumulate during aging in various human and mouse tissues, and their chronic presence is associated to tissue degeneration and age-related pathologies. Using our recently developed p16-3MR mouse model, where p16+ cells can be tracked by Renilla luciferase and RFP, and eliminated by GCV, we show that senescent cells gradually accumulate with age in various tissues, and that the age-associated accumulation is at least partly due to impaired clearance, as old p16-3MR mice required at least 3 months to re-express a significant number of senescent cells after a GCV treatment. Mice undergoing constant removal of senescent cells showed improved healthspan as measured by increased strength and endurance. Using the skin as a reference tissue, we also show that removal of chronic senescent cells is sufficient to boost tissue repair and delay tissue degeneration. The data reported here support the pro-aging function of senescent cells in vivo, and underline the potential of senolytic interventions to improve healthspan and tissue regeneration.

Investigating the systemic effects of hepatocellular senescence.

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Acute liver failure (ALF) is a rare but deadly disease. Its acute onset, rapid progression and multi-organ manifestations, make ALF a major clinical challenge. However therapeutic options are limited.
Cellular senescence is a stress response involved in multiple physiological and pathological processes including ageing, tumour suppression and tissue repair. In vitro studies have shown that senescence may be transmitted in paracrine and/or juxtacrine manner to nearby cells [Acosta et al. 2013, Hoare et al. 2016]. We and others have recently reported paracrine senescence in the liver using genetically engineered models [Bird et al. 2018]. Here we investigate whether senescence may be transmitted between organs in vivo.
Firstly, we validate a model of hepatocyte restricted cell-autonomous senescence utilising AAV-TBG-Cre mediated deletion of Mdm2 in hepatocytes. In this model we observe activation of TGFβ signalling. Then using progressive escalation of hepatocyte senescence and injury we observe renal tubular expression of p21 in response to liver senescence. We identify that renal tubular p21 expression is TGFβ dependent through inhibition in vivo. We are also examining spread of senescence to other organs in subacute models of hepatocyte senescence. These studies may provide insight for translating the inhibition of transmissible senescence in liver disease.

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Poster presentations

Identification of senescent cells in various CNS regions after injury

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The presence of senescent cells in various tissues has been reported for different developmental stages as well as in relation to aging, but also after certain types of injuries. While we have begun to understand the role of senescent cells in wound healing in organs like skin, hardly anything is known about senescent cells identity and function after injury in the nervous system.
We therefore examined the presence and function of senescent cells after injury in different regions of the murine CNS, the neocortex and spinal cord. For that purpose the expression of the senescence marker proteins p21 (CDKN1A) and pp38 MAPK were investigated using immunohistochemistry after a stab wound injury in the somatosensory cortex, after middle cerebral artery occlusion as well as after a mid- thoracic dorsal hemisection of the spinal cord. As early as one day after lesion p21 positive cells are present in the lesion area in neocortex as well as spinal cord. By day three another population of cells immuno-reactive for pp38MAPK is found in the lesion area of the different injury models. On day seven only few p21 positive cells are detectable in neocortical lesion areas, while senescent cells are still numerous at day nine post injury in spinal cord. Co-localization with different glial subtype-specific proteins revealed that most senescent cells are astrocytes.
Thus, distinct CNS regions differ profoundly in regard to the abundance of senescent cells. This seems to be independent of the injury size, as few senescent cells were present in the neocortex upon large stroke injury. We recently found profound differences between injury models involving Gray and White Matter or Gray matter only on reactive gliosis after lesion (Mattugini et al., 2018). Therefore we are presently exploring the influence of Gray Matter damage or White Matter and Gray matter damage to the emergence of senescent cells and their functional contribution after spinal cord lesion.

Investigating the role of senescence in developmental disorders

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Developmental senescence is a form of programmed, physiological senescence that occurs transiently in specific embryo locations and contributes to patterning during embryonic development. We have recently shown that the SIX1 homeoprotein is a repressor of cellular senescence (Adrados et al, 2016, PMID: 26500063). SIX1 is also a key developmental regulator, essential for organogenesis. Six1-null mice display profound abnormalities in organs like the ear, kidney and muscle, among others. In humans, mutations in members of the SIX/EYA pathway are linked to the Branquio- Oto-Renal (BOR) syndrome, a rare congenital disorder with severe defects in the ear, kidney and branchial arches. With this background, we have used Six1-deficient mice to test the hypothesis that dysfunction of senescence can underpin the developmental defects associated with SIX1 deficiency in mice and humans. We have focused on the developing inner ear, an organ where physiological developmental senescence is found and is also severely affected in Six1-deficient mice and in BOR patients. Our results show elevated senescence markers and reduced proliferation in Six1-KO developing inner ears, concomitant with defective morphogenesis of senescent structures. Collectively, these findings suggest the existence of aberrant senescence in these embryos. Transcriptomic analysis and pharmacological interventions have been used to investigate further this phenotype, in order to identify the molecular pathways involved and the impact of senescence manipulation. Our results strongly support the notion that disruption of the physiological program of developmental senescence may be linked to developmental disorders.

Poster presentations

Phospholipase A2 Receptor 1 Promotes Lung-Cell Senescence and Emphysema in Obstructive Lung Disease

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Introduction: Exaggerated lung-cell senescence is emerging as a key pathogenic factor in several chronic respiratory diseases, including chronic obstructive pulmonary disease (COPD). We previously identified phospholipase A2 receptor 1 (PLA2R1) as a master senescence regulator. Its role in pathology, however, remains unknown.
Methods: We studied lung samples and derived cultured cells from patients with COPD and controls, transgenic mice overexpressing PLA2R1, and mice injected intratracheally with a lentiviral vector encoding PLA2R1.
Results: Compared to control lungs, lungs from patients with COPD had higher PLA2R1 protein levels, with prominent PLA2R1 immunostaining in both pulmonary vascular cells and alveolar epithelial cells that also stained for p16. PLA2R1 mRNA and protein levels were also higher in cultured pulmonary-artery smooth muscle cells (PA-SMCs) and pulmonary endothelial cells (P-ECs) from patients with COPD, which exhibited early-onset cell senescence manifesting as fewer population doublings (PDLs) and higher beta- galactose-positive cell counts. PLA2R1 knockdown induced by infecting cells with retroviral vectors encoding shRNA against PLA2R1 delayed cell senescence onset and decreased inflammatory cytokine release by senescent cells. Cells transduced with the PLA2R1 gene exhibited cell senescence and JAK/STAT pathway activation. These effects were almost fully inhibited by concomitant treatment with the JAK1/2 inhibitor ruxolitinib.
To assess whether PLA2R1 upregulation caused lung lesions, we developed transgenic mice conditionally expressing PLA2R1 (LSL-PLA2R1). Moreover, we gave wild-type mice intratracheal injections of lentiviral vectors carrying the PLA2R1 gene (LV-PLA2R1) or mCherry (LV-mCherry). In both models, lung PLA2R1 overexpression induced lung-cell senescence and mimicked COPD lung alterations, with development of lung emphysema, pulmonary hypertension, and inflammation. Concomitant ruxolitinib treatment of LV- PLA2R1-treated mice attenuated lung emphysema and pulmonary inflammation.
Conclusion: These results identify the PLA2R1-JAK/STAT pathway as a new therapeutic target in COPD.

Poster presentations

Dissecting cell-autonomous and paracrine functions of cell senescence in AML response to therapy

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Background: Acute myeloid leukaemia (AML) is the most common leukaemia in adult people. First-line treatment of AML relies on cycles of intensive chemotherapy. The establishment of therapy-induced senescence (TIS) in response to anticancer drugs represents a powerful tumour suppressive mechanism that can activate a senescent program in AML cells. Senescence is characterized by cell cycle arrest and by a profound activation of the so-called Senescence Associated Secretory Phenotype (SASP) that can either promote immune-surveillance or chronic inflammation likely contributing to tumour relapse.
Materials & Method: In this work we developed a protocol to induce senescence by chemotherapy (TIS) in AML cell lines and short-term primary cultures on stromal cells and analysed accumulation of SA-βGAL by ImageStreamX technology, kinetics of DNA damage response and SASP activation. To assess SASP production, we collected media derived from senescent or not-senescent AML cells and performed protein arrays in order to identify secreted factors. In parallel, we used senescent blasts-derived conditioned medium to culture untreated AML cells in order to study the paracrine effects of SASP on tumour growth.
Results: We first conducted SA-βGAL staining on ex-vivo cultured primary AML samples at diagnosis and upon chemotherapy and found activation of a senescence program with concomitant upregulation of pro-inflammatory genes, including IL1, IL6 and IL8. Of note, SA-βGAL signal was higher in a cohort of patients that were clinically considered "responders", indicating that senescence in AML acts as a tumor suppressor mechanism. To evaluate the long-term effects of the senescence-associated secretory program upon chemotherapy we cultured AML blasts in the presence of TIS-derived conditioned medium and consistently observed a proliferative boost in AML cells, suggesting the presence of pro-tumorigenic factors released in response to senescence induction. Moreover, AML cells cultured in CM derived from senescent AML cells were able to form more colonies in semisolid media than controls. Delving into the mechanisms of the observed response, we discovered that Anakinra, the recombinant antagonist of IL1R, rescues the proliferative advantage of AML cells cultured in the presence of senescence-secreted factors, thus suggesting that senescence-induced IL1α and/or IL1β are involved in the process.

Conclusion: Overall, our study provides mechanistic insights into the biological and cellular response of AML cells to TIS and presents SASP and senescent cells as possible targets to improve AML response to therapies and counteract the onset of relapse.

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Evaluation of fetal mesenchymal stem cells senescence during in vitro amplification for therapeutic purpose: choice of cell quality parameters

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Mesenchymal stromal cells (MSCs) are fibroblast-like multipotent cells capable of proliferation, self-renewal and they are characterized by their ability to differentiate into different cell-lineage. They are known to be implicated in many key biological processes like tissue regeneration, secretion of bioactive factors which give them immunomodulatory and trophy properties and as therapeutic drugs. In order to use MSCs for therapeutic purpose, these cells must be expanded in long-term, which inevitably triggers cell senescence. In this regard, a quality control of the function and phenotype of MSCs and the evaluation of cell senescence during culture are required. Cellular senescence refers to the process of cell proliferation arrest that occurs in vivo and in vitro. This phenomenon is considered as a response of the cell to excessive endogenous end exogenous stresses, among which, telomere shortening, genomic damage, epigenomic damage, mitochondrial deterioration, oxidative stress, unbalanced mitogenic signals and several therapeutic interventions. Identification of senescent cells is not limited to permanent growth arrest, but several other features and markers are used to characterize them. These cells adopt a flattened shape with enlarged volume. In addition, they are characterized by the overexpression of β-galactosidase enzyme, and tumor suppressor proteins as p16INK4a. Moreover, senescent cells are metabolically active and exhibit a senescent-associated secretory phenotype known as SASP that can affect cell behavior.
The aim of the study was to characterize in vitro expansion of MSCs derived from Wharton's Jelly (WJ), to control the quality of MSCs in terms of function and phenotype but also to evaluate cell senescence. To induce senescence process, we used in vitro model called "replicative senescence", and cells were cultured in normoxic (N) and hypoxic (H) conditions then analyzed in early and late passages. Proliferation and senescence analysis of MSCs demonstrated a high rate of proliferation and a low rate of senescent in H condition. On the other hand, cell-surface biomarker analysis revealed that all MSCs derived from WJ expressed the common surface markers among which CD44, CD105 and that cells in late passage expressed higher expression of CD157 and CD264 but a lower expression of CD146 compared to MSCs in early passage. These cells also had important immunomodulatory properties compared to MSCs in early passage. In addition, the study of the ribosomal RNA (18S, 28S, 5.8S, 5S) methylation which are the most conserved modifications in animal cells revealed two distinct groups of methylation between early and late passages. All these results will improve the characterization of MSCs senescence and may provide a basis for establishing the quality of MSCs prior to their therapeutic application.

Poster presentations

Detailed HBV genetic map for a recent migration wave giving rise to indigenous populations in the Americas

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Objectives: Genetic studies have shown that the peopling of the Americas occurred through at least two independent migration waves from eastern Asia (1), and that some indigenous populations share genetic similarities with Australo-Melanesians (2). Our aim was to study the pathway and the dates of when this genetic signature arrived in the Americas, using the Hepatitis B virus (HBV)-human co-migration assumption (3).
Material and Methods: We analyzed all the available full-length HBV/D4 sequences (N=36) found on HBVdb. Bayesian molecular-clock and phylogeographic analyses were performed in MCMCTree4.8 and BEAST1.8, respectively.
Results: We found a common genetic ancestry for the HBV/D4 circulating in the indigenous populations of Canada, Brazil, the Caribbean, Australo-Melanesia, North-Eastern India, and Tibet. The geographic distribution of HBV/D4 correlates with human genetic similarities found previously between the indigenous people in the Americas and Australo-Melanesia. The outlier lineage of HBV/D4 consists of a clade in Tibet, with an early divergence into two clades: one consisting of two groups from North-Eastern India and Brazil, and the other comprising three subclades from Canada, Australo-Melanesia, and Caribbean people. Detection of sequences from Tibet in the outlier branch suggests that the putative origin of HBV/D4 was probably in Central Asia. The molecular-clock analysis estimated that the times to the most recent common ancestor (tMRCA) of the first and second clades were 4,752 (95% HPD: 3,230-6,210) and 5,415 (95% HPD: 4,060-6,730) years, respectively, and that the two clades diverged 7,487 (95% HPD: 5,610-9,510) years ago (Figure).
Conclusions: The monophyletic nature of HBV/D4 among indigenous people in the Americas, the Caribbean, Oceania, and Asia provides evidence of the close genetic relatedness of HBV from these populations. The inferred pattern of HBV/D4 mobility resembles the prehistoric Tibet-Burman migrations from Central Asia to North-Eastern India and further to Australo-Melanesia. Our study provides evidence that supports the utility of HBV as a tool for studying human prehistory.

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Figure. Phylogeographic tree (A), dated phylogenetic tree (B) and proposed pathways for HBV/D4 (C)

Identification of senescent cells in the intervertebral discs by using lipofuscin detection

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Low back pain (LBP) is considered a major age-related pathology affecting most of the population during lifetime. LBP is frequently associated with intervertebral disc (IVD) degeneration, a process that starts relatively early in life. The cellularity in the IVD is extremely low decreasing from the periphery to the core, most probably because of adverse nutritional conditions imposed by the avascular nature of the IVD. However, these cells seem to be extremely important for homeostasis as they regulate the production and accumulation of extracellular matrix components. We have shown that there is an increased proportion of senescent cells in herniated discs compared with non-herniated ones [Roberts S. et al Eur Spine J 15 (2006) S312–S316], a finding that has been reproduced by several other groups. Surprisingly, in several of these studies the percentage of senescent cells reported is unexpectedly high compared with other tissues. Accordingly, we estimated the number of senescent cells in IVD by using a novel staining procedure for the recognition of senescent cells in biological material including cultured cells, fresh/frozen, and archival (formalin-fixed and paraffin-embedded, FFPE) tissues, applying the GL13 immunohistochemical dye. GL13 binds on lipofuscin on perinuclear structures or in small granules in the cytoplasm of senescent cells [Evangelou K. et al. Aging Cell 16 (2017) 192–197].
In particular, we first measured the number of GL13-positive cells in IVDs from the spine and the tails of young and aged rats. In young rats the percentage of positive cells in both annulus fibrosus (AF) and nucleus pulposus (NP) was found below 2%. However, in aged rats, while in the NP the positive cells are again below 2%, in the AF positive cells were identified both in the spine and the tail (12.7% ± 2,03% and 7.7% ± 0,36%, respectively). In preliminary experiments in human samples, it was found that in pathological IVDs 16.4% ± 0,32 and 8.2% ± 1,41 positive cells in AF and NP, respectively. In contrast, in non-pathological specimens fewer positive cells were identified (8.8% ± 0.81% in the AF and below 2% in the NP). These preliminary data indicate ageing and degeneration leads to an increase number of senescent cells in the IVD. The presence of more senescent cells in AF, in comparison to NP, most probably is due to the specific microenvironment in these regions of the IVD and the different stresses these cells are exposed to.

Poster presentations

Stromal senescence induced by anti-cancer drug treatment causes a more aggressive phenotype in prostate cancer cells

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Cancer progression is strongly dependent on the cross-talk between tumor cells and their surrounding stroma, that stimulates many crucial processes of cancer malignancy, such as epithelial-to-mesenchymal transition (EMT), the enhancement of invasiveness, the acquisition of stemness features and the resistance to anti-cancer drug treatment(1). Cellular senescence, described originally as a potent tumor suppressive process, has also been correlated with deleterious effects, primarily due to the senescence-associated secretory phenotype (SASP). The SASP, indeed, is characterized by the increased levels of many pro-inflammatory cytokines, growth factors and proteases that generate a tissue microenvironment which can stimulate tumor progression(2). In this context, an interesting study demonstrated that several anti-cancer drugs, such as Doxorubicin and Paclitaxel, promotes senescence in mouse embryonic and dermal fibroblasts and the in vivo removal of the therapy-induced senescent cells reduces the short- and long-term side effects of chemotherapy(3). Docetaxel is the first and the most widely used anti-cancer drug for the treatment of metastatic and castration-resistant prostate cancer. Nevertheless, many patients develop resistance to Docetaxel with time and new studies of the mechanisms involved in the resistance to the chemotherapy and strategies to overcome this resistance are strongly necessary. The purpose of this study is to evaluate if Docetaxel induces senescence and SASP in human prostatic fibroblasts (HPFs) from benign prostatic hyperplasia. Moreover, the investigation aims to assess if the stromal senescence induced by Docetaxel treatment increases the aggressiveness of prostate cancer cells (PC3) and might be involved in the acquisition of a therapy-resistant phenotype. We demonstrated that low doses of Docetaxel induce senescence in HPFs. In particular, Docetaxel treatment causes proliferation arrest, an increase in SA-β-Gal positive cells, in the secretion of MMP-2, in ROS production and in the levels of mRNAs encoding for SASP components, such as IL-6, IL-8, VEGF-A and MMP-3. In turn, senescent fibroblasts promote prostate cancer aggressiveness, increasing the migratory and invasive abilities of PC3 cells. In conclusion, our preliminary results demonstrate that the therapy-induced senescent phenotype in stromal cells might exert a paradox effect through the induction of a more aggressive phenotype in the surrounding cancer cells.

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Poster presentations

Senescent cells identified in oral fibromas with the use of SenTraGor™: a pilot study.

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The role of cellular senescence as a tumor barrier has been extensively studied. Also there is growing evidence that senescent cells may not be exclusively "anti-oncogenic", but could also have a "pro-oncogenic role", especially the stromal senescent cells, through their senescence associated secretory phenotype. Evidence supporting the barrier role of senescence comes from studies in which cells triggered by ongogenic stimuli are arrested by senescence The in vivo evidence for its anti-tumorigenic role is indicated from the presence of senescent cells in the parenchyma of premalignant and malignant lesions (1).
In this study we intended to investigate whether the presence of senescent cells is part of the benign features of oral fibromas, (also referred as fibroepithelial polyps). Histologically these lesions show focal fibrous hyperplasia, and the etiology is usually local trauma or irritation. It is a very common oral lesion with remarkable resistance to malignant transformation (2).
To test this hypothesis we applied SenTraGor™ (a biotin-linked Sudan Black B (SBB) analogue tracing lipofuscin in senescent cells) which is a reliable, antibody-enhanced detection of senescent cells (3), in oral fibroma tissue samples (n=11) and normal oral mucosa samples (n=5). Sections from FFPE oral fibromas and normal oral mucosa were stained with SenTraGor™. In addition we tested all samples for proliferative potential using immunohistochemistry for Ki-67 expression.
Total average percentage of positive SenTraGor cells in the stroma (area of fibrous hyperplasia) was 27.5% for the oral fibromas group (n=11) in contrast to 8.6% in normal oral mucosa (normal stroma without hyperplasia) (n=5). Average expression of SenTraGor was 7.3% and 3.6% in the epithelium of fibromas and control group respectively. Of note, the percentage of stromal senescent cells was higher in the larger fibromas (size of lesion more than 0.6 cm). Also ki67 expression was almost exclusively identified in basal cells of epithelium in similar pattern in both groups.
A significant percentage of senescent cells in the area of fibrous hyperplasia were identified in this small case series of oral fibromas. Possibly these cells contribute to the benign nature of oral fibromas. Further study of this population of senescent cells in a strictly benign lesion could strengthen the current concept that there are various phenotypes of senescent cells.

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Identifying novel pan-cell type senolytics for the clearance of senescent cells

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Senescent cells accumulate during human ageing, potentially driving age-related dysfunction through depletion of mitotically active cells and stimulation of chronic inflammation. Clearance of senescent cells in mice prevents or attenuates progression of age-related disorders and significantly extends median health-and life-span, suggesting that senescent cells are causative in age-related pathologies1. As such, selective elimination of senescent cells using compounds presents an attractive therapeutic strategy for preventing age-related diseases and extending health-span2, 3. Previous work in our group has identified a group of functionally related enzymes with increased expression in deeply senescent (DS) human mammary epithelial cells (HMECs) compared to early proliferating (EP) cells. Further investigation of this group and the surrounding functional network generated using bioinformatics revealed 16 siRNAs which selectively eliminate DS HMECs but have no impact on their EP counterparts. Of note, eight of the 16 hits, including two of the functionally related enzymes, have increased expression during in vivo human skin ageing. Using targeted siRNA screening in DS human mammary fibroblasts (HMFs) and human dermal fibroblasts (HDFs) compared to EP cells, we have subsequently identified cell-type specific and multi-cell and tissue type senolytic and senostatic siRNAs. In order to identify novel senolytic small molecules that target similar processes to the senolytic siRNAs, high-throughput screening (>1,200 compounds) was performed using high-content imaging and analysis in DS HDFs and human dermal keratinocytes (DS HDKs) compared to their EP counterparts. From this, we have identified a novel family of compounds with multi-cell type senolytic activity that we have found to outperform existing senolytics, including dasatinib (D), quercetin (Q), D+Q, and navitoclax (N). Currently, top candidates are being investigated using a recently developed 3D 'aged' skin organotypic model. In summary, this work aims to identify multi-cell and tissue type genetic targets for small molecules in order to reveal novel routes to senescent cell clearance that could be used to ameliorate age-associated senescent cell accumulation and ultimately contribute to the wider goal of promoting healthy ageing.

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Poster presentations

Early growth response 2 (EGR2) is a novel master regulator of the senescence programme

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Senescence, a state of stable growth arrest, plays an important role in ageing and age-related diseases in vivo. Although the INK4/ARF locus is known to be essential for senescence programmes, the key master regulators driving p16 and ARF transcription remain largely underexplored. Previous work in our group has demonstrated that p16 siRNA knockdown can 'reverse' deep senescence (DS) in p16-positive adult human mammary epithelial cells (HMECs), thus providing a unique perspective for uncovering novel senescence drivers1. Using siRNA screening for modulators of the p16/pRB and ARF/p53/p21 pathways in DS HMECs and fibroblasts (HMFs), we identified EGR2 as a novel driver of senescence. EGR2 expression is up-regulated during senescence and its ablation by siRNA in DS HMECs and HMFs 'reverses' the senescent phenotype. We demonstrate that EGR2 activates the ARF and p16 promoters and directly binds to the ARF promoter. Loss of EGR2 downregulates p16 levels and increases the pool of p16-p21- 'reversed' cells in the population. Moreover, EGR2 overexpression is sufficient to induce senescence. Our data suggest that EGR2 is a novel master transcriptional activator of the p16/pRB and ARF/p53/p21 pathways driving senescence and a novel marker of senescence.

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Deciphering the impact of chemotherapy-induced SASP in lung cancer progression

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Lung cancer is the leading cause of cancer-related deaths in our society. Platinum-based chemotherapeutics such as cisplatin are often the treatment of choice, although it is known that they frequently result in treatment failure. Therefore, regardless of the efforts in improving lung cancer therapy, 5-year survival rate after diagnosis has remained lower than 15% for the last 40 years. Cisplatin is a DNA-damaging agent that induces cellular senescence, which restricts the propagation of cancer cells. However, discoveries over the past years have established that senescent cells implement a strong paracrine secretion, termed senescence-associated secretory phenotype (SASP), that increases the inflammatory milieu and can promote malignant phenotypes. Despite the impact this may have in lung cancer treatment and relapse, a complete understanding of the effects of the SASP in this context remains to be elucidated.

In this work, we analysed the paracrine effects of different chemotherapy-induced senescence types in human and mouse lung cancer cells. Co-cultures of senescent and lung cancer cells were performed, as well as senescent conditioned medium was collected to analyse the effect on a variety of functional assays, including proliferation, migration, stemness and mitochondrial metabolism. Our results show that platinum-induced SASP leads to an increased rate of division, migration and gain of colony and sphere-forming traits of recipient lung cancer cells, in addition to promoting a metabolic rewiring. High-throughput gene expression profiling and proteomics analyses allowed us to gain insight into the molecular mechanisms driving these effects. Of note, our results have been validated in vivo with tumour xenografts and orthotopic models of lung carcinogenesis.

Together, our data indicate that the paracrine effects of chemotherapy- induced senescence depend on the type of drug used, and suggest that platinum-based treatment of lung cancer cells promote malignant phenotypes in a cell non-autonomous manner, which might contribute to tumour relapse and an aggressive growth potential.

Poster presentations

The association between mitochondrial metabolic switch and muscle function declines according to aging

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Muscle requires continuous differentiation cycle to maintain its quality and function. In muscle differentiation, activation of muscle stem cells play critical role as it is the first step to be completed in order to start the differentiation. As aging arises, muscle stem cells activation ability diminishes and resulted in the decline of muscle function and its recovery response upon injury. On the other hand, it is eminent that mitochondrial defects are an inevitable phenomenon which occurs during aging. However, little is known about the relation of mitochondrial defects and the muscle quality during the occurrence of aging.

To get a better understanding on this matter, we compared the muscle quality and mitochondrial function between young and aged mouse. We found that muscle weights and exercise ability was significantly decreased in aged mouse compared to the young mouse. Interestingly, muscle function declines in aged mouse were accompanied by decreased oxygen consumption rate and elevated glycolysis. We then performed further confirmation using C. elegans as an aging model. In C. elegans, we observed the alteration of myofiber connectivity in age-dependent manner. In addition, we performed an age-dependent mitochondrial respiration measurement and found that the energy production pattern was also altered in the aged C. elegans. Compared to the young control worm, aged worm showed lower oxygen consumption rate. In contrast, the maximum extracellular acid production which reflects glycolytic rate was significantly higher in aged worm than in young control.

These results indicated that during aging both in mouse and C. elegans the main energy production methods by mitochondria was shifted from oxidative phosphorylation to glycolysis. We propose that this metabolic switch is most likely related to the ability of muscle quality maintenance during aging.

Role of Cellular Senescence in Skin Carcinogenesis

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Cellular senescence entails an irreversible growth arrest that evolved to prevent cancer. Senescent cells also secrete numerous pro-inflammatory molecules, termed the senescence-associated secretory phenotype (SASP). Previous studies show that senescent cells, through the SASP, can drive several age-related diseases. We recently demonstrated that genotoxic chemotherapeutic agents such as doxorubicin (DOXO) induce senescence and a SASP in mice. Although drugs such as DOXO are first-line agents for cancer treatment, their long-term side effects can be deleterious, often fueling the development of more aggressive cancers. To understand the role of senescence in cancer etiology, we subjected p16-3MR transgenic mice, which permit the identification and selective elimination of senescent cells in vivo, to the well-established two-step protocol of squamous cell skin carcinoma (SCC), in which tumorigenesis is initiated by a carcinogen and then developed by a tumor promoter. We show that the tumor promoter, but not the tumor initiator, promotes skin carcinogenesis by inducing senescence and a SASP. We also first induced senescence in p16-3MR mice with DOXO, and then followed with the two-step skin carcinogenesis protocol. Systemic treatment with DOXO exacerbated tumor growth caused by the two-step protocol, which was ameliorated by the selective removal of senescent cells. These findings suggest that senescence and the SASP can promote skin carcinogenesis, suggesting that drugs that clear senescent cells might be novel therapeutics.

Poster presentations

Characterisation of cancer cell senescence

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The most common anti-cancer treatment consists of chemotherapeutic agents that induce DNA damage in fast proliferating cells, which ultimately leads to apoptosis. It is known that cancer cells can also undergo senescence as a consequence of chemotherapy. The effect of senescent cancer cells in a tumor microenvironment still remains controversial, but chronic SASP-mediated inflammatory effects are thought to contribute to tumor relapse. For this reason, we propose to eliminate senescent cells as a consecutive treatment after chemotherapy or pro-senescence therapy. We have studied how universal the sensitivity of senescent cancer cells is to ABT-263, a potent senolytic agent that targets anti-apoptotic proteins required for the survival of senescent cells.

To do this, we tested a cell line panel of 15 cell lines consisting of melanoma, breast, lung, liver and colon cancer. To induce senescence, cells were treated with optimized concentrations of Alisertib, an aurora kinase A inhibitor that was found by our group as a potent senescence-inducer, and Etoposide, a chemotherapeutic agent with senescence-inducing potential[1]. Subsequently, we determined the IC50 values for ABT-263 treatment in senescent cells and parental cells to assess the sensitization to this senolytic. Interestingly, although the sensitization was very similar for etoposide vs alisertib-induced senescence, the response was highly divergent for different cell lines. This indicates that sensitization to ABT-263 is less dependent on the senescence trigger and is predominantly dictated by cell line intrinsic properties.

This raised the question what determines the sensitization of senescent cells to ABT-263. Since this BH3 mimetic inhibits anti-apoptotic proteins, it is feasible that cell death is mainly triggered in senescent cells that rely on anti-apoptotic signaling and harbor a functionally intact apoptotic response. To test this hypothesis, we checked the RNA and protein levels of multiple members of the apoptotic pathway. However, no correlation was found between ABT-263 sensitivity and the abundance of anti- or proapoptotic proteins.

Collectively, our data show that ABT-263 is not a universal senolytic agent and that the efficacy of senolytics depends on cell intrinsic properties. In future studies, we aim to find survival dependencies in senescent cells that are resistant to ABT-263, which will contribute to the search for novel senolytic agents.

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UVB-induced senescence in keratinocytes

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Skin is a fascinating organ to study ageing. Constituting a physical barrier of our body against external aggressions, the skin is subject to a whole series of environmental factors, constituting the skin exposome1, which can influence its ageing. Among them, UV rays appear to have the greatest impact as they are responsible for the development of specific traits leading to skin photoageing. We previously showed that repeated UVB exposures induce premature senescence of human diploid fibroblasts (HDFs)2. Here, we established a new model of premature senescence induced by repeated UVB exposures in normal human keratinocytes (NHKs). After 3 days, UVB exposures provoke the appearance of senescence biomarkers in NHKs such as growth arrest, SA-βgal and modifications of senescence-associated genes expression as observed in replicative senescent (RS) NHKs. We also showed that NHKs in UVB-induced senescence present long-term DNA damage (pyrimidine dimers and DNA double-strand breaks) associated with an activation of the DNA damage response (DDR) pathway. Finally, we showed that UVB-induced senescent NHKs display a variable expression and secretion of SASP-associated factors over time. This was confirmed by a transcriptomic analysis that highlighted the gene expression changes at different times after the last UVB stress.

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Poster presentations

Psychological Stress & Accelerated Aging in SCI Patients - A Longitudinal Pilot Feasibility Study

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There are many factors that influence aging, such as genetics, diet, and stress. The traumatic event of an spinal cord injury (SCI) and its effects on quality of life results in a high prevalence of chronic psychological distress in the SCI population, compared to the general population. Further, SCI patients often develop premature age-related morbidities, such as diabetes, cardiovascular issues and arthritis. The younger the patient and the higher the SCI lesion level the greater the loss of life expectancy. This phenomenon has been termed “accelerated aging” and occurs even if patients have access to the highest quality medical care. While the correlation, and to some degree the causal mechanism linking stress and aging is generally well understood in the general population, little is known about this link in the SCI population. As there is little information on chronic psychological distress induced accelerated aging in SCI individuals, feasibility must be assessed before future clinical trials. To assess feasibility we have established a data driven longitudinal prospective observational study to track levels of distress, and biomarkers of aging and stress. The aims of this feasibility project include a) monitoring recruitment rate, b) method validation, c) establishing aging and stress outcome reference values, d) statistical model selection, and e) power calculations given effect sizes and acquired sample sizes. Additionally, we hypothesize that there is a correlation between high distress levels and high levels of biomarkers of stress and aging. The patients in this feasibility study are participants in the Swiss Spinal Cord Injury Cohort (SwiSCI) study; a multicenter study that investigates SCI individual’s life experience; from clinical to societal outcomes, such as treatment effects and quality of life. Some SwiSCI participants also donate biospecimens at the beginning and end of rehabilitation to the SwiSCI Biobank. Outcomes assessed from the donated biospecimens, include a) telomere length in immune cells via flow cytometry, b) telomere length in whole blood gDNA via qPCR, c) plasma cortisol and d) plasma protein oxidation via ELISA, e) plasma protein glycation via fluorescent gel, and glucose and insulin in serum for f) homeostatic assessment (HOMA). Psychological distress is self-reported by participants in the SwiSCI study. Our expectations are that this project would allow us to assess if distress is correlated to accelerated aging in SCI individuals, and would allow us to determine if clinical trials with interventions against distress, to mitigate SCI accelerated aging, are feasible at our institution.

Exploring the Role of Exosome Encapsulated MicroRNAs in Senescence and Skin Ageing

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Senescent cells have been found to accumulate in chronologically aged tissues, including within the skin, and the increased presence of senescent cells has been causally linked with age-associated tissue deterioration. Exosomes are small (50-150nm) extracellular vesicles that have essential roles in intercellular communication. Exosomes contain cargo unique to their cellular origin, including specifically packaged miRNAs. Interestingly, exosome secretion from senescent cells has been shown to increase by up to 10-fold compared to early proliferative (EP) cells and these exosomes have an altered cargo, including different miRNAs (1). It has been theorised that senescent cell-derived exosomes may act as part of the senescent secretory phenotype to prematurely induce senescence in EP cells (2).

Work previously performed by our group demonstrated that miRNAs that are upregulated in senescent epithelial cells are capable of prematurely inducing senescence when transfected into EP epithelial and fibroblast cells (3). This project aims to explore the role of exosome encapsulated miRNAs in senescence and skin ageing. Human dermal fibroblasts (HDFs) were cultured to replicative senescence over ~300 days and exosomes were isolated from early passage, mid passage and senescent cells and analysed on the Nanosight 3000 for concentration and modal size. Presence of exosomal markers was determined by western blot. We aim to demonstrate cellular uptake of exosomes by means of fluorescently labelled EP HDFs and fluorescently labelled exosomes with imaging on a live cell fluorescent confocal microscope. This poster will present the current findings from this work.

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Poster presentations

Comparative proteomic profiling of replicative senescence reveals senotherapeutic targets including cytoskeletal organization, oxidative stress and mTOR signalling

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Cellular senescence is an essentially permanent proliferation arrest programme cells undergo in response to a variety of stressors including exhaustive telomere attrition, irreparable DNA damage or oncogene activation. Cellular senescence plays a number of beneficial physiological roles, such as in wound healing, embryonic development, tissue regeneration and in preventing unchecked propagation of potentially tumorigenic cells. However, with increasing age senescent cells accumulate in vivo in mammals, driving the progression of age-related diseases through causing tissue dysfunction, limiting tissue regenerative capacity and through pleiotropic, pro-inflammatory secretion. We have performed comparative proteomics analysis on human primary fibroblasts, analysing cells throughout the cellular replicative capacity until proliferative arrest. To create an unbiased profile of the molecular framework of cell senescence, we coupled pathway and network analysis to identify significant alterations in candidate proteins and pathways which accompany the onset of senescence. We have identified substantial changes in inflammatory signalling, chromatin modification, protein ubiquitination, mitochondrial fission, oxidative stress, protein translation, mTOR signalling and actin cytoskeleton organization. Informed by our proteomic profile of replicative senescence, we have evaluated the potential of a number of such altered proteins and pathways as senotherapeutic targets using repurposed compounds already known to be well-tolerated in the clinic. We have revealed that acute inhibition of mTOR signalling reverses multiple phenotypes of senescence including enlarged, granular morphology, actin stress fibres, mitochondrial accumulation, lysosomal dysfunction and pro-inflammatory secretion (Walters et al Aging 2016). These data are consistent with reports from other groups, and with promising results assessing the utility of mTOR inhibitors in the clinic for a wide range of age-related diseases (Walters and Cox IJMS 2018). We have since shown that pharmacological inhibition of the small Rho GTPase Cdc42 or associated downstream kinases PAK and ROCK impacts crucial features of senescence including senescent morphology, cell motility and intercellular communication. Furthermore, we have investigated pharmacological inhibition of antioxidant proteins upregulated upon entry into replicative senescence and observed senotoxicity across several models of senescence upon pharmacological inhibition. As such, our proteomic profile of replicative senescence has created a rich resource for senotherapy target discovery at both the protein and pathway levels, identifying promising repurposed compounds that target central molecular processes involved in senescence.

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Novel transcriptional mechanism regulating autophagy in cellular senescence

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Aging correlates with reduced autophagy, and autophagic impairment is associated with onset of age-related disease. Although the accumulation of senescent cells affects the organismal aging and the prevalence of age-related disease, the knowledge regarding roles of autophagy in cellular senescence is still limited. To identify a pathway that can coordinate autophagy in cellular senescence, we focused on a transcription factor which has been reported to promote longevity through autophagic pathway in C. elegans. In this study we found that the activity of the transcription factor goes down during senescence. siRNA knockdown of this factor accelerated cellular senescence with increased SA-β-gal positive cells and SASP expression, while the overexpression of the factor suppressed the increase of SA-β-gal positivity, suggesting the involvement of this factor during cellular senescence. Ongoing RNA-seq analysis combined with targeted gene expression studies indicates that this transcription factor contributes senescent state through regulating some autophagy gene. In our presentation we will discuss updated mechanistic insight of this transcription factor and role autophagy in cellular senescence.

Poster presentations

Regulation of myofibroblast phenotype during senescence

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Cellular senescence is an antiproliferative response involved in diverse physiological and pathological processes, which has been related to differentiation and plasticity in a variety of contexts. With this background, we set to study the impact of senescence in the differentiation potential of human primary fibroblasts, focusing on differentiation to myofibroblasts, contractile and highly fibrogenic fibroblast-like cells with an important role in wound healing and fibrosis. We have observed a clear loss of myofibroblast phenotype during fibroblast senescence, as shown by the down-regulation of myofibroblast markers such as alpha-smooth muscle actin and collagens, or changes in the extracellular matrix. The down-regulation of myofibroblast markers occurs in response to a wide range of oncogenic and DNA damage-related senescence stimuli. Furthermore, the observed phenotype is transmitted by the Senescence Associated Secretory Phenotype (SASP), as we have shown using coculture and conditioned medium experiments. Interestingly, a kinetic transcriptomic study of paracrine senescence has confirmed the non-cell-autonomous transmission of this phenotype. Pharmacological and genetic manipulations indicate that the change in myofibroblast phenotype during senescence is under a complex regulatory system that seems to involve both the early fibrogenic and the late inflammatory waves of the SASP. Our results reveal a link between senescence and myofibroblast differentiation that may have implications in the context of normal and pathological myofibroblast function.

The role of calcium and PARP signaling in cell fate decision: A choice between senescence and death

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Cellular senescence is a process of irreversible growth arrest that acts as a potent tumor suppressive mechanism. Through their secretory phenotype, senescent cells cover different biological roles besides oncosuppression either during embryogenesis or adulthood. Paradoxically, the accumulation of senescent cells with aging contributes to the creation of a microenvironment which promotes the onset of different age-related diseases, including cancer.

Reactive oxygen species (ROS) are essential messengers inside the cell, but excessive amounts can lead to irreversible damage and result in cell senescence or death. However, the mechanisms determining the decision between those two cell fates are not clearly understood.

Here, we show that treatment with hydrogen peroxide (H202), results in calcium-dependent cell death. Treating cells with H202 in the presence of an intracellular calcium chelator agent is sufficient to rescue cells from death but results into senescence. Interestingly, the same switch can be obtained by pre-incubating H202-treated cells with PARP inhibitors, suggesting one of the main mechanisms of cell death induction upon high oxidative stress derives from PARP hyperactivation. In addition, senescent cells derived from aborted cell death develop resistance to oxidative stress, suggesting that calcium and PARP signaling might be modified in a way that favors cell survival.

The kidney is a highly metabolic organ that is vulnerable to damage caused by oxidative agents such as the chemotherapy agent cisplatin. Similarly to what observed in the H2O2 model, we show that PARP inhibition prevents necrotic cell death induced by cisplatin but results into senescence in epithelial kidney cells. Switching cell fate from necrosis to senescence can protect kidney from acute damage but the chronic presence of senescent cells might lead to kidney dysfunction. For that reason, a second therapy that will eliminate senescence by apoptosis, which can restrain the inflammatory response in contrast to necrosis, might protect kidney function in the long-term. This two hit model to prevent both acute and chronic kidney disease is under development in our laboratory.

Poster presentations

Modelling gene regulatory networks in oncogene-induced senescence

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keywords: systems biology; cellular senescence; mathematical modelling; dynamical system; transcriptomics

Cellular senescence (CS) is a cell fate that arrests cell proliferation in response to numerous stresses most notably oncogenes such as RAS. The phenotypic transformations that occur in CS include cell cycle arrest, inflammatory responses and a complex metabolic shift. An emerging paradigm stipulates that senescent cells are major contributors to health and age-related illnesses, particularly cancer. As such, research on therapeutic strategies targeting senescence to treat cancer and improve healthspan has gained enormous momentum in recent years. The phenotypic and transcriptomic changes that occur in CS can be interpreted as transitions in a high-dimensional state space, where each dimension corresponds to a molecular species. These transitions are specified by the architecture of its underlying gene-regulatory network (GRN), which represents the possible molecular interactions encoded in the genome. In order to describe and predict the mechanisms governing CS, we applied the Sparse Identification of Nonlinear Dynamics algorithm (SINDy) in a high performance computing environment to datasets containing time-course gene expression data on cells undergoing senescence. Our proposed predictive modeling approach will provide a deeper understanding of cellular senescence and has the potential to unravel unknown vulnerabilities of senescent cells that may be exploited to promote healthspan.

Role of small extracellular vesicles in paracrine cellular senescence

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Ageing is a major risk factor for many human diseases. It is a complex process that progressively compromises most of the biological functions of the organisms, resulting in an increased susceptibility to disease and death. Senescence is a cellular phenotype characterized by a stable cell cycle arrest. Senescent cells are accumulated in the body during ageing. It contributes to develop age-related diseases and cancer. The alteration in intercellular communication with age has been demonstrated to be due to senescent cells developing a phenomenon denominated senescence-associated secretory phenotype (SASP). Exosomes are small extracellular vesicles (sEV) (30-120 nm) of endocytic origin whereas microvesicles are formed by shedding of the plasma membrane. They contain nucleic acids, proteins and lipid that generally reflect the status of the parental cell and can influence the behaviour of neighboring cells. In this study, we demonstrated that the small extracellular vesicles (sEV) contribute for transmitting paracrine senescence to proliferative cells. Firstly, we evaluated the presence of exosome-like particles in the sEV from senescent cells by detection of exosome markers (Alix, Tsg101 and CD63), Transmission Electronic Microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). To determine that sEV from senescent cells are mediators of the paracrine senescence, we performed functional assays using super-resolution imaging and hight-throughput. Besides, we confirmed at a single-cell level that the proliferative cells internalizing sEV from senescent cells activate senescence process using the Cre-reporter system. sEV protein analysis from senescent cells by mass spectrometry (MS) and validation of top candidates using a functional siRNA screen identify Interferon Induced Transmembrane Protein 3 (IFITM3), a component of non-canonical interferon (IFN) pathway, as partially responsible for transmitting senescence to proliferative cells. In conclusion, we found that sEV are regulators of paracrine senescence and IFITM3 contained in senescent sEV has an important role in the intercellular communication mediated through sEV during cellular senescence.

Poster presentations

Novel insights into the role of the oncometabolite R-2-hydroxyglutarate in telomere dysfunction and cellular senescence induction.

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Isocitrate deshydrogenases 1 and 2 (IDH1/2) are enzymes of the tricarboxylic acid (TCA) cycle that catalyze synthesis of α-ketoglutarate (αKG) from isocitrate. Alterations of IDH1/2 are frequent in brain cancer and occur early in the development of the disease, suggesting that abnormal metabolic function contributes to tumorigenesis. Hence, recurrent cancer-derived IDH1/2 mutations result in a gain-of-function by abolishing αKG production activity while enabling the conversion of α-KG to R-2-hydroxyglutarate (R-2HG). As a consequence, the aberrant accumulation of R-2HG leads to the inhibition of αKG-dependent enzymes, such as the lysine demethylase KDM4A. In addition to its well-established function in modulating the chromatin structure, KDM4A has been shown to play roles in various processes including the DNA damage response and cellular senescence. Interestingly, IDH mutations have been associated with defects in DNA damage sensing and repair, but whether IDH lesions can induce cellular senescence remains elusive. Here, we show that either R-2HG treatment or KDM4A depletion is sufficient to trigger cellular senescence in diploid fibroblasts. In addition, we demonstrate that abrogating KDM4A function using either RNA interference or R-2HG application give rise to telomeric defects. Telomeres are specific nucleoprotein structures protecting the ends of eukaryotic chromosomes from the induction of DNA damage. Constituted by tandem-repeated G-rich sequences, and organized in a specific T-loop structure, telomeres are challenging genomic loci to replicate. Hence, we evidence that inhibiting KDM4A increases the occurrence of fragile telomeres, indicative of replication stress at telomeres. Our data further shows that KDM4A binds to the telomeric chromatin, where it maintains low levels of H3K9me3. We are currently investigating the precise mechanism by which R-2HG and the catalytic inhibition of KDM4A regulate DNA replication at telomeres. We propose a model in which IDH oncogenic mutations and R-2HG production lead to KDM4A inhibition, resulting in telomeric defects. As telomere dysfunction causes genetic instability and is a critical step in malignant transformation, R-2HG-mediated senescence induction may initially halt tumorigenesis. However, p53 mutations are frequently detected in biopsies from patients harboring IDH mutation, which could ultimately favor the bypass of cellular senescence and tumor progression. Overall, our work will contribute to a better understanding of the mechanisms by which IDH mutations initiate a fertile ground for carcinogenesis in brain cancer. In addition, this study reveals a novel link between metabolic defects and telomere instability.

Killing of Senescent Cancer Cells by Mediators of Type I Immune Reactions is Needed for the Clearance of Senescent Cancer Cells

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Senescence induction in cancer cells is increasingly recognized as relevant for cancer therapy. Senescence can be induced in cancer cells using therapeutic compounds (therapy induced senescence; TIS) or IFNy and TNF, cytokines of type I immune responses (cytokine induced senescence; CIS). CIS can induce senescence in tumors cells in vitro and in vivo. While several in vivo data indirectly suggest that senescent cancer cells (SCC) can be cleared in vivo, their clearance has never been directly demonstrated and the potential mode of action remains unclear. Following CIS, we first found that neither macrophages nor dendritic cells were capable of phagocytosing SCC. However, killing of SCC with the Bcl2-inhibitor navitoclax rendered SCC susceptible to phagocytosis, demonstrating that killing was needed. In vivo, SCC seem to be cleared in the context of TH1 immune responses. Searching for classical effector molecules of TH1 cell associated (type I) immune responses that could induce apoptosis in SCC, we identified indeed two mediators capable of inducing apoptosis in SCC: TRAIL and NO. Further analysis uncovered that apoptosis induction in SCC by TRAIL or NO was a prerequisite to render SCC susceptible to phagocytes. Thus, TRAIL and NO mediated the clearance of SCC in the context of type I anti-cancer immune responses.

RSK3 kinase averts proliferation. Arrest and senescence during TGFβ induced epithelial-mesenchymal transition in normal epithelial cells

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TGFβ signaling is known to induce both proliferation arrest/senescence and epithelial-mesenchymal transition (EMT) in normal epithelial cells. During carcinogenesis, cells loose ability to enter proliferation arrest in response to TGFβ but they keep EMT, which contributes to metastasis formation and tumor resistance. We performed a genetic screen with 192 active kinases in normal Human Mammary Epithelial (HMEC) cells to identify kinases that could uncouple EMT from proliferation arrest/senescence after TGFβ treatment and therefore be involved in the switch between anti- and pro-tumoral effects of TGFβ in the context of breast carcinogenesis.
We found that constitutive expression of serine threonine kinase RSK3 is able to partially revert TGFβ induced proliferation arrest and senescence, while not affecting EMT in response to TGFβ treatment, therefore mimicking processes in tumor development and progression. Noteworthy, RSK3 expression decreases in response to TGFβ, implicating, that this could contribute to TGFB induced proliferation arrest.
Analysis of the public database METABRIC data on 2000 breast tumor samples show that RSK3 expression is increased in high-EMT, poor prognosis claudin-low tumors, compared with other breast cancer subtypes. Furthermore, RSK3 expression in tumors correlates with EMT score, combined from 62 genes involved in EMT. In addition we performed, knock-out of RSK3 in claudin-low breast cancer cell lines, which induced tumor cell death, conforming that RSK3 is necessary for mesenchymal tumor cell proliferation and survival.
We conclude that RSK3 is able to overcome proliferation arrest induced by TGFβ while not affecting EMT process in epithelial cells and could promote growth of claudin-low mesenchymal breast tumor. These findings improve our understanding of mesenchymal breast cancer development and suggest of potential for targeted therapy.

Interplay between melanoma cancer therapies, cellular senescence and immune responses.

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Background: Cutaneous melanoma continues to be a challenging disease. Both DNA-damaging agents and BRAF/MEK inhibitors have been shown to trigger varied therapy-induced cell fate decisions in melanoma cells, and these agents can be used in a subset of patients, often in combination with immunotherapies. To optimize or develop more effective melanoma immunotherapy strategies, it is essential to understand how the tumour microenvironment including that induced by other therapies can influence immune responses.
Hypothesis/Aim: We hypothesize that therapy-induced senescence (TIS) would have an impact on immune responses in melanoma patients. We aim to characterize cell fate decisions for treated melanoma cells and to define the interplay between TIS and immune responses.
Approaches/Results: When exposed to carboplatin/paclitaxel, irradiation or BRAF/MEK inhibitors, melanoma cells exhibit TIS in vitro (lower proliferation, reduced DNA synthesis, increased senescence-associated beta galactosidase activity and DNA damage foci). Taking advantage of CAR T-cell technology, we performed in vitro co-culture assays using NY-ESO1 recognizing T cells (NY-ESO1-T) with pre-senescent or senescent melanoma cells. NY-ESO1-T seem to be more activated (Ox40 and 4-1BB expression, and IFN-g secretion) in the presence of senescent cells. In conclusion, our data suggest that melanoma TIS may influence T-cell activation. We plan to derive primary melanoma cultures from melanoma patients' tissues and assessed the immune responses in vitro (co-culture) using matched immune cells in the context of anti PD-L1/CTLA-4 inhibitors.
Significance: Using our preclinical models, we hope to characterize molecular and cellular biomarkers that could have an impact on the immune responses as well as help to guide the therapeutic decision for melanoma patients.
Key words: Melanoma, Cellular senescence, Cancer therapies, NY-ESO1 T cells, Immune responses.

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Poster presentations

Senescent Cells Protect Against Pulmonary Hypertension Development in Mice

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Rationale. Cell senescence, defined as a stable cell-cycle arrest combined with stereotyped cell phenotype changes, is among the cellular alterations associated with pulmonary hypertension (PH) complicating chronic lung disease in humans. Here, we investigated whether cell senescence was involved in hypoxia-induced PH in mice.
Methods. Three mouse models were exposed to normoxia or hypoxia: p16LUC/+ knockin mice expressing luciferase in senescent cells served for in vivo monitoring of luminescence imaging and for assessment of p16 inactivation effects (p16LUC/LUC mice); p16GFP-INK-ATTAC mice, in which the killer gene construct driven by the p16 promoter (p16INK-ATTAC mice) was pharmacologically activated in vivo; and WT mice treated with the senolytic drug ABT263.
Results. Exposure to chronic hypoxia was associated with a time-dependent increase in lung senescent cells as assessed by thorax bioluminescence in p16LUC mice and with lung p16 and p21 protein level increases on day 8 with further elevation until day 21. Concomitantly with these changes, increases were seen in lung levels of senescence-associated secretory phenotype (SASP) components, genomic and telomeric DNA damage responses, very short telomeres (TESLA technique), and oxidative stress parameters.
Mice with genetic p16 inactivation (p16LUC/LUC mice) exposed to normoxia or hypoxia exhibited increased right ventricular systolic pressure (RVSP) and pulmonary vessel remodeling at 12-18 months, but not 4 months, of age. Elimination of p16-positive senescent cells in p16ATTAC mice increased both RVSP and vessel remodeling in both young and old mice during normoxia or chronic hypoxia. These effects occurred despite downregulated expression of SASP-associated lung inflammatory cytokines. In WT mice, continuous treatment with the senolytic drug navitoclax (50 mg/kg/day) also aggravated hypoxia-induced PH.
Conclusion. Exposure to chronic hypoxia is associated with lung-cell senescence. Accumulation of pulmonary vascular senescent cells during chronic hypoxia protects against the development of pulmonary hypertension.

Targeting senescent adipocytes in ovarian cancer to inhibit progression and chemoresistance

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Accumulating evidence suggests that aged individuals show increased accumulation of senescent adipocytes. While senescence is beneficial in some contexts, it has also been shown to play a role in chemoresistance and tumor promotion. This is due to an increase in pro-inflammatory chemokine and cytokines that are released from senescent cells, termed the senescence-associated secretory phenotype (SASP). Ovarian cancer is the most lethal gynecological cancer in which late-stage diagnoses are linked to older patient age and increased chemoresistant disease. Late stage ovarian cancer disseminates to the peritoneal cavity and preferentially seeds to the adipocyte-rich fat pad termed the omentum. However, the molecular mechanisms underlying how age affects ovarian cancer progression and chemoresistance are not fully understood. The contribution of senescent adipocytes within the omentum on ovarian cancer dissemination has not been previously investigated. We found that aged mouse omenta display senescence markers, such as increased senescence-associated beta-galactosidase (SA-β-Gal) activity, decreased LMNB1 (encoding Lamin B1), and increased BCL-2 family members and SASP gene expression. Cisplatin treatment of young mice phenocopied these senescence markers. This correlated with increased and preferential omental seeding of ovarian cancer cells. Additionally, in vitro experiments demonstrate that treatment of ovarian cancer cells with conditioned media from senescent adipocytes increases ovarian cancer colony formation ability. Together, these data suggest that the SASP from senescent adipocytes alters ovarian cancer progression. Future studies will aim to mechanistically investigate the SASP from aged omental adipocytes in ovarian cancer progression and therapeutic response and whether treatment of aged mice with senolytics inhibits omental seeding and delays chemoresistance.

Poster presentations

Cellular senescence acts as a protumoral mechanism during gliomagenesis

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Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. They are highly invasive tumors, and despite the conventional therapies always recur. Intratumoral cell heterogeneity together with the establishment of an immune suppressive microenvironment may account for the failure of the current therapeutic strategies. Cellular senescence, a permanent cell cycle arrest state, can establish an immunosuppressive microenvironment through its secretome, enhancing tumorigenesis in liver and skin mouse models. In this study we characterized and determined the function of cellular senescence during primary gliomagenesis.

First, we developed an immuno-competent mouse model recapitulating the mesenchymal GBM subtype and generated organoids derived from these mouse GBMs. Based on senescence associated-beta-Galactosidase (SA-b-Gal) staining coupled to immunohistochemistry, simultaneous analysis of resected tissue from patient surgery and mouse model of GBMs identified senescent cells both in human (SA-b-Gal+ Ki67- p16INK4A+) and mouse (SA-b-Gal+ Ki67- LaminB1- p19ARF+) GBMs. The majority of senescent cells were either tumoral, glial (GFAP+) or tumor-associated-macrophages (IBA1+) cells. Second, we eliminated the senescent cells in vivo in mouse GBMs using the p16-3MR transgene. GBMs depleted for p16INK4A+ senescent cells presented a decrease of SA-b-Gal+ cells and the corresponding mice harboured a significant longer median survival compared to control mice. Third, we undertook a single-cell RNA-seq approach on mouse GBM to unveil the mechanisms of action of senescent cells. Preliminary analysis revealed that cells expressing high levels of p16INK4A+ cells (p16INK4AHi) are mainly tumor cells and may represent the deleted cells in our p16-3MR model.

Altogether, our data showed that senescence plays a pro-tumoral role during gliomagenesis. Characterization of the secretome of the p16INK4AHi tumor cells is under scrutiny. In a broader perspective, our result might enlighten the opportunity to target and eliminate senescent cells as a potential novel therapeutic approach for GBM patients.

Differential effects of Navitoclax and Dasatinib/Quercetin on acute kidney injury (AKI)

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Acute kidney injury (AKI) and chronic kidney disease (CKD) are rising health problems contributing to an increased morbidity, mortality and global economic burden (Hoste et al. 2018).
However, preclinical therapeutic interventions comparing first-generation senolytic agents and their different mechanisms of actions and/or cell specificities in vivo within the diseased kidney are still lacking (Serrano and Barzilai 2018; van Deursen 2019).
Here, we compare the two best described first-generation senolytic agents, namely navitoclax and the drug cocktail dasatinib + quercetin (D+Q), in fibrotic acute and chronic renal disease.
The analysis of the acute kidney injury model (one intraperitoneal injection of folic acid, 250mg/kg) indicates opposing therapeutic effects of these interventions. Both senolytic treatments showed a reduction in senescent epithelial and interstitial cells based on quantitative assessment of the senescence marker senescence-associated beta-galactosidase (SA-ß-GAL) as well as p21. However, navitoclax worsened the disease outcome whereas D+Q remarkably improved renal morphology, fibrosis and function upon AKI induction. In vitro, we observe that navitoclax, but not D+Q robustly kills senescent renal epithelial cells (the main senescent renal cell type upon AKI).
We are considering several alternative mechanisms by which D+Q diminishes senescent cells in vivo and improves the disease outcome. We propose that D+Q impacts on immune cells towards a more efficient clearance and/or a general anti-inflammatory (senomorphic) effect that would reduce the damage and thus, senescent cells and fibrosis. Interestingly, the single components quercetin and dasatinib alone are not able to reproduce the therapeutic effect in vivo.

Poster presentations

Understanding the role of H2S in regulation of redox homeostasis during aging

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Aging involves time dependent deterioration of physiological functions, which can be attributed to various factors. Cellular senescence has been shown to be akin to aging which involves alteration in redox homeostasis associated with an increase in reactive oxygen/nitrogen species (ROS/RNS), inflammatory gene expression and senescence associated beta-galactosidase activity, all markers of aging. Recently, it was proposed that gaso-transmitters which includes carbon monoxide (CO), hydrogen sulfide (H2S) and nitric oxide (NO) may also play an important role in regulating redox homeostasis during senescence.
Previously it has been reported that the levels of extracellular H2S decreases during aging [1]. However, besides this not much is known about the H2S homeostasis and its role in aging. Over the years, the role of H2S has remained controversial, as it has been shown to induce DNA damage or protect against ischemia reperfusion injury [2] as well as suppress oxidative stress through Nrf2-Keap1 signalling pathway [3].
To address this conundrum, using various approaches we probed the H2S homeostasis in senescent and non-senescent cells. Using a combination of fluorescent reporter dyes for H2S and protein sulfhydration, we found that during senescence both the levels of free intracellular H2S as well as total protein sulfhydration are altered. Supplementation of H2S in cells using novel donors and depletion using targeted gene expression knock down approaches altered the levels of free radicals in the cells. Overall, we observed the levels of H2S alters the redox homeostasis during senescence, thereby affecting the inflammatory phenotype of aged cells.

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DOT1L regulates the senescence microenvironment through H3K79 tri-methylation

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Senescence, a stable cell cycle arrest, is a stress response associated with both cancer and aging. Senescent cells acquire a unique microenvironment termed the senescence-associated secretory phenotype (SASP), which can be both beneficial and detrimental. Additionally, senescent cells are characterized by robust changes in their epigenome. To globally assess the histone landscape of senescent cells, we performed epiproteomics on normal IMR90 fibroblasts induced to senesce through HRasG12V expression. Interestingly, we found that tri-methylation of histone H3 lysine 79 (H3K79me3), an active histone mark, was increased in senescent cells. To determine whether H3K79me3 contributes to increased SASP gene expression, we performed chromatin immunoprecipitation (ChIP) experiments. Indeed, H3K79me3 is increased at SASP gene loci in oncogene-induced senescent cells. H3K79me3 is methylated by disrupter of telomeric silencing 1-like (DOT1L). Consistent with the idea that increased DOT1L activity is necessary for H3K79me3 at SASP gene loci, knockdown of DOT1L during oncogene-induced senescence both decreased H3K79me3 occupancy and SASP gene expression while maintaining cells in a senescence-associated cell cycle arrest. Furthermore, to demonstrate that H3K79 methylation is sufficient for SASP induction, overexpression of DOT1L increased both H3K79me3 and SASP gene expression. Therefore, our data suggest that H3K79me3 epigenetically regulates the SASP through the methyltransferase DOT1L. These studies will allow us to understand how to restrain the harmful SASP while maintaining the beneficial senescence-associated cell cycle arrest through targeting DOT1L.

Non-autonomous induction of endothelial Icosl may underpin immune-mediated senescence surveillance

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Oncogene-induced senescence (OIS) is a tumour suppressor mechanism, with profound effects on the tumour microenvironment (TME) through the senescence-associated secretory phenotype (SASP). SASP drives immune-mediated clearance of senescent cells; failure of this clearance leads to tumourigenesis. We hypothesised that OIS cells would modulate endothelial behaviour, controlling immune recruitment and behaviour in the TME. Using transcriptomics in human liver sinusoidal endothelial cells (LSECs) cultured in control or OIS-conditioned media, we identified SASP-induced endothelial NF-kB-dependent upregulation of cytokines, chemokines, adherence molecules and the immune co-stimulatory ligand (ICOSLG). The OIS-induced NF-kB-dependent upregulation of ICOSLG was validated in multiple endothelial cell types. Using hydrodynamic tail-vein injected NRASG12V-containing transposons to generate murine hepatocyte OIS, we show hepatocyte OIS drives the upregulation of multiple NF-kB-target genes in LSECs, including Cxcl1 and Icosl, demonstrating OIS hepatocyte to endothelial signalling in vivo. Disruption of Icosl-Icos signalling, using Icosl-blocking antibodies, or deletion of Icos+ immune cells in Icos-Dtr mice, after induction of hepatocyte OIS completely abrogates the time-dependent immune-mediated senescence surveillance. Deep intrahepatic immunophenotyping reveals that OIS hepatocytes drive an intrahepatic enrichment of Icos+CD4+ T-lymphocytes and CD11b+Ly6G+ granulocytes, that was lost with Icosl blockade, suggesting that endothelial Icosl-Icos signalling may be a key regulator in senescence surveillance.

HMGB1 as a rheostat of chromatin topology and RNA homeostasis on the path to senescence

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Spatial organization and gene expression of mammalian chromosomes are maintained and regulated in conjunction with cell cycle progression. This link is perturbed once cells enter senescence. The highly abundant HMGB1 protein, known to associate with bent and looped DNA, is actively depleted from senescent nuclei to act as an extracellular proinflammatory stimulus. Despite its physiological importance, we still lack understanding of the positioning and functional roles of HMGB1 on chromatin in vivo. To address this, we mapped HMGB1 binding genome-wide in different primary cells using a tailored protocol. We then integrated ChIP-seq and Hi-C data with a graph theory approach to uncover HMGB1 demarcation at the boundaries of particular TADs. These TADs harbour genes involved in the key proinflammatory leg of the senescent transcriptional program. Moreover, we used sCLIP and siRNA- mediated knockdown to show that HMGB1 is a bona fide RNA-binding protein also affecting splicing choices. Together, our findings highlight a broader than hitherto assumed role for HMGB1 in chromatin homeostasis connected to cell cycle potency, and allow us to postulate a “rheostat” model for HMGB function with implications in cancer biology.

Poster presentations

Age-related clonal haemopoiesis is associated with increased epigenetic age and senescence

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Age-related clonal haemopoiesis (ARCH) in healthy individuals was initially observed through an increased skewing in X chromosome inactivation. More recently, several groups reported that ARCH is driven by somatic mutations [1], with the most prevalent ARCH mutations in the DNMT3A and Jak2 genes, previously described as drivers of myeloid malignancies. ARCH is associated with an increased risk for haematological cancers [1].
ARCH also confers an increased risk for non-haematological diseases such as cardiovascular disease, atherosclerosis, and chronic ischemic heart failure, for which age is a main risk factor [2]. Whether ARCH is linked to accelerated ageing and senescence has remained unexplored. The most accurate and commonly-used tools to measure age acceleration are epigenetic clocks. They are based on age-related methylation differences at specific CpG sites [3], correlating chronological age accurately with epigenetic age.
Deviations from chronological age towards an increased epigenetic age have been associated with increased risk of earlier mortality and age-related morbidities. Here we present evidence of accelerated epigenetic age in individuals with ARCH. In addition, we find evidence for clonal haemaopoiesis in mice on the transcriptional level, where haematopoietic stem cells display a strong p53 driven senescence signature. I will discuss mechanisms and possible drug interventions to eliminate senescent haematopoietic stem cells.

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Adipose tissue cellular senescence is associated with risk factors for type 2 diabetes in obese humans

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Objective
Obesity increases the risk of many aging-associated diseases, including type 2 diabetes mellitus (T2DM). Our objective was to investigate the relationship between adipose tissue cellular senescence and known risk factors for T2DM including age, adiposity, insulin resistance, and adipose tissue inflammation.
Methods
We conducted a cross-sectional study of insulin sensitive (n=11) and insulin resistant (n=13) obese humans undergoing abdominal surgery. Subjects completed a pre-surgery clinic visit for metabolic phenotyping during which we collected anthropometrics, vital signs, and fasting blood, measured body composition by a dual-energy x-ray absorptiometry scan, and conducted a 3-hour oral glucose tolerance test to assess glucose tolerance and insulin sensitivity. Subcutaneous (SAT) and visceral (VAT) adipose tissue samples were collected at time of surgery. Expression of CDKN2A, CDKN1A, and pro-inflammatory cytokines and chemokines were measured in both adipose depots. Stromal vascular cells were isolated from both depots and immunophenotyped.
Results
Expression of CDKN2A in both SAT (R^2=0.34, $p<0.01$) and VAT (R^2=0.19, $p<0.05$) was associated with glucose intolerance. Subcutaneous CDKN2A expression was also associated with age (R^2=0.19, $p<0.05$) and the oral disposition index (R^2=0.23, $p<0.05$). In VAT, CDKN1A expression was associated with age (R^2=0.34, $p<0.05$), total fat mass (R^2=0.17, $p<0.05$), and insulin resistance (R^2=0.18, $p<0.05$). Expression of VAT CDKN2A was associated with number of macrophages in VAT (R^2=0.20, $p<0.05$) and expression of the pro-inflammatory cytokines IL1B (R^2=0.11, $p=0.05$) and CXCL8 (R^2=0.13, $p<0.05$). Analyses were adjusted for age, sex, and fat mass.
Conclusions
This cross-sectional study reveals that expression of markers of cellular senescence, CDKN2A and CDKN1A, in adipose tissue is associated with several known risk factors for the development of T2DM in humans, including age, adiposity, impaired glucose tolerance, insulin resistance, and measures of adipose tissue inflammation. However, many of the associations are dependent on the adipose depot and senescence marker.
Funding Sources
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Poster presentations

WNT signaling contributes to cellular senescence in the aged alveolar epithelium

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The rapid expansion of the elderly population has led to the recent epidemic of age-related diseases, including increased incidence and mortality of chronic and acute lung diseases. Numerous studies have implicated different hallmarks of aging such as cellular senescence as a major risk factor in the pathogenesis of various pulmonary diseases such as Idiopathic Pulmonary Fibrosis (IPF). The lung epithelium that contains progenitor cells such as alveolar epithelial type II (ATII) cells represents a major site of tissue injury and aberrant activation of developmental pathways such as the WNT pathway in chronic lung diseases. However, the specific contributions of ATII cell aging in the pathogenesis of chronic lung diseases remain elusive and therapeutic targeting of age-associated pathological mechanisms has yet to be exploited in the clinical treatment of CLDs. Here, we characterized the phenotype of alveolar epithelial cells of aged mice and assessed the contribution of increased WNT signaling on cellular senescence. Methods: Primary alveolar epithelial cells were isolated from young and old (3/18 months) Tcf-GFP reporter mice. Cellular composition of lungs was assessed by FACS and characterization of epithelial cells was performed by qPCR. Chronic WNT3A treatment was used to simulate increased WNT activity in pulmonary fibrosis. Cellular senescence was monitored by qPCR and senescence-associated ß-Galactosidase activity. Results: Lungs from aged mice contained less epithelial (EpCAM+) and more inflammatory (CD45+) cells. Isolated alveolar epithelial cells showed decreased expression of the alveolar epithelial type (AT)II cell marker Surfactant Protein C and its transcription factor Nkx2.1 along with increased expression of the AT1 cell marker Hopx. Analysis of Tcf-GFP reporter mice revealed an increased WNT activity in aged alveolar epithelial cells. Chronic WNT activation induced cellular senescence in alveolar epithelial cells. Conclusions: These results show that aged alveolar epithelial cells differ phenotypically from young cells. The WNT activity in alveolar epithelial cells is increased with age and chronic WNT activation drives cellular senescence, likely contributing to tissue dysfunction.

Anti-cancer properties of mesenchymal stem cells derived from the Wharton's Jelly

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Mesenchymal stem cells (MSC) possess distinctive features, such as high proliferation rates and capability of differentiation into multiple non-hematopoietic cell types, which can be isolated from both adult and fetal tissues.1 Furthermore MSC are able of migrating towards tumors within the organism, where they interact with the local supportive microenvironment, a property that rendered them as highly appropriate candidates for use in cancer cytotherapy protocols. Towards this purpose, both naïve (unmodified) and genetically modified MSC (GM-MSC) have been employed both in vitro and in vivo, though with variable results.2 Based on the already published research work on the field, we performed a small-scale meta-analysis using a four-step strategy: compilation of a relevant publication library; deconstruction of literature methodology and reported findings; classification and organization of extracted experimental data; and data consolidation and statistical analysis.3 In turn, based on the observations and conclusions of our analysis, we evaluated the paracrine effects of various MSC populations on the proliferation and survival of selected cancer cell lines representing distinct cancer types in vitro and in vivo. Subsequently, we examined the transcriptome of two cell lines by RNA microarrays in order to exploit the expression pathways and regulatory networks contributing to the observed anti-cancer activity. Interpretation of the meta-analysis results led as to the deduction that the outcome of MSC mediated cancer cytotherapy approaches is largely dependent on various parameters. Furthermore, we were been able to highlight a set of optimal conditions, where the tumor suppressive action of MSC predominates. Mesenchymal stem cells derived from the Wharton's Jelly (WJ-MSC) were found to possess a tumour suppressive behavior, both in vitro and in vivo. mRNA analysis of cancer cells revealed a significant target dependence of the anti-tumorigenic effects displayed by MSC, which are mediated by different pathways.

1Weissman et al. Annu Rev Cell Dev Biol. 17:387–403, 2001; 2Studeny et al. J Natl Cancer Inst. 96:1593–603, 2004; 3Christodoulou et al. Stem Cell Res Ther. 9: 336, 2018.

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Poster presentations

Single cell RNA-seq analysis reveals step-wise ageing of haematopoietic stem cells

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Ageing of the haematopoietic compartment is directly correlated with a gradual decrease in stem cell function, increase in DNA damage and initiation of clonal haematopoiesis. The molecular processes by which haematopoietic stem cells (HSCs) age are still not well understood.
Using single-cell transcriptomics, we previously identified an ageing specific subcluster of HSCs in old wild-type mice, carrying a p53 and Jun/Fos transcriptional signature ("old p53" cluster), alongside with sings of cell cycle arrest, indicating functional stem cell decline (Kirschner et al. 2017). However, the majority of old HSCs showed a transcriptome similar to that of young HSCs ("young-like"). Here, we report an intermediate subpopulation of HSCs from old mice with a less defined expression of p53 signature genes ("old Intermediate"). We identified two groups of genes in old HSCs: 1) genes that gradually alter their expression from "young-like" to "old intermediate" to "old p53" subclusters and 2) genes that are stochastically upregulated or downregulated in the "old p53" specific HSC subset only.
Alongside gradual gene expression changes we found a gradual decrease in S-scores which correlated with increased p53 pathway signatures. These results indicate that HSC ageing is a step-wise process, where some cells still conserve a young-like transcriptome, and others have an increase in p53 mediated gene signatures and cell cycle arrest, indicating that these cells might progressively enter a senescent state.

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Profiling the alternative splicing landscape of senescent cells

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Cellular senescence is an irreversible cell cycle arrest in response to potentially oncogenic stimuli, characterised by a pro-inflammatory secretome that has a “bright side” when limiting the replication of preneoplastic cells and a “dark side” when the accumulation of senescent cells with ageing impairs tissue homeostasis. Despite the recent characterisation of senescent cells’ gene expression heterogeneity (1) and the suggested role of alternative splicing in the regulation of senescent phenotypes, namely through the recently found PTBP1-mediated alternative splicing program controlling the tumour-promoting secretome in oncogene-induced senescent cells (2), alternative splicing profiles associated with specific senescence inducers remain elusive. As accumulating evidence suggests some drugs are able to selectively kill specific forms of senescence, thereby improving tissue function (3), the comprehensive characterisation of senescent transcriptomes is crucial.
Next-generation sequencing of RNA (RNA-seq) allows alternative splicing quantification with unprecedented precision. Alternative sequence inclusion can be quantified from RNA-seq reads mapping to exon-exon and exon-intron junctions through the ratio between reads supporting inclusion and total reads supporting both inclusion and exclusion. However, percent spliced-in (PSI) values do not convey information on the number of reads used in the quantification (coverage), although read numbers directly reflect the evidence for measured transcript abundance.
Alternatively, by modelling alternative sequence inclusion using the beta distribution (the conjugate prior distribution of the binomial), the precision of its estimates is proportional to the associated coverage and reflected on the significance of differences in alternative splicing between samples. We employed our beta distribution-based differential alternative splicing pipeline to public and own RNA-seq datasets and were thus able to rank senescence-associated differentially spliced events according to a compromise between the magnitude of splicing changes and the amount of associated supporting evidence.
Our differential splicing analyses based on beta distribution modelling reproducibly identified, at the transcriptome-wide level, the alternative splicing changes specifically related with oncogene- or irradiation-induced senescence in multiple types of cells, providing molecular leads for therapeutically targeting senescent cells.

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Poster presentations

Restoration of senescence-associated ribogenesis defects (sard) improves senescence response in pancreatic cancer cells

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Pancreatic cancer is the 4th leading cause of death by cancer in North America with a 5-year survival rate below 7%. The best currently available treatment (FOLFIRINOX) only offers a mean survival of 11 months. Therefore, there is an urgent need for new therapeutic strategies to treat this KRas- mutant cancer. We found a high level of phosphorylation of the MAPkinases ERK1/2 in benign pancreatic intraepithelial neoplasms (PanIN), followed by a reduction in phospho-ERK levels in pancreatic ductal adenocarcinoma (PDAC). Thhis decrease correlates with progression to aggressive cancers, both in human patient samples and KRas-driven mouse models. Since PanINs are considered senescent lesions, we hypothesized that reduced phospho-ERK levels in PDAC were linked to the bypass of oncogene-induced senescence and would then allow tumor progression. We propose that a senescence program could be retrieved in PDAC by restoring high ERK signalling to engage senescence effectors and re-establish tumor suppression mechanisms lost during progression. To do so, we used a tamoxifen- inducible constitutive allele of RAF1 kinase, which acts upstream of ERK (ΔRAF1-ERT). RAF pathway activation in pancreatic cancer cells decreased their proliferation in vitro and slowed tumor growth in vivo in a xenograft model. Mass spectrometry data showed that the mechanism of action implicated a change in the phosphorylation state of nucleolar proteins that have a role in ribosome biogenesis and nucleologenesis. Depletion of several of these ribogenesis factors by shRNAs induced a senescent phenotype in pancreatic cancer cells. RAF-induced senescence was also accompanied by the formation of foci in the nucleolus containing some subunits of RNA polymerase I complex such as RPA194, TIF-1A and RPA12. The formation of RNAPI foci was also characterized as a general feature of senescence induced by RAF or RAS in both normal and cancer cells. Consistent with what our group previously published, the formation of a round large and unique nucleolus, that is typical of senescent normal cells, was also seen in senescent pancreatic cancer cells, as well as in human and mouse PanINs. We also found that this phenotype denotated a vulnerability of senescent cancer cells to inhibition of rRNA transcription by pharmacological agents such as BMH-21. Finally, through a CRISPR-Cas9 screen, we identified a pool of ribogenesis factors whose inactivation could improve RAF-induced senescence in pancreatic cancer cells. Together, our results indicate that the senescence response in pancreatic cancer cells can be improved by inhibiting ribosome biogenesis.

The long-noncoding RNA MIR31HG regulates the senescence-associated secretory phenotype via the translation factor YB-1

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Senescent cells secrete a number of factors called the senescence-associated secretory phenotype (SASP) that can impact the environment and the homeostasis of neighboring tissues. The SASP can induce senescence and activate the immune system acting as a cancer barrier, but also promote tumor progression and other aged-related pathologies. Due to the heterogeneous composition of the SASP, the identification of its regulators is of particular interest. We have identified the long non-coding RNA MIR31HG to be upregulated in primary human fibroblasts undergoing B-RAF mediated oncogene-induced senescence (OIS). We have previously shown that upon BRAF induction MIR31HG translocates to the cytoplasm where we now demonstrate it to have a role in regulating induction of the SASP. Here we show that MIR31HG knockdown in senescent fibroblasts reduces expression and secretion of pro-inflammatory components of the SASP. Breast cancer cells in contact with conditioned media (CM) from MIR31HG knockdown-senescent cells are less prone to matrigel invasion, compared to cells exposed to CM from control-senescent cells, suggesting that factors promoting invasion are reduced in the CM from MIR31HG-knockdown senescent cells. Importantly, fibroblasts receiving CM from either control or MIR31HG knockdown-senescent cells undergo senescence, suggesting that paracrine senescence is not affected by MIR31HG. We have previously shown that MIR31HG reduces translation of IL1α, one of the upstream regulators of the SASP. Mechanistically, pull down of endogenous MIR31HG followed by mass spectrometry has identified YB-1 as an interactor of MIR31HG, and depletion of YB-1 in senescence mimics the phenotype of MIR31HG knockdown. YB-1 is phosphorylated at serine 102 during senescence by the kinase Rsk and MIR31HG knockdown reduces the levels of phosphorylated YB-1 in the cytoplasm. By proximity ligation assay we have seen that the interaction of YB-1 with its kinase Rsk during senescence decreases in the absence of MIR31HG. Dissociation of the good and bad sides of the SASP is important for understanding the significance of senescence in vivo, and is crucial before considering senescence manipulation for therapeutic purposes. Our results suggest that the lncRNA MIR31HG have a dual role in senescence by suppressing p16 expression in young cells and facilitating production of a distinct subset of SASP factors in senescent cells modulating IL1α translation through the translation factor YB-1.

Age-associated alterations in murine skin involving inflammatory response with mitochondrial DNA deletions

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The skin functions not only as a first barrier to prevent invasion of pathogens on the surface of our bodies, but also to protect the interior of the living body from external environmental factors (ultraviolet (UV) radiation, chemical substances, temperature etc.). Skin aging is caused by intrinsic and extrinsic mechanisms, but it is difficult to strictly distinguish intrinsic (chronological) mechanisms from extrinsic skin aging (photoaging mainly caused by repeated exposure to UV radiation). Although research on skin aging by the photoaging has been advanced in skin aging, the relation to normal chronological aging is still not unveiled.
Recently, it has been reported in cellular senescent cells that SASP (Senescence-associated secretory phenotype) characterized by secretion of physiologically active factors such as inflammatory cytokines and matrix metalloproteases and this induces chronic inflammation which contribute to individual aging. Such senescent cells in chronologically aged skin might contribute to age-associated inflammatory responses through the SASP. However, the precise mechanisms or even relation between underlying chronological skin aging and SASP remain to be characterized in detail.
In this study, we measured age-associated alterations in dorsal skin from young (8 weeks) and old (24 months) mice by histological analysis. Age-associated alteration of gene expression patterns was determined by qPCR and immunohistochemistry. Reactive oxygen species(ROS) production was detected by confocal laser scanning microscopy. Furthermore, to elucidate the molecular mechanisms of ROS generation in chronologically aged skin, we focused on mitochondrial DNA and analyzed its mutation. We also found that chronologically aged skin had dermal atrophy caused by increased matrix-degrading enzymes and decreased collagen synthesis. Here we show that chronologically aged skin samples had increased SASP factors, elevated ROS, and a higher frequency of the mitochondrial DNA common deletion. These observations suggest that one of the physiological aging aspects, chronological skin aging is associated with increased frequency of mtDNA CD and chronic inflammation through ROS/SASP axis.

Collaborative roles of multiple SASP factors in promoting obesity-associated liver cancer

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Obesity is known to be a risk factor for several types of cancer including hepatocellular carcinoma (HCC). We have previously shown that the increased level of deoxycholic acid (DCA), an obesity-induced gut microbial metabolite, induces the cellular senescence of hepatic stellate cells (HSCs) through enterohepatic circulation (Yoshimoto et al. Nature 2013). Moreover, the senescent HSCs was producing tumor-promoting factors such as inflammatory cytokines, chemokines and proteases, so-called senescence-associated secretory phenotype (SASP) factors. In the present study, we investigated how specific SASP factors from HSCs contributed to the obesity-associated HCC development. We found that IL-33, an IL-1beta-responsive cytokine, was highly expressed in the liver tumor region and its expression was originated from the senescent HSCs. Interestingly, IL-33-deficient mice developed less tumors compared with those in wild-type mice, indicating that IL-33 plays an important role in promoting obesity-associated HCC. Moreover, a short and active form of IL-33 was found in the liver tumor microenvironment, suggesting that a protease expressed in the senescent HSCs cleaved full-size IL-33. Furthermore, in the obese mice liver, the population of regulatory T cells (Tregs) expressing ST2, a receptor for IL-33, was significantly increased, and more interestingly, the ST2-deficient mice also developed much less liver tumors, coinciding with the results using IL-33 deficient mice. Emerging evidences suggest that the activation of Tregs has a crucial role in tumor development by suppressing anti-tumor immunity. Our findings suggest that the active form of IL-33 derived from senescent HSCs could suppress anti-tumor immunity by activating ST2-positive Tregs, thereby contributing to the obesity-associated HCC progression.

Yoshimoto et al. 499:97-101, Nature, 2013
Loo et al. 7:522-538, Cancer Discovery, 2017

p21 (CDKN1A) Regulates Senescent Cell Viability and Lung Fibrosis

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Cellular senescence is a stable state of cell cycle arrest that protects the organism from tumorigenesis and regulates tissue integrity upon damage and during tissue remodeling. However, accumulation of senescent cells in tissues during aging contributes to age-related pathologies. Inhibition of CDK phosphorylation of a transcription regulator Rb contributes to cell cycle arrest in senescent cells. Notably, the CDK inhibitor p21 (CDKN1A) maintains the viability of DNA damage-induced senescent cells. Furthermore, p21 regulates the expression of ECM (extracellular matrix) components that are essential tissue components in the development of age related fibrotic diseases. Here we study the role of p21 in senescent cells and fibrosis in-vitro and in-vivo. We revealed that p21 knockdown in senescent cells leads to a CDK-dependent decrease in collagen expression and upregulation of SASP components. To test the involvement of CDK in the regulation of collagen expression and SASP secretion, we evaluated the expression of this components in senescent cells following p21 and CDK knockdown. Remarkably, while a general reduction in collagen expression and upregulation of SASP secretion was detected in senescent cells that were knocked down to p21 alone, the combined knockdown of p21 and CDK resulted in a significant upregulation in collagen levels and reduced secretion of different SASP components. Using a new inducible p21 knockdown mouse model we show that p21 knockdown in Bleomycin-induced lung fibrosis leads to a reduction in expression of senescence markers, and to a decrease in the inflammatory response. Moreover, p21 knockdown leads to an alleviation in the lung fibrosis pathology and to a reduction in collagen expression in the lungs. Overall, these findings show that p21 regulates senescent cell viability, expression of ECM and SASP components and promotes fibrosis.

Poster presentations

Exploring the role of cholesterol metabolism in retinopathies: regulation of the senescence-associated secretory phenotype.

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Ischemic retinopathies such as retinopathy of prematurity (ROP) and diabetic retinopathy (DR) affect close to 10 million North Americans and are the leading causes of blindness in developed countries. The breakdown of vascular beds in these ischemic conditions prevents nutrient and oxygen delivery and leads to hypoxic/ischemic events and a constellation of biochemical changes that compromise cellular function. Neurons that are the most closely associated with degenerated vasculature such as retinal ganglion cells in ischemic retinopathies only modestly trigger apoptosis despite a severely compromised metabolic supply rather enter a state of, a stress response in which the cells remain viable but with cellular senescence altered and compromised function. Senescent cells secrete numerous inflammatory cytokines, a phenomenon called the senescence-associated secretory phenotype (SASP), which alters the tissue microenvironment and promotes senescence in surrounding cells. We recently demonstrated that ischemic conditions cause premature cellular senescence, leading to the secretion of a SASP within the retina and alteration of the tissue microenvironment (Oubaha et al., Sci Transl Med. 2016). Moreover, limiting the SASP displays beneficial effects on reparative vascular regeneration upon ischemic stress. However, the molecular mechanisms underlying regulation of the SASP under such conditions remain to be elucidated. We have now obtained compelling data suggesting that a metabolic enzyme (namely cholesterol 25-hydoxylase, CH25H) is upregulated upon ischemic stress and promotes cellular senescence as well as the SASP. CH25H, a multi-transmembrane endoplasmic reticulum protein, converts cholesterol into an oxysterol, 25-hydroxycholesterol (25-HC). 25-HC is associated with inflammation and loss of Ch25h causes a transcriptional reduction of inflammatory genes like interleukin-6 (Gold et al., PNAS, 2014), meaning that the CH25H/25-HC axis can modulate an inflammatory response. Interestingly, CH25H is the only cholesterol synthesis enzymes upregulated upon ischemic stress in vivo and during oncogene-induced senescence in vitro, thus suggesting a conserved role of this enzyme in multiple types of cellular senescence triggered by various stresses. Lipid and cholesterol metabolism are central to retinal homeostasis and retina represents one of the most lipid rich tissues in the organism. We propose here that cholesterol metabolism coordinates the secretion of inflammatory/pro-senescence factors promoting pathological angiogenesis in retinopathy. Ultimately, our studies (combining cellular senescence, retinal biology and mRNA translation) will provide crucial insights into the molecular mechanisms regulating the inflammatory response of senescent cells and understanding the role of cholesterol metabolism in triggering senescence will provide novel therapeutic avenues to counter retinopathies.

'Modulating Senescence in the Lung Parenchyma' - ABT263 Selectively Depletes Senescent Cells in an in-vitro model of Alveolar Epithelial Senescence.

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Type 1 and type 2 alveolar epithelial cells (AEC1, AEC2) are key cell types in the distal lung. AEC1s facilitate gas exchange and AEC2s produce surfactant. Furthermore, AEC2 hyperplasia is required for the repopulation of the lung parenchyma in tissue repair.
The accumulation of senescent alveolar epithelial cells has been linked to excessive inflammation, delayed functional tissue repair and fibrosis in animal models of acute lung injury [1]. One possible intervention for this is senolytics - an emerging class of compounds that exploit specific phenotypic changes to selectively deplete senescent cells both in-vitro and in-vivo [2, 3].
This work quantified senescence in A549 cells, an AEC2-like cell line, by treating with bleomycin to trigger senescence, and X-gal staining. Quantitative PCR demonstrated an increase in expression of SASP components and pro-fibrotic genes in A549 cells treated with bleomycin. The impact of the senolytic agent ABT263 (a Bcl2/w/xL inhibitor) on senescent and non-senescent A549 cells was tested. ABT263 treatment induced apoptosis and reduced cell viability in senescent A549 cells with negligible toxicity to proliferating cells (58% reduction in viability of senescent vs 15% in proliferating with 0.5μM ABT263, n=4 p<0.0001). Senescent A549 cells demonstrate susceptibility to ABT263 in-vitro. The potential efficacy of ABT263 administration in fibrotic lung disease merits further study in vivo.

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Poster presentations

Identification of cardiac glycosides as a novel class of senolytic compounds

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Senescent cells accumulate in the organism over time and contribute to the functional deterioration of tissues, mainly due to the action of secreted factors associated with this cellular state. These secreted factors, known as SASP, may have a reinforcing effect on senescence, but in other cases it has been described that the secreted factors may have a pro-tumoral activity.
Compounds with specific cytotoxic activity against senescent cells, known as senolytics, offer an extraordinary support to the notion of a causal relationship between cellular senescence and age-related diseases, and hold the promise of effective treatments against aging.
Using a cell-based phenotypic screening, we identified Cardiac Glycosides (CGs) as a family of compounds with novel senolytic activity. CGs, by targeting the Na+/K+ ATPase pump, cause a disbalanced electrochemical gradient in which Na+ and H+ concentrations increase within the cell causing depolarization and acidification. Senescent cells present a slightly depolarized plasma membrane and higher concentrations of H+, making them more susceptible to the action of CGs. These vulnerabilities can be exploited for therapeutic purposes as evidenced by in vivo eradication of PDXs in mice treated with the combination of senogenic drugs and a senolytic, the CG Digoxin. Similarly, murine pulmonary fibrosis caused by senescent cells present in the lung is alleviated by Digoxin treatment.
This experimental approach could be a promising tool to identify new compounds with senolytic activity that could potentially be used to develop novel effective treatments against age-related diseases.

Transient p53-mediated regenerative senescence in the injured heart

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Senescence is commonly associated with aging and age-related diseases; however, transient senescence has been shown to be essential for proper development and regeneration. To follow senescence in the heart we employed injury models in zebrafish and neonatal P1 mice, which are established models for cardiac regeneration. Injured hearts of both models showed high levels of senescence, marked by SA-β-gal staining at the injured site, 4 days after injury. Interestingly, 21 days post-injury, senescence was no longer observed in either models, indicating its transient nature 1. Treatment with senolytic drugs showed the essential role of senescence in inducing cardiac regeneration. Recently, we have shown that the ECM molecule agrin promotes heart regeneration after myocardial infarction in non-regenerating mouse hearts 2. As observed in zebrafish and P1-injured hearts, agrin induced robust senescence in cardiac fibroblasts (CFs) located at the scar region and epicardium of injured hearts, which was markedly decreased after 3 weeks 1. Senescence was accompanied with transient expression of a unique phosphorylated form of p53 at Ser23, as well as with anti-apoptotic family members, Bcl-xL and Bcl-w, apparently to prevent detrimental massive cell death that may occur upon p53 activation.
Our work sheds light on the important role of CFs and induction of senescence during the repair and regeneration processes of the heart, and raises new question regarding the factors mediating the effects of senescent cells in promoting the repair of the heart.

1. Sarig et al., Circulation, 139:2491–2494, 2019
2. Bassat et al., Nature, 547:179-184, 2017

Poster presentations

The diabetic milieu speeds up the cellular senescence programme in the retinal microvasculature

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Diabetes has been suggested to induce an accelerated cellular senescence. Diabetic retinopathy (DR) is considered a vasculopathy because its sight threatening complications are associated with endothelial dysfunction triggering diabetic macular oedema and proliferative diabetic retinopathy. Therefore, here we aim to investigate the senescence programme in human retinal microvascular endothelial cells (HRMECs) and to elucidate how this is affected by the diabetic milieu. HRMECs were cultured under normal and high glucose conditions of 5mM and 25mM D-glucose respectively, until they reached their Hayflick limit. Replicative senescence was confirmed by permanent growth arrest and senescence-associated (SA)-β-galactosidase staining. Senescent HRMECs showed significant increase in cellular size and mitochondrial accumulation by flow cytometry and electron microscopy. The senescence-associated secretory phenotype (SASP) in HRMECs included IL8, IGFBP7, IL6, PAI1, and COL4A1. Interestingly, senescent HRMECs became more glycolytic than early-passage cells. The Hayflick limit reduced from 25 to 15 population doublings when HRMECs were cultured in a diabetic microenvironment. HRMECs under diabetic conditions became replicative senescent in 40 days, when compared to 55 days in controls. The number of SA-β-Gal positive cells and the expression of SASP components were significantly higher in late-passage HRMECs under high glucose conditions when compared to age-matched control HRMECs. Long-term high glucose-treated cells exhibited significantly less tube forming capacity in the 3D matrigel angiogenesis assay, and significantly less barrier function measured by trans-endothelial electrical resistance. We used Seahorse XF technology to show that long-term high glucose significantly decreased HRMECs glycolytic potential. In addition, we tested Navitoclax as a senolytic, which effectively cleared out 50% of senescent HRMECs in vitro within 3 days. The same dose induced negligible cell death in non-senescent HRMECs. Furthermore, we used the C57Bl/6 mouse as a model to investigate senescence in vivo. Optokinetics data demonstrated a significant decrease in visual acuity with age. The optokinetic response time and contrast sensitivity almost halved when comparing young (3-5 months) and old mice (18-24 months). To mimic the diabetic milieu, which includes hypoxia and high glucose, we used the oxygen-induced retinopathy (OIR) and the Streptozotocin (STZ) mouse models, respectively. Both OIR and STZ retinas showed increased SA-β-galactosidase staining coupled with increased gene expression of senescent markers, such as p53 and SASP components. Taken together, we showed evidence to demonstrate that diabetic conditions induce premature cellular senescence in the retinal vasculature and that Navitoclax acts as an effective senolytic in vitro.

Dynamic multidimensional profiling to unravel the epigenomic mechanisms underlying escape from oncogene-induced senescence

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Oncogene-induced senescence (OIS) is complex cell fate transition which blunts excessive proliferation caused by oncogenic stress and therefore acts as a potent tumor suppressor mechanism. Nonetheless, OIS is not a stable cell fate, and its subversion may be a prerequisite for overt malignant transformation. Despite the clinical relevance of this event, the underlying epigenomic mechanisms driving escape from OIS remain poorly understood. Previously, we utilized a dynamic multidimensional profiling approach to define the transcription factor network controlling the OIS cell fate transition. Building on this approach, we performed extensive time-series experiments on primary human fibroblasts expressing oncogenic RAS, and integrated transcriptome (Affymetrix gene expression microarrays), chromatin accessibility (ATAC-seq) and immunofluorescence data. Our approach revealed a continuously evolving process involving at the least two cell fate transitions: i) a senescence phase characterized by drastic changes in genome-wide transcriptional and chromatin accessibility trajectories, a typical OIS transcriptional signature (high expression of inflammatory secretory phenotype genes [SASP], repression of cell cycle genes), high levels of DNA damage and a drastic reduction in EdU incorporation; ii) a senescence escape transition involving further shifts in transcriptional and chromatin accessibility trajectories, re-expression of cell cycle genes, a shift to an epithelial-to-mesenchymal transition (EMT)/TGF-ß transcriptional signature, and resolution of DNA damage. Further analyses of transcriptome data identified transcription factors POU2F2 and PRDM1 as possible candidates mediating escape from the senescence arrest by dynamically regulating cell cycle- and SASP-associated genes. Consistent with this prediction, the protein levels of POU2F2, and to a lesser extent PRDM1, are upregulated in a temporally coincident manner with escape from the senescence arrest, and remain highly expressed during and after the escape transition.

Poster presentations

Developmentally-programmed and tissue injury-induced cell senescence are conserved features in zebrafish

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Cell senescence is a stable cell cycle arrest triggered by cells undergoing stress and their accumulation has been suggested to be the basis of aging. However, a positive physiological role as a pro- regenerative and morphogenetic force has emerged recently after the identification in mammals, birds, amphibians and fish of programmed cell senescence during embryogenesis, and during wound healing and limb regeneration. Zebrafish are evolutionary distant vertebrates with an extraordinary regenerative capacity so we considered of interest examining developmental and tissue injury-induced senescence in this organism.
Careful inspection of senescence staining in larvae (2-11dpf) showed that, apart from previously described yolk and cloaca, senescence was also identified in the developing spinal cord, intestine, stomach, liver, pronephric ducts, and crystalline. Interestingly, senescence at these structures disappeared upon treatment with senolytic compound ABT-263, supporting their senescent identity.
Regarding tissue injury-induced senescence, we observed that pectoral fin amputation in adult fish induced cell senescence, while clearance of these senescent cells by ABT-263 resulted in compromised fin regeneration, pointing to a positive role for tissue injury-induced senescence during regeneration.
Interestingly, and despite many conceptual similarities, this tissue repair response is different from developmental senescence, since fins do not show senescence during development but their amputation triggers senescence.

Targeting STING in senescence with small-molecule antagonists

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The senescence-associated secretory phenotype (SASP) has been implicated in promoting age-related diseases. Recent research has demonstrated that aberrant activation of the innate immune sensing pathway cGAS-STING contributes to senescence-associated inflammation. Thus, targeting the cGAS-STING pathway may provide beneficial effects for the treatment of senescence-associated pathologies. Yet, to what extent the SASP is regulated by the cGAS-STING pathway and its composition was unknown.

Our research aims to understand how cGAS-STING regulated SASP impacts on cellular aging and genotoxic chemotherapeutic treatments. To this end, we used a highly potent and selective small-molecule inhibitor of STING (H-151), recently discovered in our laboratory. Using H-151 allowed us to characterize the composition and understand the regulation of the cGAS-STING dependent SASP in a temporal and context-specific manner.

We found that H-151 suppressed the production of SASP factors at different stages of senescence in vitro as well as ex vivo in human adipocytes from obese patients. Bulk RNA sequencing revealed STING-dependent expression of several genes coding for antiviral proteins and chemokines in irradiation-induced senescence. Importantly, pharmacological inhibition of STING attenuated senescence-associated inflammation in aged mice in vivo.

Our findings show that cGAS-STING signaling is an important trigger of inflammation causing side effects during aging and chemotherapy. Therefore, we propose that cGAS-STING is a relevant target for the treatment of age-related diseases.

Melatonin reverses H2O2-induced premature senescence of dental pulp cells via JNK pathway

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The anti-aging activities of melatonin, a hormone secreted by the pineal gland, have been reported in senescence-accelerated mouse models and several types of cells, but its effect and mechanism on the senescence of human dental pulp cells (hHDPCs) remains unknown. In this study, we examined the effect of melatonin on premature senescence of hHDPCs. Melatonin markedly inhibited the senescence of hHDPCs after exposed to hydrogen peroxide (H2O2), including the increase in senescence-associated β-galactosidase (SA-β-gal)-positive hHDPCs and the upregulation of p21 protein, an indicator for senescence. In addition, melatonin attenuated H2O2-stimulated phosphorylation of c-Jun N-terminal kinase (JNK), while selective inhibition of JNK activity with SP600125 significantly attenuated H2O2-induced increase in SA-beta-gal activity. Together, these results reveal that melatonin antagonizes H2O2-induced premature senescence of hHDPCs via JNK pathway. Thus, melatonin might have the therapeutic potential to prevent stress-induced premature senescence of dental pulp cells across the life span.

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Uncovering the mechanism of senescence activation in cancer cells

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Pro-senescence therapy, the process of activating a stable cell cycle arrest in cancer cells, is emerging as a novel mechanism for cancer treatment. By conducting a genome-wide siRNA screening followed by further validation steps, we have identified targets that activate senescence in p16-positive cancer cell models. Interestingly, eleven of these siRNAs target genes that encode for ribosomal proteins.

Using the two most potent ribosomal protein modulators, RPS3A and RPS7, we assessed their siRNA knockdown at the cellular level by IF in p16-positive basal-like breast cancer cells, MDA-MB-468. We observed that the protein expression of RPS3A was decreased in siRPS3A treated cells and intriguingly, in siRPS7 treated cells also. This novel finding poses the question of whether these ribosomal proteins function co-dependently within the MDA-MB-468 cellular context.

Characteristically, senescent cells secrete a variety of inflammatory molecules which have been termed senescence-associated secretory phenotype (SASP). The composition of these secreted molecules differs across cell types and senescence induction mechanism. Although the SASP profile of many senescent cells have been extensively investigated, that of senescent cancer cells remains largely unexplored.

Senescent MDA-MB-468 cells induced to senesce by siRNA knockdown of RPS3A or RPS7 demonstrate translocation of p16 to the nucleus. This phenotype is associated with an increased cellular protein expression of IL-6 and IL-8 (well-known SASP components). By investigating the role of p16 in driving this process, we demonstrate that the percentage of IL-6 positive cells declines significantly following the siRNA knockdown of p16. This finding potentially implicates p16 in SASP activation and is therefore suggestive of an interplay between these two key senescence markers within MDA-MB-468 cells.

To explore the full secretory phenotype of senescent MDA-MB-468, we have defined the time point for collection of conditioned medium, which will then be applied to a cytokine array. Furthermore, we will assess the paracrine effects of the SASP from senescent MDA-MB-468 cells within their microenvironment. Overall, this investigation will contribute to our knowledge of the mechanisms and networks involved in activating senescence in p16-positive cancer cells.

Poster presentations

Identification of key factors regulated by the RAS/MAPK pathway implicated in the commitment of cells towards cellular senescence

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Cellular senescence is a powerful intrinsic anti-cancer mechanism characterized by a stable cell proliferation arrest. Therefore, elucidation of the processes regulating the onset and maintenance of senescence will help to identify pro-senescence therapies for cancer. In our laboratory, we aim to identify key factors specifically regulated by the RAS/MAPK pathway, that mediate the commitment of cells towards cellular senescence. Importantly, it is well known that 30% of all human cancers have an activating mutation in the RAS oncogene, which leads to an increased proliferation rate. However, it is also known that, through an unclear mechanism, a sustained hyper-activation of this same pathway leads to an antagonistic phenotype, which is cellular senescence. This cellular fate characterizes benign lesions, and progression into a malignant tumor is associated to the bypass of this intrinsic tumor suppressor mechanism. Recent results obtained in our lab allowed us to define a time window of activation of the RAS/MAPK pathway leading to a "pre-senescence" state where cells are negative for classic senescence markers but committed towards cellular senescence. We hypothesize that hyperactivation of RAS/MAPK pathway leads to important transcriptional changes occurring during this precise short time window, and that these changes are crucial to compromise cells towards cellular senescence. Therefore, we performed transcriptomics by mRNA-Seq to compare our ''pre-senescent'' state to a ''non-compromised'' state and found an early signature of genes coding for SASP factors and enzymes of glycolysis, lipid metabolism and the pentose phosphate pathway, suggesting a metabolic reprogramming during commitment of cells towards senescence. This also allowed identification of putative key transcription factors regulating the onset of senescence, that will be further confirmed. We believe our approach will not only lead to identification of novel senescence biomarkers, but also to identification of senescence drivers. In combination with drugs modulating SASP, this could eventually allow the design of novel therapeutic strategies aiming to promote and maintain the senescent state in cancer cells without deleterious effects associated with SASP, in order to eliminate these senescent cells afterwards via senolytic drugs.

SOD-mediated superoxide metabolism regulates cell fate in fibroblasts.

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Superoxide (O2·-) mainly originates from mitochondria and is distributed in both cytoplasm and mitochondria. The intracellular O2·--catalyzing enzymes, SOD1 and SOD2, are localized in the cytoplasm and mitochondria, respectively. Therefore, they play an pivotal role in maintaining redox balance in the physiological homeostasis. Redox imbalance has considered to induce increasing intracellular O2·- level and DNA damages, resulted in apoptotic cell death and cell senescence. However, the details of the cell fate determination mechanism by the difference in the distribution of intracellular O2·- remain unclear. In this study, using Sod1-/- or Sod2-/- fibroblasts, we examined what influence the difference in the localization of intracellular O2·- has on cell fate. Sod1-/- cells have a 3-fold increase in dihydroethidium-positive intracellular O2. On the other hand, Sod2-/- cells have a 2-fold increase in MitoSOX-positive mitochondrial O2. There is no significant difference in the increase ratio of O2·- between Sod1-/- and Sod2-/- fibroblasts. Intriguingly, the intracellular level of reactive oxygen species (ROS) including hydrogen peroxide and peroxynitrite was about 95 and 1.5 times in Sod1-/- and Sod2-/- fibroblasts, respectively. In cellular phenotype analyses revealed that Sod1-/- cells had a markedly reduced cell proliferation ability and died within 72 hours in culture, whereas Sod2-/- cells positively proliferate with reduced proliferation ability. Although both Sod1-/- and Sod2-/- cells showed a decrease in mitochondrial membrane potential, caspase3-mediated apoptosis was characteristically observed in only Sod1-/-, but not Sod2-/- cells. Furthermore, Sod2-/- fibroblasts exhibited a cellular senescence-like phenotypes such as nuclear deformation and cell cycle abnormalities. These results suggested that the total ROS levels and the difference in the subcellular localization of O2·- may be factors that regulate cell death and senescence.

Senescence is associated with alcoholic liver disease progression and alcoholic hepatitis. development of a murine model of advanced alcoholic liver disease.

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INTRODUCTION: Cellular senescence is characterized by the loss of proliferative capacity, cell cycle arrest and the acquisition of a proinflammatory senescence-associated secretory phenotype (SASP). The presence of senescent hepatocytes has been associated with poor prognosis in alcoholic liver disease (ALD) (Aravinthan et al., 2013). This study aims to analyze the role of senescence in ALD progression and in alcoholic hepatitis (AH), as well as to describe an experimental murine model that reproduces these physiopathological characteristics.
METHODOLOGY: In order to evaluate senescence in ALD progression, a previously published senescent hepatocyte gene signature (Aravinthan et al., 2014) was used and transcriptomic data from patients at different ALD stages (n=17) and AH (n=12) was compared. The model of advanced ALD was carried out in mice treated with 4% ethanol Lieber-DeCarli diet (Bertola et al., 2013) for three weeks and supplemented with 0.05% 3.5-diethoxycarbonyl-1.4-dihydrocollidine (DDC) during the third week.
RESULTS: The senescent hepatocyte gene signature was analyzed in both groups of patients by gene set enrichment analysis (GSEA). The analysis by GSEA revealed a low expression of senescence genes in patients with ALD without cirrhosis. However, senescence gene signature enrichment increased in patients with cirrhosis and was very pronounced in patients with AH. In addition, senescence markers (CDKN1A, CDKN2A, CDKN2B and ARF1) positively correlated with MELD, ABIC and Child-Pugh clinical scores. Furthermore, high expression of SASP factors was observed in patients with AH and cirrhosis. Senescence assessment by immunohistochemistry showed p21 expression in AH patients. In order to mimic advanced ALD histological characteristics, mice were treated with Lieber-DeCarli diet supplemented with ethanol and DDC. Histological analysis of the model demonstrated that livers presented ALD main characteristics: hepatocellular damage, neutrophil infiltration (MPO), ductular reaction (KRT19) and fibrosis (Sirius red). Additionally, senescent hepatocytes (p21) were observed. The combination of alcohol and DDC showed an increase in p21-positive hepatocytes compared to control mice treated with alcohol or with DDC alone, suggesting an effect of alcohol consumption on senescence induction.
CONCLUSION: Advanced ALD is characterized by the presence of hepatocellular senescence, which is especially remarkable in AH and correlates with poor prognosis clinical scores. The advanced ALD experimental murine model reproduces the main histological characteristics of AH and suggests a role for alcohol in senescence induction.

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Combining chemical and genetic screens for the discovery of novel modulators of RAF- induced cellular senescence

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Oncogene activation, as with the V600E mutation in the kinase B-RAF, can induce senescence in melanocytes and other cell types (1), and senescence is now considered an important tumour suppressor mechanism. B-RAF-V600E is expressed in 50% of nevi (beauty marks), which are composed of senescent melanocytes. 33% of melanoma nonetheless derive from nevi, suggesting the existence of senescence escape or bypass mechanisms (2). Hence, understanding how cells bypass or escape from senescence, and elaborating strategies to eliminate senescent cells has medical relevance. We are studying B-RAF-induced senescence in human cells. Our aim is to discover new ways of modulating senescence. In a pilot screen of an FDA-approved drugs repositioning library, we identified 2 classes of compounds that modulate B-RAF-V600E-induced cellular senescence in human fibroblasts: 1) glucocorticoids, that delay or bypass the onset of B-RAF senescence, and 2) cardiac glycosides, that specifically kill B-RAF senescent fibroblasts and thus are senolytic drugs. We first investigated the mechanisms by which clobetasol, a potent glucocorticoid widely used in the treatment of skin diseases, delays or bypasses B-RAF-induced senescence. We unraveled the role of transcription factor EGR1, an early target of clobetasol, in the rapid proliferative arrest associated with B-RAF senescence onset (3). We are currently investigating the molecular mechanisms underlying the senolytic action of cardiac glycoside ouabain, in the context of survival networks reorganization in B-RAF-induced senescence. We are also setting up a new screening system, allowing the co-seeding and fluorescence-based identification of 3 distinct fibroblast populations: proliferative, B-RAF-induced senescent, and etoposide-induced senescent (DNA damage). We will perform 1) a chemical screen, to identify novel senolytics against various types of senescent cells, and other modulators of senescence, and 2) a siRNA screen against druggable targets, to identify genes essential for the maintenance of senescence or for the survival of senescent cells. Identifying genes essential for the survival of senescent cells should open up avenues worth exploring for the development of novel senolytics.

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Poster presentations

T-cell senescence as an immune checkpoint in T-cell adoptive immunotherapy of cancer.

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Adoptive transfer of ex vivo-expanded T cells is a promising therapeutic approach for the treatment of viral diseases or cancers. However, the limited in vivo persistence of the transferred cells can severely compromise clinical efficacy. Culture-driven T-cell dysfunction, impeding optimal T-cell function after transfer contributes to these shortcomings. Understanding the biological underpinnings of culture-driven T-cell dysfunction could help design new approaches to optimize T-cell quality prior to adoptive immunotherapy and improve T-cell function after patient infusion. Using repeated stimulations of human T cells with anti-CD3/CD28 beads to mimic a chronic polyclonal activation or antigen-specific T-cell stimulations with clinically relevant antigens, we induced T-cell dysfunction characterized by a decrease in proliferation. Deep RNA sequencing of dysfunctional CD8 T cells in long-term cultures pointed on the modulation of various senescence-associated mediators. Phenotypic and functional assessments confirmed the accumulation over-time of cells co-expressing many "classical" exhaustion markers along with the senescence-associated-β-galactosidase. This proved to be mostly p16-mediated and in part restrained by caspase-8. Senescence-associated mechanisms are thus key players in T-cell dysfunction occurring following repeated stimulations and as such should be considered as novel immune checkpoints impeding the success of T-cell adoptive immunotherapy in humans.

Resilience to cognitive aging is associated with healthy responsiveness of adult-born dentate neurons

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During aging, some individuals are resilient to the decline of their cognitive functions whereas others are vulnerable to it. These inter-individual differences in memory abilities have been associated with differences in the rate of hippocampal neurogenesis measured at old age. Whether the maintenance of the functionality of neurons generated throughout adult life is linked to the resilience to cognitive aging remains completely unexplored.
Using the immediate early gene Zif268, we analysed the activation of dentate granule neurons born in adult (3 month-old), middle-aged (12 month-old) or senescent (18 month-old) rats in response to learning when animals reached 22 month-old. The activation of neurons born during the developmental period was also examined.
We show that neurons generated 4, 10 or 19 months before learning (and not developmentally born neurons) are activated in senescent rats with good learning abilities. In contrast, aged rats with bad learning abilities do not exhibit an activity-dependent regulation of Zif268.
Resilience to cognitive aging is associated to the responsiveness of neurons born during adult-life. In contrast, cellular senescence may be responsible for an accelerated aging of new neurons in vulnerable subjects. Understanding cellular senescence is of high clinical importance to slow aging and increase healthy cognition.

Poster presentations

The role of the nuclease EXD2 in the Alternative Lengthening of Telomeres in neoplasia

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The Alternative Lengthening of Telomeres (ALT) sustains cell proliferation in 10-15% of human cancers. ALT is a conservative DNA replication process, operated by RAD52 dependent or RAD52 independent Break-Induced Replication (BIR) DNA repair pathways. RAD52 dependent BIR, is counteracted by classical homologous recombination (c-HR) and by Non-Homologous End-Joining. However, both BIR and c-HR are considered to act synergistically to elongate telomeres in absence of telomerase. Stalled or collapsed DNA replication forks, as well as telomere dysfunction, generate DNA double-strand breaks, that are repaired either by homology-mediated recombination or classical and alternative forms of end-joining. Error- prone, end-joining pathways are prevalent at the frequently dysfunctional ALT telomere, generating unstable polycentric chromosomes that are major contributors of genomic instability in cancer. We have recently shown that the MRN-interacting exonuclease EXD2, is genome-wide essential, for the stabilization and restart of stalled DNA replication forks. In addition, EXD2 plays an important role in microhomology-mediated end-joining of DNA double strand breaks. Herein, we focus on the action of the nuclease EXD2 at the human ALT telomere. Overexpression of tagged EXD2, in the ALT human osteosarcoma cell line U2OS, revealed that EXD2 forms nuclear foci that frequently colocalize with telomeric repeats, the telomere specific proteins TRF1 and TRF2 and the ALT-associated PML bodies. Overexpression of EXD2, led to the suppression of ALT-characteristic telomeric sister chromatid exchanges, whereas EXD2 CRISPR/Cas9 knock-out, triggered classical telomeric hyper-recombination. The EXD-/- U2OS cells displayed significant -but not detrimental- telomere shortening, highly elevated C-circle levels (extrachromosomal C-rich telomeric DNA associated with ALT-activity) and increased frequencies of ALT-associated PML bodies. Immunocytochemistry, FISH, fiber-FISH and proximity ligation assays in EXD-/- cells, revealed a significant increase in telomere dysfunction foci, elevated levels of telomeric collapsed replication forks and sister-chromatid fusions (a Ligase-3-dependent phenotype). Importantly, EdU/Click-it assays and a novel modified CO-FISH protocol capable to quantify conservative telomeric replication showed that the absence of EXD2 triggers terminal BIR without concomitant increase in telomere length. Hence, the two known mechanisms of BIR-mediated telomere elongation act independently, while EXD2 appears to be an important molecule only in one of the two. Our data shed light into the complex interactions between the different terminal repair machineries and telomere elongation, highlighting the role of EXD2 as a molecular switch between RAD52 dependent or RAD52 independent telomeric Break-Induced Replication (BIR) with putative onco-therapeutic implications.

Secretions of senescent macrophages

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Senescent cells accumulate in old organisms. They secrete a variety of cytokines and extracellular matrix proteins that are responsible for their pathological effects in tissues, underlying most of age-related diseases and several premature aging conditions. In particular, senescent macrophages have been shown to promote atherosclerosis, neurodegenerative disease and the pro-inflammatory syndrome associated to obesity. It has also been demonstrated in mouse models of atherosclerosis or Tau-associated neurodegeneration, that the elimination of senescent macrophages prevented these diseases.
We developed a cell culture model of senescent macrophages to better understand the biology of senescence in this cell type and carachterize the specificity of their secretions. First, using RNAseq we described the transcriptome of senescent macrophages and identified their potential secretome. Second, we also characterized the extracellular vesicles (EVs) secreted by senescent macrophages. We isolated EVs and used small RNAseq of both EVs and cells to reveal the specific RNA repertoire of the EVs from senescent macrophages . We show that inducing senescence in macrophages increased the levels of several miRNAs previously linked to tumorigenesis and resistance to chemotherapy. We also characterized the proteome of EVs from control or senescent macrophages. Finally, we are studying the effects of the secretome from senescent macrophages on other cell types including the role of EVs RNA cargo. Of note, major RNAs upregulated in senescent EVs could be used to generate diagnostic kits to evaluate the load of senescent cells in vivo.

Poster presentations

Calcium channel ITPR2 and mitochondria-ER contacts promote cellular senescence and aging

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Upon multiple stress signals, activation of cellular senescence stops cell proliferation and ultimately leads to a pro-inflammatory secretome. Senescent cells accumulate during aging promoting various age-related pathologies. The endoplasmic reticulum ITPR2 calcium release- channel and more broadly calcium fluxes from the ER to the mitochondria have been identified as positive regulators of senescence in human cells. Nonetheless the role of ITPR2 on senescence in vivo and physiological aging has never been described. Here we show that Itpr2 knockout mice display improved aging, such as increase life span, better response to metabolic stress, less immune-senescence and both less liver steatosis and fibrosis. Cellular senescence is known to promote these age-related alterations and is accordingly reduced in both Itpr2 KO mice and Itpr2 KO mice-derived cells. Interestingly Itpr2 knockout decreases number of contacts between ER and mitochondrial membranes, that positively correlate with senescence markers both in vitro and in vivo. Furthermore, forcing these ER-mitochondria contacts induces a premature senescence response. These new findings shed light on the role of contacts and facilitated exchanges between the ER and the mitochondria through ITPR2 in regulating senescence and physiological aging.

Wild long-lived and cancer-resistant subterranean rodent Spalax undergo senescence without the acquisition of SASP components

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The blind mole rat (Spalax) is a wild, long-lived rodent that has evolved mechanisms to tolerate hypoxia and resist cancer. Old-age Spalax individuals do not show signs of age-related diseases. Cellular senescence is characterized as a state of cell cycle arrest complemented with a mechanism that limits tumorogenesis. Regardless of its tumor suppressive role, it promotes inflammation via the senescence-associated secretory phenotype (SASP). Recently we have shown that Spalax fibroblasts exhibit high DNA repair capacity. Since persistent DNA damage triggers SASP secretion, we aimed to investigate whether senescence in Spalax fibroblasts is accompanied by inflammatory response despite of their high repair capacity. We demonstrate that Spalax fibroblasts undergo replicative senescence (RS) and etoposide-induced senescence (EIS), evidenced by up-regulation of senescence markers (β-gal, p21, p53 and proliferative arrest). Yet, in contrast to the mouse and human fibroblasts, RS and EIS Spalax cells showed undetectable or decreased expression of the well-known SASP factors: interleukin 6 (IL6), IL8, IL1α, growth-related oncogene alpha (GROα), Serpin2B, intercellular adhesion molecule (ICAM-1). On the other hand, senescence in Spalax fibroblasts was associated with an increase in IL10 mRNA, an anti-inflammatory cytokine. Further investigation has shown that nuclear factor κB (NF-κB)- p65 was not translocated to the nuclei despite its activation via phosphorylation. However, when Spalax fibroblasts were subjected to inflammatory stimulation by MDA-MB231 breast cancer cells conditioned medium (CM), we found that there was a p65 nuclear translocation, mRNA expression of the inflammatory factors IL6, IL1α, ICAM-1, GROα,Serpin2B , and IL1α protein expression. IL1R antagonist attenuated the stimulating effect of MDA-MB-231 CM as was shown by the reduction of the IL-6 mRNA expression in Spalax RS cells, indicating an involvement of IL1R in the Spalax cell response to a strong inflammatory stimulus. These finding suggest that Spalax have evolved a unique mechanism to uncouple senescence and SASP. We assume that this phenomenon, which has never been discovered before, is due to the high ability of high DNA repair capacity in Spalax. Our findings may give a new aspect to prevent the so-called sterile inflammation during human aging.

Poster presentations

Multi-omics approach to study the role of transcription factor TBX2/3 in tumour senescence bypass

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The TBX2 and TBX3 T-box family transcription factors are important in embryonic development. In tumourigenesis their overexpression bypasses oncogene-induced senescence (OIS) and promote proliferation by suppressing expression of the cyclin-dependent kinase inhibitors p14, p21 and p57. They can induce invasiveness by repressing E-cadherin expression. Although depletion of TBX2 and TBX3 can restore senescence in melanoma, their full repertoire of target genes and how they control gene expression is poorly understood. Here we use a multi-omics approach (Mass spec, ChIP-seq and RNA-seq) to identify TBX2/3 cofactors and direct target genes, and undertake a screen for FDA-approved drugs that down-regulate TBX2/3 expression. The results give a novel insight into how these key developmental regulators promote tumorigenesis and identify potential strategies directed towards pro-senescence therapy.

Glial and neuronal-derived cytokines promote age-related loss of tissue homeostasis

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While inflammation is essential for tissue repair post injury, a prolonged, dysregulated and chronic inflammatory response is one of the major contributors to age-related pathologies such as cancer, heart disease, insulin resistance, Alzheimer's disease, and others. Pro-inflammatory cytokines are proteins whose secretion can become dysregulated with age, contributing to the chronic systemic inflammation observed in most age-related pathologies. To address this, we used Drosophila melanogaster as a model organism to study the sources of inflammatory cytokines and how they affect intestinal stem cell (ISC) populations in aging. Aging Drosophila exhibit a decline in ISC homeostasis demonstrated by dysplasia, an over-proliferation of stem cells—as well as metaplasia, the trans-differentiation of copper cells into enterocyte-like cells. To test whether this loss of homeostasis is driven by inflammaging, we knocked down Drosophila cytokine homologs, Unpaireds (Upd1, Upd2, and Upd3) in different tissues (muscle, fatbody, oenocytes, hemocytes, glia, and neurons), and observed the effects in the intestine of aging flies by measuring ISC proliferation rates, copper cell maintenance, and organismal lifespan. We discovered that knocking down Upd2 in neurons and glia led to a significant reduction of age-induced ISC proliferation rates. Accordingly, knocking down Upd2 in glia also significantly extended lifespan. Our findings with this project will provide important insights into the molecular mechanisms that give rise to chronic inflammation, and enhance our understanding of inter-tissue interactions in this process.

Li, H., Qi, Y., & Jasper, H. (2016). Preventing Age-Related Decline of Gut Compartmentalization Limits Microbiota Dysbiosis and Extends Lifespan. Cell Host & Microbe, 19(2), 240–253. https://doi.org/10.1016/j.chom.2016.01.008

Franceschi, C., & Campisi, J. (2014). Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 69(Suppl 1), S4–S9. https://doi.org/10.1093/gerona/glu057

Poster presentations

The stress granule protein G3BP1 is required for senescence-mediated cancer progression

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Senescence, through the senescence-associated secretory phenotype (SASP), is a driver of age-related diseases, yet senescence is required for various physiological processes. To date, the mechanisms and factors able to control senescent cells to prevent their deleterious impacts, while preserving senescence, remain elusive. Here we show that the ras-GAP-SH3-Binding Protein 1 (G3BP1) is essential for the activation of the SASP. G3BP1 depletion or pharmacological inhibition impairs the cGAS-pathway preventing the expression of SASPs without affecting cell commitment to senescence. These SASPless senescent cells impair senescence-mediated tumorigenesis in vitro and tumor progression in vivo. Together, our data demonstrate that G3BP1 is a viable target to prevent senescence-induced age-related diseases, such as cancer, while potentially preserving beneficial function of senescent cells.

Continuous transcription initiation guarantees the integrity of all active regulatory regions in response to genotoxic stress

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Transcription represents a central driving force in preserving gene expression accuracy and genomic integrity, in the face of environmental perturbations. It has been recently shown that DNA damage induces a multilayered transcription-driven response by altering the gene expression program, leading to accelerated repair of transcription-blocking DNA lesions (Lavigne et al, 2017). Nonetheless, the associated molecular events ruling these processes remain elusive. Here, we uncover a surprising gain in chromatin accessibility and preservation of H3K37ac at virtually all active Transcription Start Sites (TSSs) of mRNAs, PROMoter uPstream Transcripts (PROMPTs) and enhancers in response to UV irradiation. Importantly, we reveal the continuous and dynamic recruitment of pre-initiating RNA Polymerase II (RNAPII) as drug-inhibition of promoter-proximal pause (PPP) release replenishes the post-UV reduced levels of pre-initiated RNAPII, in all active TSSs. In addition, we characterize the fast shift of RNAPII into elongation at these regulatory loci, as demonstrated by the synthesis of short nascent RNAs proximal to TSS sites upon stress, including TSS-associated RNAs (start-RNAs). Our results highlight the necessity of continuous recruitment and engagement of RNAPII in productive transcription, ensuring maximal repair of active regulatory regions and preserving the integrity of the transcribed genome. Taken together, our work provides insights on how transcription and chromatin cooperation guarantees a healthy genome, protecting from various genetic disorders.

Lavigne, M. D., Konstantopoulos, D., Ntakou-Zamplara, K. Z., Liakos, A. & Fousteri, M. Global unleashing of transcription elongation waves in response to genotoxic stress restricts somatic mutation rate. Nature Communications 8 (1), 2076 (2017).

Poster presentations

Artificial Intelligence-Powered Drug Discovery: Age Related Diseases

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The Benevolent PlatformTM ingests biomedical data to build representations of disease biology. Using AI technology, associations between biomedical entities (e.g. diseases, genes, tissues) are extracted from the scientific literature providing a richer semantic model of the disease than provided by structured databases alone.

We aim to find putative drug targets to activate apoptotic death in senescent cells, thereby reversing disease phenotype in age-related diseases. Two methods were used to predict targets for the cellular senescence mechanism. The first used relational inference on top of the Benevolent Knowledge Graph. The second used a causal reasoning algorithm to infer the regulators of tissue-specific expression aging signatures derived from GTEx expression data.

We test if the predicted targets can kill senescent cells in primary human patient derived cell types from old patients or induced into senescence. Hits are progressed into disease relevant platforms compiled using the Benevolent PlatformTM to map senescence in cell type X to diseases.

The effect from silencing the replication licensing factor CDC6 in human breast cancer cell lines

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Cell division cycle 6 (CDC6) is essential for DNA replication in eukaryotic cells. Moreover, CDC6 plays important roles in the activation and maintenance of the checkpoint mechanisms that coordinate S phase and mitosis. CDC6 expression is deregulated from the earliest stages in many types of cancer. We have previously shown that over-expression of the replication licensing factor CDC6 leads to re-replication (a form of replication stress), genomic instability (GI) and malignant behavior promotion. Breast cancer is the second most common form of malignancy in females, in which genetic factors and hormone exposure are two main risk factors for its development. Little is known regarding the prognostic significance of CDC6 in breast cancer and despite the therapeutic progress made over the past few years, this malignancy remains an important research and therapeutic challenge. Our aim was to elucidate the role of CDC6 in maintaining malignancy in human breast cancer. We found that CDC6 is over-expressed in breast cancer cell lines, including the aggressive MDA-MB-231 (mutant p53/ triple-negative late stage breast cancer). CDC6 silencing in these cells resulted in senescence. Mitotic catastrophe, which resulted in both caspase-dependent and independent cell death, was also observed. Finally, upon CDC6 silencing, Chk1 (S345) phosphorylation was initially down-regulated. However, at later timepoints, Chk1 phosphorylation was upregulated. This finding implies that cells manage to bypass the Chk1-dependent G2/M arrest and enter mitosis under replication stress conditions. This is in accordance with our observation that Chk1 phosphorylation is upregulated in mitotic cells upon hydroxyurea treatment. Collectively, these findings suggest that CDC6 over-expression is critical for sustaining the malignant phenotype in breast cancer and imply its potential therapeutic targeting by small molecule inhibitors. We conclude by providing evidence for CDC6 as a druggable target.

Poster presentations

Uncovering genes implicated in therapy-induced senescence resistance

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Drug resistance is a major problem involved in effective response to targeted cancer therapies, and to other therapies such as radiotherapy or chemotherapy. Cellular senescence has been described as a barrier against tumour progression. Although cancer cells naturally avoid senescence, in the last years CDK4/6 inhibitors have been proposed to induce cell cycle arrest in tumour cells. Therefore, the development of pro-senescence therapies has become an attractive strategy. However, even though cancer cells are arrested after CDK4/6 inhibitors treatment, the genes regulating therapy-induced senescence resistance are still unknown limiting the development of new efficient targeted therapies. In this study, we aim to identify genes whose loss-of-function confer proliferative advantage to Palbociclib (CDK4/6 inhibitor). For this purpose, MCF7 breast cancer cells were transduced with a human genomewide sgRNA library (GeCKOv2) targeting 19,050 genes within the human genome. Cells were then treated with/without Palbociclib and deep sequencing was performed. Candidate genes whose loss-of-function prevent proliferation arrest induced by Palbociclib were selected for further validation. CRISPR-Cas9 technology was used to knockdown genes by designing 4 independent sgRNA per gene and transducing all together in MCF7. Growth curves demonstrate that MCF7 infected with sgRNAs targeting selected genes were able to bypass proliferation arrest induced by Palbociclib, Also, the senescence-like phenotype was assed by quantifying different markers of senescence (proliferation, SA-β-galactosidase) using immunofluorescence. In order to determine if the proliferation bypass observed was Palbociclib-dependent, MCF7 cells transduced with different sgRNAs were treated with other CDK4/6 inhibitors (Abemaciclib and Ribociclib) and their senescence-like phenotype was analysed using immunofluorescence. Altogether, we have identified specific genes that confer proliferation advantage to CDK4/6 inhibitors cell cycle arrest. However further studies are needed in order to fully understand the role of these genes during drug treatment resistance.These findings will be useful to design new drugs that will help us to prevent cancer relapse by proposing new cancer therapy strategies.

N-Bromotaurine and its stable analogue molecule (Bromamine T-BAT) exhibit a therapeutic effect against various cancers

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N-Bromotaurine is a physiological molecule, which arises from the neutrophil -myeloperoxidase halide system of metabolism during inflammation and constitutes the reaction product of taurine with hypobromous acid (HOBr). In order to unravel new therapeutic options in cancer, we used N-Bromotaurine as the agent with known anti-inflammatory property and anti-microbial ability. The tumor-suppressive effect of N-Bromotaurine was observed in various cancer cell types. In the case of skin cancer, we showed that N-Bromotaurine can bypass the glucocorticoid receptor (GR)-resistance of cancer cells when used in combination with cisplatin, despite the frequently mentioned glucocorticoid unresponsiveness due to GR impairment. However, due to the poor stability of the N-Bromotaurine molecule, an analogue molecule, named Bromamine T (BAT), was subsequently used. We demonstrated that cancer cell proliferation was suppressed with the use of BAT, while the anti-cancer effect of BAT appeared to be superior to that elicited by taurine. Flow cytometry and western blot experiments highlighted the intrinsic apoptotic pathway used by cancer cells following their exposure to BAT. Additional experiments proved that BAT triggered oxidative burst in cancer cells. Following treatment of cancer cells with BAT, the phosphorylation of two main arms of the MAPK family (JNK1/2 and p38) were stimulated. This implied that BAT induced ROS accumulation, which triggered the phosphorylation of stress-related MAPK kinases, via eliciting pro-apoptotic signals in cancer cells. In parallel, we indicated that BAT exerted anti-inflammatory properties by suppressing mRNA expression levels of cytokines in LPS-induced macrophages. More importantly, the in vivo experiments showed that tumor formation and distribution of immune populations in mice were impaired after treatment with BAT. Our study has produced results that demonstrate that BAT is an emerging anti-proliferative agent with favorable efficacy.

Key-words: N-Bromotaurine, Bromamine T, taurine, cancer, inflammation.

Poster presentations

SIRT1-p21 pathway negatively regulates senescence-associated secretory phenotype (SASP)

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Cellular senescence is a state of irreversible cell cycle arrest caused by various cellular stresses such as ionizing radiation (IR), ultraviolet light (UV) and chemicals; aberrant activation of oncogene; oxidative stress such as that attributable to reactive oxygen species derived from oxidative metabolism. Senescent cells develop a pro-inflammatory response termed the senescence-associated secretory phenotype (SASP). As many SASP factors, such as IL-8, IL-6, GRO-α, affect surrounding cells and alter their microenvironment, SASP may be key phenomena to link with cellular senescnece and individual aging and age-related diseases. Although the initiation and maintenance of SASP require a persistent DDR and NFκB activation, the detailed regulatory mechanism is not fully addressed. SIRT1 is NAD+-dependent protein deacetylase, which involves in various age-related diseases in mammals. We have shown that SIRT1 suppresses the expression of SASP factors. The kinetics and amount of expression of SASP factors, were drastically increased in SIRT1-depleted cells. Interestingly, the expression of p21 (Cip1) protein, a key factor of cellular senescence, is markedly decreased in SIRT1-depleted cells. Moreover, depletion of p21 also induced the enhancement of the expression of SASP factors. These results indicate that SIRT1 regulates the expression of SASP components by regulating p21 expression.

A novel in silico screening process based on Association Rule Mining for gaining mechanistic insights on drug response mechanisms and identifying novel therapeutic targets

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For full list of authors see Pharmacol Ther. 2019 Jul 30:107395. doi: 10.1016/j.pharmthera.2019.107395.

A major challenge in oncology is predicting the response to therapy on a personalised manner. The success of such a task mainly depends on developing computational pipelines that integrate various “omic” data and effectively extract actionable information. Herein, we developed such a pipeline based on Association Rule Mining, for identifying associations between driver-genes and drug response and strategies through which those associations can be utilised as actionable information for increasing therapeutic efficacy. The pipeline was developed on a publicly available pharmacogenomic profile of 1,001 cancer cell lines and non-random gene to drug associations were identified and recorded in the form of easily interpretable rules. All identified rules have been made available online via an interactive web application that allows the user to utilise multiple searching schemes for smart information retrieval. Finally a novel algorithm was developed, based on the aforementioned rules that unveils genes as targets for increasing the efficacy of already established drugs through combination therapy schemes. A number of suggested targets were validated by in silico and experimental means, suggesting that this pipeline can effectively guide the strategy of complex personalised medicine therapeutic schemes.

Poster presentations

Interaction of senescent cells with the innate immune system

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Cellular senescence is a tumor suppressor mechanism that drives cells at risk for malignant transformation into an irreversible cell cycle arrest. Senescent cells also develop a complex senescence-associated secretory phenotype (SASP), which paradoxically drives several degenerative and hyperplastic diseases of aging. In humans and mice, senescent cells increase with age and are found in inflamed and damaged tissues, premalignant lesions and chemotherapy-arrested tumors. Eliminating senescent cells by genetic or pharmacological strategies was recently shown to have a number of beneficial effects on health span. Senescent cells can be targeted for destruction by innate immune cells, primarily natural killer (NK) cells and macrophages. It is not clear, then, why senescent cells increase in numerous tissues during aging. Although the adaptive immune system is known to decline with age, the innate immune system remains robust, although it may exhibit functional changes. We are studying the interaction between senescent cells and the innate immune cells such as NK cells and macrophages that results in senescent cell elimination. Our data suggest factors that comprise the SASP and are induced in response to genotoxic stress such as matrix metalloproteinases (MMPs) can cleave NK cell receptors and ligands (expressed by senescent cells). These NK receptor-ligand interactions are essential for NK-mediated killing. We have also identified a number of SASP modules, which can be selectively induced by different senescence stimuli and are determining whether they promote immune evasion by similar mechanisms. For example, our preliminary data show that genotoxic chemotherapies such as doxorubicin also induce senescence in cell culture and in vivo. However, cells induced to senesce by doxorubicin do not show high levels of MMPs, but show decreased levels of the NKG2D ligand MICA. Thus, senescent cells induced by different stimuli likely develop different means to evade immune clearance. Our data also show increase in macrophage number with age and can be triggered by SASP. Understanding these interactions between the innate immune system and senescent cells may help develop novel therapies for the effective elimination of these cells.

About Athens

A destination with 300 days of sunshine and a coastline more than 13.000 kilometers, Greece has been a muse to poets, musicians and all form of arts for centuries. Featuring a wide range of properties inscribed on the UNESCO's World Heritage List such as the Acropolis in Athens, the archaeological sites of Delphi, Olympia, Mystras, Mycenae, the Medieval city of Rhodes and the Meteora as well as the breathtaking Caldera of Santorini (the largest caldera of the world).

Birthplace of drama, democracy, philosophy, culture, science and the Olympic Games; Greece offers a unique blend of history along with breathtaking views. Nowhere else in the world may you find 5.000 years of history in every street of every city that you visit.

The centre of economic, industrial, political and cultural life in Greece, metropolitan Athens is a bustling and cosmopolitan metropolis with an urban population of 3.3 million and a metropolitan population of about 3.8 million people. While the classical era heritage is still evident in the city - portrayed through a number of ancient monuments and artworks - a huge variety of Roman and Byzantine monuments, a small remaining number of Ottoman monuments, and significant modern landmarks can also be found. The Greek Parliament (19th century), the Athens Trilogy (Library, University, Academy) and the new (2004) Athens Olympic Sports Complex are only some of the city's modern architectural landmarks.

Athens is the historical capital of Europe, with a long history, dating from the first settlement in the Neolithic age. In 1834, it became the capital of the modern Greek state and in two centuries since it has become an attractive modern metropolis with unrivalled charm. Athens dominates the Attica region and is one of the world's oldest cities, with its recorded history spanning. A large part of the town's historic centre has been converted into a 3-kilometre pedestrian zone. The historic centre is an open-air museum, yet the city's cultural and social life takes place amid these ancient landmarks, merging past and present. The magnificent Acropolis rises above the sprawling metropolis and has stood witness to the city's many transformations.

Transportation

ATHENS INTERNATIONAL AIRPORT

One of Athens' highlights is the Athens International Airport (AIA) "Eleftherios Venizelos". Ever since it opened its "gates" back in 2001, AIA has seen an annual growth of 7% and is on the list of the fastest growing airports in the world. Year by year, new carriers and destinations are added to its network. Currently AIA has direct connections to about 65 international destinations, including all major cities around the world. Customer satisfaction is AIA's top priority, providing high security standards and excellent services to all users. The airport is located 33 km southeast of Athens and is easily accessible via a six-lane motorway (the Athens City Ring Road). Access to Athens and the Port of Piraeus is provided by express airport bus connections on a 24-hour basis, while a direct Metro line connects the airport to the city centre (Syntagma square) in just 40 minutes.

More info at www.aia.gr

TRANSPORT FOR ATHENS

THE URBAN TRANSPORT ORGANIZATION (OASA in short), is responsible for delivering and operating a well-organized and cost-effective public transport network, which facilitates the life of the citizens and visitors of Athens.

METRO Three lines serve 61 stations and connect tram, bus routes and suburban rail at hub stations.

BUSES AND TROLLEYBUSES There are 265 bus and 20 trolleybus lines that connect the Athens and Piraeus city centers with the entire metropolitan region. Airport buses run on a 24-hour basis.

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TRAM Environmentally friendly, overlooking the Saronic Gulf, the tram line connects the center of Athens with the coastal suburbs in the southeast and Piraeus (the Port city) in the southwest.

More info at www.tfa.gov.gr

What to see

ACROPOLIS

The Parthenon, a global symbol of Greece, has been standing on the "sacred rock" of Athens, the Acropolis, for thousands of years. The Parthenon along with all the other monuments of the Acropolis, are unique pieces of art, reflecting the Classical period and the Golden Age of ancient Athens during the 5th and 4th centuries B.C.

THE ACROPOLIS MUSEUM

Designed by Bernard Tschumi in collaboration with Michalis Photiadis, the sparkling new museum, has already become the city's top attraction since its opening in June 2009 and is expected to become one of the most visited and "must see" museums worldwide. The museum, which exhibits approximately 4.000 artefacts, allows the sculptures to be viewed in natural light through special glass installations along with climate-control measures that protect them from sunlight. The most impressive part of the museum is its top floor, where visitors are able to view the frieze as well as the Parthenon itself outside the windows.

ODEON OF HERODES ATTICUS

At the footsteps of the Acropolis, the Odeon was built in 161 A.D. under Tiberius Claudius Atticus Herodes. Ever since and through the ages, audiences have had the chance to enjoy world-class concerts, plays and ballets within its surroundings. The natural setting of Herodeion, with its marvelous arcades, the Parthenon as a backdrop and the moon up in the sky will certainly fascinate you.

ANCIENT AGORA

The Ancient Agora, which means "market" in modern Greek, is located at the footsteps of the Acropolis and in ancient times, served as the commercial as well as political, cultural and religious centre of the city.

PLATO'S ACADEMY

Academy was a suburb of Athens, named after the hero Academos or Ecademos. The

site was continuously inhabited from the prehistoric period until the 6th century A.D. During the 6th century B.C., one of the three famous Gymnasiums of Athens was founded here. In 387 B.C., Plato founded his philosophical school, which became very famous due to the Neoplatonists, and remained in use until 526 A.D., when it was finally closed down by emperor Justinian.

NATIONAL ARCHAEOLOGICAL MUSEUM OF ATHENS

The visit to National Archaeological Museum is a must. One of the richest collections of ancient Greek art in the world, the exhibits represent all the cultures that flourished in Greece, from prehistoric times to the period of Turkish occupation. Frescoes from prehistoric Thera and sculptures from the classical period, such as the bronze statue of Poseidon, are some of the museum's highlights.

BYZANTINE MUSEUM

The museum hosts over 25,000 artefacts divided into collections that date from the 3rd to the 20th century A.D.

MUSEUM OF CYCLADIC ART

Established in 1986 to house the private collection of the Goulandris shipping family, it focuses on ancient cultures of the Aegean, especially that of the Cyclades during the 3rd millennium B.C.

NATIONAL GALLERY OF ATHENS

Features some of Greece's and Europe's finest paintings and works of art from the 19th and 20th centuries.

BENAKI MUSEUM

The space hosts 30.000 items illustrating the spirit of the Greek world through a spectacular historical panorama, covering several time-periods from the Prehistoric, Ancient and Roman eras to the Byzantine and the contemporary Hellenic period.

What to see

PANATHENAIC STADIUM

Originally built in the 4th century B.C. for the athletic competitions of the Great Panathinaia (ancient Greek festivities), the "Kallimarmaron" Stadium (meaning "beautiful marble") was the venue of the first modern Olympic Games, in 1896.

TEMPLE OF POSEIDON AT SOUNION

This Doric temple was erected during the Golden Age of Pericles. It was devoted to Poseidon, the Olympian God of the Sea, and is located at the edge of Cape Sounion at the southern coast of Attica, with a spectacular view of the Aegean Sea. Along with the Parthenon and the temple of Aphaia, on nearby Aegina island, Poseidon's mighty monument completes the Sacred Triangle of antiquity. It was built during 444–400 B.C., probably by Iktinos, one of the two architects that built the Parthenon.

STAVROS NIARCHOS FOUNDATION CULTURAL CENTER (SNFCC)

The new SNFCC is Greece's largest cultural, educational and recreational urban complex, and globally, one of the most sustainable building complexes of its size. It promises to move its citizenry forward into the 21st century, through education, sustainability and culture. The Center includes new facilities for the National Library of Greece with a significant book collection, and for the Greek National Opera- one of the most modern opera houses in the world. Surrounding the buildings is the Stavros Niarchos Park, replete with Mediterranean planting, waterways, a Great Lawn for festivals and concerts, bike paths and children's playgrounds.

ONASSIS STEGI

This modern cultural landmark covers an entire block of Syngrou Avenue. Architecture Studio's bold design is a glass cube cloaked in rows of white marble that glow at night. Known locally as Stegi (Roof), the building hosts theatre, dance, and musical performances by Greek and leading international artists, as well as boundary-pushing exhibitions that often extend off-site. The top-floor bar and restaurant, Hytra, is a destination in its own right.

THE NATIONAL OBSERVATORY

The National Observatory of Athens is the first research Institution created in Greece (1842) after its liberation from the Ottoman (1828), the arrival of King Otto as the head of the modern Greek Kingdom (1833) and the establishment of Athens as the capital of the modern Greek state (1834). It followed the establishment of the two other oldest institutions of higher learning in the modern Greek state, the National Technical University of Athens (1836) and the University of Athens (1837).

The construction of the historic Sina's building on the hill of the Nymphs was financed by the Greek entrepreneur and national benefactor Georgios Sinas who was a successful banker in Vienna and ambassador of Greece to Austria.

The original Observatory building was designed by the renouned Danish Architect Theophilus Hansen, who also designed the Cathedral of Athens (1842) and two of the three contiguous buildings forming the so-called "classical trilogy", namely the Academy of Athens and the National Library of Greece.

EUGENIDES FOUNDATION DIGITAL PLANETARIUM

It was established in 1956, in accordance with the will left by national benefactor Eugene Eugenides. The current building was inaugurated in 1966 and completely renovated in 2003.

After more than half a century of reaching for the stars, its vision remains the same: to embrace technology and present astronomy and astrophysics in an engaging and accessible way to young minds and the general public. The planetarium's recreational and educational activities stretch far beyond its borders with numerous publications, postgraduate scholarships and donations.

The planetarium has always been at the forefront of technology, keeping pace with the advancements and challenges of the field. Since its new digital era in 2003, the planetarium boasts a 1,000m^2 dome. The foundation also hosts a library, conference centre, digital archives, the UTech creative workspace and the Athena Science and Technology Centre.

General tourist information

Greek National Tourism Organisation
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Urban Transport Organisation
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Athens International Airport
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Flight information (all airlines)
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Aegean Airlines
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www.aegeanair.com
Greek Railway Company
www.ose.gr
Ministry of Culture
www.culture.gr
Region of Attica
www.athensattica.gr
The City of Athens
www.thisisathens.org

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Fire Emergency: 199
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Poison Control: +30 210 7793777
Aids Help Line: +30 210 7222222
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PHARMACIES

Pharmacies can be found throughout Athens. For overnight service, there are always pharmacies open in most districts. For more information, call 14944.

BANKS

Major international banking institutions, credit card companies and ATMs in and around Athens are plentiful and easy to access. Banks are open to the public Monday through Thursday 08:00 to 14:30 and Fridays 08:00 to 14:00, except on public holidays. The "Open24" Bank (Eurobank) is open mornings and afternoons, as well as on Saturdays. Foreign currency may be exchanged for euros at most Greek and foreign banks and at exchange bureaus located in the city center and the Athens International Airport. Bring your passport when exchanging money as you will need it for the transaction. Traveler's checks and credit cards issued by major companies are also widely recognized and accepted as a means of purchasing items in Athens.

ELECTRICITY

In Greece the standard voltage is 230 V and the frequency is 50 Hz. You can use your electric appliances in Greece, if the standard voltage in your country is in between 220 - 240 V (as is in the UK, Europe, Australia and most of Asia and Africa). Manufacturers take these small deviations into account. If the standard voltage in your country is in the range of 100 V - 127 V (as is in the US, Canada and most South American countries), you need a voltage converter in Greece. You can also consider a combined power plug adapter/voltage converter.

Organisers and invited speakers' disclosure information

Invited speakers

Adams Peter
Aidinis Vassilis
Baker Darren J.
Benetos Athanasios
No conflict of interest
Ben-Porath Ittai
Bischof Oliver
Blow Julian
Burma Sandeep
Campisi Judith
Chondrogianni Niki
Collado Manuel
Demaria Marco
Di Micco Raffaella
Eliopoulos Aristidis
Ferbeyre Gerardo
No conflict of interest
Försch Sebastian
No conflict of interest
Fousteri Maria
Frisan Teresa
No conflict of interest
Gagos Sarantis
No conflict of interest
Garinis Georgios
Giatromanolaki Alexandra
No conflict of interest
Gonos Efstathios S
Gorgoulis G Vassilis
No conflict of interest
Hara Eiji
Harris Curtis C
Horvath Steve
Kletsas Dimitris
No conflict of interest
Kostourou Vasso
No conflict of interest
Krizhanovsky Valery
Liborio Vetrano Davide
Logothetis Christopher
Lygerou Zoi
Maier Andrea
Matsas Rebecca
Munoz-Espin Daniel
Nebreda Angel
Niedernhofer Laura
Niedzwiedz Wojciech
Nieto Isabel
No conflict of interest
Papantonis Argyris
Pavlatou Evangelia
Pefani Eleftheria-Dafni
Richard Nicholas
Robbins Paul
No conflict of interest
Schmitt Clemens
Sedivy John
Serrano Manuel
Manuel Serrano is co-founder and advisor of Senolytic Therapeutics, Inc
Shiloh Yossi
Sikora Ewa
No conflict of interest
Tavernarakis Nektarios
Townsend Paul
Trougakos Ioannis
No conflict of interest
Tsitsilonis Ourania

10 min talk

Jean-Marc Lemaitre
Alena Shen
No conflict of interest
Frédérick A. Mallette
No conflict of interest
Tamir Chandra
Andrew Young
Nicholas Rettko
Raquel Buj
Haoran Zhu
No conflict of interest
Utz Herbig
Deborah Milligan
Ana O'Loghlen
Ryan J Wallis
No conflict of interest

2 min talk

Ayush Srivastava
No conflict of interest
Covadonga Huidobro
Koji Itahana
No conflict of interest
Eva González-Suarez
EGS has served on advisory boards for Amgen and has received funding from Amgen for research
Amr Omer
Nafsika Chala
No conflict of interest
Takehiro Yamanaka
No conflict of interest
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Satotaka Omori
Wioleta Grabowska
No conflict of interest

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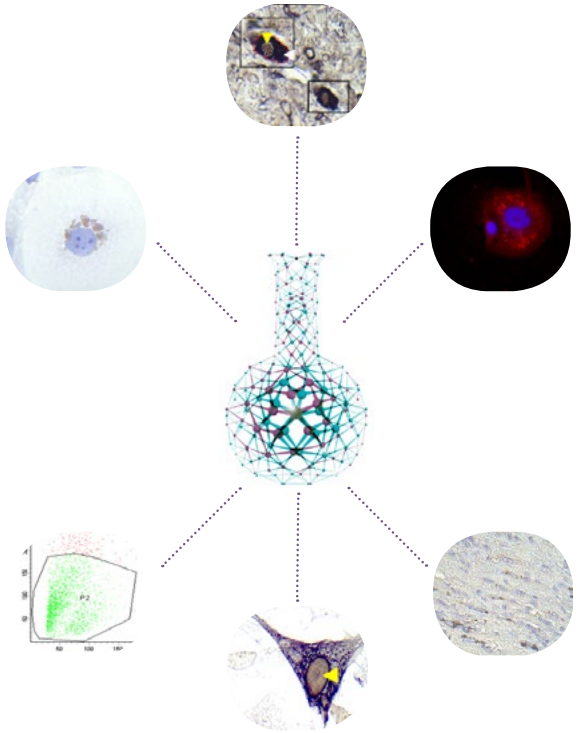

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