ICSA CONFERENCE

The Ins and Outs of Cellular Senescence : Understanding the Biology to Foster Healthy aging and suppression of diseased

Institut Pasteur, Paris
May 16 - 19 2017

ABSTRACT BOOK

Institut Pasteur

ICSA
International Cell Senescence Association

THANKS TO OUR SPONSORS

Institut Pasteur

International Cell Senescence Association (ICSA) Conference 2017

Paris, May 16-19, 2017
Institut Pasteur, Paris, France

ORGANIZERS

Oliver BISCHOF, Institut Pasteur, France
Group Leader, Molecular And Cellular Biology of Cellular Senescence and Age-related Pathologies

Peter ADAMS, Beaston Institute for Cancer Research and University of Glasgow, UK
Head of Laboratory Epigenetics of Cancer and Ageing

INVITED SPEAKERS

Juan Carlos ACOSTA	University of Edinburgh, United Kingdom
Serge ADNOT	Institut Mondor de Recherche Biomedicale, France
Andrea ALIMONTI	Institute of Oncology Research, Switzerland
Shelley BERGER	University of Pennsylvania, United States
Rene BERNARDS	Netherlands Cancer Institute, The Netherlands
Corine BERTOLOTTO	INSERM U1065, France
Marcus BOSENBERG	Yale University, United States
Judith CAMPISI	Buck Institute for Research on Aging, United States - Keynote Lecture
Kwang-Hyun CHO	KAIST, South Korea
Eric GILSON	IRCAN, France – Keynote Lecture
Myriam GOROSPE	NIH - National Institute on Aging, United States
Jürgen GROLL	University of Wuerzburg, Germany
Utz HERBIG	Rutgers University, United States
Franz JAKOB	University of Wuerzburg, Germany
Bill KEYES	IGBMC, France
Valery KRIZHANOVSKY	Weizmann Institute of Science, Israel
Masashi NARITA	Cancer Research UK Cambridge Institute, United Kingdom
Joao PASSOS	Newcastle University, United Kingdom
Alain PUISIEUX	Centre de Recherche en Cancérologie de Lyon, France
Lenhard RUDOLPH	Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Germany
Marcus RUSCETTI	Memorial Sloan Kettering Cancer Center, United States
Manuel SERRANO	Spanish National Cancer Research Centre, Spain - EMBO Keynote Lecture
Maximina YUN	University College London – IRIS, United Kingdom
Lars ZENDER	University Hospital and Faculty of Medicine Tübingen, Germany
Rugang ZHANG	The Wistar Institute, United States

THEME OF THE CONFERENCE

The 2017 « International Cell Senescence Association (ICSA) meeting » held in Paris (France) will provide a forum to present and discuss the latest developments in the field of cellular senescence.

Cellular senescence is now considered a fundamental cell fate playing important physiological roles in embryonic development, wound healing and tumor suppression, but also in pathophysiological conditions including age-related diseases, notably cancer and degenerative diseases such as sarcopenia, atherosclerosis, Parkinsons or Alzheimers disease.

As such, research on therapeutic strategies senescence for healthspan improvement has gained enormous momentum.

Topics to be covered during the conference include:

1. Mechanisms leading to onset of senescence;
2. Physiological functions of senescence, including tumor suppression, organismal and tissue development, wound healing and other tissue protective roles;
3. Pathological consequences of senescence, for example during tissue aging and chronic inflammation;
4. Avenues to exploit senescence in biomedicine, for example pro-senescence cancer therapies and strategies to suppress the pro-aging features of senescent cells.

There will be a special emphasis on evaluating current paradigms and ideas in senescence in the context of sometimes disparate ideas from the wider fields of aging, cancer, epigenetics, immunology, regenerative medicine and developmental biology.

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GENERAL INFORMATION

WELCOME DESK - OPENING HOURS

Registration desk opens at 4:00 pm on May 16th, 2017 in the hall of the CIS Auditorium.

Other days:

- Opening: 7:45-9:30 am
- Permanence during coffee breaks, lunches and cocktails.
- **Registration desk will be closed during plenary sessions.**

On the Institut Pasteur campus, the "Plan Vigipirate Attentats" is on, so please make sure to have an official ID or passport on you to enter the campus.

If your registration is fully covered, you will receive your complete congress kit including your badge, the certificate of attendance and the conference program. Please wear your badge at all time.
If registration was not fully covered, please come directly to the registration desk "on site payment". We accept payment by cash or credit card.

ORAL SESSION

Scientific sessions are taking place in the main auditorium of the "**Centre d'information Scientifique", located in the building CIS**.

All oral presenters must bring their Power Point presentation on an USB stick to the technical room as soon as possible and at least 30 minutes before the session starts. The presentation will be downloaded in the preview system and available from the computer set at the lectern on stage.

POSTER SESSIONS

Two poster sessions will be displayed in the exhibition area in the building CIS as following:

Flash talks: Wednesday, May 17th
6:30 pm – 6:50 pm

2 minutes per presentation – 2 slides maximum

Presentations to be given to technicians in preview room before afternoon session.

Poster session 1: Wednesday, May 17th
7:00 pm to 9:00 pm **All Posters**

Poster session 2: Thursday, May 18th
6:30 pm to 8:30 pm **All Posters**

NB: Poster numbers are in the program book. Check the matching number on the board to display your poster in the right place. Magnets are available at the welcome desk to mount your poster.

COFFEE BREAKS & LUNCHES

Coffee breaks and lunch buffets will be served in the entry hall of the CIS. Access to lunch buffets is strickly limited to registered participants.

WIFI

A WiFi connection is available in the auditorium and in the hall of the building.
Use your personal username and password provided with your badge.

SOCIAL EVENTS

Cocktails:

- Welcome cocktail (hall of CIS) - Tuesday 16th from 9:10 pm
- Cocktail by the posters (hall of CIS) - Wednesday 17th from 6:50 pm
- Cocktail by the posters (hall of CIS) - Thursday 18th from 6:35 pm

Conference dinner (subject to paying registration)

The dinner cruise will be held on the boat "Le Paris" on May 19th, 2017 at 8:30 pm.
Boat departure is scheduled at 9:00 pm and boat return at 11:30 pm.
Make sure you are on time.

Join us to DJ Party and Open Bar: 11:00 pm - 2:00 am

Free departure by your own means at the boat.

Participants who have registered to this conference-gala dinner will find their voucher together with their name badge in the registration envelope.

Access:

PARIS SEINE, "Le Paris" boat - Port Debilly

Entrance in front of 26 avenue de New York, Paris 16 district

Metro: Line 9, Iéna station or Alma Marceau (20 minutes from Institut Pasteur)

Bus : n°82 Stop Varsovie; n°72 stop Musée Art Moderne Palais de Tokyo

Public car park: 10 avenue George V, Paris 8 district


INSTITUT PASTEUR

SCIENTIFIC PROGRAM

Day 1 Opening session

16/05/2017
16:00-22:00

Registration

16/05/2017
16:00-18:20

16:00 **Arrival, Registration and Poster set up**

18:00 **Welcome Organizers**

1 (Em)Powering the SASP

16/05/2017
18:20-21:10

Chair: Rugang Zhang

1
18:20 **The kaleidoscope of cellular senescence**
J. Campisi
Buck Institute for Research on Aging, United States

2
19:10 **Senescence associated pattern recognition receptors in control of oncogene induced senescence**
J. Acosta
ECRC, MRC-Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

3
19:25 **Innate immune sensing of chromatin through cGAS regulates the SASP**
A. Ablasser
Global Health Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

4
19:40 **Integrin beta3 regulates senescence by activating TGF-b in a paracrine and autocrine fashion**
M. Borghesan
[1]Queen Mary University, London, United Kingdom

5
19 :55 **Uncoupling senescence-assocaited cell cycle exit from SASP production in oncogenesis**
G. David
New York University School of Medicine, New York, United States

6
20:10 **The senescence-associated secretory phenotype bridges tissue ischemia to pathological angiogenesis in retinopathy**
F. Mallette
Maisonneuve-Rosemont Research Center, Université de Montréal, Montréal, Canada

7 20:25 **p53 in bronchial Club cells facilitates chronic lung inflammation by promoting senescence**
N. Feldman
Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

8 20:40 **Metabolic basis of cellular senescence**
R. Zhang
The Wistar Institute, United States

Welcome cocktails

16/05/2017
21:10-22:00

Day 2 Senescence Triggers

17/05/2017
08:30-18:50

2.1 DNA Damage Response and Telomeres

17/05/2017
08:30-10:00

Chair: Utz Herbig

08:30 **ICSA Assembly**

9
09:00 **Epigenetic stress response and stem cell aging**
L. Rudolph
Beutenbergstraße 11, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany

10
09:30 **A fresh look at senescence associated persistent DNA breaks in mammalian cells and tissues**
A. Galbiati
IFOM, Milan, Italy

11
09:45 **Cellular senescence drives age-dependent hepatic steatosis**
D. Jurk
Newcastle University Instiute for Ageing, Newcastle University, Newcastle Upon Tyne, United Kingdom

Coffee break

17/05/2017
10:00-10:30

2.2 DNA Damage Response and Telomeres

17/05/2017
10:30-12:45

10:30 **Welcome Address by M. Christian Bréchot, General Director of Institut Pasteur**

12 10:40 **Telomere dysfunction-induced senescence and its impact on human health**
U. Herbig
Rutgers University, United States

13 11:10 **Investigating the role of neutrophil infiltrations in DNA-damage induced senescence**
A. Lagnado
Campus for Ageing and Vitality, Newcastle University Institute for Ageing, Newcastle Upon Tyne, France

14 11:25 ***Monoamine oxidases* as molecular players of cardiac senescence**
N. Manzella
UMR1048 Institute of Metabolic and Cardiovascular Diseases, INSERM, Toulouse, France

15 11:40 **Redefining entry into telomere-mediated replicative senescence: Uncapping or irreparable genome damage?**
M.A. Olivier
Centre de recherche du CHUM (CRCHUM), Montréal, Canada

16 11:55 **Frequent adaptation events in telomerase-negative cells drive genome instability in senescence**
M.T. Teixeira
Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, CNRS - Institut de Biologie Physico-Chimique, Paris, France

17 12:10 **Escaping cellular senescence by targeting telomerase expression in damaged cells: consequences for lung diseases**
V. Geli
CRCM Marseille, Inserm-CNRS, Marseille, France

18 12:25 **Length-independent telomere damage drives cardiomyocyte senescence**
J. Passos
Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle Upon Tyne, United Kingdom

Lunch

17/05/2017
12:55-14:00

3.1 Transcriptional Regulation and Gene Networks

17/05/2017
14:00-16:00

Chair: Myriam Gorospe

19 14:00 **Profound nuclear and chromatin alterations in senescence and aging**
S. Berger
Perelman School of Medicine, University of Pennsylvania, United States

20 14:30 **Single cell transcriptomics reveals heterogeneity in oncogene-induced senescence**
T. Chandra
MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

21 14:45 **Uncovering regulators of irreversible growth arrest during therapy induced senescence**
M. Klein
Andrew Koff Lab, Cornell University, New York, United States

22 15:00 **Cell-cell communication in senescence**
M. Narita
Cancer Research UK Cambridge Institute, Cambridge, United Kingdom

23 15:30 **Age-associated alterations in haematopoietic stem cell heterochromatin**
D. Garrick
INSERM UMRS 1126, Institut Universitaire d'Hématologie, Université Paris Diderot, Paris, France

24 15:45 **Signatures of senescence progression in a transcriptomic landscape analysis across the mouse lifespan**
S. Grellscheid
Department of Biosciences, Durham University, Durham, United Kingdom

Coffee break

17/05/2017
16:00-16:30

3.2 Transcriptional Regulation and Gene Networks

17/05/2017
16:30-18:30

25 16:30
Control of cell senescence by HuR and target noncoding RNAs
M. Gorospe
Laboratory of Genetics and Genomics, NIH - National Institute on Aging, Baltimore, United States

26 17:00
A senescence-specific lncRNA modulates the pro-inflammatory response of senescent cells
E. Grossi
Gene Therapy and Regulation of Gene Expression, Pamplona, Spain

27 17:15
The nuclear receptor RXRA controls cellular senescence by regulating calcium signaling
N. Martin
Senescence Escape Mechanisms team, Cancer Research Center of Lyon, UMR INSERM 1052 CNRS 5286, Centre Léon Bérard, Université de Lyon, Lyon, France

28 17:30
A dynamic transcription factor (TF) network controls oncogene-induced senescence
R.I. Martínez Zamudio
ONO, Institut Pasteur, Paris, France

29 17:45
HMGB-family members topologically bookmark the genome and control senescence-induced three-dimensional reorganization via CTCF
A. Papantonis
Center for Molecular Medicine, University of Cologne, Cologne, Germany

30 18:00
Systems biology: from understanding towards controlling of complex biological networks
K. Cho
Laboratory for Systems Biology and Bio-inspired Engineering, Department of Bio and Brain Engineering, KAIST, Daejeon, South Korea

Poster Flash Talks

17/05/2017
18:30-18:50

1P **Isolation and characterization of senescence-associated exosomes**
S. Da Silva-Alvarez
Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), Santiago De Compostela, Espagne

2P **Non-canonical inflammasome activation regulates oncogene-induced senescence**
I. Fernandez-Duran
University of Edinburgh, Edinburgh, Royaume-Uni

3P **Regulatory role of PARP-1 in the onset and maintenance of oncogene induced senescence**
L. Robinson
Institut Pasteur, Paris, France

4P **Non-autonomous regulation of chromatin structure by NOTCH1**
A. Parry
Cancer Research UK Cambridge Institute, Cambridge, Royaume-Uni

5P **Global sumoproteome analysis reveals a PML-localized network of proteins regulating cellular senescence**
G. Ferbeyre
Université de Montréal, Montréal, Canada

6P **Inhibition of the 60S ribosome biogenesis GTPase LSG1 induces senescence with a restricted senescence-associated secretory phenotype**
A. Pantazi
University of Edinburgh, Edinburgh, Royaume-Uni

7P **Investigating the role of p21 in the regulation of developmental senescence**
D. Amaya
Institut de génétique et de biologie moléculaire et cellulaire, Illkirch-Graffenstaden, France

8P **Role of integrin beta 3 in ageing**
J.A Fafián Labora
Queen Mary University of London, Blizard Institute, London, Royaume-Uni

9P **Proteotoxicity links therapy-induced cancer cell senescence to Alzheimer's disease**
D. Dhawan
Berlin School of Integrative Oncology, Berlin, Allemagne

10P **Can the inflammatory facet of SASP be controlled?**
P. D'Alessio
AISA, Villejuif, France

Poster session - Open bar

17/05/2017
18:50-21:00

Day 3 Physiological Functions of Senescence

18/05/2017
08:30-18:30

4.1 Tissue Regeneration

18/05/2017
08:30-10:00

Chair: Bill Keyes

31 08:30
Senescence induced plasticity in regeneration and cancer
B. Keyes
CNRS UMR 7104 - Inserm U 964, IGBMC, Illkirch Cedex, France

32 09:00
Rejuvenation of senescent fibroblast morphology, proteome and replicative lifepsan by modulating mTOR and cytoskeletal signalling
L. Cox
Department of Biochemistry, University of Oxford, Oxford, United Kingdom

33 09:15
Heat shock induces cellular senescence and SASP in human skin fibroblasts
S. Kerschbaum
Department of Applied Life Sciences, University of Applied Sciences, Vienna, Austria

34 09:30
Injury-induced senescence enables *in vivo* reprogramming in skeletal muscle
H. Li
Developmental and stem cells dept, Cellular Plasticity and Disease Modelling group (G5), Institut Pasteur, Paris, France

35 09:45
Cardiomyocyte-cardiac fibroblast directed crosstalk induces myofibroblast senescence to control fibrogenesis
K. Meyer
Institute of Pharmacology and Toxicology, Technical University Munich, Munich, Germany

Coffee break

18/05/2017
10:00-10:30

4.2 Tissue Regeneration

18/05/2017
10:30-12:30

36 10:30 **The interplay between senescence and regeneration: insights from the salamander**
M. Yun
University College London - IRIS, United Kingdom

37 11:00 **Identification of novel mechanisms of immune surveillance of senescent cells**
Y. Ovadya
Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

38 11:15 **IR-induced senescence of the splenic microenvironment impacts murine immune cells functions**
L. Palacio
Departement de pharmacologie, Université de Montreal, Centre de recherche du Centre Hospitalier Universitaire Ste-Justine, Montreal, Canada

39 11:30 **Ionizing radiation-induced endothelial senescence and role in normal tissue injury**
F. Soysouvanh
PRP-HOM/SRBE/L3R, Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-Aux-Roses, France

40 11:45 **Novel IL6 family member is an inducer of quiescence that augments muscle stem cell engraftment and regeneration**
A. Palla
Baxter Laboratory for Stem Cell Biology, Stanford University, Stanford, United States

41 12:00 **Accumulation of senescent cells - mechanisms and consequences**
V. Krizhanovsky
Weizmann Institute of Science, Israel

Lunch

18/05/2017
12:30-14:00

5.1 Tumor Suppression

18/05/2017
14:00-16:00

Chair: Corine Bertolotto

42 14:00 **The ZEB1 EMT-inducing transcription factor establishes a link between the escape from oncogene-induced senescence and the genetic history of breast tumorigenesis**
A. Puisieux
UMR INSERM 1052 CNRS 5286, Centre de Recherche en Cancérologie de Lyon, Lyon, France

43 14:30 **Mitotic-slippage induced senescence confers a pro-tumourigenic phenotype**
B. Cheng
Lee Kong Chian School of Medicine, Karen Crasta Lab, Nanyang Technological University, Singapore, China

44 14:45 **Senescence and immunotherapy in cancer mediated by Stat3 blockade**
M. De Martino
Laboratory of Molecular Mechanisms of Carcinogenesis, Ibyme-Conicet, Buenos Aires, Argentina

45 15:00 **The transcription factor Spi1/PU.1 limits proliferation by inducing cellular senescence during hematopoiesis, a process that is lost during early steps of leukemic transformation**
C. Guillouf
Inserm U1170, Gustave Roussy, Villejuif, France

46 15:15 **Senescent human breast fibroblasts after exposure to ionizing radiation have an altered proteoglycan expression facilitating tumor progression**
D. Kletsas
Laboratory of Cell Proliferation and Ageing, NCSR "Demokritos", Greece

47 15:30 **Oncogenic MITF^{E318K} promotes senescence delay and melanoma progression**
C. Bertolotto
INSERM U1065, Nice, France

Coffee break

18/05/2017
16:00-16:30

5.2 Tumor Suppression

18/05/2017
16:30-18:25

48 16:30 **Molecular insights of APL cure as therapy-induced senescence**
M. Ogrunc
U944, INSERM, Paris, France

49 16:45 **Interplay of CIP2A inhibition-induced senescent tumor cells and tumor infiltrating immune cells in basal-like breast cancer**
A. Laine
Division of Immunology, Netherlands Cancer Institute, Amsterdam, The Netherlands

50 17:00 **Cytokine-induced senescence proceeds via an argonaute protein 2-dependent mechanism**
M. Rentschler
Dermatology, University Medical Center Tübingen, Tübingen, Germany

51 17:15 **Identification of a new phosphorylation site on SOCS1 involved in the regulation of cellular senescence**
E. Saint-Germain
Biochemistry, Gerardo Ferbeyre lab, Université de Montréal, Montreal, Canada

52 17:30 **H3K9-active demethylases disable oncogene-induced senescence and promote melanomagenesis**
Y. Yu
Max Delbrüeck Center for Molecular Medicine, Berlin, Germany

53 17:45 **Modeling release from oncogene-induced senescence**
M. Bosenberg
Yale University, United States

54 18:15 **Advances in chromatin immunoprecipitation**
C. Proux
Active Motif Europe S.A., La Hulpe, Belgium

Group photo

18/05/2017
18:25-18:35

Poster session - Open bar

18/05/2017
18:35-21:30

Day 4 Pathologies and Therapies

19/05/2017
08:30-19:00

6.1 Age-related Diseases and Pathological Consequences

19/05/2017
08:30-10:00

Chair: Serge Adnot

55 08:30
Role of cellular senescence in chronic lung diseases
S. Adnot
Institut Mondor de Recherche Biomedicale, France

56 09:00
Contribution of *in vivo* senescent cells to age-related diseases
D. Baker
Mayo Clinic, Rochester, United States

57 09:15
Antioxidant treatment can promote lung tumor formation during chronic oxidative stress
M. Breau
Team 8, INSERM U 955, Créteil, France

58 09:30
Targeted therapy-induced senescence enhances immune surveillance of KRAS mutant lung cancers
M. Ruscetti
Department of Cancer Biology and Genetics, New York, NY 10065, United States

Coffee break

19/05/2017
10:00-10:30

6.2 Age-related Diseases and Pathological Consequences

19/05/2017
10:30-12:15

59 10:30 **HDAC inhibitors impair the pro-inflammatory senescence-associated secretory phenotype by blocking the nuclear-cytoplasmic translocation of damage DNA fragments**
M.G. Vizioli
Epigenetics, The Beatson Institute for Cancer Research, Glasgow, United Kingdom

60 10:45 **Insights into haematopoietic stem cell ageing and disease from single cell studies**
K. Kirschner
Beatson Institute for Cancer Research, University of Glasgow, Glasgow, United Kingdom

61 11:00 **Neuregulin-1 attenuates stress-induced vascular senescence *in vitro* and *in vivo***
H. Shakeri
dep. medicine, pharmacology, physiopharmacology, university of Antwerp, Antwerp, Belgium

62 11:15 **Osteoporosis**
F. Jakob
Orthopedic Center for Musculoskeletal Research, University of Wuerzburg, Würzburg, Germany

63 11:45 **Combining senescence with biomaterials research and biofabrication for regenerative medicine**
J. Groll
University of Wuerzburg, Germany

Lunch

19/05/2017
12:15-13:30

7.1 Biomedical Exploitation

19/05/2017
13:30-15:30

Chair: Andrea Alimonti

64 13:30 **Targeting the cancer genome and the tumour immune response for pro-senescence therapy**
A. Alimonti
Institute of Oncology Research, Bellinzona, Switzerland

65 14:00 **Evaluation of a novel ß-Galactosidase specific PET tracer for in vivo imaging of tumor senescence**
M. Krueger
Werner Siemens Imaging Center, University Hospital Tuebingen, Tuebingen, Germany

66 14:15 **Nanotechnology-based approaches for targeting senescent cells: defining novel therapeutic strategies**
S. Macip
University of Leicester, Leicester, United Kingdom

67 14:30 **Selectively targeting the ribosome: a novel pro-senescence therapeutic strategy for p16+ Basal-like breast cancer**
M. Moore
Cell Biology and Cutaneous Research, The Blizard Institute, Queen Mary, University of London, London, United Kingdom

68 14:45 **Induction of ribosomal checkpoint induced senescence (RCIS) for the treatment of liver cancer**
L. Zender
Department of Internal Medicine VIII, University Hospital Tübingen, Tübingen, Germany

69 15:15 **Novel diagnostic tools for lung cancer early detection**
D. Munoz-Espin
Oncology, University of Cambridge, Cambridge, United Kingdom

Coffee break

19/05/2017
15:30-16:00

7.2 Biomedical Exploitation

19/05/2017
16:00-17:30

70 16:00 **A one-two punch model for the treatment of cancer**
R. Bernards
Netherlands Cancer Institute, Amsterdam, The Netherlands

71 16:30 **Defects specific to progeroid Cockayne syndrome cells are recapitulated during normal cellular senescence**
M. Ricchetti
Stem Cells & Development, CNRS UMR3738 Team Nuclear & Mitochondrial DNA Stability, Institut Pasteur, Paris, France

72 16:45 **Closing Lecture:** **Keynote Lecture**
Tissue repair: an integrated view of senescence and reprogramming
M. Serrano
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

17:15 **Poster prize**

Sponsored by

Public Session

19/05/2017
17:30-19:00

Chair: Eric Gilson

Panel Experts: Peter Adams, Serge Adnot, Oliver Bischof, Myriam Gorospe, Maximina Yun, Clemens Schmitt, Manuel Serrano

Banquet "Senescence-on-the-Seine"

19/05/2017
20:30-02:00

ORAL PRESENTATIONS

The kaleidoscope of cellular senescence

J. Campisi
Buck Institute for Research on Aging, United States

Cellular senescence was first formally described more than half a century ago, when Hayflick and colleagues connected the senescence response to two important physiological processes: tumor suppression and aging. Since then, cellular senescence has been implicated in a much wider range of physiological and pathological processes. These processes range from embryonic development and cellular reprogramming to a growing list of diseases, many of which are among the most common pathologies associated with aging. As the physiological and pathological roles of cellular senescence expand, many new questions have arisen in the field.

How diverse are senescent phenotypes, particularly the senescence-associated secretory phenotypes (SASPs)?

How dynamic are these phenotypes, and how do they vary depending on the species, tissue and cell type?

What are the main molecular mechanisms by which senescent cells are established and maintained *in vivo*, and how stable are these cells and their phenotypes during both physiological and pathological processes?

And what new insights into molecular mechanisms, physiology and pathology will senescent cells provide in the near future?

Senescence associated pattern recognition receptors in control of oncogene induced senescence

J. Acosta
ECRC, MRC-Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

Oncogenic activation (i.e. RAS) induced a powerful fail safe mechanism in normal somatic cells termed Oncogene Induced Senescence (OIS), that is characterized by a permanent cell cycle arrest with altered metabolism (SA-bGal), the induction of the p16-Rb and p53-p21 tumour suppressor pathways and the activation of a characteristic secretome (Senescence Associated Secretory Phenotype, SASP).

Previously, we identified the inflammasome as a key regulator of the activation of the SASP and OIS. Inflammasomes are molecular platforms for the activation of Caspase-1, an inflammatory Caspase that cleaves and activate IL1B. Importantly, inflammasomes are assembled by the activation of Pattern Recognition Receptors (PRRs) of the NOD-like receptor family. PRRs are Receptors of the Innate Immune Response, which are the first step in the process of recognition of pathogens and the activation of Immunity. PRR recognize Pathogen Associated Molecular Patterns (PAMPs) abundant in pathogens but absent in the host (i.e. LPS of Gram negative bacteria). Finally, some endogenous molecules from the host could activate PRR during cellular damage and stress acting as Danger Associated Molecular Patterns (DAMPs) (i.e. HMGB1 or alarmin).

Here we identified PRRs from the Toll-Like Receptor (TLR) family regulating Prolifferation and the SASP in OIS. We will provide some evidence of how TLR signaling activates the downstream inflammasome to establish a feed-back loop for the regulation of the SASP and the cell cycle arrest. Finally, we will discuss which specific DAMP for TLRs is activated during OIS.

Innate immune sensing of chromatin through cGAS regulates the SASP

A. Ablasser
Global Health Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Cellular senescence is a process triggered by a variety of distinct cellular damages or stresses and characterized by an irreversible cell-cycle arrest. Senescent cells are metabolically active and secrete various inflammatory factors collectively referred to as the senescence-associated secretory phenotype (SASP). Despite its relevance, the mechanism(s) underlying the regulation of cytokine production from senescent cells remains incompletely understood. Here we would like to discuss a role for innate immune DNA sensing in the regulation of paracrine senescence. We show that the intracellular DNA receptor cyclic GMP-AMP synthase (cGAS) recognizes chromatin from leaky nuclei in senescent cells. The activation of cGAS in turn triggers the production of soluble cytokines and chemokines, thereby controlling the secretion of the SASP.

Multiple distinct stimuli of cellular senescence engage cGAS signalling *in vitro* and we show a cGAS-dependent senescence response upon irradiation *in vivo*. Our findings provide novel insights into the molecular mechanisms underlying senescence by proposing the cGAS-STING signalling pathway as a crucial initiator of the cytokine response triggered in senescent cells.

Integrin beta3 regulates senescence by activating TGF-b in a paracrine and autocrine fashion

M. Borghesan[1], V. Rapisarda[1], V. Miguela[3], V. Encheva[2], P. Snijders[2], A. Lujambio[3], A. O'loghlen[1]
[1]Queen Mary University [2]The Francis Crick Institute, London, United Kingdom [3]Icahn School of Medicine at Mount Sinai, New York, United States

Cellular senescence is governed by intra- and extra-cellular signals and strongly depends on the interaction of the cells with (extracellular matrix) ECM ligands. We have previously established that dysfunction of the polycomb repressor component CBX7 is associated with the induction of senescence (O'Loghlen et al, *Aging Cell* 2014). To explore signalling mechanisms that may be related to this, we used a SILAC analysis to detect altered pathways in CBX7-knockdown (shRNA) primary human breast fibroblasts (BFs).

Altered proteins were highly related to ECM receptor-interacting and focal adhesion pathways in the KEGG database. Consistently, knockdown of CBX7 is associated with a strong increased expression of Integrin beta 3 or ITGB3, a hetero-dimeric transmembrane receptor forming focal adhesion complexes. Furthermore, chromatin immunoprecipitation analysis revealed that CBX7 binds to ITGB3 Transcriptional Start Site. Here we demonstrate the relevance of ITGB3 forming active focal adhesions in senescence activation using human primary cells overexpressing ITGB3, oncogenic RAS, etoposide DNA damage and Palbociclib therapy inducing senescence models.

A panel of small molecule inhibitors was employed to determine the pathway activation dependent upon ITGB3 ectopic expression. Among this panel the inhibitors targeting TGF-β-receptor 1 (TGFBR1 or ALK5), avb3/avb5 integrin (cilengitide), Rho-associated kinases 1/2 (ROCK1/2), and integrin-linked kinase (ILK) were capable of reversing the proliferation arrest induced by ITGB3 expression and the upregulation of p21CIP. Conditioned media (CM) from cells expressing ITGB3 induced the stabilization of p53 protein, the nuclear translocation of SMAD2/3 and a reduced proliferation rate in normal BFs. More important the use of cilengitide, an avb3 antagonist was able to uncouple the SASP release from the proliferation arrest in OIS.

All together, our results establish the integrin-β3 subunit as a marker and regulator of senescence by activation of the TGFB pathway and demonstrate the importance of cellular adhesion during senescence.

Uncoupling senescence-assocaited cell cycle exit from SASP production in oncogenesis

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Cellular senescence has been defined as a stable cell cycle exit triggered by different stress, including oncogene activation and telomere attrition. In addition to cell cycle exit, hallmarks of senescence include the secretion of a specific set of proteins collectively referred to as SASP (for Senescence-Associated Secretory Phenotype). As it limits the proliferation of damaged cells, senescence was first hypothesized to serve as a barrier against cancer progression.

We had previously demonstrated that the chromatin-associated Sin3B protein is required for oncogenic KRas-driven senescence. Sin3B is an essential component of the Sin3-HDAC co-repressor complex that is recruited by sequence specific transcription factors in order to repress the expresso of their target genes.

To directly investigate the contribution of senescence in cancer progression, we have generated a mouse model of pancreatic cancer, where Sin3B can be specifically inactivated in the pancreas. In stark contrast with the expected role of senescence as a barrier against cancer progression, our results indicate that Sin3B inactivation led to delayed pancreatic cancer progression, correlating with an impaired oncogene-associated inflammation.

Conversely, in a mouse model of prostate cancer, Sin3B inactivation promotes cancer progression. Together, these observations suggest that senescence can modulate cancer progression in a context dependent manner, and its effects on tumorigenesis likely extend beyond the control of cellular proliferation. Finally, we will present our approach to uncouple cell cycle exit from SASP production, in order to decipher the specific contribution of the SASP on cancer progression.

The senescence-associated secretory phenotype bridges tissue ischemia to pathological angiogenesis in retinopathy

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Pathological angiogenesis is the hallmark of diseases such as cancer and retinopathies. Although tissue hypoxia and inflammation are recognized as central drivers of vessel growth, relatively little is known about the process that bridges the two. In a mouse model of ischemic retinopathy, we found that hypoxic regions of the retina showed only modest rates of apoptosis despite severely compromised metabolic supply.

Using transcriptomic analysis and inducible loss-of-function genetics, we demonstrated that ischemic retinal cells instead engage the endoplasmic reticulum stress inositol-requiring enzyme 1a (IRE1a) pathway that, through its endoribonuclease activity, induces a state of senescence in which cells adopt a senescence-associated secretory phenotype (SASP).

We also detected SASP-associated cytokines (plasminogen activator inhibitor 1, interleukin-6, interleukin-8, and vascular endothelial growth factor) in the vitreous humor of patients suffering from proliferative diabetic retinopathy. Therapeutic inhibition of the SASP through intravitreal delivery of metformin or interference with effectors of senescence (semaphorin3A or IRE1a) in mice reduced destructive retinal neovascularization *in vivo*.

We conclude that the SASP contributes to pathological vessel growth, with ischemic retinal cells becoming prematurely senescent and secreting inflammatory cytokines that drive paracrine senescence, exacerbate destructive angiogenesis, and hinder reparative vascular regeneration. Reversal of this process may be therapeutically beneficial.

p53 in bronchial club cells facilitates chronic lung inflammation by promoting senescence

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In response to various forms of stress, p53 limits tumorigenesis by inducing apoptosis, cell cycle arrest and senescence. It also restrains inflammation to fulfill its tumor suppressor function in a non-cell autonomous manner. The role of p53 in regulation of chronic inflammation following repeated tissue damage is less understood. To understand the role of p53 in chronic lung inflammation we have constructed a mouse model where p53 is specifically knocked out in bronchial epithelia Club cells and subjected these mice to repetitive lipopolysaccharide (LPS) inhalations.

This chronic exposure resulted in severe bronchitis within weeks. Surprisingly, lungs deficient in p53 in their Club cells were significantly protected from the chronic inflammatory processes, as reflected in reduced accumulation of neutrophils, macrophages as well as CD3, CD4 and CD8 T cells, in both bronchio-alveolar space and lung interstitium.

LPS stimulated accumulation of bronchus associated lymphoid tissue (BALT)-like T cell and B cell aggregates adjacent to bronchioles also required Club cell p53 expression. Chronically inflamed bronchioles expressed senescence, but not apoptosis markers, which were significantly attenuated in Club cell specific p53 knockout mice. Moreover, pharmacological elimination of senescent cells with a Bcl2 family inhibitor, protected wild type mice from chronic LPS induced bronchitis and BALT generation nearby bronchioles.

We propose that p53 is necessary for the induction of cellular senescence in Club cells exposed to repeated LPS induced injury. Our data therefore provides the first positive link between p53 induced Club cell senescence and progression of airway inflammation.

Metabolic basis of cellular senescence

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Cellular senescence is a state of stable growth arrest. Two key hallmarks that define the role of senescence in human health are the stable senescence-associated growth arrest and the senescence -associated secretory phenotype. While the senescence-associated growth arrest is critical for tumor suppression, the role of the senescence-associated secretory phenotype in cancer is context-dependent. In addition, both of these phenotypic characteristics contribute to the aging process. Thus, senescence offers a unique interface to study interventions for both cancer and aging. As such, understanding the mechanistic basis for the senescence-associated growth arrest and secretory phenotype will aid in devising strategies to harness the tumor suppressive function of senescence while eradicating the tumor and aging promoting aspects of senescent cells. We will discuss our recent discoveries regarding the role of metabolic reprogramming in regulating these phenotypes.

Epigenetic stress response and stem cell aging

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Adult tissue stem cells contribute to the lifelong maintenance of 0rgan homeostasis and regeneration. However, the functionality of stem cells declines during aging and there is emerging evidence for the clonal dominance of mutant stem cells. Both processes contribute to the evolution of aging associated dysfunctions and diseases but molecular mechanisms that impair the function of stem cells during aging remain incompletely understood. Our recent work revealed that alterations in epigenetic stress responses lead to an aberrant activation of developmental pathways that impair the self renewal and regenerative capacity of muscle stem cells. Recent studies from the laboratory of David Scadden indicate that heterogeneity of epigenetic memory in hematopoietic stem cell is linked to the number of cell divisions and determines the heterogeneity in the functionality of HSCs during aging. Interestingly, the vast majority of gene mutations leading to clonal dominance of HSCs during aging affect epigenetic regulators. Together, these data indicate that alteration in epigenetic memory and stress responses represent driving forces for the decline of stem cell function and the selection of stem cell mutation during aging. During my talk I will present new data on physiological conditions and molecular mechanisms that may contribute to alterations of the epigenome and gene regulation in aging stem cells.

A fresh look at senescence associated persistent DNA breaks in mammalian cells and tissues

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The DNA-damage response (DDR) arrests cell-cycle progression until DNA lesions, like DNA double-strand breaks (DSBs), are repaired. The presence of DSBs in cells is usually detected by indirect techniques that rely on the accumulation of proteins at DSBs, as part of the DDR. Such detection may be biased, since some factors and their modifications may not reflect physical DNA damage. The dependency on DDR markers of DSB detection tools has left questions unanswered. In particular, it is known that senescent cells display persistent DDR (Fumagalli et al, 2012; Fumagalli et al, 2014), that we and others have proposed to be persistent DSBs, resistant to endogenous DNA repair activities. Others have proposed that these peculiar DDR foci might not be sites of damaged DNA per se but instead stable chromatin modifications, termed DNA-SCARS (Rodier et al, 2011).

We developed a method, named “DNA damage *in situ* ligation followed by Proximity Ligation Assay” (DI-PLA) for the detection and imaging of DSBs in cells (Galbiati et al, 2017). DI-PLA is based on the capture of free DNA ends in fixed cells *in situ*, by ligation to biotinylated double-stranded DNA oligonucleotides, which are next recognized by anti-biotin antibodies. Detection is enhanced by PLA with a partner DDR marker at the DSB. We validated DI-PLA by demonstrating its ability to detect DSBs induced by various genotoxic insults in cultured cells and tissues. Most importantly, by DI-PLA, we demonstrated that both senescent cells in culture and tissues from aged mammals retain true unrepaired DSBs associated with DDR markers.

We are further investigating the accumulation of DNA breaks, by DSB Labeling In Situ and Sequencing (BLISS), which allows genome-wide single nucleotide resolution of mapping DNA breaks by Next Generation Sequencing (Yan et al, in press).

Preliminary results show that damage-induced senescent cells accumulate DNA DSBs at telomeres, consistently with our previous conclusions (Fumagalli et al, 2012). Analysis of NGS data obtained by BLISS will allow us to understand whether there are any other regions, in the genome of senescent cells, that accumulate persistent DSBs.

Cellular senescence drives age-dependent hepatic steatosis

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Cellular senescence is a state of irreversible cell-cycle arrest characterised by a pro-inflammatory phenotype and mitochondrial dysfunction. It is a major contributor to age-related tissue degeneration. In this study, we found a close correlation between hepatic fat accumulation and markers of hepatocyte senescence in ageing mice exposed to different dietary interventions and in NAFLD patients. Furthermore, global gene expression analysis by RNA-seq revealed that senescent markers and fat accumulation correlated with aberrant expression of lipid metabolism and inflammatory genes.

Elimination of senescent cells by suicide gene-meditated ablation of p16Ink4a-expressing senescent cells in INK-ATTAC mice or combined treatment with senolytic-drugs dasatinib plus quercetin (D+Q) reduced overall hepatic steatosis. Conversely, inducing hepatocyte senescence caused fat accumulation in vitro and in vivo. Mechanistically, mitochondria in senescent cells lose the ability to metabolise fatty acids efficiently. Thus, our study demonstrates that cellular senescence drives hepatic steatosis and targeting senescent cells may offer a novel pharmacological strategy to reduce steatosis.

Telomere dysfunction-induced senescence and its impact on human health

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In somatic human cells telomeres can function as “replicometers”, capable of counting cell division cycles as they progressively erode with every round of DNA replication. Once they are critically short, telomeres become dysfunctional and consequently activate a proliferative arrest called replicative senescence (RS). It is becoming increasingly evident, however, that telomeres not only count cell divisions, but also function as sensors of genotoxic stresses in order to stop cell cycle progression prematurely and long before cells would have entered RS. This persistent proliferative arrest is triggered by dysfunctional telomeres that are not necessarily short and is therefore called telomere dysfunction-induced cellular senescence (TDIS). Although RS and TDIS are generally thought to reduce the fitness of an organism as they likely promote aging and the development of aging-associated diseases, more recent studies suggest that they evolved to benefit the organism. I will present and discuss new data on the damaging as well as on the beneficial aspects of telomere based senescence responses, including data that reveal an unexpected role for TDIS in wound healing in humans.

Investigating the role of neutrophil infiltrations in DNA-damage induced senescence

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Background and Objectives: Neutrophils have been shown to be key players in the recognition and elimination of pathogens, however, recent data has revealed that they may play other roles in disease, notably the development of cancer.

Senescence, the state of irreversible arrest observed in somatic cells is characterised by a Senescent Associated Secretory Phenotype (SASP) which includes pro-inflammatory cytokines, chemokines and extracellular matrix proteases. The SASP is believed to play a role in the recruitment and activation of immune cells, including macrophages, CD4 T and NK cells which have been shown to play a role in clearance of senescent cells. However, the relationship between neutrophil recruitment and senescence has not been completely investigated.

Results and Conclusions: We show that co-culture between young human fibroblasts and young neutrophils for 3 days leads to a significant reduction in the replicative lifespan of fibroblasts. Human fibroblasts pre-cultured with neutrophils experienced accelerated telomere shortening and increased expression of a variety of senescent markers. Pre-treatment with the enzyme catalase or ectopic overexpression of telomerase prevented the effects of neutrophils on senescence of human fibroblasts, suggesting a role for oxidative stress-mediated telomere shortening in the process. Consistent with a role for neutrophils in telomere dependent senescence, we found an association between neutrophil infiltrations and telomere dysfunction in ageing mice.

Furthermore, we showed that induction of liver injury induced by CCl4 which resulted in increased neutrophil infiltrations contributed to telomere dysfunction in hepatocytes. Consistent with a role for neutrophils in the process, inhibition of neutrophil recruitment using the neutralising antibody Ly6G or in mice lacking TLR2 prevented CCl4 induced telomere dysfunction. Our results suggest that neutrophils as a consequence of their role in the immune system may inadvertently induce senescence in young cells via oxidative stress-mediated telomere dysfunction.

Monoamine oxidases **as molecular players of cardiac senescence**

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Several scientific evidences have implicated senescence as an important component in the etiology of the age-dependent pathologies. With reference to cardiovascular diseases, it was well investigated that excess of reactive oxygen species (ROS) contribute to trigger and accelerate the cardiac senescence processes and a new role of monoamine oxidases, MAO A and MAO B, is emerging in this context. MAO-A expression substantially increases with ageing especially in heart, and its involvement in cardiac degenerative diseases is supposedly related to the formation of hydrogen peroxide produced during the degradation of its substrates norepinephrine and serotonin.

Using H9c2 cardiomyoblasts as a cellular model, here we show that chronic MAO-A activation mediated by physiological (Norepinephrine) and synthetic substrate (Tyramine), induces an increase in ROS levels leading to DNA damage response (DDR) activation accompanied with an irreversible cell cycle arrest (p53/p21/pRb pathway) as well as an increase in cell size and SA-β-gal typical of senescence.

To define the role of MAO–dependent H_2O_2 production in senescence activation, we designed a mutant MAO-A (K305M) specifically altered in its reactivity with O_2 as electron acceptor but not with monoamine substrates. Our findings show that K305M failed to induce H_2O_2 generation and consequently to activate senescence pathway compared to MAO-A WT, confirming the triggering role of H_2O_2 in activation of cellular senescence mediated by MAO-A activity. Mechanistically, we show that the activation of AKT-mTORC1 cascade induces dysfunctional mitochondrial accumulation, contributing to ROS-mediated stabilization of the DDR and cell cycle arrest.

Finally, we demonstrate that mTORC1 inhibition or mitophagy induction by Parkin overexpression decrease dysfunction at mitochondria level and prevent senescence, suggesting that mitochondria are central players in cellular senescence.

In conclusion, the present study is the first to show a possible link between MAO-A and premature senescence in cardiac cells. The work provides mechanistic insights into the role of MAO-dependent oxidative stress on development of age-related pathologies.

Redefining entry into telomere-mediated replicative senescence: uncapping or irreparable genome damage?

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Introduction: Replicative senescence is the permanent growth 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) growth arrest (SAGA). Since telomeres are heterogeneous in length within single cells, the number of short telomeres necessary for senescence onset remains poorly defined. Furthermore, accumulating evidences suggest that normal cells can tolerate a certain level of TU before entering SAGA, which directly lead us to hypothesize that TU in itself is not sufficient to trigger stable SAGA.

Methodology: We use controlled shelterin inactivation to trigger TU and subsequent SAGA in normal human fibroblasts and validate our observations during natural replicative senescence.

Results: We show that telomere dysfunction per se is not sufficient to trigger senescence. While continued TU generates stable DDR activation at telomeric ends, this overall weak DDR allows the rapid bypass of a primary growth arrest and thereby, a re-entry into the cell cycle.

The subsequent return into S phase and particularly 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 chromosomes bridges or micronuclei, which then sustain a definitive p53-mediated secondary SAGA. 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 SAGA display GI, while cells captured in mitosis with TU have not yet undergone telomere fusions explaining their continued, albeit slowed, proliferation.

Progresses for the field: 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 leads to permanent irreparable genome damages.

Frequent adaptation events in telomerase-negative cells drive genome instability in senescence

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Failure to maintain telomeres leads to their progressive erosion at each cell division. This heterogeneous process eventually triggers replicative senescence, a pathway associated with increased genome instability, which may contribute to the early steps of tumorigenesis. On the other hand, senescent cells activate the DNA damage checkpoint at telomeres, preventing the accumulation of unstable chromosomes. By analyzing lineages of single cells using a microfluidic approach, we found that another process, called adaptation, occurred frequently in telomerase-negative budding yeast cells.

Adaptation was defined in S. cerevisiae as the process that allows the cell to eventually complete mitosis despite the presence of an unrepaired DNA damage. This is achieved via a bypass of the DNA damage checkpoint activated state and leads to mitosis completion at the expense of genome instability. We previously showed that individual telomerase-negative lineages frequently displayed transient cell cycle arrests, followed by normal cell divisions, suggesting the involvement of adaptation in senescence. Here, we developed a fluorescent reporter assay to monitor checkpoint activation in single live cells. We found that telomerase-negative cells actively maintained the checkpoint factor Rad9 in a phosphorylated state while escaping the transient arrests, as predicted for adaptation events. Consistently, adaptation-deficient mutants (*cdc5-ad* and *tid*Δ) suppressed the transient arrests. Surprisingly, using fluctuation assays to assess genome instability, we found that *cdc5-ad* telomerase-negative cells showed a two-fold decrease in mutation rate compared to wild-type *CDC5* telomerase-negative cells specifically at senescence crisis.

Thus, not only does adaptation shape senescence dynamics and contribute to its heterogeneity by allowing cell survival after transient arrest, but we also demonstrate that it strongly promotes genome instability in the context of replicative senescence. Overall, adaptation could be an essential mechanistic link between ageing and cancer emergence.

Escaping cellular senescence by targeting telomerase expression in damaged cells: consequences for lung diseases

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A key regulator of cellular arrest in response to telomere shortening and DNA damage is the cyclin-dependent kinase inhibitor $p21^{Cdk1a}$ that interacts with the cyclin E/Cdk2 complex to block the G1/S transition.

P53-dependent upregulation of $p21^{Cdk1a}$ is thought to be the primary event inducing replicative senescence. We have created a knock-in mouse model in which a cassette encoding mCherry-2A-mTert (telomerase) has been inserted after the first exon of $p21^{Cdk1a}$. Our results indicate that upon conditions that induced $p21^{Cdk1a}$, Tert and mCherry are specifically produced and telomerase activity can be detected. The aim is to create a fine-tuned regulatory loop allowing expression of telomerase thus avoiding replicative senescence in cells in which telomeres are damaged. We have asked whether expression of telomerase driven by the $p21^{Cdk1a}$ promoter counteracts the development of lung alterations, namely lung emphysema and pulmonary hypertension in mice exposed to hypoxia.

Our results indicate that targeting telomerase expression through the $p21^{Cdk1a}$ promoter prevents emphysema in mice experiencing hypoxia. Our results suggest that telomerase is primarily a DNA repair enzyme that heals damaged telomeres experiencing replication stress.

Length-independent telomere damage drives cardiomyocyte senescence

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Cellular senescence, a process driven in part by telomere shortening, has been implicated in age-related cardiac dysfunction. However, the role of cellular senescence and its underlying mechanisms in slowly dividing/post-mitotic cardiomyocytes is not understood.

In this talk, I will show that during ageing, human and murine cardiomyocytes acquire a senescent-like phenotype characterised by persistent DNA damage at telomere regions which can be driven by mitochondrial dysfunction, and occurs independent of cell-division, telomere length and telomerase activity. Telomere damage in cardiomyocytes activates the classical senescence-inducing pathways, p21CIP and p16INK4a, but not the senescence-associated secretory phenotype (SASP).

Crucially, clearance of p16INK4a-positive senescent cells in mice alleviates myocardial hypertrophy, a detrimental feature of cardiac ageing. Our data demonstrates a key role for telomeres in senescence, ageing and disease of a largely post-mitotic tissue, the heart.

Profound nuclear and chromatin alterations in senescence and aging

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We are investigating chromatin alterations during senescence and aging, to identify potential points of intervention to prevent negative consequences, such as loss of regenerative capacity and tissue damage. In previous surprising findings, we discovered that autophagy, a well-known cytoplasmic process, initiates in the nucleus and degrades portions of the nuclear lamina and associated chromatin (Ivanov et al., 2013, *JCB*; Dou et al., 2015, *Nature*).

Here we show that the resulting cytoplasmic chromatin fragments (CCF) activate the innate cellular immunitycytosolic DNA sensing cGAS-STING pathway, triggering short-term beneficial inflammation to suppress activated oncogenes, but long-term tissue destructive inflammation. Hence, disruption of the CCF-cGAS-STING pathway reduces expression of the senescence-associated secretory phenotype (SASP) in primary human cells, and impairs immuno-surveillance that suppresses oncogenic Ras *in vivo*. However, the CCF-cGAS-STING pathway remains activated in cancers that escape senescence, correlating with cancer-associated inflammation. Also destructive, the CCF-cGAS-STING pathway contributes to aging-related chronic inflammation that is commonly associated with age-related diseases. Hence, mice deficient in STING show reduced hair loss and reduced inflammation in skin, liver, and intestine during physiological aging. Overall our findings indicate that genomic DNA serves as a reservoir to initiate cytoplasmic signaling that triggers inflammation. Targeting the cytoplasmic chromatin-mediated pathway in older individuals may hold promise in treating destructive age-associated phenotypes including inflammatory diseases such as cancer.

Single cell transcriptomics reveals heterogeneity in oncogene-induced senescence

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A key event in a healthy cell turning into a cancer cell is the activation of an oncogene. To prevent transforming to a cancer cell, the cell harbouring the oncogene activates a tumour suppressive programme, pushing itself into an irreversible growth arrest, called oncogene induced senescence (OIS). Everyone carries OIS cells, for example in the benign lesions (for example moles) that never progress to malignant cancer. Most of the time these lesions stably exist over decades, but sometimes individual cells escape and progress to cancer. What enables individual cells to turn malignant and how are they different from the cells around them? Here we present single cell transcriptomes of a time-course of human fibroblasts on their way to senescence after oncogene activation. Applying machine learning to order cells along a senescence trajectory, we find an unexpected bifurcation, leading to two distinct senescence endpoints. Each of these endpoints exclusively expresses sets of canonical senescence genes. Most importantly, one population failed to regulate key genes thought essential for the stability of the senescent state, leading to a scenario where the heterogeneity of the benign state might enable escape to malignancy.

Uncovering regulators of irreversible growth arrest during therapy induced senescence

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Senescence is the culmination of a dynamic process in which a number of phenotypes are acquired over time. The strictest definition of cellular senescence is irreversible cell cycle arrest and elaboration of a growth factor and cytokine secretion program known as the SASP.

Insight into how cells become irreversibly arrested is limited. We suspect that this is because of the different contexts in which cells become senescent, and the lack of cellular systems in which this phenotype can be temporally separated from the other phenotypes.

Understanding CDK4i therapy induced senescence (TIS) provides an opportunity to change this. CDK4 inhibition induces cell cycle exit, and, once arrested, some cells can progress into senescence. We previously showed that MDM2 degradation is the first molecular event during this transition. Stabilizing MDM2 can prevent cells treated with CDK4i from progressing into senescence. Thus, to create a system in which we could study the temporal nature of changes associated with acquisition of irreversible growth arrest, we generated cells in which FLAG-MDM2 was expressed from a tetracycline inducible promoter. Treatment with doxycycline and CDK4i induced cell cycle exit but prevented senescence; cells remained quiescent. After removing doxycycline the cells progressed synchronously into senescence. The number of SA-β-gal and SAHF positive cells increased abruptly from a minimum at four days to a maximum at five days. Irreversible arrest occurred at day 14. A SASP profile was first detected at day 21 and elaborated further by day 28. Thus, we separated the acquisition of early and late phenotypes from irreversible growth arrest.

Using transcriptional profiles generated at different times in this system we identified a senescence associated gene expression (SAGE) profile that is changed across the transition from reversible to irreversible growth arrest. We are currently in the process of manipulating these genes and regulatory networks to determine which are needed to establish irreversible growth arrest. Additionally, we are integrating this data with companion studies to analyze the epigenetic landscape in an effort to understand the transition between reversible and irreversible growth arrest, and metabolic profiling to understand the networks that underlie the transition from quiescence into senescence.

Cell-cell communication in senescence

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Cellular senescence is a highly stable state of cell cycle arrest induced by various pathophysiological stimuli. Senescent cells typically exhibit distinct morphological changes, and a number of biochemical and molecular markers of senescence have been described, which are typically associated with the effector mechanisms of senescence. Our group is interested in various aspects of such senescence effectors, and how they are related with each other. Senescence is recognised as an 'autonomous' tumour suppressor mechanism, and we speculate that the stable nature of the senescence arrest might be, in part, regulated through a fundamental re-organisation of the high-order chromatin structure. Senescent cells also have 'non-autonomous' activities that exhibit diverse effects upon neighbouring cells and the surrounding tissues through the senescence -associated secretory phenotype (SASP). In addition, we recently showed that NOTCH-mediated direct cell-cell contact plays a role in the non-autonomous functions of senescence: NOTCH high senescent cells can transmit their phenotype to neighbouring 'normal' cells. Strikingly, this cell-cell contact provides a profound impact on the chromatin structure in the neighbouring cells, suggesting that NOTCH signal contributes to 'non-autonomous epigenetic regulation'.

Age-associated alterations in haematopoietic stem cell heterochromatin

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Recent studies indicate that a characteristic feature of cellular senescence and aging is widescale remodeling of chromatin structure, and in particular a decline in the integrity of heterochromatin domains. These changes in heterochromatin integrity are believed to contribute to the aging phenotype in a number of ways, including by upregulating genomic retrotransposable elements, increasing genomic instability and perturbing gene expression patterns, including the induction of a pro-inflammatory signature. In this study we have investigated the contribution of heterochromatin structure to the functional changes observed with age in haematopoietic stem cells (HSC), which lead to impaired generation of antibody-producing B lymphocytes and contribute to reduced immune function in the elderly. We demonstrate that in both humans and mouse, HSC aging is associated with a global reduction in histone H3K9me3, a modification critical for heterochromatin formation, and relaxation of heterochromatin structure, as indicated by the transcriptional derepression of genomic repeats and endogenous retroviral elements.

Age-related changes in HSC heterochromatin is associated with decreased expression of the H3K9 methyltransferase SUV39H1 and upregulation of miR-125, a microRNA family which directly targets the SUV39H1 transcript in HSC. We show that overexpression of miR-125b and inhibition of SUV39H1 in HSC from young individuals reduces B lymphoid potential. Conversely, both inhibition of miR-125 and enforced expression of SUV39H1 improved the capacity of HSC from elderly individuals to generate B cells. Overall these findings in chronologically aging HSC add to a growing body of evidence in senescent cells suggesting that heterochromatin decline and the concomitant deregulation of genomic repeat elements is a common feature of the aging process.

Signatures of senescence progression in a transcriptomic landscape analysis across the mouse lifespan

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Ageing is characterised by deterioration of healthspan and increased mortality rate. The ageing process is affected by both genotype and environment. Dietary restriction has been shown to increase lifespan and healthspan across many organisms.

Here we present a landscape analysis of ageing under normal or 30% calorie restricted diets in C57BL/6 mice across multiple time-points spanning 3 months till 30 months of age. RNA was extracted from livers of 3 biological replicates per time point, DNase treated and stranded libraries were prepared and sequenced on an Illumina platform to obtain paired end reads of 100 bases, with an average of 27 million reads per time point replicate. The resulting data was analysed to interrogate changes in gene expression regulation at multiple levels: transcriptional, post-transcriptional RNA processing such as alternative splicing as well as long non-coding RNA.

We focus on illuminating the possible mechanisms that provide dietary restricted organisms with improved healthspan and lifespan. Our data shows strong correlations between age and diet related gene expression changes and senescence markers. We also report tight regulation across the lifespan for specific biological processes, at multiple levels of gene expression regulation, with specific time-points showing major changes potentially representing turning points.

Control of cell senescence by HuR and target noncoding RNAs

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Senescent cells accumulate in aging tissues, and their metabolic and gene expression profiles are linked to cancer and other age-associated pathologies. Our recent studies have focused on the RNA-binding protein HuR [1], a suppressor of senescence, and its interaction with different classes of noncoding RNAs – long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs). I discuss the ribonucleoprotein (RNP) complexes that HuR forms with several noncoding RNAs that influence protein abundance and modulate the senescent phenotype. HuR association with lncRNAs such as *LINCP21* suppresses the translation of select mRNAs [2], its interaction with lncRNA *HOTAIR* promotes the ubiquitin-mediated proteolysis of select proteins [3], and its association with *RMRP* enhances mitochondrial homeostasis [4], while its competition with *7SL* modulates *TP53* mRNA translation [5]. Likewise, HuR can bind microRNAs like let-7, which can function as tumor suppressors [6], modulating their availability to repress mRNAs bearing let-7 sites. Finally, our recent studies have shown that HuR can also bind circRNAs and these interactions reduce the expression of select proliferative proteins and reduces cell division [7]. In summary, HuR is well known for regulating target mRNAs encoding proteins that modulate many cellular functions, including carcinogenesis and senescence [1], but growing evidence indicates that HuR also regulates many target noncoding RNAs that govern protein expression patterns affecting growth arrest and cell senescence.

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A senescence-specific lncRNA modulates the pro-inflammatory response of senescent cells

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Senescence is a condition of permanent growth arrest caused by different potentially oncogenic insults. Beside the effect on cell proliferation, during senescence many pro-inflammatory factors, collectively known as senescence-associated secretory phenotype (SASP), are produced to activate the immune response and reinforce senescence in a paracrine and autocrine manner. While the role of many protein factors (as p16INK4a and p53) in senescence is well established, only recently the involvement of non-coding transcripts (ncRNAs) in this process is emerging.

In the last years, genome-wide analysis demonstrated that the eukaryotic genome is pervasively transcribed and that the majority of the transcripts do not encode proteins. Among them, long non-coding RNAs (lncRNAs) refer to non-coding transcripts generally longer than 200 nucleotides with a crucial role in several biological processes such as proliferation and differentiation whereas their involvement in senescence remains poorly investigated.

To functionally characterize novel lncRNAs able to modulate cellular senescence, we took advantage of an in vitro cellular system resembling senescence response through the activation of H-RAS, a process called oncogene-induced senescence (OIS). We evaluated the expression profile of primary human fibroblasts during OIS and we identified a robust set of 30 lncRNAs significantly induced in senescence. Among them, we found that a cytoplasmic lncRNA, thereafter named senescence-induced lncRNA (sin-lncRNA), was upregulated not only in OIS but also in irradiation-induced and replicative senescence. Sin-lncRNA expression is highly specific of senescent cells and it's driven by one of the master regulators of senescence, C/EBPβ, that directly activates sin-lncRNA transcription. Interestingly, despite its strong induction in senescence, sin-lncRNA functions as negative regulator of senescence response since its depletion increases growth arrest and promotes senescence features without affecting apoptosis. Genome-wide analysis of senescent cells depleted of sin-lncRNA revealed that the absence of this transcript increases the expression of several genes involved in matrix remodelling and recruitment of immune cells, suggesting that sin-lncRNA may have a role in controlling the pro-inflammatory response in senescence.

Further studies will be carried out to elucidate the molecular mechanisms by which sin-lncRNA exerts its function, in order to provide new insights on the regulatory network linking lncRNAs and senescence response.

The nuclear receptor RXRA controls cellular senescence by regulating calcium signaling

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Cellular senescence is a stable cell proliferation arrest that plays a fundamental role in contexts such as embryonic development, wound healing, tumor suppression and aging. However, the molecular and cellular mechanisms controlling senescence are still incompletely understood.

We recently identified the inositol 1,4,5-trisphosphate receptor type 2 (ITPR2) as a senescence regulator in a loss-of-function genetic screen. In response to senescence-inducing stresses such as oncogene activation and telomere shortening, calcium is released from the endoplasmic reticulum (ER) through ITPR2 and accumulates in the mitochondria through the mitochondrial calcium uniporter (MCU). This leads to a decrease in mitochondrial membrane potential and a subsequent accumulation of reactive oxygen species which triggers senescence. ITPR2 expression is reduced in a number of tumors but how its expression is regulated is barely known. To gain insights into the regulation of ITPR2 expression, we performed a siRNA screen targeting 195 transcription factors and epigenetic regulators. The retinoid X receptor alpha (RXRA), which belongs to the nuclear receptor family, was thereby identified as a transcriptional repressor of ITPR2. We observed that knockdown of RXRA triggers cellular senescence in primary human fibroblasts in an ITPR2- and MCU-dependent manner. Knockdown of RXRA and increased calcium signaling through the ITPR2-MCU axis induces DNA damage and ultimately senescence by activating p53. Altogether our work identifies RXRA as a senescence regulator and sheds light on a new mechanism controlling calcium signaling and cellular senescence.

A dynamic transcription factor (TF) network controls oncogene-induced senescence

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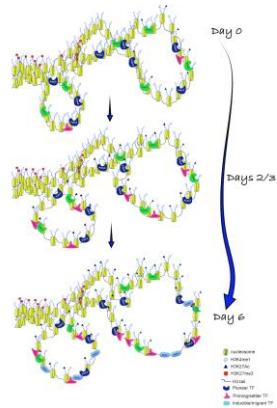
Oncogene-induced senescence (OIS) is a complex cell fate transition that blunts proliferation caused by oncogenic stress while preserving some level of cellular function. Despite its demonstrated biological and clinical significance, the underlying (epi)genomic mechanisms driving this cell fate transition remain largely unknown. To address this issue, we applied a dynamic approach using high throughput sequencing and Affymetrix gene expression microarrays coupled to sophisticated computational analyses to identify and integrate histone modifications (ChIP-seq), chromatin accessibility (ATAC-seq) and transcriptome changes during RAS-induced senescence in primary fibroblasts. This approach revealed a pre-established genomic configuration composed of near-mutually exclusive 'silent' (H3K27me3) and 'active' (H3K4me1/3, H3K27Ac, ATAC) megabase-sized chromatin domains that appear inert to RAS signalling. In contrast, highly dynamic yet ordered behaviours for chromatin accessibility and active histone marks are observed at the kilobase range, which are mirrored by a dynamic transcriptional output. Dynamic footprinting analyses of ATAC and active histone modification profiles identified a hierarchical transcription factor (TF) network controlling the OIS gene expression program. We identified at least three tiers for TFs which act in concert: i) top tier pioneer TFs which shape the fibroblast genome via pervasive binding on chromatin, ii) second tier local pioneers which act in concert with top tier pioneers to open specific chromatin regions in response to RAS signalling and iii) inducible TFs which drive RAS-specific gene expression by binding to this previously opened DNA. Collectively, our approach unravelled a modular yet plastic TF network that selectively responds to the RAS oncogenic stress by modulating the access to relevant genomic regulatory regions controlling the OIS-specific transcriptome, and identified novel candidates for subsequent perturbation studies.



HMGB-family members topologically bookmark the genome and control senescence-induced three-dimensional reorganization via CTCF

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Primary human cells eventually exhaust their replication potential and, thus, enter senescence. This is a physiological mechanism common to cells of different developmental origins and is linked to such processes as wound healing, tumorigenesis constraint, or age-associated disruption of homeostasis. We sought to understand whether a common molecular backbone regulates entry into senescence. We used three human primary cell types – umbilical vein endothelial cells (HUVECs), fetal lung fibroblasts (IMR90s), and mesenchymal stromal cells (MSCs) – and hypothesized that this backbone will be functionally linked to changes in the three-dimensional organization of the genome; hence, we performed RNA sequencing (RNA-seq) and whole-genome chromosome conformation capture (Hi-C) in cells from multiple single donors, and complement these with single-cell transcriptomics using a drop-seq based platform. Integrative data analyses showed that a group of chromatin conformation regulators are markedly suppressed across the three cell types, and that most changes in genome conformation occur at the Mbp-level to involve rearrangement of topologically-associating domains (TADs). We focused on proteins of the HMGB family, HMGB1 and HMGB2, as these abundant nuclear factors are known to be involved in gene regulation and DNA bending, yet they become extruded from cells upon entry into senescence. We reasoned that this can explain the observed changes in TAD organization and gene expression, and combined knock-down and ChIP-seq experiments to show that each HMGB factor takes up a specific role by either binding gene promoters involved in cell cycle regulation, or by bookmarking TAD boundaries and causing formation of prominent CTCF foci upon depletion. Then, our work offers novel insight into senescence-entry control via chromatin reorganization.

Systems biology: from understanding towards controlling of complex biological networks

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Most biological processes are governed by physical and chemical interactions between molecules. Cells handle environmental changes through various signal transduction pathways, which forms signaling network. In addition, proteins are expressed from genes, and genes are regulated by proteins such as transcription factors. The relationship between proteins and genes can therefore be abstracted as a gene regulatory network.

Components (nodes) of these networks are activated/inactivated or increased/decreased in amount according to their interactions (links) over time; this is called network dynamics. To trace the dynamics of a network, we can define a network state as a tuple of values of network components at a specific time point. When there is no change in the input signal of the network system, the network state will follow the inherent network dynamics determined by interactions between network components. Eventually, the network state without any input signal will converge to a steady state called an attractor state while the network state with sustained input signals will converge to another steady state called a pseudo-attractor state. The trajectories from all the initial states to attractor states form an attractor landscape. Recent studies have shown that the attractor states correspond to the cellular phenotypes in response to external stimuli. A molecular regulatory network uniquely determines the corresponding attractor landscape, and thus the attractor landscape can be reshaped by network rewiring in our framework. Network rewiring occurs by perturbations that change the network dynamics or transform the network topology through induction of constant changes of node activity or link connections. Different biological processes including differentiation, tumorigenesis, and cellular aging can be represented by reshaping of the attractor landscape. In this talk, I will introduce some case studies about complex molecular interaction networks to discuss how the emergent properties of cellular functions can be induced by complicated interaction of numerous molecules and how we can understand and control the dynamical cellular functions by perturbing some target molecules in the network.

Senescence induced plasticity in regeneration and cancer

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Senescence is a form of cell cycle arrest that was first discovered to be induced by stress such as aging, DNA-damage and oncogenic signaling. Moreover, even while arrested, senescent cells interact with their microenvironment through the secretion of a variety of proteins collectively known as the senescence-associated secretory phenotype (SASP). Functions for the SASP in reinforcing cell-cycle arrest and promoting immune cell-mediated clearance supported tumor suppressive functions. However, beneficial physiological roles for senescence and the SASP were found in tissue growth and repair, such as during embryonic development or wound healing. This suggests more complex biological roles than currently understood. Recently, we uncovered a primary function of the SASP in promoting cellular plasticity and tissue regeneration. Surprisingly, upon transient exposure to the SASP, primary mouse keratinocytes exhibited increased expression of stem cell markers and displayed enhanced regenerative properties when transplanted *in vivo*. This finding further supports how cellular senescence primarily acts as a beneficial and regenerative biological process, and that only when misregulated, does it have pathological consequences in cancer and aging. Here I will discuss our ongoing studies investigating the biological basis for senescence and the SASP, and how genetic perturbations can have detrimental effects in cancer and aging.

Rejuvenation of senescent fibroblast morphology, proteome and replicative lifepsan by modulating mTOR and cytoskeletal signalling

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Cellular senescence, a programme of cell proliferation arrest, occurs in response to various stressors including telomere attrition, other types of DNA damage, oncogene induction, ER stress and oxidative stress. Senescent cells contribute to organismal ageing, through inability to repair dysfunctional tissues and through secretion of the pro-inflammatory SASP. We have recently demonstrated reversal of senescent cell phenotypes on treatment of primary human cells with dual mTOR inhibitors, which is notable as mTOR inhibition has been shown to increase mouse lifespan and healthspan and reduce the SASP; we propose that mTOR inhibition impacts particularly on signalling pathways that drive senescence and the SASP. We have now shown that pulsed dosing using pan-mTOR inhibitors allows cells to undergo multiple cycles of senescence and 'reversal', strongly countering the view that senescence is irreversible. Both pulsed dosing and chronic long term treatment with the mTOR inhibitor result in greatly extended replicative lifespan of skin fibroblasts. To investigate the molecular basis of senescence and the impact of mTOR inhibition, we have conducted quantitative comparative proteomics analysis of proliferating and senescent cells treated with various mTOR inhibitors. Our novel results demonstrate rejuvenation of the senescent cell proteome, particularly with regard to proteins important in gene expression control, morphology and cellular architecture. We are starting to investigate these pathways more fully using both pharmacological agents and CRISPR.

Heat shock induces cellular senescence and SASP in human skin fibroblasts

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Burn wounds are divided into three zones. The first cell layer usually goes into immediate necrosis after heat exposure while the underlying cells manage to survive but are severely damaged. In this „zone of stasis", most cells are alive but under immense stress. This often leads to the phenomenon called burn wound progression: In the days following the initial incident the zone of necrosis will spread and affect more and more tissue. Unfortunately the deeper the burn wound the lower are chances of recovery while chances of delayed wound healing, hypertrophic scarring, infection and shock increase simultaneously. Thus the survival of the cells of the zone of stasis and the inhibition of burn wound progression is one of the main goals when treating burn injuries.

We hypothesize that some cells in the "zone of stasis" undergo cellular senescence. Senescence is caused by many stresses some of which are closely associated with the damage caused by heat injuries. Beside the initial heat stress, low pH, lack of oxygen and nutrients and the production of ROS are hallmarks of thermal injuries. A major problem of burn wound treatment is an overshooting immune response, which could be further enhanced by a senescence induced SASP, thus intensifying burn wound progression.

In order to mimic the "zone of stasis" we established a cell culture based model. We use human fibroblasts and apply a 10 minute heat shock protocol that ensures that while the cells are severely damaged they do not undergo apoptosis. To assess senescence we looked at b-galactosidase, p16 and IL-6 levels and currently we extend the analysis to other senescence markers. Depending on the temperature we found large numbers of senescent cells indicating that senescence is involved in burn wound progression.

Injury-induced senescence enables in vivo reprogramming in skeletal muscle

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In vivo reprogramming is a promising approach for tissue regeneration in response to injury. Several examples of in vivo reprogramming have been reported in a variety of lineages, but some including skeletal muscle have so far proven refractory. Here, we show that acute and chronic injury enables transcription-factor-mediated reprogramming in skeletal muscle. Lineage tracing indicates that this response frequently originates from Pax7+ muscle stem cells. Injury is associated with accumulation of senescent cells, and advanced aging or local irradiation further enhanced in vivo reprogramming, while selective elimination of senescent cells reduced reprogramming efficiency.

The effect of senescence appears to be, at least in part, due to the release of interleukin 6 (IL-6), suggesting a potential link with the senescence-associated secretory phenotype. Collectively, our findings highlight a beneficial paracrine effect of injury-induced senescence on cellular plasticity, which will be important for devising strategies for reprogramming-based tissue repair.

Cardiomyocyte-cardiac fibroblast directed crosstalk induces myofibroblast senescence to control fibrogenesis

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Introduction: An important feature and prognostic hallmark of cardiovascular disease is cardiac fibrosis, marked by an increased formation of connective tissue that finally leads to deteriorated organ structure and function (Leask, 2015). Importantly, efficient therapies for the treatment of cardiac fibrosis are not available (Gourdie et al., 2016). Our previous findings uncovered a yet unrecognized role of myofibroblast senescence in restraining cardiac fibrosis and established the cellular senescence program as a novel anti-fibrotic mechanism and potential therapeutic target in the heart (Meyer et al., 2016). However, the triggers and mechanisms that induce and maintain cardiac myofibroblast senescence have not yet been investigated.

Objectives: To investigate if cardiomyocyte (CM) damage, particularly hypertrophy induces myofibroblast (CF) senescence, and whether senescent CF propagate senescence within the CF population in order to control fibrogenesis.

Materials & methods: Co-culture and conditioned medium based mono-culture of primary CM and CF were employed to study CM-CF directed crosstalk and senescence induction under physiological and pathophysiological conditions. CM were either untreated or stimulated with the hypertrophy and CM damage inducing agents phenylephrine (PE), isoproterenol (Iso) or angiotensin II (AngII).
Senescence was quantified by histochemical/immunofluorescence staining of senescence-associated beta-galactosidase (SA-ß-GAL), $p21^{CIP1/}$WAF1, HP1ß as well as the proliferation marker Ki67. The fibrotic potential of CF was determined by qPCR and Western Blot based collagen and alpha-smooth-muscle actin expression analysis examining the underlying changes in mRNA and protein expression of proliferative profibrotic synthetic CF versus senescent antifibrotic CF.

Results: We observed an initial CF hyperproliferation phase that was followed by a pronounced decline of proliferating CF co-cultured to damaged (hypertrophied) CM when compared to the mock treated condition (4-6% vs. 17%, respectively). In line with an advanced CM hypertrophy and thus, CM damage, robust CF senescence was noticed. SA-ß-gal was increased 2-2.5-fold and 13-20% of CF were positive for the senescence marker $p21^{CIP1/WAF1}$ (mock: 5%). Similar results were obtained with the conditioned medium based mono-culture system indicating an intercellular and paracrine mediated CM-CF directed crosstalk leading to (primary) myofibroblast senescence in a time-dependent fashion. Mechanistically, the matricellular and senescence-inducing protein CCN1 was identified as one of the key regulators of the CM-CF directed communication. Moreover, fibrotic genes such as Col1a2, Col3a1 and fibronection were downregulated in late-stage senescent myofibroblasts wheras no changes could be observed in the proliferating CF fraction. In addition, CF senescence was robustly inducing (secondary) cellular senescence of CF in a paracrine fashion. Conditioned medium of hypertrophy-induced senescent CF led to a 50% increase in CF SA-ß-gal when compared to CF treated with conditioned medium of non-senescent CF. In alignment with this, there was a 1.5-3-fold increase of the senescence markers $p21^{CIP1/WAF1}$ and HP1ß.

Conclusion: Our results provide first evidence for a critical role of CM damage, particularly hypertrophy, to induce myofibroblast senescence by CM-CF directed communication via CCN1 in a time-resolved fashion. Moreover, senescent CF have the potential to propagate the senescence program within the CF population.

The interplay between senescence and regeneration: insights from the salamander

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Salamanders are the only vertebrates capable of regenerating complex structures such as limbs in the adult stage. Limb loss triggers the generation of a mass of cells that dedifferentiate and re-enter the cell cycle to proliferate, named a blastema, and the set up of a morphogenetic programme which leads to the restoration of the missing structures. Remarkably, salamanders are able to stage numerous rounds of regeneration throughout their lives without losing their regenerative capabilities, suggesting that regeneration is a process unaffected by aging. In addition, cells derived from the blastema exhibit an indefinite lifespan in culture, without ever undergoing replicative senescence. This raises the possibility that mechanisms for the regulation of senescence may operate in salamanders. Indeed, our research has shown that salamanders have a highly efficient immune-dependent mechanism to clear senescent cells. Paradoxically though, our work also suggests that senescent cells have important functions during regeneration of complex structures.

In this talk, I will discuss our recent evidence supporting a requirement for transient senescent cells during limb regeneration, as well as insights into how replicative senescence is controlled in these remarkable organisms.

Identification of novel mechanisms of immune surveillance of senescent cells

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Cellular senescence, a stable irreversible cell-cycle arrest, prevents propagation of damaged cells in the organism. Immune system recognizes and eliminates senescent cells to facilitate their removal from tissues. In order to unravel the molecular mechanisms behind this process we evaluated the cell surface proteome specific to human senescent fibroblasts. We identified nine proteins that were expressed preferentially on the surface of senescent cells. One of these proteins is Grp94, an ER chaperone which can translocate to cell surface, and acts there as a potent regulator of the immune response. We validated the presence of the full-lenght Grp94 protein on the surface of senescent cells and we show that Grp94 is co-localized with senescence markers in sites of pathologies.

Importantly,cell-surface Grp94 is being accumulated in senescent cells in a time-dependent manner. This accumulation is inhibited by GPM1, a small-molecule which specifically promotes Grp94 dimerization and retention in the ER. To evaluate the functional contribution of cell surface Grp94 we studied its role in the NK cell-mediated elimination of senescent cells. We have previously shown that NK cells specifically recognize and eliminate senescent cells *in vitro* and *in vivo*. Down-regulation of cell-surface Grp94 decreases NK cell-mediated cytotoxicity toward senescent cells. In liver fibrosis, inhibition of cell-surface Grp94 by administration of GPM1 blocked immune clearance of senescent cells, halted tissue regeneration and led to increased fibrosis. Understanding the functional role of cell-surface Grp94 will enable harnessing the immune system to eliminate senescent cells in different pathological conditions.

IR-induced senescence of the splenic microenvironment impacts murine immune cells functions

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Senescent cells accumulate in various tissues over time and following exposure to genotoxic stresses such as ionizing radiation (IR). Their accumulation, in part through their specific secretory phenotype, was shown to contribute to the aging phenotype and to the development of cancer. Expression of the p16INK4a gene, a cyclin dependent kinase inhibitor, is a marker and inducer of aging/senescence.

We previously showed that IR-induced p16INK4a expression alters lymphopoiesis, a hallmark of aging (Carbonneau et al. Blood 2012). Whether the accumulation of p16INK4a senescent cells also interferes with immune cell functions is unknown. We here provide evidences that IR-induced p16INK4a expression negatively impacts immune cell functions in the spleen. In particular, we found F4/80+ macrophages isolated from irradiated mice to express high levels of p16INK4a and to have a reduced phagocytosis activity. We also identify a defect in the capacity of isolated T cells to proliferate in response to allogenic stimuli, an effect that was dependent on the splenic microenvironment. Interestingly, elimination of p16INK4a expressing cells, using the 3MR-p16 transgenic mouse model, allowed for the restoration of T cell proliferation in vitro. We are currently evaluating these effects in vivo in response to viral pathogens. Our data suggests that it may be possible to pharmacologically enhance immune cell function during aging and in cancer treated patients.

Ionizing radiation-induced endothelial senescence and role in normal tissue injury

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Radiotherapy is the main locoregional modality in cancer treatment. However, its major limitation is the radiation damages to neighboring healthy tissues. The endothelial compartment appears to play an important role in the initiation, progression and maintenance of radiation-induced normal tissue injury. Under the effect of ionizing radiation, the endothelial cells (ECs) acquire a senescent phenotype. Cellular senescence is a powerful tumor suppressor mechanism but, paradoxically, long-term senescence can have contradictory effects on tissue damages and wound healing process. Despite the fact that recent studies have shown the existence of senescent cells within the radiation-induced lesions, their role has never been demonstrated. In this study, we aim to identify the role of senescent ECs in radiation-induced pathophysiological process. After an in vitro comparative analysis on 44 genes involved in senescence phenotype of 6 human primary endothelial cell types after high dose irradiation, it appears that HUVEC (Human Umbilical Vein Endothelial Cell) cell type is the most relevant in term of radiation-induced gene expression. Senescence was followed using C12FDG labelling and flow cytometry. The dynamic molecular profile (44 genes including p21 and p16) associated with the radiation-induced senescent EC phenotype was analyzed after 9 doses of radiation exposure and 7 time points. By a combination of statistical and mathematical methods adapted for temporal expression, we decipher the dynamical transcriptional program involved in radiation-induced endothelial senescence. In vivo, using luciferase knock-in mice (p16LUC) which reports the expression of p16INK4a, we show the overexpression of p16INK4a after a high-dose pulmonary 3x3 mm² stereotactic irradiation using bioluminescence imaging. By a combination of double immunolabeling of different pulmonary cell types associated with p21, p16INK4a expression and SA-b-Gal staining, we will determine senescent cell types after a lung irradiation. In parallel, with an endothelium specific knockout for PAI-1 (VeCADCre-PAI-1Flox/Flox), an important player in senescence phenotype and mainly express in the endothelium, we aim to elucidate the implication of the endothelial PAI-1 pool in the progression of radiation-induced senescence and consequently its contribution in lung injury.

Novel IL6 family member is an inducer of quiescence that augments muscle stem cell engraftment and regeneration

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Knowledge of the regulators that maintain a quiescent state is lacking, yet such cues are crucial to maintaining a muscle stem cell reservoir that can meet the needs of regeneration throughout life. Regeneration of skeletal muscle is dependent on the function of tissue-resident muscle stem cells (MuSC), known as satellite cells. MuSC dysfunction is central to muscle pathophysiology, including in age-associated loss of muscle regenerative capacity and congenital disorders such as Duchenne muscular dystrophy. Despite the central role of satellite cells in muscle regeneration, the signals controlling the balance between muscle stem cell quiescence, proliferation, and differentiation remain incompletely understood. To discover regulators of quiescence we developed a novel *in vivo* imaging-based screening strategy allowing identification of proteins that do not induce *in vitro* proliferation, but instead maintain MuSCs in a non-mitotic state, poised for rapid and robust expansion upon transplantation. We demonstrate that a member of the IL6 family of cytokines induces reversible exit from the cell cycle and induction of a global transcriptional program significantly enriched within a newly established satellite cell “quiescence signature”. Genetic ablation of the receptor for this IL6 family member in mice demonstrates that signaling via this Gp130 receptor family member is essential for maintenance of satellite cell quiescence, and for proper skeletal muscle regeneration *in vivo*. Given that aberrant activation and exhaustion of stem cells is feature of aging, this IL6 cytokine family member constitutes an attractive therapeutic target in muscle disease states.

Accumulation of senescent cells - mechanisms and consequences

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Senescent cells are present in pre-malignant lesions, tumors following therapy and sites of tissue damage. At these places cellular senescence limits tumorigenesis in response to activated oncogenes and DNA damage. However, when senescent cells accumulate in tissues they can contribute to pathology of age-related diseases. The accumulation of senescent cells in tissues can result from the resistance of these cells to pro-apoptotic stimuli. Molecular mechanisms underlying this resistance are not well understood. We show that senescent cells from both human and mouse origin upregulate the anti-apoptotic proteins members of Bcl-2 family. Joint knockdown or inhibition by a small molecule of these proteins induced selective apoptosis of senescent cells. Notably, treatment of mice with the small molecule efficiently eliminated senescent cells from sites of tissue damage and might led to subsequent entrance of tissue stem cells into cell cycle. When senescent cells gradually accumulate in tissues with age they promote a chronic “sterile” inflammation via secreted pro-inflammatory and matrix-remodeling factors. These factors lead to immune-cell recruitment and senescent-cell clearance. The extent of immune-system involvement in regulating age-related accumulation of senescent cells, and its consequences, are unknown. We show that mice with impaired immune surveillance of senescent cells exhibit both higher senescent-cell tissue burden and chronic inflammation. Consequently, they exhibit multiple pathologies and significantly lower survival than control animals. Similarly, impaired immune surveillance in progeroid mice is associated with accumulation of senescent cells and shorter lifespan.

Our finding that pharmacological elimination of senescent cells increases survival in mice sheds new light on mechanisms governing senescent-cell presence in aging.

The ZEB1 EMT-inducing transcription factor establishes a link between the escape from oncogene-induced senescence and the genetic history of breast tumorigenesis

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The plasticity of cancer cells underlies their capacity to adapt to the selective pressures they encounter during tumor development. Aberrant reactivation of epithelial-mesenchymal transition (EMT), a normally latent embryonic transdifferentiation program, promotes cancer cell plasticity and fuels tumor development and metastatic spread. Consistent with a prominent role in tumorigenesis, we have shown that EMT-inducing transcription factors of the TWIST and ZEB families act as genuine oncoproteins,fostering cell transformation and primary tumor growth by preventing oncogene -induced senescence and apoptosis. Recently, we have further demonstrated that EMT inducers are expressed in normal mammary stem cells and that their expression influences the entire natural history of breast tumorigenesis.

In contrast with differentiated cells, human mammary stem cells have the innate capacity to withstand an aberrant mitogenic activation. This property is based upon an antioxidant program driven by the ZEB1 EMT inducer and the methionine sulfoxide reductase MSRB3. This pre-emptive program prevents the formation of oncogene-induced DNA damage, a major cause of genomic instability, and influences the emergence of cancer-associated events. Overall, our data suggest that malignant transformation of mammary stem cells does not hinge on genomic instability and indicate that intrinsic properties of the cell-of-origin dictates the genomic landscape of breast cancers.

Mitotic-slippage induced senescence confers a pro-tumourigenic phenotype

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Antimitotic drugs are often administered as first-line chemotherapy in several malignancies. However, their clinical success is often limited by acquired chemoresistance and disease relapse. The anti-proliferative basis of these drugs is induction of mitotic arrest culminating in cell death. Mitotic slippage occurs when cells exit mitosis and “slip” into interphase without chromosome segregation and cytokinesis. Little is known about how cells post-slippage influence outcome of treatment.

Here, we demonstrate that post-slippage cells exhibit paracrine pro-tumourigenic potential following senescence and senescence-associated secretory phenotype development. This occurs in an autophagy-p53 axis-dependent manner, with autophagic inhibition in post-slippage cells bypassing senescence and leading to cell death. Indeed, the autophagy inhibitor Chloroquine and microtubule poisons synergistically inhibited tumour growth in mice. Hence, regimens bypassing/targeting the senescence phenotype could provide a potential effective combinatorial strategy with antimitotic drugs. Sensitivity to this combinatorial treatment was dependent on p53 status, an important factor to consider before treatment.

Senescence and immunotherapy in cancer mediated by Stat3 blockade

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Constitutive activation of Stat3 in diverse cancers is associated with tumor progression and immune evasion. We described in murine breast cancer (BC) models that Stat3 inhibition induces senescence and that immunization of mice with Stat3-blocked BC cells induces an antitumoral immune response that involves $CD4^+$Th and NK cells.

Here, we studied the mechanism of senescence induced by Stat3 inactivation and the use of the supernatant (SN) from Stat3-blocked cells to formulate an immunotherapy (IT).

Knockdown of Stat3 with siRNA increased SA-β-gal staining in triple negative (4T1, MDA-MB-231 and MDA-MB-468 cells) and ErbB2 positive (C4HD, JIMT-1 and KPL-4 cells) BC models, in colon cancer and melanoma. However, in cells that have low levels of Stat3 activation (BT-474, T47D and MCA101), the inhibition of Stat3 did not produce changes in this marker. In senescent cells, we observed an increase in trimethylation of histone H3 at Lys9 and in cell cycle inhibitors expression ($p16^{INK4a}$ (p16) or $p21^{CIP1}$). Interestingly, Stat3 inhibition *in vivo* increased SA-β-gal staining and p16 expression in 4T1 tumor.

Then, we embedded the SN of C4HD or 4T1 cells transfected with Control siRNA (SN-Control), Stat3 siRNA -senescent cells- (SN-Stat3) or the combination of Stat3 and p16 siRNAs -non senescent cells- (SN-Stat3+p16) in a slow delivery pellet. The IT protocol was to implant s.c. these pellets together with an injection of irradiated wild-type tumor cells. Prophylactic IT with SN-Stat3 and SN-Stat3+p16 using C4HD tumor model, decreased tumor growth (72% and 51%, respectively vs. SN-Control). Therapeutic IT with SN-Stat3 and SN-Stat3+p16 in mice bearing 4T1 tumor decreased tumor growth (51% and 41%, respectively vs. SN-Control) and pulmonary metastasis (70% and 50%, respectively vs. SN-Control).

In both IT protocols the antitumor effect was associated with greater activation and cytotoxic activity of NK cells and an increase in memory $CD4^+$-T cells vs. SN-Control. We observed that SN-Stat3 and SN-Stat3+p16 inhibited proliferation of 4T1 cells but increased the proliferation of T lymphocytes and the number of IFNγ producing $CD4^+$-T cells *in vitro*. These results suggest that Stat3 blockade induces senescence in tumor cells with high activation of Stat3 and the SN-Stat3 is an effective adjuvant for IT.

The transcription factor Spi1/PU.1 limits proliferation by inducing cellular senescence during hematopoiesis, a process that is lost during early steps of leukemic transformation

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The involvement of aberrant transcription factor activity in human acute myeloid leukemia (AML) has been largely reported. However, the protective effect of oncogene-induced senescence (OIS) against AML apparition has been poorly studied. The Spi1/PU.1 transcription factor (TF) is a key regulator of many steps of hematopoiesis, and limits self-renewal of hematopoietic stem cells. The deregulation of its expression or activity contributes to AML, in which Spi1 can be either an oncogene or a tumor suppressor. Here, we explored whether OIS is a mechanism by which the Spi1 limits hematopoietic cells expansion, and thus prevents the development of AML. We show that Spi1 overexpression triggers cellular senescence in primary fibroblasts and primary hematopoietic stem and progenitor cells.

Cellular senescence and hematopoietic differentiation are two independent processes triggered by Spi1 and several hematopoietic lineages are prone to Spi1 induced-OIS. In hematopoietic cells, Spi1-induced senescence requires a functional p38MAPK14 pathway but is independent of a DNA-damage response. In contrast, in fibroblasts, Spi1-induced senescence is triggered by a DNA-damage response. Importantly, using our well-established Spi1 transgenic leukemia mouse model, we demonstrate that Spi1 overexpression also induces senescence in the bone marrow *in vivo* before the onset of the pre-leukemic phase of the disease. Remarkably, progression of the disease was concomitant to the loss in the senescence process. Overall, our results demonstrated that senescence is an important safeguard against the development of AML induced by aberrant expression of TF.

Senescent human breast fibroblasts after exposure to ionizing radiation have an altered proteoglycan expression facilitating tumor progression

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Cancer research is traditionally focused on the neoplastic cell *per se*, however numerous studies have revealed that stroma plays an important role in tumor development and progression. Stromal fibroblasts, the most abundant cell type of tumor microenvironment, synthesize extracellular matrix (ECM) molecules and soluble mediators, highlighting their importance on paracrine interactions between them and tumor cells. We have already reported that repeated non-cytotoxic, curative, doses of ionizing radiation provoke senescence of stromal lung fibroblasts in a p53-dependent mode. These cells express an inflammatory phenotype and they further enhance significantly the growth of cancer cells in co-cultures in vitro and in immunocompromised mice in vivo. This effect seems to be largely due to the overproduction of MMPs by senescent fibroblasts, as an MMP inhibitor significantly reduced tumor growth. As radiotherapy is often used also in breast cancer treatment, we further studied the effect of ionizing radiation on breast stroma fibroblasts. We have found that ionizing radiation provokes the accumulation of prematurely senescent breast stomal fibroblasts both in vitro and in vivo. These cells express a catabolic and inflammatory phenotype. Interestingly, senescent cells overexpress also syndecan-1, a poor prognostic factor in breast cancer development. This overexpression seems to be independent of the activation of two major pathways in senescence, i.e. those regulated by p53 and p38 MAPK, and it is rather the effect of TGF-β, which in an autocrine fashion activates the Smad pathway and in collaboration with the transcription factor Sp1 activates syndecan-1. Interestingly, invasive breast cancer cells enhance further syndecan-1 expression in stromal cells, again via the paracrine action of TGF-β, showing a positive feedback loop in tumor progression.

In addition, senescent fibroblasts are also characterized by a decrease of decorin expression, the latter having an antitumorigenic effect. These data indicate that the ionizing radiation-mediated accumulation of senescent stromal fibroblasts may represent a long-term side-effect of this anticancer therapeutic approach.

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Oncogenic MITF^{E318K} promotes senescence delay and melanoma progression

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MITF encodes an oncogenic lineage-specific transcription factor in which a germline mutation (*MITF*^{E318K}) was identified in human patients predisposed to both nevus formation and, among other tumor types, melanoma. We previously showed that this mutation reduces the SUMOylation of MITF. In humans, environmental stresses that influence melanoma progression, such as ultraviolet radiation and hypoxia, trigger the production of reactive oxygen species (ROS), which alters the process of SUMOylation. SUMOylation modulates the activity of a large number of proteins, mostly transcription factors, and therefore is involved in many biologically important functions, including the response to oxidative stress and cellular senescence. Cellular senescence is a critical tumor-suppressing mechanism that restrains cancer progression at premalignant stages. Importantly, this topic appears central to melanoma development, because in human, several known melanoma susceptibility genes (CDKN2A, CDK4, RB1, TERT, POT1, ACD) are linked to cellular senescence.

Themolecular mechanisms underlying the oncogenic activity of MITF^{E318K} remained uncharacterized. Using a new mouse model harboring endogenous MITF^{E318K} mutation from its own promoter and melanocytes isolated from MITF^{E318K} carriers, we discovered how this mutation exerts its pro-melanoma effect. We've demonstrated that MITF^{E318K} delays the program of senescence to potentially favor melanoma progression in vivo.

These results support the notion that carriers of the MITF^{E318K} mutation should benefit increased surveillance, allowing an early and more effective management in case of melanoma.

Molecular Insights of APL cure as therapy-induced senescence

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Treatment of acute promyelocytic leukemia (APL) with retinoic acid (RA) and arsenic trioxide (As_2O_3) is associated with differentiation of leukemic blasts and leads to the cure of most patients. This led to the widespread belief that RA and As_2O_3 mediated their effects via induction of differentiation. However, we previously showed that the differentiating effect of RA on APL leukemic blasts is uncoupled from its ability to eradicate disease.

Instead, disease clearance was clearly associated with PML-RARA degradation, mediated by these agents. Loss of self-renewal of leukemia-initiating cells (LICs) in APL depends on both intact PML and P53 with features of therapy-induced senescence (Ablain et al., 2014).

At the molecular level, we observe with endogenous proteins *in vivo* upon RA- or As_2O_3-induced PML/RARA degradation favours acetylation of P53 in a PML-dependent manner. This results in enhanced P53 binding onto specific promoters *in vivo* and activation by therapies of a subset of senescence-associated P53 target genes.

We further investigated the importance of downstream targets of PML-mediated P53 activation via knocking out those genes in APL *in vivo*. Our preliminary results suggest that PAI1 and NR4A1 may both contribute to therapy-induced disease regression *in vivo*, stressing the importance of the senescence process in APL cure.

Ablain, J., Rice, K., Soilihi, H., de Reynies, A., Minucci, S., and de The, H. (2014). Activation of a promyelocytic leukemia-tumor protein 53 axis underlies acute promyelocytic leukemia cure. **Nat Med** *20*, 167-174.

Interplay of CIP2A inhibition-induced senescent tumor cells and tumor infiltrating immune cells in basal-like breast cancer

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Basal-like breast cancer (BTBC) is one of the most aggressive breast cancer molecular subtypes, and it lacks efficient therapy options. Oncoprotein CIP2A expression associates clinically with BTBC, and our results demonstrate that high CIP2A expression significantly predicts for poor patient survival in triple negative breast cancer, that constitutes a major subset of BTBC. Further, my yet unpublished results demonstrate that CIP2A-deficient mouse model is severely impaired in tumor initiation in basal-like breast cancer model. CIP2A inhibition also significantly impairs xenograft tumor growth of human BTBC cells. Together, these results suggest that CIP2A is a novel driver oncoprotein, and a potential therapy target, in human BTBC.

Previously, we have shown that CIP2A promotes breast cancer by inhibiting senescent growth arrest (Laine et al. *Cancer Discov* 2013). Induction of senescence has been shown to cause cancer cell-extrinsic signals altering function of tumor infiltrating immune cells. These can lead to either tumor promotive or suppressive immune responses. Based on this we hypothesize that in addition to effects of CIP2A-mediated senescence inhibition to tumor cell-intrinsic mechanisms, these, and effects of CIP2A targeting directly on immune cells, may impact infiltrating immune cells and thus therapy response. To test this hypothesis we have set up mouse mammary tumor cell and organoid cultures from a spontaneous basal like breast cancer mouse model. These cultures are genetically manipulated by CRISPR/Cas9 system to knock out CIP2A prior to transplantation into recipient mice. Thus far we have validated tumor cell-intrinsic role of CIP2A in the selected mouse model as loss of CIP2A induces senescent growth arrest in mammary gland tumor cells *in vitro*. Currently, we are studying the interplay between the immune system and CIP2A-inhibited tumors *in vitro* and *in vivo*. Overall, this study will address feasibility of CIP2A targeting as a novel approach to combat basal-like breast cancer. Results of this project will also enhance our general understanding of cross-talk between senescent tumor cells and tumor environment in this clinically challenging human cancer type.

Cytokine-induced senescence proceeds via an argonaute protein 2-dependent mechanism

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Overexpression of oncogenic HRAS ($HRAS^{G12V}$) or BRAF ($BRAF^{V600E}$) may permanently arrest cell division, a phenomenon named oncogene-induced senescence (OIS).

OIS also occurs in premalignant lesions *in vivo*, and it is now well accepted as an intrinsic tumor suppressor mechanism. We have shown previously that antigen-specific T helper 1 (Th1) cells drive developing cancers into dormancy. Further mechanistic studies revealed that this immunity-driven tumor dormancy is based on the senescence-inducing effect of the Th1 cell cytokines interferon-gamma (IFN-gamma) and tumor necrosis factor (TNF). Cytokine-induced senescence (CIS) is an extrinsic senescence pathway and, in the case of IFN-gamma and TNF, strictly depends on cytokine signaling via Stat1 and TNF receptor 1. Together, IFN-gamma and TNF activate the p16INK4a/Rb senescence pathway. Consecutive inactivation of the E2F family of transcription factors then leads to permanent growth arrest. Until now, the molecular mechanisms of transcriptional regulation during the course of CIS remained enigmatic. As argonaute protein 2 (Ago2) in association with Rb corepresses E2F target genes throughout OIS, we analyzed its role in CIS. Treatment of different human cancer cell lines with IFN-gamma and TNF for 96 h permanently stopped their proliferation in the absence of excessive cell death, and time-dependently increased senescence-associated β-galactosidase activity reaching a maximum after 72–96 h. We then tested the expression and localization of Ago2 protein after cytokine challenge using immunofluorescence staining for Ago2/Ki67.

As expected, cancer cells showed Ago2 expression independent of the culture conditions. Yet, following treatment with the cytokine cocktail, Ago2 translocated from the cytoplasm into the nucleus in Ki67-negative cancer cells. Ago2 translocation already occurred after 24–48 h of treatment, and can thus be considered as an early event in CIS. In further experiments, we investigated the functional role of Ago2 in CIS. In these experiments, siRNA-mediated knockdown of Ago2 released the cancer cells from cytokine-induced growth arrest. Taken together, CIS is an important tumor suppressor mechanism that permanently stops the proliferation of human cancer cells. As Ago2 rapidly translocates into the nucleus after cytokine treatment, probably acting as a corepressor of the E2F/Rb complex, this protein contributes to senescence induction in human cancers.

Identification of a new phosphorylation site on SOCS1 involved in the regulation of cellular senescence

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Our research is focused on the tumor suppressor SOCS1 (Suppressor of Cytokine Signaling 1), a protein classically known to act as a negative regulator of the cytokine response pathway. The SOCS1 gene is frequently silenced in numerous cancers.

Our laboratory has shown that the SOCS1 protein can interact with the tumor-suppressor p53 and promote its activation. This mechanism is necessary for the induction of cellular senescence, an important tumor suppressor mechanism. Understanding the regulation of p53 by SOCS1 could be important to modulate and reactivate p53 activity in tumor cells.

Our first aim was to map precisely which domains and residues were involved in the interaction between p53 and SOCS1. We used many punctual mutants which allowed us to identify a region corresponding to the N-terminal part of the SH2 domain of SOCS1 as important for interacting with p53. More precisely, Y80 and W81 mutations resulted in impaired interaction with p53. To test the pertinence of these mutants, we overexpressed them in cancer cells. Overexpression of SOCS1 in U2OS cancer cells results in growth arrest, but the SOCS1 mutants that we identified as interacting less with p53 are incapable of performing this growth arrest, suggesting this function of SOCS1 is important for tumor suppression. In normal cells, overexpression of SOCS1 leads to cellular senescence, but these same mutants cannot induce this process as the wild-type.

Our principal aim is to identify the role of phosphorylation in the regulation of the SOCS1-p53 interaction. Indeed, we showed by mass spectrometry that Y80 is phosphorylated. We also show that phosphomimetics or non-phosphorylatble mutants of SOCS1 regulate its tumor-suppressor function by acting on SOCS1's ability to induce senescence and p53 target genes. We also discovered that the Src family of kinases, notably YES1 and Src, are capable of phosphorylating SOCS1 in vitro and that Src-family inhibitors combined with SOCS1 improve the SOCS1 phenotype. We have developed a phospho-specific monoclonal antibody against tyrosine 80 that works in Immunohistochemistry.

SOCS1 is mostly downregulated in cancers, but in the subset of cases where it is overexpressed, we hypothesize the protein could be inactivated by post-translational modifications such as the phosphorylation we identified. If this is the case, phosphorylation of SOCS1 could become a diagnostic marker used for cancer patients. We are currently screening TMAs from various cancers in order to find a correlation.

H3K9-active demethylases disable oncogene-induced senescence and promote melanomagenesis

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Oncogene-induced senescence (OIS) serves as a failsafe program against imminent transformation and further propagation of malignant cells. In senescence, H3K9me3-governed silencing of S-phase-promoting E2F target genes is essential for the sustained cell-cycle arrest, as well as other significant features of senescence. Given the importance of H3K9me3, as well as the frequently observed overexpression of H3K9-demethylating moieties in various cancer types, we studied the role of H3K9-active demethylases LSD1 and JMJD2C in OIS. MEF cells co-expressing oncogenic Ras and LSD1 or JMJD2C failed to enter Ras-induced senescence. Moreover, enforced LSD1 or JMJD2C expression promoted melanomagenesis in Braf-V600E knock-in mice.

To test the therapeutic potency of LSD1 and JMJD2C inhibition, we took advantage of Ras/BRaf-driven melanoma cell lines, which show high expression of LSD1 and JMJD2C on both transcriptional and protein levels. Inhibition of LSD1 or JMJD2C by RNA interference or chemical inhibitors restored senescence and significantly reduced xenograft growth in nude mice. Notably, by targeting LSD1 and JMJD2C, we were also able to prohibit the growth of BRAF inhibitor *(i.e.* Vemurafenib)--resistant melanoma cells.

Taken together, our data underscore the essential role of the H3K9me3 mark in OIS and suggested H3K9-targeting demethylases as potential novel targets for novel anti-cancer treatment strategies.

Modeling release from oncogene-induced senescence

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Melanocytic nevi have been proposed as a model of oncogene-induced senescence. Clonal growth of nevic melanocytes occurs due to BRAF-activating mutations. Growth arrest with features of senescence almost always occurs following a period of growth and typically is life-long. Partial biopsies of nevi typically result in partial regrowth and pigmentation of the nevi, suggesting that the growth arrest is not irreversible. Furthermore, melanocytic nevi can also serve as precursor lesions for roughly 25% of melanoma. We have utilized genetically-engineered mouse models of melanoma to evaluate oncogene-induced senescence and release from growth arrest/progression to melanoma. Based on these models, constitutive activation of mTORC1 signaling is sufficient to bypass Braf-induced growth arrest, but is insufficient for full progression to melanoma, which appears to require concurrent mTORC2/AKT activation. The relevance of these and related studies to oncogene-induced senescence will be discussed.

Advances in chromatin immunoprecipitation

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Chromatin Immunoprecipitation or ChIP has provided many important insights into a variety of biological processes and diseases. However as we ask more complex questions, the limitations of traditional ChIP have impeded our scientific advancements. Active Motif has developed a variety of tools and services to overcome many of these challenges, including challenging samples such as low cell numbers, normalization across samples, and identification of binding partners.

Many global differences in histone modification levels cannot be seen when performing ChIP-Seq due to sample and condition variations. To resolve this problem, we have developed a Spike-In strategy specifically for ChIP, which not only normalizes for biological variation across samples but also for technical variation that can occur during the ChIP procedure.

DNA binding proteins typically form complexes when binding to chromatin and the subunits of a given complex can impact which genomic regions are bound. To identify potential chromatin protein complexes, Active Motif has commercialized RIME (Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins) developed by Dr. Jason Carroll. This protocol enables the unbiased Mass Spec based detection of chromatin based protein-protein interactions.

These tools along with a variety of other epigenetic related assays and services developed by Active Motif enable scientists to answer a variety of biological questions in unprecedented details.

Active Motif capabilities include:

- Innovative products for Chromatin Immunoprecipitation and DNA Methylation
- Epigenetic Services
- Antibodies for ChIP and ChIP-Seq
- Recombinant Proteins and substrates
- Multiplex Histone PTM Quantitation products and services
- Luciferase Reporter Assays

Role of cellular senescence in chronic lung diseases

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Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality in most countries. The dominant risk factor for COPD in industrialised countries is smoking, but ageing is also important, along with factors such as occupational exposures, respiratory infections early in life, and a history of asthma. COPD is characterised by slowly progressive airflow obstruction and emphysema due to destruction of the lung parenchyma. Chronic inflammation is a hallmark of COPD that favours development of co-morbidities. The mechanisms leading to lung destruction and inflammation in COPD are not well understood and therefore, no curative treatment is available for this disease. One recent concept is that cell senescence is a key process in the pathophysiology of COPD.

That telomere dysfunction and exaggerated lung-cell senescence are major players in the pathogenesis of the lung alterations in COPD is supported by the following facts: -i) Subjects with COPD or with emphysema exhibit short telomeres in lung and circulating cells; ii) some individuals with telomerase gene mutation develop early lung emphysema or lung fibrosis, especially in the setting of smoking, such lung diseases are therefore considered to be telomere disorders; iii) there is an accumulation of senescent alveolar-epithelial (AECs) and endothelial cells (P-ECs) and fibroblasts in lung specimens from patients with emphysema and COPD8; iv) pulmonary vascular endothelial, smooth muscle cells, and lung fibroblasts derived from lungs of patients with COPD exhibit increased susceptibility to replicative or stress-induced senescence when compared to controls; v) mice with telomerase deficiency have an increased susceptibility to develop pulmonary emphysema after exposure to cigarette smoke; vi) mediators and inflammatory cytokines released from senescent cells contribute to sustained inflammation in COPD and affect lung tissue remodelling; vii) remodelling of pulmonary vessels at the origin of pulmonary hypertension (PH) in COPD is linked to the SASP of vascular senescent cells.

In current studies, we identified two major molecular pathways that are required to induce cell-senescence in COPD, the mTOR (mechanistic target of rapamycin) pathway and the PLA2R1 (phospholipase A2 receptor 1) signalling pathway.

Using lung specimens and derived cultured cells, namely pulmonary vascular endothelial cells (P-ECs) and smooth muscle cells from patients with COPD and from age- and sex-matched control smokers, we show that cell senescence is linked to the activation of mTOR and that inhibiting mTOR by low dose of rapamycin prevents cell senescence and inhibits the SASP. To explore whether mTOR activation is causal in lung pathology, we developed transgenic mice exhibiting increased mTOR activity in different lung cells. We show that mTOR activation is sufficient to induce cell senescence in the lung and to mimic the lung alterations that occur in COPD, with rapid development of lung inflammation and emphysema.

To explore the PLA2R1 pathway in lungs and cultured cells from patients with COPD and controls, we infected cells from patients with COPD and controls by retroviral vectors encoding PLA2R1 or shRNA against PLA2R1, in collaboration with D Bernard'team. Our results show that constitutive expression of PLA2R1 induces premature senescence whereas PLA2R1 knockdown delays replicative senescence in cells from patients with COPD. Our results also support the view that JAK1/2 are downstream effectors of PLA2R1 on senescence.

These results suggest that the drugs tested here, mTOR inhibitors and JAK1/2 inhibitors, may be proposed as therapeutic interventions for cell senescence in COPD without increasing the risk of cancer.

Contribution of *in vivo* senescent cells to age-related diseases

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One of the major influences underlying chronic diseases in people is chronological age. One reason for this predisposition may be that the fundamental processes of normative aging actively drive age-related diseases. If this were to be true, interventions that interfere with mechanisms of aging may therefore hold promise for therapeutic intervention in many human pathologies. Senescent cells, which are characterized by a permanent cell-cycle arrest and unique pro-inflammatory secretory phenotype, have been shown to accumulate with age in several tissues of mice and people. It was unknown if these cells were simply bystanders in the process or active contributors to tissue dysfunction.

Through the use of a combination of *in vivo* mouse models and therapeutics to eliminate senescent cells, it was found that senescent cells are drivers of several late-life diseases, including cancer, atherosclerosis and osteoarthritis. These findings, along with unpublished studies on the impact of senescent cells to neurodegenerative and lung diseases that will be discussed, indicate that senescent cells contribute to a host of age-related pathologies and therapeutic interventions that attenuate the detrimental impacts of senescence may hold promise for many applications.

Antioxidant treatment can promote lung tumor formation during chronic oxidative stress

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Rationale: Oxidative stress is a major contributor to the lung alterations associated with cigarette smoke exposure and chronic lung disease. Antioxidants are broadly viewed as cancer-protective molecules that prevent the accumulation of mutations. However, this view has been strongly challenged in recent years. Antioxidants, including N-acetylcysteine (NAC), have been shown to increase the risk of developing lung cancer in humans and to promote lung cancer progression in mice with oncogenic RAS or RAF mutations. Furthermore, antioxidants, such as NAC, are known to promote escape from cell senescence under various conditions.

Objective: To analyze lung alterations associated with chronic oxidative stress, we studied JunD-deficient mice at various ages, with or without chronic NAC treatment. This murine model was chosen because JunD expression is decreased in lung cancer and loss of JunD expression results in the production of reactive oxygen species and in cell senescence

Methods and results: JunD knockout mice (JunD-ko) were treated with NAC (in drinking water, 40 mg/kg) or vehicle from birth to the age of 4 months or 12-18 months and compared to same-age control mice. The proportion of mice with lung adenocarcinoma at or after 12 months of age was 50% in NAC-treated JunD-ko mice and only 10% in NAC-treated control mice. Lung cancer was not detected in any of the vehicle-treated JunD-ko or control mice, even at the age of 24 months. Compared to their young counterparts, old mice exhibited increased biomarkers of oxidative stress and cell senescence, including p16 and p21 expression and acid β-galactosidase activity. These alterations were more marked in JunD-ko than in control mice. Chronic NAC treatment led to a decrease in these biomarkers with a reduction in p16, p21, and acid β-galactosidase activity in the lung.

Conclusion: Treatment with the antioxidant NAC can promote lung tumor formation during aging. The loss of JunD magnifies this effect and increases cell senescence. We suggest that NAC treatment, by protecting against cell senescence, may promote the development of lung cancer during chronic oxidative stress.

Targeted therapy-induced senescence enhances immune surveillance of KRAS mutant lung cancers

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Advances in immune oncology, and, in particular, immune checkpoint blockade, have revolutionized the treatment of lung cancer. However, as the effectiveness of these T cell-centric therapies is dependent upon appropriate MHC expression of neo-antigens produced during tumor evolution, not all patients respond. Induction of cellular senescence, a two-component process that involves cell cycle arrest and activation of the immunomodulatory senescence-associated secretory phenotype (SASP), has been shown to promote clearance of tumor cells by NK cells, macrophages, and neutrophils in a neo-antigen independent manner. Nonetheless, whether pharmacological targeting of oncogenic signaling pathways may also lead to senescence-related immune surveillance and tumor regressions remains an outstanding question. Here, we set out to identify targeted therapies that could induce senescence in KRAS mutant lung tumors, and interrogate whether this senescence induction could lead to immune surveillance and subsequent tumor responses in vivo. While CDK4/6 inhibitors as single agents induce a reversible cytostatic response, combinations with other targeted therapies synergize in KRAS mutant lung and pancreatic cancer cell lines through induction of RB-mediated cellular senescence. In vivo, combination treatment leads to significantly reduced tumor burden in PDX models of lung cancer and increased survival in murine lung cancer models, and is accompanied by SASP induction, increased NK cell ligand expression, and infiltration and activation of NK cells. Consistent with triggering an anti-tumor immune response, combination treatment leads to SASP-dependent NK cell cytotoxicity in vitro. Depletion of NK cells or suppression of SASP abolishes the efficacy of combination treatment by inhibiting NK-mediated immune clearance. These studies establish that targeted therapy-induced senescence can trigger a distinct means of antigen-independent immune surveillance that can lead to enhanced tumor responses, and, importantly, provide one mechanism by which cytostatic agents can cause tumor regressions in patients.

HDAC inhibitors impair the pro-inflammatory senescence-associated secretory phenotype by blocking the nuclear-cytoplasmic translocation of damage DNA fragments

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Cellular senescence is a highly stable cell cycle arrest elicited by replicative exhaustion or stresses such as DNA damage, oxidative stress and aberrant oncogenic activation. The senescence response is now considered a potent barrier to tumorigenesis. Senescent cells secrete a group of factors known as the senescence-associated secretory phenotype (SASP). Expression of the SASP requires activation of NFkB signalling.

The SASP reinforces senescence and promotes immune-mediated clearance, but it can also alter tissue microenvironment contributing to age-related pathologies including cancer. We show that HDAC inhibitors (HDACi), specifically TSA and Vorinostat/SAHA, suppress SASP in senescent irradiated fibroblasts at a very low non-toxic doses, without reversing the senescence growth arrest. In senescent cells, low-dose HDACi also induce a marked decrease of senescence-associated beta-galactosidase activity and suppress formation of senescence-associated heterochromatin foci (SAHF). In proliferating cells, the same dose is non-toxic and non-cytostatic. We also showed previously that senescent cells extrude fragments of γH2AX-positive chromatin from the nucleus into the cytoplasm, so-called cytoplasmic chromatin fragments (CCFs), dependent on disruption of a nuclear lamin B1-LC3 interaction [1, 2]. We found that HDACi do not directly down-regulate NFkB signalling, but do so indirectly linked to suppression of mROS, improved DNA repair, reduction of intranuclear DNA damage foci and complete abolition of γH2AX-positive CCF thought to activate NFkB and the SASP via the cytoplasmic DNA sensing pathway, cGAS/STING. Importantly, we found that low doses of HDACi suppress SASP in two independent mouse models of senescence: ionizing radiation and acetaminophen induced liver injury. Together, these data suggest that HDACi improve repair of DNA damage, suppress trafficking of damaged chromatin into the cytoplasm and therefore induction of the SASP. These results provide a mechanistic underpinning for the reported pro-longevity/healthy aging activities of HDACi and support improved investigation of these molecules in this regard.

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Insights into haematopoietic stem cell ageing and disease from single cell studies

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Haematopoietic stem cells (HSC) are essential to replenish the blood system. With age, HSCs accumulate DNA damage and expand in numbers, but lose functionality. However, little is known about the heterogeneity within the HSC compartment, especially during ageing. Here, we used single cell RNA-Sequencing to elucidate the heterogeneity within the aged HSC compartment in mice. We found an ageing specific sub cluster of cells that carry a p53 and Jun/Fos transcriptional signature and show signs of cell cycle arrest. Moreover, the majority of HSC in the aged mouse still express a young HSC signature, leading us to conclude that HSC ageing might be asymmetrical in the bone marrow niche. This ageing specific sub cluster is expanded when HSCs are subjected to hyper proliferation *in vivo* by an oncogenic Jak2 mutation, namely Jak2V617F, which is important in the onset of myeloproliferative disease. In summary, single RNA-sequencing data enable us to identify thus far unknown transcriptional heterogeneity in the HSC compartment.

Neuregulin-1 attenuates stress-induced vascular senescence *in vitro* and *in vivo*

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Aims: Cardiovascular ageing is a key determinant of life expectancy. Cellular senescence, a state of irreversible cell cycle arrest, is an important contributor to aging due to the accumulation of damaged cells. Targeting cellular senescence could prevent age-related cardiovascular diseases. In this study, we investigated the effects of neuregulin-1 (NRG-1), an epidermal growth factor with powerful cardioprotective and anti-atherosclerotic effects, on cellular senescence.

Methods & results: Senescence was induced in cultured rat aortic endothelial cells (ECs) and aortic smooth muscle cells (SMCs) by 2h exposure to 30 µM hydrogen peroxide (H2O2) . Cellular senescence was confirmed after 72h using senescence associated-β-galactosidase staining (SA-β-gal), cell surface area, and western blot analyses of senescence associated pathways (acetyl-p53, p21).

Recombinant human NRG-1 (rhNRG-1, 20ng/ml) significantly reduced H2O2-induced senescence, as shown by a lower number of SA-β-gal positive cells, smaller surface area and lower expression of acetyl-p53. Consistently, aortic SMCs isolated from SMC-specific ErbB4 deficient mice ($ErbB4^{f/+}$ SM22α cre+) showed earlier cellular senescence in vitro compared to wild type ($ErbB4^{+/+}$ SM22α cre+) SMCs. Next, C57BL/6, ErbB4f/+ SM22α cre+ and their wild-type littermates mice were rendered diabetic with streptozotocin (STZ) and randomized to receive rhNRG-1 (20 µg/kg) or vehicle.

In all mouse strains, diabetes resulted in a significant induction of cell senescence in the aorta, which was attenuated by rhNRG-1 treatment. Also, diabetic $ErbB4^{f/+}$ SM22α cre+ mice showed significantly more vascular senescence than diabetic wild type littermates.

Conclusions: This study is the first to explore the role of the cardioprotective growth factor NRG-1 in vascular senescence. Our data demonstrate that NRG-1 markedly inhibits senescence induced by oxidative stress in vascular cells in vitro and in the aorta of diabetic mice in vivo. Consistently, deficiency in the NRG-1 receptor ErbB4 provokes cellular senescence in vitro as well as in vivo.

Osteoporosis

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Osteoporosis in the elderly is an age-related multifaceted syndrome that precipitates in fragility fractures of the spine, the hip and the radius as the major osteoporotic fractures. Healthy bone responds to mechanical forces and adapts to the requirements of daily living, but this adaptation is inadequate in osteoporosis. Besides gender, bone mineral density and a series of clinical risk factors age is a major contributor. To date there are a few bone related candidate genes where mutations in genes of key osteogenic pathways may account for an early onset of osteoporosis in the 4th and 5th decades of life, but otherwise no master gene has been identified and the general believe is that osteoporosis in the elderly is a complex polygenic syndrome. It is yet unclear if primary osteoporosis is a premature aging syndrome and the influence of epigenetic phenomena on the pathophysiology is just recently being evaluated.

However recent studies did not identify significant changes in the methylome of DNA from peripheral blood mononuclear cells in patients with osteoporosis indicating that also in this respect there are no specific master switches that might explain the syndrome. Several groups including our own have characterized the transcriptome of multipotent skeletal precursors (so called mesenchymal stem cells MSC) from osteoporotic patients. Skeletal precursors from patients with osteoporosis harbor a characteristic clustering signature which is to a certain extent related to populations after in vitro aging and is also significantly different compared to the profile of cells from healthy aged 80+ probands. *In vitro* cells from osteoporotic donors display some features of premature senescence. This may already indicate that epigenetic changes related to mesenchyme and mesenchymal precursor cells account for the alterations in tissue regeneration and maintenance. Methylome analyses from these populations were very recently described and there were differentially methylated loci situated in genomic regions with enhancer activity. Downstream of such genomic regions mechanisms of stem cell expansion and osteogenic differentiation appeared to be altered. Both the transcriptome and the methylome analyses reveal promising candidate targets for treatment of osteoporosis. Sophisticated analyses of epigenetic changes and evaluation of candidate mechanisms in vitro and in mouse models are in progress and will be discussed.

Combining senescence with biomaterials research and biofabrication for regenerative medicine

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Biomaterials research has in the last decade focused on the development of strategies for materials that actively interact with the host system, combining biodegradability with specific cell adhesion and materials degradation. Most recent trends comprise interaction with the immune system and activation of endogenous repair and regenerative mechanisms, for example through the recruitment of resident tissue and especially stem cells. Another recent trend is the exploitation of 3D printing technologies to recapitulate hierarchical tissue structures with cell-material constructs in the research field of biofabrication.

The more and more obvious potential to exploit senescent cells and their secretome for regenerative purposes raises the question to which extend the fields of biomaterials research and biofabrication can synergize with senescence research for new stimuli in regenerative medicine. Thislecture will recapitulate some recent biomaterials trends and introduce the field of biofabrication. It will then, at examples of work pursued in my lab, lay out potential strategies how recent findings in senescence could be exploited for regenerative medicine through combining them with biomaterials.

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Targeting the cancer genome and the tumour immune response for pro-senescence therapy

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Senescence induction in cancer works as a potent weapon to eradicate tumorigenesis. Therapies that enhance senescence not only promote a stable cell growth arrest but also work as a strong stimulus for the activation of the tumor immune response.

However, cancer cells can bypass senescence by either acquiring new genetic alterations or by disrupting the tumor immune response. Here, I will discuss the identification of oncogenic pathways that bypass Pten-loss induced cellular senescence thereby promoting prostate cancer progression and metastasis. Next, I will present evidence demonstrating that tumor-associated macrophages (TAMs) control senescence induction in prostate cancers and that immunotherapies that interfere with the polarization of TAMs promote senescence in Pten null; Trp53 null tumors by increasing the number of TNFα– releasing M1-like macrophages in the tumor microenvironment.

Evaluation of a novel *ß-Galactosidase* specific PET tracer for *in vivo imaging* of *tumor senescence*

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Background: Senescence has essential effects on cancer treatment and outcome. Therefore the development of a diagnostic tool to detect senescent cells in cancer patients is of great interest. Here we describe the development of a PET tracer for non-invasive imaging of b-galactosidase as a surrogate marker for senescence.

Material/methods: Two different models were used to test the tracer *in vitro*. In HCT116 cells doxorubicin was used to induce senescence and in an HRas driven liver progenitor cell line a doxycycline-dependent p53-specific shRNA was used to elicit senescence. Both cell lines were incubated with the tracer after induction of senescence. Subsequently the activity in the cells was measured in a gamma-counter.

For *in vivo* testing mice bearing *s.c.* HCT116 or HRas driven liver progenitor xenografts were used. Mice were treated with and without doxorubicin or doxycycline respectively to induce senescence. Afterwards the tracer was injected *i.v.* and PET/MR scans were performed. The tracer uptake in the tumors (%ID/cc) and tumor-to-muscle ratios were determined.

Results: While *in vitro* senescent HCT116 cells showed an uptake of 11 kBq/10^6 cells, non-senescent control cells showed an uptake of only 4 kBq/10^6 cells. Senescent cells in the HRas driven liver progenitor model showed an increased tracer uptake of 192 kBq/10^6 cells compared to non-senescent cells (63 kBq/10^6 cells).

In vivo we could show 1.7+/-0.7 (n=7) vs 1.1+/-0.4 (n=5) %ID/cc tracer uptake in senescent and non-senescent HCT116 cells respectively. In the HRas model the tracer uptake in senescent tumors was significantly increased compared to control tumors (1.5+/-0.3 (n=16) vs 0.9+/-0.3 (n=11) %ID/cc). *Ex vivo* b-galactosidase staining and immunohistology of Ki67, Caspase3, HP1γ, p53 and p16 confirmed induction of senescence.

Conclusions: We could show here an increased uptake of our novel b-galactosidase specific PET tracer in senescent cells and tumors *in vitro* and *in vivo*. Therefore our tracer is a promising tool for non-invasive detection of b-galactosidase as a surrogate marker for senescence. Clinical translation of our tracer is in preparation. It could become the first tool for non-invasive detection of senescence in patients. It therefore has potential to significantly improve cancer diagnostics.

Nanotechnology-based approaches for targeting senescent cells: defining novel therapeutic strategies

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Accumulation of senescent cells has been associated with the pathogenesis of many diseases, including age-related pathologies and cancer. Consequently, targeting senescent cells has been proposed as a potentially relevant therapeutic strategy. Consistent with this, it was recently demonstrated that clearing senescent cells in a transgenic mouse model increased their healthspan and longevity.

The major problem with the therapeutic targeting of senescent cells in humans is the lack of specificity and selectivity of classic senescent markers, which creates a high risk of off-target effects. Here, we describe the use of molecularly imprinted nanopolymers (nano-MIPs) designed to bind to B2MG, a membrane protein recently characterized as a novel marker of senescence, and propose it as a proof-of-concept method for the specific detection of senescent cells. We show that fluorescein-tagged nano-MIPs displayed a higher affinity for senescent cells compared to non-senescent ones, as assessed by microscopy and flow cytometry. The nano-MIPs were internalized after binding to B2MG on the cell membrane and accumulated inside the cell. We show that this had no intrinsic toxic effect, since the cells were able to survive after incubation with the nano-MIPs.

Our results show the feasibility of senescent cell detection by Nano-MIPs using a membrane marker. Moreover, they suggest that specific nano-MIPs laden with a toxic payload could be a selective mechanism for drug delivery into senescent cells. These nano-MIPs are currently being generated and could provide a new nanotechnology-based method for senescent cell clearing.

Selectively targeting the ribosome: a novel pro-senescence therapeutic strategy for p16+ basal-like breast cancer

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The discovery of oncogene-addiction and the demonstration that senescence can be induced in established tumours has paved the way for pro-senescence therapies in cancer. Here, we identify a specific subset of ribosomal proteins (RPs) as novel pro-senescence therapeutic targets in p16 positive basal-like breast cancer (BLBC), a highly aggressive disease subtype and implicate dysregulated ribosomal biosynthesis in senescence evasion in cancer.

Ribosomal Stress-Induced Senescence (RSIS) is associated with a panel of well-established senescence markers and results in a highly stable cell cycle arrest followed by establishment of a senescence-associated secretory phenotype. Mechanistically, RSIS initiation is dependent on endogenous p16 triggering a re-sensitisation to the p16/p107/p130 tumour suppressor axis and is independent of elevated DNA damage. In the absence of p16, specific RP knockdown results in caspase-mediated apoptosis together with p53 stabilisation, further confirming the requirement for p16 in senescence activation. Importantly, ribosomal protein loss is well tolerated by normal human mammary epithelial cells and represents a novel cancer-cell specific route to future therapies.

Examination of the METABRIC dataset (2,000 matched breast cancer samples) revealed that the hits identified here are frequently dysregulated in BLBC, with overexpression associated with a poor prognosis. Strikingly, a $RPS3A^{HIGH}$ $RPS7^{HIGH}$ expression signature correlates with an earlier age of onset across all breast cancers and $RPS3A^{HIGH}$ $RPS7^{HIGH}$ $p16^{HIGH}$ tumours are associated with the very worst patient outcomes, supporting a role for these RPs as drivers of disease and potential prognostic biomarkers.

Here we will present detailed analysis of the mechanism of senescence activation following RP knockdown, as well as our latest findings on the utility of these RPs as prognostic biomarkers for breast cancer and improved patient stratification.

Induction of ribosomal checkpoint induced senescence (RCIS) for the treatment of liver cancer

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While ribosomal proteins are generally considered to be essential genes, the existence of ribosomopathies suggests that a therapeutic window for exploiting ribosomal proteins as targets for cancer therapy may exist. We report distinct stress responses of tumor cells and normal cells upon knockdown of Rpl15, a ribosomal protein which was identified in a direct *in vivo* shRNA screen for new therapeutic targets in liver cancer. While normal cells undergo a reversible cell cycle arrest, we found that in tumor cells, the knockdown of Rpl15 triggers a ribosomal stress response followed by induction of cellular senescence, designated ribosomal checkpoint induced senescence (RCIS).

Importantly, Rpl15 suppression triggered RCIS independent of any reduction in global protein translation. Using well established therapy resistant murine HCC models, we show that shRNAs targeting Rpl15 potently suppress tumor development in genetically diverse murine HCCs. Using Rpl15 shRNA transgenic mice, allowing for ubiquitous shRNA mediated suppression of Rpl15, we show that systemic Rpl15 suppression can be tolerated for up to 5 days, thus revealing a therapeutic window for metronomic Rpl15 inhibitory therapies. However, molecular modeling analyses revealed that Rpl15 is not druggable by small molecule inhibitors and we thus set out to explore whether RCIS can be induced via interference with other ribosomal proteins or other factors involved in ribosome biogenesis. We generated and screened a focused shRNA library targeting 41 ribosomal proteins and 19 ribosome biogenesis factors and found that apart from Rpl15, only a small subset of shRNAs scored. From a druggability point of view it was interesting, that shRNAs targeting components of the RNA polymerase I complex had scored, as recently a pharmacological RNA polymerase I inhibitor became available. Both genetic and pharmacological inhibition of RNA polymerase I induced RCIS and mediated an excellent prolongation of survival (far superior to the clinically used standard therapy Sorafenib), however in contrast to targeting Rpl15, targeting RNA polymerase I failed to achieve full long term tumor suppression. Mechanistically we found a less efficient immune mediated clearance of senescent cells as a possible explanation. In line with the idea that distinct secretory profiles underlie differences in immune mediated clearance of RCIS cells, cytokine arrays revealed distinct secretory profiles of RCIS induced by Rpl15 suppression and RCIS induced via shRNA or pharmacological RNA polymerase I inhibition.

Novel diagnostic tools for lung cancer early detection

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Lung cancer is the leading cause of cancer-related deceases worldwide. Most of the lung cancer cases escape diagnosis until tumours are at an advanced stage, which results in more than 90% of patients having a 5-year mortality from first diagnosis. Patients diagnosed with early lung cancer have much better outcomes, so one approach to reduce mortality from lung cancer is to detect it earlier. Cellular senescence is characterised by a stable cell cycle arrest that occurs when a single cell is afflicted by different types of damage or stress. Senescent cells are accumulated in a wide variety of precancerous lesions, including lung premalignant tumours. Therefore, approaches to trace senescent cells can result in potent new tools to detect lung tumours earlier.

We are implementing the use of galacto-encapsulated fluorophores that are preferentially released within senescent cells. Galacto-coated beads (GalNP) are internalised via endocytosis and, after fusion with lysosomal vesicles, the beads are released by exocytosis. The high lysosomal β-galactosidase activity of senescent cells allows a preferential release of the cargo by a β-galactosidase-mediated hydrolysis of the sugar layer. We have validated the specific activation of GalNP carrying a fluorophore in human lung fibroblasts undergoing oncogene-induced senescence, and in human lung cancer cell lines undergoing chemotherapy-induced senescence. Systemic administration of diagnostic GalNP labels lung tumour xenografts undergoing senescence in mice. Importantly, we have preliminary data that diagnostic GalNP are preferentially activated in lung adenomas enriched in senescent cells in a KRas-driven mouse model of lung cancer. GalNP have the potential to be developed as a cancer theranostic tool, by combining the simultaneous encapsulation of tracers and cytotoxic drugs.

Inaddition,we are developing two-photon fluorescent probes, consistent of different galactopyranosyl -histidine-naphtalimide (AHG) formulations, able to detect senescent cells. These probes are suitable for highly sensitive in vivo imaging, and are characterised by a deep penetrance into the sample and a low photo damage.

Collectively, our approaches open new diagnostic modalities to detect lung tumours, as well as other cancers and age-related disorders, accumulating senescent cells.

A one-two punch model for the treatment of cancer

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Cellular senescence is generally considered to be a stress response that restricts proliferation. It also has an anti-cancer effect, as activation of oncogenes can lead to a stress response known as “oncogene-induced senescence”, which has been shown to restrict oncogenic transformation in vivo.

More recently, it has been demonstrated that even full-blown cancer cells can be triggered to undergo senescence. Senescent cells often produce a number of inflammatory cytokines (known as the “senescence-associated secretome”). This begs the question whether this inflammatory response is helpful in cancer therapy (immune clearance) or detrimental in that it can enhance dissemination.

We have developed a genetic screening model to find genes whose suppression induces senescence in cancer. We use a reporter gene that is activated in senescent cells in combination with CRISPR/Cas9 libraries to screen for novel drug targets for induction of senescence. We use senescent cancer cells to screen compound libraries for novel agents having senolytic activity. We test the efficacy of these drugs in sequential treatments of cancer: The first therapy aims to induce senescence, the second therapy aims to kill senescent cells. We refer to this approach as the “one-two punch” approach to cancer therapy.

Defects specific to progeroid cockayne syndrome cells are recapitulated during normal cellular senescence

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Ageing is dramatically accelerated in some rare genetic disorders like the Cockayne syndrome. Dissecting the defect(s) of these diseases is critical to develop treatments and elucidate dysfunctions that might lead to normal ageing.

Cockayne syndrome (CS) is characterized by dramatically precocious ageing, neurodegeneration, and UV hypersensitivity. We discovered a specific alteration in cells from CS patients. Indeed, in these cells mitochondrial ATP production is redcued due to depletion of the catalytic subunit of the mitochondrial DNA polymerase (POLg), in turn resulting from accumulation of the little-known serine protease HTRA3 (Chatre et al, 2015, PNAS 112: E2910-9). HTRA3 overexpression in CS cell depends on nitrosative and oxidative stress, but the underlying mechanism is still unknown. These defects seem independent from the UV sensitivity defect. Importantly, we rescued alterations in CS patient cells by scavenging reactive oxygen and nitrogen species, thereby restoring physiological HTRA3 and POLG1 levels, and opening possibilities for treatments, which are presently lacking for CS patients.

We wondered whether alterations specific to CS cells were also present during processes linked to normal ageing. We discovered that HTRA3 is overexpressed in normal senescent cells and events leading to its expression precede established senescence markers like beta galactosidase activity and p21 expression, indicating that they may represent early cell senescence determinants. Our data also show that factors able to rescue alterations in CS cells delay senescence in normal cells.

Thus, our data suggest that factors specifically implicated in progeroid cellular defects are implicated in cellular senescence, a process linked to ageing.

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Keynote Lecture

Closing lecture:
Tissue repair: an integrated view of senescence and reprogramming

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Reprogramming of differentiated cells into pluripotent cells can occur in vivo, but essentially nothing is known about the mechanisms, processes, and mediators involved. We have generated mice where we can induce ubiquitous expression of the four Yamanaka reprogramming factors. These factors, when expressed continuously during 1 week, produce widespread de-differentiation in multiple tissues. Upon switching off the reprogramming factors, de-differentiated tissues re-differentiate and homeostasis is restored. We have found that senescence participates in the process of in vivo reprogramming. Senescence is a cellular response to damage characterized by an abundant production of cytokines and other extracellular factors, which recruit inflammatory cells and can orchestrate tissue remodeling. I will present an integrated view of tissue repair whereby tissue injury, through senescence, primes surviving cells to undergo partial reprogramming and initiate tissue repair.

POSTER FLASH TALKS

Isolation and characterization of senescence-associated exosomes

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Cell senescence was originally identified as a stable proliferative arrest undergone by normal human cells after extensive in vitro culture. Although initially related to aging, it was afterwards described as a process that can be triggered by many potentially dangerous situations, acting as a protective response.

In particular, cells challenged by oncogene activation trigger cell senescence as an effective tumor suppressor mechanism, opposing tumor initiation and progression. Moreover, chemotherapeutic treatment of cancer might restrict tumor growth by promoting tumor cells to engage in the senescence response. The power of this antiproliferative mechanism led many to propose the development of a prosenescence therapy of cancer.

However, in recent years many laboratories have described the pleiotropic effects of factors secreted by senescent cells, known as SASP. These SASP factors have been shown to be released from senescent cells and to alter neighboring cells. Some activities of the SASP seem to contribute to the senescent phenotype, but others could have detrimental activities, with some reports even suggesting a potential protumorigenic effect. Apart from these secreted factors we became interested in the isolation and characterization of exosomes released from senescent cells. Exosomes are cell-derived vesicles that are present in many biological fluids, are actively secreted by cells, and play key roles in intercellular signaling.

Using different systems to induce senescence, based on oncogene expression or chemotherapeutic treatment of cancer cells, we isolated senescence-associated exosomes. After sequential ultracentrifugation of conditioned media, we verified the identity of these exosomes by electron microscopy, size determination, and exosome marker detection, and we further confirmed that they were internalized when incubated with target cells.

Finally, we have performed a comparative proteomic analysis of the senescence-associated exosomes with the aim of identifying specific components and potentially define the activity of these vesicles.

Non-canonical inflammasome activation regulates oncogene-induced senescence

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Senescent cells display a senescence-associated secretory phenotype (SASP) which reinforces both autocrine and paracrine senescence. Caspase-4 (Cysteine-aspartic acid protease 4 or CASP4) is an inflammatory caspase core of the non-canonical inflammasome. Because of the emerging evidence showing a key role for CASP4 in inflammatory responses including IL-1b regulation (a major component of the SASP), we hypothesized that CASP4 may play a role in senescence.

Firstly, we found that CASP4 is upregulated at the transcriptional and protein level in human primary fibroblasts overexpressing RASV12. Next, to study whether CASP4 regulates senescence, we used a well-established inducible model of RASV12-induced senescence, in which IMR90 cells have been stably transformed to express a ER:RASV12 construct. Upon addition of tamoxifen (4OHT), these cells undergo oncogene-induced senescence (OIS) through activation of RASV12. siRNA-mediated downregulation of CASP4 in ER:RASV12 plus 4OHT caused a bypass of senescence as we observed increased 5-bromo-2'-deoxyuridine (BrdU) incorporation compared to control senescent cells. Moreover, downregulation of CASP4 in ER:RASV12 plus 4OHT decreased the levels of IL-1a, IL-1b, IL-8 and IL-6.

Overexpression of CASP4 in IMR90 cells induced b-galactosidase associated activity (SA-bgal) and cell proliferation arrest, as seen by reduced BrdU incorporation and colony formation. However, the sole overexpression of CASP4 was not able to induce the transcription of SASP components such as IL-1a, IL-1b, IL-6 or IL-8. CASP4 is as a pattern recognition receptor for LPS. Therefore, in order to mimic the activation of CASP4 in senescence, we transfected LPS into IMR90 cells. As previously described in other cell types, intracellular LPS caused a rapid cell death response. However, the fraction of cells surviving pyroptosis showed features of senescence such as higher levels of p16 and p21, increased SA-bgal, decreased BrdU incorporation and expression of IL-1b, suggesting that it is necessary to activate the non-canonical inflammasome for a full senescence response, including expression of the SASP. The senescence response to LPS transfection was enhanced when cells were stimulated with Toll-like receptor agonists to prime the inflammasome prior to transfection and in cells ectopically expressing CASP4. Altogether these results evidence a role for CASP4 in regulating OIS.

Regulatory role of PARP-1 in the onset and maintenance of oncogene induced senescence

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Cellular senescence arrests cell proliferation in response to various genetic and epigenetic stresses. The stable cellular arrest is accompanied by widespread changes in chromatin structure, metabolism and gene expression. Senescence acts dichotomously - both protective and degenerative. On the one hand, it protects young organisms against cancer, promotes tissue repair, regeneration and development however, senescence also promotes age-related pathologies, contributes to aging and tumorigenesis. Intimately linked to this functional dichotomy is the senescence associated secretory phenotype (SASP) which releases numerous cytokines, and inflammatory factors. However, the transcriptional regulation of the SASP remains poorly understood. An emerging paradigm suggests Poly(ADP-ribose) polymerase 1 (PARP-1), a very well-studied protein established in DNA repair, plays a key role in transcriptional regulation, regulation of genes involved in inflammation and cancer, as well as contributing to chromatin stricture. PARP-1 is functionally linked with the NF-kB transcription factor, known to regulate many SASP factors, potentially implicating PARP-1 in regulation of the SASP. The general activity of PARP-1 in senescence, regulation of the SASP or the chromatin remodeling are unclear. Here, we aim to define the function of PARP-1 in the SASP gene regulation and role in chromatin structure. First, we integrate of the genomic locations of PARylation (post-translational modification catalyzed by PARP-1) and the binding of PARP-1 across the genome using ChIP-seq. Secondly, we integrate the changes to global chromatin structure and gene transcription using the assay for transposase accessible chromatin (ATAC) and transcriptomics in the context of PARP-1 knock-down or pharmacologically inhibited senescent cells. These data sets will provide comprehensive insight into the contributions of PARP-1 and PARylation to the onset and maintenance of senescence and the SASP.

Non-autonomous regulation of chromatin structure by NOTCH1

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Senescent cells interact with the surrounding microenviroment achieving both pro-oncogenic and tumour-suppressive outcomes. In addition to autocrine and paracrine signalling mediated by factors of the Senescence-Associated Secretroy Phenotype (SASP) we have recently identified that NOTCH1 can drive a unique "NOTCH-induced" form of senescence in adjacent cells via cell contact dependant juxtacrine signalling (Hoare et al. 2016). In the context of RAS-induced senescence (RIS) we discovered a cell-autonomous role for NOTCH1 in regulation of the SASP - a central hallmark of senescence.

These data prompted us to investigate the impact of NOTCH1 signalling on other key features of the senescent cell - namely chromatin structural alterations. In fibroblasts undergoing RIS there is a dramatic reorganisation of chromatin structure that can result in the formation of Senescence-Associated Heterochromatic Foci (SAHF), microscopic heterochromatic structures visible via DAPI staining. We find that NOTCH1 inhibits the formation of SAHF at least partially through transcriptional repression of a critical structural component, High-Mobility Group A (HMGA). Next, we show that nucleosome positioning is substantially altered in RIS and that this re-distribution is antagonised by NOTCH1 expression. Finally, we illustrate non cell-autonomous and cell-contact dependent regulation of chromatin structure mediated by lateral induction of NOTCH1 signalling.

Global sumoproteome analysis reveals a PML-localized network of proteins regulating cellular senescence

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Several regulators of SUMOylation have been previously linked to senescence but most targets of this modification in senescent cells remain unidentified. Using a two-step purification of a modified SUMO3, we profiled the SUMO proteome of senescent cells in a site-specific manner. We identified 25 SUMO sites that were significantly regulated by senescence on 23 proteins. We found that most targets whose SUMOylation levels were altered in the senescent phenotype were PML nuclear body (PML-NB) associated, which correlates with the increased number and size of PML-NBs in senescent cells. UBC9, the sole SUMO E2 enzyme, was among the targets whose SUMOylation was upregulated in senescence. However, SUMOylation of UBC9 at Lys-49 increased its association to PML bodies inhibiting senescence. . We thus propose both pro- and anti-senescence functions for SUMOylation and discuss our findings in light of the well-known association between UBC9 expression and cancer.

Inhibition of the 60S ribosome biogenesis GTPase LSG1 induces senescence with a restricted senescence-associated secretory phenotype

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Ribosome biogenesis represents a highly conserved biological process that is required to fuel cell division and growth. Under normal conditions, ribosome biogenesis is tightly regulated to maintain adequate ribosomal content in the cell. However, several oncogenes promote ribosome biogenesis and elevated levels of this process are often found in cancer cells, to support their high biosynthetic demands. Hence, ribosome biogenesis is a pathway that might provide candidate targets for therapeutic intervention. Our research aimed at assessing whether inhibition of late stage biogenesis of the 60S ribosomal subunit would result in tumour suppressive cellular responses. We focused upon the GTPases EFL1 and LSG1 that catalyse the last two cytoplasmic reactions in the biogenesis of the 60S subunit.

Our results demonstrate that RNAi-based silencing of the two GTPases in human lung fibroblasts triggers a potent induction of ribosomal stress-induced senescence, characterised by activation of the p53 and p16/RB pathways. Inhibition of p53 allowed initial bypass of senescence, but was unable to rescue the long-term growth arrest, indicating that the ribosomal stress-induced senescence may halt tumourigenesis even when p53 has been lost. Preliminary data obtained in a 3D mammosphere culture model also indicate that inhibition of 60S biogenesis could elicit an antiproliferative response in transformed cells, potentially in a p53-independent manner. Further investigation utilising gene expression analysis revealed a minimal Senescence-Associated Secretory Phenotype (SASP) that lacks the canonical pro-inflammatory signature of the oncogene-induced senescence (OIS) SASP and is restricted to members of the TGF-β family.

In summary, we introduce a novel form of ribosomal stress-induced senescence with a restricted SASP that holds promise as a cancer therapy.

Investigating the role of p21 in the regulation of developmental senescence

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The identification of the senescence program in the non-pathological setting of the developing embryo has generated exciting new questions in both senescence and in developmental biology research. Indeed, senescence has been demonstrated to be widespread in the embryo with suggested physiological roles in development. Such roles may include the promotion of plasticity and tissue growth to guide normal embryonic patterning through SASP signaling, as well as tissue remodeling through the timely elimination of senescent cells.

In this project, we investigate how senescence contributes to the complex patterning of the embryo by studying the CDKN1A (p21) cell-cycle inhibitor, which has previously been shown to be a key regulator of developmental senescence. In particular, we are interested to investigate if p21 is sufficient to induce developmental senescence, or if it contributes to the survival of senescent cells. First, to gain insight into the functional role of p21 during developmental senescence, we investigate its endogenous expression and correlate this with developmental senescence patterning. This is performed by studying SAß-gal activity and in situ hybridization for p21 and other developmental and senescence markers. To investigate further, we are in the process of studying in vivo models where we manipulate p21 in the embryo. Specifically, we induce p21 expression in ovo via the administration of Trichostatin A or via p21 electroporation into the neural tube and the wing bud in chick. In another model, we acutely shut down p21 expression at specific developmental stages via an inducible p21 knock-down mouse model. Together, we anticipate the identification of the mechanisms by which p21 contributes to the developmental senescence program.

Role of integrin beta 3 in ageing

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Ageing is characterized by a decline in tissue homeostasis. Cellular senescence is defined as an induced proliferative arrest to prevent the propagation of damaged cells in a tissue. Besides, it has been associated with the normal ageing process. Integrin signalling regulates diverse functions in cancer, angiogenesis, stemness, drug resistance, fibrosis and wound healing. Recent studies of our group have demonstrated that integrin beta 3 regulates cellular senescence by activating the TGF-β pathway. However, the molecular and cellular mechanisms implicated in ageing process are not clear. Here, using an in vitro model of human primary fibroblasts from young and old donors we investigated the expression levels of integrin beta 3. We found that old primary fibroblasts express higher levels of integrin beta 3 than the young ones. Next, we decided to determine whether reducing integrin beta 3 mRNA levels from old donors could attenuate ageing. Using RNAi technology in old fibroblasts, reveal that integrin beta 3 is a regulator of ageing in human primary fibroblasts derived from old donors. Furthermore, we found increased expression of integrin beta 3 in a subset of mouse tissues from old mice. Altogether, our results suggest that an increase in integrin beta 3 levels is a hallmark of tissues during ageing. Due to these, we anticipate our findings to be the starting point to study the role that integrin beta 3 plays in an ageing model of intercellular communication.

Proteotoxicity links therapy-induced cancer cell senescence to Alzheimer's disease

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Senescence is associated with secondary features such as the senescence-associated secretory phenotype (SASP) that comprises pro-inflammatory cytokines, immune modulators and extracellular matrix factors. SASP contributes to the proteotoxic stress associated with senescence by exhausting the capacity of cells to maintain accurate protein synthesis, post-translational processing, vesicular transport and secretion. Autophagy induced under these conditions appears as an effort to cope with this proteotoxic stress (Dörr-JR et al., Nature, 2013). Notably, similar cellular stresses can be observed in Alzheimer's disease (AD), which can be referred to as a protein misfolding disease due to the accumulation of abnormally folded beta amyloid (Aβ) and Tau amyloid proteins in the brain, triggering neuron degeneration. Interestingly, two apparently unrelated cellular processes – accumulation of Aβ peptides in AD and extensive production of SASP factors in cellular senescence – both lead to proteotoxicity in their respective conditions.

The aim of this project is to probe whether a link between cellular senescence and AD might exist. Intriguingly, our data, based on gene set enrichment analyses and RQ-PCR, indicates an upregulation of AD-associated genes in therapy-induced senescence (TIS) in Eμ-myc-driven B-cell lymphomas, suggesting a functional role of the AD machinery in TIS. This upregulation was only seen in the presence of SASP, underscoring proteotoxicity as the link between these two pathologies. Additionally, we were able to show that senescence precedes plaque formation in the AD-inflicted mouse brain. We also observed a correlation between the APP status and markers of cellular senescence. Ongoing research aims at finding common links between AD and the senescence machinery that could serve as therapeutic targets to facilitate clearance of senescent cells in a patient after chemotherapy.

Proteotoxicity induced by misfolded proteins can be considered as the common denominator of neurodegenerative diseases and TIS of cancer cells. This systems medicine-driven approach will provide novel insights into both pathologies, and may lead to re-purposing of therapeutic strategies across distinct disease entities.

Can the inflammatory facet of SASP be controlled?

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Senescence is an engrained phenomenon inducing cell proliferation arrest once a certain checkpoint has been reached, controlling the programmed number of divisions inherent to each cell-type. Senescence can also be accelerated by various types of stress, including inflammation. Besides, senescent cells themselves can develop a specific secretion profile promoting inflammation (SASP). Sustained inflammation, intrinsically deleterious on the microenvironment, moreover can contribute to the degeneration of stem cells and their niche, compromizing tissue regeneration.

Cell senescence translates, among other effects, in an increased expression of surface adhesion molecules, notably on endothelial cells, thereby increasing leukocyte and metastasis recruitment, sustaining chronic inflammation and favoring cancer spreading. Concomitantly, stressed/senescent cells display increased actin polymerization, giving rise to intracellular “stress fibers” that facilitate gene transcription by several transcription factors, including NF-kB. An interesting way to counteract deleterious/pro-inflammatory senescence would be to decrease the expression of adhesion molecules and regulate actin metabolism. Here we report that a natural monoterpene indeed displays such activities.

Monoterpenes have long been shown to display anti-tumoral and soothing effects. We have demonstrated that d-Limonene exerts significant anti-stress and anti-inflammatory properties. Indeed, in animal and human models, this monoterpen decreases systemic levels of such pro-inflammatory cytokines as TNF-alpha and IL-6. These cytokines activate the NF-kB pathway, which can be inhibited by d-Limonene.

Both in vitro and in vivo, d-Limonene was also demonstrated to inhibit the NF-kB dependent expression of adhesion molecules (ICAM-1, VCAM-1, P-selectin) on endothelial cells. Furthermore, d-Limonene significantly interferes with actin polymerization leading to reverse F-actin fibers into G-actin monomers.

Thus, d-Limonene induces both an actin-dependent reinternalization of surface adhesion molecules and a blockade of their synthesis by inhibition of the NF-kB pathway. d-Limonene thus strategically inhibits major pro-inflammatory cellular mechanisms.

Since actin is also strongly involved in the generation of autophagic phagophores, d-Limonene also modulates autophagy, known to be blocked by an inflammatory microenvironment. This could explain how d-Limonene promotes cell repair/rejuvenation.

From these studies, it appears that d-Limonene, a non-toxic monoterpene with anti-inflammatory and anti-tumoral properties, is able to control a number of related cell-mechanisms involved in cell senescence, including SASP.

POSTER SESSION

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mTOR inhibition ameliorates senescence independently of the SASP in a mouse model of chronic inflammation

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Cellular senescence is characterised by enhanced secretion of pro-inflammatory mediators; the so-called senescence-associated secretory phenotype (SASP). Senescence is causally implicated in ageing and in the development of age-related diseases, including various respiratory disorders. The mechanistic target of rapamycin (mTOR) plays important roles in ageing and inhibition of mTOR with rapamycin extends lifespan in several model organisms and delays onset of age-related pathology. It was suggested that rapamycin may exert these effects by supressing senescence-associated inflammation. We aimed to determine whether a rapamycin-supplemented diet could ameliorate phenotypes of accelerated senescence and premature ageing in a mouse model of chronic inflammation, whereby NF-κB activity is enhanced due to knockdown of the inhibitory nf-kb1 subunit (*nfkb1-/-*). While no significant differences in the mean and maximum lifespan were found, overall healthspan was improved in *nfkb1-/-* mice fed with a rapamycin-supplemented diet, including improvements in neuromuscular coordination and long-term memory. Various tissues were also improved by a rapamycin-supplemented diet, including the heart and skin. In the lung, enlargement of alveolar airspaces (indicative of emphysema) observed in *nfkb1-/-* mice was significantly reduced with rapamycin and a number of senescence- associated markers were decreased including telomere-associated damage and p16 expression. Moreover, *in vitro* treatment with rapamycin decreased senescence markers in mouse adult fibroblasts (MAFs) from *nfkb1-/-* mice, including mitochondrial-derived reactive oxygen species (ROS), following X-ray irradiation. However, there were no changes in SASP-associated pro-inflammatory mediators in the serum and lungs of *nfkb1-/-* mice following rapamycin supplementation. Our data suggest that pathways regulating the SASP and cell-cycle arrest are independent when NF-κB activity is enhanced. Beneficial effects of rapamycin may be due to improved mitochondrial function in the *nfkb1-/-* mouse model.

Senescence of renal stromal mesenchymal-like cells contributes to generate a pro-inflammatory micro-environment associated with ageing

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Incidence risk of diseases associated with chronic inflammation such as chronic kidney diseases increases in the elderly. Age-related renal changes are now associated with the induction of cellular senescence programs. The accumulation of senescent cells during ageing and the senescence-associated secretory phenotype leading to inflammaging appear deleterious and account for progressive organ dysfunction. To date, the cellular actors implicated in the chronic inflammation in the kidney during ageing are unknown. The evolution of kidney macrophage pool with aging is not fully characterized and the tissue-derived signals controlling macrophage dynamics are unknown. The objectives of this project are to determine the role of resident kidney mesenchymal-like stromal cell and macrophage crosstalk as regulator of kidney physiology during ageing and kidney injury recovery. Deregulation of this partnership with ageing could participate to the increased risk of renal failure in the elderly.

New proteins discovery in senescence induction and their diverse outcomes

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A complete reorganization of the cellular genome occurs early during senescence induction. These changes manifest as a redistribution of chromatin marks at specific genes. However which proteins are necessary to remodel the chromatin, and whether they are connected to senescence signaling remains a real black box. Therefore a screen has been set up in the laboratory in order to answer this fascinating question. Of these preliminary siRNA hits, we decided to focus on three interesting candidates that are implicated in chromatin dynamics. I confirm the role of these three proteins in senescence by establishing several stable WI-38 hTERT cell lines exhibiting pTRIPZ doxycycline inducible shRNA vector. I use SA-β-galactosidase (SA-β-gal) staining as a readout for cellular senescence as well as EdU incorporation. Moreover, preliminary gene profiling arrays already confirm that cells are entering cellular senescence as I observe down-regulation of cell cycle genes and up-regulation of senescence marker genes. We are currently determinating i) specific pathways involved in triggering JMJD2C, HDAC7a and UBE2N senescence ii)what are the different outcomes of those three different senescence types, particularly regarding the secreted factors.

Extracellular citrate is a robust marker of cellular senescence induced via both p53 dependent and independent mechanisms but is regulated in a distinct manner from the senescence-associated secretory phenotype

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Citrate has been identified as a marker of human ageing, we hypothesise that the rising blood concentration of citrate in humans over the age of 50 is a result of increasing numbers of senescent cells which also accumulate with age. Plasma citrate is resistant to haemolysis and stable at room temperature for at least 26 hours making it a potential clinical biomarker of senescent cell status. Previously we have demonstrated using untargeted and targeted metabolic profiling that accumulated extracellular citrate (EC) is a marker of irreparable DNA double strand break (IrrDSB) induced premature senescence and proliferative exhaustion induced senescence in fibroblasts. We now show that extracellular citrate (EC) is repressed by the ectopic expression of the catalytic subunit of telomerase, *TERT*, but not by a variant of *TERT*, *TERT-HA*, that does not lengthen telomeres or immortalise the cells showing the EC is regulated by telomere length and supporting its role in detecting irreparable DNA double strand breaks. EC is also induced when senescence is induced by low dose (0.5 mM) sodium butyrate (NaB) which we previously showed was mediated by $p16^{INK4A}$ and not by p53 or $p21^{WAF}$. Therefore, EC is a generic marker of fibroblast senescence and not just limited to senescence-induced by irreparable DNA double strand breaks. The mechanism behind the rise in EC is not yet understood, however we have begun to investigate the relationship between EC and the senescence-associated secretory phenotype (SASP). The kinetics of EC accumulation is similar to the SASP protein interleukin 6 (IL-6) and it is repressed by p53 independently of $p21^{WAF}$ and senescence itself. However, EC in senescent fibroblasts cannot be blocked as efficiently as IL-6 by addition of corticosteroids, suggesting the regulation of EC differs from the regulation of the SASP. Furthermore the induction of IL-6 by high dose (4 mM) NaB, which has been attributed to a DNA double strand break-independent mechanism, does not induce EC. EC therefore is potentially a robust marker of senescent growth arrest that is useful in the study of ageing and related pathologies *in vivo* but is not a surrogate marker of the SASP.

Protein and chemotherapy profiling of extracellular vesicles harvested from therapeutic induced senescent triple negative breast cancer cells

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Triple negative breast cancer (TNBC) is an aggressive form of breast cancer with relatively poor clinical outcomes and limited treatment options. Chemotherapy, while killing cancer cells, can result in the generation of highly chemoresistant therapeutic induced senescent (TIS) cells which have the potential to form stem cell niches resulting in metastases. Our aim was to identify mechanisms by which TIS TNBC cells maintain survival in the face of chemotherapy. TIS was induced and confirmed in Cal51 TNBC cells. Mass spectroscopy (MS) analysis was carried out on extracellular vesicles (EVs) harvested from control compared to senescent Cal51 cells with in silico analyses of the MS data undertaken using STRING, Ingenuity Pathway Analysis and Innate DB programs. We demonstrate that TIS Cal51 cells treated with 75nM paclitaxel for 7 days became senescent (β-galactosidase [SA-β-Gal] positive, Ki67 negative, increased p21 and p16, G2/M cell cycle arrest) and released significantly more EVs than non-senescent control cells ($p \leq 0.01$). Moreover, TIS cells displayed an increased expression of the multidrug resistance protein 1/p-glycoprotein (MDR1/P-gp). MS analysis demonstrated that EVs derived from senescent Cal51 cells contained 142 proteins with a significant increased fold change compared to control EVs. A fluorescent analogue of paclitaxel (Flutax-2) was utilised to show the removal of chemotherapy in EVs from senescent cells. The main findings of this study are: (1) TIS cells release significantly more EVs than control cells and (2) TIS cells maintain viability via upregulation of MDR1/P-gp and the removal of key proteins and chemotherapy in their EVs.

Function and Regulation of the IL-1 Pathway in the Senescence-Associated Secretory Phenotype

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Preventing benign lesions from progressing to malignant transformation remains a priority in the field of cancer therapy. An increasingly attractive strategy to enhance the barrier to malignancy is the induction of cellular senescence, a stable form of growth arrest induced by stresses including DNA damage and oncogene activation. Although senescent cells no longer divide, they remain metabolically active and secrete a defined set of cytokines, growth factors, and proteases, collectively termed the senescence-associated secretory phenotype (SASP). These factors paradoxically have both pro- and anti-tumorigenic properties depending on tissue context. Notably, the *in vivo* effect of the SASP is poorly understood, as it has been difficult to study the effects of the SASP independently of other senescence-associated phenotypes, such as cell-cycle exit. Consistent with previous data, we show here that the SASP factor IL-1α acts as an upstream regulator of the SASP, and is necessary and sufficient to induce SASP factor expression. IL-1α acts through the IL-1 receptor (IL-1R) to initiate the SASP, and is required for SASP maintenance. Importantly, ablation of IL-1α or IL-1R does not affect the cells' ability to senesce, providing us with a system to study the effects of the SASP independently of cell-cycle exit. Transcriptome profiling of IL-1R-depleted senescent cells indicate that IL-1R controls the late arm of the senescence secretome, which consists of pro-inflammatory cytokines mediated through the transcription factor complex NF-κB. Finally, genetic inactivation of IL-1α in the mouse impairs pancreatic cancer progression and suggests that abrogation of the SASP may delay immune cell infiltration or activity, thereby hindering the progression of malignant lesions. These findings may provide insight into the therapeutic potential of SASP manipulation in inflammatory cancers.

Clinically used T-type Ca2+ channel antagonists impede extracellular vesicle (EV) release from Triple Negative Breast Cancer (TNBC) cells

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Triple-negative-breast-cancers (TNBCs) are complex, aggressive tumours with a high recurrence risk, partially attributable to the development of chemo-resistant therapeutic–induced-senescent (TIS) cells. Senescent cells secrete not only the Senescence-Associated-Secretory-Phenotype (SASP), but also release more extracellular vesicles (EVs) than non-senescent cells thereby impacting on the tissue microenvironment, both locally and distally. Importantly, modulation of intracellular calcium (Ca2+) levels with clinically used Ca2+ channel antagonists, impacts on cell-to-cell communication via EV secretion. Our hypothesis is, that impeding EV release abrogates the ability of TIS cells to interact with the TME and potentially results in their demise.

Following induction of senescence in CAL51 TNBC cells with a 7 day 75nM Paclitaxel-treatment, TIS cells demonstrated increased EV secretion compared to non-senescent cells ($p \leq 0.01$). Low doses of T-type Ca2+ channel antagonists, Mibefradil and NNC-55-0396, resulted in (i) a decrease in the basal intracellular Ca2+ levels in CAL51 cells and (ii) reduced the levels of EVs released compared to controls. This identifies clinically used T-type Ca2+ channel antagonists as potential treatments to impede extracellular vesicle (EV) release from Triple Negative Breast Cancer (TNBC) cells, establishing it as a novel mechanism to reduce potential EV-derived effects.

In summary, our data highlights a potential new way to repurpose Ca2+channel antagonists to target EV release from TIS cancer cells and suggests their use in novel mono or polytherapies for blocking the senescence-induced transfer of chemo-resistance in TNBC by EVs thereby abrogating in part, the continued communication of senescent cancer cells with the tumour microenvironment (TME).

Collaborative regulation of the senescence secretory program by DNA-damage independent ATM activity and acetyltransferases

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Cellular senescence is a tumor suppressor mechanism that prevents proliferation of damaged cells following exposure to genotoxic stresses like telomere shortening, ionizing radiation or cytotoxic drugs. The senescent phenotype is characterized by different senescence-associated (SA) programs including a permanent growth arrest (SAGA) and production of a pro-inflammatory secretory phenotype (SASP). Cancer therapies can trigger SA programs in cancer and non-cancer cells, including the SASP, which can strongly influence tumor microenvironment evolution with both beneficial and deleterious effects on cancer progression. A better understanding of the mechanisms regulating the SA programs could help to improve beneficial aspects of the therapy-induced senescence.

Activation of the DNA damage response (DDR) is critical for promoting varied SA programs including SAGA and SASP. Interestingly, the regulation and impact of the DDR is different between SAGA and SASP. While the DDR and cell cycle checkpoints leading to SAGA occur within minutes after DNA lesions, the DDR-dependent SASP requires days to develop. Here we show that histone deacetylase inhibition triggers an accelerated ATM-dependent SASP, implicating chromatin remodeling and acetylation events in SASP maturation. Indeed, in addition to MRN/ATM, chromatin recruitment of KAT5/TRRAP, CBP/p300, and PCAF/GCN5 complexes coincided with SASP induction, whereas their depletion compromised the SASP. Importantly, hyper-acetylation combined with low level DNA damage triggered a synergistically accelerated SASP within a few hours, implying that the SASP program relies on both low DDR activity and an acetyltransferases network. Thus, delayed chromatin recruitment of acetyltransferases cooperates with residual low-level DDR activity to ensure activation of the SASP in the context of senescence, not in response to transient DNA damage-induced growth arrest. This sheds light on a temporal layer of complexity during SA programs that could be exploited to target selected SA phenotypes including SASP

Chronic treatment with resveratrol and olive oil phenols on MRC5 fibroblast SASP: inhibition of pro-tumoral microenvironment

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Cellular senescence can be defined as “a double-edged sword”: even if it is considered a tumor-suppressive process, it is characterized by a strong increase in secreted factors termed the “senescence-associated secretory phenotype” (SASP). SASP from stroma senescent fibroblasts provides a possible target for anticancer interventions creating a cancer-favoring microenvironment. SASP factors induce the expression of epithelial-to-mesenchymal transition (EMT) markers and drive endothelial cells toward a pro-angiogenic phenotype, emerged as integral process in promoting carcinogenesis.

Polyphenols are well-known anti-senescence compounds and have been proposed as potential anti-cancer therapeutics.

We used the chronic treatment (5 weeks) with low resveratrol concentration (5μM) to modulate senescence-related pro-tumoral features of human MRC5 fibroblasts. The expression of SASP factors was increased in senescent fibroblasts, while resveratrol chronic treatment was capable of reducing levels of interleukins (1α, 1β, 6 and 8), TGFβ, MMPs (2 and 3), uPA, receptors (uPAR, TGFβ-R2, IGF-1R and CXCR4) and of NF-kB, a master regulator of SASP.

TGFβ-mediated activation of fibroblasts to myofibroblasts, detected by alpha-SMA expression, was inhibited by MRC5 pre-treatment with resveratrol.

After resveratrol treatment, conditioned media (CM) were collected from MRC5 cultures and delivered to A375 and A375-M6 melanoma cell cultures to mimic tumor microenvironment. After 48h incubation with CM from resveratrol-treated fibroblasts, a reduction in tumor cell proliferation and invasiveness and in the expression of epithelial-to-mesenchymal transition markers was found in both melanoma cultures, compared to incubation with CM from senescent untreated MRC5.

Also endothelial progenitor cells (EPC) were incubated overnight with CM from MRC5. A negative modulation in the expression of angiogenetic markers was observed in EPC after incubation with CM from resveratrol-treated fibroblasts compared to CM from senescent fibroblasts. Accordingly, a reduction in tubular-like structures and in the capillary network was detected as well as in the number of migrated cells.

By using the same chronic protocol, MRC5 fibroblasts were treated with other polyphenols such as oleuropein and dihydroxytyrosol and similar results were obtained in preliminary experiments.

On the whole, chronic treatment with polyphenols might be useful to counteract the detrimental effects of cellular senescence, decreasing a wide range of SASP factors.

Senescence associated secretory phenotype is DNA damage and DDR independent in human vascular smooth muscle cells undergoing senescence upon NOX4 depletion

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One of the very important effector mechanisms of cellular senescence is senescence associated secretory phenotype (SASP). A number of SASP components have been identified and they belong to the family of interleukins, chemokines, growth factors, proteases etc. Both detrimental and beneficial aspects of SASP has been reported depending on the cell type or senescence-inducing stimuli. Among several different signaling pathways and molecules that were shown to regulate SASP, induction of double strand DNA breaks (DSB) and DNA damage response (DDR) activation are considered as the most common trigger of both senescence associated growth arrest (SAGA) and SASP. Indeed, using human vascular smooth muscle cells cultured *in vitro*, we have shown that doxorubicin or H_2O_2 treatment led to the induction of senescence accompanied by increased secretion of IL-6, IL-8 and VEGF. As expected, inhibition or downregulation of ATM kinase – a master DDR signaling molecule, facilitated lowering of SASP factors secretion. Interestingly, downregulation of NOX4 oxidase, a ROS producing enzyme, which stimulates vascular smooth muscle cells proliferation, resulted in permanent growth arrest and induction of senescence. Importantly, NOX4 depletion-induced senescence was neither accompanied by DSB nor DDR induction. However, the increased secretion of selected SASP factors was detected in conditioned medium from NOX4-depleted, senescent cells. The SASP induction correlated with activation of p38 kinase. Moreover, the amount of IL-6 secreted by cells that underwent DDR-independent senescence was comparable with the amount of the interleukin produced by cells forced to senescence by DSB induction and DDR activation. Secretion of IL-8 was even higher in NOX4-depleted senescent cells. Altogether, our results showed that inhibition of important signaling pathways stimulated by NOX4-derived ROS contributes to senescence of smooth muscle cells, which potentially could increase local inflammation. Since specific NOX isoform inhibitors are considered as potential drugs for treatment of vascular pathologies, the possibility that they may induce senescence of cells building the vasculature should be carefully considered.

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Extracellular prostaglandins E1 and E2 are upregulated in a subset of oral premalignant keratinocytes and senescence but differentially regulated by p53

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Aneuploid oral premalignant lesions (OPMLs) have a greater probability of progression to malignancy than their diploid counterparts. We showed using an unbiased metabolomic screen of conditioned medium from confluent cultures that diploid OPML keratinocytes have increased levels of prostaglandins (PGE1 and PGE2) that are almost exclusively absent in their aneuploid counterparts, the exception being line D4. These results were confirmed using competitive and sandwich ELISAs and extended to include the cytokines IL-1 alpha, IL-1 beta, IL-6 and IL-8. We also showed that keratinocytes rendered immortal by the defined disruption of p53 and $p16^{INK4A}$ coupled with ectopic telomerase expression (OKF4) or diploid OPML cells treated with the ROCK inhibitor Y-27632 had reduced or absent PGE1/2 over-expression, indicating that one or more mutations involved in keratinocyte immortalisation may regulate the PGEs and ILs. However, a survey of DNA damage response pathways revealed that the diploid OMPL did not show elevated phosphorylation of p53 or the Ataxia Telangiectasia Mutated protein indicating that other mechanisms of senescence or Y-27632 are involved in PGE regulation. Surprisingly, OKF6-TERT1 that differed from OKF4 only in that it had retained wild type p53 continued to over-express the PGEs and ILs and was used as a model to study their regulation further. p53 knockdown (80%) in OKF6-TERT1 reduced PGE1 levels but not PGE2, IL6 and IL8 indicating that the loss of p53 in aneuploid OPMLs may only be partially responsible for the regulation of the PGEs and ILs. OKF6-TERT1 was also treated with cyclooxygenase 1 and 2 inhibitors and whilst as expected the PGEs were almost eliminated, the ILs remained high showing that in OKF6-TERT1 cells at least, PGEs are not necessary for the high levels of ILs. However, a closer examination of microarray data mined from the cell line panel revealed that D4 over-expressed IL-1 alpha which is known to be a driver of the senescence-associated secretory phenoptype (SASP) and an inducer of PGE2 and D4 also over-expressed other SASP transcripts which were also transcriptionally downregulated by ectopic telomerase. The role of IL-1 alpha in regulating the PGEs in diploid and aneuploid dysplasias is currently being investigated.

Integrin beta 3 induces a TGF beta rich secretome during senescence activation

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Senescent cells are characterised by a permanent cell cycle arrest and the release of a secretome formed by soluble factors, growth factors and enzyme-degrading proteins, termed SASP (senescence-associated secretory phenotype). The SASP is the main responsible for inducing a variety of non-cell autonomous responses including enhancing proliferation, senescence, immune responses and cellular plasticity. However, how the SASP is regulated is still largely unknown. Here, we have identified the integrin subunit beta 3 (*ITGB3* or beta3) as a novel marker and regulator of senescence. We find that *ITGB3* expression accelerates senescence in human primary fibroblasts by inducing a TGF beta-rich secretome. This is mediated by enhancing the expression of the metalloproteases (MMPs) 1 and 9, which promote the release of TGF beta from the latent TGF-beta binding proteins (LTBPs). Altogether, TGF beta induces senescence in an autocrine and paracrine fashion, where pan-specific neutralizing anti-TGF beta 1–3 antibodies and TGF beta receptor inhibitors block non-cell autonomous senescence induced by *ITGB3* expression. Surprisingly, an alpha-v-beta-3 antagonist, cilengitide, uncouples the senescence response induced by H-RAS^{G12V} expression. While, genetic manipulation of *ITGB3* overcomes senescence, cilengitide, only prevents the induction of the SASP without altering the proliferation arrest or cell cycle inhibitors. Altogether, our data reveal the importance of cellular adhesion during senescence and identify integrin as regulators of the SASP.

Senescent human fibroblasts show extracellular metabolomes that overlap with those of irreparable DNA damage, ageing and disease; evidence for the potential clinical utility of extracellular citrate and its independence from terminal differentiation

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Cellular senescence can modulate various pathologies and is associated with irreparable DNA double strand breaks (IrrDSBs). To identify novel non-invasive markers of senescent cells, extracellular senescence metabolomes (ESMs) were generated from five lines of human fibroblast rendered senescent by proliferative exhaustion (PEsen) or 20 Gy of γ rays (IrrDSBsen) and compared with those of young proliferating cells, confluent cells, quiescent cells and cells exposed to repairable levels of DNA damage. ESMs of PEsen and IrrDSBsen overlapped and showed increased levels of citrate, molecules involved in oxidative stress, a sterol, mono-hydroxylipids, tryptophan metabolism, phospholipid and nucleotide catabolism, as well as reduced levels of dipeptides containing branched chain amino acids. The ESM overlaps with the ageing and disease body fluid metabolomes, supporting their utility in the non-invasive detection of human senescent cells *in vivo* and by implication, the detection of a variety of human pathologies. One ESM metabolite, citrate, is readily detectable in human serum and plasma and was investigated further. Extracellular citrate (EC) is not merely related to the increase in senescent cell biomass; levels inside the cell decline relative to protein and stay the same on a per cell basis, whilst EC accumulates to about 11 fold in the same experiments. To test cell types other than fibroblasts we induced IrrDSBs in human keratinocytes in serum-free medium low calcium medium (0.09mM) and also tested the effect of terminal differentiation by raising the calcium concentration of the medium to 0.4 mM permit both optimum proliferation and stratification. Under these conditions the keratinocytes detached following 10 Gy of ionising radiation and EC either fell (0.9 mM calcium) or remained the same (0.4 mM calcium) suggesting the induction of apoptosis or terminal differentiation of the senescent cells. However, despite a considerable increase in cell size and increased differentiation in 0.4 mM calcium there was no reproducible increase in EC showing that it is not induced by terminal differentiation and also emphasising that increased EC is not simply a consequence of the increase in biomass seen in senescent fibroblasts.

Loss of glycoproteins in the inflammatory secretome of normal senescent fibroblasts is associated to ER-stress-related altered trafficking routes

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background: Senescence is a cellular process activated by oxidative and oncogenic stresses, pharmacological treatments or telomere shortening. Although growth-arrested, senescent cells are biochemically active, and accumulate in ageing tissues. Secretomes of senescent breast and prostate fibroblasts showed dramatic increases of inflammatory molecules and affect immune responses. Alternately inflammation can propagate senescence.

Non-Melanoma Skin Carcinomas (NMSC) are age-related diseases that may be considered as warning cancers as linked to a higher risk of being diagnosed for another carcinoma. We showed that senescent secretome from Normal Human primary Dermal Fibroblasts (NHDF) promotes early carcinogenesis of ageing autologous normal epidermal keratinocytes. This involved Matrix MetalloProteinases (MMPs) among the NHDFs inflammatory secretome. Defects in the synthesis or the maturation of secreted proteins lead to endoplasmic reticulum (ER) stress detected by a quality-control machinery that refines protein synthesis, folding or secretion.

Objectives: Better characterize the secretome of senescent NHDFs. Investigate a link with altered secretory compartments. Correlate in vitro defects with in vivo expression in biological samples.

methods: Comparative proteomic analysis of the culture media of young and senescent human normal dermal fibroblasts (NHDFs). Paraffin-embedded skin samples from young and aged healthy people used to verify by immunohistochemistry the differential expressions of secreted molecules in the dermal compartments. In vitro immunofluorescence studies to investigate the different secretory compartments affected by senescence. The involvement of the different ER stress sensors was investigated by transcriptional analysis of their target genes expression, western blotting and manipulated by siRNA transfection in senescent NHDFs.

results and conclusions: Albeit common inflammatory features with that of other organs (high interleukins, cytokines and MMPs secretions), the secretome of senescent NHDFs presents a severe reduction in several glycoproteins with anti-tumoral properties. Their absences were validated in aged healthy human skin samples. Their secretions rather than expressions were altered in senescent NHDF. The organelles and vesicular compartments were affected, and glycoproteins targeted to the lysosomal degrading pathway. The secretory defects were associated to an ER stress. Senescence-related ER stress may then influence the composition of the secretome of senescent cells and perturbate tissue function and promote carcinogenic progression.

Exosomes Can Be Isolated From the Conditioned Media of Senescent Human Mammary Fibroblasts

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Extracellular vesicles are becoming increasingly 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 miRNAs and mRNAs. Here, we demonstrate that exosomes may be isolated from the conditioned media of proliferative (passage 16) Human mammary fibroblasts (HMFs) as well as those cultured to replicative senescence (passage 29), via a protocol of successive centrifugation steps (300 x g, 2000 x g, 10,000 x g and 100,000 x g). Characterisation of exosomal size and concentration was determined using both LM20 Nanosight nanoparticle tracker (Malvern UK) and transmission electron microscopy.

The established paracrine signalling of the senescent-associated secretory phenotype (SASP) is hypothesised to be a major mechanism through which senescent cells influence their local environment and may contribute to the propagation of disorders associated with age. Increased production of exosomes may represent an additional signalling mechanism which may complement the actions of the SASP and constitute a further mechanism through which senescent cells may influence their microenvironment and potentially contribute to age related disorders. Our latest findings comparing conditioned media investigations with isolated exosome preparations will be presented.

Regulation of senescent cell viability by ROS-NO balance

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One of the well accepted theory of aging as well as cellular senescence is the Free-Radical Theory of Aging (FRTA). The theory state that accumulation of free radicals overtime causes genomic damage, which drives cellular senescence and aging. The goal of our study was to identify the genes which regulate ROS and NO levels during cellular senescence, especially when the DNA damage driver is not oxidative stress. To achieve this goal, we utilized an agent which causes direct DNA damage and triggers senescence. Using this model, we evaluated the modulation of ROS and NO levels in cells, where the expression of one the 50 genes known to alter ROS and NO levels which was knocked down using specific shRNAs. Almost all the genes affected levels of free radicles but we were also able to identify the genes which regulate free radical balance specifically during cellular senescence. Our screen allowed us to identify few genes, which included genes like Nrf2, iNOS, STAT, AP1, AKT, PI3KCD, CAT, which modulated the levels of free radical in non-damaged cells and accelerated senescence onset. Furthermore, we confirmed the role of these genes using microarray data analysis and we found that most of genes which regulate antioxidant genes significantly downregulated during cellular senescence. To sum up, we found ROS-NO are highly critical for cellular senescence induction and maintenance of viability of damaged cells.

Transcriptional repression of DNA repair genes is a hallmark and a cause of cellular senescence

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Cellular senescence response is (i) activated by numerous stresses, (ii) is characterized by a stable proliferation arrest, and (iii) results in the acquisition of a specific secretome. Timely regulated senescence is thought to be beneficial, whereas chronic senescence such as during normal or premature aging is deleterious as it favors most, if not all, age-related diseases. In this study, using in-house or publicly available microarray analyses of transcriptomes of senescent cells, as well as analyses of the level of expression of several DNA repair genes by RT-qPCR and immunoblot, we show that repression of DNA repair gene expression is a hallmark of senescent cells. This repression is mediated by the RB/E2F pathway and it may play a causal role in senescence induction, as single DNA repair gene repression by siRNA was sufficient to induce premature senescence. Importantly, activating RB independently of direct DNA damage also results in repression of DNA repair genes and in the subsequent induction of DNA damage and senescence. The dogma is that DNA damage observed during cellular senescence is directly provoked by DNA lesions following genotoxic attack (UV, IR, ROS) or by induction of replicative stress upon oncogenic activation. Our results support a largely unsuspected contribution of the loss of DNA repair gene expression in the induction and the accumulation of the DNA damage observed in most, if not all, kinds of cellular senescence, and thus in the induction of cellular senescence.

Connection between nuclear envelope dysfunction and DNA damage response at telomeres

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Telomeres are nucleoprotein complexes, which protect chromosome ends from damage or from fusion with neighboring chromosomes. In human cells, a subset of telomeres are anchored to the nuclear envelope during postmitotic nuclear assembly. In metazoans, the nuclear envelope is lined by a thin meshwork composed of A- and B-type lamins, which gives structural support to the nucleus and plays major roles in genome organization and stability. Cells expressing a mutated form of lamin A called progerin, show strong nuclear envelope abnormalities accompanied with DNA damage foci at telomeres and accelerated telomere shortening, which leads to premature senescence entry. Mechanism of this harmful effect is still unclear.

Our aim is to elucidate the connection between nuclear envelope dysfunction and DNA damage, particularly at telomeres. First we want to determine when in the cell cycle and where in the nucleus the DNA damage occurs after progerin expression. To this aim, I set up tissue culture models to study the impact of progerin expression on telomere dynamics and integrity. I confirmed that Progerin expression led to major nuclear morphology defects and DNA damage. Next, the accumulation of the DNA damage marker gH2AX was followed in cells during culturing and the timing of senescence entry compared to cells expressing wt Lamin A or a control vector. These experiments will help set up the conditions to perform live cell imaging experiments to follow the occurrence of DNA damage after induction of progerin expression in real time. At the same time we observed that progerin-expressing cells displayed an impaired recruitment of DNA damage protein 53BP1.

Recent work shows that over-accumulation of the nuclear envelope protein SUN1 in progerin expressing cells is pathogenic. We want to determine whether SUN1 mislocalization is also responsible for telomere dysfunction in progerin cells. Using immunofluorescence and co-IP experiments, I verified that SUN1 had a stronger affinity for progerin than Lamin A that led to its accumulation at sites of nuclear envelope blebbing. SUN1 depletion experiments will be performed to assess the pathogenicity of this recruitment for telomere dysfunction.

Molecular beacons to visualize the rhythmicity of the DDR during the senescence program

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Introduction: Genome stability is essential to prevent cancer development. In normal cells, genotoxic stresses like radiotherapy trigger a cellular DNA Damage Response (DDR) leading to cell cycle arrest, notably via the activation of p53 and its transcriptional target, the cyclin-dependent kinase inhibitor $p21^{waf1}$ (CDKN1A). The feedback regulation of the Mdm2-p53 loop during the DDR results in a series of cycles of their activities (rhythmic undulations), allowing cells that have received sub-lethal damages to recover from the DDR. Alternatively, when irreparable DNA lesions activate the senescence program, whether DDR rhythmicity influences senescence-associated (SA) phenotypes remains unknown.

Research Objectives: Our hypothesis is that DDR rhythmicity plays a role in modulating entry into and stability of the SA growth arrest (SAGA). Our data shows that soon after synchronous DNA damage initiation, individual cells become DDR-dephased, and thus the rhythmicity of p53-p21 cannot be assessed in the overall cell population. To solve this hurdle we developed new fluorescent molecular beacons to permit DDR visualization and manipulation in individual cells via the regulation of $p21^{waf1}$.

Progress: These CDKN1A reporter cassettes contain various promoter-protein combos including a $p21^{waf1}$ protein fused to a fluorescent label (p21-Fluo) for analysis by live stage microscopy. We have validated the p21-Fluo beacon using multiple functional DDR tests and found that the rhythmicity of p21 during the early DDR is not due to (post-)translational regulation but rather exclusively depends on p53 to regulate $p21^{waf1}$ mRNA levels. p21-Fluo reporters in combination with cell cycle fluorescent tags (Fucci System) allows us to track DDR rhythmicity during senescence establishment and to study their interplay during long-term cell fate decisions following anticancer therapies.

Contribution to Knowledge Advancement: Several clues have already been acquired in understanding cell fate decisions in response to DNA damaging therapies, but the role of DDR rhythmicity remains unclear. We propose that the concomitant study of DDR and cell cycle rhythmicity in a chronological manner will enable us to further dissect and understand key temporal moments during the senescence program, and highlight new therapeutic avenues to manipulate SA phenotypes.

Investigating the differential effect of mono- and dimeric G4 ligands on telomere dynamics using Single Telomere Length Analysis

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Telomeres are nucleoprotein structures that cap and protect the natural ends of chromosomes by preventing them from being recognized as DNA double strand breaks. Human telomeres are composed of the DNA sequence TTAGGG tandemly repeated into array up to 25 kb and terminates by a 3'-overhang. The G-rich strands of telomeres are capable to fold into contiguous G-quadruplex (G4) structures, which are four-stranded helices arising from the formation of tetrads of hydrogen-bonded Gs. Small molecules that bind G4 structures have been developed in order to target telomeres and interfere with telomere function. G4 ligands can be used as a tool to investigate telomeres biology and they represent potential anti-cancer drugs. A pyridine dicarboxamide ligand, named 360A, previously developed in the lab, can inhibit cancer cell proliferation and induce cell death. While 360A exhibits a preferential binding at telomeres (Granotier C. *et al.*, 2005); this ligand can also bind non-telomeric G4s. A dimer of 360A ($[360]_{2A}$) has been synthesized in order to target contiguous G4 structures. In vitro, $[360]_{2A}$ has a better affinity for telomeric G4s than 360A. G4 formation at telomeres can lead to replication fork stalling resulting in single strand gaps. If unrepaired, these gaps can lead to fragile telomeres and stochastic deletion events. In this study we compared the differential effect of 360A and $[360]_{2A}$ on cell proliferation and on telomere dynamics in a cancer cell line. To this end, we used Single Telomere Length Analysis (STELA) (Baird D.M. *et al.*, 2003), which is a high-resolution single molecule method allowing to determine the full spectrum of telomere length from different chromosome ends and to detect telomere deletion events.

The role of telomeres in melanocyte senescence and skin ageing

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Cellular senescence is an irreversible cell cycle arrest, associated with the secretion of pro-inflammatory molecules, also known as the senescence-associated secretory phenotype (SASP), which can act in a paracrine manner, inducing senescence in neighbouring cells. It is thought that accumulation of senescent cells contributes to loss of tissue function during ageing. Melanocytes positive for senescence markers have been shown in the skin of middle-aged human donors, however, very little is actually known about the mechanisms underlying melanocyte senescence during skin ageing. In this study, I aimed to investigate whether telomere dysfunction is a driver of melanocyte senescence, and whether senescent melanocytes contribute to skin ageing phenotypes by acting in a paracrine manner.

I performed immuno-FISH combining immunofluorescence against γH2AX (a marker of DNA damage) and *in situ* hybridisation for telomere specific PNA probe in skin biopsies from young and older human donors, and found a significant increase in telomere-associated foci (TAF) in melanocytes in skin of older donors. However, by conducting quantitative-FISH I did not find evidence for telomere shortening in melanocytes. In fact, damaged telomeres were significantly longer than telomeres that did not signal a DNA damage response, suggesting that long telomeres may be more susceptible to damage in melanocytes *in vivo*. Furthermore, telomeric damage was significantly higher in keratinocytes surrounding melanocytes with a higher number of dysfunctional telomeres in skin of young and older donors. These results indicate that melanocytes may exert a bystander effect, and contribute to telomere-associated damage in neighbouring cells.

In order to further explore the bystander effect, I co-cultured young and senescent melanocytes with dermal fibroblasts *in vitro*, and found that senescent melanocytes induce higher frequencies of TAF in these cells. Interestingly, conditioned media from senescent melanocytes was sufficient to induce TAF in fibroblasts, suggesting that soluble factors can mediate paracrine damage induction. This corroborates the observations made *in vivo*, supporting the idea that factors secreted by senescent melanocytes can contribute to paracrine telomeric damage induction.

This study provides novel evidence showing that telomere dysfunction is a feature of human melanocyte ageing, and that melanocytes contribute to telomeric damage formation in neighbouring cells.

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Inhibition of oncogene-induced senescence by glucocorticoïds and implication of the EGR1 transcription factor

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Senescence is a cellular response to stress, and corresponds to a stable cell cycle arrest associated with characteristic changes in gene expression or morphological modification. Several types of stress are capable of inducing senescence, including loss/deprotection of telomeric sequences, oxidative stress, DNA damage and oncogene expression (Oncogene Induced Senescence). All of these stresses may occur during human oncogenesis and the senescence response is an important tumor-suppressor mechanism that blocks the proliferation of potentially cancerous cells.

We use a docycycline-inducible B-RAF-V600E where we can finely control expression to trigger Oncogene Induced Senescence (OIS) in human immortalized fibroblast. B-RAF-V600E is a mutation commonly found in various types of cancer (80% of malignant melanoma). We noticed that when we induce senescence through the expression of our oncogene and at the same time treat the cells with glucocorticoïds, the cells were able to continue to proliferate. We have been able to show that the glucocorticoïds effects on the proliferation were due to the regulation of p15-CDKN2B and p21-CDKN1A. By using transcriptomic analysis and a screening of SiRNA, we identified EGR1 as an important regulator of senescence induced by B-RAF-V600E.

Impaired ferritinophagy in senescent cells is associated with iron accumulation

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The healthspan of mice can be enhanced through the removal of senescent cells, which accumulate with age and contribute to chronic diseases and age-related dysfunctions.[1, 2] Iron (Fe) is utilized by many biochemical reactions in the body, but is also implicated in ageing due to accumulating in tissues and its propensity to generate oxidative stress.[3] We describe a novel biological activity of senescent cells relating to altered Fe acquisition and storage. Intracellular Fe accumulation was coupled to cellular senescence irrespective of stimuli (irradiation, replicative or oncogenic) and occurred in senescent cells derived from both human and mouse. Fe accumulation in senescent cells was associated with dramatic changes in the expression profile of relevant homeostatic proteins; with increased transferrin receptor 1 (TfR1) (Fe uptake) and ferritin (Fe storage) coupled with diminished ferroportin (Fe efflux) at the membrane. The magnitude of ferritin overexpression represents a robust biomarker of cellular senescence and of associated Fe accumulation, and conferred resistance to Fe-induced toxicity. Sustained Fe chelation using deferiprone inhibited both Fe accumulation and ferritin upregulation, but did not obstruct cellular senescence development. The Fe accumulation phenotype of senescent cells was associated with impaired ferritinophagy; the process of ferritin being sequestered into autophagosomes and delivered to lysosomes for degradation, which is essential for liberating Fe from ferritin to increase its bioavailability. Impaired ferritin degradation provides an explanation for the Fe accumulation phenotype of senescent cells, by effectively trapping Fe in ferritin and thereby creating a perceived cellular deficiency. Promoting ferritin degradation by using the autophagy activator rapamycin averted the Fe accumulation phenotype of senescent cells, preventing the increase of TfR1, ferritin and intracellular Fe. Furthermore, the enrichment of senescent cells in mouse ageing hepatic tissue was found to accompany Fe accumulation, an elevation in ferritin and mirrored our observations *in vitro* using cultured senescent cells. We have identified consistent and robust characteristics of senescent cells that may provide tangible opportunities to develop biomarkers and senolytic therapeutics.

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The Senesceome: Data Mining System for Investigating Temporal Pathways, Functional Modules and Gene Networks in Cellular Senescence

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Oncogene-induced senescence is a highly dynamic process accompanied by global alterations in gene and protein expression levels: HRAS-G12V activation in human diploid fibroblasts leads to the immediate response of a mitotic stage with enhanced proliferation before progressive transition into a senescent state. It is conceivable that this transition is governed by the timely modulation of distinct gene sets and sequential interaction between individual 'regulatory modules'. However, the inherent heterogeneity of the senescence phenotype has led to a difficulty in identifying a core senescence signature in the establishment and maintenance of the senescence phenotype. This is despite increasing availability of large gene expression datasets from senescence-associated transcriptomic experiments, in part due to the lack of a unifying system in which gene expression data can be compared across datasets. The objective of the Senesceome is to collect these expression datasets and compile them into an open-access database where the data can be queried by a variety of supporting computational tools. By integrating senescence-associated gene expression datasets with 'purpose-built gene sets', along with functional annotation from publicly available databases, the Senesceome would provide a platform for identifying senescence signatures from user-defined gene lists and subsequent computational data mining for their functional relevance.

Histone variant H2A.J accumulates in senescent cells and promotes inflammatory gene expression

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The senescence of mammalian cells is characterized by a proliferative arrest in response to stress and the expression of an inflammatory phenotype. We discovered that histone H2A.J (Uniprot Q9BTM1), a poorly studied H2A variant found only in mammals, accumulates in human fibroblasts in senescence with persistent DNA damage. H2A.J also accumulates in mice with aging in a tissue-specific manner and in human skin. Knock-down of H2A.J inhibits the expression of inflammatory genes that contribute to the senescent-associated secretory phenotype (SASP), and over-expression of H2A.J increases the expression of some of these genes in proliferating cells. H2A.J accumulation may thus promote the signaling of senescent cells to the immune system, and it may contribute to chronic inflammation and the development of aging-associated diseases.

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A Proteomic Atlas of Oncogene Induced Senescence

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Oncogene induced senescence is a stress response pathway characterized by cell cycle exit, metabolic rewiring and the provocation of a secretory program. While many studies have examined regulatory events in the biological process, a global view of proteomic changes across senescence induction has remained to be seen. Therefore, here we present quantitative proteomic data mapping thousands of proteins across ten distinct time points to reveal temporal changes at the protein level following $HRAS^{G12V}$ over-expression in early passage fibroblasts. Further, employing top down proteomics—the study of intact proteins by mass spectrometry—we characterize and quantify differences in mitochondrial protein complexity in growing and $HRAS^{G12V}$-induced senescent fibroblasts. Given the power of top down proteomics to resolve the combinatorial complexity of protein modifications, proteolytic cleavages, and SNPs, this study reveals novel insights regarding primary metabolism and illuminates the intricacy of the mitochondrial proteome. Together this work comprises the largest dynamic, time-resolved proteomic dissection of senescence induction.

HMGBs expression and their relevance in Endothelial Progenitor Cell Function

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Endothelial Progenitor Cells (EPCs) play a significant role in homeostasis and repair of the vascular system. Endothelial Colony Forming Cells (ECFCs) represent a subset of endothelial progenitors which are consistently isolated from human cord blood. Importantly, ECFC reparative capacity can be harnessed as a promising therapeutic option for ischaemic diseases. Accumulating evidence indicates that smoking, diabetes and an inflammatory tissue microenvironment reduce ECFC vasculogenic potential, and this has been associated with premature senescence. Understanding the mechanisms leading to the loss of vasoreparative ability and establishment of premature senescence in these cells may facilitate design strategies to restore their functionality.

HMGB proteins are a family of non-histone chromatin associated proteins which regulate transcription via mechanisms that expose promoters and facilitate binding of transcription factors. HMGB1 and HMGB2 also have a role as secreted proteins, mainly upon cellular stress, where they regulate innate immune response and cytokine release. Further roles have been described in senescence, autophagy and angiogenesis.

Here, we studied HMGBs in ECFCs. Gene expression microarray data indicated high expression of HMGB2 and moderate to low expression of HMGB1. HMGB3 and HMGB4 were not significantly expressed in ECFCs. Therefore, we further investigated HMGB1 and HMGB2 under conditions relevant to endothelial cell biology such as differentiation, senescence, autophagy, serum starvation and hypoxia.

Expression of HMGB1 in ECFCs was lower compared to endothelial cells from the aorta, saphenous vein, and dermal microvasculature. In contrast, HMGB2 was higher in ECFCs than in these mature endothelial cells. HMGB2 also showed downregulation during both replicative and stress-induced cell senescence, autophagy and serum starvation. The effect of recombinant HMGB1 protein on cell proliferation and endothelial barrier formation was assessed using a clonogenic assay and the xCELLigence (ACEA Biosciences) system. Treatment with recombinant HMGB1 reduced ECFC clonogenic capacity but had no significant effect on vascular permeability.

Regulated expression of HMGBs in various physiological conditions suggests a role in several aspects of endothelial cell biology. In particular, we described a significant decrease in HMGB2 expression during ECFC senescence and differentiation. Further research should reveal whether manipulation of HMGB proteins may be used to modulate the function of endothelial progenitors.

Glycolysis in senescence and metabolites in ageing

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Enhanced glycolysis, the Warburg effect, is well-known property of cancer, while it is not clear how it is coupled with the other hallmark of cancer, immortalization. Several recent reports established the significance of phosphoglycerate mutase PGAM both in the Warburg effect and cancerous proliferation. We reported that Mdm2 ubiquitin ligase binds to and promotes proteolysis of PGAM under senescence-inducing stress, DNA damage and oncogenic insults (1). Moreover, the recent analysis of PGAM-TG and KO mice disclosed the impact of PGAM on the Warburg effect. Interestingly, the tissue-context dependent and cooperative manner are observed on the impact of PGAM *in vivo* and *in vitro*.

Additionally, our efforts (2, 3) was to develop standard experimental procedures to identify and precisely quantify human age-related blood metabolites, in corporation with Dr. Mitsuhiro Yanagida in OIST. While human aging is a highly complex biological process exhibiting great individual variation, it is still not possible to distinguish human blood samples from young and elderly donors. To this end, our intent was to increase the reproducibility and accuracy of measuring blood metabolites, which are highly unstable. Our deliberate exclusion of middle-aged donors (40~70 years old) from the study also gave us a clearer age-difference between young (average; 29yr) and elderly (average; 81yr). The combination of comprehensive CV (coefficient of variation) analysis and RBC (red blood cell) metabolomics enabled us to identify 51 previously unreported metabolite CVs. Ultimately, we identified 14 age-related metabolites. Six of these 14 candidates are RBC-enriched, suggesting that RBC metabolomics is highly valuable. Furthermore, Pearson's correlation coefficients demonstrated significant correlations among several age-related compounds, including citrulline, N-acetyl-arginine, and dimethyl-guanosine (implicated in renal function), NAD, NADP (redox-related), leucine, and isoleucine (muscle maintenance). Their correlation strongly supports the physiological significance of our findings.

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Dissecting the molecular basis and signalling pathways underlying cellular senescence and aging

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Cellular senescence is a stable cell cycle arrest that normal cells undergo in response to a variety of intrinsic and extrinsic stimuli. Being implicated in ageing and age related diseases including cancer it is of great importance to elucidate the signalling pathways involved in regulating the senescent state. The p53-p21 and p16-pRB tumour suppressor pathways have clearly been implicated in senescence but the critical downstream targets of these pathways are unclear.

My primary goal is to identify and characterize the transcription factors (TFs) that act downstream of the p16-pRB and p53-p21 pathways to regulate senescence growth arrest. TFs regulate gene expression at different stages of embryonic development and are key to the establishment and maintenance of specific cell fates. Previous research in the group has compared mitotic and senescent cells by microarray analysis, and identified genes that are differentially expressed upon senescence. It was found that B-MYB, FOXM1, LIN9 and LIN52 were all significantly down-regulated upon senescence and the down-regulation was reversed when senescence was bypassed upon inactivation of the p16-pRB and p53-p21 pathways. It has been shown that the multi-vulval class B (Muv) genes (LIN9, LIN37, LIN52, LIN54 and RBBP4) form a variety of complexes by sequentially recruiting B-MYB and FOXM1 through various phases of the cell cycle to promote mitotic gene expression. Therefore, we were interested to determine if loss of B-MYB-MuvB-FOXM1complexes plays a direct role in cellular senescence and to identify what are the downstream targets of these complexes in senescence particularly those causally related to the stability of the growth arrest. Our studies have demonstrated that ectopic expression of B-MYB bypasses senescence more efficiently than constitutively active FOXM1 in our conditionally immortal human fibroblasts. Our goal is to determine if they synergize and act in complex with MuvB proteins. Initial results from the synergy experiments are highly encouraging.

Together these studies may enable us to assemble a cocktail of TFs that can efficiently bypass senescence and lay the foundation for a better understanding of the pathways underlying cellular senescence.

Signatures of Senescence Progression in a Transcriptomic Landscape Analysis Across the Mouse Lifespan

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Ageing is characterised by deterioration of healthspan and increased mortality rate. The ageing process is affected by both genotype and environment. Dietary restriction has been shown to increase lifespan and healthspan across many organisms.

Here we present a landscape analysis of ageing under normal or 30% calorie restricted diets in C57BL/6 mice across multiple time-points spanning 3 months till 30 months of age. RNA was extracted from livers of 3 biological replicates per time point, DNase treated and stranded libraries were prepared and sequenced on an Illumina platform to obtain paired end reads of 100 bases, with an average of 27 million reads per time point replicate. The resulting data was analysed to interrogate changes in gene expression regulation at multiple levels: transcriptional, post-transcriptional RNA processing such as alternative splicing as well as long non-coding RNA.

We focus on illuminating the possible mechanisms that provide dietary restricted organisms with improved healthspan and lifespan. Our data shows strong correlations between age and diet related gene expression changes and senescence markers. We also report tight regulation across the lifespan for specific biological processes, at multiple levels of gene expression regulation, with specific time-points showing major changes potentially representing turning points.

A novel lncRNA, linc-ASEN prevents cellular senescence by suppressing transcription of p21 tumor suppressor gene

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Long non-coding RNAs (lncRNAs) play important roles on regulation of diverse biological processes such as cell proliferation, apoptosis, and cellular senescence. Although many lncRNAs have been identified, only a small number of lncRNAs have been characterized functionally until now. Here, we firstly identified a novel long noncoding RNA, named Linc-ASEN (Associated with SENescence) from senescent cancer cells which were induced by doxorubicin or radiation treatment. Linc-ASEN played a critical role to prevent cellular senescence in both p53- and p21-dependent manners. We found that Linc-ASEN regulated p53 binding activity on proximal p53-responsive element of p21 promoter, and specifically increased p21 transcription without transcriptional alternations of other p53-induced genes such as Noxa, Puma, and Gadd45. Moreover, we figured out that Linc-ASEN involved in chromatin modification by the assessment of histone acetylation and methylation status. Finally we found that Linc-ASEN levels are inversely correlated with p21 mRNA levels in human cancer patient tissues including lung, breast, and colon cancer. Together, we revealed that Linc-ASEN has a critical role to prevent cellular senescence through the fine-tuning of p21 expression.

Novel features of senescence induced by overexpression of the epigenetic regulator, UHRF1

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Acute DNA damage or oncogene overexpression can lead to cellular senescence. Using zebrafish as a novel model for cancer gene discovery, our published work demonstrated for the first time that the overexpression of an epigenetic modifier, UHRF1 (ubiquitin-like with PHD and ring finger domains 1), was sufficient to cause cellular senescence in hepatocytes followed by rapid onset of liver cancer in the absence of any other sensitizing mutations. UHRF1 is a multifunctional protein that couples DNA methylation with histone-modifications, such as trimethylation of lysine 9 on histone H3 (H3K9me3), by coordinating the recruitment of histone methyltransferases with DNA methyltransferases. While many oncogenes induce senescence when overexpressed, UHRF1-induced senescence only partially recapitulated the principal features of oncogene-induced senescence. UHRF1 overexpression in zebrafish hepatocytes caused global loss of DNA methylation by redistributing DNMT1 and induces intranuclear structures reminiscent of Senescence-Associated Heterochromatic Foci (SAHFs). We report the surprising finding that, these structures were negative for H3K9me3, a marker of heterochromatin. In fact, hepatocytes expressing high levels of UHRF1 were devoid of any H3K9me3 and had reduced levels of H3K9me2, suggesting that high levels of UHRF1 promoted global euchromatinization. This massive reorganization of the chromatin in UHRF1-overexpressing hepatocytes was accompanied by modest levels of DNA damage, a landmark of oncogene-induced senescence. Our model challenges actual dogma that oncogene-induced senescence creates heterochromatin foci marked by H3K9me2/3 and suggests that senescence caused by UHRF1 overexpression has features that distinguish it from classical oncogene-induced senescence.

UHRF1 regulates DNMT1 transcription via p53 in cellular senescence

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Global loss of DNA methylation has been implicated in chronological aging and cell senescence, implying the involvement of overall decreased DNA methyltransferase (DNMT) activities. In our previous study, we demonstrated that Ubiquitin like with PHD and ring finger domains 1 (UHRF1), a member of a subfamily of RING finger-type E3 ubiquitin ligases, regulated DNMT1 expression and subsequent gain of senescent phenotypes of Human diploid fibroblast (HDF). Unexpectedly, UHRF1 regulated DNMT1 expression at transcription level. Up to date, UHRF1 is known to have E3 ligase activity and also directly recruit DNMT1 to its specific DNA sequences, but no clear evidence supports its direct role as a transcription regulator, implying the involvement of additional transcription factor. Interestingly, DNMT1 promoter regions possess p53 binding sequence and p53 knockdown increased DNMT1 promoter activity and its expression. Moreover, UHRF1 suppression increased p21 expression without alteration of p53 expression and induced HDF senescence. These results indicate that DNMT1 transcription is regulated by UHRF1-mdiated p53 transcriptional activity in cellular senescence. Further detailed study on the regulation of p53 activity by UHRF1 is currently under investigation.

Scorpion venom components trigger cell cycle arrest of colorectal tumor cells through DNA signaling damage

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Scorpion venom was a rich source of bioactive peptides and toxins which exhibit an antitumor effect *in vitro* and *in vivo*. Thus, the aim of the present study was to investigate the propensity of Scorpion Venom Component III (SVCIII) isolated from *Androctonus australis hector* venom to induce oxidative damage and cell cycle arrest on colorectal tumor cell line HCT116.

The effect of SVCIII on HCT116 proliferation was analyzed by MTT assay. Expression of cyclins was evaluated by western blot and P53 and P21 mRNA by RTQPCR. ROS (reactive oxygen species) generation was estimated by fluorescent dye (DCFH-DA) along with assessment of DNA damage by comet assay and H2AX phosphorylation

The obtained results showed that SVCIII induced a significant decrease in cell growth of colorectal tumor cells associated with a DNA damage effect particularly double strands break due to phosphorylation of histone γH2AX. Moreover, enhancement in intracellular ROS level was detected and can be a key early signal to SVCIII-induced cytotoxicity and genotoxicity on HCT116. In addition; we found that mRNA expression of P53 and cyclin dependent kinase inhibitor, p21Waf1/Cip1 was significantly upregulated while protein expression of cyclin D1, E and B1 was significantly decreased. These results suggest that the inhibition of tumor growth by SVCIII in HCT116 cells occurs through oxidative damage and G1-S transition pathway. Furthermore, the expression of molecular target of scorpion toxins in colorectal membrane tumor cells can be also incriminated in theses antitumor activity. Hence, these properties make SVCIII a promising candidate for development of new anticancer peptides.

Keywords: Scorpion venom, DNA damage, Senescence, cycline D1, HCT116 cells.

A Genome-Wide RNAi Screen Identifies Novel Regulators of AKT-Induced Senescence

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Dysregulation of the PI3K/AKT/mTORC1 signalling pathway occurs in up to 70% of sporadic human cancers. Paradoxically, oncogenic signalling in normal cells results in oncogene-induced senescence (OIS), which acts as a significant barrier to restrain aberrant proliferation. Understanding the mechanisms of OIS in normal cells and how this is subverted in cancer cells will provide insight into the development of cancer and how it can be targeted therapeutically. We have previously demonstrated that hyperactivation of AKT results in OIS via a p53 and mTORC1-dependent mechanism (Astle et al, *Oncogene* 2011). To further understand the mechanism underlying this response, we performed RNA-sequencing (RNA-seq) of proliferating cells and cells made senescent by hyperactive AKT. Gene ontology analysis demonstrated significant upregulation of NF-kB target genes associated with the senescence-associated secretory phenotype (SASP), which is consistent with recent studies implicating mTORC1 in SASP regulation.

To determine the genetic changes required to overcome PI3K/AKT/mTORC1-induced senescence, we performed a genome-wide RNAi senescence escape screen using multiparametric readouts and established a novel machine learning approach to phenotypically classify senescent cells (Chan et al., *Assay and Drug Dev Tech* 2016). We identified and validated 98 candidates, several of which are known and potential tumour suppressors. Using various genetic approaches, we are investigating their role and specificity for PI3K/AKT/mTORC1- or RAS-induced senescence, which will be critical to determine how they can be exploited to treat cancer.

Molecular pathways of senescence in syncytiotrophoblast regulate placenta structure and function

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The placenta is an autonomous organ, essential for the maintenance of fetal growth and development during pregnancy. The multinucleated syncytiotrophoblast layer of the placenta, intended for nourishing the fetus during gestation, exhibits characteristics of cellular senescence. Yet, the functional role of molecular pathways regulating senescence in syncytiotrophoblast for placenta structure and function is not understood. Using Dynamic Contrast-Enhanced MRI (DCE-MRI), we found an altered contrast agent dynamics in placentas of p53-/-, Cdkn2a-/- and Cdkn2a-/-;p53-/- mice, with the most significant changes in Cdkn2a-/-;p53-/- placentas. These functional alterations in Cdkn2a-/-;p53-/- placentas were accompanied by histopathological changes. Gene set enrichment analysis (GSEA) of human primary syncytiotrophoblast showed upregulation of senescence markers associated with cell cycle regulation and senescence associated secretory phenotype (SASP). Senescence pathways regulating SASP, including MAPK, NFκB and JAK-STAT were induced in this population. Syncytiotrophoblast exhibited an elevated expression of gelatinases MMP2 and MMP9, which are essential for placental invasiveness and function. Furthermore, activity of gelatinases is reduced in placentas of senescence attenuated mice, suggesting that senescence regulatory pathways control gelatinase activity in the placenta. Therefore, molecular pathways upregulated in human syncytiotrophoblast were compromised in mice with an attenuated senescent program, and may lead to morphological and functional abnormalities observed in their placentas. Interestingly, human placentas, derived following pregnancy complicated with Intrauterine Growth Restriction (IUGR) show a marked reduction in the expression of key regulators of senescence, in gelatinase activity and in expression of the secretory components that were altered in senescence attenuated mice. Altogether, we propose that molecular mediators of senescence regulate placental structure and function.

FANCD2 modulates oncogene-induced-senescence (OIS)

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Fanconi anemia patients present bone marrow failure, developmental abnormalities, cancer predisposition with cellular and chromosomal hypersensitivity to drugs inducing DNA interstrand cross-links. Biallelic mutations have been identified in 21 genes. Eight of these FA proteins constitute the nuclear core complex which is activated by blocked DNA replication forks and which ubiquitinates two key proteins FANCD2 and FANCI leading to activation homologous recombinaison pathway.

Since the unscheduled activation of oncogene leads to senescence (OIS), considered as anticancer barrier, we wondering determine if the FANC pathway was involved in senescence process.

We used two cellular models, the IMR90-hTERT/ER:RAS^{V12} and the Wi38-hTERT/ER:GFP:RAF1 cells, in which oncogenes induction by 4-HT exposure leads to cellular senescence, ie growth arrest and SAHF formation, after 6 days or 72 hours of induction, respectively. Oncogene activation, is followed by a transitory increase of both FANCD2 and FANCA (a core complex protein) followed by their degradation concomitantly to the raise in SAHF-positive cells.

siRNA-mediated FANCD2 depletion, in absence of oncogenes induction, leads per se to a pre-senescent phenotype revealed by p16, p53, p21 expression, and, ROS and SAHF positive cells accumulation.

Oncogene induction in FANCD2-depleted cells drives anticipation in both ROS and SAHF accumulation process. On the contrary, the increasing in molecular markers induced by OIS and cell cycle blockage appeared to be independent of the FANCD2 presence.

Whereas the anti-oxidant agent NAC reduces both ROS and SAHF formation in FANCD2-proficient cells, the frequency of SAHF-positive cells remains unchanged in NAC-treated FANCD2-depleted cells.

Increased level and maturation of the protease, cathepsin L (CTSL1) was observed during OIS leading to the histone H3 cleavage. In FANCD2-depleted cells, in spite of an increased accumulation of active CTSL1, H3 is less processed than control cells and the exposure to CTSL1 inhibitor (Z-FF-FMK) failed to modify the higher rate of SAHF-positive cells observed in FANCD2-depleted cells.

These results suggest (1) a FANCD2 role in the maintenance of the chromatin landscape, somehow delaying the molecular modifications that lead to SAHF and (2) that ROS accumulation in FANCD2-depleted cells could modulate the CTSL1 function or modify the histone H3 cleavage.

Targeting MEK/ERK pathway decreases expression of senescence markers and results in apoptotic death of HDACi-senescent tumor cells

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Cellular senescence is considered to be a tumor suppressor program as it leads to complete inhibition of proliferation. However, tumor cells induced to senescence remain viable and develop a SASP that can contribute to inflammation and transformation of surrounding cells. Senescent cells are metabolically active, and stringent balance between synthesis and degradation of proteins is required to maintain senescent phenotype and viability. The present work was performed using *E1A+cHa-Ras* transformed fibroblasts. It is known that Ras/Raf/MEK/ERK cascade contributes to activation of mTOR kinase – a key regulator of cellular senescence. We have studied involvement of MEK/ERK pathway in development of senescent phenotype and in regulation of cell viability of Ras-transformed cells induced to senescence by HDACi sodium butyrate (NaBut). PD0325901 (PD) was used to inhibit MEK/ERK pathway.

We showed that when senescence is induced upon suppressed MEK/ERK pathway, such markers of senescence as cell cycle arrest, SA-beta-galactosidase activity, cell size and protein content and levels of lactate are reduced in comparison with NaBut treatment alone. Despite ERK1,2 inhibition, the level of mTOR Complex 1 activity remains high, preventing complete decrease of senescence markers expression. However, mTORC1 activity fails to maintain cellular viability, and NaBut + PD treated cells die due to massive cell death. In NaBut + PD-treated cells mitochondria undergo destruction of their internal structure without damage of outer membranes, thereby they are not removed by mitophagy. In cells treated with PD alone activation of AMPK favors AMPK-mediated autophagy and helps *Ras*-transformed cells to overcome treatment with PD. In NaBut + PD-treated cells, which have a high level of mTORC1 activity and mitochondrial dysfunction, activation of AMPK leads to a change in metabolic homeostasis that is essential for viability of senescent cells. Thus, we show that inhibition of MEK/ERK pathway in cells induced to senescence activates form of autophagy that is unable to restore the energy deficit and apparently destroys the complicated metabolic balance, which supports of viability of *Ras*-transformed senescent cells.

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Common Delayed Senescence in Melanocytes from Multiple Primary Melanoma Patients

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Multiple primary melanoma (MPM), the gain of more than one independent melanoma, occurs in approximately 5% of melanoma patients. Reasons for developing MPM are unknown but evidence so far suggests that delayed or defective melanocyte senescence is involved. MPM patients are significantly more likely to be found in melanoma susceptibility pedigrees and germline mutations found so far exist primarily in *CDKN2A* (encoding *p16*) and components of the telomere shelterin complex. These genes are known or predicted to play important roles in maintenance of normal replicative lifespan.

Owing to these previous observations, we hypothesised that normal melanocytes from *CDKN2A* wild-type MPM patients might display significant extensions in replicative lifespan compared to cells from single primary melanoma (SPM) patients. Melanocytes were established from sun-protected skin biopsies and serially passaged until they reached replicative senescence, confirmed with senescence associated beta-galactosidase immunocytochemistry.

Remarkably, melanocytes from MPM patients displayed over a 4-fold higher mean cellular lifespan (16.3 doublings, n = 10) than those from SPM patients (3.7 doublings, n = 8) ($p = 0.0057$). Adult melanocytes from unaffected individuals have been previously reported to undergo a maximum of 10 population doublings, similar to the maximum among these SPM cultures, suggesting SPM patients are suitable controls. Donor age was found not to contribute to the difference in lifespans. However a significant correlation existed between donor age and melanocyte lifespan in the MPM cohort only. A significant correlation was also seen when melanocyte lifespan was analysed with age at first diagnosis of melanoma in our MPM patients. These results provide further evidence that melanocyte lifespan influences MPM susceptibility.

To elucidate which pathways are involved in senescence, p16 and p21 levels were examined in some MPM lines. Puzzlingly some did not display increased expression of the two proteins at senescence. It is suggested that polymorphisms in the 3'UTR influence this finding but more evidence is needed.

Overall these results provide additional evidence that delayed senescence plays an important role in melanoma susceptibility and that MPM patients could make useful candidates for elucidation of novel melanoma susceptibility genes.

Integrin beta 4 activation is crucial for the ionizing radiation-induced premature senescence

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Cellular senescence refers to the irreversible growth arrest occurred by various intrinsic and extrinsic stresses. Recent many studies demonstrated that cellular senescence plays a crucial role in tumor regression after exposure to ionizing radiation (IR) in vitro and in vivo. However, the mechanism whereby signaling molecules regulate IR-induced cellular senescence remains uncertain. In this study, we provided evidence that integrin β4 (ITGβ4) activation was essential for the IR-induced cellular senescence. IR directly phosphorylated ITGβ4 at a tyrosine residue 1510, leading to activation of ITGβ4-Src-AKT pathway. We further determined that ITGβ4 phosphorylation is regulated by membrane fluidity and cholesterol content. Simultaneously, IR induced p53-caspase pathway through DNA damage response (DDR) signal in an ITGβ4-independent manner. When we inhibited ITGβ4-Src-AKT signaling pathway using specific siRNAs or inhibitors, cell fate switched from senescence to apoptosis both in cancer cells and in tumor tissues of xenograft mice, which were exposed to IR. Collectively, these results demonstrated that activation of ITGβ4/Src/AKT signaling pathway is critical for the induction of premature senescence, instead of apoptosis, by IR exposure. The identification of ITGβ4-related specific signaling pathway in IR-induced cellular senescence may provide novel targets for the efficacy of radiotherapy.

KLF6 tumor suppressor activity is associated to the induction of cellular senescence

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Krüppel-like factor 6 (KLF6) is a transcription factor involved in cell-cycle regulation and cellular differentiation. Indeed, KLF6 is considered a tumor suppressor whose expression is frequently lost in various human cancer types. In previous work, we shown that KLF6 knockdown in normal fibroblast led to cell transformation. As cellular senescence is a crucial anticancer mechanism that prevents growth of cells at risk for neoplastic transformation, we aimed to elucidate the impact of KLF6 on cellular senescence. This cellular process could be elicited through several mechanisms including oncogene activation, DNA damage and oxidative stress. Through H_2O_2-treatment we induced NIH3T3 fibroblast cells to become senescent, detected by senescence-associated beta galactosidase activity and heterochromatin foci (SAHF) formation. Then, stable transduction of shRNA for downregulation of KLF6 expression led to a decreased of the senescent cells index together with chromatin instability signs, thereby bypassing H_2O_2-induced cellular senescence. In addition, ectopic expression of KLF6 in HeLa cervical carcinoma cells was able to induce cellular senescence. This surprising finding, along with its cytostatic function upon oncogenic activation, suggests that KLF6 tumor suppressor activity could be mediated by cellular senescence as an alarm signal in response to certain stimuli that produce exacerbated proliferation or cell transformation.

The implication of glycogen in cellular proliferation

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Glycogen is a multi-branched polysaccharide that serves as the main form of glucose storage in the body, where the main reserves are in the liver and muscle. Hepatic glycogen serves as a buffer of blood glucose levels, whereas in the muscle, it acts as an energy supply during contractions. It has been observed that glycogen metabolism is altered in many tumor types, and that glycogen content is inversely correlated with proliferation rate. In addition, it has recently been described that when glycogen accumulation is forced in glioblastoma U87 cells in hypoxia, senescence is induced and tumor growth is inhibited *in vivo.* Interestingly, we have observed that glycogen levels are increased in human and mouse fibroblasts under replicative senescence, and that MEFs depleted of glycogen bypass senescence and immortalize faster than WT counterparts. We have also demonstrated that senescence pathways are down regulated in MEFs lacking glycogen, compared to controls. Furthermore, mice lacking hepatic glycogen (GYS2 KO) treated with N-nitrosodiethylamine (DEN), a well-known hepatocarcinogen, show higher tumor burden and mortality than control mice. We also evaluated the effect of glycogen on hepatocyte proliferation after the surgical removal of two thirds of liver mass. GYS2 KO mice present faster proliferation and liver regeneration rates, when compared to WT counterparts. Collectively, our preliminary data suggest that glycogen metabolism plays a crucial role in the regulation of cell cycle in both pathological states, such as hepatocellular carcinoma, and in physiological roles, such as in senescence and liver regeneration after partial hepatectomy.

Small molecule-mediated inhibition of cyclin-dependent kinases or methyltransferases reinforce DNA damage-induced cellular senescence in human ovarian cancer cells

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Senescence is an important tumor suppression mechanism and senescent cells develop many senescence-associated (SA) phenotypes that are responsible for carrying their biological functions. Among those, the SA growth arrest (SAGA) removes damaged cells from the proliferative pool of cells, thus preventing their expansion. We have previously showed that human ovarian cancer cells (OC) retain a capacity to undergo therapy-induced senescence (TIS) following exposure to first line agents used in the clinic. In this study, we investigate how the manipulation of cell cycle and epigenetic regulators can impact TIS and other cell fate decision like continued proliferation or apoptosis taken by high-grade serous OC (HGSOC) cells in response to DNA damage.

Using a panel of HGSOC cell lines, we find that pretreatment with cyclin-dependent kinase inhibitors (CDKi) or epigenetic regulators (ER) can switch cell fate decision following exposure to ionizing irradiation (IR). In contrast to IR alone, CDKi and ER pre-treatment can re-orient the primary IR response from apoptosis or mitotic catastrophe to senescence. Our data also suggests that senescence induced by IR-CDKi combos is not only due to the repression of phosphorylation of pRB but could also be related to indirect epigenetic regulation of gene expression as observed using methylation blockers. We also note that instead of a synthetic lethal effect, concomitant treatment using IR-CDKi combos promote long-term cancer cells survival in most cell lines, suggesting that the re-direction to senescence can have context-dependant detrimental effects. In general, our results illustrate the possibility of manipulating cell fate decision to benefit anti-cancer mechanism including senescence after first-line OC DNA damaging treatments. We also find that the combined usage of two different treatments might not always lead to a more effective synergising effect during cancer therapy. Because different HGSOC cell lines have diverse responses to the same drug combos, we project that a similar approach to cell fate manipulation in patient using drug combos will require personalized testing of individual tumors characteristics before applying treatment.

Senescence is critical for injury induced *in vivo* reprogramming in skeletal muscle

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In vivo reprogramming is an emerging strategy for tissue repair, while senescence has been proposed to promote regeneration. However, it is unknown whether senescence could facilitate the reprogramming process during tissue repair and regeneration. We focused on skeletal muscle, a highly relevant tissue for examining the regeneration process. We have found that senescence induced by tissue damage, both acute (cardiotoxin) and chronic (muscular dystrophy), is critical for *in vivo* reprogramming in skeletal muscles of reprogrammable mice. We further demonstrated that $Pax7^+$ satellite cells (muscle stem cells) are the cell of origin of muscle teratomas. *Moreover*, by blocking IL-6, an important component of senescence-associated secretory phenotype, *in vivo* reprogramming was successfully hindered, clearly linking senescence to the effects of injury on cellular plasticity. These findings will have an impact on the fields of tissue regeneration and reprogramming. In addition, they provide a fascinating link with the reports in the last year showing that senescence is beneficial for tissue regeneration.

Low and high energy X rays lead to different levels of senescent phenotype on HUVECs

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The evaluation of radiosensitivity is historically linked to the survival fraction measured by the clonogenic assay, which is until now the gold standard in such evaluation. The representation of the survival fraction as a function of the dose leads to survival curves which are modelled by the linear-quadratic model (LQ-model). Nevertheless, the clonogenic assay is a quite restrictive method which does not take into account cell-cell interactions and the phenotype of surviving cells as well. Radiation-induced damage to the vascular endothelium is potentially involved in the initiation and the development of normal tissue injury in radiotherapy concerns. Thus, in this study we compared the biological effects on HUVECs (Human Umbilical Vein Endothelial Cells) exposed to low energy x-rays (generated at 220 kV on a SARRP) and high energy x-rays (generated at 4 MV on a LINAC). Cell survival fractions were measured/calculated by using clonogenic assay while morphological changes, cell viability/mortality, cell cycle analysis and β-galactosidase activity were evaluated by flow cytometry. Finally molecular footprinting of 44 genes involved in senescence process were measured by RT-qPCR. While the clonogenic assay showed very similar survival fraction curves for both conditions, we found highly significant differences between the two conditions of irradiation, when considering other biological outputs when cell were irradiated at confluence. Cell number and survival, morphological changes, cell cycle analysis, molecular footprinting and β-galactosidase activity were measured for doses up to 20 Gy. For all the assays, we observed and demonstrated stronger effects on HUVECs irradiated with the LINAC (4 MV) compared to the same irradiation performed with the SARRP (220 kV). All together these results strongly support the fact that the clonogenic assay is not sufficient alone and that we need to implement new models with multi-parametric biological outputs to accurately estimate the biological cellular fate. Moreover, by using our multi-parametric biological outputs approach, we have demonstrated that, in similar irradiation exposure (same dose and dose rate), low and high energy X rays lead to different levels of senescent phenotype on HUVECs.

Effects of *N*-acetyl-L-cysteine on lipid peroxidation and fatty acid profile of human exfoliated deciduous teeth stem cells

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Introduction: Human exfoliated deciduous teeth stem cells (DTSC) could be potentially used to repair teeth and bones. Their extensive capacity to proliferate and differentiate might be decreased during *in vitro* cultivation - when oxygen applied in supraphysiological concentration generates reactive oxygen species (ROS). Excessive amount of ROS leads to lipid peroxidation and decrease in polyunsaturated fatty acids (PUFA) content of cell membranes. The oxidative damage could be decreased by the addition of an antioxidant to the cell culture medium.

Aim: We investigated the effects of different concentrations (0.1 mM, 1 mM, 2 mM) of the *N*-acetyl-L-cysteine (NAC) on lipid peroxidation and PUFA status of DTSC.

Methods: The dental pulp was extracted from healthy exfoliated deciduous teeth of 5 children (6 - 8 years) with the informed consent of the parents. Cells were expanded to the 6th passage, then treated with different NAC concentrations (0.1 mM, 1 mM, 2 mM) or not (control), and analysed after 48h. The concentration of thiobarbituric acid-reactive substances (a measure of cellular lipid peroxidation) was determined spectrophotometrically. Fatty acids methyl esters from complete lipid extract were analysed by gas-liquid chromatography. Statistical analysis was performed between values before and after treatments, using paired Student's t-test for normally distributed variables, and Wilcoxon signed rank test for non-normally distributed variables. The level of statistical significance was set to $p < 0.05$.

Results: Lipid peroxidation was decreased ($p < 0.05$) upon 0.1 mM NAC. Total PUFA were increased ($p < 0.05$) upon both 0.1 mM and 1 mM NAC. Linoleic acid (18:2n-6) and total n-6 PUFA were increased ($p < 0.05$) upon 0.1 mM NAC. Docosahexaenoic acid (22:6n-3) was decreased ($p < 0.05$) upon 2 mM NAC.

Conclusion: When applied at 0.1 mM dose, NAC reduced oxidative damage of DTCS by decreasing lipid peroxidation and increasing PUFA in stem cells. This dose could be used in DTSC culturing in order to improve stem cell quality and quantity.

Impact of reprogramming in lung regeneration

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Differentiated cells can achieve full pluripotency in a process known as cellular reprogramming. Cellular senescence promotes the reprogramming of neighbour cells in vivo upon the ectopic expression of the reprogramming factors Oct4, Sox2, Klf4 and c-Myc (OSKM). However, it is unknown whether cell reprogramming in turn promotes tissue repair and regeneration. Pulmonary fibrosis is a degenerative disease currently lacking effective treatments and it is characterised by the accumulation of senescent cells and by impaired lung regeneration. Here, we show that the induction of OSKM after bleomycin-induced pulmonary fibrosis promotes the expansion of p63+ Krt5+ lung progenitor cells leading to enhanced regeneration and functional recovery. Cellular reprogramming opens up new possibilities for the development of novel therapeutic strategies in lung fibrosis.

Cellular senescence during skeletal muscle regeneration

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Adult skeletal muscles can regenerate after repeated trauma, yet our understanding of how adult satellite cells restore muscle integrity and homeostasis after regeneration is limited. In the adult mouse, satellite cells are quiescent and located between the basal lamina, and the myofibre. After injury, they re-enter the cell cycle, proliferate, differentiate and fuse to restore the damaged fibre. A subpopulation of myogenic cells then self-renews for future repair. When satellite cells are removed from their niche, they rapidly express the Myod protein and proliferate. We identified Notch/Rbpj as a major regulator of muscle stem cell quiescence. Compromised Rbpj function results in depletion of muscle stem cells from their niche. The multifunctional adaptor protein Numb was reported to inhibit Notch activity in several organisms and tissues, therefore we examined the role of this protein during muscle regeneration.

To investigate the respective roles of Notch and Numb we generated conditionally mutant *Numb:Numblike* double mutant mice. Unexpectedly, we found that Numb function did not modulate Notch activity during regeneration, although muscle regeneration was compromised in Numb mutant mice. Unexpectedly, we uncovered a novel function for Numb in suppressing cellular senescence of muscle stem cells. Notably, we observed two types of senescence in regenerating muscle: a transient senescence in wild type mice found in endothelial cells, and an additional persistent senescence in *Numb* mice in muscle stem cells. Anti-oxidant treatment or p53 ablation resulted in functional rescue of the senescence phenotype as well as regenerative potential in *Numb* mutants, but not in the wild type mice. These findings suggest that the regulation of senescent cells might depend on distinct pathways during muscle regeneration. Ongoing studies are focused on the functional role of senescent cells and their impact on the muscle regeneration process using strategies that promote suppression of senescent cells in vivo.

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Adherence of monocytes to endothelial cells involves CD44 in replicative senescence but not in stress-induced senescence

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Introduction: Endothelial senescence has been implicated in vascular ageing. The characteristic senescence-associated secretory phenotype (SASP) associated with senescent cells is believed to be pro-inflammatory. Yet our proteomics data on the secretome of senescent endothelial cells did not identify increased levels of pro-inflammatory species. Moreover, secretome from senescent endothelial cells did not promote monocyte adhesion while the SASP from human dermal fibroblasts, an established model, promoted monocyte adhesion. Therefore, this study investigated the hypothesis that the pro-inflammatory effect of senescent endothelial cells is independent of SASP.

Methods: A static adhesion assay was carried out investigating the binding of human THP-1 monocytes to Human Umbilical Vein Endothelial cells (HUVECs). HUVECs were grown in tissue culture to induce replicative senescence (REPS) or early passage HUVECs were treated with 100μM of tertiary butyl hydrogen peroxide (t-BHP) for 1h on 3 consecutive days to induce stress-induced premature senescence (SIPS). Senescence was confirmed by SA-β gal staining and p53 expression, 1x106/ml of THP-1 cells were incubated with senescent HUVECs for 1hr at 37°C in 5%CO2 atmosphere. In some experiments HUVECs were pre-treated with TNF-α (10ng/ml). Adhered THP-1 cells were counted with light microscopy and fold change calculated compared to control. To better mimic in vivo conditions, continuous flow of fluorescent labelled THP-1 cells, 1x106/ml at a sheer rate of 5 dynes/cm2 and 37°C was passed over a monolayer of senescent or young HUVECs for 45 minutes.

Results: A significant increase in the adherence of THP-1 cells to REPS HIVECS (4.4 fold) and SIPS HUVECs (5.4 fold) compared to young HUVECs (1.9 fold) was observed both in a static assay and under flow ($p<0.01$ and <0.05 respectively by ANOVA). In Western Blots REPS significantly increased CD44 protein levels compared with untreated cells. This was further investigated in the presence of TNF-α. However, SIPS did not affect CD44 levels, even in the presence of TNF-α.

Conclusion: A significant increase in adherence of leucocytes was observed in senescent HUVECs under both static and flow conditions in response to TNF-α treatment. The pro-inflammtory role of REPS is, in part, due to CD44.

Hyperhomocystenemia and methylenetetrahydrofolate reductase polymorphism in Alzheimer´s disease

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Background and aim: The implication of MTHFR gene polymorphisms as risk factors for the occurence of Alzheimer's disease (AD) is still controversial. Our study was carried out to investigate the possible association between C677T polymorphism in MTHFR gene, total plasma homocysteine concentration, and Alzheimer´s disease. Our results have reported an association between increased plasma Hcy level and AD. However the results show no association between the SNP C677T and AD.

Methods: Our study includes 75 patients with Alzheimer disease and 80 non-demented controls. Total plasma homocysteine (Hcy) levels were determined using fluorescence polarization immunoassay (Abbott AxSYM system, Abbott Laboratories) and genotyping for MTHFR by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR/RFLP).

Results: Univariate logistic regression showed that AD patients presented higher total plasma Hcy levels than controls and the difference was statistically significant (p<0.001).The genotypes distribution in cases was 21% CC (wild homozygous genotype), 53% CT (mutant heterozygous genotype) and 26% TT (mutant homozygous genotype) .In the control group, the CC genotype frequency was 46%, CT 39% and TT 15%.This difference was not statistically significant (OR=1.78, p=0.28).

Conclusion: Our data confirms the association between increased plasma Hcy concentration and AD.No significant differences in the C677T MTHFR polymorphisms distributions was found between patients and controls.

Key words: C677T MTHFR gene polymorphism, homocysteine, Alzheimer disease.

Modeling Down Syndrome in a humanized telomere mouse strain

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Down syndrome (DS) is a complex developmental disorder. It is caused by trisomy of chromosome 21 (T21) and affects 1/750 live births. DS patients display multiple pathological conditions such as mental retardation, muscle hypotonia, craniofacial abnormalities, congenital heart disease and gastrointestinal alterations. In addition, DS patients show certain features associated with premature ageing such as earlier skin wrinkling and hair greying, hypogonadism, accelerated menopause, hypothyroidism, immunosenescence and earlier onset of neurodegenerative diseases. However, the mechanisms underlying accelerated ageing in DS remain largely unknown. Recently it has been shown that Ts65Dn DS mouse model show impaired cell renewal in different adult stem cells compartments and accelerated entry into senescence of proliferating fibroblasts through activation of the INK4/ARF locus. Nevertheless the DS ageing phenotypes are not well recapitulated in any of the current DS mouse strains. Interestingly, the rate of telomere shortening is accelerated in blood lymphocytes from DS patients and short telomeres are associated with the appearance of dementia. We hypothesize that species-specific differences in telomere length between human (short telomeres) and mouse (long telomeres) might affect the penetrance of DS phenotypes. To test this notion, we are introducing a transchromosomic DS mouse model (Tc1) into a telomerase-deficient background (mTerc-/-). Preliminary results indicate that Tc1:mTR-/- mice show reduced lifespan. This new DS mouse model will provide us with a valuable tool to better understand the mechanisms that drive premature ageing in DS patients. Moreover, it would facilitate the development of therapeutic interventions to ameliorate DS-associated pathologies

mTOR Pathway Activation Drives Lung-Cell Senescence and Emphysema

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Chronic obstructive pulmonary disease (COPD) is a highly prevalent and devastating condition for which no curative treatment is available. Exaggerated lung-cell senescence may be a major pathogenic factor. Here, we investigated the potential role for mTOR signaling in lung cell senescence and alterations in COPD, using lung tissue and derived cultured cells from patients with COPD and from age- and sex-matched control smokers. Cell senescence in COPD was linked to mTOR activation, and mTOR inhibition by low-dose rapamycin prevented cell senescence and inhibited the pro-inflammatory senescence-associated secretory phenotype. To explore whether mTOR activation was a causal pathogenic factor, we developed transgenic mice exhibiting mTOR overactivity in lung vascular cells or alveolar epithelial cells. In this model, mTOR activation was sufficient to induce lung-cell senescence and to mimic COPD lung alterations, with the rapid development of lung emphysema, pulmonary hypertension, and inflammation. These findings support a causal relationship between mTOR activation, lung cell senescence, and lung alterations in COPD, thereby identifying the mTOR pathway as a new therapeutic target in COPD.

Defining Reed-Sternberg cells in Hodgkin's Lymphoma as senescent

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Hodgkin's lymphoma (HL), a B-cell originated malignancy of the immune system, is among the most frequent malignancies in young adults, with a second peak in the elderly. Although the rate of cure is high, 20-35% of patients relapse. Interestingly, the malignant cells are quite rare within the lymphoid mass, and usually account for only about 1-5% of all cells in the tumor tissue. The remaining cells are recruited to the lymph node by pro-inflammatory signals. In classical Hodgkin's lymphoma, the tumor cells are composed of small, mononucleated Hodgkin (H) cells, and large mono/multinucleated Reed-Sternberg (RS) cells. The Reed-Sternberg cells have distinctive large cell morphology, are characteristic of the disease and their presence is essential for diagnosis. Enlarged cells, is one of the hallmarks of senescence, but whether RS cells are senescent has not been previously investigated. We show that RS cells have characteristics of senescent cells; RS cells in HL biopsies specifically express the senescence markers and cell cycle inhibitors p21Cip1 and p16INK4a and are negative for the proliferation marker Ki-67, suggesting that these cells have ceased to proliferate. Moreover, the RS-like cells in HL lines, stained specifically for senescence-associated ß-galactosidase (SA-ß-gal). Oxidative stress promoted senescence in these cells as demonstrated by their staining for p21Cip1, p16INK4a, p53 and gH2AX. Senescent cells produce copious amounts of inflammatory cytokines termed 'senescence associated secretory phenotype' (SASP), primarily regulated by Nuclear Factor kB (NF-kB). Indeed, we show that NF-kB activity and NF-kB-dependent cytokines production (e.g. IL-6, TNF-α, GM-CSF) were elevated in RS-like cells. Furthermore, NF-kB inhibitors, JSH-23 and curcumin reduced IL-6 secretion from RS-like cells. By defining RS cells as senescent, our studies demonstrate for the first time, to our knowledge, the occurrence of pathologically-relevant senescent cells in a primary, untreated, tumor mass. This offers now new insights on the origin of the pro-inflammatory microenvironment in HL and for new modalities for treatment. Further understanding the pathways important for the establishment of senescence in HL, as well as gaining insight into targetable mechanisms for the eradication of these cells, will provide new therapeutic approaches for HL patients with recurrent or chemo-resistant disease.

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, and has been linked to numerous age-associated pathologies. Morphological changes associated with senescence disrupt tissue homeostasis by acting on surrounding cells and matrices,for 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 successfully 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 senescence 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 2D model of senescence has been established by serially passaging human dermal fibroblasts (HDFs) to DS. These DS HDFs display a panel of well-established senescence markers, including SA-β-Gal, p16 and p21. HDFs were also induced into premature senescence using UVB and similarly exhibited senescence markers.

However, 2D systems provide limited information about senescence *in vivo* and do not, for example, consider the role of a surrounding cell matrix. To examine how senescence contributes to the ageing in a tissue-like environment, a 3D living skin equivalent (LSE) of ageing is in development. Skin has a clear intrinsic ageing phenotype and can be modelled *in vitro* using organotypic methods. EP or mixed EP/DS HDFs are suspended in a dermal matrix while proliferating primary human epidermal keratinocytes (HEKs) are seeded on the surface to create an epidermis. Using this model, we can analyse the effect of DS on the skin phenotype, and, in the future, will investigate if by reversing senescence the skin ageing phenotype can be alleviated. LSEs using EP HDFs show clear stratification of the epidermis and spatial expression of proteins corresponding to human skin controls. The development of quantitative analyses has created high-throughput, unbiased data extraction platforms from immunofluorescence experiments. The latest findings with regards to the contribution of senescent fibroblasts to skin ageing will be presented.

Involvement of lamin B1 in COPD pathogenesis

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Introduction: Cellular senescence has been implicated in chronic obstructive pulmonary disease (COPD) pathogenesis in relation to mTOR activation and dysregulated autophagy. Downregulation of lamin B1 has been recognized to be associated with development of full senescence through modifying chromatin conformation and gene expression accompanied by senescence-associated secretory phenotype. Therefore, we sought to determine if lamin B1 downregulation is involved in the process of cigarette smoke (CS) extract-induced cellular senescence in COPD pathogenesis.

Methods: Lamin B1 protein levels in lungs from patients were evaluated. Using human bronchial epithelial cells (HBEC), gene, protein expression, histone modification, and cellular senescence were evaluated during CSE exposure and lamin B1 knockdown. To elucidate the autophagic degradation of lamin B1, confocal microscopic evaluation and immunoprecipitation were performed. To further clarify the physiological relevance of lamin B1 expression levels in COPD pathogenesis C57BL/6J mice were exposed to CS over a 6-months period.

Results: The expression levels of lamin B1 were decreased in COPD lungs compared with non-COPD lungs, which were inversely correlated with pulmonary function test. CS extract induced HBEC senescence with concomitant decrease in lamin B1 expression levels. LaminB1 reduction during CS extract exposure was recovered by lysosomal protease inhibitors and ATG5 knockdown. Confocal microscopic evaluation detected cytoplasmic colocalization between lamin B1 and LC3, reflecting autophagic degradation of lamin B1. DNA microarray and mass spectrometry demonstrated decrease in DEPTOR expression, an mTOR inhibitor by lamin B1 knockdown, which was responsible for subsequent autophagy impairment and cellular senescence. Lamin B1 knockdown enhanced histone modification of H4K20me3 but reduced H3K27me3 and H3K9me. Development of emphysematous change was detected in mouse lungs exposed by CS. Decreased lamin B1 and DEPTOR with concomitantly increased p62 reflecting insufficient autophagic degradation were demonstrated in lung homogenate from CS exposed-mice.

Conclusion: These findings suggest that autophagic degradation of lamin B1 might be involved in COPD pathogenesis through accelerating cellular senescence. Phenotypic alterations linked to cellular senescence can be attributed to epigenetic histone modifications and subsequent insufficient autophagy by reduced laminB1.

PAK4 controls the non-canonical NFkB component RelB to prevent senescence-like growth arrest in breast cancer

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PAK4 is frequently overexpressed in human tumours and contributes to multiple hallmarks of cancer. However, the functional role of PAK4 in tumourigenesis *in vivo* remains largely unknown and the signaling pathways controlled by PAK4 in cancer remain poorly understood.

Here, we report that PAK4 is overexpressed in human breast cancer and associated with poor prognosis.

In multiple *in vitro*, *in vivo* and *ex vivo* cancer models, we demonstrate that PAK4 inhibition almost invariably arrests cancer cell growth while inducing several senescence-like features. MMTV-PyMT-driven mammary tumourigenesis is also impaired in a mouse model with conditional PAK4 depletion in mammary epithelial cells.

RNA sequencing and subsequent mechanistic exploration reveal that PAK4 inhibits NFkB signaling and target gene expression. Further, subunits of the non-canonical NFkB family were required for senescence-like growth arrest upon PAK4 depletion. Importantly, PAK4-mediated phosphorylation of RelB inhibits RelB-DNA interactions.

These findings establish PAK4 as a pro-tumourigenic regulator of breast cancer acting through the non-canonical NFkB subunit RelB. Our study thus uncovers a vulnerability of cancer cells to PAK4 inhibition that may be explored as a therapeutic strategy.

Methylene blue increases expansion and differentiation potential in low oxidative stress populations of human mesenchymal stem cells

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Human bone marrow derived-mesenchymal stem cells (MSC) have potential for use in a range of cell-based therapies. Clinical use of MSC requires large numbers of cells expanded in vitro over a significant amount of time. This in vitro expansion induces cellular senescence, impacting culture quality and application. The study's aim was to decrease cellular senescence of MSC in vitro by modulating oxidative stress and reactive oxygen species (ROS) production by methylene blue (MB) supplementation in MSC in vitro culture media.

We investigated the effect of MB on MSC expansion and differentiation in vitro. Methylene blue is an alternative mitochondrial electron transfer chain molecule (redox modulator) that is known to enhance cell bioenergetics. As such, we analyzed changes in functional aspects of mitochondria and, in conjunction, examined MB effects on cellular senescence pathways.

MSCs were stratified based on in vitro culture fitness, determined by number of colony forming units (CFU) and longevity. This resulted in two groups; (i) weak MSC and (ii) vigorous MSC. Mitochondrial function analysis determined that the two groups differed in oxidative status. MB application significantly improved in vitro MSC expansion and differentiation potential in the vigorous MSC group in vitro, but had little effect on the weak group. While MB application had no effect on mtDNA copy number rate or telomere attrition rate in either group, it reduced the number of senescent cells in the vigorous group.

MSC can be stratified based on oxidative status which can be used for quality control screening. More importantly, MB is able to modulate oxidative stress in low oxidative (vigorous) MSC populations, inhibiting their senescence and increases their expansion and differentiation potentials. MB cannot however rescue MSC with advanced mitochondrial dysfunction (weak group), thus MB should be used as a senescence preventing agent in low oxidative status MSC.

Elucidating how novel pathways cooperate to mediate MDM2 degradation and senescence upon CDK4 inhibition

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CDK4 is an essential cell cycle kinase that phosphorylates Rb and drives cells through the restriction point and into the mitotic cycle. Inhibiting CDK4 activity in cycling cells can induce quiescence, senescence, or apoptosis, depending on the cell type. Which outcome occurs can have implications for therapeutic efficacy. We have previously reported that MDM2 status following treatment with CDK4 inhibitors (CDK4i) can affect whether cells undergo quiescence or senescence, and in a pilot study, we showed that this was associated with patient outcome in well-differentiated and dedifferentiated liposarcoma.

The transition of quiescent cells to senescent cells is called geroconversion. MDM2 down-regulation is associated with and necessary for CDK4i therapy-induced senescence. Thus, we focused on understanding the molecular mechanism controlling MDM2 accumulation in quiescent cells.

In quiescent cells, the deubiquitinase HAUSP dissociates from MDM2. In some cells, reducing HAUSP is sufficient for MDM2 degradation, and these cells undergo geroconversion. However, in other cells, reducing HAUSP is not sufficient for MDM2 degradation, and the cells remain quiescent. Examining the importance of previously described negative regulators of MDM2 turnover, we found that PDLIM7 associates with MDM2 in quiescent cells, where the protein is stabilized. PDLIM7 is sequestered in other complexes in cells in which MDM2 turnover occurs. Sub-cellular localization of PDLIM7 determines whether a cell will undergo senescence or quiescence. In untreated tumor cells that will undergo senescence following treatment with CDK4i, PDLIM7 is in cytoplasmic puncta, while in cells that exit the cycle but fail to senesce, it is localized diffusely throughout the cell.

Co-staining with organelle markers indicates that these puncta are localized at lysosomes and are dependent on the expression of cadherin 18. Cells lacking CDH18 have a modest growth deficit and fail to undergo senescence when treated with CDK4i, although they exit the cell cycle with similar kinetics.

Thus, CDH18 expression and interaction with PDLIM7 contribute to the determination of cell fate following CDK4i, which suggests that monitoring the expression of CDH18 and its interaction with PDLIM7 might be useful as a pretreatment biomarker for CDK4 inhibitor therapy.

Robust, universal biomarker assay to detect senescent cells in biological specimens

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Cellular senescence contributes to organismal development, aging and diverse pathologies. Moreover, genetic or pharmacological elimination of senescent cells, by senolytic drugs, was recently shown to rejuvenate tissues and extend health span in animal models, paving the way for new therapeutic modalities. Consequently, accurate senescence detecting markers are a primary necessity. Nevertheless, available assays to detect senescent cells remain unsatisfactory. Recently, we demonstrated the specificity of the histochemical stain Sudan-Black-B (SBB) to reveal senescent cells, exploiting its ability to react with lipofuscin, an established hallmark of senescence, even in archival material. Yet, SBB staining possesses several technical challenges. Here, we designed and synthesized a lipophilic, biotin-linked Sudan Black-B (SBB) analogue suitable for sensitive and specific, antibody-enhanced detection of lipofuscin-containing senescent cells in any biological material. This new hybrid histo-/immuno-chemical method is easy to perform, reliable and universally applicable to assess senescence in biomedicine, from cancer research to gerontology.

Therapy-induced senescence in melanoma increases mitochondrial bioenergetics, altering both mitochondrial mass and dynamics

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Melanoma is one of the most aggressive types of cancer and its incidence has increased over the last decades. Despite promising advances in therapies, the 5-year survival of patients with metastatic melanoma is still low. Senescence is an end-point of chemotherapy and targeted therapies; and the senescence associated secretory phenotype (SASP) can affect tumor growth and microenvironment influencing the outcome of treatment.

Previous reports have shown that metabolic interventions can modulate the SASP and mitochondrial metabolism appears to play a relevant role in resistance to therapy in melanoma. We sought to assess the bioenergetics of therapy-induced senescent cells. With this purpose B16-F1 mouse melanoma cells were incubated with the alkylating agent temozolomide (TMZ). TMZ triggered the DNA damage response, increased p53 and p21 levels and inhibited cell proliferation. Increases in SA-ß-Gal activity, cell size and gene expression of SASP components were also observed. Approximately 60 % of the culture became senescent after the treatment.

The induction of senescence was accompanied by a substantial increase in mitochondrial basal, ATP-linked and maximum respiration rates; as well as in coupling efficiency, spare respiratory capacity and respiratory control ratio. Assays in permeabilized cells confirmed that mitochondrial electron transport and ADP phosphorylation were higher and more coupled in senescent cells. Further assessment of citrate synthase activity, Mitotracker green staining and mtDNA/nDNA ratios revealed an increase in mitochondrial mass. Besides, more elongated mitochondria and a decrease in the levels of Fis1 and Drp1 recruitment to mitochondria could be observed in senescent cells. These results pointed to alterations in mitochondrial fission that possibly account for the changes in shape and function.

To establish that alterations in mitochondrial function were specifically linked to senescence we studied mitochondrial alterations in isolated senescent cells (separation based on cell size) and used the p53 inhibitor pifithrin-α. Both experimental approaches confirmed that the increase in mitochondrial mass and bioenergetics were associated with senescence.

In sum, our results indicate that the induction of senescence in melanoma by TMZ promotes a p53-dependent reprogramming of melanoma energy metabolism, which involves alterations in both mitochondrial mass and dynamics.

Automated Image Analysis detects aging in Clinical-grade Mesenchymal Stromal Cell Manufacturing

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Background: Senescent cells are undesirable in cell therapy products due to reduced therapeutic activity and risk of aberrant cellular effects, and methods for assessing senescence are needed. Early passage mesenchymal stromal cells (MSC) are known to be small and spindle-shaped but become enlarged upon cell aging. Indeed, cell morphology is routinely evaluated during MSC production using subjective methods. We have therefore explored the integration of automated image analysis of cell morphology into clinical cell manufacturing.

Methods: An automated imaging system was adopted for analyzing changes in cell morphology of bone marrow-derived MSCs during long-term culture. The manifestations of aging and initiation of senescence were monitored by population doubling numbers, expression of $p16^{INK4A}$ and $p21^{Cip1/Waf1}$, β-galactosidase activity and telomeric terminal restriction fragment analysis.

Results: Cell area was the most statistically significant and practical parameter for describing morphological changes. MSCs from passages 1 (p1) and 3 (p3) were remarkably uniform in size with cell areas between 1800-2500 μm^2. At p5 the cells began to enlarge resulting in a 4.5-fold increase at p6-9 as compared to p1. Congruently, the expression of $p16^{INK4a}$ and activity of β-galactosidase increased markedly reaching highest levels at p6-7, after which cell proliferation ceased.

Conclusions: Automated image analysis of cell morphology is a useful tool for evaluating aging in cell cultures throughout the lifespan of MSCs. Our findings suggest that automated morphology analysis can be included in the quality control of clinical-grade MSCs and that cell morphology is still a supreme measure of cell quality.

Chlamydia pneumoniae infection associated to atherosclerosis in Moroccan patients

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Background: Atherosclerosis is a multifactorial vascular inflammatory process; the inflammation of the vessel wall is an important mechanism, responsible for initiation, progression, sclerosis, erosion, and rupture of atherosclerotic plaques, representing therefore a real mechanism of cell death. *Chlamydia pneumoniae* (*C. pneumoniae*) has been suggested as a possible etiologic agent for this inflammatory mecanism, it has been shown that *C. pneumoniae* infects human mononuclear cells, and transmigrate into circulation and infect endothelial cells by cell-to-cell transmission of *C. pneumoniae*.

Objectives: The main objective of our study was to evaluate the association between *C. pneumoniae* and atherosclerosis in Moroccan patients by a case/control approach and strain genotyping.

Methods: A total of 137 patients with cardiovascular diseases and 124 controls were recruited at university hospital CHU IbnRochd, Casablanca, Morocco. Clinical data (pathological and behavioral risk factors) were recorded, blood samples were collected in EDTA tubes and 37 atherosclerotic plaques were obtained from patients subjected to surgery. *C. pneumoniae* DNA detection in peripheral blood mononuclear cells (PBMCs) and atherosclerotic plaques was performed by nested PCR. For strains molecular characterization, a second nested PCR was carried out to amplify a 366 bp fragment of the ompA variable domain 4 gene. The PCR products were sequenced and the sequences were processed by Seaview MUSCLE and statistical analysis was done by SPSS.

Results: The Nested PCR results showed 54% and 18% respectively for positivity in case's and control's PBMCs and 86.5% in atheroma plaques, the difference being significant between the two groups (p<0.001, OR_a=8,580, CI, 95% [3,273-22,491]).

Sequencing of the detected strains showed more than 98% homology with human *C. pneumoniae* reference strains. However, the phylogenetic study and polymorphism analysis of *C. pneumoniae* sequences revealed various genotypes. The comparison between our strains and *C. pneumoniae* strains retrieved in GenBank showed an identity with human strains and some new genotypes were also detected.

Conclusion: Our case/control study revealed *C. pneumoniae* involvement in cardiovascular diseases development especially in atherosclerosis, suffering of vascular tissue in the studied patients; the genotyping showed that most of the detected strains were identical to the human strains circulating worldwide. However, some new strains were also revealed.

Cellular senescence is a major response to cytotoxic chemotherapy in high-grade serous ovarian cancer

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Introduction: High grade serous ovarian cancer (HGSOC) remains the most lethal gynecological malignancy in countries that routinely screen for cervical neoplasia. Given the minimal improvements in treatments and outcomes over the past few decades, a better understanding of cancer cell fate decisions in response to therapy is necessary. In particular, the evaluation of the role of cellular senescence in response to therapy could guide new therapeutic opportunities.

Methods: Key senescence hallmarks, including altered morphology, senescence-associated beta-galactosidase (SA-β-Gal), DNA-SCARS, cell cycle arrest, cyclin dependent kinase inhibitor expression, and the presence of a senescence-associated secretory phenotype (SASP) were evaluated in serially passaged and in carboplatin and paclitaxel- or ionizing radiation-treated primary HGSOC cells. A tissue microarray (TMA) consisting of pre- and post-treatment HGSOC tissue samples was used to evaluate the importance of the senescence response *in vivo* and to assess its impact on clinical outcomes of patients. Senescent primary HGSOC cells were treated with Bcl-2/Bcl-xL inhibitors to evaluate the possibility of redirecting senescent cells to death after standard chemotherapy.

Results: A majority of serially passaged HGSOC primary cultures ultimately undergo replicative senescence, as evidenced by an enlarged and flattened morphology, expression of $p16^{INK4A}$, cell cycle arrest, and positive SA-β-Gal staining. Ionizing radiation and carboplatin/paclitaxel treatments triggered therapy-induced senescence (TIS) in proliferating primary HGSOC cells without causing cell death, suggesting cellular senescence is the major response of primary HGSOC cells to radio- and chemotherapy. Comparison of pre- and post-chemotherapy HGSOC patient tissue samples revealed changes in senescence biomarkers suggestive of TIS in post-treatment samples. Additionally, a stronger TIS response post-chemotherapy correlated with better patient overall survival. Lastly, Bcl-2/Bcl-xL inhibitors synergized with standard chemotherapy to ablate senescent HGSOC primary cultures.

Conclusion: Our data reveal that primary HGSOC cells primarily undergo senescence in response to standard chemotherapy *in vitro*, and that this phenomenon accounts at least in part for the observed *in patient* cellular response. HGSOC TIS could in turn be targeted by senolytic drugs to eliminate residual senescent cancer cells, suggesting a new avenue to enhance current HGSOC chemotherapeutic strategies.

Replicative senescent bone marrow mesenchymal stem cells spotting by surface protein expression

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Mesenchymal Stem Cells (MSC) hold great potential in the field of regenerative medicine in a wide variety of applications thanks to their self renewal capacity, immunosuppressive properties and paracrine effect. Currently, they are the most used in clinical trials with cell-based treatments. However, even with this important research effort, results remain very heterogeneous and far from what was expected. One challenge with this kind of therapy is to ensure cell homogeneity and quality with an extensive characterization of their potential. Previous studies suggested the importance to consider chronological aging and senescence that could incapacitate MSC therapeutic effect. Indeed, it was shown that some MSC functional capacities decline as donor age increases. On top of that, to obtain enough purified MSC, an amplification step is needed with *in vitro* culture that can also induce replicative senescence. In order to better understand MSC senescence and to counteract its deleterious effect, this study focus on surface protein expression to find a way to sort living cells.

As a first step, a proteomic study on MSC surface proteins was conducted on bone marrow MSC from young donors that were brought to replicative senescence by extended *in vitro* culture. This model has the advantage to be close to *in vivo* replicative senescence. These cells were compared to early passage cells coming from the same donors. This study showed that almost all surface proteins expression decreased, didn't change or were variable on replicative senescent MSC except one marker that always increased : CD157. These results were confirmed by flow cytometry with an in-depth analysis considering expression intensity and with the study of various sub-type MSC markers (CD146, CD200, CD106, CD71, CD140b) in addition to the classical MSC surface phenotype (CD105, CD166, CD73, CD90). These results could conduct to the possibility to sort senescent MSC with the association of several cytometric characteristics adjusted for each sample. This could lead to study purified senescent MSC and to improve the therapeutic potential of MSC samples by removing senescent MSC.

Reversal of phenotypes of cell senescence through modulation of mTOR signalling and the actin cytoskeleton

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Cellular senescence is a state of essentially irreversible proliferative arrest, with senescent cells accumulating *in vivo* with increasing chronological age. Senescent cells both fail to contribute to tissue regeneration, and moreover secrete a pro-inflammatory SASP. These changes drive pathological ageing and the progression of age-related disease. Senescent cells are characteristically enlarged, granular, flat and vacuolated cells with visible stress fibres and lipid droplets. Continued cell growth as a consequence of overactive mTOR signaling coupled with a failure of cell division enforced by tumour suppressors p53 and Rb and cyclin kinase inhibitors p21 and p16 may account for many of these phenotypes. Hence modulation of such pathways may be a route to alleviating senescence phenotypes. While it has already been shown that rapamycin, a well characterized mTORC1 inhibitor, can delay the onset of senescence in cell culture, suppress the SASP and lead to improved health and longevity in mice, it does not alter other phenotypes of senescence. We have recently demonstrated reversal of senescence phenotypes, including loss of SAβGAL staining, reduction in cell size, granularity and mitochondrial mass and reacquisition of fibroblastic spindle morphology, by acute treatment of replicatively senescent (RS) human fibroblasts with nanomolar doses of a dual mTORC1/2 inhibitor [1]. We have now extended these studies to demonstrate that dual mTORC inhibition can reverse DNA-damage induced and oncogene-induced senescence as well as RS, highlighting a common pathway of senescence modulation. We have further explored the molecular basis for such reversal, using a range of techniques including live cell imaging and comparative proteomic analysis on both chronic and acute drug dosing. Our results strongly support the notion that mTORC signalling underpins the SASP, and additionally we identify for the first time a key role in cellular senescence of biochemical pathways regulating the actin cytoskeleton.

1. Walters, H.E., S. Deneka-Hannemann, and L.S. Cox, Reversal of phenotypes of cellular senescence by pan- mTOR inhibition. Aging Vol 8 No. 2, pp 231-244 (Albany NY), 2016

Long-term Treatment with Exogenous CDK4/6 Inhibitors Induces Cellular Senescence in Primary Non-tumoral Cells

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Cellular senescence is a state of irreversible growth arrest, which relies on the activity of cyclin-dependent kinase (CDK) inhibitors p16 and p21. Moreover, cells induced to senescence by DNA damage activate the senescence-associated secretory phenotype (SASP), a complex secretory program that promotes many physiological and pathological processes, including age-related diseases and cancer. However, the overexpression of p16 is sufficient to induce cellular senescence without the activation of DNA damage and a SASP. Palbociclib (PD-0332991) and abemaciclib (LY-2835219) are selective CDK4/6 inhibitors (CDKi), similar to endogenous p16, and are currently used for treating breast cancer patients. To explore the roles of exogenous CDK4/6 inhibitors in cellular senescence and SASP regulation, primary human foreskin (BJ) and lung (WI38) fibroblasts treated with palbociclib or abemaciclib were analyzed. During the treatment, cells were growth arrested and displayed enlarged cell size. The growth arrest was maintained even 1 week after removal of the CDKi, and associated with elevated SA-β-galactosidase activity. Importantly, functional p53 was essential for the irreversible growth arrest induced by palbociclib or abemaciclib. Similar to p16-overexpression, exogenous CDK4/6 inhibitors induced a lower SASP when compared with genotoxic drugs such as doxorubicin. Conditioned medium (CM) collected from doxorubicin-induced senescent cells promoted cancer cell migration, while exposure to CM from palbociclib- or abemaciclib-treated cells did not exert any effect. In addition, these drugs induced irreversible growth arrest in mouse embryonic fibroblasts without p16 up-regulation, implying different mechanisms for cellular senescence induction between human and mouse cells. Pharmacological interventions aimed at interfering with the activity of CDKs might represent a less toxic strategy for cancer treatment.

RNAi and CRISPR/Cas9 based *in vivo,* models for drug discovery

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With the advent of CRISPR/Cas9 technology, the speed and precision in which genetically engineered mouse models can be created is unprecedented. We now have at our disposal a genetic toolbox that will enable the rapid generation of sophisticated mouse models of human disease. We have reproducible RNA interference (RNAi) technology, which can be exploited experimentally to effectively and reversibly silence nearly any gene target not only *in vitro* but also in live mice. Here, we take advantage of these powerful technologies and combine both CRISPR/Cas9 and inducible RNAi-mediated gene silencing to not only model disease, but also mimic drug therapy via RNAi in the same mice, giving us advanced capabilities to perform preclinical studies *in vivo*. Furthermore, we have harnessed the power of CRISPR/Cas9 to create a novel platform for more rapid and cost-effective production of RNAi models that will serve as entry into the generation of higher organisms such as rats and guinea pigs with reversible gene silencing capabilities in the near future. Undoubtedly, extending our RNAi technology into other species will transform cancer research by providing model systems in which disease and therapeutic outcomes more closely resemble their human counterparts. The ability to better model clinical disorders and evaluate genetic and environmental stimuli in advanced mammalian species will increase our confidence in predicting drug responses in humans and push drug discovery research into a new era.

Last minute

Development of a novel ChIP-Seq spike-in normalization strategy to enable detection of genome-wide differences in histone modification levels in the presence of epigenetic inhibitors

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Small molecule inhibitors of EZH2 have great therapeutic potential for cancer. In order to fully understand how inhibitors of EZH2 function therapeutically, it is necessary to understand how inhibitor treatment alters H3K27me3 occupancy across the entire genome and how this may affect gene expression. Genome-wide maps of histone modification patterns can be generated using ChIP-Seq, however our experience has shown that current ChIP-Seq methodology and available ChIP-Seq data analysis approaches do not detect the expected genome-wide decrease in H3K27me3 following EZH2 inhibitor treatment. To overcome this challenge we developed a novel chromatin spike-in strategy for ChIP-Seq. Specifically, we introduced drosophila chromatin as a minor fraction of total chromatin into our ChIP reactions and added a drosophila specific antibody as a way to consistently pull out drosophila chromatin as a minor fraction of the total ChIP DNA. The drosophila ChIP-Seq reads are used to normalize the human ChIP-Seq data from DMSO and inhibitor treated samples. Using this strategy we now detect dramatic reductions in H3K27me3 levels in the presence of EZH2 inhibitors.

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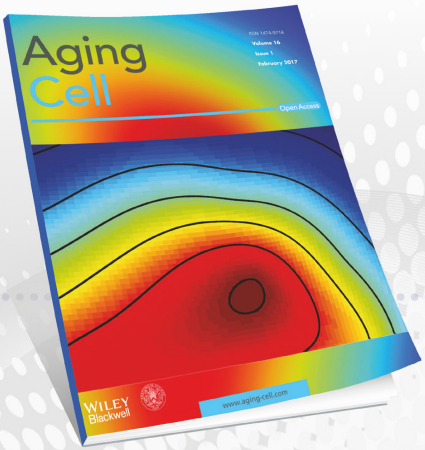

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