

ISF Workshop

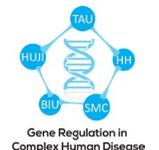
Annual Meeting of the
International Cellular Senescence Association

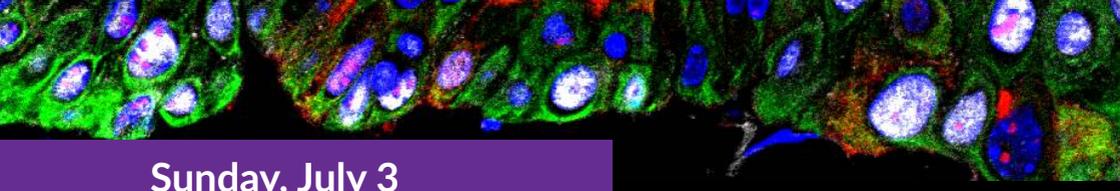
Cellular Senescence: From Mechanisms to Therapeutic Opportunities

July 3-6, 2016

The David Lopatie Conference Centre, Weizmann Institute of Science

Program





Sunday, July 3

15:30-21:00 **Opening session: Immune surveillance and inflammation**
Chair: **Dorothy Bennett**

15:30-16:00 Registration

16:00-16:15 **Dorothy Bennett**, ICSA President
Welcome and introduction

16:15-17:00 **Scott Lowe**, Memorial Sloan-Kettering Cancer Center
Immune surveillance of premalignant senescent cells

17:00-17:25 **Angela Santoni**, Sapienza University of Rome
NK cell-mediated immunosurveillance against drug-induced senescent tumor cells

17:25-17:40 **Juan-Carlos Acosta**, University of Edinburgh
The activation of the Inflammasome by Toll-like receptors (TLRs) regulates Oncogene Induced Senescence

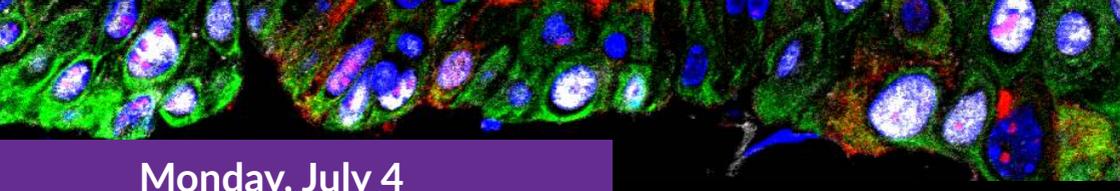
17:40-18:00 Coffee break

18:00-18:25 **Yinon Ben-Neriah**, The Hebrew University of Jerusalem
Senescence-associated parainflammation

18:25-18:40 **Ana O'Loghlen**, Queen Mary University
Role for integrin beta 3 regulating cellular senescence and ageing through focal adhesion formation

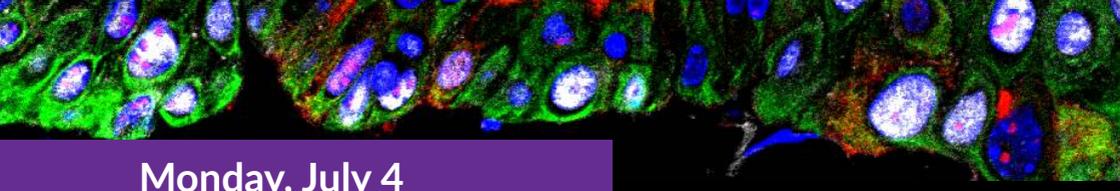
18:40-18:55 **Andrew Koff**, Memorial Sloan Kettering Cancer Center
Biomarker discovery through a molecular understanding of CDK4 inhibitor therapy induced senescence

19:00-21:00 Cocktail Dinner



Monday, July 4

- 09:00-14:15 **Session 1:** Senescent cells in physiological and pathophysiological conditions
Chair: **Manuel Serrano**
- 09:00-09:25 **Jan van Deursen**, Mayo Clinic
How senescent cells drive aging and age-related disease
-
- 09:25-09:50 **Jennifer Gamble**, University of Sydney Medical School & Centenary Institute
Inflammation and Endothelial Senescence
-
- 09:50-10:05 **Tom Bird**, University of Edinburgh
Inhibition of TGF beta reduces paracrine senescence and improves hepatic regeneration in acute liver injury
-
- 10:05-10:20 **Maximina Yun**, University College London
Conserved and novel functions of programmed cellular senescence during vertebrate development
-
- 10:20-10:45 Coffee Break
-
- 10:45-11:10 **Martin Bennett**, University of Cambridge
Vascular smooth muscle cell senescence in atherosclerosis
-
- 11:10-11:35 **Victor J. Thannickal**, University of Alabama
Myofibroblast Senescence in Fibrotic Lung Disease
-
- 11:35-12:00 **Valery Krizhanovsky**, Weizmann Institute of Science
The role of club cells senescence in chronic lung disease
-
- 12:00-12:15 **Antonio Sarikas**, Technical University of Munich
Essential role for premature senescence of myofibroblasts in myocardial fibrosis
-
- 12:15-14:15 Lunch and Poster session
-



Monday, July 4

14:15-19:05 **Session 2: Senescence, epigenetics and metabolism**
Chair: **Scott Lowe**

14:15-15:00 **Daniel Peeper**, Netherlands Cancer Institute
EMBO Lecture: Oncogene-induced senescence: what if it fails?

15:00-15:25 **Peter Adams**, University of Glasgow
Epigenetics of cell senescence ? insights into aging and cancer

15:25-15:50 **Oliver Bischof**, Institut Pasteur
Dynamic epigenomic and transcriptomic profiling reveal stress-specific senescence states

15:50-16:05 **Hannah Walters**, University of Oxford
Reversal of phenotypes of senescence by pan-mTOR inhibition

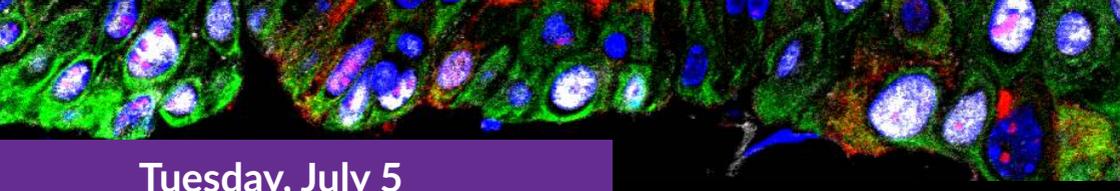
16:05-18:00 Coffee and Poster session

18:00-18:25 **Gerardo Ferbeyre**, University of Montreal
Defective ribosome biogenesis in senescence reveals a novel checkpoint pathway to control cyclin-dependent kinases

18:25-18:50 **Jesus Gil**, Imperial College London
The SWI/SNF complex prevents liver cancer by regulating senescence

18:50-19:05 **Aaron Mendez-Bermudez**, Institute for Research on Cancer and Aging (Nice)
Role of the telomeric protein TRF2 in heterochromatic replication and cellular senescence

19:05-21:00 Cocktail Dinner



Tuesday, July 5

09:00-10:45 **Session 3: Senescence and stem cells**

Chair: **Clemens Schmitt**

09:00-09:30 **Manuel Serrano**, Spanish National Cancer Research Center
An integrated view of senescence and reprogramming

09:30-09:55 **Pura Muñoz-Cánoves**, ICREA/UPF (Barcelona)
and CNIC (Madrid)
Stem cell senescence in aging muscle

09:55-10:10 **Han Li**, Institut Pasteur
Senescence is critical for injury-induced in vivo reprogramming in skeletal muscle

10:10-10:25 **Jacob Goodwin**, Karolinska Institutet
Loss of CDK2 Inhibits BRAFV600E/MYC-induced mouse lung tumor development

10:25-10:45 Coffee Break

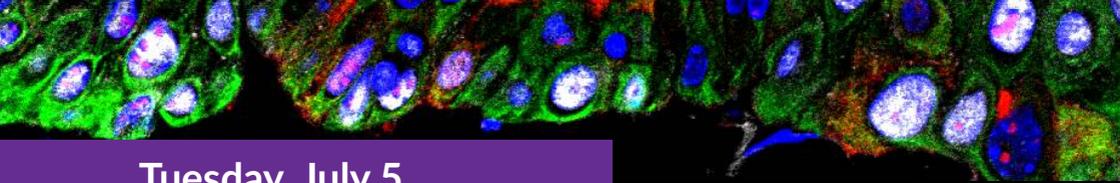
10:45-12:15 **Session 4: DNA damage**

Chair: **Judith Campisi**

10:45-11:10 **Jan Hoeijmakers**, Erasmus University Rotterdam
Maintaining Nature's Perfection: impact of DNA repair and possibilities for healthy aging

11:10-11:35 **Fabrizio d'Adda di Fagagna**, IFOM (Milano)
The role of DNA damage response modulation in cellular senescence

11:35-12:00 **Yossi Shiloh**, Tel Aviv University
The DNA damage response at the crossroads of genome stability, aging and cell senescence

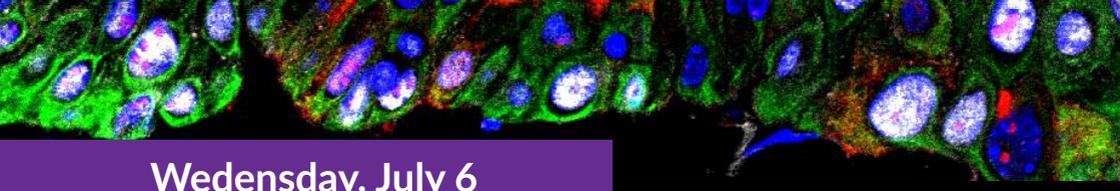


Tuesday, July 5

12:00-12:15 **Corinne Abbadie**, CNRS (Paris)
A new DNA damage route to senescence and neoplastic escape

12:15-14:15 Lunch & Posters

14:15-21:00 Tour



Wednesday, July 6

09:00-10:45 **Session 5:** Senescence in the hematopoietic system
Chair: **Angela Santoni**

09:00-09:25 **Clemens Schmitt**, Max-Delbruck-Center for
Molecular Medicine
**Therapeutic implications of primary and secondary
senescence in cancer**

09:25-09:50 **Daohong Zhou**, University of Arkansas for Medical Sciences
**Clearance of senescent cells rejuvenates aged
hematopoietic stem cells**

09:50-10:05 **Peter de Keizer**, Erasmus University Medical Center
**Selective targeting of senescence through a FOXO4-based
peptide to counteract features of aging, chemotoxicity and
cancer therapy resistance**

10:05-10:20 **Muge Ogrunk**, INSERM (Paris)
**Molecular Insights of APL cure as therapy-
induced Senescence**

10:20-10:45 **Coffee Break**

10:45-12:45 **Session 6:** Senescence –how many faces after all?
Chair: **Valery Krizhanovsky**

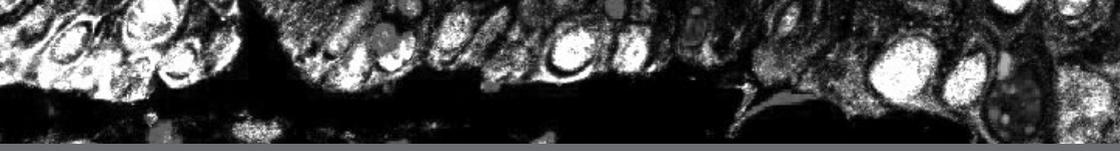
10:45-11:10 **Andrei Gudkov**, Roswell Park Cancer Institute
**Biological sense of senescent cells: value and price of their
pharmacological eradication**

11:10-11:35 **Ittai Ben-Porath**, The Hebrew University of Jerusalem
The benefits of aging-associated beta cell senescence

11:35-12:20 **Judith Campisi**, Buck Institute for Research on Aging
Cellular senescence: Quo vadis?

12:20-12:45 **Prizes/Fellowships & Wrap up**

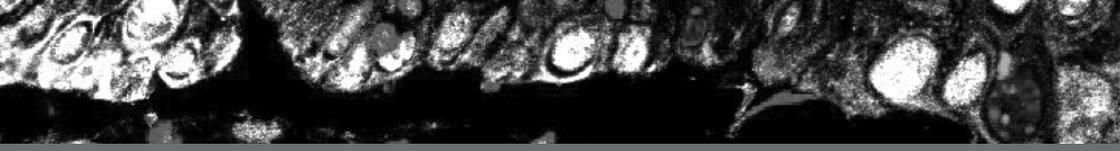
12:45-14:00 **Lunch**



Immune surveillance of premalignant senescent cells

Scott W. Lowe, Howard Hughes Medical Institute, Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065 USA.

Our laboratory is interested in the roles and regulation of cellular senescence during the processes of tumorigenesis, therapy response, wound healing, and aging. Senescent cells display a repressive chromatin configuration thought to stably silence proliferation-promoting genes, while simultaneously activating an unusual form of immune surveillance involving a secretory program referred to as the senescence-associated secretory phenotype (SASP). We demonstrate that senescence also involves a global remodeling of the enhancer landscape with recruitment of the chromatin reader BRD4 to newly activated super-enhancers adjacent to key SASP genes. Transcriptional profiling and functional studies indicate that BRD4 is required for the SASP and downstream paracrine signaling. Consequently, BRD4 inhibition disrupts immune cell mediated targeting and elimination of premalignant senescent cells in vitro and in vivo. Our results identify a critical role for BRD4-bound super-enhancers in senescence immune surveillance and in the proper execution of a tumor-suppressive program. More broadly, they help firmly establish senescence as a two component process of gene repression and gene activation programs that give rise to the stable arrest and secretory phenotype, respectively.



The activation of the Inflammasome by Toll-like receptors (TLRs) regulates Oncogene Induced Senescence.

Priya Hari, Irene Fernandez-Duran, Nuria Tarrats and Juan Carlos Acosta.

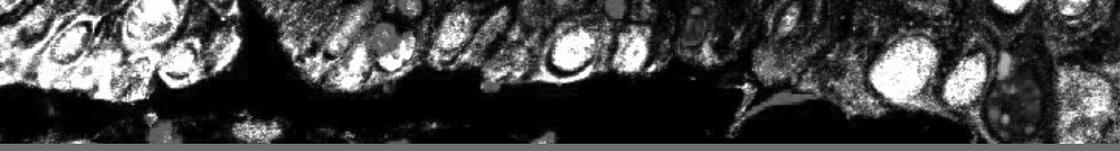
Edinburgh Cancer Research UK Center, The Institute of Genetics and Molecular Medicine, University of Edinburgh. Crewe Road, Edinburgh EH4 2XU, UK.

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 Damage Associated Molecular Patterns (DAMPs) (i.e. HMGB1 or alarmin).

Gene Set Enrichment Analysis of genes induced during OIS showed the enrichment of several groups of genes belonging to distinct PRR families. In a candidate-based loss of function siRNA screen searching for potential new PRRs regulating OIS, we identified a member of the Toll like receptor (TLR) family, TLR2, as a key regulator of OIS. TLR2 expression is upregulated during OIS, and Knockdown of TLR2 inhibited the induction of both p16-Rb and p53-p21 tumour suppressor pathways, the activation of IL1 signalling and the SASP. Activation of TLR2 during OIS primed a specific inflammasome by induction of its components and IL1B. Moreover, activation of OIS also induced the cleavage of other important functional substrates of the inflammasome. Knockdown of this specific inflammasome strongly bypassed the senescent response, the priming and cleavage of inflammasome substrates and the activation of the SASP, indicating a key role of the TLR2-inflammasome pathway in the regulation of OIS. Finally, the analysis of human cancer data sets have shown that mutations and deletions of inflammasomes are associated significantly with worst overall survival. Furthermore, High expression of TLR2 and its counterparts TLR1, TLR2 and TLR10, as well as the associated inflammasome is linked with better prognosis (Overall survival) in lung adenocarcinoma and gastric cancer, what is a characteristic of tumour suppressor genes. In summary, we are proposing for the first time a model where the activation of TLR signaling by oncogenic Ras regulates the onset and maintenance of OIS by regulating the activation of the inflammasome.



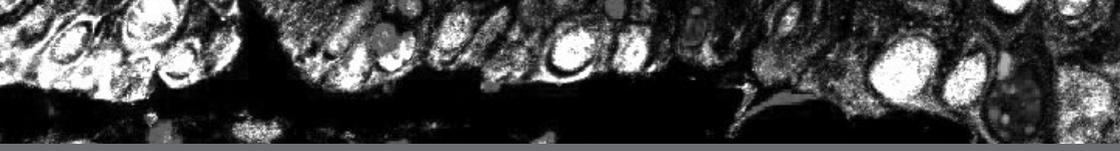
Senescence-associated parainflammation

Audrey Lasry, Dvir Aran, Adar Zinger, Upasana das Adhikary, Eliran Kadosh, Asaf Hellman Eli Pikarsky, Atul Butte, Yinon Ben-Neriah

The Lautenberg Center for Immunology and Cancer research, Hebrew University-Hadassah Medical School, Jerusalem and Institute for Computational Health Sciences, University of California, San Francisco, California

Inflammation has many faces, most commonly observed as an acute reaction in response to pathogen or another insult, or a chronic phase, accompanying chronic infection and chronic remittent inflammatory disease, such as inflammatory bowel disease. Yet, there is another type of smoldering inflammation, which is harder to notice or monitor; it is called parainflammation and appears to underlie some of the major human diseases, cancer, diabetes type 2 and certain neurodegenerative diseases¹. We have developed mouse models of cancer based on inducible CK1 α knockout, which exhibit smoldering inflammation, and demonstrate how a low grade, infiltrate-free inflammatory reaction to persistent DNA damage response translates to an aberrant growth². To that end we determined the inflammatory repertoire of the knockout mice, demonstrated its association with cellular senescence and showed how in the absence of p53, a senescence-inflammatory response (SIR) is converted from a growth inhibitory to growth promoting mechanism, both in vitro and in vivo³. Anti-inflammatory reagents capable of blocking SIR prevent carcinogenesis in mutant mice. We determined the transcriptome signature of parainflammation, explored its prevalence in human cancer and determined its relationship to certain molecular and clinical parameters affecting treatment and prognosis. We show that parainflammation-positive tumors are enriched for p53 mutations and associated with poor prognosis. NSAID treatment suppresses parainflammation in both murine and human cancers, possibly explaining a protective effect of NSAIDs against cancer⁴. Our data suggests that parainflammation may be a driver for p53 mutagenesis and a guide for cancer prevention by NSAID treatment⁵.

1. Medzhitov, R. Origin and physiological roles of inflammation. *Nature* 454, 428-35 (2008).
2. Elyada, E. et al. CK1 α ablation highlights a critical role for p53 in invasiveness control. *Nature* 470, 409-13 (2011).
3. Pribluda, A. et al. A senescence-inflammatory switch from cancer-inhibitory to cancer-promoting mechanism. *Cancer Cell* 24, 242-56 (2013).
4. Aran et al, Widespread parainflammation in human cancer, *Genome Biol*, In Press.
5. Lasry A, Zinger A and Ben-Neriah Y, Inflammatory networks underlying colon cancer. *Nature Immunology*, 2016, 17:230-40



Role for integrin beta 3 regulating cellular senescence and ageing through focal adhesion formation

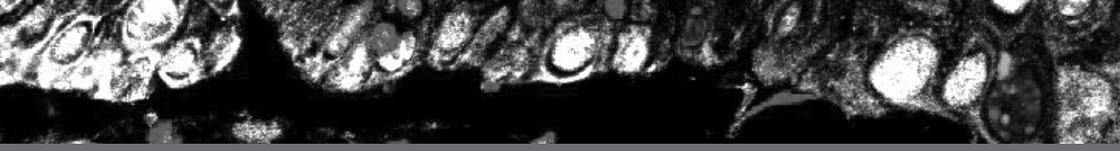
V Rapisarda¹, S Sodini¹, M Borghesan¹, W Alazawi², A Lujambio³ and A O'Loghlen¹

¹Epigenetics and Cellular Senescence Group, Blizard Institute, Queen Mary University of London, 4 Newark Street, London E1 2AT, UK

²Immunobiology Group, Blizard Institute, Queen Mary University of London, 4 Newark Street, London E1 2AT, UK

³Department of Oncological Sciences, Mount Sinai Hospital, NYC, USA

Cellular senescence is an important *in vivo* mechanism to prevent the propagation of damaged cells. It induces a permanent cell cycle arrest, where the cells are metabolically and transcriptionally active. Senescent cells undergo extensive changes in gene expression, including the expression of cell cycle inhibitors and of a distinctive secretory phenotype named SASP, which affects the tissue homeostasis. Activation of senescence during ageing has been demonstrated to be detrimental, due to the reduced clearance of senescent cells by the immune system. However, the mechanisms regulating senescence and ageing and affecting the surrounding microenvironment are not well characterised. Here, we show a role for integrin beta 3 (ITGB3) regulating senescence and ageing. ITGB3 expression accelerates the onset of senescence in human primary fibroblasts, by activating the latent form of TGF β that binds to the extracellular matrix (ECM). This induces the activation and release of soluble TGF β to the media, which in turn activates senescence and induces the SASP. Blocking ITGB3 binding to the extracellular matrix overcomes the senescence arrest and the secretion of the SASP. Furthermore, endogenous ITGB3 expression is increased by different triggers of senescence in human fibroblasts, but also along human and mouse ageing. Our results demonstrate the importance of focal adhesion formation during senescence activation and ageing, and identify integrins as new therapeutic targets to promote healthy ageing.



Biomarker discovery through a molecular understanding of CDK4 inhibitor therapy induced senescence.

Mary E. Klein^{1,2}, Marta Kovatcheva^{1,2}, Jossie Yashinski¹, Mark Dickson³, William D. Tap³, Samuel Singer⁴, and Andrew Koff^{1,2}

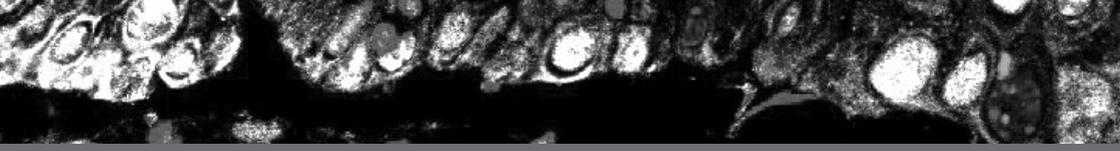
¹Laboratory of Cell Cycle Regulation, ²The Louis V. Gerstner Graduate School of Biomedical Sciences, and the Departments of ³Medicine and ³Surgery, Memorial Sloan Kettering Cancer Center, NY, NY USA 10065.

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 had previously reported that what happens to MDM2 following treatment with CDK4i can affect whether cells undergo quiescence or senescence, and in a pilot study showed 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 CDK4 inhibitor therapy induced senescence. Thus we focused on 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 will 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. The components of these complexes are being identified and include a cadherin molecule.

While we further our efforts to understand this interaction, we are simultaneously developing a proximity ligation based assay for use in the clinic as a companion diagnostic capable of predicting whether CDK4 inhibition will result in cellular senescence.

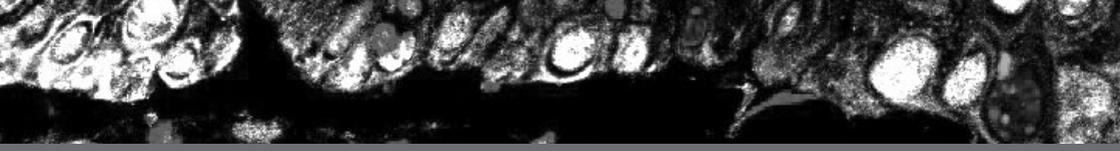


Title: Inhibition of TGF β reduces paracrine senescence and improves hepatic regeneration in acute liver injury.

Authors: Thomas G Bird^{1,2,3}, Rachel A Ridgway³, Luke Boulter¹, Wei-Yu Lu¹, Thomas Jamieson³, David F Vincent³, Olivier Govaere⁴, Sofia Ferreira-Gonzalez¹, Alicia M Cole³, Trevor Hay⁵, Kenneth J Simpson¹, Mairi Clarke⁶, Ana Sofia Rocha³, Robert J B Nibbs⁶, Nico Van Rooijen⁷, John P Iredale², Alan R Clarke⁵, Tania A Roskams⁴, Owen J Sansom³ and Stuart J Forbes^{1,2}

Affiliations: ¹MRC Centre for Regenerative Medicine, University of Edinburgh, UK. ²MRC Centre for Inflammation Research, University of Edinburgh, UK. ³Cancer Research UK Beatson Institute, Glasgow, UK. ⁴Department of Imaging and Pathology, KU Leuven and University Hospitals Leuven, Belgium. ⁵School of Biosciences, Cardiff University, UK. ⁶Institute for Infection Immunity and Inflammation, University of Glasgow, UK. ⁷Vrije Universiteit Medical Center (VUMC), The Netherlands.

Abstract: Liver injury results in rapid regeneration through hepatocyte division. However, following acute severe injury, effective regeneration of the liver may fail. We investigated whether and how senescence underlies this regenerative failure. In human disease and murine models of liver injury we observed acute hepatocellular senescence marker expression (SA- β Gal, p21^{WAF1+}, p16^{INK4a}, γ H2Ax and DCR2) occurring in proportion to the severity of necrosis. We then utilized an *in vivo* murine model where hepatocyte-specific deletion of *Mdm2* ($\Delta Mdm2$) induces hepatocyte senescence. Using numerous strategies to target $\Delta Mdm2$ to discrete populations of hepatocytes we analysed direct versus indirect induction of senescence. Following hepatocyte $\Delta Mdm2$ other hepatocytes without $\Delta Mdm2$ display activation of p21^{WAF1+}. This occurs in response to either local $\Delta Mdm2$ senescent hepatocytes, or to recipient $\Delta Mdm2$ hepatocytes in a transplantation model. Having observed TGF β pathway activity in senescent hepatocytes in human disease and murine models we examined TGF β R1 as a tractable target to prevent paracrine senescence induction *in vivo*. Paracrine senescence was dependent upon macrophages, CCL2 and TGF β R1 in the $\Delta Mdm2$ model. Inhibition of TGF β R1 in liver injury reduces hepatocellular senescence and improves hepatocellular regeneration. It additionally restores the regenerative response to heightened injury which is typically lost during severe liver disease. Thus, this mechanism of acute spreading senescence through which solid organ injury impairs local regeneration offers a therapeutic target in severe acute tissue injury.



Conserved and novel functions of programmed cellular senescence during vertebrate development

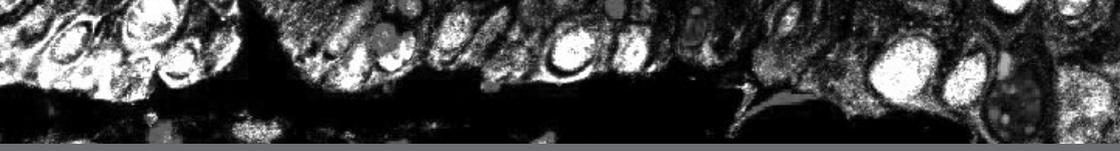
Hongorzul Davaapil^{1,2}, Jeremy P. Brockes¹ and Maximina H Yun^{1*}

¹ Institute of Structural and Molecular Biology, Division of Biosciences, University College London, Gower Street, London, WC1E 6BT, UK

² Institute of Ophthalmology, University College London, 11-43 Bath Street, London, EC1V 9EL, UK

Cellular senescence is an anti-tumourigenic mechanism which, in the long-term, can lead to disruption of tissue structure and function. Accumulation of senescent cells has recently been shown to contribute to age-related disorders in mammals, establishing senescence as a hallmark of ageing. However, in recent years it has become clear that senescent cells can contribute to physiological processes, as illustrated by the seminal finding that cellular senescence is a programmed mechanism that contributes to mammalian development. These findings have led to the proposal that developmental senescence may have predated the evolutionary origin of stress-induced senescence. However, the degree of conservation of developmental senescence through phylogeny, as well as the extent of its functions during development, remains unknown.

Here, we show that cell senescence is an intrinsic part of the developmental programme in amphibians. Programmed senescence occurs in specific structures at defined time-windows during amphibian development. It contributes to the physiological degeneration of the amphibian pronephros and to the development of the cement gland and neighbouring regions such as the oral cavity. In both contexts, senescence depends on TGF β signalling but is independent of ERK/MAPK activation. Furthermore, elimination of senescent cells through temporary TGF β inhibition leads to developmental defects in the embryonic kidney, cement gland and primary mouth. Our findings uncover conserved and new roles of senescence in vertebrate organogenesis and support the view that cellular senescence may have arisen in evolution as a developmental mechanism.



Vascular smooth muscle cell senescence in atherosclerosis

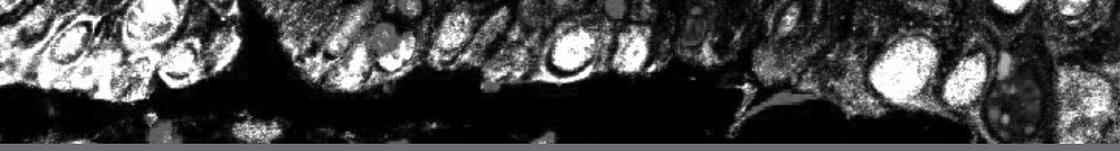
Martin Bennett, Division of Cardiovascular Medicine, University of Cambridge, Cambridge

Background: Cardiovascular disease is the commonest cause of death in industrialised countries, and will soon be the commonest cause of death worldwide. Most myocardial infarctions (heart attacks) and strokes result from rupture of atherosclerotic plaques; the factors that determine plaque stability are therefore of fundamental importance. Plaques comprise an accumulation of vascular smooth muscle cells (VSMCs) and inflammatory cells (macrophages and T lymphocytes), dead cells, and intracellular and extracellular lipid. VSMCs in advanced plaques and cultured from plaques show evidence of VSMC senescence and DNA damage, including telomere shortening, mitochondrial DNA damage, and oxidative stress, all of which can directly induce senescence. Senescence can have multiple effects on plaque development and morphology; however, the consequences of VSMC senescence or the mechanisms underlying VSMC senescence are mostly unknown.

Methods and Results: We examined expression of proteins that protecting telomeres from shortening in VSMCs derived from human plaques and normal vessels. Plaque VSMCs showed reduced expression and telomere binding of Telomeric repeat-binding factor-2 (TRF2), associated with increased DNA damage. DNA damage induced loss of TRF2 by increased ubiquitin-mediated degradation of TRF2 protein. To examine the functional consequences of loss of TRF2, we expressed TRF2 or a TRF2 functional mutant (T188A) as either gain or loss of function studies *in vitro* and in ApoE^{-/-} mice. TRF2 overexpression bypassed senescence, reduced DNA damage, and accelerated DNA repair, whereas TRF2^{T188A} showed opposite effects. Transgenic mice expressing VSMC-specific TRF2^{T188A} showed increased atherosclerosis and necrotic core formation *in vivo*, whereas VSMC-specific TRF2 increased relative fibrous cap and decreased necrotic core areas. TRF2 protected against atherosclerosis independent of secretion of senescence-associated cytokines.

MtDNA damage occurred early in the vessel wall in Apolipoprotein E null (ApoE^{-/-}) mice, before significant atherosclerosis developed. MtDNA defects were also identified in circulating monocytes and liver, and associated with mitochondrial dysfunction as determined by Complex I activity. To determine whether mtDNA damage directly promotes atherosclerosis, we studied ApoE^{-/-} mice deficient for mitochondrial polymerase- γ proofreading activity (polG^{-/-}/ApoE^{-/-}). polG^{-/-} mice show accelerated ageing across a range of tissues, but without increased ROS. polG^{-/-}/ApoE^{-/-} mice showed extensive mtDNA damage and defects in oxidative phosphorylation, but no increase in ROS. polG^{-/-}/ApoE^{-/-} mice showed increased atherosclerosis, associated with impaired proliferation and apoptosis of vascular smooth muscle cells, and hyperlipidaemia. Transplantation with polG^{-/-}/ApoE^{-/-} bone marrow increased features of plaque vulnerability, and polG^{-/-}/ApoE^{-/-} monocytes showed increased apoptosis and inflammatory cytokine release.

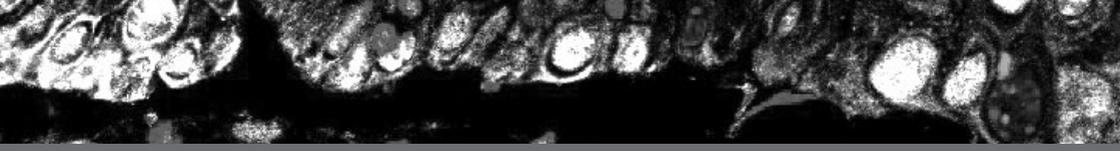
Conclusions: We conclude that plaque VSMC senescence in atherosclerosis is associated with loss of TRF2. VSMC senescence promotes both atherosclerosis and features of plaque vulnerability, identifying prevention of senescence as a potential target for intervention. mtDNA damage is present, widespread, causative and indicates higher risk in atherosclerosis. Protection against mtDNA damage and improvement of mitochondrial function are potential areas for new therapeutics.



"Myofibroblast Senescence in Fibrotic Lung Disease"

Victor J. Thannickal, University of Alabama

Fibroblast senescence has been shown to be essential for the termination of wound healing responses in the liver and skin, primarily in murine models of acute tissue injury. In aged mice subjected to lung injury, we observed a deficiency in the resolution of fibrotic repair in comparison to young mice. This deficiency was associated with the acquisition of a senescent and apoptosis-resistant phenotype of tissue myofibroblasts. Myofibroblast senescence is mediated, in part, by redox imbalance involving the reactive oxygen species (ROS)-generating enzyme, NADPH oxidase 4 (NOX4), and the Nrf2-dependent antioxidant response pathway. Human fibrotic lung disease, in particular idiopathic pulmonary fibrosis (IPF), replicates many of these features seen in aged mice with non-resolving fibrosis. Together, our data indicate that myofibroblast senescence in the context of aging is more sustained and contributes to fibrotic tissue remodeling. Senolytic therapies targeting myofibroblasts may be an effective therapeutic strategy for chronic fibrotic diseases, including those of the lung.

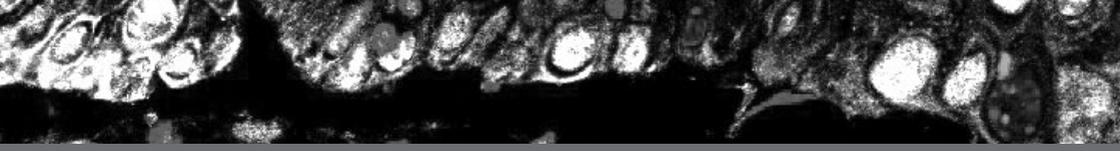


The role of club cell senescence in chronic lung disease

Adi Sagiv¹, Amir Bar-Shai², Miki Hatzav², Naama Feldman¹, Lior Zada¹, Ezra Vadai¹, Ronen Alon² and Valery Krizhanovsky¹

¹Department of Molecular Cell Biology, ²Department of Immunology, Weizmann Institute of Science, Rehovot, Israel

Repeated airway injury gives rise to severe bronchitis and chronic obstructive pulmonary disease (COPD). Club (Clara) cells are multifunctional exocrine cells of the distal airways (bronchioles) involved in airway protection from air-borne toxins and epithelial regeneration following injury. In response to various forms of stress, p53 limits tumorigenesis by inducing apoptosis, cell cycle arrest and senescence. We have therefore hypothesized that p53 in club cells would protect these cells and their surrounding tissue from chronic inflammation. To that end we have constructed a mouse model where p53 is specifically knocked out in club cells and subjected these mice to repetitive lipopolysaccharide (LPS) inhalations which mounted severe bronchitis. Surprisingly, lungs deficient in p53 in their club cells were significantly protected from this chronic inflammatory response reflected in reduction of accumulation in both bronchio-alveolar space and lung interstitium of neutrophils, macrophages as well as CD4 and CD8 T cells. Moreover, in control mice we observed accumulation of bronchus associated lymphoid tissue (BALT)-like T cell and B cell aggregates adjacent to bronchioles following repeated LPS exposure. These aggregates were dramatically reduced in their amount and size in Club cell specific p53 knockout mice. Importantly, these Club cell specific p53 knockout mice were as sensitive as control mice to acute lung inflammation elicited by a single LPS exposure. In order to understand the role of club cell p53 in chronic inflammation caused by repeated LPS exposure we analyzed these cells for molecular markers of senescence and apoptosis, two key processes regulated by p53. Chronically inflamed bronchioles were found to express senescence, but not apoptosis markers, which were significantly attenuated in the club cell specific p53 knockout mice. We propose that p53 is necessary for the induction of cellular senescence in club cells exposed to repeated LPS and smoke induced injury and inflammation. Our data therefore provides the first positive link between p53 induced club cell senescence and progression of airway inflammation.



Essential role for premature senescence of myofibroblasts in myocardial fibrosis

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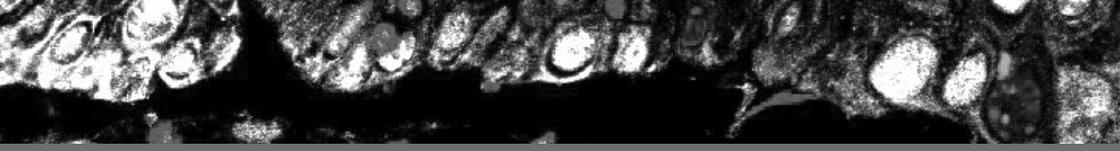
Background: Fibrosis is a hallmark of many myocardial pathologies and contributes to distorted organ architecture and function. Recent studies have identified premature senescence as regulatory mechanism of tissue fibrosis. However, its relevance in the heart remains to be established.

Objective: To investigate the role of premature senescence in myocardial fibrosis.

Methods: Murine models of cardiac diseases and human heart biopsies were analyzed for characteristics of premature senescence and fibrosis. Loss-of-function and gain-of-function models of premature senescence were used to determine its pathophysiological role in myocardial fibrosis.

Results: Senescence markers p21^{CIP1/WAF1}, senescence-associated β -galactosidase (SA- β -gal) and p16^{INK4a} were increased 2-, 8- and 20-fold ($n=5-7$; $P < 0.01$), respectively, in perivascular fibrotic areas after transverse aortic constriction (TAC) when compared to sham-treated controls. Similar results were observed with cardiomyocyte-specific β 1-adrenoceptor transgenic mice and human heart biopsies. Senescent cells were positive for platelet derived growth factor receptor α ($92.5 \pm 1.8\%$), vimentin ($92.1 \pm 1.5\%$), and α -smooth muscle actin ($65.2 \pm 10.5\%$), specifying myofibroblasts as the predominant cell population undergoing premature senescence in the heart. Inactivation of the premature senescence program by genetic ablation of p53 and p16 INK4a (Trp53^{-/-} Cdkn2a^{-/-} mice) resulted in aggravated fibrosis after TAC when compared to wild-type controls ($49 \pm 4.9\%$ vs. $33 \pm 2.7\%$, $P < 0.01$), and was associated with impaired cardiac function. Conversely, cardiac-specific expression of CCN1 (Cyr61), a potent inducer of premature senescence, by adeno-associated virus serotype 9 gene transfer, resulted in $\sim 50\%$ reduction of perivascular fibrosis after TAC when compared to mock- or dominant-negative CCN1-infected controls ($11.9 \pm 1.4\%$ vs. $22.4 \pm 4.0\%$ and $22.1 \pm 1.8\%$, respectively; $P < 0.01$), and improved cardiac function.

Conclusions: Our data establish premature senescence of myofibroblasts as essential anti-fibrotic mechanism and potential therapeutic target in myocardial fibrosis.



Oncogene-induced senescence: what if it fails?

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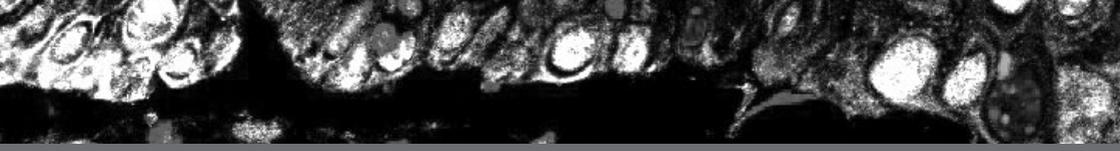
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In response to various stress signals, such as the unscheduled activation of oncogenes, cells can activate tumor suppressor networks to avert the hazard of malignant transformation. We have shown previously that oncogene-induced senescence (OIS) acts as such a break *in vivo*, withdrawing cells from the proliferative pool virtually irreversibly, thus acting as a vital pathophysiologic mechanism protecting against cancer. A prime example *in vivo* is the melanocytic nevus (mole), in which the common BRAF^{V600E} mutant oncoprotein initially drives expansion but subsequently causes cellular senescence.

In spite of the established role of OIS in preventing tumorigenic expansion both in animal models and humans, we have only begun to define the underlying mechanism and identify the key players. We observed numerous phenotypic changes occurring during OIS, both in the cytoplasm and in the nucleus. These include the activation of autophagy, a catabolic process operating in the cytoplasm, and downregulation of lamin B1, a component of the nuclear lamina (NL). We also studied the regulation of lamins in connection with autophagy and nuclear envelope integrity during OIS. Furthermore, we performed DamID to investigate the changes in genome-NL interactions in a model of OIS triggered by the expression of the common BRAF^{V600E} oncogene.

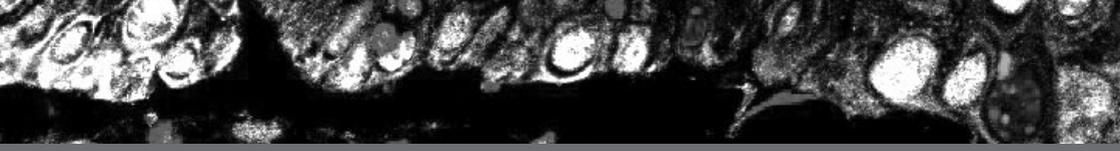
We subsequently studied how melanomas that arise upon OIS escape can be managed clinically. Until recently, most advanced stage melanomas were refractory to the available therapeutic options, but there are encouraging developments. For example, BRAF^{V600E} can be targeted by specific inhibitors, which cause unprecedented melanoma regression. However, relapse eventually occurs, owing to a variety of resistance mechanisms. To identify novel targets for (combinatorial) therapy, we set out to do negative selection RNAi screens *in vivo*. The problem of drug resistance and results from these screens will be discussed.



Dynamic epigenomic and transcriptomic profiling reveal stress-specific senescence states

Dr. Oliver Bischof - Institut Pasteur

In this talk we will discuss our recent efforts to dissect the transcriptional and epigenomic landscape of senescence to provide means to better identify it. We provide evidence that senescence comes in many flavours and that to fully understand senescence and exploit its therapeutic potential for healthspan extension we must study it in all its flavours and should refrain from making swift global judgements.



Reversal of phenotypes of senescence by pan-mTOR inhibition

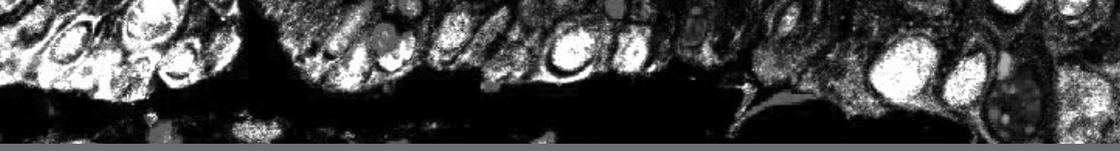
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Cellular senescence is a state of essentially irreversible proliferation arrest, and a hallmark of ageing. Through limiting tissue replicative capacity, causing tissue dysfunction and secretion of SASP components, accumulation of senescent cells with chronological age *in vivo* drives both ageing and the progression of age-related diseases. A crucial biochemical modulator of cellular senescence and longevity is mTOR, the Ser/Thr kinase present in two complexes (mTORC1 and mTORC2) that is responsible for coordination of diverse nutrient and growth signals into a cellular response. Genetic or pharmacological inhibition of mTOR as the functional component of mTORC1 (most notably with rapamycin) can extend lifespan, delay age-related diseases and blunt secretion of the SASP [1, 2]. mTORC1 has diverse signalling roles in the cell, including inhibition of autophagy and activation of protein and lipid synthesis, as well as lysosome biogenesis. mTORC2 is significantly less well understood than mTORC1, though responds to growth factors and controls cell metabolism, survival and organisation of the actin cytoskeleton. Using several mTOR inhibitors with different inhibitory profiles for mTORC1 and mTORC2, we have shown that acute pan-mTORC inhibition in primary human fibroblasts can reverse many phenotypes of senescence [3]. These include loss of SA- β -gal staining, reduction in cell size and granularity and reacquisition of fibroblastic spindle morphology. We provide evidence for a possible mechanism of action for the observed rejuvenation through induction of the actin cytoskeleton rearrangement pathway. We have extended this analysis to examine cell motility in migration assays comparing old, young and rejuvenated cell populations using live cell imaging techniques. Our results suggest that simultaneous, acute inhibition of both mTORC1 and mTORC2 may provide a promising strategy to reverse the development of senescence-associated features in senescent cells.

1. Laberge, R.M., et al., *mTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation*. Nat Cell Biol, 2015. **17**(8): p. 1049-61.
2. Herranz, N., et al., *mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype*. Nat Cell Biol, 2015. **17**(9): p. 1205-17.
3. Walters, H.E., S. Deneka-Hannemann, and L.S. Cox, *Reversal of phenotypes of cellular senescence by pan-mTOR inhibition*. Aging (Albany NY), 2016. **8**(2): p. 231-44.

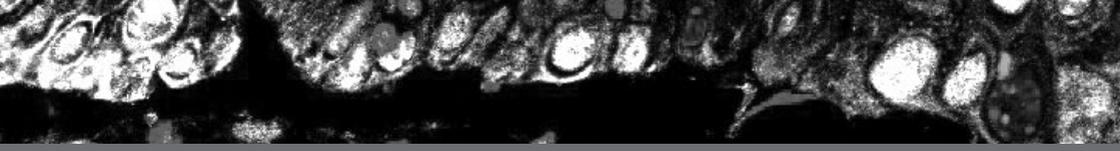


Defective ribosome biogenesis in senescence reveals a novel checkpoint pathway to control cyclin-dependent kinases

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Cellular senescence is a tumor suppressor program characterized by a stable cell cycle arrest. Here we report that senescence triggered by a variety of stimuli both in cell culture and *in vivo* involves diminished ribosome biogenesis and accumulation of both rRNA precursors and ribosomal proteins. These defects were associated with reduced expression of several ribosome biogenesis factors. Depletion of ribosome biogenesis factors was sufficient to induce senescence with activation of the tumor suppressors p53 and RB. However, genetic analysis revealed that RB but not p53 was required for the senescence response to altered ribosome biogenesis. Mechanistically, a ribosomal protein binds and inhibits the cyclin-dependent kinase CDK4 in senescent cells, thereby inducing accumulation of hypophosphorylated RB, cell cycle arrest and senescence. Moreover, overexpression of this ribosomal protein induces senescence and genetic analysis revealed that RB but not p53 was required. We thus describe a mechanism maintaining the senescent cell cycle arrest relevant for cancer therapy.



Role of the telomeric protein TRF2 in heterochromatic replication and cellular senescence

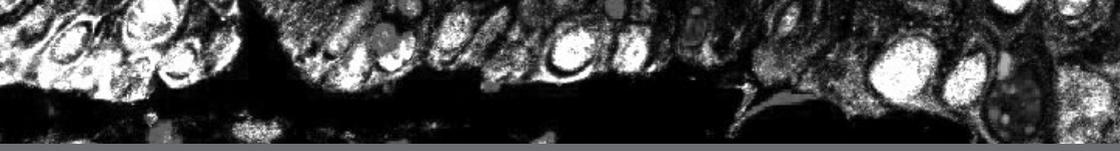
Aaron Mendez-Bermudez^{1,2}, Liudmyla Lototska¹, Serge Bauwens, Marie-Josephe Giraud-Panis¹, Karine Jamet¹, Stephan Koundrioukoff³, Michelle Debatisse³, Jing Ye² and Eric Gilson¹

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Telomeres are essential for maintaining chromosomal stability by preventing unwanted chromosome fusions and DNA damage response (DDR) activation. These events are mediated by a series of factors that interact with telomeric DNA such as the TRF2 protein. TRF2 ChIP-seq studies from our lab have shown that TRF2 can also bind outside telomeres (Simonet et al., 2011). In this work, we investigate whether TRF2 could be involved in the protection of these extra-telomeric TRF2-bound sequences. First, ChIP-seq analysis using γ -H2AX antibodies revealed that, in addition to telomeric sequences, a reduced expression of TRF2 triggers γ -H2AX accumulation at H3K9me3 rich regions, in particular at pericentromeric Satellite III (Sat3) DNA. These Sat3 repeats were indeed found to be bound par TRF2 in our previous study (Simonet et al., 2011). We confirmed this TRF2-Sat3 binding in several cell lines. Interestingly the pericentric Sat3 repeats of cells suffering from topological stress exhibit an increased binding of TRF2 as well as an increased ATM-dependent DDR activation. Conversely, the Sat3 repeats of cells treated with TSA or compromised for the histone methyltransferase responsible for H3K9me3, Suv39H1 or G9a, exhibit a reduced TRF2 binding. In support of a role for TRF2 in the protection of pericentromeric chromatin against replicative DNA damage, the binding of TRF2 to Sat3 pericentromeres increases during S phase as well as upon replicative stress (HU and aphidocolin treatment). In addition, we showed by DNA combing and a BrdU assay that TRF2 inhibition causes a delay in the replication timing of Sat3 repeats. Finally, we found by comet assay that in pre-senescent cells, when the level of TRF2 declines, Sat3 DNA is highly broken.

Together these results show that TRF2 is recruited to heterochromatic pericentromeric regions to facilitate their replication. We propose that the reduced expression of TRF2 observed during replicative senescence leads to pericentromeric damages that synergize with uncapped telomeres to trigger senescence.

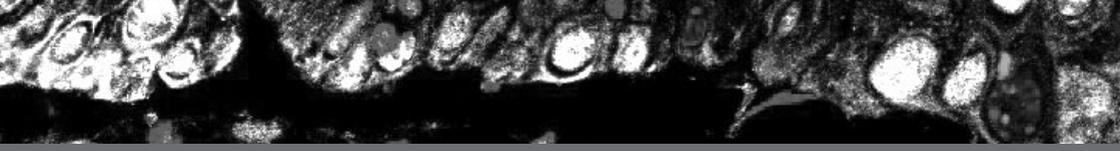


Senescence is critical for injury-induced *in vivo* reprogramming in skeletal muscle

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3. These authors contribute equally to this work

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. Here we find that senescence induced by tissue damage is critical for *in vivo* reprogramming in both acute and chronic skeletal muscle injury models in mice. Using Pax7-specific lineage tracing model, we demonstrate that muscle stem cells are the cell of origin for *in vivo* reprogramming. Interestingly, *in vivo* reprogramming occurs faster in aged mice (2 years) than young mice (2 month) upon acute muscle injury, mostly due to the age-dependent accumulation of senescent cells. Moreover, by blocking IL-6, an important component of senescence-associated secretory phenotype (SASP), we successfully hindered *in vivo* reprogramming, clearly linking senescence to the effects of injury on cellular plasticity. These findings provide a fascinating extension of our understanding of how senescence is beneficial for tissue regeneration.

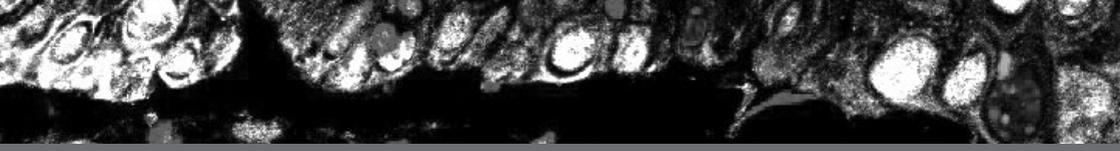


Loss of CDK2 Inhibits BRAF^{V600E}/MYC-induced Mouse Lung Tumor Development

Dept. of Microbiology, Tumor and Cell Biology (MTC)

Nyosha Alikhani, Jacob Goodwin & Lars-Gunnar Larsson

We have shown previously that MYC plays an important role in senescence suppression during malignant transformation. This MYC activity requires CDK2 function in cell cultures. Here we investigated whether CDK2 is required for MYC-driven initiation and progression of tumors *in vivo* and whether this is related to senescence regulation. We have established a conditional BRAF^{V600E}/MYC-driven mouse lung tumor model in which the oncogenic BRAF^{V600E} mutant and a MYC-estrogen receptor fusion protein (MycER) are conditionally activated in the lungs of mice by inhalation of Ad-Cre virus. In addition, MycER activity is regulated by administration of tamoxifen. We have shown previously that activation of BRAF^{V600E} induces benign lung adenoma limited by extensive senescence induction, while simultaneous activation of MYC by tamoxifen synergistically accelerated tumor development. This was due to MYC-mediated senescence suppression as evidenced by reduced senescence-associated p16INK4A, p21CIP1, H3K9me3 and β -gal staining and increased Ki-67 staining. To study the role of CDK2 in this process, we have generated BRAF^{V600E}/MycER/CDK2^{lox/lox} mice with the conditional Cre-mediated knockout of CDK2. Our preliminary results show that CDK2 depletion does not prevent BRAF^{V600E}/MYC-induced tumor initiation, but delays tumor progression. This results in a significant increase in survival of the mice from a median of 50 days in tamoxifen-treated BRAF^{V600E}/MycER mice to a median of 220 days in BRAF^{V600E}/MycER/CDK2^{lox/lox} mice. In fact, 40% of the latter group survived until the experiment was terminated after 240 days. Further, the acceleration of BRAF^{V600E}-induced tumor development observed after MYC activation by tamoxifen was completely wiped out in BRAF^{V600E}/MycER/CDK2^{lox/lox} mice, suggesting that CDK2 is required for this MYC function. Our preliminary data suggest that MYC activation and loss of CDK2 is linked to suppression and activation of senescence in tumors, respectively. Treatment of tumor-bearing mice with pharmacological CDK2 inhibitors is ongoing and will be discussed. The results so far suggest that CDK2 activity is required for MYC-driven tumor development and senescence suppression *in vivo*, and that inhibition of the MYC/CDK2 axis potentially has clinical relevance for combating cancer.



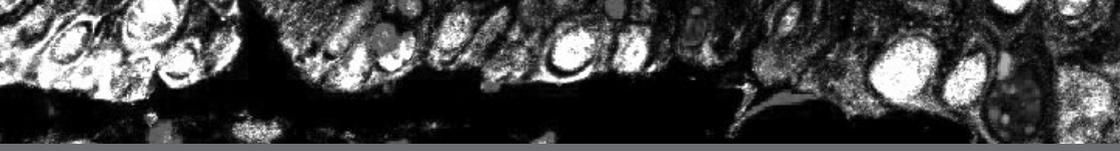
Dr. Fabrizio d'Adda di Fagagna

TITLE:

DNA damage and cellular senescence

ABSTRACT:

Cellular senescence in the context of ageing and cancer is often associated with DNA damage generation and DNA damage response (DDR) activation. We will discuss the evidence in support of the presence of DNA damage in senescence cells and the emerging role of non coding RNA in DDR signaling.



**The DNA Damage Response:
Where Genome Stability Meets Human Morbidity**

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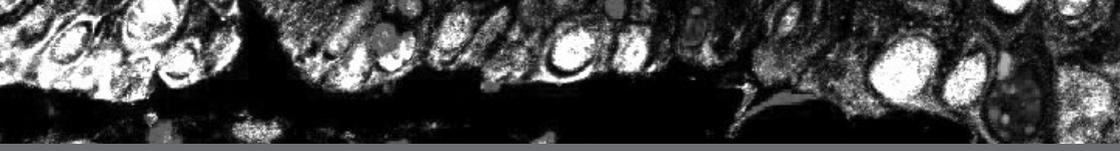
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Maintenance of genome stability is critical for cellular homeostasis. A central axis in maintaining genome stability is the DNA damage response (DDR) – a complex signaling network that is most vigorously activated by critical DNA lesions such as double strand breaks (DSBs). The DDR's breadth is based on a core of DDR-dedicated proteins and temporary recruitment of hundreds of proteins from various cellular circuitries. The primary transducer of the DSB response – the serine-threonine kinase, ATM – mobilizes this network by initiating a multi-level cascade of protein post-translational modifications, most notably, phosphorylation. Recent evidence suggests that ATM may also play a role in the response to many other genotoxic stresses.

Sequence alterations in the multitude of genes encoding DDR players may affect DDR effectiveness. Thus, the efficiency with which genome stability is maintained in human populations may vary over a broad continuum. At the extreme end are embryonic lethal genotypes and the well-documented 'genome instability syndromes' – severe phenotypes that typically combine degeneration of specific tissues, cancer predisposition and segmental premature aging. A prototype syndrome is ataxia-telangiectasia (A-T), in which the primary genetic defect is *ATM* mutations. A-T involves neurodegeneration, immunodeficiency, cancer predisposition, genome instability, segmental premature aging, and acute radiation sensitivity.

Further along this spectrum are presumed conditions that are variably expressed throughout life and not recognized as distinct clinical entities, but collectively may constitute a major determinant of public health. The links being uncovered between the DDR and cellular metabolism are still more evidence of the possible effects of variations in genome instability on human health. Another emerging link is between genome stability and aging pace. An important component of these phenotypes is revealed when patients react adversely to treatments with DNA damaging agents, such as radiation therapy or certain chemotherapies. Thus, disclosure of the DDR's nuts and bolts has profound ramifications in many areas in medicine.

Using mouse models to substantiate this contention, we are combining mutations in the *Atm* and *Wrn* genes in mice. The human WRN gene is mutated in Werner syndrome (WS) – a genomic instability disorder characterized primarily by segmental premature aging. WRN is a member of the RecQ family of helicases, and also possesses 3'-5' exonuclease activity. WRN is involved in many DNA-related transactions and in resolving various DNA intermediate structures. Notably, *Wrn*^{-/-} mice have no distinct phenotype, while *Atm*^{-/-} mice exhibit a moderate phenotype characterized by small body size, sterility, cancer predisposition and radiation sensitivity. Strikingly, the *Atm*^{-/-}/*Wrn*^{-/-} double knockout genotype was embryonic lethal, leading to severe developmental retardation in utero. *Atm*^{-/-}/*Wrn*^{+/-} animals were born in frequency less than Mendelian expectation and exhibited runted appearance, reduced life expectancy, and a late-developing cerebellar demise. Interestingly, *Atm*^{+/-}/*Wrn*^{+/-} double heterozygous animals showed early signs of segmental aging beginning at the age of 6 months, and early death, typically between 8 and 14 months of age. We attribute great importance to this genotype, which exists in humans and may lead to early appearance of ailments that are usually associated with older age. Notably, cells with this genotype exhibit elevated senescence, which ties this process to the organismal aging.



A new DNA damage route to senescence and neoplastic escape

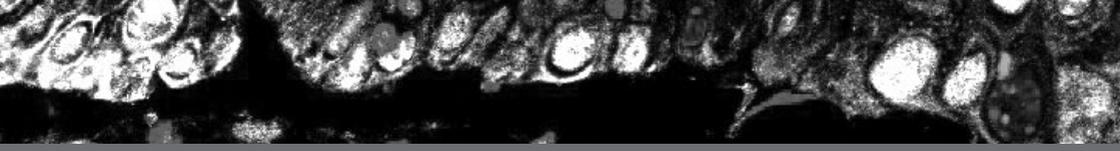
Joe NASSOUR, Nathalie MARTIN and Corinne ABBADIE

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Senescent cells display unrepaired DNA damages, including deprotected telomeres in the context of replicative senescence and DNA double strand breaks or stalled replication forks in the context of oncogene- or stress-induced senescence. These unrepaired DNA damages persistently activate the ATM/ATR-dependent DNA Damage Response (DDR) pathway which leads to the activation of p53, the up-regulation of p21 and cell cycle arrest at the G1/S transition. The persistence of the activation of this pathway is at the origin of the stability and irreversibility of senescence. The p16/Rb pathway is also activated at senescence, alternatively or in addition to the p53/p21 pathway. Although this pathway seems to be activated in almost all forms of senescence, the signals which induce it are not clearly established.

We show that unlike fibroblasts undergoing replicative senescence, senescent epithelial cells (namely keratinocytes and mammary cells) do not display shortened telomeres or DNA double strand breaks (DSBs) and, in accordance, do not activate the DDR and the p53/p21 pathways. Instead, they accumulate DNA Single-Strand Breaks (SSBs). These breaks remain unrepaired because of a decrease in PARP1 expression and activity. This defect in the SSB repair pathway leads to the formation of abnormally large and persistent XRCC1 foci that engage a signaling cascade involving the p38MAPK and leading to p16 up-regulation, Rb hypophosphorylation and cell cycle arrest. The oxidative stress plays a major role in this mechanism by acting as the clastogenic agent and also by leading to the down-regulation of PARP1 (Nassour et al, Nature Commun, 2016).

Importantly, the default in the SSB repair pathway, which let the SSBs unrepaired, is also causal of the emergence of post-senescent transformed and mutated precancerous cells. We are now working on the mechanisms which lead some senescent cells to re-enter cell cycle. We are also trying to understand how the senescent cells deal with their unrepaired SSBs to generate mutated daughter cells.



Selective targeting of senescence through a FOXO4-based peptide to counteract features of aging, chemotoxicity and cancer therapy resistance

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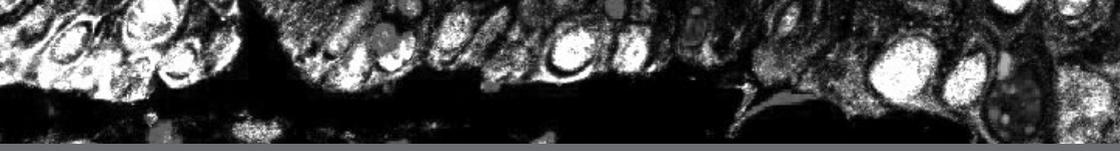
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Senescence is associated with numerous pathologies, including cancer. Clearance of senescent cells in a genetic fashion was shown to be beneficial in delaying aging in models for accelerated and natural aging. Therapeutic options for targeting senescence are limited, however, and are either only mildly selective over non-senescent cells or show varying degrees of off-target toxicity. In an attempt to circumvent these challenges and prospectively design a new class of anti-senescence compounds, we sought to identify molecular pivots crucial for maintaining senescent cell viability.

We observed FOXO4 to fulfill those criteria and designed a unique FOXO4-based cell penetrating peptide which specifically disrupts senescent cell viability in various settings *in vitro* and *in vivo*. We identified the specific cellular conditions under which this peptide is, and is not, effective.

Excitingly, under conditions where it was well tolerated, the FOXO4 peptide potently counteracted chronic features of aging in fast aging *Xpd*^{TTD/TTD} and naturally aged mice by improving fitness, responsiveness, hair density, and organ function. Moreover, this peptide counteracted hallmarks of chemotoxicity and could resensitize therapy resistant Metastatic Melanoma, Mammary Carcinoma and Glioblastoma multiforme cells to anti-cancer treatment.

Thus, FOXO4 is a weak-spot in senescent cell viability and a peptide to influence FOXO4 signaling may be employed to improve the healthspan of a plethora of senescence-related diseases, including subsets of treatment resistant cancer.



Molecular Insights of APL cure as Therapy-induced Senescence

Müge Ogrunc, Hugues de Thé

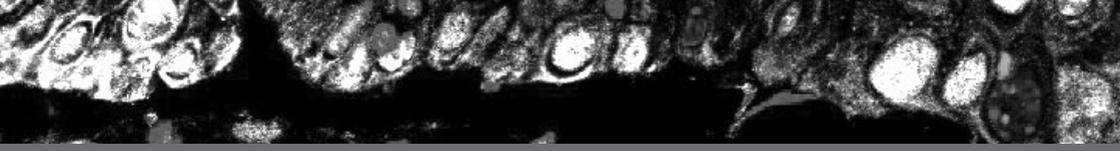
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Although stress signals operate via distinct signaling pathways for the establishment of senescence, oncogenic stimuli interestingly seem to activate major tumor suppressor networks via promyelocytic leukemia protein (PML) nuclear bodies (NBs). In strong support of this notion, we recently demonstrated that upon PML/RARA-targeted therapies, acute promyelocytic leukemia eradication depends on both intact PML and P53, which drive therapy-induced senescence (Ablain *et al.*, 2014). While PML NBs are considered as typical phenotypic senescence markers, the precise mechanism through which they activate the P53 tumor suppressor signaling remained largely elusive. Efficiency of several PTMs of P53 have been linked with PML NBs. Since PML's involvement in RAS-induced senescence was discovered, there has been circumstantial evidence that PML enhances acetylation of P53, although multiple other, and often contradictory, mechanisms were proposed. However, due to the lack of endogenous setting and *in vivo* manipulations, PML's molecular mechanism of action remains elusive.

In the context of APL cells, we found that P53 signaling is activated upon PML/RARA-targeted therapy -retinoic acid (RA)- treatment. Unexpectedly little or no P53 stabilization was observed upon RA treatment. Yet, we observed a remarkable elevation of Lys379 in mice (corresponding to Lys382 in human) acetylation of P53. PML presence is required for P53 acetylation at Lys379 and to activate a distinct set of senescence-associated genes, such as PAI-1. Those display a greatly enhanced, PML-dependent, binding of acetylated P53 onto their promoters. Hence, our results unravel a non-canonical P53 activation, which requires PML nuclear bodies for transcriptional induction of specific senescence genes. Complete elucidation of this mechanism is required to harness this PML/P53 pathway in other human cancers.

Reference:

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

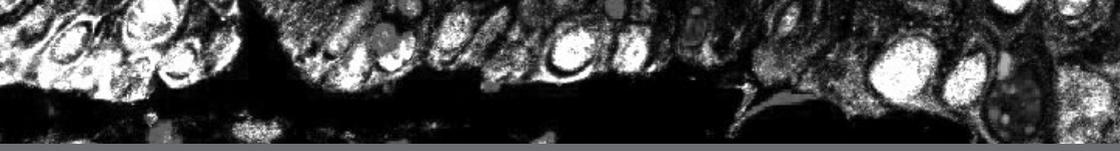


The benefits of aging-associated beta cell senescence

Aharon Helman, Agnes Klochendler, Narmen Azazmeh, Yael Gabai, Reba Condiotti, Yuval Dor and **Ittai Ben-Porath**

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Senescent cells form and accumulate within tissues during normal aging. While such cells contribute to aging-associated pathology, the direct consequences of senescence on cell and tissue function are mostly poorly characterized. The senescence effector p16^{Ink4a} (Cdkn2a) is expressed in pancreatic beta cells during aging and limits their proliferative potential, yet whether these cells undergo senescence, and how this affects their capacity to secrete insulin, is unknown. We found that beta cell-specific activation of p16^{Ink4a} in transgenic mice enhances glucose-stimulated insulin secretion (GSIS). In diabetic mice this leads to improved glucose homeostasis, providing an unexpected functional benefit. p16^{Ink4a} expression in beta cells induces hallmarks of senescence, including cell enlargement as well as increased glucose uptake and mitochondrial activity, which all promote insulin secretion. GSIS increases during normal aging of mice, driven by elevated p16^{Ink4a} activity. We found that islets of human adults contain p16^{Ink4a}-expressing senescent beta cells, and that senescence induced by p16 in a human beta cell line increases insulin secretion, in a manner dependent in part on the activity of mTOR and PPAR- γ . Our findings reveal a novel role for p16^{Ink4a} and cellular senescence in promoting insulin secretion by beta cells, and in regulating normal functional tissue maturation with age.

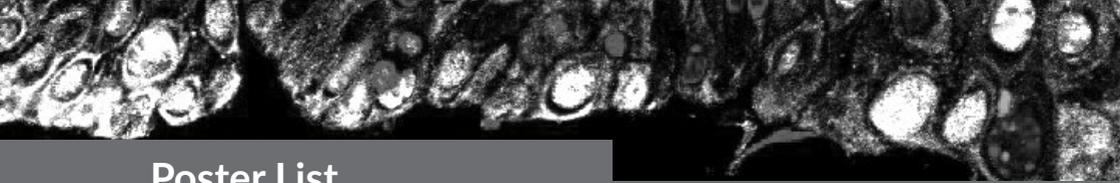


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Cellular senescence: Quo vadis?

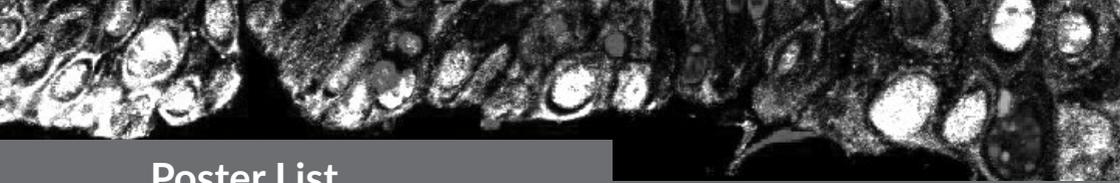
Judith Campisi, Buck Institute for Research on Aging and Lawrence Berkeley National Laboratory

Cellular senescence is an essentially irreversible arrest of proliferation (growth) by cells that experience certain stresses. First described in the 1960's as the failure of normal human cells to divide indefinitely in culture, and based mostly on intuition, the senescence response was predicted to subserve two important physiological functions: suppressing cancer and promoting aging. Both functions were thought to rely on the growth arrest: cancers cannot form without cell growth, and aging mammals lose growth capacity for tissue repair and regeneration. Six decades later, we appreciate the foresight of those early predictions. There is little doubt now that the senescence growth arrest suppresses cancer in young organisms. And there is mounting evidence that the age-dependent accumulation of senescent cells can drive many age-related pathologies and phenotypes, ranging from neurodegeneration to, ironically, late life cancer. But is the growth arrest really responsible for myriad aging pathologies -- especially cancer, the process it evolved to suppress? We now know that the answer to this question resides largely in the SASP -- the senescence-associated secretory phenotype -- which develops to varying degrees in senescent cells, depending on their origin and the senescence-stimulating event. The SASP includes numerous chemokines, growth factors, proteases, pro-inflammatory cytokines and signaling metabolites. It is now known to have striking effects on neighboring normal, stem and premalignant cells, as well as on the tissue microenvironment and systemic milieu. Moreover, the SASP is now known to be both deleterious -- promoting age-related pathology -- and beneficial, notably stimulating tissue repair and optimizing certain developmental processes. Thus, the senescence response is a kaleidoscope -- a constantly changing pattern of causes and effects. Our challenge now is to understand this process in enough detail to harness its benefits, while mitigating its deleterious effects.



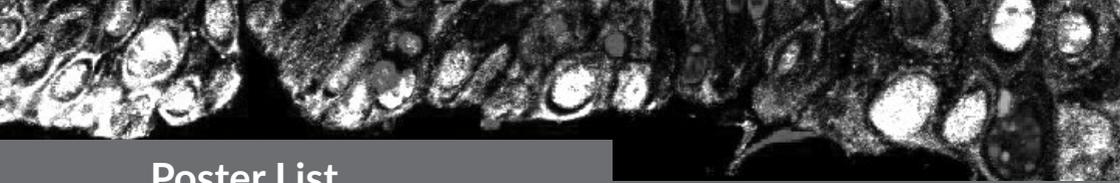
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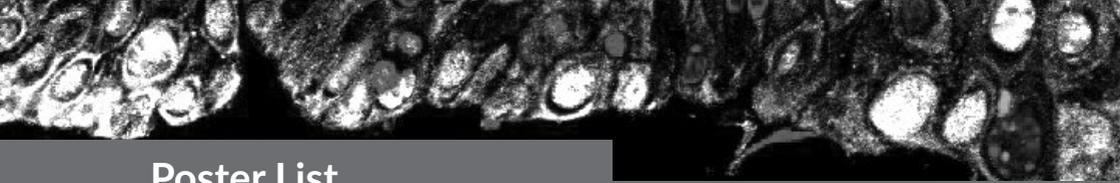
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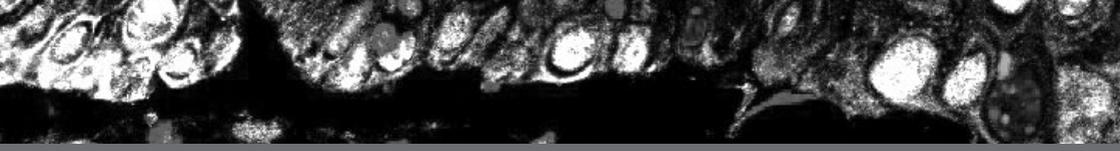
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Role of protein kinase C in DNA damage response and the implementation of senescence

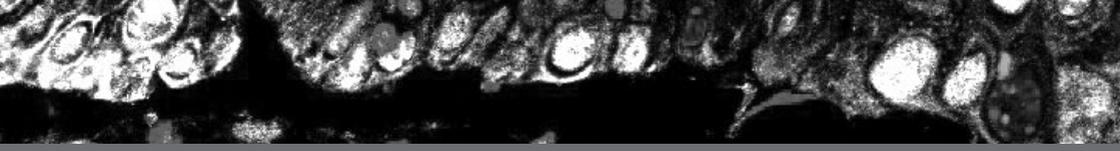
Assaf Ben-Ari, Udi Zurgil and [Etta Livneh](#).

Department of Microbiology, Immunology and Genetic. Faculty of Health Sciences. Ben Gurion University of the Negev, Beer Seeva 84105

Senescence is characterized by permanent cell cycle arrest and loss of proliferative capacity, despite continued viability and metabolic activity. Senescent cells undergo massive genome modulation and exhibit characteristic features including secretion of complex mixtures of extracellular proteins and soluble factors, resulting in the senescence-associated secretory phenotype (SASP). Components of SASP reinforce or implement cell cycle arrest and contribute to tumor suppression by signaling and recruiting the immune system to clear senescent cells. However, some secreted molecules, associated with inflammation and malignancy, act as pro-tumorigenic agents. Hence, cellular senescence could be tumor suppressive, but may also be pro-tumorigenic. Therefore, it is important to understand the complex nature of senescence and the signaling pathways of its activation, as some chemotherapy drugs function also through senescence induction.

Very few studies showed a role for PKC in senescence. Here we show that PKC η , an epithelial specific and anti-apoptotic kinase, promotes senescence induced by oxidative stress and DNA damage. Using PKC η -knockdown breast adenocarcinoma MCF-7 cells we show that PKC η promotes senescence induced by oxidative stress and DNA damage via its ability to upregulate the expression of the cell cycle inhibitors p21^{Cip1} and p27^{Kip1} and to modulate transcription of major components of SASP such as IL-6 and IL-8. Moreover, we demonstrate that PKC η creates a positive loop for reinforcing senescence by increasing the transcription of both IL-6 and IL-6 receptor. Thus, the presence/absence of PKC η modulates major components of SASP. Furthermore, our studies demonstrate that PKC η interferes with γ H2AX phosphorylation, which mark DNA double-strand breaks for repair. The phosphorylation of γ H2AX may reflect the extent of DNA breaks but also the cellular response to DNA damage. Our experiments suggest that the response to DNA damage (repair processes) was more efficient in PKC η -knockdown cells. The phosphorylation on ATM and Chk2 was also lower in PKC η expressing cells. This could provide a clue for an underlying mechanism that promotes senescence.

Revealing the molecular regulators of senescence will improve our ability to develop new therapeutic strategies for clearing tumor cells. Our studies showing a role for PKC η in senescence could identify PKC η and/or its activated signaling cascades as targets for intervention senescence induction. Since PKC η is highly expressed in epithelia that are the origin of about 90% of human tumors, this may provide critical inhibitors as therapeutic adjuvants for different types of carcinomas.

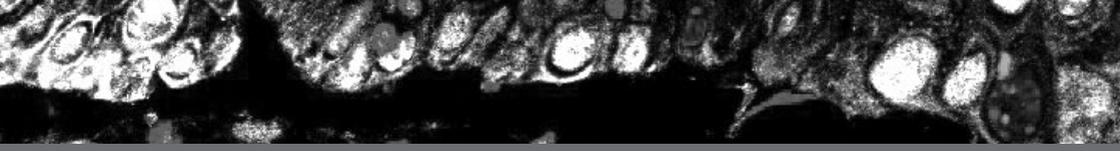


Cellular Senescence in Hodgkin's Lymphoma

Gopas J, Stern E, Zurgil U, Ozer J, Ben Ari A, Shubinsky G, Braiman A, Sinay R, Ezratti R, Dronov V, Balachandran S, Benharroch D and [Livneh E](#).

Microbiology, Immunology and Genetics, Ben Gurion University, Beer Sheva, Israel , Department of Oncology and Department of Pathology, Soroka University Medical Center, Beer Sheva, Israel

Hodgkin's lymphoma (HL), a B cell originated malignancy of the immune system, is one of the most frequent lymphomas in the Western world. Although the rate of cure is high, about 20-35% of patients relapse, and about half of them eventually die of the disease or treatment-related late toxicities and secondary malignancies. Conceptually, novel treatment strategies are thus needed, particularly for this category of patients. The malignant cells, called Hodgkin and Reed-Sternberg (HRS) cells, only make up 1-2% of the total tumor cellularity; the remaining mass comprising a mixed infiltrate population is thought to be recruited to the lymph node by HRS-driven pro-inflammatory signals. The origin of the pro-inflammatory signals remains elusive, and represents a critical gap in our knowledge of the pathogenesis of HL. We propose that a sub-population of HRS cells, which we call herein large RS cells, have characteristics of senescent cells, and, thus produce large amounts of inflammatory mediators (the so-called "senescence-associated secretory phenotype", or SASP). Senescence is characterized by permanent cell cycle arrest and loss of proliferative capacity, despite continued viability and metabolic activity. We show that the senescence markers the cell cycle inhibitors p16INK4a and p21Cip1 are expressed in large RS cells in all HL patient's biopsies examined. Moreover, the large RS cells are negative for Ki-67 staining, demonstrating that these cells have ceased to proliferate. We also show that large, 'RS-like' cells in a HL-derived line, L428, stained for the characteristic senescence marker β -galactosidase (β -gal). Oxidative stress and chemotherapy increased the proportion of β -gal positive large RS cells. Furthermore, we show that the large RS cells secrete high levels of cytokines. We suggest that these senescent RS cells may be responsible, at least in part, for creating a pro-inflammatory microenvironment, promoting HL pathogenesis, and mediating chemo-resistance in relapsed disease. Understanding the pathways important for the establishment of senescence in HRS cells, as well 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.



Developing new potential treatments for senescence-induced biliary disease.

Sofía Ferreira-González¹, Wei-Yu Lu¹, Tak Yung Man¹, Benjamin Dwyer¹, Alex Raven¹, Eoghan O'Duibhir¹, Luke Boulter², Juan-Carlos Acosta³, Stuart Forbes¹.

¹ MRC Centre for Regenerative Medicine, Edinburgh University, UK

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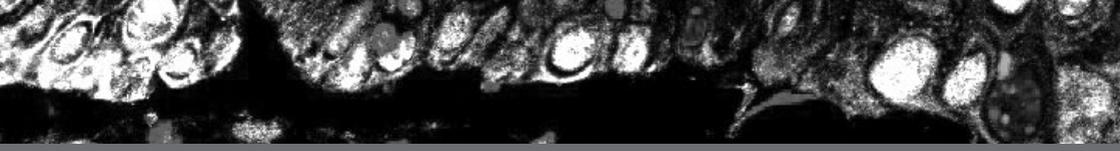
³ ECRC, Edinburgh University, UK

Senescence has proved to be an essential mechanism in the beginning and development of cholangiopathies like Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis. We hypothesize that senescence affects the outcome of biliary disease through three mechanisms:

- Release of SASP factors that affect the parenchyma and its function.
- Increase of fibrotic elements that impair proper liver regeneration
- Impaired proliferation of the progenitor compartment, which decreases the regenerative potential of the liver.

We have developed a highly efficient model based in the conditional deletion of MDM2 in cholangiocytes, that mimics the clinical outcome of cholangiopathies. Using this model we can focus on the development of different strategies against the three different senescence-related mechanisms present in biliary disease.

Our preliminary results allow us to disclose the underlying mechanisms of senescence, to understand how it disseminates through the tissue and to find therapeutic options for future treatments based on the blockage of TGF β response, anti-fibrotic treatment and the transplant of progenitor cells.



Primary Cilia loss in cholangiocytes during Senescence.

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Primary Cilia are evolutionary conserved structures present in almost all human cell types. With only one copy per cell, these non-motile organelles are central for the understanding of cell cycle regulation and the transmission of extracellular signals (like TGF β) to the interior of the cell.

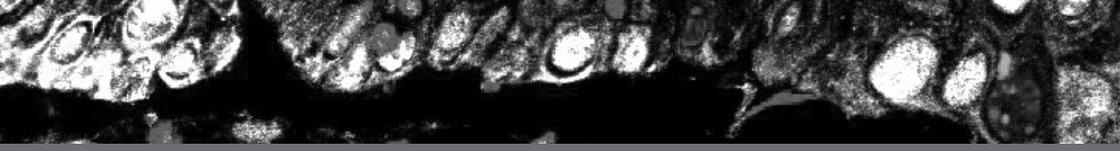
In the liver, the role of primary cilia in cholangiocytes remains elusive, but our preliminary data in human suggest that the primary cilia are lost during senescence-associated biliary disease.

We have developed a highly efficient model based in the conditional deletion of MDM2 in cholangiocytes, accumulation of p53 and activation of p21, that mimics the clinical outcome of cholangiopathies, in which senescence is an essential feature of the damage.

Our mouse model revealed a complete loss of Primary Cilia in cholangiocytes, which can be also reproduced *in vitro* during TGF β -induced senescence in a cholangiocyte line.

We have also developed a mouse model that conditionally eliminates the primary cilia in cholangiocytes. This model will allow us to study if the loss of primary cilia is a consequence of senescence during biliary disease, or if senescence is generated by the loss of the organelle.

In any case, whether the loss of primary cilia in cholangiocytes is a consequence or a cause of senescence, it is clear that defining the hallmarks of this process is essential to understand the mechanisms of senescence during biliary disease and ameliorate the prognosis of these pathologies.



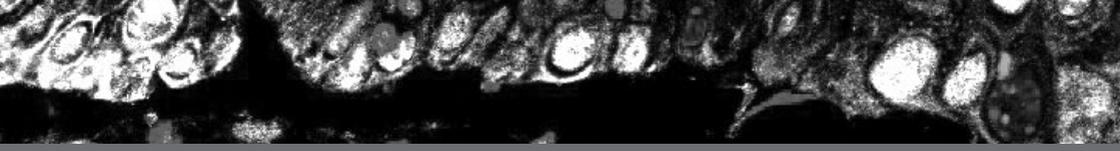
Identification of senescence mediators using shRNA library screen

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We have previously reported that ectopic expression of the cancer/testis antigen SSX2 induces an oncogene-induced senescence (OIS) response in MCF7 breast cancer cells and HEK293 cells. This is highly contrary to the endogenous expression of this antigen in melanoma cell lines where it sustained growth and shows oncogenic potential, and suggests that mediators of SSX2 OIS are present in MCF7 and HEK293 cells. We hypothesized that the knockdown of these mediators of SSX2 OIS therefore would lead to sustained growth where SSX2 expression normally would induce senescence. To identify these mediators, we carried out a functional shRNA library enrichment screen targeting about 5000 genes encoding nuclear proteins. Among shRNAs that mediated sustained growth during SSX2-induced senescence were identified several targeting MED1, a gene encoding Mediator of RNA polymerase II transcription subunit 1. In initial validation experiments, about 50% reduced expression of MED1 was shown to suppress or postpone the observed SSX2 induced senescence response. Although cells with MED1 knockdown acquired an enlarged cytoplasm after a period of 6 days of SSX2 expression, similarly to control cells transfected with non-targeting controls, they did not undergo a senescence growth arrest. Furthermore, initial experiments with HEK293 cells showed that MED1 knockdown sustained proliferation in response to SSX2 expression, whereas cells transduced with non-targeting controls appeared apoptotic/senescent. At present, it is not clear whether MED1 knockdown interferes with the senescence response in general or interferes with specific mechanisms associated with SSX2 expression/function. Therefore we will also study the role of MED1 in the senescence response to the HRAS oncogene. Interestingly, reduced MED1 expression in cancer patient tumors or cancer cell lines was reported to be associated with increased invasiveness and metastasis in non-small cell lung cancer and melanoma. This was due to MED1-mediated alterations in expression levels of many metastasis-related genes. Based on these results, we speculate that a reduction in Med1 expression could play a crucial role in cells to tolerate the expression of oncogenes and, in turn, promote cancer progression.



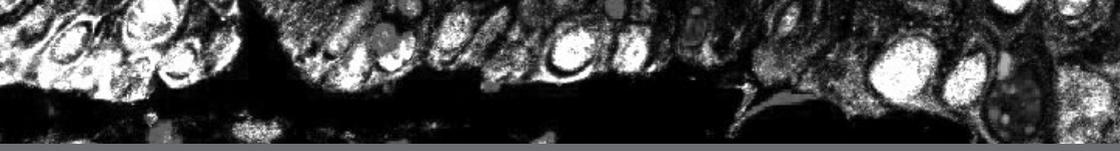
Exploiting senescence-associated proteotoxicity as a target in cancer therapy.

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Cellular senescence is a growth arrest fail-safe mechanism triggered by stress signals like oncogene activation and DNA damage induced by chemotherapy. Previously, we were able to show that cells undergoing therapy-induced senescence (TIS) undergo massive proteotoxic stress as a consequence of the increased protein production associated with the senescence-associated secretory phenotype (SASP) (Dörr-JR et al., Nature, 2013).

Here, we address the role of (toxic) protein degradation by the proteasome in senescent vs. non-senescent cancer cells. We present here that drug-senescent Eμ-myc transgenic mouse lymphoma cells display, as previously shown for autophagy inhibition, enhanced vulnerability to proteasome inhibition *in vitro* compared to their equally treated but genetically senescence-impaired counterparts. Importantly, when we simultaneously blocked proteasome and autophagy activity with moderate doses of both agents, TIS lymphomas exhibited overadditive susceptibility and particularly rapidly died. Adding to our previous findings that senescent cells rely on a hypercatabolic metabolic state to fuel energy-consuming autophagy as a mechanism to cope with toxic senescence-associated peptides, we now demonstrate a similar dependency on an intact proteasomal degradation machinery. Our therapeutic approach converts the rather detrimental implications of largely pro-inflammatory SASP factors into a novel strategy to eliminate malignant cells in a synthetically lethal manner.



Involvement of the Unfolded Protein Pathway in senescence and a pre-transformation process by senescence escape in normal human epidermal keratinocytes.

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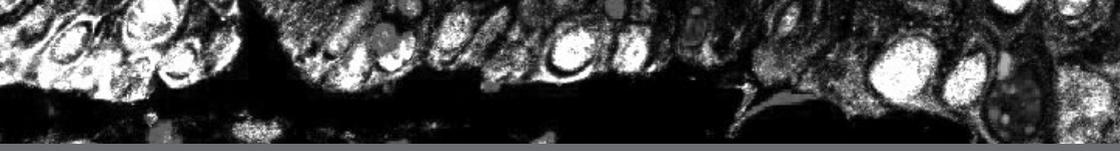
*Corresponding author

Aging is considered as the main risk factor in most of chronic diseases in humans. However, the mechanisms that drive aging remain largely unknown. Aging is accompanied by an increase in senescent cells, which are viable but have lost their ability to divide. It is now demonstrated that cellular senescence is causally implicated in generating age-related phenotypes and that removal of senescent cells can prevent or delay tissue dysfunction and diseases such as cancer.

To better understand the relationship between senescence and the early steps of transformation, we used a model of normal human epidermal keratinocytes (NHEKs). Senescent NHEKs have two opposite outcomes: either they die by autophagic programmed cell death or they spontaneously and systematically escape from senescence to give rise to pre-neoplastic emerging cells (a process we called Post Senescence Neoplastic Emergence, PSNE). We identified a specific gene expression signature of this pre-transformed phenotype and used it to construct a molecular function map, where we identified the Unfolded Protein response (UPR) as a process that crucially regulates senescence and neoplastic escape. The UPR is an adaptive mechanism set up by cells to respond to environmental or intrinsic changes.

Here, we show that the three branches of the UPR are activated during senescence but not in the neo-formed transformed cells (PSNE). Interestingly, individual silencing by siRNA on the PERK, ATF6a, and IRE1a genes showed that only invalidation of PERK altered the onset of the senescent phenotype. We previously demonstrated that when over activated, macroautophagy induces senescent-cell death, whereas when only moderately activated, macroautophagy promotes senescent cell-death escape and PSNE. In this study we revealed that autophagy markers such as ATG5 and ATG8 are under the dependence of PERK. Besides, our data revealed that knock-down of PERK and to a less extent ATF6a and IRE1a at the beginning of the senescence plateau significantly decreased the frequency of PSNE.

Our findings highlight for the first time the crucial role of the UPR in regulating the antagonistic role of autophagy in the context of replicative senescence and senescence escape.



Is senescence-associated β -galactosidase a marker of neuronal senescence?

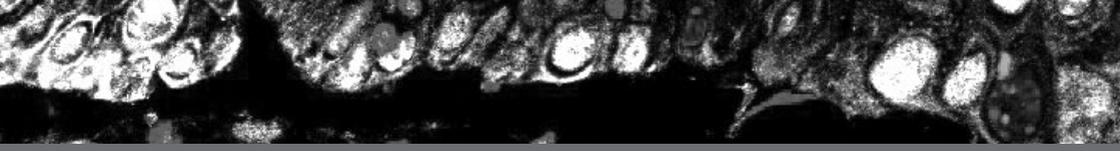
Malgorzata Piechota¹, Piotr Sunderland¹, Adrianna Wysocka¹, Maria Nalberczak², Malgorzata Sliwinska², Katarzyna Radwanska², Ewa Sikora¹

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Senescence is associated with irreversible arrest of cell proliferation and persistent activation of DNA damage response (DDR). One of its features is the presence of senescence-associated- β -galactosidase (SA- β -gal). Interestingly, studies in the last decade detected SA- β -gal in aging neurons *in vitro* and *in vivo* and revealed that neuronal senescence-like phenotype in aging mouse brains was driven by DDR activation. Here, we would like to propose that neuronal SA- β -gal activity is not exclusively linked to neuronal senescence both *in vitro* and *in vivo*. Firstly, we found noticeable SA- β -gal activity in cortical neurons quite early in culture, many SA- β -gal- positive neurons were negative for another canonical marker of senescence, which are double-strand DNA breaks (DSBs) and DDR signalling triggered by low doses of doxorubicin did not accelerate the appearance of neuronal SA- β -gal. As regards *in vivo*, we observed evident induction of SA- β -gal activity in hippocampus of the oldest 24 month-old mice, which is consistent with previous findings and support the view that at this advanced age neurons developed a senescence-like phenotype. Surprisingly, however, we also detected relatively high SA- β -gal activity already in much younger 3 month-old mice, which we link with non-senescent process.

Additionally, we showed induction of nuclear REST protein in aging neurons in long-term culture, which resembles molecular changes taking place in neurons of the aging brain and we propose that REST induction could be a good marker of neuronal senescence *in vitro*.



REST: A new regulator of the INK4-locus in senescence?

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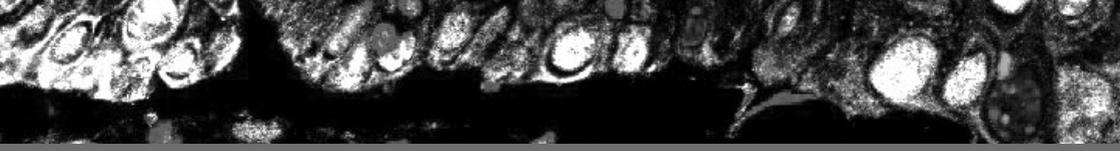
The Polycomb Group (PcG) proteins are essential for maintenance of normal and cancer stem cell populations, mainly because of their ability to bind and repress tumor suppressor loci (Bracken et al., Nature reviews. Cancer, 2009, Dietrich et al., Embo J., 2007). An example of an important locus, which is regulated by PcG proteins, is the INK4B-ARF-INK4A tumorsuppressor locus (Dietrich et al., Embo J., 2007, Bracken et al., Genes & development, 2007). The expression of genes encoded by this locus changes when the cells age, differentiate or enter senescence (Kia et al., Epigenetics Chromatin, 2009).

We have previously shown that the transcription factor Rest interacts with Polycomb Repressive Complex (PRC) 1 and 2, and is required for recruitment of these two complexes to a subset of Rest target genes in mES cells (Dietrich et al. 2012).

Interestingly, like the PcG complexes, we find that REST regulates the INK4B-ARF-INK4A locus in human fibroblasts. Moreover, Rest appears in several cases to regulate genes from promoter distal binding sites through RE1 elements.

In this project we try to understand if distal REST binding sites, through PcG interactions, leads to promoter looping that repress transcription of the *INK4B-ARF-INK4A* locus.

As a model, we study oncogene-induced senescence using a TIG3-BRAF-ER cell line, which upon tamoxifen stimulation leads to increased BRAF activity and derepression of the INK4A-ARF-INK4B tumor suppressor locus. We found that REST binds to a RE1-site upstream INK4B and that knock down of REST leads to growth-arrest and derepression of the INK4B-ARF-INK4A locus, correlating with reduced RNF2 binding.



Braf^{v600E} induced senescence is irreversible *in vivo*

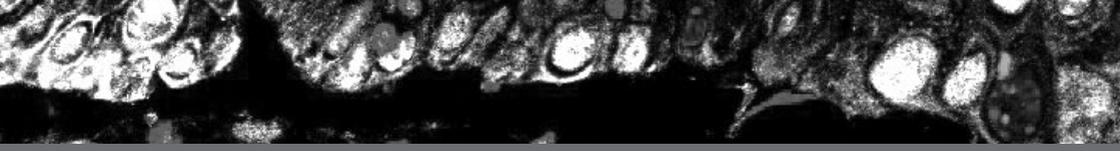
Sam Garnett¹, Rosalie V. McDonough¹, Anthony N. Karnezis³ and David Dankort^{1,2,‡}

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Lung cancer arises through the acquisition of a number of genetic lesions with a preponderance of activating mutations in the canonical MAPK cascade (RTK-RAS-RAF-MEK). Direct pathway activation through Braf^{v600E} expression induces benign lung adenomas that fail to progress to adenocarcinoma due to oncogene-induced senescence (OIS). The simultaneous ablation of the *Tp53* tumour suppressor gene permits bypass of senescence and progression to adenocarcinoma. Human tumors however sustain mutations in a temporally separated fashion. Here, we use a lung cancer model where oncogene activation (Braf^{v600E}) and tumor suppressor loss (*Tp53*) are independently controlled through the actions of Flp and Cre recombinases. We show that *Tp53* loss early in the genesis of disease, prior to OIS, is permissive for lung tumor (adenoma) progression to cancer (adenocarcinoma). Conversely *Tp53* loss after senescence is established has no additional consequence to disease progression. This study demonstrates that Braf^{v600E} induced senescence is not reversible *in vivo*, suggesting that therapy-induced senescence would stabilize disease.



In vitro removal of senescent fibroblasts by activated monocytes

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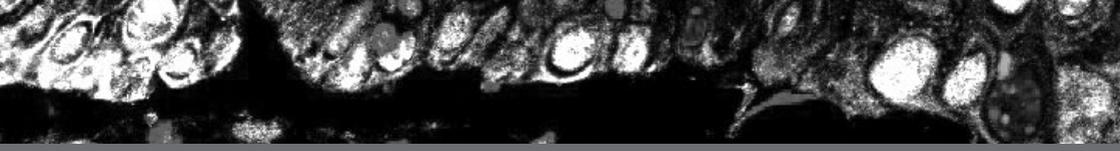
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Immune surveillance of senescent cells aims at removal of damaged, (or) precancerous cells and helps to maintain tissue integrity. Senescent cells are removed by immune cells – Sagiv *et al.* (2013) demonstrated in vitro clearance of senescent fibroblasts by NK cells and Lujambio *et al.* (2013) documented immunosurveillance of premalignant hepatocytes by monocytes and macrophages. We investigated, if monocyte-derived macrophages are able to clear senescent fibroblasts.

WS1 fibroblasts were rendered senescent after growth with 500 μ M BrdU for at least a week. THP-1 monocytic cell line was induced to differentiate into macrophages with PMA in the culture media for three days. The fibroblasts were labeled with calcein to assess viability. The macrophages were dyed with Dil to track the cell position and coincubated with the fibroblasts. Nonsenescent fibroblasts served as control. The fibroblasts were counted under a microscope at about 2, 5, 23 and 30 hours. To investigate engulfment of senescent cells by the macrophages, the fibroblasts and macrophages were dyed with membrane dyes (either Dil or DiO) and colocalization of the signal was measured with flow-cytometry. Also, influence of the factors secreted by the senescent cells on the process was investigated by incubating senescent cells with media from nonsenescent fibroblasts and *vice versa*.

We observed decrease in number of senescent fibroblasts when incubated with monocyte-derived macrophages. On the other hand, nonsenescent fibroblast counts rised when incubated with the macrophages, however less rapidly than the numbers of fibroblasts grown without the macrophages. Macrophages engulfed the fibroblast cell debris in higher amount in senescent cells than in control fibroblasts. Preliminary results suggest that the factors produced by senescent cells into culture media induced higher removal of both senescent and control cells by macrophages. On the other hand, medium from nonsenescent cells mitigated removal of both senescent and nonsenescent fibroblasts.

We demonstrated that senescent fibroblasts may be removed by monocyte-derived macrophages *in vitro*. Although the relevance to *in vivo* situation remains to be elucidated, we believe that this model may contribute to research on the mechanisms of senescent cell removal.



Selectively targeting the ribosome as a therapeutic strategy for pro-senescence therapy

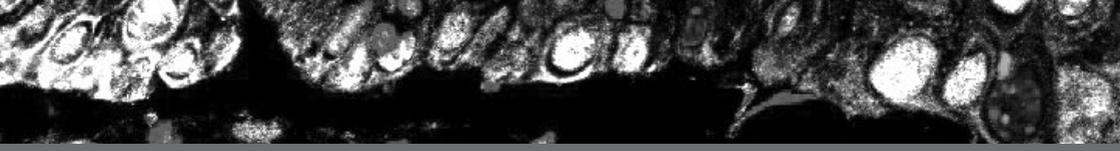
Madeleine Moore, Mike Philpott, Cleo Bishop

The Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, London E1 2AT

The discovery of oncogene-addiction and the demonstration that senescence can be induced in established tumours has paved the way for pro-senescence therapies. p16+ve basal-like breast cancers (BLBC) are particularly aggressive with poor patient prognosis. They have early onset, are frequently triple negative and have a current unmet clinical need. Therefore, we sought to identify genes that govern senescence evasion in p16+ BLBC and identify new pro-senescence targets for this subset of breast cancer.

Following genome-wide siRNA screening and filtering for potential side effects using normal human mammary epithelial cells, we identified 20 siRNAs that activated a stable senescence arrest in p16+ve cancer cell models. Strikingly, 11 ribosomal proteins (RPs) emerged from this analysis implicating dysregulation of ribosomal biosynthesis in cancer, and suggesting that targeting specific subunits of the ribosome could be a highly effective pro-senescence strategy.

Analysis of the METABRIC dataset (2,000 matched breast cancer samples) revealed a coordinated dysregulation of these RPs, and elevated expression of a number of these RPs results in a reduced overall survival in p16+ BLBC patients. We will present detailed analysis of the mechanism of senescence activation following RP knockdown, and our latest findings on the utility of these RPs as prognostic biomarkers for breast cancer and improved patient stratification.



Molecular mechanisms of senescence regulate placental structure and function

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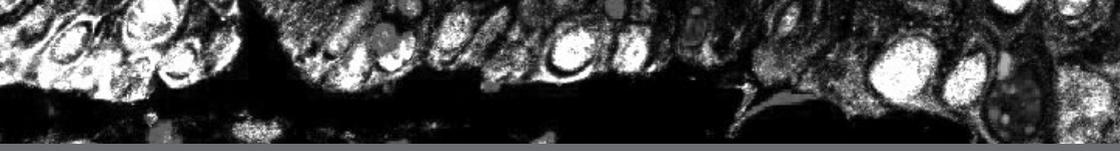
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The placenta is an autonomous organ, essential for the maintenance of fetal integrity during pregnancy. The multinucleated syncytiotrophoblast layer of the placenta intended for feeding and oxygenating the fetus during intrauterine life, exhibits characteristics of cellular senescence. The functional role of molecular pathways regulating senescence in syncytiotrophoblast is not understood. To gain this understanding, we studied placentas of $Cdkn1a^{-/-}$, $p53^{-/-}$, $Cdkn2a^{-/-}$ and $Cdkn2a^{-/-};p53^{-/-}$, compared to placentas of wild-type (WT) mice. Using Dynamic Contrast-Enhanced MRI (DCE-MRI), we found an altered contrast agent dynamics in placentas of the knockout mice, with the most significant changes in the $Cdkn2a^{-/-};p53^{-/-}$ placentas. These placentas also exhibited histopathological aberrations, compared to placentas of WT mice. Microarray analysis of human primary syncytiotrophoblast showed upregulation of senescence markers associated with cell cycle regulation and senescence associated secretory phenotype (SASP). We propose that senescence maintains placental function by the inhibition of cell cycle in syncytiotrophoblast and by the secretion of SASP components. These molecular effects were compromised in mice with an attenuated senescent program, ultimately leading to morphological and functional abnormalities in the placenta. Importantly, human placentas derived from pregnancies complicated with Intrauterine Growth Restriction (IUGR) show a marked reduction in the expression of key regulators of senescence. Overall, molecular mediators of senescence regulate placental structure and function.

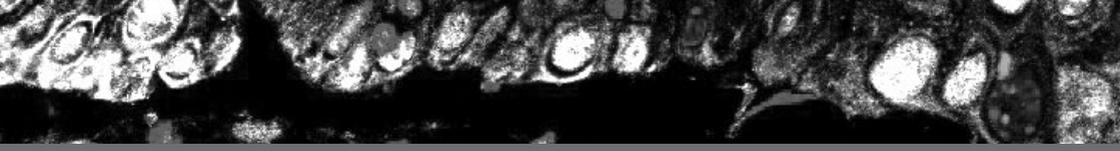


Identification of novel SWI/SNF-regulated genes mediating a coordinated senescence response to prevent oncogenic transformation

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We previously identified the SWI/SNF component ARID1B, which is frequently mutated in hepatocellular carcinoma, as a regulator of senescence. Our data *in vitro* and *in vivo* (mouse liver model) indicated that ARID1B knockdown allows bypass of oncogene-induced-senescence (OIS) partially via modulation of the well-known senescence effectors p16^{INK4a} and p21^{CIP1}, but also by restraining p53-activating stimuli such as DNA damage and oxidative stress. Consistent with this, ARID1B ectopic expression activated p53 to induce senescence. To identify the SWI/SNF effectors mediating this effect, we performed a bypass-of-senescence screen using a focused shRNA library targeting ARID1B-induced genes in OIS. To allow the identification of strong candidates and minimize the number of false positive hits we used an average of six shRNAs targeting each gene. The screen identified multiple ARID1B target genes regulating OIS. We validated *in vitro* and *in vivo* three of these SWI/SNF targets, ENTPD7, SLC31A2 and NDST2, showing that their downregulation, in the context of oncogenic RAS, dampens DNA damage and oxidative stress and allows senescence bypass. Consistent with their role downstream SWI/SNF, their knockdown also prevented ARID1B-induced senescence. Amongst these targets, ENTPD7, an enzyme involved in nucleotide metabolism, partially phenocopied the effect of ARID1B suggesting that the nucleotide metabolism could be a liability for targeting SWI/SNF mutated tumors. Nevertheless we also found that *in vivo* the concurrent regulation of multiple targets is needed to closely phenocopy the effect of ARID1B deletion on senescence. In conclusion, we identified several SWI/SNF targets with the ability to regulate OIS, implying that the program of senescence controlled by SWI/SNF is intricate and requires the coordinated regulation of multiple effectors.



Coupling shRNA screening with single cell RNA-seq to identify genes and processes mediating senescence during reprogramming

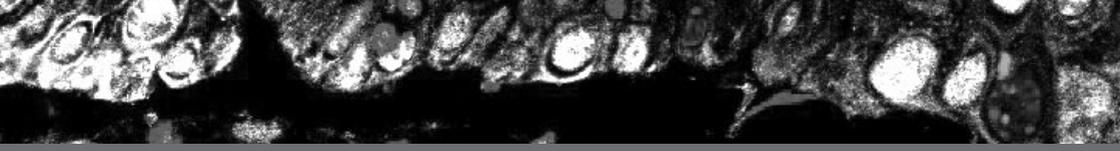
Marieke Aarts¹, Patrizia Beolchi¹, Ana Banito¹, Tamir Chandra², Thomas Carroll¹, Gopuraja Dharmalingam¹, Nadine Martin¹, Johannes Zuber³, Keisuke Kaji⁴, Jesús Gil¹

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Somatic cells can be reprogrammed to a pluripotent state by ectopic expression of the transcription factors Oct4, Sox2, Klf4 and c-Myc (OSKM). The resulting induced pluripotent stem cells (iPSCs) are highly similar to embryonic stem cells and can be used for regenerative medicine, drug screening and disease modelling. Despite recent advances in identifying the underlying mechanisms, reprogramming is slow and inefficient, suggesting the presence of safeguarding mechanisms to counteract cell fate conversion. One of the initial barriers cells need to overcome to become successfully reprogrammed is senescence, which is mediated through activation of the tumour suppressors p53/p21^{CIP1}, p15^{INK4B} and p16^{INK4A}.

To identify modulators of this reprogramming-induced senescence, we performed a genome wide short hairpin RNA (shRNA) screen in primary human fibroblasts expressing OSKM. shRNAs that allowed bypass of the OSKM-induced growth arrest were identified by next-generation sequencing. In parallel, we used single cell RNA-sequencing to profile the effect of the identified shRNAs on the transcriptome.

The combined shRNA screening and single cell RNA-seq approach revealed novel mediators of this senescence response and validated previously implicated genes such as *CDKN1A* and *MTOR*. Single cell transcriptome analysis has suggested novel pathways that could be involved in inducing pluripotency. Candidate shRNAs were tested for their ability to improve iPSC reprogramming. The identified candidates may represent common repressors of cell fate conversion, thereby potentially improving the efficiency of somatic cell reprogramming towards other fates than pluripotency.



Induction of cellular senescence and hair follicle stem cell dysfunction upon p16^{INK4a} expression in the skin

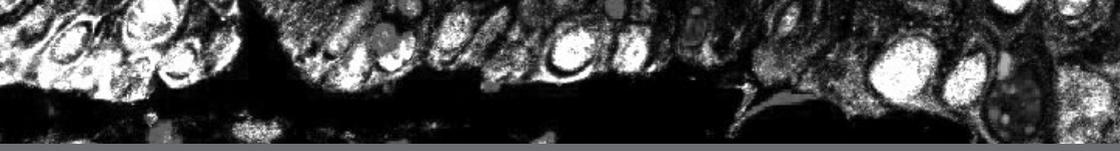
Narmen Azazmeh¹, Ronit Tokarsky-Amiel¹, Aharon Helman¹, and Ittai Ben-Porath¹.

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Cellular senescence is a physiologic stress response program, in which cells cease to proliferate and undergo dramatic alterations in morphology, metabolic activity and gene expression. Senescence is mediated mainly by the p14^{ARF}/p53 and p16^{INK4a}/Rb tumor suppressor pathways, which are interconnected, yet their distinct contributions to the different components of the senescence program are not fully understood. Senescence is involved in tumor suppression, aging, multiple pathologies and normal embryonic development, however, many fundamental aspects of its beneficial and detrimental roles in tissue remodeling and physiology remain poorly elucidated. We have developed mice that allow inducible activation of p16^{INK4a}, a senescence activator, in the epidermis of the skin. Using this model, we set out to study the effects of p16^{INK4a} activation on senescence induction, on skin structure and hair follicle stem cell function.

We found that p16^{INK4a} activation led to cell cycle arrest and senescence formation within the epidermis. Although expressed only in a subset of cells, p16^{INK4a} caused pronounced epidermal alterations, including an increase in thickness and keratinocyte enlargement, and dysfunction of hair follicle stem cells. Upon long-term induction, we found that p16^{INK4a} activation led to expansion of differentiated epidermal layers, alopecia, and dermal structure modification, that were distinct from those observed during normal aging. p16^{INK4a} activity induced substantial changes in wound closure, and in overall epidermal proliferation rates.

Our findings indicate that p16^{INK4a} activity can induce dramatic structural and functional changes in the epidermis, most likely acting through both cell autonomous and non-autonomous mechanisms. Our data shed light on one of the most important cellular genes and mechanisms regulating aging and cancer.



Targeting the senescence-associated secretory phenotype with HDAC inhibitors.

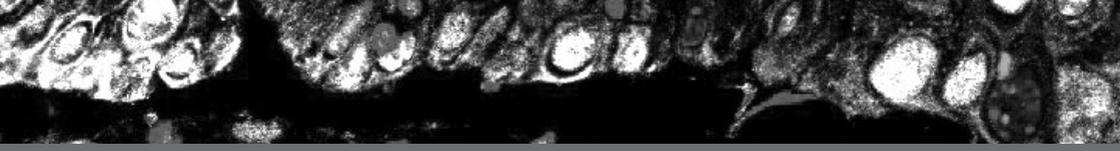
Maria Grazia Vizioli¹, Neil Robertson¹, Clara Correia Melo², David Schultz³, Zhixun Dou³, Joao F. Passos², Shelley L. Berger³, and Peter D. Adams¹

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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). 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 Trichostatin A (TSA), a potent HDAC inhibitor, suppresses SASP in senescent irradiated fibroblasts at a very low non-toxic dose, without reversing the senescence growth arrest. In proliferating cells, the same dose is non-cytostatic. In senescent cells, TSA induces a marked decrease of senescence-associated beta-galactosidase activity and suppresses formation of senescence-associated heterochromatin foci (SAHF). Maintenance of the SASP requires persistent DNA Damage Response (DDR) signalling and activation of NFkB signalling. We also showed previously that senescent cells extrude fragments of γ H2AX-positive chromatin from the nucleus into the cytoplasm, so-called cytoplasmic chromatin fragments (CCFs), dependent on disruption of a nuclear lamin B1-LC3 interaction. We found that TSA does not directly down-regulate NFkB signalling, but inhibits DDR signalling, as evaluated by improved DNA repair, intranuclear foci and complete abolition of γ H2AX-positive CCF. Importantly, taking advantage of an irradiated mouse model we found that low doses of TSA suppress SASP *in vivo*. Together, these data suggest that TSA improves repair of DNA damage to suppress intranuclear and cytoplasmic DDR signalling and therefore induction of the SASP. Ongoing experiments are testing the chemopreventative activity of TSA and other clinical grade HDAC inhibitors against cancer and other age-related diseases via their ability to suppress senescence-associated inflammation.

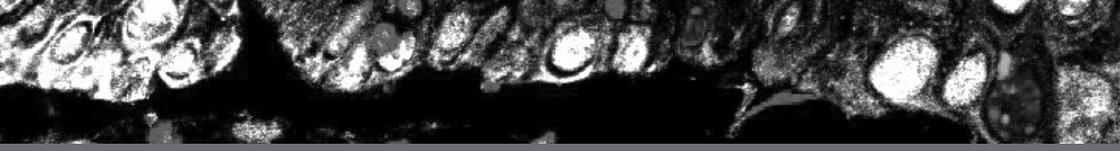


Histone chaperone HIRA maintains dynamic chromatin and prevents DNA damage in cellular senescence

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Cellular senescence is a stable cell cycle arrest and tumor suppressor mechanism. We previously presented evidence that, despite being non-replicative, chromatin of senescent cells is maintained in a state of dynamic equilibrium with continuous synthesis and turnover of core histones. The DNA replication-independent histone chaperone HIRA deposits newly synthesized histones H3.3/H4 into chromatin of senescent cells, maintains chromatin integrity and contributes to suppression of neoplasia. However, the precise mechanism and importance of long term chromatin maintenance by HIRA remains to be elucidated. We set out to better define the role of HIRA in senescent cells. Knockdown of HIRA in an ionizing radiation (IR) model of senescence led to accumulation of cells with unrepaired DNA damage and depleted of histones H3.3/H4. Exposure of cells to a low dose of IR (2Gy) supported the notion that HIRA has an important role in repair of DNA damage by non-homologous end joining (NHEJ). While HIRA-deficient senescent cells exhibited a high persistent DNA damage response, based on γ H2AX, phosphoCHK2 and 53BP1 foci, they showed low levels of p53 signalling, based on phosphop53 and p21 expression. Intriguingly, HIRA deficient senescent cells also had profound nucleo-cytoskeletal and centrosomal abnormalities and increased ploidy. Together, these results suggest that two features of senescent cells, persistent DNA damage and depletion of histones, might conspire to drive increased cell ploidy a feature of aging and a potential precursor to malignancy.



ATRX is a new regulator of senescence.

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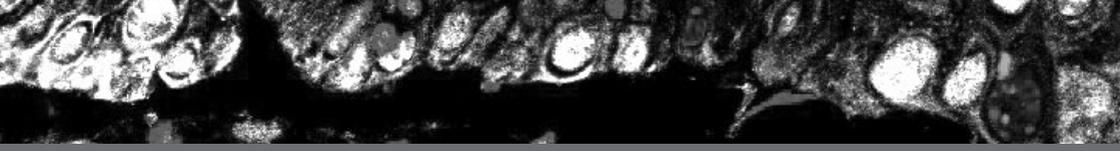
ATRX is a member of the SWI/SNF family of ATP-dependent helicases implicated in the maintenance of telomeric and pericentromeric heterochromatin, site-specific transcriptional regulation and X chromosome inactivation. Loss or mutation of ATRX is frequently seen in cancer cells that employ the alternative lengthening of telomeres (ALT). Here we report that ATRX plays a role in senescence.

In both primary and transformed cycling cells ATRX accumulates at telomeres and a few discrete nuclear foci. However, when cells are induced to enter senescence the number of foci increases substantially. Foci form soon after cells exit the cell cycle, well before the classic hallmarks of senescence (SAHF, SASP, SA- β -gal). This is observed in normal human diploid fibroblasts undergoing replicative, DNA damage induced and oncogene induced senescence, as well as in transformed cells treated with DNA damaging agents or CDK4 inhibitors (CDK4i) that induce senescence. Foci do not increase in quiescent or differentiated cells, or in cells that are induced to undergo autophagy by serum starvation. Thus, the increase in ATRX foci is an early indicator that cells have embarked on a pathway to senescence.

ATRX is necessary for senescence. Knocking down ATRX prevents transformed cells from accumulating SAHF, SA- β -gal or elaborating the SASP following treatment with chemotherapy. Conversely, reconstituting an ATRX deficient ALT cell line (U2OS) with ATRX is sufficient to convert CDK4i induced quiescence into senescence. This is dependent on ATRX's ability to interact with both the HP1 family of proteins and the histone modification H3K9Me3, as well as on a functional helicase domain. Reducing ATRX in already senescent cells does not reverse senescence; however, the SAHF are lost. Thus, ATRX is not required for maintaining cells in a senescent state but is necessary for the formation of the SAHF.

We performed ChIP-seq and RNA-seq in transformed cells that were induced to senesce following either CDK4i or DNA damage to determine whether there were additional ways that ATRX participated in senescence. ATRX bound to a number of discrete, gene-associated loci regardless of the senescence stimulus and the expression of a subset of these genes was specifically repressed in senescent cells. Furthermore, ectopic repression of one such gene, *HRAS*, was sufficient to drive cells from quiescence into senescence.

Thus, ATRX is a novel regulator and an early marker of senescence.



Targeting senescence-associated phenotypic promiscuity for selective cancer cell depletion

Maja Milanovic¹, Dimitri Belenki¹, Katharina Pardon¹, Clemens A. Schmitt^{1,2}

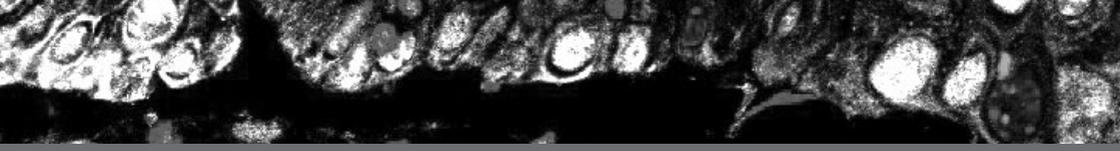
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Senescence is primarily described as an irreversible cell cycle arrest associated with tissue aging and is accompanied by morphological, metabolic, proteomic and epigenetic cellular changes. A senescence response to oncogenic or cytotoxic stress has been shown to limit expansion of premalignant lesions and contribute to the outcome of cancer therapy. Whether oncogene- and therapy-induced senescence (OIS and TIS), respectively, are truly irreversible *in vivo* is an issue of ongoing debate. *Ex vivo* and *in vitro* evidence from our laboratory intriguingly shows that tumor cells in TIS can escape the cell cycle arrest in conditional systems where essential mediators of senescence can be turned on and off. Paradoxically, we found cells in TIS to acquire characteristics of stem and progenitor cells (collectively termed senescence-associated stemness (SAS)). Upon reversal of senescence, these cells display enhanced self-renewal capacity and aggressiveness *in vitro* and *in vivo*, which depends on potentiated, cell-intrinsic β -catenin signaling. Considering that standard chemotherapeutic regimens induce senescence, in particular in apoptosis-resistant cells, it seems desirable to eradicate TIS cells before they can reemerge and drive an even more malignant disease.

To extend our understanding of the epigenetic events that enable SAS in senescent cells, we studied and present here the genome-wide redistribution of chromatin marks in the $E\mu$ -*myc* transgenic, *Bcl2*-overexpressing B-cell lymphoma model of TIS. We were particularly interested in chromatin traits that are associated with re-entry of TIS cells into the cell cycle. We show that the TIS-specific chromatin state is associated with permissiveness to stem cell-related transcriptional programs and transcription factor-mediated reprogramming, and uncover potential vulnerabilities that may be exploited by conceptually novel, epigenetic treatment strategies.

In addition, we uncovered profound plasticity regarding the phenotypic presentation of $E\mu$ -*myc*;*bcl2* TIS lymphoma cells, and further investigated the functional implications of the associated changes by pharmacological intervention.



NOX4 in senescence and chronic wound healing

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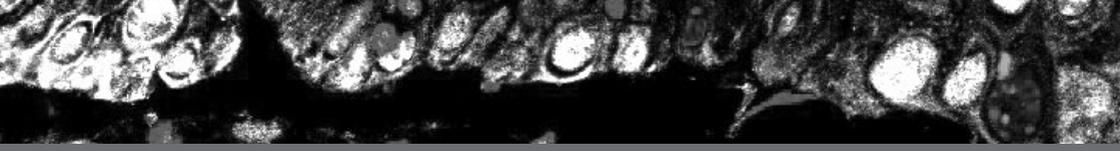
Chronic wounds, among them venous and ischemic ulcers, belong to age-related diseases and their prevalence is higher within diabetic patients. Venous ulcers typically exhibit chronic inflammation and ischemia. A common denominator of both conditions is excessive levels of NADPH oxidase 4 (NOX4), a ROS producing protein linked to cellular senescence induction and expansion onto bystander cells. (Hubackova et al. 2012) The ability of NOX4 to induce senescence is ascribed to inactivation of mitochondrial respiratory chain complex I and subsequent ROS-associated damage. (Kozielec et al. 2013)

We were interested whether venous ulcers have increase in NOX4 and whether such increase translates to cellular senescence.

Biopsies of venous ulcers were collected for RNA isolation and histological examination. Transcription was analyzed by microarrays and the selected genes were confirmed by qRT-PCR. The presence of senescence in venous ulcers was visualized by novel technique. The method combines SA β -galactosidase assay with fluorescent substrate DDAOG and immunohistology using fluorescence detection system. The stained frozen cryosections were examined by confocal microscopy, the photon counting mode enabled to distinguish senescent cells from non-senescent. This approach allows to visualize senescent cells by favored enzymatic activity in high resolution and investigate them in the context of the tissue.

We proved that *NOX4* is significantly increased in venous ulcers on transcriptional level compared to healthy skin and concordantly, several genes of mitochondrial respiratory chain complex I were significantly decreased. *IL1A*, *IL1B* and *TNFA*, which are known NOX4 drivers, were correspondingly increased. Furthermore, senescent cells were observed within NOX4-rich lesions.

As cellular senescence is considered to be aggravating already impaired healing in chronic wounds, the above data suggest detrimental role of excessive induction of NOX4. (Mendez et al. 1999, Stanley & Osler 2011) We have presented increase of NOX4 in venous ulcers in context of chronic wound healing and introduced new technique employable in histological research.

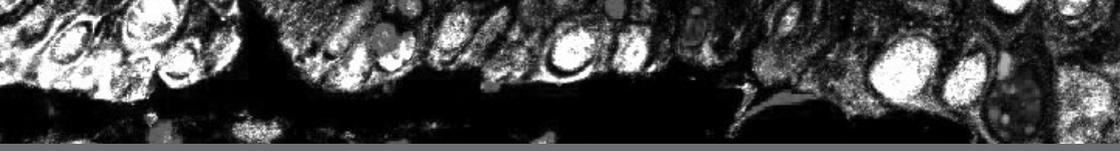


Dynamic multidimensional profiling defines the oncogene- and DNA damage-induced senescence gene expression programs

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Cellular senescence is currently viewed as a cell fate decision arising from a variety of (epi)genomic stresses and developmental cues, and is known to play crucial roles as an anti-tumour and in age-related pathologies. However, our present view of senescence is a rather static one that does not describe the dynamic genomic regulation that orchestrates this cell fate transition. To address this gap, we applied a dynamic approach using high throughput sequencing and Affymetrix gene expression microarrays coupled to bioinformatics analyses to monitor and integrate histone modifications (ChIP-seq), chromatin accessibility (ATAC-seq) and transcriptome changes during oncogene-induced senescence (OIS) and DNA damage induced senescence in primary fibroblasts. Our approach reveals a remarkably organised yet highly responsive genome whose modulation gives rise to the gene expression signature. A pre-established genomic configuration composed of near-mutually exclusive 'silent' (H3K27me3) and 'active' (H3K4me1/3, H3K27Ac, ATAC) megabase-sized chromatin domains appears inert. In contrast, highly dynamic behaviours for chromatin accessibility and active histone marks are observed at the kilobase range, where most of the variable gene transcription takes place. Genome-wide, ATAC signals correlated with the presence of H3K4me1/3 and H3K27Ac, identifying promoters and enhancers and thus defining the fibroblast identity as well as the senescence transition. Our results also suggest that, depending on the senescence inducer, even if eventually leading to a stable cellular cycle arrest, the transcriptomic trajectories are distinct, suggesting that cellular senescence is a heterogeneous cellular process, involving stressor-dependent transcriptional programs. Additional dynamic integrative analyses of transcriptomic and epigenome profiling identified known factors as well as novel candidates regulating each type of senescence and their temporal hierarchy, generating a systems map linking regulators to specific transcriptional paths. Collectively, our results provide a dynamic view of different types of senescence, and identify candidates for phenotypic modulation studies, as well as core pathways concomitantly involved in the regulation of gene expression modulation during senescence independently of the stressor.



Assessment of health status by molecular measures in middle-aged to old persons, ready for clinical use?

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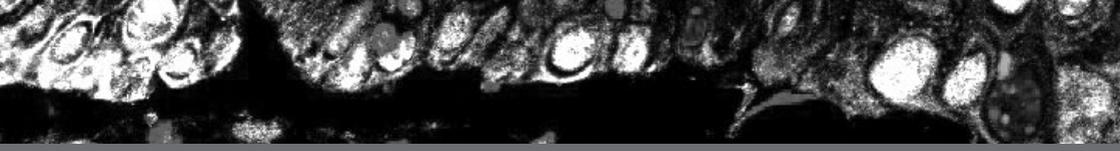
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In addition to measures already used in clinical practice, molecular measures have been proposed to assess health status, but these have not yet been introduced into clinical practice. We aimed to test the association of functional capacity measures used in current practice and molecular measures with age and health status.

The cohort consisted of 178 middle-aged to old participants of the Leiden Longevity Study (range 42-82 years). We tested associations between functional capacity measures (physical tests: grip strength, 4-meter walk, chair stand test; cognitive tests: Stroop test, digit symbol substitution test and 15-picture learning test) with age and with cardiovascular or metabolic disease as a measure of the health status. These associations with age and health status were also tested for molecular measures (C reactive protein (CRP), numbers of senescent p16INK4a positive cells in the epidermis and dermis and putative immunosenescence (presence of CD57+ T cells)).

All functional capacity measures were associated with age. CRP and epidermal p16INK4a positivity were also associated with age, but with smaller estimates. Grip strength and the Stroop test were associated with cardiovascular or metabolic disease, as was epidermal p16INK4a positivity. All associations with cardiovascular or metabolic disease attenuated when adjusting for age.



Do senescence markers correlate *in vitro* and *in situ* within individual human donors?

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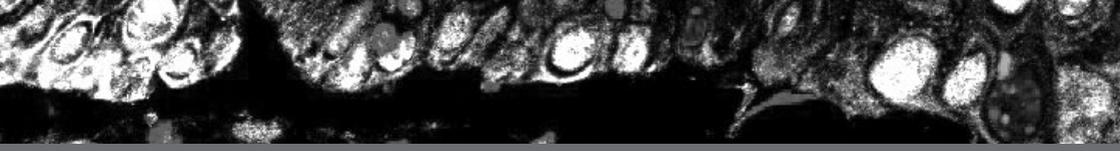
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Cellular senescence can be detected by several markers both *in vitro* and *in situ*, but little is known on how well senescence markers correlate within individual donors. By using data from highly standardized experiments, correlations between the same *in vitro* senescence markers were studied in duplicate short-term experiments, and between short-term and long-term experiments. In addition, different *in vitro* senescence markers measured within the short-term and long-term experiments were tested amongst each other for correlation. The different *in vitro* senescence markers were also tested for correlations with *in situ* p16INK4a cell positivity.

From a total of 100 donors (aged 20-91 years), cultured dermal fibroblasts were assessed for reactive oxygen species (ROS), telomere-associated foci (TAF), p16INK4a and senescence-associated β -gal (SA β -gal), both in non-stressed conditions and after supplementing the medium with 0.6 μ M rotenone for 3 days (short-term experiment). In cultured fibroblast from 40 of the donors, telomere shortening, levels of ROS and SA β -gal were additionally assessed, with or without 20 nM rotenone for 7 weeks (long-term experiment). In skin tissue from 52 of the middle-aged donors, the number of p16INK4a positive dermal cells was assessed *in situ*.

More than half of the correlations of the same senescence markers *in vitro* between duplicate



Markers of cellular senescence and chronological age in various human tissues: a systemic review of the literature

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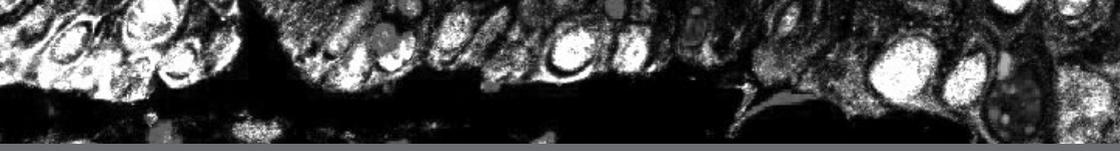
Background: Cellular senescence, a stable growth arrest of cells, is increasingly recognized as a driver of the aging process. Several studies report higher numbers of senescent cells in various tissue samples of older humans when compared to the young.

Objective: To systemically describe the present literature on the association between markers of cellular senescence and chronological age dependent on the type of tissue.

Methods: Systematic search in Pubmed, Web of Science and Embase for relevant articles that reported on senescence markers dependent on chronological age in any human tissue. Out of 3833 unique articles 43 articles were identified including 44 human cohorts. Data were extracted on the origin of tissue, the type of markers being used, and the age and gender distribution of the individuals. A total of 78 associations between senescence markers and chronological age were reported.

Outcomes: Cohort sizes ranged from 3 to 176 individuals, and varied widely in their age distribution. Out of the 78 associations, 34 were positive and statistically significant associations ($p < 0.05$) between senescence markers and chronological age, six showed positive trends ($0.05 < p < 0.10$), 27 associations were inconclusive ($p > 0.10$) and one association showed a negative statistically significant association ($p < 0.05$). A large proportion of the positive associations were based on studies conducted in blood, whereas it was less often the case in kidney and skin.

Conclusion: Almost half of the associations between markers of cellular senescence and chronological age show a positive significant association. This can be interpreted as proof of an evident biological phenomenon but it is unclear to what extent publication bias explains for these outcomes.



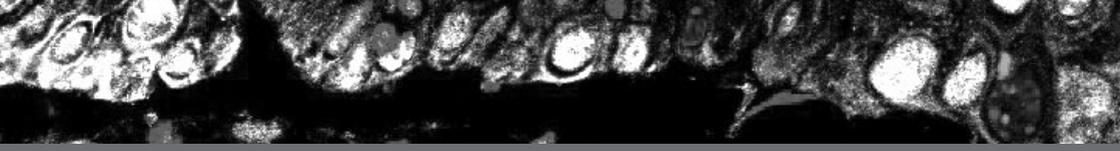
**14th March 2015 Abstract Submission for
Cellular Senescence:
From Molecular Mechanisms to Therapeutic Opportunities**

Autophagy-induced senescence and chemoresistance following anti-mitotic therapy

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**Lee Kong Chian School of Medicine, Nanyang Technological University
Institute of Molecular and Cell Biology, A*STAR**

Anti-mitotic drugs such as paclitaxel and vinblastine have been used as front-line therapies for the treatment of many cancers. These agents induce activation of a spindle assembly checkpoint (SAC), which leads to a prolonged mitotic arrest that culminates in cell death. However, this anti-proliferative mechanism is often impeded by the development of chemo-resistance. A major cause of resistance is mitotic slippage. Mitotic slippage is a process whereby cells exit mitosis and undergo an abnormal cytokinesis failure despite an active SAC. A large population of these post-slippage cells undergo senescence, largely thought to serve as an anti-proliferative mechanism. Here we find autophagy plays an important role in senescence induction, which can induce pro-tumorigenic signaling. Inhibition of autophagy via pharmacological drugs or silencing of autophagy-associated genes could over-ride senescence and promoted cell death upon anti-mitotic drug treatment. Taken together, our data suggest that autophagy mediated-senescence contributes to chemo-resistance and could serve as a potential combinatorial target with anti-mitotic drugs in future clinical trials.

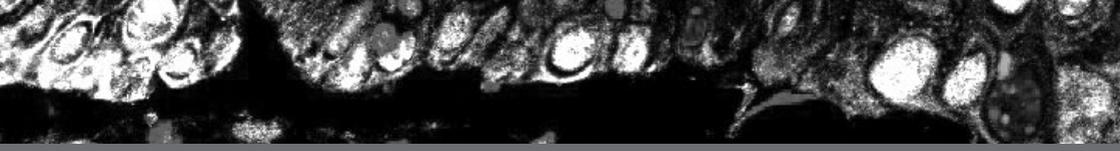


The long-noncoding RNA MIR31HG regulates the senescence-associated secretory phenotype

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Biotech Research and Innovation Centre, University of Copenhagen, Denmark.

Senescent cells secrete a number of factors called the senescence-associated secretory phenotype (SASP) that can impact the environment and the homeostasis of neighboring tissues. The SASP can induce senescence and activate the immune system acting as a cancer barrier, but also promote tumor progression and other aged-related pathologies. Due to the heterogeneous composition of the SASP, the identification of its regulators is of particular interest. We have identified the long non-coding RNA MIR31HG to be upregulated in primary human fibroblasts undergoing BRAF mediated oncogene-induced senescence (OIS). We have shown that nuclear MIR31HG in proliferating cells represses p16^{INKA} expression by recruiting Polycomb group proteins. Interestingly, upon BRAF induction MIR31HG translocates to the cytoplasm where we now demonstrate it to have a role in regulating induction of the SASP. MIR31HG knockdown in senescent fibroblasts reduces expression and secretion of pro-inflammatory components of the SASP. Breast cancer cells in contact with conditioned media (CM) from MIR31HG knockdown-senescent cells are less prone to matrigel invasion, compared to cells exposed to CM from control-senescent cells, suggesting that factors promoting invasion are reduced in the CM from MIR31HG-knockdown senescent cells. Importantly, fibroblasts receiving CM from either control or MIR31HG knockdown-senescent cells undergo senescence, suggesting that reinforcement of senescence is not affected by MIR31HG. Mechanistically, the protein levels of CEBP β and activated-NF κ B, main regulators of the SASP are reduced, and the upstream regulator, IL1 α , is decreased at protein level but not mRNA level in MIR31HG knockdown-senescent cells. Besides, addition of recombinant IL1 α restores the phenotype observed, suggesting that MIR31HG affects IL1 α regulation at the protein level. To elucidate the molecular mechanism, we are performing RIA-seq to detect RNA-RNA interactions and optimizing a pull-down protocol using the nuclease Csy4 to identify proteins interacting with MIR31HG. Dissociation of the good and bad sides of the SASP is important for understanding the significance of senescence *in vivo*, and is crucial before considering senescence manipulation for therapeutic purposes. Our results suggest that the lncRNA MIR31HG have a dual role in senescence by suppressing p16 expression in young cells and facilitating production of a distinct subset of SASP factors in senescent cells.

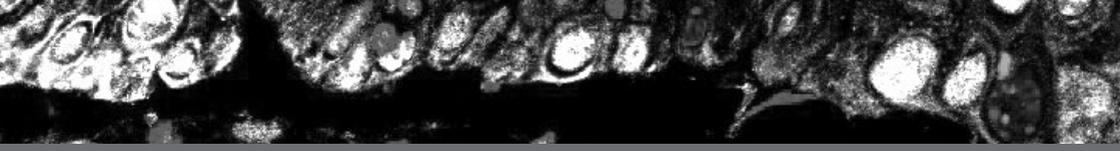


Intracellular Ca^{2+} chelation rescues human mesenchymal stem cells from stress-induced senescence via autophagy initiation

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Intracellular calcium has been reported to play an important role in the induction of apoptosis and necrosis, however a little is known about its impact in the premature senescence. Previously we have shown that human endometrium-derived mesenchymal stem cells (hMESC) via activation of the canonical ATM/Chk2/p53/p21/Rb pathway enter the premature senescence in response to sublethal oxidative stress (Oxid Med & Cell Longev, 2013; Aging, 2014; Cell Cycle, 2016). The present study aimed to investigate the possible correlation between intracellular calcium levels and senescence progression in H_2O_2 -treated hMESC. Firstly, we revealed a rapid increase in intracellular calcium levels ($[\text{Ca}^{2+}]_i$) in H_2O_2 -stimulated cells using fluo-3AM imaging. Further by omitting Ca^{2+} from staining solution we showed that Ca^{2+} release from intracellular stores rather than Ca^{2+} entry across the plasma membrane might be responsible for the $[\text{Ca}^{2+}]_i$ elevation during H_2O_2 treatment. To validate the role of this intracellular calcium increase in hMESC senescence, we applied a specific intracellular calcium chelator – BAPTA-AM. As expected, cell pretreatment with 10 μM BAPTA-AM had no significant effect on cell viability, but effectively decreased H_2O_2 -induced $[\text{Ca}^{2+}]_i$ levels. Interestingly, intracellular calcium chelation in H_2O_2 -treated hMESC was sufficient to alter the senescence phenotype, to avoid G0/G1 cell cycle arrest, and finally to resume proliferation. Accordingly, BAPTA-AM affected the p53/p21/Rb pathway in H_2O_2 -treated hMESC, leading to the decrease of p53 phosphorylation, down-regulation of p21 expression and slight increase in Rb phosphorylation. Next, we revealed that BAPTA-AM induced an early onset of autophagy in H_2O_2 -treated hMESC as indicated by the appearance of the LC3-II band, an enhanced phosphorylation of Ulk1 at Ser555 and reduced phosphorylation of Ulk1 at Ser757. Summarizing the obtained data we can conclude that BAPTA-AM-induced decrease of intracellular Ca^{2+} levels in H_2O_2 -treated hMESC leads to the premature senescence prevention via autophagy initiation.

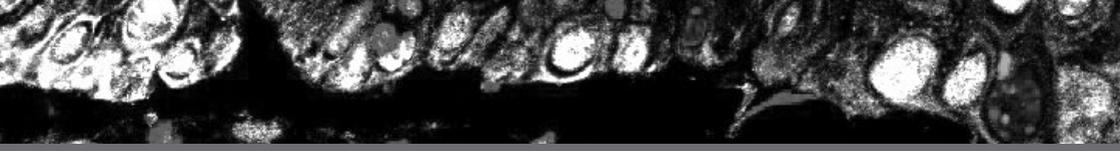


Fibroblasts of wild long-lived and cancer resistant subterranean rodent *Spalax* undergo senescence without acquisition of pro-inflammatory response: an essential role of NF- κ B signaling.

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Cellular senescence is a stress response program aimed to arrest cell cycle in the response to DNA damage. Despite the beneficial tumor suppressive role for individual cells, cellular senescence typically exert adverse pro-tumorigenic effect on adjacent microenvironment via acquisition of senescent-associated secretory phenotype (SASP). NF- κ B signaling has a crucial role in SASP via activation and nuclear translocation of NF- κ B p65 subunit leading to expression of the pro-inflammatory and pro-growth factors. Here we demonstrate that normal fibroblasts isolated from newborn's skin and lung of wild cancer resistant subterranean rodent *Spalax* undergo senescence either replicative or etoposide-induced without acquiring SASP. Senescent fibroblasts showed increased activity of senescence-associated beta-galactosidase (SA- β -gal), reduced cell growth and cellular hypertrophy. Western blot analysis demonstrated stimulation of signaling pathways responsible for senescence. Nonetheless phospho-p65 decreased compared with early-passage fibroblasts. Mass spectrometry revealed that senescent fibroblasts of *Spalax* secrete increased levels of multiple proteins with anti-tumorigenic and anti-angiogenic activity. Further, we treated cancer cells (Hep3B, HepG2) with conditioned medium (CM) of senescent *Spalax* fibroblasts. Cancer cells exposed to senescent *Spalax* secretome acquire the flattened shape and display SA- β -gal activity. *Spalax* CM induces growth arrest in cancer cells via retinoblastoma (Rb) activation. Surprisingly, NF- κ B p65 phosphorylation in cancer cells, undergoing senescence under exposure to *Spalax* senescent CM, gradually decreased reflecting the repression of inflammatory response. These findings demonstrate that 1) SASP is not necessarily required for acquisition of senescence phenotype; 2) targeting senescence in cancer cells can be achieved without acquiring inflammatory secretory response. Finally, our evidences support the hypothesis that senescence can be transmitted in a paracrine fashion that may have a therapeutic application in cancer.

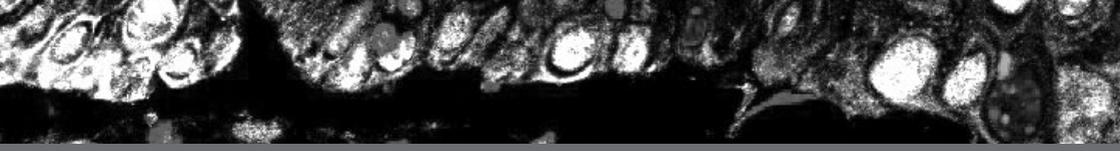


Defective ribosome biogenesis in senescence reveals a novel checkpoint pathway to control cyclin-dependent kinases

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Cellular senescence is a tumor suppressor program characterized by a stable cell cycle arrest. Here we report that senescence triggered by a variety of stimuli both in cell culture and *in vivo* involves diminished ribosome biogenesis and accumulation of both rRNA precursors and ribosomal proteins. These defects were associated with reduced expression of several ribosome biogenesis factors. Depletion of ribosome biogenesis factors was sufficient to induce senescence with activation of the tumor suppressors p53 and RB. However, genetic analysis revealed that RB but not p53 was required for the senescence response to altered ribosome biogenesis. Mechanistically, a ribosomal protein binds and inhibits the cyclin-dependent kinase CDK4 in senescent cells, thereby inducing accumulation of hypophosphorylated RB, cell cycle arrest and senescence. Moreover, overexpression of this ribosomal protein induces senescence and genetic analysis revealed that RB but not p53 was required. We thus describe a mechanism maintaining the senescent cell cycle arrest relevant for cancer therapy.

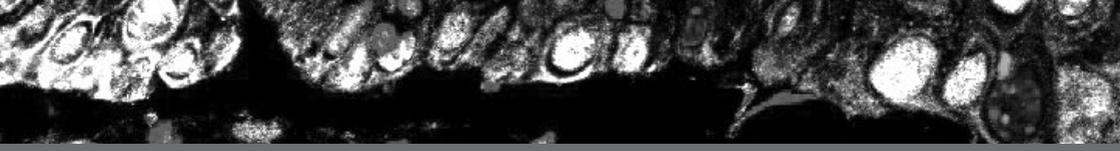


Quantitative identification of senescent cells in ageing and disease

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Senescent cells are present in pre-malignant lesions, sites of tissue damage, and they accumulate in tissues with age. In-vivo identification, quantification and characterization of senescent cells is currently challenging, limiting our understanding on the role of senescent cells in ageing and age related diseases. Here we present a new method to precisely quantify and identify senescent cells in tissues on a single cell basis. This method combines senescence-associated-beta-galactosidase assay with staining of molecular markers for cellular senescence and markers of cellular identity. The evaluation is performed by using ImageStreamX, which combines flow cytometry and high content image analysis. Using this method we were able to quantify senescent cells in tumors, fibrotic tissues and tissues of aged mice. Our approach identified for the first time that senescent cells in tissues of the aged mice have larger cell size when compared to non-senescent cells. Our study allows quantitative evaluation of senescent cells in tissues and thus provides a basis for quantitative assessment of their impact on pathologies and ageing.



DISSECTING THE MOLECULAR BASIS AND SIGNALLING PATHWAYS UNDERLYING CELLULAR SENESCENCE

Ruchi Kumari¹, Parmjit Jat²

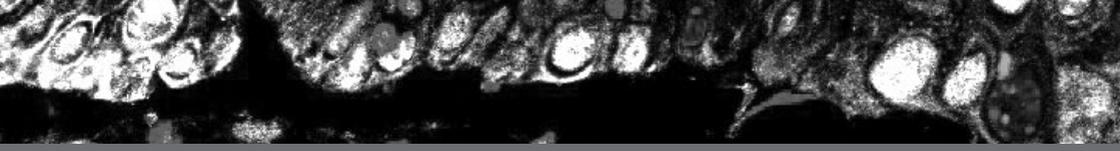
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Cellular senescence is a stable cell cycle arrest that normal cells undergo in response to a variety of intrinsic and extrinsic stimuli. Being implicated in ageing and age related diseases including cancer it is of great importance to elucidate the 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. Previous research in the group has compared mitotic and senescent cells by microarray analysis, and identified genes that are differentially expressed upon senescence. TFs regulate gene expression at different stages of embryonic development and are key to the establishment and maintenance of specific cell fates. To identify the TFs that act downstream of the p16-pRB and p53-p21 pathways to regulate senescence growth arrest, we overlaid the list of differentially expressed transcripts with the 1391 manually curated sequence-specific DNA binding factors, identified by Luscombe and colleagues. This list was further refined by examining what happened to their expression, when senescence was bypassed upon inactivation of the p16-pRB and/or p53-p21 pathways.

To determine if silencing expression of up-regulated TFs individually bypasses senescence, lentiviral pGIPZ shRNAmir constructs, corresponding to the up regulated TFs are being tested for their ability to bypass senescence. To determine if ectopic expression of down-regulated TFs individually bypasses senescence we have prepared, full length lentiviral expression constructs for all down-regulated TFs. These are now being systematically tested for their ability to bypass senescence in a conditionally immortal human fibroblast cells as well as other senescence assays.

This will enable us identify key downstream TFs that have a causal role in senescence laying the foundation for a better understanding of the pathways underlying cellular senescence and may thus serve as molecular targets for therapeutic intervention against cancer.

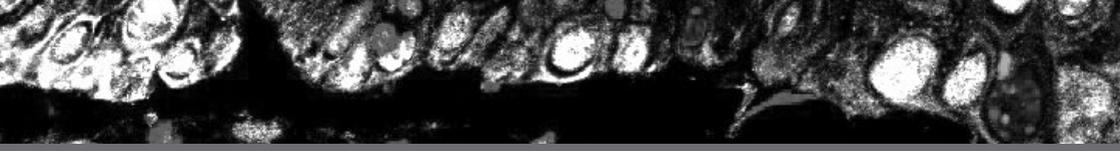


Impaired immune clearance of senescent cells promotes aging in mice

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Cellular senescence is a cell-intrinsic stress response programmed to impose a stable cell-cycle arrest in damaged cells and prevent their propagation in tissues. However, long-term presence of senescent cells might have deleterious consequences, as they can promote tissue degeneration and malignant transformation via the secretion of pro-inflammatory and matrix remodeling factors. These factors might also facilitate immune clearance of senescent cells soon after their formation, and allow regeneration of the tissues. Still, it is not clear to what extent the immune system regulates the number of senescent cells during aging, when these cells tend to accumulate in tissues. Here we show that mice with impaired immune surveillance of senescent cells due to Perforin-knockout exhibit higher tissue burden of these cells and suffer from chronic inflammation. As a result, these mice exhibit poor health and survive significantly less than wild-type animals. Similarly, Perforin-knockout mediated impaired immune surveillance leads to increased accumulation of senescent cells in progeroid mice and shortens their lifespan. In an opposite manner, pharmacological elimination of senescent cells increases median survival in progeroid mice, providing a tool to modulate aging process.



CD4+ and CD8+ senescence associated subsets differ between anti-TNF α responders and non-responders in Rheumatoid Arthritis.

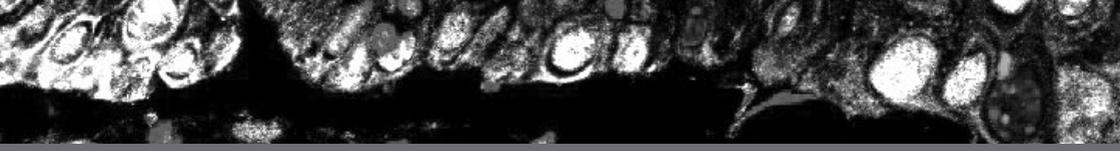
S. McCartan, R. Hedley, D. Ahern, F. Carlucci, S. Kirakidis and P.C. Taylor

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In Rheumatoid Arthritis (RA) senescent T-cells are reported to be disproportionately increased to levels incommensurate with age; however, reports differ regarding the effects of DMARDs and biologic treatments perhaps due to the cell type investigated (CD4+ or CD8+ cells) and/or the markers used to define senescence. Anti-TNF α therapy is known to increase IL-17A production in the peripheral blood. Using mass cytometry (CyTOF) to phenotype PBMCs from healthy controls (N=10), RA responders and non-responders to anti-TNF α therapy (N=5 of each; defined by improvement of DAS28 from baseline to 12 weeks), we were able to confirm this finding and also discovered that non-responders to therapy have an increased proportion of IL-17A producing cells in CD4+ and CD8+ senescence associated subsets at baseline.

A senescent profile was elucidated within both the CD4+ and CD8+ compartments, considering CD28+ CD27+ as a non-senescent subset, CD28+ CD27- and CD28- CD27+ as cells advancing towards senescence, and CD28- CD27- as fully senescent cells. Although RA patients had higher numbers of cells in all CD4+ and CD8+ senescence associated subsets compared to healthy, age-matched controls, these results were not statistically significant. Investigation of subset functional phenotype showed CD4+ and CD8+ fully-senescent cells had a higher proportion of TNF α producing cells compared to non-senescent cells ($p < 0.05$); but interestingly, it was the CD4+ CD28+ CD27- subset that had the most pro-inflammatory profile with the highest number of IL-17A and TNF α producing cells ($p < 0.01$). This subset was increased in non-responders only at 12 weeks post anti-TNF α treatment ($p < 0.05$), a change that could be attributable to a loss of CD27 expression by the CD28+ CD27+ subset. At baseline, non-responders had a greater proportion of IL17A producing cells within senescence associated subsets compared to healthy controls (CD4+ [$p < 0.05$] and CD8+ CD28- CD27- cells [$p < 0.01$], and CD4+ CD28- CD27+ cells [$p < 0.01$]), whereas in responders an increase was found within the non-senescent subset only (CD4+ CD28+ CD27+; $p < 0.05$).

In conclusion, not only do non-responders have a higher number of IL17A producing cells across a number of senescent subsets at baseline, anti-TNF α therapy also increases the size of the senescence associated CD4+ CD28+ CD27- subset, potentially through loss of CD27, a receptor which can inhibit Th17 differentiation. These results provide additional proof for the association of IL-17A with a lack of response to anti-TNF α therapy.

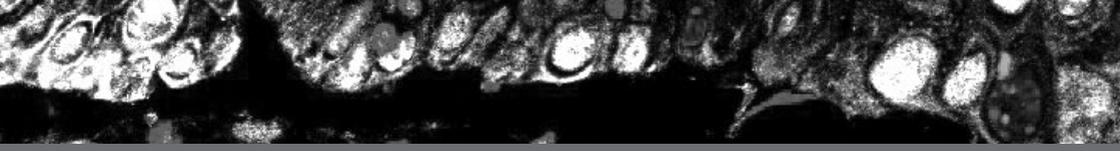


Astrocytes overexpressing transforming growth factor β 1 show characteristics of cellular senescence

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Astrocytes, the most abundant cells in the brain, play a crucial role in neural circuit formation and for the homeostasis and maintenance of the brain. Recent studies have demonstrated that astrocytes are vulnerable for premature stress-induced senescence results from oxidative stress and β -amyloid peptide, which accumulates in Alzheimer's disease. Senescent astrocytes accumulate with age and show characteristics of senescence-associated secretory phenotype (SASP). TGF- β 1, a multifunctional protein, was found to increase in aging. Moreover, TGF- β 1 is secreted as part of the SASP components and triggers senescence in neighboring cells. Here, we aimed to investigate the role of TGF- β 1 in astrocyte senescence using a mouse model overexpressing TGF- β 1 under the promoter of astrocytes. Our results show that TGF- β 1 astrocytes exhibit characteristics of senescence, seen by the increase in p16^{ink4a}, p21 and p19 expression. TGF- β 1 astrocytes also show characteristics of SASP, meaning increased IL-6 and IL-8 secretion, increased levels of GFAP and S100 β and enlarged nuclei. Furthermore, these cells fail to provide neuronal support. Understanding the mechanisms leading to astrocyte senescence may improve our understanding about the mechanisms leading to neurodegenerative diseases.

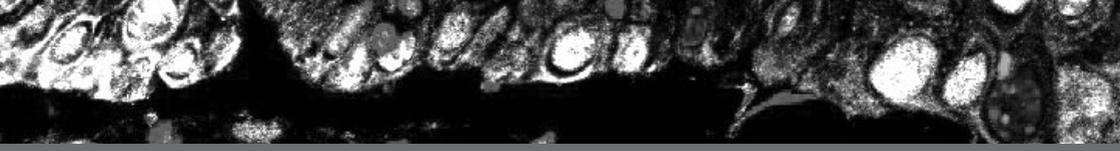


UHRF1 is an upstream regulator of WNT5A-mediated senescence

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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 this study, we investigated expression profiles of DNMT1-interacting proteins and their effects on DNMT1 expression. In the progress of replicative senescence of human diploid fibroblast (HDF), DNMT1 expression specifically decreased from the earlier stage before gain of SA- β -gal activity, a typical senescence marker, whereas DNMT3 expression did not change. Similar results were obtained in the stress-induced senescence triggered by exogenous subcytotoxic dose of H₂O₂. By analyzing expression patterns of 53 known DNMT1-interacting proteins using cDNA microarray data of the two different cell senescence systems, we identified that 7 genes, such as CBX5, SUV39H1, EZH2, PARP1, UHRF1, CHEK1 and HELLS, were commonly down-expressed at the same time point as DNMT1 suppression, suggesting that these genes may cooperate with DNMT1 to regulate DNA methylation pattern in the progress of senescence. When individually suppressed the seven genes using siRNAs, only UHRF1 regulated DNMT1 expression. In addition, knockdown of UHRF1 effectively induced senescent phenotype of HDF. Finally, we identified WNT5A as the downstream effector of UHRF1/DNMT1-mediated DNA methylation. These results indicated that UHRF1 is the effective upstream regulator of DNA methylation-linked cell senescence through regulating DNMT1 expression.



Activity of HSF1 in connection with stress induced cellular senescence

Ortner V.*, Riegel E.*, Unterberger C.*, Wegrostek C.*, Dunzinger S.* and Czerny T.*

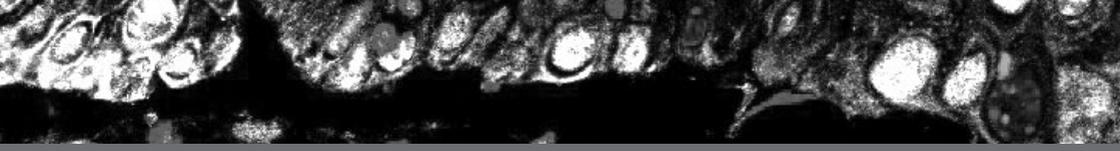
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The heat shock response (HSR) pathway is a highly conserved cellular mechanism to escape from unfavourable conditions (e.g. heat, heavy metals, proteasom inhibition) and recover normal protein homeostasis within the cell. The key transcription factor of this pathway is heat shock transcription factor 1 (HSF1). Upon activation HSF1 hyperphosphorylates, trimerizes and localises to the nucleus where it binds to specific DNA binding sites, the so called heat shock elements (HSEs), in the promoter region of its target genes. This stress activated expression of heat shock proteins (Hsps) and other chaperones helps to clear the cell of denatured proteins and evade proteotoxic stress. We developed a highly sensitive reporter with multimerized HSEs and luciferase as reporter gene to detect the activity of HSF1 under various conditions in cell culture cells. Kinetic studies of the activation levels of HSF1 revealed a strong correlation between severity of heat (temperature) and the duration of the impact (time of heat treatment)¹. Heat treatment is one way to reliably induce premature cellular senescence² and the role of Hsps in the establishment of senescence is not completely understood³. We try to correlate the activity of HSF1 after heat stress to the induction rate of cellular senescence. Therefore we use data from a HSE reporter assay and qPCR target gene analysis together with β -galactosidase activity and cell cycle analysis for the detection of cellular senescence after treatment of the cells at different temperatures and different recovery times.

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Pulmonary fibrosis and cellular senescence

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It was Élie Metchnikoff who first proposed that “aging is the replacement of noble elements by ignoble ones”, i.e., a progressive replacement of specialized cells by connective tissue/fibroblasts. Nowadays, tissue fibrosis is considered a major cause of progressive organ failure in aging and to be involved in numerous chronic age-related pathologies. The lungs are among the organs most susceptible to excessive fibroproliferative processes, with a strong predisposition to fibrosis with advancing age. One of the most aggressive and enigmatic manifestations of dysbalanced fibroproliferative repair is Idiopathic Pulmonary Fibrosis (IPF), an age-related disease with unknown etiology and no effective treatment.

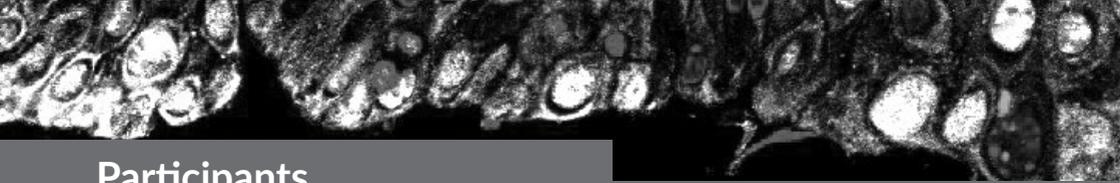
We found that primary cultures of fibroblasts derived from lung biopsies of IPF patients exhibited (i) accelerated replicative cellular senescence (CS); (ii) high resistance to oxidative-stress-induced cytotoxicity or CS; (iii) a CS-like morphology even at the proliferative phase; and (iv) a rapid accumulation of senescent cells expressing the myofibroblast marker α -SMA. Our findings suggest that CS could serve as a bridge connecting lung aging and pulmonary fibrosis, and be an important player in the disease progression. Consequently, targeting senescent cells has the potential to be a promising therapeutic approach.

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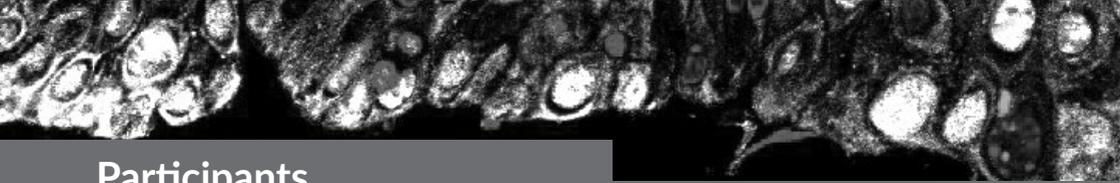


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