Cellular Senescence in the Lung

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Contributor: Diana Klein

Cellular senescence is a key process in physiological dysfunction developing upon aging or following diverse stressors including ionizing radiation. It describes the state of a permanent cell cycle arrest, in which proliferating cells become resistant to growth-stimulating factors. Senescent cells differ from quiescent cells, which can re-enter the cell cycle and from finally differentiated cells: morphological and metabolic changes, restructuring of chromatin, changes in gene expressions and the appropriation of an inflammation-promoting phenotype, called the senescence-associated secretory phenotype (SASP), characterize cellular senescence. The biological role of senescence is complex, since both protective and harmful effects have been described for senescent cells. While initially described as a mechanism to avoid malignant transformation of damaged cells, senescence can even contribute to many age-related diseases, including cancer, tissue degeneration, and inflammatory diseases, particularly when senescent cells persist in damaged tissues. Due to overwhelming evidence about the important contribution of cellular senescence to the pathogenesis of different lung diseases, specific targeting of senescent cells or of pathology-promoting SASP factors as potential therapeutic approach has been suggested. In this review, we summarize recent advances regarding the role of cellular (fibroblastic, endothelial, and epithelial) senescence in lung pathologies, with a focus on radiation-induced senescence. Among the different cells here, a central role of epithelial senescence is suggested.

Keywords: Senescence-associated secretory phenotype ; SASP ; lung injury ; pulmonary disease ; radiotherapy ; ionizing radiation ; cancer therapy ; pulmonary fibrosis ; lung

1. Introduction

Cellular senescence and in particular the ‘cellular senescence phenotype’ was initially discovered by Leonard Hayflick in 1961, who observed that the number of cell divisions in fibroblasts was limited and these normal, non-transformed cells reached the end of their replicative life span upon prolonged culturing, because the telomeres had reached a critical length [1][2]. In contrast to embryonic cells and stem cells, normal human cells divide approximately 52 times (the so-called Hayflick limit) before cell aging finally begins by entering a state of permanent growth arrest. Herein, the gradual shortening of telomeres (30–200 bp with each mitotic event) is thought to manifest an increased incidence of double-strand breaks (DSBs) at the DNA ends (known as ‘the end replication problem’) [3][4]. DSB (and even DNA single-strand breaks) in turn trigger the activation of DNA damage response (DDR) pathways, finally leading to the activation of the downstream kinases CHK2 and CHK1 through the protein kinases Ataxia-telangiectasia-mutated (ATM) and Ataxia-telangiectasia and Rad3-related (ATR), respectively [5]. The resulting cell cycle arrest allows for effective DNA repair, and thus for the resumption of normal cell functioning [5]. Increased DNA damage and/or inefficient damage removal then results in chronic DDR signaling that can foster apoptotic cell death or a stable cell cycle arrest—cellular senescence [5][6]. This senescence-associated cell cycle arrest (mostly G1) depends on the activation of the cyclin-dependent kinase (CDK) inhibitors p21/WAF1 and p16/INK4A, the decisive components of tumor-suppressor pathways that are governed by the p53 and retinoblastoma (Rb) proteins, respectively [7][8].

The cellular senescence phenotype bears morphological as well as characteristic gene expression alterations. Senescent cells have an enlarged, flattened and irregular shape bearing more vacuoles, an increase in senescence-associated β-galactosidase (SA-β-gal) activity (due to more and bigger lysosomes), and (partially) an altered chromatin organization known as senescence-associated heterochromatin foci (SAHF) [9]. The stable and irreversible form of cell cycle arrest is due to accumulation of the p16, p15/INK4b, p27/Kip1 and p21 CDK inhibitors, while the cell’s metabolic activity is maintained. Furthermore, tumor suppressor proteins, such as phosphatase PTEN (phosphatase tensin homolog), p53 or hypo-phosphorylated Rb, can be used to detect cellular senescence. Even the absence of markers can be used, including the absence of the proliferation marker Ki-67 or the lack of bromodeoxyuridine (BrdU) incorporation. Thus, there are reliable methods to specifically detect senescent cells, but due to the lack of a ‘master senescent cell marker’, usually a combination of markers is necessary to detect cellular senescence in vitro and in vivo in isolated tissues of interest (Table 1). Of note, most of these tools lack the capability of real-time imaging of senescence particularly in living subjects [10].

This is a critical issue with respect to practical applications such as image-guided surgical removal of senescent cells, as well as monitoring senescence during different pathologies [11][12][13]. Non-invasive biomarkers for cellular senescence could include the senescence-associated secretory phenotype (SASP), that involves the production of secretory growth factors and cytokines, reinforce the senescence arrest, and alter the cell's microenvironment, e.g., changes in extracellular matrix composition and the immune environment [9][14][15][16]. Interleukin-6 (IL-6) and IL-8 for example are key SASP factors boosting the senescence growth arrest by acting in an autocrine feedback loop. Further examples of well-known SASP factors are: the CC-chemokine ligand 2 (CCL2), a monocyte chemotactic protein with even angiogenic potential, as well as factors like transforming growth factor beta (TGFβ), a multifunctional and pro-fibrotic cytokine, plasminogen activator inhibitor-1 (PAI-1), also known as endothelial plasminogen activator inhibitor (or serpin E), and insulin-like growth factor 1 (IGF-1), which plays an important role in childhood growth, and has anabolic effects in adults [17][18][19][20][21]. Upon secretion from senescent cells, these SASP factors usually act in a paracrine manner to stimulate proliferation and/or transformation of adjacent immortalized cells, or even might trigger the senescence of other cells in the microenvironment.

Table 1. Summary of methods for the detection of senescent cells.

Target	Marker	Method of Detection
Lysosomes	SA-β-gal	Histochemical detection of β-galactosidase activity at pH 6 [9][20][22][23][24][25][26][27][28][29]
		Fluorogenic probes (e.g., C12FDG) [30][31]
		Near-infrared molecular probe (in vivo and in vitro) [32]
		Two-photon fluorescent probe (in vivo and in vitro) [33]
	Lipofuscin	Lysosomal aggregates stained with Sudan Black B [34]
Cell cycle inhibitors	$p16^{INK4a}$, $p21^{Cip/Waf1}$, $p15^{INK4b}$, p27	Western blot [9][18][19][20][23][24][29][35]
		RT-PCR [20][35][36][37]
		Immunofluorescence [35][38]
		Immunohistochemistry [29][39]
		Western Blot [40]
Cell proliferation	Ki-67 (absence)	RT-PCR [40]
		Immunofluorescence [38]
	BrdU incorporation (absence)	Immunofluorescence [18]
	Telomere shortening	FISH [41][42]
SASP factors	Cytokines (e.g., IL-6, TNFα) Chemokines (e.g., IL-8, MIPs, CCLs) Proteases (e.g., MMPs) Candidates: TGFβ, GM-CSF, PAI-1, IGF-1	Immunofluorescence [19][20]
		RT-PCR [9][20][25][26][37][38][43]
		Western Blot [9][19][20]
Tumor suppressors	pPTEN, p53, hypo-phosphorylated Rb, FOXO4	Western blot [9][18][24]
		RT-PCR [36]
		Immunofluorescence [6]
Chromatin organization	SAHF	Immunofluorescence [9]
	NFκB p65 subunit	Western Blot [9]
		RT-PCR [9]
	reorganization of DNA structure by DAPI, antibodies against facultative heterochromatin	Immunofluorescence [44]

Target	Marker	Method of Detection
DNA damage marker	γH2AX	Western blot [45] Immunofluorescence [27][28]

It is thought that cellular senescence contributes to developmental processes including promoting remodeling, inflammation, infectious susceptibility, and angiogenesis as well as fundamental processes, such as wound healing and tissue regeneration. Herein, senescent cells which fulfilled their action are removed from the interfered tissue via infiltrating immune cells. However, if senescent cells persist, these cells might foster age- and disease-associated physiological dysfunction particularly through their progressively changing secretory profile [46].

With this respect, cellular senescence is now considered an important driving force for the development of chronic lung pathologies, particularly chronic inflammation observed in lungs of aging patients and of patients suffering from asthma, chronic obstructive pulmonary diseaseor pulmonary fibrosis. The accumulation of senescent cells in lungs has disadvantageous consequences [47][48]. Understanding the mechanisms driving induction of cellular senescence as well as the mechanisms mediating pathology-promoting effects of senescence may offer new treatment strategies for chronic lung diseases. In this review, we summarize recent findings about the different senescent lung cells with respect to their potential contribution to inflammation and remodeling/fibrosis, and with a special focus on the contribution to radiation-induced pneumopathy.

2. Radiation-Induced Cellular Senescence in Lungs

Radiotherapy is used in more than half of all cancer patients, both for curative and palliative purposes [49]. Although modern and precise radiotherapy techniques substantially improved the delivery of energy (summarized as ionizing radiation) in the form of electromagnetic waves (gamma- or X-rays) or particles (neutrons, beta or alpha) used for cancer eradication, damage healthy cells and can lead to severe early and late complications in the tumor microenvironment with an increased risk of morbidity in patients after radiotherapy (RT) [50]. Radiation-induced lung disease (inflammation and fibrosis) is a major hurdle in the successful treatment of thorax-associated tumors [51][52]. Radiation-induced pulmonary fibrosis affects up to 25% of cancer patients receiving radiotherapy to tumors of the thoracic region [53]. The radiosensitivity of lung tissue is also dose-limiting when the whole body is irradiated prior bone marrow transplantation [54] [55]. The mechanism of radiation-induced normal tissue damage, however, is not fully understood; no causal strategy for the prevention or treatment of radiation-induced damage to the lungs is available so far [56][57]. Highly conformal radiation techniques such as stereotactic body radiation therapy (SBRT) or intensity-modulated radiotherapy (IMRT) are suited to minimize the irradiated lung volume. For example, SBRT is applied to patients with early stage inoperable non-small cell lung cancer [58]. Through image-guided precise targeting of very small volumes, relatively high dose-per-fraction sizes were delivered to the tumors. However, persisting adverse effects such as chest wall pain, rib fracture, esophagitis, brachial plexopathy, and in particular pneumonitis and fibrosis were reported [58][59]. In contrast to conventional dose rates (1–4 Gy/min), the so called ‘‘Flash” radiotherapy (> 40 Gy/s; Flash-RT) was shown to enhance the differential effect between normal tissue and tumor in lung models [60]. Herein, it was hypothesized that the protective effect of Flash irradiation was related to the high dose rate delivery. Indeed, Flash irradiation was shown to minimize persistent DNA damage, to reduce the inflammatory response and to facilitate radiation recovery [61].

Cellular stress, and in particular DNA-damaging drugs and ionizing radiation, can induce senescence in most lung cell types. Persistence of senescent cells in turn remains a major problem in certain lung diseases as these senescent cells or more precisely their altered secretory profile, the so-called SASP, might foster lung injury. However, the precise role of each senescent cell type within lung (radiation) injury remains elusive because it depends on the pathological trigger (e.g., dose and fractionation for RT) and the temporality of the observation [22].

3. Senescence of Lung Epithelial Cells

Repetitive injury, especially to the pulmonary epithelium, is considered a central factor in the development of various lung diseases. Herein, the senescence of the respiratory epithelium either of the ciliated pseudostratified columnar epithelium, the cuboidal epithelium or the squamous epithelium in the alveolar ducts and alveoli is regarded as a central process for the initiation and progression of related lung diseases, particularly in pulmonary fibrosis and experimental lung fibrosis models [10][20][35]. Human lung tissues from lung fibrosis (IPF) patients were shown to harbor numerous senescent epithelial cells as revealed by prominent SA-β-gal and p16 staining [35]. IPF related epithelial senescence was closely

associated with the SASP factors IL-1β, IL-6, IL-8 and TNF-α, which were already correlated with pulmonary fibrogenesis [62]. Of note, using an in vitro model of (bleomycin) stress-induced epithelial cell senescence, senescent lung epithelial cells-derived SASP factors were able to mediate the activation of pulmonary fibroblasts [35]. Therefore, the current hypothesis is that alveolar epithelial injury imposed on senescent epithelial cells leads to aberrant wound healing and the secretion of high levels of growth factors and chemokines that foster the activation of adjacent cells, including endothelial cells and fibroblasts, and the deposition of the extreacellular matrix [63]. Among the 'activating' epithelial-derived SAPS factors, increased levels of MMP12, SERPINE1, SPP1, and fibrotic mediator Wnt-inducible signaling protein (WISP) 1 were determined [64]. Moreover, pharmacological clearance of senescent lung epithelial cells by the induction of apoptosis in fibrotic alveolar (type II) epithelial cells or ex vivo three-dimensional lung tissue cultures (using dasatinib and quercetin) reduced SASP factors and extracellular matrix markers (e.g., collagen1a1, collagen5a3 and fibronectin) clearly indicating that senolytic drugs may be a viable therapeutic option for IPF [64].

In a preclinical model of radiation-induced pneumopathy, clearance of senescent cells with a senolytic drug (ABT-263) efficiently reduced senescent cells and reversed pulmonary fibrosis [26]. This, of course, would even limit the diminishing epithelial regenerative capacity, as well as associated SASP-mediated effects on adjacent lung cells as a central aspect in the development of lung injury. Therefore, targeting particularly senescent lung epithelial cells was suggested as a promising option for pulmonary fibrosis. Furthermore, radiation-induced senescence of lung epithelial cells was also closely connected to radiation-induced vascular dysfunction and associated extravasation of pre-metastatic immune and circulating tumor cells in a mouse model of radiation-induced pneumopathy [20][65]. Adoptive transfer of mesenchymal stem cells during the early phase after irradiation efficiently counteracted epithelial senescence (as well as vascular dysfunction) [21][65]. RT-induced senescence of bronchial-alveolar epithelial cells was further accompanied by the up-regulation of the SASP factor CCL2 [65]. Importantly, abrogation of certain aspects of the secretome of senescent lung cells, in particular signaling inhibition of the SASP factor CCL2, secreted predominantly by RT-induced senescent epithelial cells, limited inflammation as well as fibrosis progression [20]. This radioprotective action by addressing or modulating the SASP phenotype or senescent lung cells can have important implications in oncology, because higher doses of radiation might improve both local tumor control and survival. Moreover, treatment of thoracically irradiated mice with ABT-263 almost completely reversed pulmonary fibrosis, even when the initiation of ABT-263 treatment was delayed until fibrosis was established [26]. This means that unlike other known radiation protectants and mitigators, which were usually needed to be applied before or shortly after RT, senolytic drugs such as ABT-263 have the potential to be used as an effective, novel treatment of radiation-induced side complications such as inflammation and fibrosis, even after the lung injury develops into a progressive disease [53].

Although studies in animal models and patient samples show a complex response with multiple interactions between resident epithelial cells, fibroblasts and endothelial cells, and, in addition, infiltrating immune cells to radiation-induced diseased lung states, senescent epithelial cells and accompanied SASP contribute to the alteration and 'activation' of the lung microenvironment. Persistent or irreparable DNA damage following XRT in lung epithelial cells in turn can induce an irreversible cell cycle arrest that lead to apoptosis or to the establishment of cellular senescence. Radiation-induced epithelial senescence leads to increased SASP factor production. Very close by, the hitherto quiescent healthy endothelium that usually provides an efficient barrier to liquids or cell extravasation becomes activated and/or "angiogenic" phenotype (acute effect) in response to certain SASP factors. Increased endothelial permeability associated with increased leakage of blood stream components into the lung interstitium then fosters inflammation and/or metastasis formation. Normal fibroblasts will also be activated by SASP factors potentially resulting in a phenotypic change into pro-fibrotic myofibroblasts and/or cancer-associated fibroblasts that foster tissue remodeling by extracellular matrix deposition and thus fibrosis progression.

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References

1. L. Hayflick; P.S. Moorhead; The serial cultivation of human diploid cell strains. *Experimental Cell Research* **1961**, *25*, 585-621, 10.1016/0014-4827(61)90192-6.
2. L. Hayflick; The limited in vitro lifetime of human diploid cell strains. *Experimental Cell Research* **1965**, *37*, 614-636, 10.1016/0014-4827(65)90211-9.
3. Victoria Lundblad; The end replication problem: more than one solution.. *Nature Medicine* **1997**, *3*, 1198-1199, 10.1038/nm1197-1198.

4. Jonathan A. D. Wattis; Qi Qi; Helen Byrne; Mathematical modelling of telomere length dynamics.. *Journal of Mathematical Biology* **2019**, *80*, 1039-1076, 10.1007/s00285-019-01448-y.

5. Stephen Jackson; Jiri Bartek; The DNA-damage response in human biology and disease. *Nature* **2009**, *461*, 1071-8, 10.1038/nature08467.

6. Brian Kennedy; Shih-Yin Tsai; Baar Mp; Brandt Rmc; Putavet Da; Klein Jdd; Derks Kwj; Bourgeois Brm; Stryeck S; Rijksen Y; et al. Faculty Opinions recommendation of Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging.. *Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature* **2017**, *169*, , 10.3410/f.727432103.793536572.

7. Judith Campisi; Fabrizio Daddadifagagna; Cellular senescence: when bad things happen to good cells. *Nature Reviews Molecular Cell Biology* **2007**, *8*, 729-740, 10.1038/nrm2233.

8. Masashi Narita; Sabrina Nũnez; Edith Heard; Masako Narita; Athena W. Lin; Stephen A. Hearn; David L. Spector; Gregory J. Hannon; Scott W. Lowe; Rb-Mediated Heterochromatin Formation and Silencing of E2F Target Genes during Cellular Senescence. *Cell* **2003**, *113*, 703-716, 10.1016/s0092-8674(03)00401-x.

9. Chien, Y.; Scuoppo, C.; Wang, X.; Fang, X.; Balgley, B.; Bolden, J.E.; Premsrirut, P.; Luo, W.; Chicas, A.; Lee, C.S.; et al. Control of the senescence-associated secretory phenotype by NF-kappaB promotes senescence and enhances chemosensitivity. Genes Dev. 2011, 25, 2125–2136.

10. Bennett G Childs; Matej Durik; Darren J Baker; Jan Van Deursen; Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nature Medicine* **2015**, *21*, 1424-1435, 10.1038/nm.4000.

11. Ying Wang; Jun Liu; Xiaowei Ma; Chao Cui; Philip R. Deenik; Paul K. P. Henderson; Ashton L. Sigler; Lina Cui; Real-time imaging of senescence in tumors with DNA damage. *Scientific Reports* **2019**, *9*, 2102, 10.1038/s41598-019-38511-z.

12. Beatriz Lozano-Torres; Irene Galiana; Miguel Rovira; Eva Garrido; Selim Chaib; Andrea Bernardos; Daniel Muñoz-Espín; Manuel Serrano; Ramón Martínez-Máñez; Félix Sancenón; et al. An OFF–ON Two-Photon Fluorescent Probe for Tracking Cell Senescence in Vivo. *Journal of the American Chemical Society* **2017**, *139*, 8808-8811, 10.1021/jacs.7b04985.

13. De Keizer, P.L. The Fountain of Youth by Targeting Senescent Cells? Trends Mol. Med. 2017, 23, 6–17.

14. Suresh Rattan Is Peter Jorgensen; Peter Jorgensen And Suresh Rattan Is; Extracellular Matrix Modulates Morphology, Growth, Oxidative Stress Response and Functionality of Human Skin Fibroblasts during Aging In Vitro. *Journal of Aging Science* **2014**, *2*, , 10.4172/2329-8847.1000122.

15. Sarah Hibbert; Extracellular matrix is an influential force in ageing. *British Journal of Dermatology* **2017**, *177*, 1160-1161, 10.1111/bjd.15980.

16. Larissa G.P. Langhi Prata; Inna G. Ovsyannikova; Tamara Tchkonia; James L. Kirkland; Senescent cell clearance by the immune system: Emerging therapeutic opportunities. *Seminars in Immunology* **2018**, *40*, 101275-101275, 10.1016/j.smim.2019.04.003.

17. Sun, Y.; Coppe, J.P.; Lam, E.W. Cellular Senescence: The Sought or the Unwanted? Trends Mol. Med. 2018, 24, 871–885.

18. Francis Rodier; Jean-Philippe Coppe; Christopher K. Patil; Wieteke A. M. Hoeijmakers; Denise P. Muñoz; Saba R. Raza; Adam Freund; Eric Campeau; Albert R. Davalos; Judith Campisi; et al. Erratum: Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nature* **2009**, *11*, 1272-1272, 10.1038/ncb1009-1272a.

19. Jean-Philippe Coppé; Pierre-Yves Desprez; Ana Krtolica; Judith Campisi; The senescence-associated secretory phenotype: the dark side of tumor suppression.. *Annual Review of Pathology: Mechanisms of Disease* **2010**, *5*, 99-118, 10.1146/annurev-pathol-121808-102144.

20. Alina Wiesemann; Julia Ketteler; Alexis Slama; Florian Wirsdörfer; Thomas Hager; Katharina Röck; Daniel R. Engel; Jens W. Fischer; Clemens Aigner; Verena Jendrossek; et al. Inhibition of Radiation-Induced Ccl2 Signaling Protects Lungs from Vascular Dysfunction and Endothelial Cell Loss. *Antioxidants & Redox Signaling* **2019**, *30*, 213-231, 10.1089/ars.2017.7458.

21. Diana Klein; Jennifer Steens; Alina Wiesemann; Florian Schulz; Farnusch Kaschani; Katharina Röck; Masahiro Yamaguchi; Florian Wirsdörfer; Markus Kaiser; Jens W. Fischer; et al. Mesenchymal Stem Cell Therapy Protects Lungs from Radiation-Induced Endothelial Cell Loss by Restoring Superoxide Dismutase 1 Expression.. *Antioxidants & Redox Signaling* **2016**, *26*, 563-582, 10.1089/ars.2016.6748.

22. Nunzia Caporarello; Jeffrey A Meridew; Dakota L Jones; Qi Tan; Andrew J Haak; Kyoung M Choi; Logan J Manlove; Y S Prakash; Daniel J Tschumperlin; Giovanni Ligresti; et al. PGC1α repression in IPF fibroblasts drives a pathologic metabolic, secretory and fibrogenic state. *Thorax* **2019**, *74*, 749-760, 10.1136/thoraxjnl-2019-213064.

23. Shruthi Hamsanathan; Jonathan K Alder; Jacobo Sellares; Mauricio Rojas; Aditi U. Gurkar; Ana L. Mora; Cellular Senescence: The Trojan Horse in Chronic Lung Diseases.. *American Journal of Respiratory Cell and Molecular Biology* **2019**, *61*, 21-30, 10.1165/rcmb.2018-0410TR.

24. Peter J. Barnes; Jonathan Baker; Louise E. Donnelly; Cellular Senescence as a Mechanism and Target in Chronic Lung Diseases.. *American Journal of Respiratory and Critical Care Medicine* **2019**, *200*, 556-564, 10.1164/rccm.201810-1975TR.

25. O Toussaint; Véronique Royer; Michel Salmon; José Remacle; Stress-induced premature senescence and tissue ageing.. *Biochemical Pharmacology* **2002**, *64*, 1007-1009, 10.1016/s0006-2952(02)01170-x.

26. Florence Debacq-Chainiaux; Emmanuelle Boilan; Jérémie Dedessus Le Moutier; Geoffroy Weemaels; Olivier Toussaint; p38(MAPK) in the senescence of human and murine fibroblasts.. *Advances in Experimental Medicine and Biology* **2010**, *694*, 126-137, 10.1007/978-1-4419-7002-2_10.

27. Dawne N. Shelton; Edwin Chang; Peter S. Whittier; Nghee Choi; Walter D. Funk; Microarray analysis of replicative senescence.. *Current Biology* **1999**, *9*, 939-945, 10.1016/s0960-9822(99)80420-5.

28. Patrick Dumont; Maggi Burton; Q M Chen; Efstathios S Gonos; Christophe Frippiat; Jean-Baptiste Mazarati; François Eliaers; José Remacle; O Toussaint; Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast.. *Free Radical Biology and Medicine* **2000**, *28*, 361-373, 10.1016/s0891-5849(99)00249-x.

29. Jean-François Dierick; François Eliaers; José Remacle; Martine Raes; Stephen J. Fey; Peter Mose Larsen; O Toussaint; Stress-induced premature senescence and replicative senescence are different phenotypes, proteomic evidence.. *Biochemical Pharmacology* **2002**, *64*, 1011-1017, 10.1016/s0006-2952(02)01171-1.

30. Efstathios S Gonos; Anastasia Derventzi; Marie Kveiborg; Georgia Agiostratidou; Moustapha Kassem; Brian F.C Clark; Parmjit S. Jat; Suresh Rattan; Cloning and Identification of Genes That Associate with Mammalian Replicative Senescence. *Experimental Cell Research* **1998**, *240*, 66-74, 10.1006/excr.1998.3948.

31. Thierry Pascal; Florence Debacq-Chainiaux; Aline Chrétien; Coralie Bastin; Anne-France Dabée; Vincent Bertholet; Jose Remacle; Olivier Toussaint; Comparison of replicative senescence and stress-induced premature senescence combining differential display and low-density DNA arrays. *FEBS Letters* **2005**, *579*, 3651-3659, 10.1016/j.febslet.2005.05.056.

32. Kamil C. Kural; Neetu Tandon; Mikhail Skoblov; Olga V. Kel-Margoulis; Ancha Baranova; Pathways of aging: comparative analysis of gene signatures in replicative senescence and stress induced premature senescence. *BMC Genomics* **2016**, *17*, 1030-224, 10.1186/s12864-016-3352-4.

33. Pavan Parikh; Sarah Wicher; Karl Khandalavala; Christina M. Pabelick; Rodney D. Britt; Y. S. Prakash; Cellular senescence in the lung across the age spectrum. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **2019**, *316*, L826-L842, 10.1152/ajplung.00424.2018.

34. Scott L. Weinrich; Ron Pruzan; Libin Ma; Michel Ouellette; Valeric M. Tesmer; Shawn E. Holt; Andrea G. Bodnar; Serge Lichtsteiner; Nam W. Kim; James B. Trager; et al. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nature Genetics* **1997**, *17*, 498-502, 10.1038/ng1297-498.

35. Linlin Sun; Jeffrey Y. Chiang; Ji Young Choi; Zheng-Mei Xiong; Xiaojing Mao; Francis S Collins; Richard J. Hodes; Kan Cao; Transient induction of telomerase expression mediates senescence and reduces tumorigenesis in primary fibroblasts. *Proceedings of the National Academy of Sciences* **2019**, *116*, 18983-18993, 10.1073/pnas.1907199116.

36. Mary Y. Armanios; Julian J.-L. Chen; Joy Cogan; Roxann G. Ingersoll; Cheryl Markin; Mingyi Xie; Irma Vulto; Carol W. Greider; James Loyd; Jonathan K Alder; et al. Telomerase Mutations in Families with Idiopathic Pulmonary Fibrosis. *New England Journal of Medicine* **2007**, *356*, 1317-1326, 10.1056/nejmoa066157.

37. Alberto Diaz De Leon; Jennifer T. Cronkhite; Anna-Luise A. Katzenstein; J. David Godwin; Ganesh Raghu; Craig S. Glazer; Randall L. Rosenblatt; Carlos E. Girod; Edward R. Garrity; Chao Xing; et al. Telomere Lengths, Pulmonary Fibrosis and Telomerase (TERT) Mutations. *PLOS ONE* **2010**, *5*, e10680, 10.1371/journal.pone.0010680.

38. Allison R. Tsang; Haley D.M. Wyatt; Nicholas S.Y. Ting; Tara Beattie; hTERT mutations associated with idiopathic pulmonary fibrosis affect telomerase activity, telomere length, and cell growth by distinct mechanisms. *Aging Cell* **2012**, *11*, 482-490, 10.1111/j.1474-9726.2012.00810.x.

39. Juan Manuel Povedano; Paula Martinez; Rosa Serrano; Agueda M Tejera; Gonzalo Gómez-López; Miriam Bobadilla; J. M. Flores; Fatima Bosch; Maria A. Blasco; Therapeutic effects of telomerase in mice with pulmonary fibrosis induced by damage to the lungs and short telomeres. *eLife* **2018**, *7*, , 10.7554/eLife.31299.

40. Tianju Liu; Francina Gonzalez De Los Santos; Yuyue Zhao; Zhe Wu; Andrew E. Rinke; Kevin K. Kim; Sem H. Phan; Telomerase reverse transcriptase ameliorates lung fibrosis by protecting alveolar epithelial cells against senescence.. *Journal of Biological Chemistry* **2019**, *294*, 8861-8871, 10.1074/jbc.RA118.006615.

41. Jin Young Sung; Ji-Hyun Bae; Jong-Ha Lee; Yoon-Nyun Kim; Dae-Kwang Kim; The Melatonin Signaling Pathway in a Long-Term Memory In Vitro Study. *Molecules* **2018**, *23*, 737, 10.3390/molecules23040737.

42. Leila T Tchelebi; Nicholas G Zaorsky; Jennifer C Rosenberg; Navesh K Sharma; Leonard C Tuanquin; Heath B Mackley; Rodney J Ellis; Reducing the Toxicity of Radiotherapy for Pancreatic Cancer With Magnetic Resonance-guided Radiotherapy. *Toxicological Sciences* **2020**, *175*, 19-23, 10.1093/toxsci/kfaa021.

43. Hoang Quy Nguyen; Nhu Hanh To; Patricia Zadigue; Stéphane Kerbrat; Alexandre De La Taille; Sabine Le Gouvello; Yazid Belkacemi; Ionizing radiation-induced cellular senescence promotes tissue fibrosis after radiotherapy. A review. *Critical Reviews in Oncology/Hematology* **2018**, *129*, 13-26, 10.1016/j.critrevonc.2018.06.012.

44. Matthias Guckenberger; Kurt Baier; Bülent Polat; Anne Richter; Thomas Krieger; Juergen Wilbert; Gerd Mueller; Michael Flentje; Dose–response relationship for radiation-induced pneumonitis after pulmonary stereotactic body radiotherapy. *Radiotherapy and Oncology* **2010**, *97*, 65-70, 10.1016/j.radonc.2010.04.027.

45. Gerben R. Borst; Masayori Ishikawa; Jasper Nijkamp; Michael Hauptmann; Hiroki Shirato; Rikiya Onimaru; Michel M. Van Den Heuvel; José S.A. Belderbos; Joos V. Lebesque; Jan-Jakob Sonke; et al. Radiation pneumonitis in patients treated for malignant pulmonary lesions with hypofractionated radiation therapy. *Radiotherapy and Oncology* **2009**, *91*, 307-313, 10.1016/j.radonc.2009.02.003.

46. Yonghan He; Dinesh Thummuri; Guangrong Zheng; Paul Okunieff; Deborah E. Citrin; Zeljko Vujaskovic; Daohong Zhou; Cellular senescence and radiation-induced pulmonary fibrosis.. *Translational Research* **2019**, *209*, 14-21, 10.1016/j.trsl.2019.03.006.

47. Chris R. Kelsey; Mitchell E. Horwitz; Junzo Chino; Oana Craciunescu; Beverly Steffey; R J Folz; Nelson J. Chao; David A. Rizzieri; Lawrence B. Marks; Christopher Kelsey; et al. Severe Pulmonary Toxicity After Myeloablative Conditioning Using Total Body Irradiation: An Assessment of Risk Factors. *International Journal of Radiation Oncology*Biology*Physics* **2011**, *81*, 812-818, 10.1016/j.ijrobp.2010.06.058.

48. Julian D. Down; Jacquelyn C. Yanch; Identifying the High Radiosensitivity of the Lungs of C57L Mice in a Model of Total-Body Irradiation and Bone Marrow Transplantation. *Radiation Research* **2010**, *174*, 258-263, 10.1667/rr2149.1.

49. Jing, H.; Lee, S. NF-kappaB in cellular senescence and cancer treatment. Mol. Cells 2014, 37, 189–195.

50. Sonia G. Rao; James G. Jackson; SASP: Tumor Suppressor or Promoter? Yes!. *Trends in Cancer* **2016**, *2*, 676-687, 10.1016/j.trecan.2016.10.001.

51. Zhanhuai Wang; Yang Tang; Yinuo Tan; Qichun Wei; Wei Yu; Cancer-associated fibroblasts in radiotherapy: challenges and new opportunities.. *Cell Communication and Signaling* **2019**, *17*, 47, 10.1186/s12964-019-0362-2.

52. Alexander Aliper; Marine E. Bozdaganyan; Philipp S. Orekhov; Alex Zhavoronkov; Andreyan N. Osipov; Replicative and radiation-induced aging: a comparison of gene expression profiles.. *Aging* **2019**, *11*, 2378-2387, 10.18632/aging.101921.

53. Guanghong Jia; Annayya R. Aroor; Cassie Jia; James R. Sowers; Endothelial cell senescence in aging-related vascular dysfunction.. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **2018**, *1865*, 1802-1809, 10.1016/j.bbadis.2018.08.008.

54. Matthew J. Rossman; Rachelle Elizabeth Kaplon; Sierra D. Hill; Molly N. McNamara; Jessica R. Santos-Parker; Gary L. Pierce; Uglas R. Seals; Anthony John Donato; Endothelial cell senescence with aging in healthy humans: prevention by habitual exercise and relation to vascular endothelial function. *American Journal of Physiology-Heart and Circulatory Physiology* **2017**, *313*, H890-H895, 10.1152/ajpheart.00416.2017.

55. Bjorn Baselet; Pierre Sonveaux; Sarah Baatout; An Aerts; Pathological effects of ionizing radiation: endothelial activation and dysfunction. *Cellular and Molecular Life Sciences* **2018**, *76*, 699-728, 10.1007/s00018-018-2956-z.

56. Carolyn M. Roos; Bin Zhang; Allyson Palmer; Mikolaj Ogrodnik; Tamar Pirtskhalava; Nassir M. Thalji; Michael Hagler; Diana Jurk; Leslie A. Smith; Grace Casaclang-Verzosa; et al. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell* **2016**, *15*, 973-977, 10.1111/acel.12458.

57. Khan, S.Y.; Awad, E.M.; Oszwald, A.; Mayr, M.; Yin, X.; Waltenberger, B.; Stuppner, H.; Lipovac, M.; Uhrin, P.; Breuss, J.M. Premature senescence of endothelial cells upon chronic exposure to TNFalpha can be prevented by N-acetyl cysteine and plumericin. Sci. Rep. 2017, 7, 39501.

58. Audrey LaFargue; Charlotte Degorre; Isabelle Corre; Marie-Clotilde Alves-Guerra; Marie-Hélène Gaugler; François Vallette; Claire Pecqueur; Francois Paris; Ionizing radiation induces long-term senescence in endothelial cells through mitochondrial respiratory complex II dysfunction and superoxide generation. *Free Radical Biology and Medicine* **2017**, *108*, 750-759, 10.1016/j.freeradbiomed.2017.04.019.

59. Sae Aratani; Masako Tagawa; Shinya Nagasaka; Yukinao Sakai; Akira Shimizu; Shuichi Tsuruoka; Radiation-induced premature cellular senescence involved in glomerular diseases in rats. *Scientific Reports* **2018**, *8*, 16812, 10.1038/s415

98-018-34893-8.

60. Diana Klein; The Tumor Vascular Endothelium as Decision Maker in Cancer Therapy. *Frontiers in Oncology* **2018**, *8*, , 10.3389/fonc.2018.00367.
61. Hellweg, C.E. The Nuclear Factor kappaB pathway: A link to the immune system in the radiation response. Cancer Lett. 2015, 368, 275–289.
62. R B Effros; Telomerase induction in T cells: A cure for aging and disease?. *Experimental Gerontology* **2006**, *42*, 416-20, 10.1016/j.exger.2006.11.005.
63. Tareq Saleh; Liliya Tyutynuk-Massey; Emmanuel K. Cudjoe Jr.; Michael O. Idowu; Joseph W. Landry; David A. Gewirtz; Non-Cell Autonomous Effects of the Senescence-Associated Secretory Phenotype in Cancer Therapy. *Frontiers in Oncology* **2018**, *8*, , 10.3389/fonc.2018.00164.
64. Nathan Basisty; Abhijit Kale; Ok Hee Jeon; Chisaka Kuehnemann; Therese Payne; Chirag Rao; Anja Holtz; Samah Shah; Vagisha Sharma; Luigi Ferrucci; et al. A Proteomic Atlas of Senescence-Associated Secretomes for Aging Biomarker Development. **2019**, , 604306, 10.1101/604306.
65. Christopher D. Wiley; Alexis N. Brumwell; Sonnet S. Davis; Julia R. Jackson; Alexis Valdovinos; Cheresa Calhoun; Fatouma Alimirah; Carlos A. Castellanos; Richard Ruan; Ying Wei; et al. Secretion of leukotrienes by senescent lung fibroblasts promotes pulmonary fibrosis. *JCI Insight* **2019**, *4*, , 10.1172/jci.insight.130056.
