Immunotherapy and Senolytics: Alleviating radiation-induced senescence

Hendrik Veltman

S4590791

Prof. Dr. R.P. Coppes

Rijksuniversiteit Groningen (RUG)

Bachelor of Biology (Pre-master)

Abstract

According to data published in 2018 by the World Health Organisation (WHO), cancer is the second major cause of death globally. Treatment of malignant tumours often involves radiotherapy, sometimes in combination with chemotherapy and/or surgery. Radiotherapy however, leads to DNA damage and stress resulting in the emergence of senescent cells in the tumour and the surrounding healthy tissue. Cellular senescence is a state in which the cell undergoes permanent cell cycle arrest. The senescence-associated secretory phenotype (SASP) contributes to induction of more senescent cells which in term may lead to many diseases a few of which are fibrosis, neurodegeneration, osteoarthritis and atherosclerosis. Furthermore, senescence in the tumour can lead to transformed radio-resistant cells which sporadically exit this cell-cycle arrest, leading to recurrence. For these reasons, it is important to obtain a better understanding of the molecular mechanisms of senescence and the way in which immunotherapy and senotherapy may aid in eliminating senescent cells thus alleviating degenerative diseases and minimizing recurrence rates of cancer. Upon cellular stress, senescence is induced via the p53 and retinoblastoma protein pathways leading to oxidative stress and the activation of anti-apoptotic pathways. Senescence surveillance is primarily initiated by the innate immune system involving NK cells, neutrophils and macrophages. However, $CD4^+$ T cells take part as well using APCs. Although senescent cells can evoke an immune response, in some occasions the SASP can create an immunosuppressive micro-environment mediated by IL-1 and IL-6 leading to the inhibition of NK cell function. Recent studies have identified a number of different strategies in targeting senescent cells. Senolytic drugs primarily focus on inhibiting anti-apoptotic pathways. Furthermore, immunotherapy is used to activate NK cells selectively targeting senescent cells using surface markers. Another approach is to design chimeric antigen receptors (CAR) T cells. Although these novel strategies hold great potential. However, most senolytic drugs are still in clinical trial and some have unwanted off-target effects. Furthermore, the use of CAR T cells as well as antibody-dependent cytotoxicity (ADCC) are still in its infancy. Therefore, a lot more research has to be conducted in order to alleviate radiation-induced senescence.

Introduction

According to data published in 2018 by the World Health Organisation (WHO), cancer is the second major cause of death globally. Roughly 70% of cancer deaths occur in second and third world countries. [1] In addition, in developed countries, cancer is the primary cause of death, due to a number of aspects including a decrease in cardiovascular diseases, better access to health care and thus an ageing population. Treatment of malignant tumours often involves radiotherapy, sometimes in combination with chemotherapy and/or surgery. [2] Recent developments have resulted in new techniques such as stereotactic body radiotherapy (SBRT) with which it is possible to deliver higher radiation doses to the tumours with increased accuracy and less damage to surrounding healthy tissue. In Berg et al. a look was taken at patients with non-small-cell lung cancer (NSCLC) which is often inoperable due to poor lung function as a result of COPD and life-long smoking. Large cohorts of patients suffering from NSCLC treated with SBRT had higher survival rates compared to patients treated with 3D-conformal radiotherapy. In this study, healthier, often younger, people underwent surgical removement whereas older people with poor lung function received SBRT. Here, no difference in survival rate was observed between the two groups meaning that SBRT, after adjusting for group-differences, was approximately as effective as surgery. However, recurrency after treatment with SBRT is still high. [3] Recent studies have suggested that one of the reasons for this higher recurrency rate can be attributed to an increase of senescent cells emerging after radiotherapy.

Cellular senescence is a state in which the cell undergoes permanent cell cycle arrest. Interestingly however, senescent cells still maintain their vital metabolic processes. [4] The senescence secretome, often referred to as the senescence-associated secretory phenotype (SASP) contributes to induction of more senescent cells which in term may lead to many diseases a few of which are fibrosis, neurodegeneration, osteoarthritis and atherosclerosis. [5] The SAPS is accountable for paracrine signalling using growth factors, cytokines and chemokines and it can have a pernicious effect on its surrounding healthy tissue. Senescence can be induced by a number of stress factors including telomere dysfunction, oxidative damage and DNA damage due to carcinogenic agents and irradiation. [4,5] Through a phenomenon that is called the non-targeted bystander effect, cells that are not radiated can still indirectly be affected. Radiation induces this non-targeted bystander effect of senescent cells through a protein called 5'-adenosine monophosphate-activated protein kinase- (AMPK) and Nuclear Factor B (NF-kB)-dependent bystander signalling pathways. Resulting in the emergence of more senescent cells from previously healthy tissue surrounding the tumour. Both AMPK and NF-kB play an important role in cellular senescence. AMPK inhibits cell growth and proliferation, and its expression level rises when energetic stress occurs. NF-kB is a group of transcription factors contributing to a whole range of processes including inflammatory response, oxidative stress response, differentiation and apoptosis. [4]

Senescent cells can, in some contexts, be beneficial, although this possibly depends on how long the cells are preserved within the tissue and the way in which senescence is induced. Previous studies have shown that senescence can induce expression of tumour suppressor molecules thus preventing unregulated proliferation of damaged cells. Ritschka et al. described how exposure of healthy liver cells in vivo to signalling molecules that are secreted by senescent cells promoted regeneration and induced cell plasticity, both important in tissue regeneration and wound healing. However, cells started to inhibit these regenerative signals, switching to paracrine signalling, when they were exposed for longer periods of time to SASP molecules. It was proposed that senescent cells can prevent proliferation whilst also activating the immune system to promote elimination of these damaged cells. Furthermore, the SASP induces regeneration and plasticity in surrounding cells. Taken together this mechanism would further encourage tissue regeneration. [6] Since senescence inhibits uncontrolled proliferation, bypassing senescence may in fact result in more aggressive forms of cancer. This idea has been elucidated in Achuthan et al., where it was argued that chemotherapy-induced senescent cells occasionally exit the senescent arrest giving rise to a cancer subpopulation exhibiting stem cell-like properties with an increased resistance to antitumour agents. This might be due to epigenetic

alterations caused by stress-induced mutagenesis. However, the exact underlying mechanisms for this phenomenon are unclear. This conveys that the inability for permanent cell cycle arrest can be detrimental to tumour elimination and promotes recurrence. [7]

In recent years, researchers have been focussing on a number of different approaches to try and alleviate this problem by eliminating senescent cells or inhibiting SASP secretion, to avoid recurrence of cancer and the development of degenerative diseased emerging from previously healthy tissue surrounding the tumour. These approaches are thought to be of additional value by mitigating the negative side effects of senescence induced by conventional treatments with radio- and chemotherapy. One of these fields which is currently in its infancy is called senotherapy. This type of therapy focusses on a group of drugs called senolytics which selectively target and kill senescent cells. Senostatic drugs on the other hand inhibit the function of senescent cells, primarily by affecting the SASP. However, senostatic drugs have not been studied as extensively as senolytic drugs. [8] In addition to senotherapy, several immunotherapy strategies have been proposed. One of these strategies is chimeric antigen receptor (CAR) therapy, which focusses on receptor editing of T cells in order to recognise and target specific ligands on the cell surface of senescent cells. Another approach makes use of an immune mechanism called antibody-dependent cell-mediated cytotoxicity (ADCC). In ADCC, antibodies designed to target cell surface markers of senescent cells are capable of activating natural killer (NK) cells to induce clearance. A third technique to effectuate clearance of senescent cells is to isolate senescence specific antigens (SSA) and present them to antigen presenting dendritic cells. However, there are currently no universal senescence specific antigens identified to create a senescence vaccine. [9] Rejuvenation of senescent cells may also be a future solution to the problem. Attempting to reprogram senescent cells rather than clearing them could be a potential therapy. There has already some promising data been published in Latorre et al. in which a look was taken at altering the spliceosome using small molecules to rescue senescent cells. This research was based on the claim that the expression of splicing factors is dysregulated in several different lineages of senescent cells. This method may aid in alleviating primary fibrosis and reverse degenerative phenotypes. [10]

All things considered; radiation-induced senescence can be initially beneficial. However, when these growth arrested cells evade the immune system or escape the senescent cell state, it may lead to recurrence of tumour growth or the development of degenerative diseases in surrounding tissue. Therefore, the goal of this review is to investigate what molecular mechanisms play a role in radiation-induced senescence and how current advancements in immunotherapy and senotherapy in the future may aid in alleviating both emergence of degenerative disease and recurrence of cancer.

Molecular mechanisms of radiation-induced senescence

It is important to have a clear oversight of the molecular mechanisms behind radiation-induced senescence in order to analyse new approaches to reduce senescence and thereby mitigating harmful side effects. Studies have shown that radiotherapy increases the risk of recurrence of tumour growth due to the emergence of senescent cells. [8] It is pivotal to address this problem considering the fact that roughly half of cancer patients will receive radiotherapy during their treatment. [11]

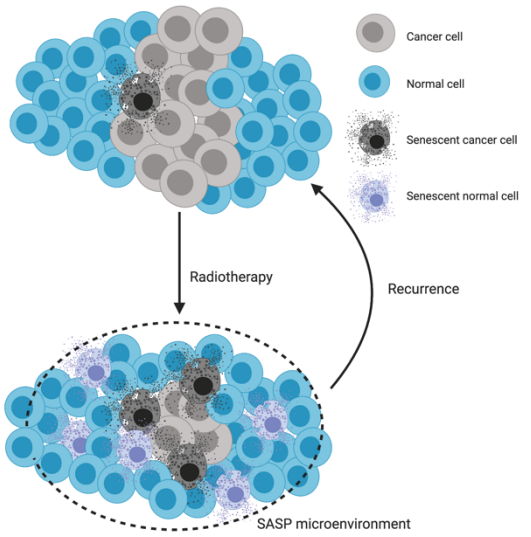

Fig 1. *Radiotherapy leads to the emergence of senescent cancer cells (Black) and in its surrounding tissue to senescent normal cells (Purple). Therefore, increased recurrence due to the SASP microenvironment is seen in patients treated with radiotherapy. Furthermore, emergence of senescent cells in surrounding healthy tissue occurs leading to diseases like pulmonary fibrosis.*

Radiotherapy leads to DNA damage to irradiated cells which will then initiate the DNA damage response (DDR). If the cell cannot ensure the integrity of the DNA it will initiate apoptosis. However, when the DNA is not damaged enough to undergo apoptosis, cells may initiate senescence. This leads to a group of transformed senescent cells that are radio resistant. Occasionally these cells escape the permanent cell-cycle arrest evolving into so-called stem cell-like cancer cells and as a consequence may lead to a more aggressive recurrence of the tumour *(figure 1)*. Furthermore, radiotherapy and the SASP also evoke an immune response in healthy non-cancer cells surrounding the tumour. This type of radiation-induced senescence has also been linked to fibrosis which can be deadly by itself. [8] Senescence is primarily effectuated by the p53 pathway which shows increased levels of activity upon induction of senescence. [2] The p53 protein plays a crucial role in cellular proliferation and cell cycle regulation. After the cell is exposed to radiation, activity of a transcription factor called TBX2 is decreased. This will lead to a rise of p14ARF expression which will result in the inhibition of mouse double minute 2 homolog (MDM2), thereby hindering ubiquitination of p53. [2,12] Furthermore, the formation of a complex called promyelocytic leukaemia protein acetyltransferase complex (PML) is also able to increase p53 activity. This increased p53 activity leads to the transcription of a whole range of downstream targets resulting in cell cycle arrest and senescence. Other signals aid in this process as well including reactive oxygen species (ROS), cyclin-dependent kinase inhibitor p21 and Bruton's tyrosine kinase. The latter is responsible for increasing the stability of p53 thereby aiding in its function.

In addition to p53, another pathway closely interacting with p53 called the retinoblastoma protein (pRB) pathway assists in cell cycle arrest. The RB protein by itself causes cell-cycle arrest and inhibits MDM2 activity thereby also stimulating p53 activity. Due to responses to stress p16 activity is increased inhibiting cell-cycle regulators called cyclin-dependent kinases (CDKs). Under normal circumstances, these CDKs are responsible for inhibiting RB protein. Furthermore, the activation of p21, due to increased p53 activity, also results in inhibition of CDKs. Therefore, both pathways interact at different levels and inactivation of either pathway may inhibit induction of senescence *(figure 2)*. [2]

In another study, the metabolic processes of radiation induced senescence were investigated. It was previously ascertained that an MDA-MB-231-2A breast cancer cell line lacked sufficient securin expression and initiated senescence upon irradiation. A securin-knockdown possibly inhibits apoptosis thereby creating a bias for senescence. The data showed that irradiated cells had elevated glycolysis activity compared to MDA-MB-231 irradiated cells that were not deficient for securin. This was assessed by measurement of the enzyme called glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using a 2D gel electrophoresis. GAPDH is responsible for producing energy and is a crucial protein in glycolysis. The extracellular lactate concentration were also measured, which again confirmed their hypothesis

that increased glycolysis induces senescence. To ensure that this was not a cell line specific phenomenon, another human breast cancer cell line MCF-7 was included. This cell line also had low securin levels compared to the MDA-MB-231 cells and showed increased extracellular lactate levels upon irradiation. Finally, the MDA-MB-231-2A cancer cell line was treated with a glycolytic inhibitor to determine whether senescence was induced by an increase in glycolytic activity. Cells were exposed to dichloroacetate (DCA) before irradiation which resulted in a reduced level of radiation-induced senescence, again confirming the hypothesis. Thr-172 phosphorylation analysis showed that the energy sensor AMPK was responsible for the enhanced glycolysis. In addition to AMPK, NF-kB activity increased as well. NF-kB is known to be activated and enhanced by glycolysis. Both AMPK and NF-kB play an important role in cellular senescence. AMPK inhibits cell growth and proliferation, and its expression level rises when energetic stress occurs. NF-kB is a group of transcription factors contributing to a whole range of processes including inflammatory response, oxidative stress response, differentiation and apoptosis *(figure 3)*.[4] Taken together this data shows a complex mechanism of different transcription factors and cell-cycle regulators all contributing to the senescence phenotype and leading to the bystander effect through the increase of ROS. However, the P53 and pRB pathways appear not to be redundant and inhibiting either one of them may lead to apoptosis instead of senescence.

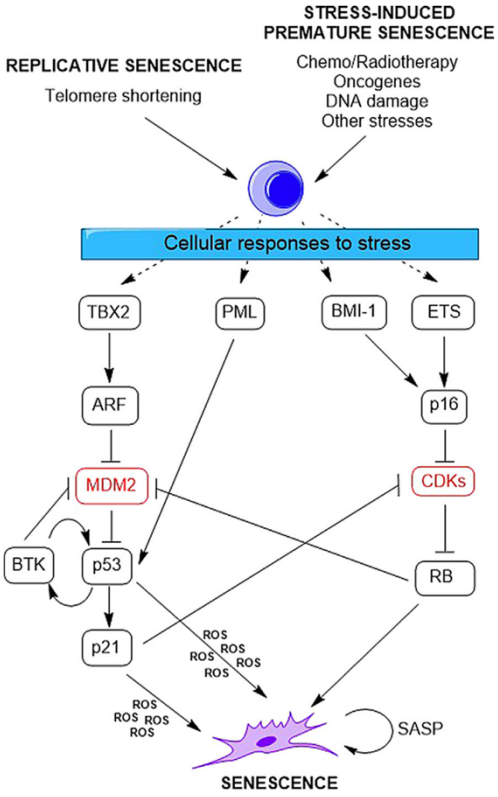

Fig 2. *Summary of the main pathways involved in senescence.* (Derived from A.F.S. Tabasso et al., 2019)

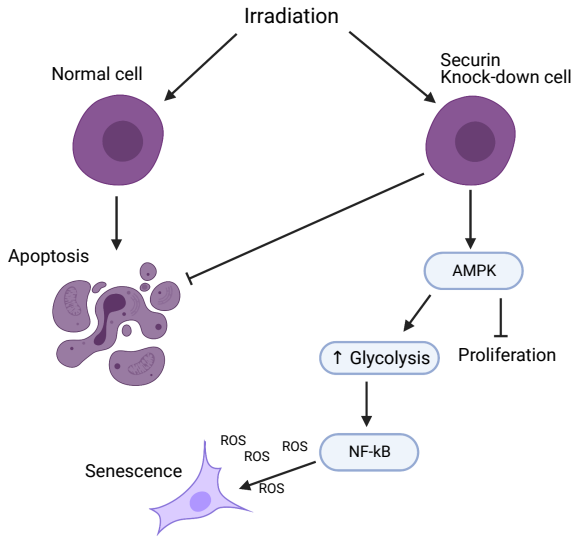

Fig 3. *Role of securin in radiation-induced senescence. Insufficient securin prevents the cell from initiating apoptosis. Upon irradiation, AMPK expression increases, inhibiting proliferation and leading to an increase in glycolysis. NF-kB is then activated resulting in oxidative stress and senescence.*

Innate and adaptive immune responses to senescent cells

As described previously, senescent cells secrete proinflammatory cytokines, chemokines and growth factors responsible for immune clearance and initiating tissue repair systems. Initiating senescence by reactivating p53 in mouse liver carcinomas using RNA interference was associated with increased activity of the innate immune responses including NK cells, neutrophils and macrophages. [13] This was further investigated in Sagiv et al., where it was proposed that the NKG2D ligands MICA and ULBP2 were upregulated in senescent cells. These ligands are essential for NK cell activation and clearance of the senescent cells. Interestingly, the molecular mechanisms of this upregulation were attributed to the DDR, which is known to play a crucial role in inducing senescence by controlling the SASP phenotype. Furthermore, activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway was also involved in elevated expression of the MICA and ULBP2 ligands. It was suggested that ERK1/2 may be responsible for stabilizing mRNAs coding for NKG2D ligands by downregulating microRNAs. [14]
In addition to NK cell activity, other studies have shown that the recruitment of macrophages in mice is also important for clearance of senescent cells, possibly due to the SASP. [15] In another study, conducted in mice, clearance of senescent hepatocytes was mediated by antigen specific $CD4^+$ T cells. However, $CD4^+$ T cell activation depended on the presences of antigen presenting cells (APCs). MHC class II expression on the senescent hepatocytes cell surface alone was insufficient for $CD4^+$ T cell proliferation. Suggesting that the innate immune system is crucial in immune surveillance of senescent cells. [16] In another study, oncogene-induced senescence in primary human melanocytes resulted in upregulated MHC class II expression induced via an autocrine loop of the SASP. These cells were able to evoke an adaptive immune response through activation of $CD4^+$ T cells in vitro. Furthermore, in vivo, these senescent melanocytes migrated into the local lymph nodes, suggesting that these cells exhibit an antigen-presenting role. [17]
All things considered; it appears that the innate immune system plays a key role in senescence surveillance. However, the adaptive immune system takes part in this process as well although it is dependent on APCs for senescence surveillance. It can be said that more research is needed to accurately assess the adaptive immune response to senescent cells.

Senescent cells defy the immune system

Albeit data has been established showing how immune surveillance of senescent cells can have a positive effect on tissue resurgence, other research proposed that it can suppress the immune system and even promote tumour growth. In Ruhland et al., a look was taken at the mechanism behind this immunosuppressive pro-tumorigenic microenvironment using an immune-competent mouse model. This mouse model made it possible to control senescence activation in mesenchymal cells. It was discovered that the SASP factor Interleukine-6 (IL-6) was responsible for the perceived local immunosuppression, by inducing suppressive myeloid cells and thereby preventing $CD8^+$ T cell activation. This immunosuppressive phenomenon results in a tumour permissive microenvironment that is very different from the anti-tumorigenic characteristics some other senescent cells may have. It is believed that this primarily depends on the way in which the senescence is induced and the type of cell it concerns, since this affects the SASP. [18] In another study published in Cancer Cell, a look was taken at the immune responses to senescent hepatocytes and found similar immunosuppressive occurrences. It was proposed that the SASP of these senescent hepatocytes create a permissive environment for hepatocellular carcinomas. Two interleukins playing an important role in this process are IL-1 and IL-6, promoting immunosuppressive myeloid cells to accumulate which leads to the inhibition of NK cell function. As described previously, NK cells play an important role in senescence surveillance. Furthermore, a chemokine called CCL2 was identified as part of the SASP secreted by these senescent hepatocytes attracting immune cells that express a CCR2 receptor. However, some tumour cells secreted cytokines inhibiting maturation of monocytes into macrophages, which resulted in

unrestrained tumour growth. This study again shows the contradictory function of senescence and the SASP in different stages of tumour development. [19] Another study also based on the hypothesis that senescent cells can create an immunosuppressive pro-tumorigenic microenvironment looked at a naturally occurring senescent cell population called $p16^{Ink4a}$. This type of senescence is known to be induced by p16 and caused by age related changes. In Baker et al., apoptosis of these cells was induced in a mouse model by injection of a senolytic drug called AP20187. The clearance of $p16^{Ink4a}$ cells increased the lifespan of this mice significantly and decreased tumour development and cardiac aging. In addition, it also had beneficial effects on other age-related alterations including changes in fat and eye function. [20]
Taken together, these findings suggest that senescence can both have a beneficial and detrimental effect on the tissues microenvironment through promoting or inhibiting immune surveillance respectively. This again elucidates the importance of developing new techniques to target senescence as a means to prevent cancer development.

Senolytics

Persistent senescence can have detrimental effects on the tissue it resides in and increases the likelihood of cancer recurrence and the development of many diseases including fibrosis, neurodegeneration, osteoarthritis and atherosclerosis. [5] Therefore, researchers have been trying new approaches to tackle this problem. One way to achieve senescent cell clearance is through the use of senolytics. These are small molecules that induce apoptosis of senescent cells. Senescent cells can persist in tissue via activation of anti-apoptotic pathways. Senolytic drugs selectively target these pathways thereby inducing apoptosis. There is increasing evidence that administrating senolytic drugs in addition to more conventional cancer treatments including radiotherapy, can decrease the recurrence rate thus increasing survival prospects of patients with cancer. [2] In order for senolytic drugs to be effective it has to be selective in only killing senescent cells, but simultaneously is should have a broad spectrum, meaning that not only one particular senescent subpopulation is affected. One of these senolytic drugs that holds great potential is ABT263 also known as navitoclax. This agent inhibits anti-apoptotic proteins BCL-2 and BCL-xL thus selectively killing senescent cells. It has shown to be effective against radiation-induced senescence and suppressed the expression of a number of SASP factors. According to the data published in Chang et al., this novel potential drug can be orally administered and is an efficient senolytic agent against radiation-induced senescence. [21] However, according to a number of studies, its toxicity limited clinical studies. Navitoclax can be toxic, especially in patients with thrombocytopenia as it also targets platelets. In order to increase specificity, researchers have tried to combine it with galactose. As senescent cells have higher amounts of the galactosidase enzyme, this approach results in increased accumulation of navitoclax in these cells.
Two other senolytic drugs that have been shown to be successful in targeting senescent cells in vitro and in vivo mouse models are Dasatinib and Quercetin. Dasatinib is a tyrosine kinase inhibitor and Quercetin is a flavonoid responsible for the bitter taste in apple peals. These flavonoids showed to be less senolytic on their own and for that reason are used in combination. This is possibly due to redundancy of anti-apoptotic pathways. Another senolytic flavonoid drug is Fisetin. Flavonoids are chemicals present in numerous fruits and vegetables known for their antioxidant effects. Both Quercetin and Fisetin inhibit BCL-xL, HIF-1alpha and also likely target other anti-apoptotic pathways. Studies have shown that Dasatinib plus Quercetin reduced senescence significantly with high specificity in vivo. The drug combination has also been shown to reduce radiation-induced senescence that caused skin ulcerations in mice. However, although these results are promising, very limited research data is available on its efficacy in humans. [2,22] Fisetin has been shown to be approximately twice as potent as Quercetin. In vivo and in vitro studies have shown that it is involved in suppressing inflammation, the cell cycle, apoptosis, angiogenesis and metastasis. [2] Fisetin is already sold as a dietary supplement to enhance cognitive brain function at a dose of 100mg. In contrast, it is estimated that a regular diet

contains approximately 0.4 mg of Fisetin. A clinical trial, using a dose of 100mg for patients with colorectal cancer, showed positive effect although minimal. Therefore, an ongoing phase 2 randomized clinical trial is currently being conducted by the Mayo clinic in Rochester, Minnesota to look at the effects of Fisetin at a much higher dose (20mg/kg). [2,23]

Another drug that is used to treat diabetic patients is called metformin. This drug has been shown to be responsible for reducing the secretion of pro-inflammatory cytokines of senescent cells, thereby affecting the SASP. Drugs that do not explicitly eliminate senescent cells but only affect its secretion pattern are called senostatic drugs. It is believed that the decrease in the secretion of pro-inflammatory cytokines was accomplished by inhibiting the NF-kB transcription factors. As mentioned before, this group of transcription factors plays a key role in determining the SASP. Interestingly, their data showed that while pro-inflammatory cytokine secretion was reduced, anticancer cytokines appeared to be unaffected. [24]

There are currently a number of other potential senolytic and senostatic drugs being investigated. However, as of yet, none are approved for clinical use. [2] Therefore it can be said that more clinical trials are needed. Fortunately, some of these drugs are already sold as supplements or approved by the FDA as a treatment for other illnesses. This would allow for faster approvement if these drugs turn out to be of significant value in targeting senescent cells.

Immunotherapy: Eliminating senescent cells

Another approach, in addition to senolytic and senostatic therapy, is immunotherapy. There are a number of different approaches similar to immunotherapy in cancer treatment. The goal of these approaches is to improve the immunosurveillance of senescent cells. As mentioned previously, senescence surveillance by the immune system occurs through membrane receptors of NK cells, macrophages and $CD4^+$ T cells. [16,17,25] For this reason, one considered approach is to design antigen receptors to activate T cells specifically targeting the senescent cells. The so-called chimeric antigen receptor (CAR) T cells are already used in anti-cancer therapies with promising results. [25] These synthetic receptors consist of an extracellular single-chain variable fragment (scFv) responsible for recognizing the specific antigen of interest, a transmembrane domain and intracellular tyrosine-base activation motifs (ITAMs). The second and third generations of CARs also contain co-stimulatory domains mediating increased T cell persistence, cytotoxicity and cytokine secretion. Binding of these CARs to an antigen of interest leads to the activation of the CAR T cell and subsequent initiation of cytotoxicity through the release of perforin and granzyme granules and activation of death receptor signalling. Using this method, it is possible to precisely eliminate tumour cells utilizing the tumour-associated antigens (TAAs). [26] Recently, researchers have been focussing on using this strategy to specifically target senescent cells. In Amor et al., CAR T cells were tested that targeted senescent cell populations expressing the urokinase-type plasminogen activator receptor (uPAR) on its cell surface. [27] As of yet, there are no universal markers known that are solely expressed by senescent cells. [9] Therefore, a surface marker that is broadly expressed by senescent cells had to be identified first. To achieve this, three types of senescent cells were examined: therapy-induced senescence in mouse lung adenocarcinoma, oncogene-induced senescence in mouse hepatocytes and culture-induced senescence in mouse hepatic stellate cells. The uPAR receptor was found to be broadly expressed and highly specific for senescent cells. This receptor is involved in degradation of extracellular matrix, wound healing and tumorigenesis. Furthermore, it promotes persistence of tumour cells. After identification, an anti-mouse uPAR CAR T cell line was constructed. This cell line showed to be senolytic in vitro as well as in vivo against senescent lung adenocarcinomas and reduced liver fibrosis induced by senescence. [27]

Another approach that holds great promise is ADCC. This technique relies on inducing cytotoxicity in NK cells by utilizing antibodies that target specific cell surface markers in the senescent cell population. In Kim et al., a cell surface marker called dipeptidyl peptidase 4 (DPP4) also known as CD26 was

identified, which was expressed in senescent cells and not in proliferating cells. DPP4 is a protease that inhibits the hormones glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). These hormones also known as incretins are responsible for the quick release of insulin upon a sudden increase in blood-glucose levels therefore playing a crucial role in treatment of diabetes. However, it is not yet fully known what the significance of this information is due to the sudden increase in expression of DPP4 in senescent cells. It was hypothesised that DPP4 might play a role in the blood-glucose homeostasis that changes with age. After identification of this surface marker, an immunoassay was conducted. The ADCC assay revealed selective elimination of DPP4 positive senescent WI-38 fibroblasts. This showed that the DPP4 surface maker may be a promising new target for the ablation of senescent cells using ADCC.

In addition to CAR editing and ADCC, another strategy would be to rejuvenate the immune system to make it more vigorous in detecting senescent cells. [9] In older adults, NK and T cells appear to decrease in number and functionality due to the increase in AMPK activity. A study conducted in mice showed that a lack of cytotoxicity of NK and T cells further increased senescence. Therefore, rejuvenating NK and T cell cytotoxicity and inhibiting the AMPK pathway would be a potential strategy. [25]

All three strategies hold potential in eliminating senescent cells. However, CAR T cells are labour intensive and costly due to the fact that it has to be patient specific. ADCC on the other hand is not as costly or labour intensive, but a lack of research and identified surface markers makes it complicated to specifically target a senescent population. Therefore, the best aim would be to focus on identifying these surface markers in order to precisely target senescent cells without any off-target effects.

Conclusion

Cellular senescence can be induced in many different ways, one of which is radiotherapy during cancer treatment. Although senescence can be beneficial and anti-tumorigenic, if it persists it can in fact result in recurrence of even more aggressive malignancies or lead to diseases including, but not limited to fibrosis, neurodegeneration, osteoarthritis and atherosclerosis.

Persistent senescent cells use the SASP to create an immunosuppressive and tumour permissive microenvironment. For these reasons it is important to develop a better understanding of the molecular mechanisms behind radiation-induced senescence and to find new techniques to target these cell-cycle arrested populations to mitigate their harmful effects on its surround tissue.

One such approach to eliminate senescent cells is through the use of senolytic drugs. As of yet, no senolytic agents are used for clinical treatment. In addition, most of these agents either lack potency (Flavonoids), are unspecific, or lead to undesired side effects (Navitoclax). [22,27, 28] Therefore, more research needs to be conducted on this matter. Another strategy that already has been proven to hold great potential in the fight against cancer are CAR T cells. Repurposing CAR T cells to target senescent cells showed promising results (Amor et al., 2020). However, this approach would only be effective if it is personalized to the patient's condition. This would be a tedious process and highly expensive. Although some molecules have been identified, there is still a lack of universal markers. Utilizing NK cell cytotoxicity in ADCC on the other hand makes use of the patients own immune system without having to synthetically design antigen receptors. This approach appears to be less time consuming, although again a lack of specific surface markers makes it challenging to target very specific senescent cell populations. It should also be noted that, especially in patients treated with chemotherapy in addition to radiotherapy, are often immunocompromised, meaning that utilizing their innate immune system is not always a possibility.

All things considered, there is not one silver bullet tackling this problem. Senescent cells are characterized by the type of cell and the stress factors leading to induction. This has to be taken into account when looking for a clinical treatment. Most senolytic strategies are in its infancy and lack sufficient research. However, recent data shows that there are promising ways in which this problem can be alleviated in the future. For future references, it appears that identifying specific surface markers could hold the key to development of more efficient immunotherapies with less off-target effects. These surface markers could then be used for ADCC as well as designing more CARs. Furthermore, focussing on those senolytics that are already FDA approved for other diseases may help in a faster utilization of these agents.

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