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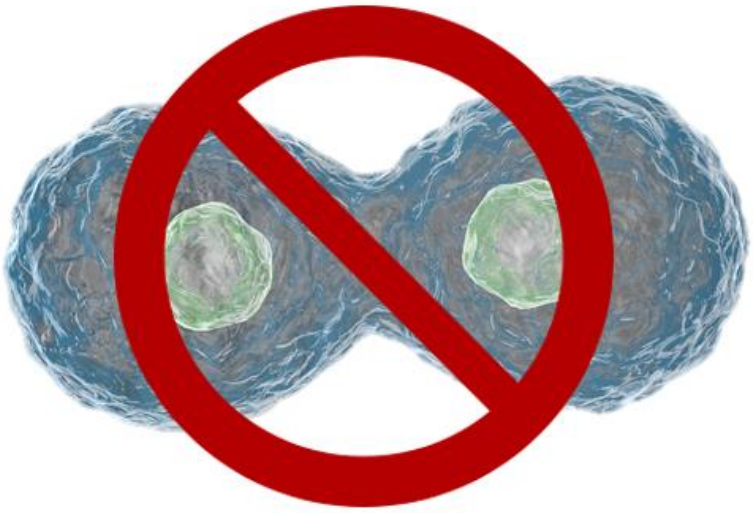

Senescence as cancer treatment

THE DOUBLE-EDGED SWORD OF CELLULAR SENESCENCE IN CANCER PREVENTION AND CANCER PROMOTION

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Abstract

Cellular senescence is seen as a mechanism that prevents cancer by irreversibly stopping the cell cycle when an oncogenic mutation is obtained or when the cell has exceeded a certain number of cell divisions. Numerous studies have suggested that this halt on proliferation induced by senescence might be exploitable for the treatment of cancer. However, inducing senescence in cancer cells also has its downsides since cancer cells might be able to escape senescence and because of the senescence associated secretory phenotype (SASP). Recent studies have shown that the SASP stimulates cancer development and causes age associated pathologies. In this review, I will first discuss the characteristic and triggers of senescence. Then we will look at how senescence can be induced and how to determine the effectiveness of the drugs in tumors. However, the SASP and genomic instability in senescent cells pose a risk for recurrence of cancer. Therefore, I will also discuss the potential of combining senescence induction with elimination of the senescent cells as cancer treatment.

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introduction

Cellular senescence

Cellular senescence is traditionally defined as an irreversible cell-cycle arrest. This phenomenon was first described by Moorehead and Hayflick, who observed that human fibroblasts in culture stopped proliferating after a finite number of cell divisions despite the presence of a mitogen (Hayflick et al. 1961). Although senescent cells have stopped replicating they are still viable and remain metabolically active (Campisi et al. 2005).

In vivo senescence is seen in human naevi (moles) (Michaloglou et al. 2005). These naevi are premalignant tumors of cutaneous melanocytes which only rarely progress into malignancy (Kuwata et al. 1993). Almost all naevi carry a mutation in the oncogenic B-Raf which could lead to uncontrolled cell proliferation (Michaloglou et al. 2005). However, because these cells have become senescent the proliferation is halted and the progression to malignancy has been averted for many decades (Bennett et al. 2003). This possible prevention of malignant tumor development gives us a potential function of senescence which is the prevention of cancer. Interestingly, this is strengthened by the observation that cells in lung adenomas, pancreatic intraductal neoplasias and prostate intraepithelial neoplasias, all premalignant tumors, turn out to be senescent, whereas in the malignant forms senescence was absent (Collado et al. 2010). This suggests that premalignancies have to overcome senescence to become cancerous.
Moreover, the limited number of cell divisions Hayflick and Moorhead observed could also serve as a protection against cancer. If a cell reaches around 50 division, which happens relatively fast in cancer cells, the proliferation will automatically stop by senescence induction and the cancer cell will thus stop proliferating making it harmless.

Senescence characteristics

Growth arrest is an important characteristic of senescence. However, quiescent cells are in the G_0-phase or resting phase of the cell cycle and are therefore also growth arrested, albeit temporalily, so there must be other factors that distinguish senescence from quiescence. One of these factors is the presence of senescence associated β-galactosidase activity (SA β-gal). The increase in SA β-gal results from the increased activity of GLB1 gene in senescent cells which encodes the lysosomal β-gal enzyme. This increase in SA β-gal is widely used as a senescence biomarker (Dimri et al. 1995). However, SA β-gal might not be the most precise biomarker as some cell types express SA β-gal but do not show any other factors associated with senescence (Piechota et al. 2016). It should therefore be considered to use multiple markers to assess senescence. So, for example, the activity of tumor suppressor proteins such as p53, p21, p27 or p16 could be used as senescence marker since their activity is needed for the induction of the cell cycle arrest (Gonzalez et al. 2016). Another important characteristic of senescence is the senescence associated secretory phenotype (SASP). The SASP results from an alteration in protein expression and protein secretion and consists of interleukins, inflammatory cytokines and growth factors affecting the surrounding tissue (Coppé et al. 2010).

Triggers of senescence

The arrest in cellular proliferation Hayflick and Moorehead described more than half a century ago is now known as the Hayflick limit. But what caused this arrest? As was later discovered, the ends of chromosomes are protected by a cap of a repetitive DNA sequence called telomeres. Each time a cell replicates these telomeres become shorter until a critical

point is reached where the chromosome ends resemble a double strand break. To prevent the fusion of uncapped chromosome ends and thus extensive DNA anomalies, senescence is induced. This form of senescence is called replicative senescence (d'Adda di Fagagna et al. 2003). The trigger for the induction of senescence is the unprotected chromosome ends which lead to a DNA damage response resulting in the activation of ATM/ATR and CHK1/CHK2 (fig. 1c). These proteins then lead to the activation of the tumor suppressor protein p53 which in turn activates a cyclin-dependent kinase (CDK) inhibitor leading to the activation of retinoblastoma protein (pRB). pRB induces elevated levels of reactive oxygen species (ROS). The elevated levels of ROS can cause DNA damage by oxidative stress which leads to a high frequency in mutations called genomic instability (Waris et al. 2006). However, ROS also has a function in inducing senescence by causing an activation of PKC-δ which eventually leads to the inhibition of DNA replication and cytokinesis and so makes a cell senescent (Ohtani et al. 2012).

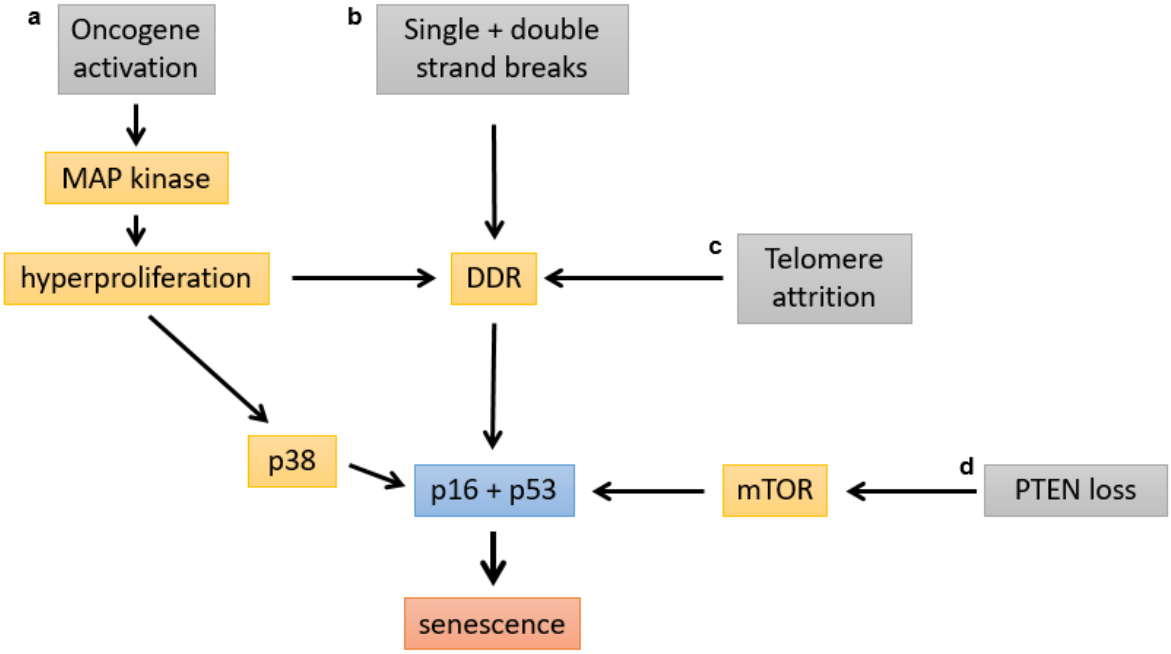

Figure 1. Pathways triggering senescence. *The activation of an oncogene, a high amount of single or double strand breaks, telomere attrition or loss of a tumor suppressor gene can all induce senescence via different pathways. However, senescence is always induced by the activation of p53 and/or p16. A) Oncogene activation leads to hyperproliferation which can lead to p53 and p16 activation via a stress response or via a DNA damage response (DDR). B) A high amount of single and double strand breaks leads to a DDR activating p53 and p16. C) Telomere attrition leads to uncapped chromosome ends causing a DDR again activating p16 and p53. D) Loss of PTEN leads to activation of mTOR causing an activation of p16 and p53.*

Another form of senescence is not caused by too many replications of the cell but by an unrepairable amount of DNA damage, which could lead to the development of cancer, and is therefore called stress induced premature senescence (SIPS) or accelerated senescence. There are three types of triggers in SIPS. The first is a direct reaction to an extensive amount of single and double strand breaks which again leads to the activation of the DDR (fig. 1b). The second trigger is the activation of an oncogene like RAS or B-Raf. The activation of these oncogenes hyperactivates the MAP kinase pathway, resulting in increased proliferation called hyperproliferation. The hyperproliferation activates a stress response via p38 which is able to activate p53 and p16 causing a cell cycle arrest (fig. 1a) (Di Micco et al. 2006). The third trigger is also caused by the activation of oncogenes. The hyperproliferation that results

from the oncogenes not only causes a stress response but also leads to the collapse of replication forks. These collapses then lead to a DDR and thus again induces a cell cycle arrest (fig. 1a).

The last form of senescence is characterized by the loss of the tumor suppressor gene PTEN, which again can lead to cancer. This form of senescence is called PTEN loss-induced cellular senescence (PICS) and not triggered by a stress response or DDR caused by hyperproliferation. Instead PICS is induced by the activation of mTOR, which leads to the activation of p16 and p53 thus inducing senescence (fig. 1d) (Alimonti et al. 2010).

In conclusion, the induction of senescence after oncogene activation, telomere attrition or DNA damage has a crucial role in preventing cancer progression. Furthermore, transgenic mice with an extra copy of an important senescence factor like p53 show an increased protection against cancer (Matheu et al. 2004). Moreover, once a cell does progress to a tumor the outcome of treatment with traditional chemotherapeutics is determined by the ability of the tumor to become senescent. Since mice with tumors that are capable of becoming senescent following chemotherapy show a much better survival than those with tumors that are not capable of becoming senescent (Schmitt et al. 2002). Taken together, these findings suggest an important role for senescence in cancer even after the formation of a tumor. The main question of this review is therefore: can senescence serve as a treatment for cancer?

Senescence as cancer treatment

Inducing senescence

The key principle behind using senescence as a cancer therapy is putting a halt on the uncontrolled proliferation of cancer cells. However, cancer cells have already bypassed the senescence induced by the oncogene activation. So how can these cells still become senescent? The theory behind this is a redundancy in the pathways regulating senescence which was also shown in figure 1 (Fridman et al. 2008). Because of this overlap between the pathways inducing senescence it becomes highly unlikely that all pathways are simultaneously mutated in cancer cells and thus senescence can still be induced. This hypothesis is strengthened by the observation that in cell lines where p53 activity, one of the important tumor suppressor genes, is comprised by the viral E6 are still sensitive to senescence induced by the chemotherapeutic doxorubicin indicating that other pathways besides p53 can induce senescence (Chang et al. 1999).

The usage of the chemotherapeutic doxorubicin by Chang et al. as a senescence inducer seems a bit peculiar since doxorubicin and other traditional chemotherapeutics but also irradiation are known to function via causing extensive DNA damage in rapidly dividing cells leading to cell death. However, it is observed that not all tumor cells become apoptotic but that a small part of the cells can become senescent (Jones et al. 2005). This might be due to the fact that not all cancer cells maintain the ability to become apoptotic but do remain sensitive to senescence. For instance, tumors where the apoptosis pathway is blocked by apoptosis blocker BCL2 can still become senescent via p53/p16 induction (Schmitt et al. 2002). The ratio between apoptosis or senescence induction can also be modulated by the amount of chemotherapeutics that is being administered. For example, a high dose of doxorubicin induces apoptosis in human cancer cells whereas a lower dose induces senescence (Ewald et al. 2009). The different response to a lower dose compared to a higher dose of doxorubicin might be due to less extensive DNA damage caused by the lower dosage which makes the cancer cells able to survive the amount damage albeit in a

senescent state. However, because chemotherapeutics target all rapidly dividing cells the side effects of traditional chemotherapy are severe and may also lead to permanent tissue damage (Lee et al. 2014). Even lower doses of these drugs might also cause a reaction in non-tumor tissue causing long-term tissue damage. It is therefore necessary to find more specific drugs that induce senescence by directly targeting one of the downstream effectors of senescence.

There are several downstream targets that can be exploited to induce senescence (fig. 2). Four of them will be discussed in this review. The first target is telomerase (fig. 2a). Most tumors acquire telomerase activity which prevents the attrition of the telomeres. This makes tumor cells able to proliferate beyond their expected normal lifespan. As described earlier uncapped chromosomes by loss of the telomeres lead to senescence induction. however, with telomerase activity the chromosomes remain capped preventing a replicative senescence response. Inhibition of telomerase could therefore be a target for senescence induction. Consistent with this theory is the reduced cancer susceptibility in telomerase deficient mice (Blasco. 2005). In humans, small molecule enzyme inhibitors can inhibit telomerase activity in tumor cells. Such a compound named imetelstat is already in phase II clinical trials. However, all patients had moderate to severe side effect making this therapy not much better than traditional chemotherapeutics (Salloum et al. 2016, Baerlocher, 2015).

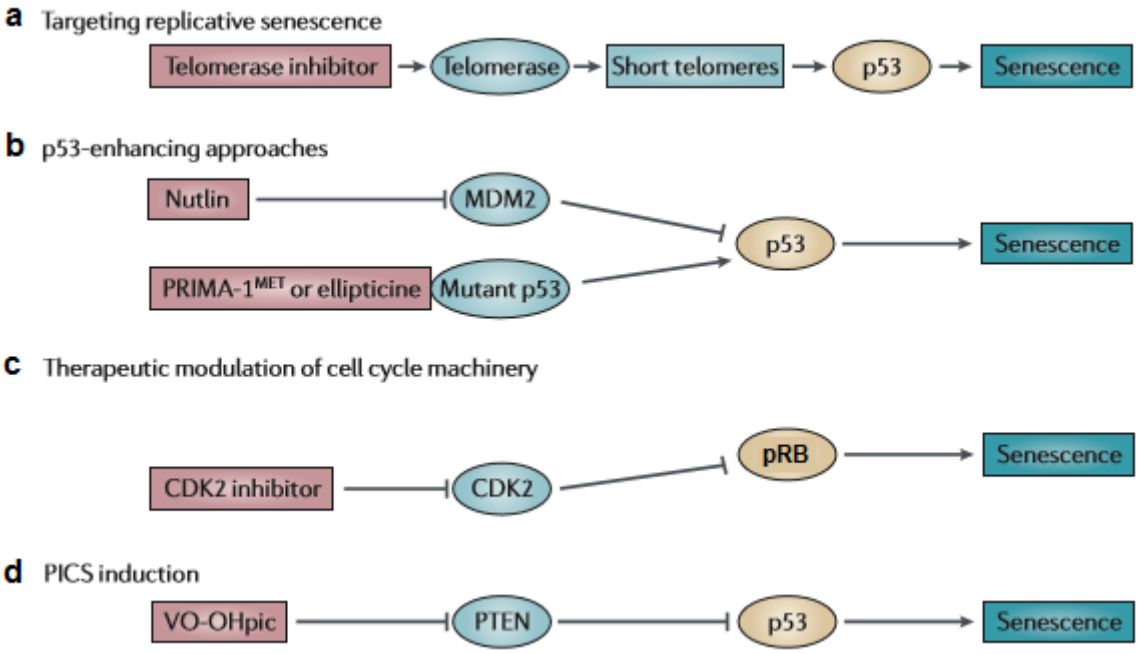

Figure 2. possible targets to induce senescence. *Overview of approaches that can induce senescence. Inhibitors are shown in red and targets are shown in blue. A) inhibiting telomerase leads to short telomeres which activates p53 via the DDR. B) enhancing p53 via either inhibiting MDM2 or via restoring mutant p53 activity. C) targeting the cell cycle machinery to activate pRB. D) inducing PICS via PTEN inhibition. (Figure adapted from Nardella et al. 2011)*

The second target is restoring p53 activity (fig. 2b). Since many tumors have already inactivated the p53 and p16 pathway to evade accelerated senescence by oncogene activation a potential way to induce senescence is reactivation of p53. In tumors that have wild type p53 activity left, MDM2 inhibition could be an effective target. MDM2 is an oncogene that can be upregulated in tumor cells, it then interacts with p53 leading to its inactivation. Therefore inhibiting MDM2, with for example nutlin, could lead to the reactivation of p53 and an induction of senescence (Hong et al. 2014). However, when p53 is mutated, there will only be more mutant p53 if you inhibit MDM2. Therefore, the restoration of wild type

p53 activity could be more effective. Mutant p53 is folded in a wrong conformation leading to the dysfunctionality of the protein. Consequently restoring the wild type function, with for example PRIMA-1, could give a normal p53 activity (Liang et al. 2009). Reactivation of p53 has been shown to be effective in mice. Ventura et al. made a Cre-loxP based mouse model so they could temporarily regulate the expression of the tumor suppressor gene p53. They found that endogenous expression of p53 lead to the induction of senescence in sarcomas without affecting the normal tissue (Ventura et al. 2007). This suggest that restoring p53 could effectively induce senescence.

The third target is the CDKs involved in senescence induction (fig. 2c). In normal cells when the DNA damage response is activated CDK2 and CDK4/6 will be inhibited by p16 or p21. However, in many cancer cells the p16/p21 pathway is compromised so the CDKs lose their brake which in turn leads to the uncontrolled progression of the cell cycle. So therapeutically blocking these CDKs should be a potential target in inducing senescence. Surprisingly, clinical trials where CDK inhibitors were used had limited success (Lapenna et al. 2009). However, when CDK inhibitors were used in a more specific genetic context the inhibitors turned out to be more effective. For example, a synthetic lethal interaction was observed in non-small cell lung carcinoma mouse model if the oncogene K-Ras was expressed in combination with a selective CDK4 inhibitor (PD0332991) (Puyol et al. 2010). This synthetic response that induced senescence was not seen in normal tissue indicating its specificity and so limiting the chance of side effects.

The fourth target is PTEN (fig. 2d). As described earlier loss of PTEN can induce senescence. So if a tumor cell has not already lost PTEN in its process of becoming a tumor cell, inhibiting PTEN could induce senescence. Although inhibiting a tumor suppressor might not seem the most wise thing to do in a cancer cell, proof of principle studies have shown the feasibility of this approach to induce senescence (Alimonti et al. 2010). A compound that can inhibit PTEN is VO-OHpic, which is a small molecule. By inhibiting PTEN the mTOR pathway is activated which activates p16 and p53 and induces senescence.

effectiveness therapy

Although multiple drugs have been shown that can induce senescence, it still has to be determined how effective these drugs are in individual patients. A very high efficiency is needed to ensure that all tumor cells become senescent. If this is not the case the remaining tumor cells could form a new tumor leading to recurrence of the tumor. To determine the effectiveness and prevent recurrence, good biomarkers for senescence in vivo are needed. As described previously SA β-gal is a commonly used marker for senescence. However, SA β-gal is not always exclusive to senescent cells and could therefore give the wrong impression that the tumor cells have become senescent when this might not be the case. To this date there are no robust senescence biomarkers, many of the in vivo markers have been shown to unreliable and to some degree nonspecific. Nevertheless, the use of multiple markers as confirmation of senescence in vivo is accepted (Baek et al. 2017). So, for example, the combination of SA β-gal staining with p16 levels is considered reliable. This combination could therefore serve as a reliable way to determine the effectiveness of the therapy in individual patients.

Senescence associated secretory phenotype

If there are compounds that are able to induce senescence effectively in the whole tumor of an individual then the tumor will remain in the patient's body for the rest of his life. This will make cancer a chronic disease. However, the diagnosis of cancer was probably made because the patient was suffering from the symptoms of the tumor and he or she will have to live with these symptoms for the rest of their life. Interestingly, the SASP secreted by the

senescent tumor cells might solve this problem on its one. The SASP has a proinflammatory nature that can attract immune cells to the site where the tumor originally was. These immune cells can then clear some of the senescent and the possible surviving tumor cells. Indeed, Kang et al. showed that immune surveillance initiated by the SASP is important to prevent the progression of pre-malignant hepatocytes to hepatocellular cancers. In addition, IL-6 and IL-8, important components of the SASP, are known to autonomously and non-autonomously reinforce senescence. Likewise, another SASP component named Insulin-like growth factor binding protein 7 (IGFBP7) can induce and reinforce senescence in melanocytes preventing the progression to malignancies (Wajapeyee et al. 2008).

However, in elderly people where the immune system is becoming less active the immune clearance of the senescent tumor cells will disappear (Denkinger et al. 2015). Therefore, the senescent tumor will remain in these patients after senescence induction. Elderly people in general already have an increase in senescence and they reveal the downside of senescence, the sustained chronic inflammation by the SASP proteins promote chronic inflammation and thereby give rise to diseases such as osteoarthritis, pulmonary fibrosis, Alzheimer's disease, and contradictory also cancer (Watanabe et al. 2017). This increased cancer risk may in part be the result of the effect of different components of the SASP. First, IL-6 and IL-8 that reinforce senesce and in that way have an anti-tumorigenic function can contradictory also work pro-tumorigenic via the activation of the Ras signaling pathway (Ancrile et al. 2007). This contradiction makes it difficult to predict the long-term outcome of senescence as therapy. Moreover, IL-6 and IL-8 are also able to promote the epithelial to mesenchymal transition in tissue surrounding the senescent cells (Laberge et al. 2012). In this process epithelial cells lose their polarity and obtain migratory and invasive properties promoting metastasis. Secondly, one of the proteins secreted by senescent cells is matrix metalloproteinase 3 (MMP3). This enzyme is capable of degrading the extracellular matrix and so promotes the migration of cancer cells (Liu et al. 2007). Finally, another SASP factor is vascular endothelial growth factor (VEGF). As a growth factor it can promote tumor progression by stimulating the proliferation of endothelial cells. Furthermore, VEGF can also increase vascularization and so supply the growing tumor with enough vessel formation, thus maintaining oxygen and nutrition supply to the tumor.

Genomic instability in senescent cells

Not only the SASP will increase the risk of the recurrence of cancer if not all the senescent cancer cells are cleared by the immune system. The senescent cells might not be genomically stable which is, as mentioned earlier, caused by elevated ROS that is needed to activate PKC-δ, an important component to stop the cell cycle. A positive feedback loop between PKC-δ and ROS even further elevates the levels of ROS in the senescent cell. Via oxidative DNA damage ROS can lead to a high frequency in mutation which is known as genomic instability (Waris et al. 2006). This genomic instability can lead to their escape from senescence and their continuation as the cancer cells they were before becoming senescent. The escape can be facilitated by mutations in key regulatory genes involved in the maintenance of senescence. An escape facilitated by a mutation in key regulatory gene was shown by Dirac et al. They have shown that in mouse embryonic fibroblasts (MEF) senescence can be escaped by suppressing p53 (Dirac et al. 2003). They developed a lentiviral vector that directs the expression of a p53-specific short hairpin which suppresses p53 through RNA interference. These MEF's then rapidly re-entered cell cycle and lost their senescence associated phenotype indicating the importance of functional p53 in maintaining the senescent cell cycle arrest. In addition, not only mutations can lead to the loss of senescence, when Restall et al. induced senescence by inhibiting HSP90 in small cell lung cancer cells the senescent phenotype disappeared upon removal of the inhibitor (Restall et al. 2010). The ability of a cancer cell to escape senescence might not be so surprising if the

consideration is made that all cancers have to escape senescence induced by the oncogene activation prior to becoming a cancer cell.

Senescence as cancer treatment?

Taken everything together it is doubtful that senescence induction on its own can serve as a successful cancer therapy. Especially in elderly people in whom the immune system becomes less and less active and thus immune clearance of the senescent cells is compromised, the remaining senescent cells pose a real treat in recurrence of cancer. This is because the senescence associated phenotype can also promote tumorigenesis and thus stimulate the development and migration of secondary tumors. Moreover, the presumed irreversible cell cycle arrest is probably more reversible than previously thought. This could have serious consequences if a senescent tumor cell loses its cell cycle arrest and continues to proliferate uncontrollably. Even in younger individuals where the immune system should be efficient enough to clear senescent cells the question remains whether a whole massive senescent tumor can be cleared by the immune system on its own. It might be the case that even in these patients senescent cells will remain thus also increasing the risks of recurrent cancer. So, for all patients, just making the tumor senescent will not be a reliable treatment.

Combining senescence induction with senolytics

Since the remaining senescent cells pose such a risk in recurrence for the patient a combination of senescence induction with a therapy that removes senescent cells may be an attractive alternative. Drugs that target senescent cells and induce cell death are called senolytics. The proof of principle that such drugs could potentially work was established in a genetically modified progeroid mouse model. These mice had a loss of function in the BubR1 gene. This gene is needed for the mitotic checkpoint ensuring an accurate chromosome separation. Without the function of this gene these mice have a shortened lifespan and show age-related phenotypes. Furthermore, they have an increase in senescent cells, making them a good model for testing the principle of senolytics. They carried a drug inducible suicide gene that upon induction selectively activated apoptosis in cells that were p16 positive. These cells were thus senescent so only senescent cells were effectively killed (Baker et al. 2011). Although genetic modification in humans is ethically not possible, this study did show the possibility to selectively kill senescent cells. Interestingly, other cellular differences between senescent cells and non-senescent cells have now become targets to selectively kill senescent cells (fig. 3).
For instance, the metabolic activity differs in senescent cells . Senescent cells upregulate their glucose intake and glucose conversion making them vulnerable to blocking glucose utilization which could be exploited by using small molecules (fig.3a) (Dörr et al. 2013). A second potential target can be the induction of apoptosis (fig. 3b). Senescent cells are surprisingly resistant to apoptosis comparable to cancer cells. They become so resistant by upregulating the anti-apoptosis genes and in that way increase their survival (Campisi et al. 2007). The anti-apoptosis genes that are upregulated are part of the Bcl-2 family and particularly consist of Bcl-W and Bcl-XL (Zhu et al. 2015). Factors that would normally induce apoptosis will now have no effect on the senescent cells because of the shifted balance between pro- and anti-apoptotic factors. So to overcome this shifted balance and thus induce apoptosis inhibiting members of the Bcl-2 family could be a potential target (Croce et al. 2016). A promising compound that inhibits the Bcl-2 family is a BH3 mimetic called ABT-737. In mice that suffered from irradiation induced lung damage, treatment with ABT-737 lead to an increase in caspase 3, a key apoptosis regulator, and a significant decrease of senescence in the damaged lungs (Croce et al. 2016). A different way to target the increased reluctance of senescent cells to go into apoptosis is the use of the already clinically used

drugs dasatinib and quercetin. Dasatinib is an inhibitor of tyrosine kinase which interferes with the of ENFB-dependent suppression of apoptosis. Quercetin is a flavonoid which is naturally present in some foods. It can induce apoptosis by inhibiting the pro-survival PI3 kinase. The combination of these drugs was shown to reduce senescence in the muscles of mice after irradiation (Zhu et al. 2015). A third strategy to eliminate senescent cells could be the use of oncolytic viruses like the measles vaccine virus (fig. 3c). This virus shows a better infection and lysis of senescent cancer cells than non-senescent cancer cells and can thus selectively kill senescent cells (Weiland et al. 2014). A fourth strategy was recently published by researchers of the Erasmus MC who identified FOXO4 as an important molecule in senescent cell viability (fig. 3d). They developed a peptide, FOXO4-DRI, that is able to interrupt the interaction of FOXO4 with p53 which in senescent cells leads to exclusion of p53 from the nucleus. This nuclear exclusion resulted in cell intrinsic apoptosis of senescent cells in aged mice (Baar et al. 2017). These diverse strategies show that selectively killing senescent cells is possible in mice. However, the translation to humans still has to be made.

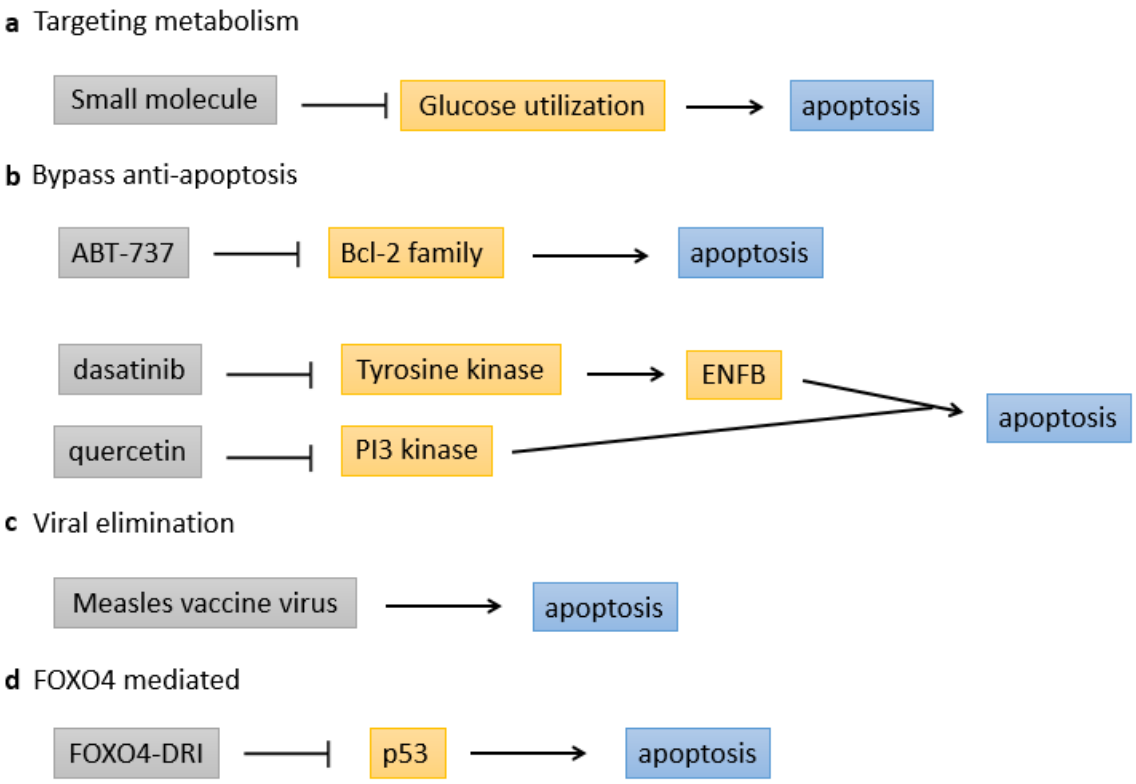

Figure 3. pathways to eliminate senescent cells. *Several mechanisms can induce apoptosis selectively in senescent cells. A) small molecules can inhibit the use of glucose leading to apoptosis, senescent cells are especially sensitive to this. B) the anti-apoptosis genes upregulated in senescent cells can be bypass by the use of ABT-737 which inhibits the Bcl-2 family and so induces apoptosis or by the combination of dasatinib and quercetin which also induces apoptosis. C) the measles vaccine virus has a higher preference for senescent cells in infecting and lysing them. D) FOXO4-DRI inhibits the interaction between p53 and FOXO4 leading to apoptosis.*

Conclusions

Since senescence was first described by Moorhead and Hayflick in the 1960's the field of senescence now begins to discover the full potential of being able to induce senescence and eliminate senescent cells. The ability of senescence to stop the cell cycle gained a lot of interest for its potential in putting a halt on the rapid proliferation of cancer cells. Furthermore, the disability to clear senescent cells by the immune system in elderly people is associated with multiple age related diseases. For example, diabetes type 2 and atherosclerosis, typical age diseases, are associated with senescence. The senolytics described in this review for the elimination of senescent cancer cells could therefore also have extra beneficial effect in elderly patients who already had elevated senescence levels before starting the senescence inducing therapy. Studies in mice have indeed shown that senolytics can increase lifespan and health. For instance, the combination of dasatinib and quercetin in mice improved the cardiac function, exercise capacity, extended the health span and delayed age-related symptoms and pathology (Zhu et al. 2015). Another example is the FOXO4-DRI peptide which restored fitness, hair density and renal function in fast and naturally aged mice (Baar et al. 2017). Interestingly, a Dutch newspaper very recently reported the use of FOXO4-DRI by humans. They stated that a life threatening rejuvenation drug was already being sold without being thoroughly tested by its developers. Less than a month after the article by Baar et al. appeared companies started to develop FOXO4-DRI on its own and made it accessible to individuals. Companies were able to develop FOXO4-DRI because it is a simple peptide consisting of 46 amino acids that is producible with standard biochemical techniques. At least one person was reported to have actually taken the drug. A man, who passed his 50s, injected himself with FOXO4-DRI for a month and he made the observation that he had an increase in hair density and an increased fitness (Volkskrant, 2017). However, of course, this is scientifically not reliable. The effect seen could be a placebo effect or it could be the result of other drugs the man had taken before. Moreover, the researchers have not yet determined whether FOXO4-DRI is safe enough to be used by humans and therefore they have some concerns. They are especially worried about the long term effects of the drug in humans since mice do not live that long, making the research difficult to translate to humans. Therefore, since this drug has only been tested on mice and dosage and possible side effects have to be determined first in clinical trials, which could take years, the use of this drug as senolytic in humans is still far away.

Another consideration that has to be made is whether the current senolytics are able to diminish the amount of senescent cells enough to effectively remove the senescent tumor. As seen in the mouse models there was enough removal of senescent cells to decrease age-related pathology. However, to decrease the risk of senescent cancer cells escaping senescence as much as possible, all the senescent cancer cells should be removed. This is also important for the mental wellbeing of the patient. Cancer survivors described the fear of recurrence as the greatest concern in their life and a lower quality of life was associated with an increased fear of recurrence (Simard et al. 2013). The survivors in this study received normal treatment where the tumor was fully removed and were considered cured. However, if the senolytics are not able to fully remove the senescent tumor then the patients cannot be considered cured. It is imaginable that this leads to an increased fear of recurrence and thus a decreased quality of life.

A different concern that comes with the use of senolytics is that the normal functions of senescence will be comprised. A function of senescent cells is to stop proliferation of cells that have obtained an oncogene and thus prevent the progression to cancer. A concern therefore is that the use of senolytics might interfere with this process. Short term treatment with BH3 mimetics or activation of a drug inducible gene which lead to suicide of senescent cells, both in mice, was not associated with enhanced tumorigenesis but was associated with reduced cancer rates (Schmitt. 2017). However, the translation to the more long lived human

still has to be made. Nevertheless, because removal of a senescent tumor probably only requires a short term treatment, instead of the long term treatment that is probably required to reduce age associated pathologies, long term effect caused by reduced senescence function are likely not that severe.

Senolytics might not be able to clear the whole tumor. The consideration could therefore be made to combine senescence induction and removal with a surgery. With this surgery the vast majority of the tumor could be removed. The remaining cells of the tumor could then be more easily reached by the senescence inducing and removing drugs, making it more likely that the whole tumor disappears. However a major downside to this is that senescence is needed for tissue repair (Demaria et al. 2014). The appearance of senescent fibroblasts and endothelial cells was observed very early in the response to a cutaneous wound. These senescent cells secrete platelet-derived growth factor AA (PDGF-AA) in their SASP and so stimulate the differentiation of myofibroblasts which accelerates wound closure. The function of senescence in wound closure makes senolytics incompatible with surgery since the risk of bad wound healing is high. Even if the patient did not have surgery prior to senolytic treatment caution is needed to not obtain any injury after the senolytic treatment since it might prove difficult to recover from this.

In conclusion, the use of cellular senescence induction in combination with senolytics as a cancer treatment is a very promising future therapy, despite the hurdles that still have to be taken. For instance, there is still a need for a drug that effectively induces senescence in every tumor cell and the same applies for a drug that effectively kills every senescent cell. After these drugs have been identified the translation from mouse model to humans has to be made. Furthermore, the side effects of senolytic therapy have to be determined in humans especially the effect on wound healing and cancer risks should be investigated. Taken everything together, it will be exciting to watch the future progression this field will make in battling a devastating disease like cancer.

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