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Science of the Human Body publishes research papers and literature reviews written by biomedical and premedical students of the university colleges of Amsterdam, Utrecht, Groningen, Maastricht, Rotterdam, and Middelburg. The aim is to create a platform where students can read about each other's research, learn about and experience the process of publication and peer reviewing, but most importantly the students will get the credit they deserve and inspire others with their research.

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LETTER FROM THE EDITORS

Dear Reader,

I am extremely proud to announce the release of the first issue of our third volume at the end of our fourth year in business. I want to thank the editors, peer-reviewers, support IT team for all their hard work and enthusiasm during the past year. Next to that, I would like to thank you, the reader for staying active within our community and reading the research of our authors.

If you are new to our work, I will give a short introduction. The journal is a shared platform for the six UCs that provide classes in (bio)medical and life sciences where you can share, discuss and most importantly improve their research skills. This will help the students in the future for their master's degree and prospective career as a biomedical researcher or medical doctor.

This issue will have a premier, as it will be the first time that it only includes original research, which will hopefully inspire the young researchers out there. Also, this issue will again represent the wide array of topics that the biomedical and medical field covers. For instance, Ilva Noa Stellingwerf and Nuno Martinho Charneca Martins discuss FcγRIIIa and its importance for the cytotoxic function of the natural killer cell in cancer, while Marie Øiahals Dokken discusses the modifications of cellular senescence to treat age-related diseases.

As the process of publishing this issue comes to an end, my time as Editor-in-Chief is also coming to an end. Together with the new Editor-in-Chief I will put a call out for editors and peer reviewers. If you are interested in biomedical research and want to become involved in the publishing process, make sure to follow us on Facebook. Other changes about the structure of the journal itself be published soon on our website and Facebook page. Enjoy your well-deserved summer break!

Sincerely yours,

Joost Hoekstra
Editor-In-Chief Science of the Human Body 2018-2019

ORIGINAL RESEARCH

FcγRIIIa and its importance for the cytotoxic working of the natural killer cell in cancer

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Abstract

Natural killer (NK) cells play an important role in the detection and killing of stressed cells, including those of a cancerous origin. This process is done via initiation of antibody-dependent cellular cytotoxicity (ADCC). FcγRIIIa, also known as CD16a, is expressed on the surface of these NK cells and is a vital receptor in the activation of ADCC. Therefore, this receptor has been used as a target in cancer drug development. Current research should focus more on the activation of FcγRIIIa or inhibition of factors that downregulate CD16a, most notably ADAM17 and PTEN. While monoclonal antibody therapy is widely used, inhibition of these cancer immuneditors, which results in higher levels of ADCC, has not yet reached the spotlight. Thus, ADAM17 and PTEN inhibitors should be further researched to uncover their full potential.

Introduction

The immune system is composed of a complex network of cells that can identify foreign agents, promote their destruction, and most importantly discriminate between self and non-self molecules, an ability known as self-tolerance [1]. Although the mechanisms involved in the differentiation between self and non-self are similar, two different branches, the innate and the adaptive immune systems, have developed at different stages in evolution and are conceptually distinct [2]. The innate immune system equips the host cells with immediate and short-lasting defence against pathogens without requiring prior exposure to such microorganisms [2]. The adaptive immune response gives the host cells the capacity to produce a specific immune response mechanism every time a particular microorganism is re-encountered, and therefore can provide protective immunity [3]. The identification of cells of the immune system has long been established, but recently the innate lymphoid cells (ILCs) have been recognized to execute effector functions that overlap those of some adaptive immune system cells [4]. ILCs are important players in tissue homeostasis and inflammation, which happens via production of effector cytokines. ILCs consist of three groups denominated group 1, 2, and 3 ILCs, based on their capability to generate effector cytokines related to Th1, Th2, or Th17cells, respectively [5]. In conjunction with ILC1s, the natural killer (NK) cell composes group 1 ILCs, which are distinguishable by their expression of cytokines, namely transcription factor T-beta and interferon (IFN)-γ [6].

NK cells are non-differentiated cells that only need stimulation by IL-12 and IL-15; they are derived from hematopoietic progenitor cells, CD34+ cells, which further develop in the bone marrow, lymph nodes and tonsils, as well as in the blood and mucosa-associated lymphoid tissue [7]. Depending on the potency of the cell, NK cells are commonly found in the blood and the spleen, in the liver and in the gravid uterus [8]. NK cells can regulate killing of invading cells, a task also performed by B and T-lymphocytes, but are also involved in the removal of stressed or damaged cells. Such a process is initiated by detection of a lack of major histocompatibility complex (MHC) class I molecules, which is also commonly missing on cancer cells to evade recognition by CD8+ T cells. Subsequent induction of antibody dependent cellular cytotoxicity (ADCC), a process chiefly mediated by the CD16a (or FcγIIIa) receptor, leads to removal of stressed cells [7]. The presence of the CD16a receptor is a key factor in distinguishing between the two subsets of NK cells: the cytotoxic and immunomodulatory NK cells. NK cells not expressing CD16a ($CD56^{high}$ NK cells)

are immunomodulatory, as they are unable to induce ADCC, and those expressing CD16a are cytotoxic. NK cells can, therefore, kill cells that have down-regulated MHC I, since they can express inhibitory receptors that attach to MHC I molecules. Cells with down-regulated MHC I disinhibit NK cells and come to be NK cell-mediated cytotoxicity targets. This may play a crucial role in cancer therapy, as transformed and virally infected cells are removed and NK cells may potentially generate less immunopathology, such as graft-versus-host disease (GVHD) [9]. This literature review hence further looks into the possibilities CD16a expressed on NK cells offers for the prevention, termination and treatment of cancer and viral infections, putting the focus on ADCC induction.

Results

CD16a, through binding of the constant part of an IgG bound to antigen, is able to start signal transduction. Antigen-binding results in clustering of CD16a, which leads to signal transduction initiating ADCC [10]. Clustering leads to phosphorylation of the conserved tyrosine motifs present in the Fcγ subunit, which contains the two YxxI/L consensus sequences characteristic of ITAM [11]. Phosphorylation of the Fcγ chain induces a signaling cascade in the cell, which includes members of the Src family, e.g. Lyn and Fyn [12]. Sequential activation of the Syk kinase family results in activation of the PI3K-PKC (phosphatidylinositol 3-kinase protein kinase C) pathway. The ensuing $^{2+}$ mobilization leads to increased levels of cytoplasmic $^{2+}$, with resulting cellular activation [13]. Nuclear transcription and release of stored vesicles containing lytic enzymes, such as perforin, granulysin and granzymes (mainly granzyme B) by the NK cell happens [14]. Receptor aggregation and phosphorylation of the conserved tyrosine residues can lead to activation of ZAP-70, Fak and f-chain subunits, which results in actin polymerization, phagocytosis and receptor internalization [15].

CD16a is a moderate affinity receptor, with the highest affinity for $_1$, even though it can interact with $_2$ and $_3$ [16]. NK cells bind the constant part of IgG, and are therefore not specific and more potent than the specific cytotoxic T-cell, which is able to kill tumour cells in a less efficient way. Ianello and Ahmad (2005) concluded that there are five ways in which monoclonal antibodies (mAbs/IgG) can combat cancer, the first and most potent being ADCC [14]. The production of mAbs often focuses on production of $_1$, as this IgG has the highest affinity for FcγRIIIa.

The importance of FcγRIIIa signaling in induction of ADCC can be seen from a study involving FcγR deficient mice, which were unable to activate ADCC [17]. Another study supported this observation; FcγR deficient mice were unable to respond to trastuzumab and rituximab, two monoclonal antibodies used to treat cancer [18]. mAbs lacking the Fc region, hence unable to interact with FcγRs, were shown to be less effective in inducing ADCC [19], again showing the necessity of FcγRs, mainly that of FcγRIIIa in the treatment of cancer. In addition to this, certain mutations in FcγRIIIa are classified as primary immunodeficiencies, an example of which is the L66H mutation [20].

Cancer can, as aforementioned, be treated using monoclonal antibodies (mAbs). Examples of this are trastuzumab and rituximab, which interact with the tumour via their Fab arms, while interacting with CD16a on the NK cell to induce ADCC in the cancerous cells. The use of monoclonals is currently a well-known therapy and has proven to be effective, depending on the type of mAb used. Their specificity directs which cancer they are able to treat, thereby making it more difficult to fully treat cancer, which has a heterogeneous character.

Another way in which cancer can be treated focuses on the inhibition of ADAM17 and PTEN. ADAM17 and PTEN are both potent 'cancer immuneditors', which causes functional exhaustion of NK cells [21]. Functional exhaustion is seen in reduced FcγRIIIa expression and a resulting reduced cytotoxicity in tumour-associated NK cells [22, 23].

ADAM17, a disintegrin and metalloprotease-17, is a member of the metalloproteinase superfamily, which is characterized by the well-conserved methionine adjacent to a zinc-binding motif in its catalytic region. It is a primary protease in CD16 cleavage and is constitutively expressed on NK cells [24]. Inhibitors of ADAM17, better known as TNF-alpha converting enzymes, prevent cleavage of CD16a upon activation of the NK cell [25]. ADAM17 appears to be a regulatory checkpoint for CD16a in healthy cells, however, tumour cells are able to exploit immune checkpoints like ADAM17 to suppress antitumour immunity. Blocking ADAM17 might enhance therapeutic efficacy, e.g. in combination with tumour-targeting mAbs that aim to enhance

levels of ADCC. Research by Blum et al. and Jing et al. does not take ADAM17 as its target, but focuses on adapting the structure of CD16a in such a way that it is able to initiate ADCC, while being less susceptible to ADAM17 [26, 27]. Recent efforts to develop efficient small-molecule ADAM 17 inhibitors were made, but no clinical success has been achieved, although advancements in blocking the function of ADAM17's epitope region have showed promising results due to a higher specificity and longer half-life [28].

PTEN is a protein encoded by the PTEN gene, present on chromosome 10, that can act as a tumour suppressor gene [29]. Activation of the phosphatase associated with PTEN is a common phenomenon in cancers that causes a decline of NK cell's ability to attack tumor targets. In addition to the inhibition of CD16a via dephosphorylation of the 3' phosphate of the inositol ring of PIP3, PTEN is involved in cell cycle regulation and it can negatively regulate the Akt/PKB signaling pathway [30]. Upregulation of PTEN leads to decreased actin accumulation, polarization of the microtubule organizing centre and induction of convergence of cytolytic granules at the NK-target cell interface [31]. A PTEN inhibitor is an extract of *Sarcopoterium spinosum*, which is mainly used for its antidiabetic activity [32], but may be useful in cancer as well. Apart from this, currently developed inhibitors of PTEN include bpV(phen), bpV(pic), VO-OHpic and SF160 [33]. PTEN inhibitors seem to not be used in cancer treatment, but might become useful additions in the future, due to their role in cell cycle regulation, cell proliferation, survival, and motility.

Tumour killing by NK cells can lead to an increased response upon surreptitious contact with tumorous cells; it can also promote increased production and release of IFN-γ [34]. The increased response does require NK cells getting in contact with IL-2 and IL-15. Making use of this property of NK cells could provide a target for future therapy, however, this requires accurate knowledge about the location of tumours. In addition to this, it might damage the surrounding tissue more than it would harm the tumour, which shows the need for further research into this potential therapy.

Cancer cells are able to resist immune system killing which they can do in multiple ways, e.g. by upregulating expression of programmed cell death protein 1 (PD-1). This protein is expressed on the cell surface and has the ability to downregulate the immune response, affecting both T-cells and NK cells. This is seen in e.g. chronic HIV-1 infection, which is able to downregulate the cytotoxic working of the NK cell via upregulation of PD-1 on NK cells [35]. In addition to decreased cytotoxicity, NK cell proliferation is reduced. PD-1 inhibitors are a new class of drugs with the ability to upregulate immune responses to attack tumours, they are therefore already used in cancer. IL-18 plays an important role in the upregulation of PD-1, therefore RNAi-mediated knockdown of IL-18 or binding via the IL-18 binding protein are used to reactivate NK cells [36].

The promise of the future

While FcγRIIIa shows a lot of potential, there are still many unknowns. This paper focuses on the possibilities of FcγRIIIa in the destruction of cancerous cells, but FcγRIIIa is not the only receptor involved in ADCC. The NKG2 receptor is another important activating target in the cytotoxic pathways of the NK cell [16]. Furthermore, the exact role of MHC class I has not yet been fully elucidated: changes in the normal working of this receptor might also interfere with killing of cancerous cells via ADCC. Overcoming this barrier constitutes another problem. The so-called missing self is a signal for ADCC, but certain tumours are able to influence their MHC class I receptors.

Research and development of drugs appears to mainly focus on the production of mAbs able to target both NK cells and tumours, meaning inhibitors like ADAM17 and PTEN are at times overlooked in their potential. mAbs need to be specific; they bind a marker or antigen on a tumour, thereby possibly missing other tumours in the body. This is in part due to tumour heterogeneity, which majorly complicates cancer treatment, especially if metastasis occurs. Development of multivalent mAbs is a possible solution, but brings along other difficulties, namely immunogenicity and the weight of these mAbs.

FcγRIIIa is mainly expressed on NK cells, a cell not potent at infiltrating solid tumours [37] Targeting NK cells is, therefore, generally a better attempt for treatment of metastases or minimal residual diseases. NK cell manipulation can also influence the outcome of organ transplantation [38] and might be involved in reduction of inflammation, e.g. in autoimmune

diseases [39]. NK cells are not the only cells with the ability to induce ADCC, but they are more successful than macrophages and eosinophils, which express different types of Fc receptors. The creation of a more resilient or reactivating CD16a receptor, which has proved to play a pivotal role in ADCC, is the focus of multiple studies, such as the research by Sung et al. (2018) [40], where a new assay permits the determination of CX 1:1 measurements of ADCC lytic capacity (without NK cell isolation), instead of antibody recognition.

Cancer remains a disease too complex to solve at once. In contrast with viral infections, where large percentages of cytokines (such as IL-2, IL-12, IL-18) and IFNs are released and can hence stimulate NK cells, in tumour microenvironments such cytokines are most often absent. Therefore, since NK cells that are active cannot release growth factors of autocrine nature in these microenvironments, NK cells could be targeted to tumours, or growth factors could be given to intensify NK cells' functions so that NK cells could engage more in cancer immunotherapeutic treatments. Other attractive approaches, including intervention in specific stages of the signalling pathways, such as cell surface receptors and cytokines to strengthen the NK cells role in cancer cells is still limited to animal models or to *in vitro* studies. However, the development of new inhibitors of signaling pathways and the synergy of different treatments, including the use of cytokines and receptor-ligand pairs, may contribute to the successfulness of cancer immunotherapy using NK cells.

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ORIGINAL RESEARCH

Modifying Cellular Senescence to Treat Age-Related Diseases

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Abstract

Age may bring a series of degenerative changes which cause illness, ultimately resulting in death. The cause of many of these degenerative changes can be attributed to cells entering a state of senescence and the accompanying senescent associated secretory phenotype. Previous research has looked into how to remove senescent cells and their ramifications. However, cellular senescence also carries beneficial properties, such as tumor suppression, and can be useful for cancer treatment. Therefore, complete removal of senescence is not a rational approach to treating age-related diseases and other approaches must be investigated. This paper reviews literature regarding relevant developments in modifying cellular senescence so as to answer the research question "How can modification of cellular senescence delay aging by treating age-related diseases?". Recent research on suppressing and inducing senescence to benefit health are explored. Furthermore, the feasible applications of methods senescence alteration to treat age-related diseases are discussed. The results of this paper conclude that aging, in essence, cannot be reversed or cured. However, with the modification of cellular senescence, prevention of age-related diseases, as well as their treatment is attainable. To this degree, aging is interpreted as having been delayed along with healthy aging being feasible. This paper explores novel advancements within the field of senescence alteration to add to existing literature, as well as give a new perspective on the debate surrounding the treatment of aging.

Introduction

Modifying Cellular Senescence to Treat Age-Related Diseases

Aging is a major risk factor for prevalent diseases affecting our population such as cancer, neurodegenerative disorders, cardiovascular disease and metabolic disease. Since degenerative diseases are correlated with aging, it can be presumed that there is an underlying common process causing these diseases in relation to aging. Presently, these diseases are treated individually, and in many cases symptomatically [1]. This raises the question; could these diseases be treated targeting a specific common mechanism? This would lead us to target the mechanism of aging. Targeting aging could result in a great number of other illnesses being treated as degeneration would be affected. Thus, the suppression of aging has the potential to delay or treat age-related diseases, as well as resulting in maximal longevity [2].

Cellular senescence has shown to have a key role in aging [3] and thus this field of research holds a successful future within gerontology and the treatment of age-related diseases. Senescence is the physiological deterioration resulting in mortality and decline in fertility with age [4]. Cellular senescence occurs when cells enter a state of irreversible growth arrest [5]. Furthermore, senescent cells promote neighboring cells to enter a state of senescence through the senescence-associated secretory phenotype (SASP), spreading the effect of senescence within local tissue. The mechanisms that drive cellular senescence are correlated with DNA damage, telomere erosion, oxidative stress, and inflammation (Toh et al., 2016). With a fully functional immune system, senescent cells will stay at a constant value, either by self-induced apoptosis or removal by the immune system. However, as people age, the immune system degenerates, resulting in accumulation of senescent cells [3]. Thus, the build-up of senescent cells with age contribute to degenerative changes in tissue which further promote a range of age-related diseases.

Although seemingly contradictory, cellular senescence does encompass beneficial aspects such as

having a role in tumor suppression and wound healing [5]. Therefore, simply eliminating all senescent cells is not an appropriate action in treating age-related diseases. Since cellular senescence can produce both detrimental and beneficial effects, both approaches of inhibiting and inducing senescence to intervene with disease are being investigated. The current challenge lies on suppressing the detrimental aspects whilst enhancing the positive aspects of cellular senescence [6]. This paper will focus on the modification of senescent cells in order to treat age-related diseases. In this context, it will also be concluded how these modifications can result in delayed aging. Strategies regarding suppression of cellular senescence as well as enhancing the beneficial aspects of cellular senescence will be discussed. With these different aspects of research being covered, a discussion will be formed to answer the research question "How can modification of cellular senescence delay aging by treating age-related diseases?".

Methods

In order to answer the research question, a literature review was constructed. Given the various research published, a discussion was formed on how attainable it is to treat age-related diseases by means of modifying cellular senescence. As a primary source, an interview was conducted with Peter de Keizer at Utrecht Medical Centre. Keizer was involved in research during which they were able to reverse aging phenotypes in mice by targeting cellular senescence. Both his research and the information gathered from the conducted interview (see Appendix A) are discussed and referred to in this review.

Additionally, relevant research papers were found using the PubMed database with specific criteria, and chosen according to their applicability to the topic of this paper. PubMed was initially used to gather expansive literature. The PubMed database was later used with MeSH search tags to acquire specific research papers referred to in the literature review. These articles were chosen to represent the newest findings within each sub-section of the review. Only articles published in English and containing the search terms shown in Table 1 or Table 2 fit the selection.

Literature Review

Strategies Suppressing Senescence

Cellular senescence, as well as the SASP, have been shown to promote a great number of degenerative changes in tissue. This is due to senescence and the SASP disrupting normal tissue structure and function [7]. In addition to promoting a range of age-related diseases, cellular senescence increases the risk of cancer as it stimulates neoplastic transformations and tumor progression through the SASP [8]. Therefore, the removal of senescent cells and the suppression of SASP can be seen to be an appropriate method to counteract changes which may result in cancer as well as other degenerative diseases. Cellular senescence contains several unique properties which can be exploited for counteracting its detrimental effects. Hereby, modifications that suppress senescence will be explored through the newest discoveries within the field.

Self-induced apoptosis

In order to remain viable after DNA damage and maintain a stable state of growth arrest, senescent cells make use of pro-survival pathways [9]. These factors can be exploited as senescent cells are heavily reliant on pathways of pro-survival compared to normal cells. Therefore, senolytic drugs, which remove senescent cells without targeting normal cells, may be an option for eliminating senescent cells [8].

A recently published research paper by Baar et al., demonstrates how a synthetic peptide can initiate self-induced apoptosis in senescent cells using in vivo mice models [3]. Following damage in normal cells, foxo4 favors a senescent response, through binding with p53, over an apoptotic response thus keeping senescent cells viable. The designed compound, foxo4-DRI, intervenes with the foxo4-p53 interaction. The foxo4-DRI peptide reduces the viability of senescent cells by competing with foxo4-p53 binding, triggering the release of active p53 being expelled from the nucleus into the cytosol. The protein p53 is known to stimulate an apoptotic response. Following administration of the synthetic peptide, free moving p53 may end up in the mitochondria, resulting in cell-intrinsic apoptosis through caspase-3/7, rather than senescence [3]. These interactions are illustrated in Figure 1 and 2 below. Following the administration of the synthetic peptide, foxo4-DRI, the mice subjects regained hair and tissue growth, in addition to a 25% lifespan extension. Foxo4-DRI was also observed to have removed chemotoxicity

Table 1: PubMed Search Strategy

Strategy	Search Terms	Results
1	Senescent cells	7020
2	Senescence age related disease	16543
3	Senescence	392379
4	Age related disease	16415
5	Aging impact society	911
6	Modification senescence	9515
7	Treatment senescence	123325
8	Senescence cancer therapy	9684
9	Pro-senescence	54
10	Reactivation of proliferation in senescent cells	91

Table 2: PubMed MeSH Search Strategy

Strategy	Search Terms	Results
1	“Cellular Senescence"[Mesh] AND "Neoplasms"[Mesh]	1087
2	"Cellular Senescence"[Mesh] AND "Drug Therapy"[Mesh]	105

[3]. From the results of this study, it can be seen that self-induced apoptosis of senescent cells efficiently removes a significant amount of senescent cells resulting in improved fitness. This can therefore be further investigated and applied to the treatment of age-related diseases.

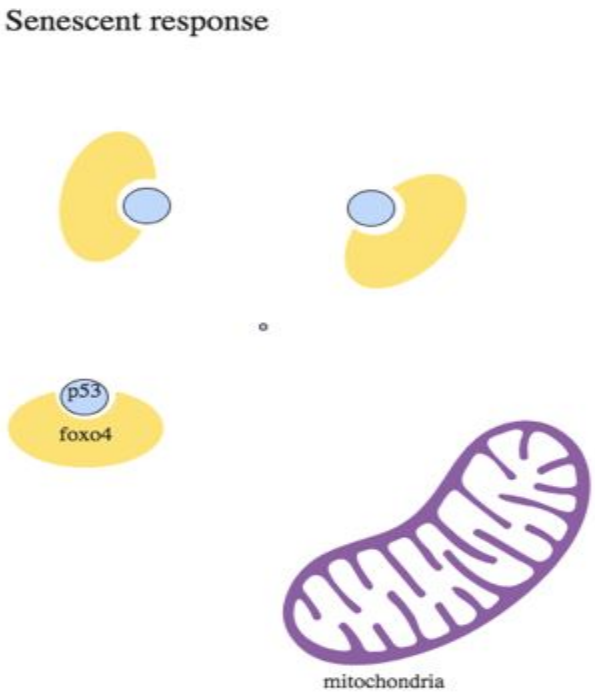

Figure 1: Foxo4-p53 interaction in senescent cells.

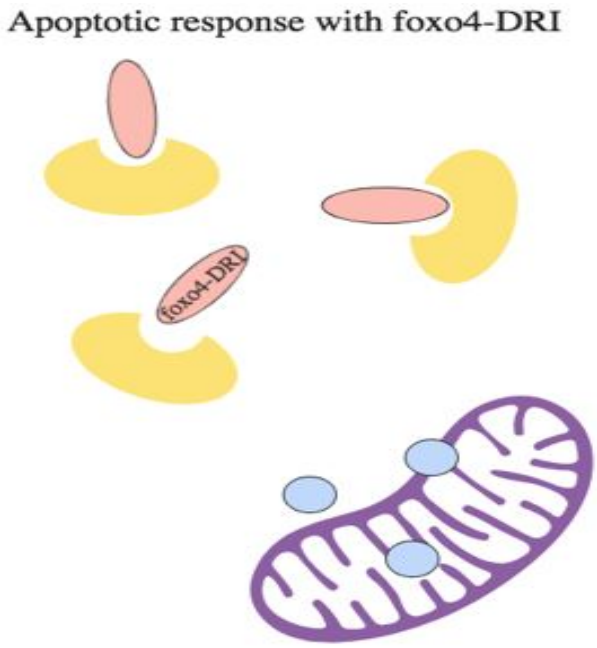

Figure 2: Foxo4-p53 interaction in senescent cells followed by treatment with foxo4-DRI to self-induce apoptosis.

Suppression of SASP

An alternative approach to targeting senescence without the use of senolytics would be to interfere with the SASP [10]. The SASP can promote neoplas-

tic transformation of premalignant cells and induce senescence in neighboring cells [8]. Thus, suppression of the SASP can be an attainable option for control and prevention of senescence as well as tumorigeneses.

Rapamycin, a macrolide compound produced by the bacterium *Streptomyces hygroscopicus*, is shown to increase lifespan in all of the model organisms to which it has been administered [11]. It does so by suppressing the SASP through binding with the FK506-binding protein 12 (FKBP121), resulting in inhibition of the activity of the mammalian target of rapamycin (mTOR) [2]. The mTOR network has been shown to regulate a great number of aging hallmarks, including cellular senescence. It can therefore be said that mTOR contributes as a regulator of lifespan. This effect of inhibition of mTOR is directly linked to mTOR complex 1 (mTORC1) inhibition. Recent data has identified promotion of the SASP as a main function of mTORC1 [12]. mTORC1 controls the translation of mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MK2), and during senescence, MK2 is responsible for phosphorylating the RNA-binding protein ZFP36L1. This phosphorylation inhibits the ZFP36L1 from degrading transcripts of various SASP components. However, when rapamycin is administered, ZFP36L1 is activated and can induce the degradation of the SASP components [13]. These causal sequences of the administration of rapamycin can be seen in Figure 3 below. Given the large range of research covering suppression of the SASP, it can be said that the use of synthetic inhibitors to subdue the detrimental effects of senescence holds a promising future.

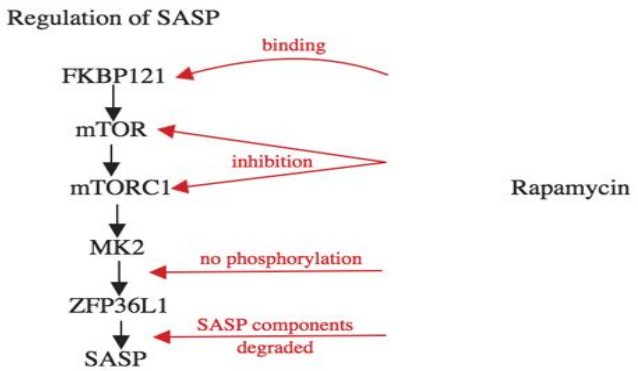

Figure 3: Effects of administering rapamycin on SASP regulation.

Strategies Inducing Senescence

Despite its detrimental consequences, cellular senescence can also have beneficial effects, especially in regard to cancer treatment. Inducing senescence has the potential to be used as a defense against cancer progression due to its permanent loss of the ability to undergo cell division [14]. Cancer cells have the capability to avoid senescence and thus resulting in cancer and tumor progression. Nonetheless, cancer cells can be induced to undergo senescence due to subjecting to specific stressors [15]. Therapies which induce senescence promote a stable cell growth arrest as well as activate an antitumor immune response [16]. Hereby, recent developments regarding inducing senescence will be explored.

Therapy-induced senescence

Traditional chemotherapy has been seen to initiate cellular senescence in normal cells and tumor cells. This process, termed (chemo)therapy-induced senescence (TIS), falls under one of the alternatives that can be used to enhance cancer therapies [16]. Traditional cancer therapies rely on high doses of cytotoxic treatments and radiation which result in killing cancer cells. However, these treatments also target normal cells, as well as cancer cells developing resistance to the treatment. Subjecting cancer cells to chemotherapy at a lower dosage than traditionally used, can promote TIS, rather than the destruction of cells. This treatment promotes senescence through a stable growth arrest and thus prevents tumorigenesis [17]. TIS, compared to traditional chemotherapy, carries the added benefit that it may result in reduced toxicity [16]. Seeing that therapeutic interventions, such as chemotherapy, inadvertently induces senescence in cancer cells as well as normal cells, researchers have directed their focus to explore other strategies which induce senescence solely in cancer cells.

Pro-senescence

Calcinotto and Alimonti put forward a novel form of cancer therapy termed pro-senescence, which entails inducing senescence solely in cancer cells. Cyclin-dependent kinase (CDK) inhibitors are pro-senescence compounds thought to be the most promising currently being tested [15]. O´Leary and colleagues have gained promising results in the clinic when researching the effect of CDK4/6 inhibitors on inducing senescence in cancer cells [18]. In normal cells, the cyclin D protein binds

to CDK4/6 at low levels resulting in the majority of retinoblastoma protein (RB) being unphosphorylated and thus active. This active RB binds to and blocks E2F transcription factors, resulting in inhibition of effectors promoting cell progression. On the other hand, in cancer cells there is over-expression of cyclin D and CDK 4/6 due to the upregulation of oncogenes. This increase in cyclin D-CDK4/6 complexes can lead to increased phosphorylation of RB inactivating it. This allows action of the E2F family, through transcription factors which drive cell progression in an uncontrolled manner. CDK4/6 inhibitors promote senescence of cancer cells due to the decrease of phosphorylation of RB, resulting in inactivation of E2F transcription factors. These processes are illustrated in Figure 4 and 5 below. This way cell progression is prevented and cells are stuck in a senescent state [19].

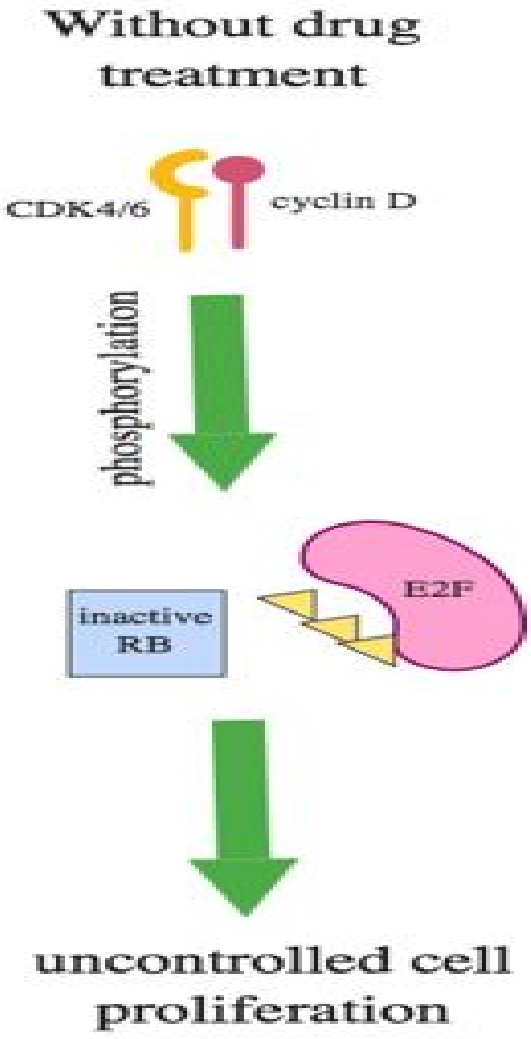

Figure 4: Effect of CDK4/6 inhibitors on cancer cell proliferation

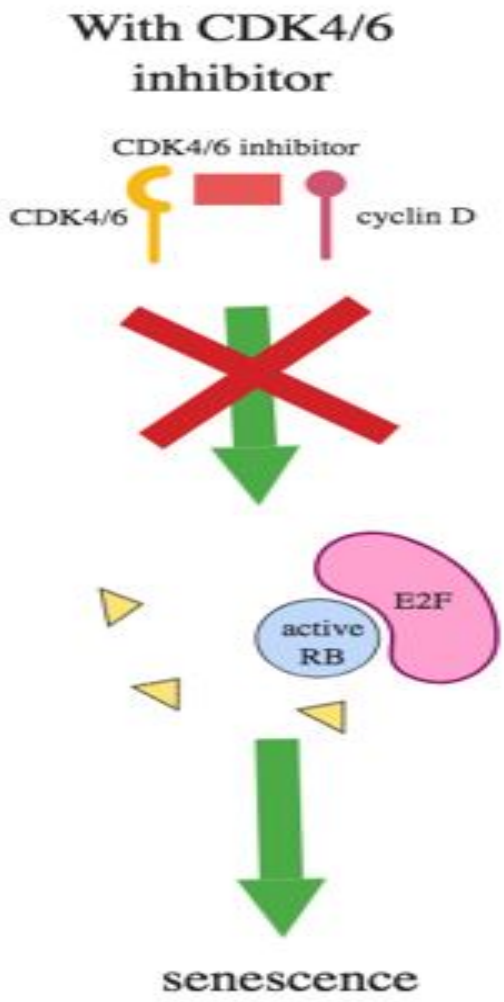

Figure 5: Effect of CDK4/6 inhibitors on cancer cell proliferation

Discussion

Modifying Cellular Senescence Can Delay Aging by Treating Age-Related Diseases

There have been several scientists claiming that intervening with cellular senescence could open doors to delay aging and even attaining immortality. As mentioned in the introduction, the concept of treating aging either by reversal or prevention is not a realistic view. There is currently no evidence suggesting that we can push lifespan past its current limit. Considering these remarks, we must continue to move our scope of research from the concept of preventing aging to achieving healthy aging. Healthy aging, achieved through targeting age-related diseases, can potentially bring the average life expectancy closer to the maximum age currently recorded. Accordingly, the results spec-

ified above have aimed to answer the formulated research question "How can the modification of cellular senescence delay aging by treating age-related diseases?".

Given the studies discussed, it seems that we can exploit cellular senescence based on the context of which it is to be applied. Interfering with the pathways which regulate the SASP of existing senescent cells, through inhibition of mTORC1 can keep senescent cells at a tolerable level [12]. With the buildup of senescent cells during the process of ageing, removal of these cells can be an alternative through the use of senolytics which interact with the pro-survival pathways of senescent cells, such as foxo-DRI [3].

Although seemingly contradictory, health interventions which promote cellular senescence, such as TIS and pro-senescence, can stall the progression of tumorigenesis by locking cancer cells in a senescent state of growth arrest [15]. The use of low doses of cytotoxic compounds and radiation, or CDK4/6 inhibitors such as palbociclib, offers a novel direction in cancer therapy in which target selectivity is enhanced and toxicity is lowered. Moreover, approaches inhibiting and inducing cellular senescence can be combined to work together. Senolytic drugs, such as foxo-DRI, can be administered simultaneously or after pro-senescent therapies so to eradicate senescent cancer cells. Furthermore, foxo-DRI was seen to self-induce apoptosis in senescent cells as well as therapy-resistant cancer cells [20]. Hence, cellular senescence also sheds light onto future interventions for patients with therapy-resistant cancers which do not show much improvement with traditional health interventions. In this way, the opposing effects of cellular senescence can be manipulated to reach the similar goal of treating cancer and can revolutionize the approach currently used in cancer treatment.

Cellular senescence has been deemed the "golden bullet" to treating age-related diseases [20]. This can be deduced from the vast amount of research seen within this field. However, senescence acts antagonistically within different circumstances [7], thereby, altered approaches must be taken for separate conditions. Depending on the disease and health of the patients. Hence, it can be concluded that cellular senescence cannot be controlled to cure the aging process in its entirety, as of yet. However, it can be confidently presumed that the modification of cellular senescence has the potential to treat and prevent a wide range of diseases associated with aging. With the prevention and treatment of age-related diseases, maximal longevity and healthy aging will be more attainable and thus this will cause a delayed effect on aging.

Conclusion

Manipulating cellular senescence has shown great potential in research in relation to healthy aging. Age is a main risk factor for disease and thus reduces average lifespan, with cellular senescence playing a key role in aging phenotypes and age-related diseases. This brings to light the formulated research question "How can the modification of cellular senescence delay aging by treating age-related diseases?" Treatment and prevention of age-related diseases, as well as age-associated phenotypes, may lengthen average life span, thus delaying the aging process through achieving healthy aging. This has been confirmed in a great number of research studies. Modification of cellular senescence, including, but not limited to, suppression and/or promotion of cellular senescence, has shown to have a positive effect on treating age-related diseases and aging phenotypes. Accordingly, aging can be said to be delayed by attaining healthy aging and thus a longer life span. Hence, it can be seen that the diverse mechanisms of cellular senescence can be manipulated to our advantage in treating and preventing age-related diseases and delaying aging.

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Appendix

Summary: Interview with Peter de Keizer On November 16 of 2018, a personal interview with Peter de Keizer took place at Utrecht Medical Centre. The research paper "Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging", in which he was a contributor, was discussed. Keizer expanded on the mechanisms involved when administering foxo4-DRI to senescent cells causing self-induced apoptosis. He revealed the approaches which are now being explored to improve efficacy and reduce toxicity of the synthetic peptide in hopes to begin with human clinical trials in the near future. Keizer also expressed his personal opinions on the debate regarding curing the process of aging. Furthermore, different topics within the field of senescence were discussed such as other pathways which can be targeted, current research taking place and potential future research. Keizer provided some useful articles which were taken into consideration when constructing this paper. Information extracted from the interview has been referred to where appropriate.