

Senescent Cells

Sandra Kaufmann, MD (Main Author), Jacob Cerny-Garcia (Contributing Editor)

E-mail: KaufmannAAI@gmail.com

Introduction

In the never-ending quest for improved health and longevity, the most recent scientific endeavors have revolved around the idea of cellular senescence and senolytic therapies. Scientists have been aware of senescent cells for a decade or so, but the idea of eradicating them to improve health is rather new. At the moment, there are innumerable cell culture and small mammal studies demonstrating the potential benefits of senolytic therapy, but the translation into humans is just emerging. Therefore, at the moment, it is popular to talk about senolytic therapy, but few people actually know what senescence is. In addition, there are very few scientific recommendations available. Therefore, this diatribe begins with a review and interpretation of what we presently know about senescence, and then delves into the potential molecular agents that may or may not be clinically available. Of note, this is written in Kaufmann style, which is a hybrid of a scientific paper and a popular article. The goal is to provide enough information for scientific scrutiny, while allowing non-scientists to understand the information as well.

What is cell senescence?

Senescence, in technical terms, is the condition or process of deterioration with age. On a cellular level, it is a state whereby a perfectly functioning cell is found to have DNA damage, and in response, places itself in a pseudo “shut down” mode until the problem can be sorted out. A cell in this state is called quiescent. If the problem is fixable, the cell repairs what it can and gets released from quarantine to return to cellular normality. If the damage is not fixable, it can either become apoptotic and die, or become senescent.

If one were to personify a senescent cell, it would be the grumpy, old employee who portrays a constant negative outlook. This guy was a great worker in his day, but now, not only does he not do his own job, he poisons the minds of his fellow employees. Furthermore, his morphology also changes, as his once trim and active physique has morphed into one of obesity and dysfunction. Others like to refer to these cells as zombie cells, but I prefer my analogy. Rather, these cells become dysfunctional, alter their morphology and are disruptive to their surroundings.

The process begins when a cell undergoes DNA damage, but this can be precipitated by innumerable insults. To put it simply, however, a double-stranded break gets recognized by the cell and the repair process is initiated. This can be caused by shortened telomeres, which resemble a double-stranded break to the repair proteins, and is referred to as replicative senescence, or it can be from an actual mid-DNA, double-stranded fracture.

Double-stranded DNA breaks can occur from a litany of insults, including “oncogenic activation, oxidative and genotoxic stress, mitochondrial dysfunction, irradiation, or chemotherapeutic agents.”⁽¹⁶⁾ Senescence may also be precipitated by “excessive reactive oxygen species (ROS), strong mitogenic/oncogenic signaling, loss of certain tumor suppressors, mitotic stress, stalled DNA replication, and chromatin disruption.”⁽³⁾

The timing of the senescent change is also not the same in every case. A cell can enter damage-induced cellular senescence either by a gradual accumulation of damage that occurs in replicative aging, or by a sudden outburst of damage induced by radiation or chemotherapeutics.⁽²⁶⁾

Because of the large variability in the pathological progression, the entire process deserves a closer look.

When the cell perceives damage to its DNA, a cascade of events begin to occur, starting with the induction of the DNA damage response system (DDR).

The DDR places the cell into the aforementioned “shut down” mode, which is prudent as a cell should cease replication if there is a possibility of creating new cells with DNA damage.

The cell cycles thru 4 phases, the first 3 are a part of interphase.

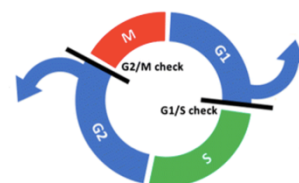
G1: (gap 1): cell increases in size

S: (synthesis stage): DNA gets replicated

G2: (gap 2): cell prepares to divide

M: (mitosis phase):

The 4th phase is mitosis where the cell actually divides.



Cell cycle arrest can occur in either of two places; either at the G1/S junction or the G2/M junction. Different insults determine where the cell arrests; for example replicative senescence generally causes cessation in the G2/M phase.⁽³⁴⁾ As well, different markers or cellular cytokines may be produced depending on the phase of cessation.

While the cell is in an arrested state, multiple events are occurring. For example, at this time, the cell produces Cyclin Dependent Kinase inhibitors (CDK inhibitors), such as p53, p21 and p16. These CDK inhibitors block or inhibit CDK- mediated phosphorylation of the retinoblastoma tumor suppressor protein (Rb). While Rb is present and not phosphorylated, cell progression is blocked.

General cell cycle arrest:

Temporary arrest

- 1) Increasing levels of p53
- 2) p53 increases transcription of p21 (cyclin-dependent kinase inhibitor p21)
- 3) Leads to initial transient arrest
- 4) Inhibits cyclin E-CDK2
- 5) Prevents phosphorylation/ inactivation of Rb
- 6) Achieve high levels of Rb
- 7) Leads to steady repression E2F genes
- 8) Cell cycle progression blocked

Permanent arrest

- 1) Increasing levels of p53 (level dependent)
- 2) p53 leads to increasing levels of p16
- 3) p16 Inhibits cyclin D-CDK 4/ D-CDK 6
- 4) Prevents phosphorylation/ inactivation of Rb
- 5) Achieve high levels of Rb
- 6) Leads to steady repression E2F genes
- 7) Cell cycle progression blocked

The relative quantity or level of p53 seems to help determine the fate of the cell. It appears that transient, lower quantities of p53 facilitate quiescence and repair, while higher levels or prolonged levels correspond with senescence. However, the even more elevated levels appear to be associated with apoptosis as well.

“Transient low-grade p53 activation facilitates repair; however, prolonged activation can drive cells into senescence.”⁽³⁾

“The absolute levels of p53 expression seem to be decisive in determining cell fate. High levels of p53 lead to apoptosis and lower levels lead to temporary CCA. However, the effects of p53 on senescence remains unclear.”⁽³⁴⁾

Some insight can be obtained by examining the senescence-associated CDK-inhibitors (cyclin dependent kinases) that are indicative of cell cycle arrest.

p16INK4a, a key cyclin-dependent kinase inhibitor, is considered a tumor suppressor protein in that it assists in cell arrest. In humans, it is encoded by the CDKN2A gene on chromosome 9. p16INK4a selectively inhibits CDK4 and

CDK6 (Cyclin dependent kinases) and acts by decelerating the cell's progression from the G1 to the S phase. It is known to be a tumor suppressor for melanoma, oropharyngeal squamous cell carcinoma, cervical cancer, and esophageal cancer.

Without p16, CDK4/6 binds to cyclin D and forms an active protein complex that then phosphorylates retinoblastoma protein (pRB). Interestingly, but somewhat out of the scope of this diatribe, once phosphorylated, pRB dissociates from the transcription factor E2F1. This liberates E2F1 from its bound state in the cytoplasm and allows it to enter the nucleus. Once inside, E2F1 promotes the transcription of target genes that are essential for transition from the G1 to the S phase.

p14ARF is a human tumor suppressor protein as well, but is unusual in that it is encoded in the same gene locus as p16, CDKN2A. The frame or base pair reading, however, is shifted so that the translation and subsequent protein product is different. This accounts for the name Alternate Reading Frame protein product. Because it codes from the same piece of DNA, it is also located on the short arm of chromosome 9. p14ARF acts as a tumor suppressor much like p16, but it functions by promoting p53 and subsequently p21 activation. p19ARF is the equivalent in mice.

p21 (WAF/ CIP1) is a cyclin-dependent kinase inhibitor (CKI) that is capable of inhibiting all of the cyclin/CDK complexes, although it is primarily associated with inhibition of CDK2. p53 increases the levels of p21, and thus is associated with linking DNA damage to cell cycle arrest. p21 is encoded by the CDKN1A gene, located on chromosome 6.

The other key point to note is the difference between quiescent and senescent cells, as one is a temporary situation, and the other is not. This is much like the difference between being temporarily sedated and unconscious for a procedure, versus being unconscious and vegetative. In both situations, the person or cell is still alive; but clearly the ultimate outcomes are very different.

Unlike quiescent cells, senescent cells are non-responsive to mitogenic or growth factor stimuli; thus, they are unable to reenter the cell cycle, even in advantageous growth conditions.

The markers are a bit different as well. For example, p16INK4A is generally indicative of senescence, p21 is present in both, while the markers p27 and HES1 tend to be only in quiescent cells.

The key here, which should be reiterated, is that cellular quiescence is a reversible growth arrest state. Quiescent cells are capable of resuming proliferation and normal cell function, while senescent cells are not.

What happens to a cell when it's senescent?

Two major things happen once a cell becomes senescent. First, the cell changes on the inside; namely the cell's morphology and internal workings are significantly altered. Secondly, the cell alters its external environment by exuding innumerable cytokines.

The most apparent of these changes is physical; the cell becomes flat, enlarged, vacuolized, and can produce multiple or enlarged nuclei.

Within the nucleus, the chromatin undergoes several metamorphoses, including the formation of PML (promyelocytic leukemia protein) bodies, and Senescence-Associated Heterochromatin Foci (SAHFs).

The organelles meanwhile, increase in mass with functional defects. The mitochondrial dysfunction is characterized by decreased respiratory activity and membrane potential together with increased mitochondrial ROS production. This is referred to as Senescence-Associated Mitochondrial Dysfunction (SAMD).⁽³²⁾

To actually detect these changes, it is possible to measure the chemical signatures from organelles. For example, the respiratory defects and reactive oxygen species in mitochondria are quantifiable, as is Senescence-Associated- β -galactosidase activity (SA- β -Gal) and lipofuscin in lysosomes.

Meanwhile, from a metabolic point of view, the cell demonstrates overall increased glycolysis, increased mitochondrial metabolism, and increased autophagy.

In addition to these changes, the cell activates specialized methods for self-preservation. Specifically, the cell creates Senescent Cell Anti-apoptotic Pathways (SCAPs) which promote cellular survival.

The exterior alterations reflect what is referred to as the Senescent-Associated Secretory Phenotype (SASP), or the production and secretion of proinflammatory and pro-fibrotic proteins. This includes a combination of cytokines, chemokines, extracellular matrix proteases, growth factors, and other signaling molecules. It also includes membrane-bound cell surface ligands and receptors that exacerbate the cellular response.

The specific composition of the excreted factors varies depending on the cell type and the senescence inducers. As well, the reaction of the environment to these factors can vary based on their specific niche, and the genetic context of the cells being exposed to the senescent secretome. In general, the SASP contributes to an increasing local, as well as systemic, inflammatory state.

In addition to the SASP, the senescent cell can influence adjacent cells through juxtacrine NOTCH/JAG1 signaling, ROS production, or by cargo transfer, which occurs via formation of cytoplasmic bridges or release of exosomes.⁽¹⁶⁾

The SASP has innumerable effects, both beneficial and detrimental. On the plus side, the recruitment of the immune system serves as a useful mechanism to eradicate these cells, and in fact, explains how the senescent cell population remains minimal early in life.

As well, the SASP affects the cell from which it came, functioning in an autocrine loop, which reinforces the cell's arrested state.

“Indeed, knockdown of IL-6R, insulin-like growth factor-binding protein 7 (IGFBP7), or CXCR2, a receptor for IL-8 and related chemokines, prevents senescence. This autocrine loop contributes to the tumor-suppressive function of senescence.”⁽¹⁶⁾

The detrimental effects, on the other hand, are profound. The bad neighbor or bystander effect is referred to as paracrine senescence, where a senescent cell can encourage nearby, normal and active proliferating cells to become senescent. By creating a toxic environment around the senescent cell, it is not difficult to imagine a penumbra of senescence cells developing around the original cell. Because one senescent cell can influence more than one or two surrounding cells, the increase in senescent cells exceeds a pure linear relationship. This essentially magnifies the effect of the small number of the original senescent cells.

“These data suggest that a ‘one-off’ localized induction of senescence (e.g. by chemo- or irradiation tumour therapy) may be expected to result in a continuously accelerated accumulation of senescent cells. Conversely, ablation of senescent cells should not just reduce senescent cell numbers but in addition reduce rates of senescent cell accumulation to a youthful state.”⁽³²⁾

As well, the SASP precipitates an inflammatory response that can both remain local or become systemic and affect other parts of the body. This ever-increasing inflammatory state, or inflammasome, has huge medical consequences.

In addition, the SASP secretome precipitates cellular homeostatic aberrations, deterioration of the stem cell niche, angiogenesis, induction of epithelial-to-mesenchymal transition, fibrosis and the establishment of conditions that promote survival and proliferation of cancer cells.

How exactly is the SASP controlled? Internally, the SASP is managed by multiple signaling pathways, including the DNA Damage Repair system or DDR, p38 MAP kinase, and cGAS/STING. Most of these cascades seem to converge on the activation of Nuclear Factor- Kappa Beta (NF- κ β) and CCAAT/enhancer-binding protein-β (C/EBPβ). These are activated and enriched in the chromatin and regulate the SASP by directly controlling the transcription of key regulators such as IL-8 or IL-6. In turn, these interleukins enhance the activity of C/EBPβ and NF- κ β and amplify SASP signaling. This process has innumerable levels of increasing complexity, but further specifics are not necessary for this discussion.⁽³²⁾

The SASP can include some or all of the following⁽³⁴⁾:

Cytokines: IL-1a, 6, 8, GROa, GROb, PAI-1 (Plasminogen activator inhibitor -1), CCL2/MCP-1

Growth factors: GM-CSF, G-CSF, HFG/SF, IGF, TGF-B, CCN2/CTGF, VEGF

Proteases: MMP- 1, 2, 3, 7, 9

chemokines: CXCL-1, 3,10

Non-soluble extracellular proteins: collagen, fibronectin, laminin

“Bioactive molecules released from senescent cells are potent inducers of cell senescence in bystander cells. NF- κ B-dependent SASP components are sufficient to aggravate autocrine and paracrine senescence, however, it appears highly probable that additional factors released from senescent cells including exosomal miRNAs and pro-oxidants may also contribute to senescence-induced bystander senescence.”⁽³²⁾

How do we identify senescent cells?

As we now know, a senescent cell undergoes innumerable changes, and thus it should be easy to identify. Unfortunately, every cell or cell type is a bit different. Some cells exhibit phenotypes or exude markers that resemble markers of senescence on a regular basis, and not every senescent cell has all of the properties we have examined. Therefore, it has become clear that it takes several markers in conjunction to clearly distinguish a senescent cell.⁽³⁾

“Although a single universal marker for cellular senescence is still yet to be unveiled, senescent cells present several distinguishing features in vitro, such as (i) flattened morphology and enlarged nuclear size, (ii) increased senescence-associated B-galactosidase (SA-b-Gal) activity, (iii) activation of p53 and p16INK4a-pRB tumor suppressor pathways that block cell cycle progression; (iv) activation of DNA damage response (53BP1 and γH2AX foci); and (v) the formation of heterochromatin foci, enriched in chromatin modifications, such as S83-HP1γ, HIRA, ASF1, macroH2A, and H3K9me3, which remodel the transcriptional landscape.”⁽²⁴⁾

There are however, two markers that have become more prevalent than the others; i.e. p16INK4a and Senescence-Associated Beta- Galactosidase (SA-β-Gal).

p16INK4a

The most commonly used marker at present is p16INK4a, which gets produced at the G1/ S phase break and is associated with irreparable DNA damage. Its levels are almost undetectable in young healthy organisms, but it markedly increases during tumorigenesis and aging.⁽¹⁶⁾

On the other hand, it has been noted that there is a high expression in non-senescent pancreatic Beta cells and some neurons.⁽²⁶⁾

Senescence-Associated Beta-Galactosidase (SA-B-Gal)

Senescence-Associated β-Galactosidase is an enzyme which is normally found in many different cells under standard physiological conditions, i.e. between a pH range of 4.0 - 4.5. It is, however, significantly amplified in senescent cells as a result of increased lysosomal content. Because of this, histochemical detection of β-gal activity at a pH 6.0 allows for improved specificity of senescent identification.

The enzyme, elevated secondary to an increased lysosomal activity, is also elevated in some normal cells. This can be typical for active macrophages, Kupffer cells, and osteoclasts. This reiterates the need for a multiple marker approach for identification. The other limitation is that the tissue being studied must be fresh in order to retain its enzymatic activity. Unfortunately, most biopsied tissues get placed in a preservative.

Abbreviated list of potential senescent markers per the literature:

- p53
- ARF, CDKs (p16INK4a, p15INK4b, p21WAF,/CIP1, p27KIP1)
- DDR markers (ATM, 53BP1, γH2AX, MBC1, CHK2)
- DEC1
- DCR2
- PML nuclear bodies
- SAHF (senescence-associated heterochromatic foci)
- HMGA proteins
- TIF (telomere dysfunction-induced foci)
- DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence)
- Cell surface proteins (DEP1, B2MG, DPP4)
- Lipofusion accumulation
- α-Fucosidase: another lysosomal enzyme

Why do our cells undergo senescence?

Our cellular DNA is constantly under stress, and even under normal conditions, the rate of DNA damage can reach as high as 10^5 DNA errors/cell/day.

If we destroyed every cell with DNA errors, over time, we would have very few cells remaining. Therefore, the body repairs these errors, but puts the cell into a holding pattern until the damage is addressed. This system is cell sparing, as well as serving in a protective capacity against mutagenesis and cancer. The SASP is important in this regard, as it attracts the immune system to remove the unsalvageable cells. Therefore, the system is credited with being a defense against cancer.

There is also evidence that the senescence cell system is vital to growth and remodeling of tissue under normal embryonic and childhood growth, which is referred to as developmental senescence. It is also pertinent to wound healing and helps control the fibrotic response to tissue injury. ⁽⁴⁾

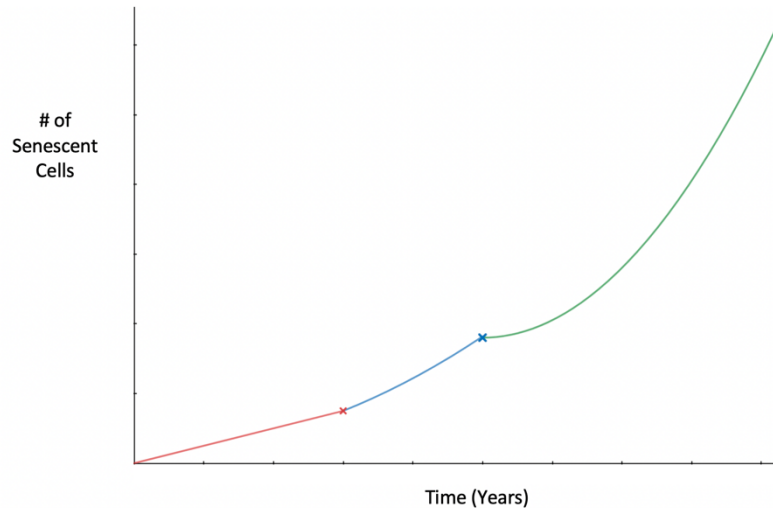
Like many cellular processes, things that were once beneficial become detrimental with aging.

How many Senescent cells are there?

At the moment, there is really no way of actually knowing how many senescent cells one has; however, the number of these cells starts out very low and this number remains low, as the immune system is capable of clearing out the cells in an expedient fashion. Over time, however, this number slowly increases as several things occur. First, individual senescent cells have a negative effect on their surroundings, and more senescent cells are created. Age, injury and concomitant medical problems create more and more senescent cells. We know, for example, that senescent cells accumulate around areas of pathology. Lastly, the immune system becomes unable to remove the cells in a timely fashion.

Examining the theoretical curve of senescent cells over time, one can imagine that it is linear with a slightly slope until somewhere in early or middle age range. In the graph, this is represented by the first, linear segment. At some point in time, which can vary tremendously between individuals, the slope becomes more pronounced, but perhaps maintains a somewhat linear form; this is the second section. Then, in the third section, the slope becomes an upward curve, perhaps approaching an exponential increase. This is a cumulative function of ever-increasing senescent cells occurring on their own from advancing age or insult, the additional burden of cells converted secondarily, and a failing immune system or clearance ability.

At the far right of the curve, there is no way to know if the curve levels off, or if it keeps increasing until expiration.



“This accumulation is partly counteracted by immune-mediated turnover of senescent cells, resulting in a slow accumulation as a net effect. In mouse liver, this accumulation is remarkably linear with age at least over the first half of lifespan suggesting that rates of accumulation and degradation may be approximated as constants over short time spans.”⁽¹⁰⁾

“Senescent cell burden is usually low in healthy people through middle age but increases across several years after the late 60s. The number of senescent cells in adipose tissue in when in their early 70s correlates with extent of frailty and disability.”⁽³³⁾

These cells accumulate in a heterogeneous fashion throughout both the entire body and within individual organs and tissues, such that some body parts are more effected than others. The pattern is determined by the individual and their unique situation. For example, a person with intervertebral disc disease is going to have more senescent cells in their injured discs than in the non-injured ones. People with lung or heart disease are going to have more in their areas of concern. The clearance of such cells is not homogenous either. There is evidence that while some senescent cells, i.e. liver cells, can be cleared quickly by the immune system, other tissues evade immune clearance, such as the senescent cells from melanocytic nevi.⁽¹⁶⁾

The overall, actual number of these cells is thought to be fairly low, even as the numbers increase with age. However, even low numbers of cells can have significantly detrimental effects.⁽⁴⁾

In a mouse transplant model, where individual senescent cells were placed into normal, middle-aged mice at a cell ratio of 1 in 10,000 cells, the effect was sufficient to cause profound physical dysfunction within 2 months and precipitated early death due to accelerated onset of age-related diseases.⁽³³⁾

So, how many cells are there? There is truly no way of knowing. We do know, however, that in very old primates, they have demonstrated upwards of 15% of cells being senescent.⁽³⁷⁾

In a very creative study, researchers examined human intervertebral discs that were biopsied either during surgery or autopsy. They compared two tissue types within the intervertebral disc, the nucleus pulposus (NP) and the annulus

fibrosis (AF), both between different people and within the same individual, but at different sites. Ages ranged from the 30s to the 70s. The numbers of senescent cells were identified by both p16INK4a and SA- β -gal markers. Not surprisingly, there were more senescent cells in degenerate discs. Whereas we don't have exact numbers, we do have percentages.

When using p16INK4a as a marker, the more normal NP tissue had 6% senescent cells, while the degenerate discs had 11%. The AF tissue had 10% senescent cells in the healthier tissue, compared to 18% in the degenerate discs.

When using SA- β -gal as a marker, the more normal NP tissue had 43% senescent cells, while the degenerate discs had 69%. The AF tissue had 40% senescent cells in the healthier tissue, compared to 69% in the degenerate discs.

The two methods of senescent identification clearly give us very different results; however the percentages are impressive. Even if the lower number is accurate, 6% of senescent cells in healthy tissue is still a large number. As well, the 40% increase in number secondary to disc injury is impressive.

Where are these cells?

At the moment, it appears that senescent cells are present in pretty much every tissue examined to date. This includes fat, muscle, bone, eyes, skin, kidney, liver, cardiovascular tissue, blood vessels, lung and brain. ^(3, 8, 10, 33, 36)

In the brain, there is evidence that not all cells can become senescent. We do know however, that astrocytes, a subtype of brain cells, are capable of such conversion. ⁽³⁾

“With age, human brain tissue displays an increase in the number of astrocytes positive for p16INK4A and matrix metalloproteinase 3 (MMP3), a common SASP-associated protease. Astrocytes undergo senescence in vitro in response to ROS exposure, characterized by the presence of SA β -gal activity, growth arrest, and increased expression of both p16INK4A and p21.” ⁽³⁾

Clearance of cells

It has now been clearly established that senescent cells are generally detrimental over time and they accumulate in an ever-increasing pattern throughout the body. The remaining question asks if the removal of these cells is beneficial. It appears, at least in a plethora of animal models, that the answer is yes. Whereas the removal has yet to confirm the extension of life, it has confirmed, for the most part, that many of the scourges of aging can be reduced. ⁽²⁶⁾

How many need to be cleared out? Again, more questions than answers. Despite clearing only 30% of the senescent cells, however, Zhu noted a profound improvement in age-related phenotypes. ⁽³⁷⁾

Another interesting thing occurs after senescent cells are eradicated; normal and new proliferating cells seem to fill in the newly vacated areas, returning the tissue to a normal, homeostatic state.

Animal models

The place to start examining the effects of senescent cells and their clearance is in animal models, and there is a plethora of studies supporting this theory.

“A seminal study demonstrated clearance of approximately 30% of senescent cells and improvement of heart, kidney, and adipose tissue function. Subsequent studies focused on specific age-related conditions, such as frailty, idiopathic pulmonary fibrosis (IPF), atherosclerosis, osteoporosis, liver steatosis, and osteoarthritis, where senescent cell clearance proved beneficial, revealing the common denominator of these various, age-related conditions.”⁽²⁶⁾

Bone and Joints

“In old mice, the senolytic intervention improved bone mass, strength, and microarchitecture, thereby reducing age-related osteoporosis.”⁽²⁶⁾

“We demonstrated that dasatinib alleviated arthritis symptoms and histopathological destruction in CIA mice. Dasatinib treatment inhibited the production of proinflammatory cytokines including IL-1 β , TNF- α , and IL-6, and promoted the production of the anti-inflammatory cytokine IL-10.”⁽¹⁵⁾

Cardiac

“SNC clearance resulted in a sex- and strain-independent increase in median lifespan with corresponding reduction of SNC markers in several vital organs, including heart and kidney.”⁽⁸⁾

Adipose tissue

“Although senescent cells accumulate in adipose tissue of obese humans and rodents, a direct pathogenic role for these cells in the development of diabetes remains to be demonstrated. Here, we show that reducing senescent cell burden in obese mice, either by activating drug-inducible “suicide” genes driven by the p16Ink4a promoter or by treatment with senolytic agents, alleviates metabolic and adipose tissue dysfunction.”⁽²⁸⁾

Intervertebral discs

“Here, we show that year-long clearance of senescent cells mitigates age-associated increases in disc protease, PG matrix fragmentation and PG loss. These results provide compelling evidence that senescent cells are responsible for generating inflammatory cytokines and catabolic proteases that are known to increase with aging in disc, and thus support a causal relationship between cellular senescence and age-associated disc degeneration.”⁽²⁹⁾

Human studies

There are several studies in humans as well, demonstrating the presence of senescent cells, but with significantly less research investigating the clearance of these cells.

Intervertebral discs

As discussed previously, there are published reports that demonstrate an increased number of senescent cells with age and degeneration in human discs. ⁽²⁹⁾ When this tissue was removed from humans, placed in culture, and treated, Curcumin and o-Vanillin successfully cleared senescent intervertebral disc (IVD) cells and reduced the senescence-associated secretory phenotype (SASP). ⁽⁷⁾

Adipose

“In humans, the pre-adipocytes are thought to contain the most abundant types of senescent cells.” ⁽³⁷⁾

Molecular agents

Tackling this senescent cell problem can be done in several ways. Clearly, the first thing to do is to prevent them in the first place by maximizing cellular health. In addition to that approach, which may minimize but certainly not completely prevent senescence, there are several ways of dealing with these cells. In addition to just getting rid of them, it is possible to alter their behavior, as well as their secretory signature.

Thus, the category of medications or molecular agents that work on senescent cells are called senotherapeutics, which allows for a broad spectrum of cellular manipulations. These fall into several categories, with two major outcomes. Either the cell is eradicated, or it is controlled to decrease its negative influence. There is obvious overlap and lack of clarity in the following definitions, but the newness of this field has generated non-specific lingo. As different researchers have utilized a variety of terms in this field, to be inclusive, they are all included.

- 1) Senolytics: Agents that eliminate senescent cells
- 2) Senoptotics: Agents that also eliminate senescent cells, but work through apoptosis
- 3) Senomorphics: Agents that can reverse cellular senescence, or suppress the markers of senescence and the secretory phenotype of senescent cells, without inducing cellular death.
- 4) Senostatics: Agents that inhibit paracrine signaling and thus block the ‘proliferation’ of senescence due to the bystander effect. Again, the cell is not eliminated.

Senostatics

Senostatics do not kill senescent cells, but rather create a more static or controlled environment. This occurs by the inhibition of paracrine signaling, thus blocking the bystander effect. There is evidence that antioxidants, NF-κB

inhibitors, flavonoids, polyphenols and other phytochemicals may have senostatic activity. ⁽³²⁾ In addition, both metformin and rapamycin are considered senostatics.

Metformin

“In recent years, metformin has been demonstrated to function as a senostatic, inhibiting the pro-inflammatory secretory phenotype of senescent cells.” ⁽³²⁾

Rapamycin

“Rapamycin did not act as a senolytic but rather a potent SASP suppressor through a complex mechanism including inhibition of its main target mTOR kinase.” ⁽²⁵⁾

Senomorphics

Senomorphics act by altering the cell and its SASP in some fashion, thus reducing the negative effect of the senescent cell. There is overlap between the senomorphic and senostatic categories in the literature, however, frequently cited examples of senomorphics include free radical scavengers, and inhibitors of I κ B kinase (IKK), Nuclear Factor Kappa-Beta, and the Janus kinase (JAK) pathways.

Senolytics

Interest in senolytic therapy has grown tremendously in the last year or so, and like any new promising ideas, technology companies have blossomed. At the present, these include UNITY Biotechnology, Oisin Therapeutics, and Antoxerene.

Because this review will examine many, if not most of the recognized senolytic agents. Some of these are promising and commercially available, some are in developmental stages but not yet available and the rest are mentioned simply for the sake of completeness.

The major challenges facing senolytic therapy revolve around 3 major issues.

- The different molecular agents influence senescent cells via different molecular pathways.
- The various molecular agents are effective against different subsets of experimental cells. No one individual agent or family of agents has yet been shown too effective on all senescent cells.
- The dose and timing of any particular agent has yet to be solidified. Any specifics may need to be tailored for an individual as determined by where that person falls on the senescent cell curve, any medical co-morbidities they demonstrate, and how aggressive they elect to be.

An overall senolytic therapy plan must involve multiple agents, given at appropriate intervals for maximum benefit as well as risk reduction. Emerging therapies will continuously evolve, and thus in several years, this information will be only historically interesting.

Because different cell types respond differently to different senolytics, it is not good enough to simply say that an agent is senolytic. It is important to identify what cells are specifically being targeted. In addition, combinations of agents may be synergistic as well as complementary.

Therefore, within the following individual molecular agent reviews, the mechanisms of action and the known affected cell types are listed. This review is going to be incomplete however, as not every agent has been adequately tested versus every cell type. As well, we do not yet know how this information applies to living people.

Dosing and timing of senolytics is also important as it is yet unclear how much is required, and how frequently it should be administered. Senolytics have been reported to be effective after a brief exposure, and can clear cells within 18 hours. They do not, however, have to be present continuously. This has led to what is called the “hit-and-run” intermittent dosing strategy. Some agents are both senolytic and senomorphic, and the ratio of which should dictate dosing guidelines.

Presently available agents

Fisetin

Fisetin, a polyphenol and flavonoid, is present in low concentrations in many fruits and vegetables such as apples, persimmon, grapes, onions, cucumbers, and at higher concentrations in strawberries.

In a cell culture study comparing ten flavonoids, fisetin was determined to be the most potent senolytic, even proving to be superior to quercetin. Acute or intermittent fisetin treatment studies in both old and progeroid mice significantly reduced senescence markers in multiple tissues. The agent also reduced senescence in a subset of cells in murine and human adipose tissue. ⁽³⁶⁾

It is important to note that fisetin can be bipolar; it can be senolytic to some cells, while only being senomorphic to others.

Mechanism of Action

- Regulation of P13K/AKT/ mTOR and NF- κ B
- Inactivates ERK1/2
- Targets BCL-2 family

Cell type (s)

- HUVECs (senescent human umbilical vein endothelial cells)
- Human fetal lung (IMR-90) cells: myofibroblasts with smooth muscle-like contractile properties. (Senomorphic Only)
- MEFs (Murine embryonic fibroblasts): (Senomorphic) more potent than Quercetin in cell cultures

Does NOT work in

- Preadipocytes (senomorphic in some studies)

Statistics of note

Rapid and terminal half-lives: 0.09 and 3.1 h

Whole body mouse models ⁽³⁶⁾

In a model of progeroid mice given 60 mg/kg/day and exposed in two time periods, i.e. from 6 to 8 weeks, and then from 8 to 12 weeks; the number of senescent cells were significantly reduced as compared to controls.

In a normal mice model, animals were treated at the age of ten weeks for ten weeks and fisetin was shown to reduce the expression of SASP markers significantly in all tissues, including fat, spleen, liver, kidney and CD3+T cells.

In naturally aged mice (22 to 24 months), 100 mg/kg of fisetin was administered for five days and then sacrificed three days later. The short course of fisetin reduced the number of p16INK4a expressing cells in subcutaneous white adipose tissue including mesenchymal stem/progenitor, immune, and endothelial cells.

Lastly, very old mice, roughly equivalent to seventy-five human years, were fed a diet containing 500 ppm fisetin. This resulted in an extension of median, as well as maximal, lifespan.

Overall, studies in mice have demonstrated benefits both from intermittent, short-term therapy, intermittent longer-term therapy and simple long-term treatment.

Human tissue

Greater omental adipose tissue resected from patients during surgery was treated for 48 h with 20 μ M fisetin. This therapy precipitated a significant reduction in the percent of SA- β -gal positive cells, and the expression of IL-6, IL-8, and MCP-1.

In summation, the only present recommendation for fisetin in the literature is extrapolated from a mouse model. Aged mice given a one-time treatment were administered 100 mg/kg daily for five days and based on standard conversions to humans, recommendations included 500 mg per day for five days for a 60 kg human.

However, fisetin tends to be almost as senomorphic as senolytic, and therefore there is no clear answer as senomorphics need to be administered on a more continuous basis, while senolytics do not.

I think the clinical answer hinges on if fisetin is taken alone or in combination with other senotherapeutics. If taken alone, it should be used as a high dose, intermittent therapy. If used in combination, it can be used at a lower, but more continuous therapy. The third option is to combine strategies and use a daily small dose consistently, with intermittent large dose boluses.

Quercetin

Quercetin a.k.a (3,3,4,5,7- pentahydroxyfavone) is also a plant-derived polyphenol. The highest amount of quercetin is found in cappers (233 mg/100gm), raw yellow chili peppers (50 mg/100gm) and onions (22mg/100gm). Quercetin is present in some fruits as well, mostly apple and mostly in the peel. It is also found in black tea and white wine.

Discovered as a senotherapeutic very early on in the search for effective molecular agents, quercetin has become a “classic” in this category. Because of this status, it has been tested on more cells and in more situations than most of the other agents.

Mechanism of Action

- Regulation of P13K/AKT/ mTOR and NF- κ B
- Induction HIF-1a
- Targets BCL-2 family

Cell type (s)

- Human umbilical vein endothelial cells (HUVECs)
- Mouse BM-MSCs (bone marrow-derived mouse mesenchymal stem cells)
- Human mesenchymal stem cells (hMSCs) of the Werner syndrome (WS)
- Premature aging model⁽²⁴⁾
- MEFs (Murine embryonic fibroblasts) (mixed results/ study dependent)

Does NOT work in

- Primary adult endothelial cells⁽¹⁸⁾
- Human senescent mesenchymal stromal cells ⁽¹⁴⁾
- Preadipocytes (senescent)
- Mouse embryonic fibroblasts (mixed results)

There are presently no dosing recommendations for this agent on its own, as most of the time it is paired with Dasatinib. However, as it is clearly senolytic in some cell types, an intermittent, high dose strategy is not unreasonable. The structural similarity and mechanism of action are close to that of fisetin; therefore, it is also possible that quercetin may be senomorphic as well as senolytic to other cells. Following this train of thought, a lower dose, continuous therapy is not unwarranted. Its lack of toxicity also permits a wide range in dosing options, including a lower daily dose punctuated by intermittent higher, bolus doses.

Specific dosing and timing of dosing for any individual need to consider how this agent fits into the overall senotherapeutic strategy. Presently, the only recommendations presently available come from the Age Reversal Network, which suggest two doses of 25 mg/kg separated by a week when combined with dasatinib.

Dasatinib

Dasatinib, (SPRYCEL, Bristol-Myers Squibb), a multiple kinase inhibitor, is currently used to treat chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL) tumors in patients who are resistant or intolerant to imatinib mesylate. It is approved for treatment of pediatric patients one year and above for newly diagnosed Philadelphia chromosome positive lymphoblastic leukemia in combination with other chemoagents.

Because this medication is a known chemotherapy agent, it comes with both the baggage of sounding threatening, but also provides the benefit of clinical experience and pharmacodynamic information.

Mechanism of Action

Interacts with P53

Inhibits PAI-2

Tyrosine kinase inhibitor (multikinase inhibitor)

inhibits BCR-ABL gene

inhibits SRC kinase family

Inhibits platelet-derived growth factor receptor Beta (PDGFR)
kinases

inhibits c- KIT (protooncogene)

Inhibits ephrin-A receptor kinases

Cell type(s)

Senescent human pre-adipocytes

Statistics of note

Half-life: 3–5 hours

“Kinases are a class of enzyme that mediates phosphate transfer from adenosine triphosphate (ATP) onto certain amino acid residues to produce cell signal transduction resulting in a range of cellular processes. The discovery of their overexpression in various cancers, particularly the receptor tyrosine kinase subtype has led to the development of several tyrosine kinase inhibitors (TKIs). Their binding to TKIs is usually via competitive inhibition at the ATP binding pocket, stopping cell proliferation signaling.”⁽⁶⁾

Metabolism

Dasatinib accounted for <1% and 19% of the dose in urine and feces, respectively, suggesting that dasatinib was well absorbed after p.o. administration and extensively metabolized before being eliminated from the body.⁽⁹⁾

The maximum plasma concentrations (C_{max}) of dasatinib are observed between 0.5 hours and 6 hours (T_{max}) following oral administration.

Distribution

Some of the medication is able to reach the brain by crossing the blood brain barrier, however, the concentration is low and quite variable. In mice, 1- 4 hours after dosing, dasatinib brain concentrations were on average 5.5% of those found in the plasma (range, 3.2%-8.6%).

In 22 human patients, 15 adults and 7 kids, detectable levels were found in the CSF in only two adults and four kids three hours after an oral dose. In the three patients for whom plasma and CSF concentrations were available, the brain penetrance was 5%, 8%, and 28%, respectively. Therefore, CNS penetrance is extremely variable.⁽³⁰⁾

This information is important when considering the possibility of using dasatinib as a treatment for senescent brain cells. As the penetration is variable, the clinical outcome of such a treatment would likely be difficult to predict.

Side effects

The known side effects of this medication come from studies where the medication is taken on a daily basis for months, if not years, in cancer patients. Determination of the effects of one to three-day dosing in non-cancer patients is hard to determine from the literature. In non-official interviews with heme-oncology specialists, the likelihood of significant side effects with the abbreviated doses are minimal.

Regardless, product labeling currently includes warnings in reference to fluid retention, cardiac ischemia, pulmonary hypertension, and QT prolongation. Reports also include mild proteinuria or nephrotic syndrome, pleural effusions, fungal sepsis, hepatotoxicity, thrombocytopenia, pneumonia, nausea, enteritis, fever, neutropenia, mucositis, diarrhea, sepsis, hypotension, abdominal pain, skeletal pain and cytopenias.

Generally speaking, these side effects take a month or more to appear but may be unpredictable.

Traditional doses⁽¹³⁾

To determine the dose of the medication, several trials in cancer patients have been conducted with the following dose recommendations:

100 mg QD: chronic phase CML

140 mg/QD or 70 mg BID: accelerated or blast phase CML

up to 240 mg/day: ALL (acute lymphoblastic leukemia)

Things to know

- Concurrent medications that may increase plasma concentrations of dasatinib:
 - Clarithromycin
 - Erythromycin
 - Ketoconazole
- Concurrent medications may decrease plasma concentrations of D:
 - Antacids/H2 blockers/proton pump inhibitors⁽¹¹⁾
 - Carbamazepine
 - Dexamethasone
 - Phenobarbital
 - Phenytoin
 - Rifampin
 - St. Johns wart

Dosing

The dosing of Dasatinib for non-cancer patients for senolytic therapy has not been established. Clearly, it is a potent senolytic that needs to be dosed on an intermittent basis and not continuously. Researchers from the Age Reversal Network have suggested the dose of 2.5 mg/kg combined with quercetin at a one day per week, for two consecutive weeks. This daily weight-based dose can be higher than the 100 to 140 mg recommended for cancer treatment, but presently there are no significant trials to support either dose.

Further discussion of this can be found under the D/Q combination category.

D/Q combination

Based on early studies, the combination of the two molecular agents has proven to be effective in many models, and may be synergistic rather than simply complimentary.

“In mice equivalent to 80-year-old humans, treatment with dasatinib plus quercetin increases survival by 36% and does so without increasing the period of morbidity at the end of life.”⁽³³⁾

Cell types (s)

- senescent MEFs: Combination therapy works better
- senescent bone-marrow derived murine mesenchymal stem cells
- senescent primary fibrotic alveolar epithelial type II cells

“This suggests that the combination of D+Q selectively targets a broader range of senescent cell types than either agent alone.”⁽³⁷⁾

Animal trials

Single and intermittent treatment in innumerable mice models at a dose of D 5mg/kg & Q 50mg/kg demonstrated the following:

- Reduced osteoporosis
- Improved cardiac function in aged mice/improved left ventricular function
- Improved vasomotor function in aged mice
- Improved lifespan in progeroid Erec1 mice
- Reduced fatty liver in aged mice
- Reduced senescent markers in a variety of tissue
- Improved exercise tolerance

Human D/Q trials

The first and to date, only, human trial of D/Q senolytic therapy was published in 2018. In a two-center, open-label study, a combination therapy of D @ 100mg/day and Q @ 1250 mg/day was administered to older patients with

idiopathic pulmonary fibrosis. The dose was given for 3 days continuously in one week with a four-day break, for a total of 3 weeks and nine doses.

The dose of dasatinib was selected based on the FDA-approved dose for chronic administration from patients that was effective for inducing apoptosis in human cancer cells.

The intermittent dosing pattern was based on “studies in mice with pulmonary fibrosis, the effects of DQ on different lung cell types in culture, senescent cell clearance rate from freshly isolated human explants treated with DQ, peak concentrations, and elimination half-lives of DQ in humans, and dose escalation studies in old rhesus monkeys.”⁽²¹⁾

The trial included 14 patients, ages 55 to 84 (mean 70.8), who were mostly caucasian. Side effects were divided into serious, moderate and mild. Of these, there was one serious, 31 moderate and 37 mild. The mild complaints were mostly skin irritation and nausea, and the moderate included a cough, feeling tired and/or weak, and nausea. The serious event was pneumonia.

The study did not measure senescent cells directly, but looked at cellular markers in the plasma, and physical ability. Measures that improved included the six-minute walk distance, 4-m gait speed, and chair stand time. Pull function, clinical chemistries, frailty index, and reported health did not change while the levels of circulating SASPs were inconclusive.

The good news

- The medications were generally well tolerated. There were no detectable effects on renal or hepatic function and no evidence of cell lysis syndrome. There were a substantial number of side effects, but the paper did not report specifically when these side effects occurred. Therefore, we do not know how many doses were administered prior to any negative issues. It is certainly possible that the three doses repeated over three weeks was a bit aggressive for the older, somewhat frail population.
- Physical improvements were demonstrated. Despite there being no actual measurement involving senescence, we know that something beneficial did occur.

Dose

I believe the clinical recommendations for this senolytic agent combination need to be based on the individual patient, and their position on the senescence cell graph.

If a patient falls into section one of the graph, this therapy is probably not necessary. If they fall into the second section, meaning the slope of the cellular senescence graph has increased, I recommend two to three sequential, daily doses (pending toleration or side effects) of 100 to 120 mg. This dose range has been shown to be therapeutic for cancer cells, with minimal or tolerable side effects. As the side effect profile for quercetin is much more tolerable, the suggested dose of 25 mg/kg is not unreasonable. This combination of D/Q can be repeated 2 to 3 times a year, with a total yearly dosing between 4 and 9 total dose days.

If the patient falls into the third graph section with an almost exponential increase in senescent cells, I believe the patient would benefit from more treatments, but they are more likely to be frail with concomitant medical problems. The key would be to eradicate the senescent cells in a slow, but steady fashion that prevents tumor lysis syndrome, electrolyte imbalances, organ damage or any other unforeseen complications. Thus, the idea is to slowly shift their cell curve to the right. Therefore, one to two-day sequential doses should be administered at a more frequent rate of every three (4x/year) to two months (6x/year), for a total dose number between 4 and 12 total dose days.

With these recommendations in mind, clearly each patient must be evaluated, and an appropriate treatment plan developed based on age, concomitant medical problems, administration of other senotherapeutics, and side effects.

Clinically unavailable Senolytic agents

Navitoclax (ABT-263)

This medication is an orally bioavailable, synthetic small-molecule antagonist of a subset of the B-cell leukemia 2 (Bcl-2) family of proteins. It has been trialed for the treatment of solid tumors, Non-Hodgkin's Lymphoma, Chronic Lymphoid Leukemia, and Hematological Malignancies.

Senescent cells, much like many cancers, generally resist apoptosis, in part, by up-regulating the antiapoptotic proteins BCL-2, BCL-XL, and BCL-W.

Navitoclax has been shown to remove senescent cells involved with cardiac atherogenesis and can decelerate further lesion development. It can also eradicate hematopoietic senescent cells and helps to rejuvenate aged or irradiated bone marrow in animal models. ⁽⁸⁾

Mechanism of Action

Targets BCL-2 family: BCL-XL, BCL-W

Cells type (s)

murine senescent hematopoietic stem cells

Known side effects

Neutropenia and thrombocytopenia (even after small doses)

Other Related BCL inhibitors

ABT-737

A1331852 inhibits BCL-XL

A1155463 inhibits BCL-XL

Venetolax

UBX1967 *BCL-2 inhibitor*

UBX1967 is being evaluated for the potential treatment of age-related diseases of the eye, including age-related macular degeneration, diabetic macular edema and proliferative diabetic retinopathy.

Forkhead box protein 04 (FOXO4)-interacting protein

*FOXO4-DRI peptide FOXO4 D-Retro-Inverso (FOXO4-DRI).

FOXO4-DRI, a cell-penetrating peptide (CPP), is a completely artificial, human-engineered peptide, comprising the amino acid sequence LTLRKEPASEIAQSILEAYSQNGWANRRSGGKRP, wherein the amino acids are D-amino acid residues.

With D-amino acids substituted for L-amino acids, the chirality of the molecule is reversed; therefore it cannot be processed in the usual way, precipitating apoptosis of senescent cells. This peptide was evaluated in aged mice in 2017, showing destruction of senescent cells without side-effects, and producing the usual array of benefits to measures of health and age-related decline as a result.

Cleara Biotech, a reasonably new tech company from the Netherlands, was launched in 2018 to specifically develop senolytic therapies based on the FOXO4 peptide inhibition. Presently, there are no actual medications available on the market. The third-generation agent, Proxofim has shown good results, although it's not yet ready for prime time. According to the company website, they are working on a fourth generation, improved version of Proxofim.

Mechanism of Action

By competing with endogenous FOXO4 for p53 binding, FOXO4-DRI disrupts senescence-associated FOXO4/PML/DNA-SCARS and causes nuclear exclusion of active p53.

Cell type (s)

IMR 90
BJ
Senescent human WI-38 fibroblasts

Statistics of note

In cell cultures, FOXO4-DRI was taken up after 2- 4h after administration, and remained detectable for at least 72h.

FOXO4-DRI effectively disrupts the p53-FOXO4 interaction, but the importance of the FOXO4 protein itself is more complicated in DNA damage and senescence. As FOXO4-DRI causes nuclear exclusion of active p53, the levels of p21 Cip1 decline. However, the loss of p21 Cip1 alone is insufficient to induce apoptosis and was actually shown to induce a senescence-escape instead. Rather, the exclusion of p53 itself has been reported to induce apoptosis directly when relocated to mitochondria, thereby explaining the FOXO4-DRI effects. ^(1,2)

Mice models

The protein was administered in three mouse scenarios, with fast-aging mice, naturally aged mice, and in chemotherapy-treated animals as all situations are characterized by an abundance of senescent cells. In all instances,

significant improvements were noted after the intervention including liver and kidney tests, fur density, exploratory behavior, and voluntary running wheel activity. Importantly, administration of FOXO-DRI did not lead to any of the negative side effects reported for other “senolytics,” such as ABT compounds that target BCL2/W/XL anti-apoptotic proteins. ⁽⁵⁾

Dosing

There are no human studies, no available agents, and thus no actual recommendations. However, it may become important in the future to note that, at least in culture, the potency of FOXO4-DRI was more pronounced when applied in consecutive rounds at reduced doses versus the single, high dose strategy. ⁽¹⁾

UBX0101

UBX0101 is an investigational, new drug that was cleared by the U.S. FDA for clinical trials of joint injection. The agent is produced by Unity Biotechnology, who co-developed UBX0101 with Johns Hopkins Technology Ventures (JHTV), the commercialization arm of The Johns Hopkins University. The University licensed the intellectual property around senescent cell technology to Unity Biotechnology Inc. and presently, both entities jointly own the patent.

Mechanism of Action

UBX0101 interrupts the interaction of MDM2 with p53

Cell(s) ?

Mouse models

Young mice were surgically manipulated to mimic injury to the anterior cruciate ligament (ACL). Injections of UBX0101 into the mice’s joints 14 days after the trauma demonstrated roughly a 50% reduction in the number of senescent cells. In addition, gene expression representing reparative cartilage growth was activated in the joint after the treatment.

Older mice, known to have thinner joint cartilage and increased pain levels as compared to the younger mice, were treated as well. After the injections, the older mice exhibited reduced pain similar to their more youthful counterparts but did not exhibit signs of cartilage regeneration.

Human cultures

Cultures of human cartilage cells, removed from donors with clinically severe osteoarthritis, were exposed to treatment with 43 μ M UBX0101 for four days. The number of senescent chondrocytes, as determined by the SA- β -gal assay, were dramatically reduced and new, non-senescent cartilage began forming in place of the senescent cells.

Humans

In the fall of 2019, doctors began injecting UBX0101 into the knees of older human patients suffering from moderate to severe femoro-tibial osteoarthritis. Presently, the results of these preliminary treatments remain unavailable to the public. ⁽²⁰⁾

Hsp90 inhibitors

Hsp90s are proteins that serve as protein chaperones and whose expression are induced by heat shock, thus the name, Heat Shock Proteins. They are also numbered after their molecular size in kDa, and are generally ATP dependent.

Hsp90 plays a role in innumerable cellular functions including assisting client proteins with folding, stabilizing them against heat stress, and chaperoning these proteins through degradation. Hsp's are ubiquitous throughout the cell and come in multiple isoforms and homologs with distinct functions. In mammals, for example, the Hsp90 chaperone family comprises four homologs: HSP90 α , HSP90 β , Grp94 and Trap1. Inducible Hsp90 α and constitutively expressed Hsp90 β are the major homologs, sharing approximately 90% of their sequences.

HSP90 and its homologs are localized in different cellular compartments and interact with different co-chaperones and client proteins. HSP90a and b are mainly localized in the cytoplasm or the nucleus and phosphorylated by DNA-PK (P-HSP90). HSP90 can also be found secreted into the extracellular matrix or are membrane associated (eHSP90). The endoplasmic reticulum homolog of HSP90 is GRP94. Interaction of different co-chaperones and client proteins makes it a versatile platform engaged in a range of important cellular signaling pathways.

In order to influence very isolated cell activities, specific HSP inhibitors must be identified. A broad based, or pan-Hsp90 inhibitor could potentially precipitate catastrophic injury throughout the cell. The search, therefore, is on for Hsp Inhibitors that can act as senolytics without causing other cell injury.

Mechanism of Action

Down regulation of the anti-apoptotic PI3K/AKT pathway

Cells

Different Hsp90 inhibitors have demonstrated different effects in different cell types.

Geldanamycin

Senescent Ercc1 $-/-$ MEFs

17AAG (tanespimycin),

Senescent Ercc1 $-/-$ MEFs

17-DMAG (alvespimycin)

Murine mesenchymal stem cells (MSCs) (Ercc1-deficient mice)

IMR90 fibroblasts

WI38 cells.

Upregulation of phosphorylated AKT, a client protein of HSP90, has been shown to stabilize senescent cells from apoptosis. Inhibition of cytoplasmic HSP90 leads to AKT de-phosphorylation, making senescent cells susceptible to apoptosis, similar to what is seen in cancer cells.

Geldanamycin

Geldanamycin is a specific inhibitor of eHSP90, an antibiotic originally discovered in the bacterium *Streptomyces hygroscopicus*. eHsp90 (Secreted or membrane bound HSP90) client proteins include the matrix metalloproteinases MMP-2 and MMP-9, LDL receptor-like protein (LRP1) and EGFR2/Her/ErbB2 receptors. Geldanamycin cannot be used *in vivo* because of its high toxicity and potential to cause liver damage.

17AAG (Tanespimycin) 17-Demethoxygeldanamycin

The first semisynthetic derivatives of Geldanamycin, 17 AAG has a lower toxicity but the same potency as Geldanamycin and is currently in clinical trials.

17-DMAG (Alvespimycin)

17-DMAG is a more water soluble, geldanamycin-derived HSP90 inhibitor also starting clinical trials.

Radicicol (Monorden)

A macrocyclic antifungal structurally unrelated to Geldanamycin, that also specifically binds to Hsp90. Radicicol does not deplete cells of Hsp90, but rather increases synthesis as well as the steady-state level of this protein, similar to a stress response.

Mouse model

Using the *Ercc1* progeroid syndrome mouse model, 17-DMAG was administered three times per week, every three weeks at a relatively high concentration beginning at six weeks of age. Generally, these mice spontaneously develop age-related degenerative diseases and have a maximum lifespan of seven months. Treatment mice, however, had a significant reduction in a kyphosis, dystonia, tremor, loss of forelimb grip strength, coat condition, ataxia, gait disorder, and overall body condition. The therapeutic effect of 17-DMAG on healthspan by intermittent treatment was confirmed in a second, short term treatment cohort ^(12,13)

As yet, there are no commercially available Hsp90 inhibitors.

Agents with limited activity

Curcumin

The only study addressing this question reported that curcumin acted as a senolytic in mouse and human Intravertebral Discs (IVDs). ⁽⁷⁾

Piperlongumine

Piperlongumine, derived from the fruit of the Long pepper plant (*Piper longum*), comes from southern India and southeast Asia. It is known as a potent anti-inflammatory, anti-atherosclerotic and anti-tumor agent. Specifically, Piperlongumine suppresses the production of Tumor Necrosis factor- α and interleukin-6 and inhibits the activation of nuclear factor- κ B (NF- κ B) against proinflammatory responses. Importantly as a senolytic, it inactivates the phosphatidylinositol-3-kinase/protein kinase B (Akt)/mammalian target of rapamycin, mitogen-activated protein kinase 14/c-Jun N-terminal kinase, NF- κ B, and signal transducer and activator of transcription 3 (STAT3) pathways.

Macrolide antibiotics

In a fishing expedition for senolytic agents, researchers discovered that one commonly utilized antibiotic, Azithromycin, and one unknown antibiotic, Roxithromycin, were senolytic.

Mechanism of Action

Unclear

Cell type (s)

Both:

Human lung and skin fibroblast cell lines (MRC-5 for screening and BJ for validation)

“However, the effects of Azithromycin on mitochondrial oxygen consumption rates (OCR) were biphasic, showing inhibitory activity at 50 μ M and stimulatory activity at 100 μ M. These autophagic metabolic changes induced by Azithromycin could mechanistically explain its senolytic activity.”⁽²⁷⁾

Roxithromycin

More effectively killed senescent MCR -5 fibroblasts (70%), but also had a small, negative effect on the viability of normal MRC-5 fibroblasts.

In addition, Roxithromycin has been shown to effectively promote hair re-growth, possibly by stimulating the production of normal hair follicle stem cells. Mechanistically, this hair re-growth phenomenon could be due to the highly efficient removal of neighboring senescent skin fibroblasts. Moreover, Roxithromycin has also been reported to have stronger anti-inflammatory effects than both Azithromycin⁽²⁷⁾

Recommendations

The issue with taking antibiotics as senolytics is that they are antibiotics. Thus, the microbiome of the human body is unnecessary altered, which is not necessarily a good thing. However, if a patient does require treatment with these specific antibiotics, the benefits become twofold.

Histone deacetylase inhibitors

Panobinostat, a histone deacetylase (HDAC) inhibitor, was approved in 2015 for use with bortezomib (Velcade) and dexamethasone for resistant or advanced multiple myeloma.

In general, Histone deacetylases (HDAC) remove acetyl groups in the lysine residues of histones and non-histone proteins and thus regulate important cellular functions including gene expression, differentiation, proliferation and survival.

The medication has never been used individually in humans as a senolytic, but as part of a therapy for multiple myeloma. It was during these studies that the beneficial senolytic properties were discovered, demonstrating that Panobinostat eradicated persistent senescent cancer cells that arose post-chemotherapy. Unfortunately, there is no other information available.

Mechanism of action

- Pan-HDAC inhibitor
- Decreases BCL-XL expression
- Increases acetylation of Histone 3

Cell type(s)

- Chemotherapy-induced senescence of cancer cells, non-small cell lung cancer, and head and neck squamous cell carcinoma cell lines

Side effects: diarrhea, cardiac arrhythmias, especially QT prolongation

Conclusions

- There is reasonably clear evidence that senescent cells are omnipresent as people get older and have more pathologies, and that these cells are generally, but not entirely, detrimental.
- We do not know how many we have, nor how many we can eradicate with any particular treatment agent, but the reduction of said cells should be beneficial to improved or healthy aging.
- If we choose to use and recommend these therapeutics, it will be necessary to create educated guidelines as to what age or degree of infirmity to initiate the treatments, what agent(s) to utilize, and what the optimal dosing schedule will be.
- At the present time, the only agents that are available are fisetin, quercetin, and Dasatinib. In the next few years, this will undoubtedly be out as date as new therapies are emerging on the horizon.

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