Senolytics: A Potential Fountain of Youth

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ABSTRACT

Senolytics are groups of natural compounds, small molecules drugs, and methods that eliminate senescent cells in the body of an organism. This review illustrates the pathogenesis or factors that lead to cellular senescence. As cellular senescence is the main culprit for age-associated diseases, the development of various classes of senolytics is of paramount importance in maintaining or even extending our health-span. Different classes of senolytics with some perspective of its potential for clinical applications will also be discussed.

Keywords: Senescent cells, senolytics

ABSTRAK

Senolyitcs merupakan kelompok senyawa alami, obat dan metode yang melenyapkan sel-sel tua dari tubuh. Tinjauan ini membahas patogenesis dan faktor-faktor yang menyebabkan proses penuaan sel. Mengingat penuaan sel merupakan penyebab utama penyakit-penyakit akibat penuaan, pengembangan berbagai jenis *senolytics* sangat penting dalam upaya mempertahankan dan memperpanjang harapan hidup. Akan dibicarakan pula beberapa kelas *senolytics* beserta potensi klinisnya. **Khing S. Ong, Zack ST. Lim, Boenjamin Setiawan. Senolytics: Sumber Peremajaan yang Potensial**

Kata kunci: Penuaan sel, *senolytics*

INTRODUCTION

Cellular senescence is one of the hallmarks of aging.[1] As such, it is also multi-factorial in nature. Evidence from researches established that senescent cells are associated with most age-related diseases.[2] Reducing age-related diseases in older people, not only extend the health-span of senior citizens, will also has big impact on our socio-economic well being. Therefore, it would be most productive, that researches are looking on an array of possible ways to intervene the accumulation of senescent cells in different tissues or organs where the pathology is located.

Recently, there are a flood of studies on screening and or developing of a variety of potential candidates for senolytics.[3,4] Senescent cells arising from different stimuli exhibit distinct proteomes.[5] In addition, gene expression profiles of senescent cells are cell type specific.[6] Hence senescent cells are groups of heterogenous cells. As such, it is likely that no one senolytic can effectively eliminate all types of senescent cells.

PATHOGENESIS OF CELLULAR SENESCENCE

Cellular senescence is a process of cell aging, in which the cell loses its replicative capability via the shortening limitation of telomere, but the cell become resistance to apoptosis, with enhanced pro-survival phenotype, increase metabolic activity, and development of senescence associated secretory phenotype (SASP) that consists of various pro-inflammation cytokines, chemokines, factors effecting stem cell function, and growth factors, that causing tissue damaging microenvironment.[7]

There are a variety of stimuli that can induce cell senescence. Telomere erosion as mentioned induces a stable growth arrest. Loss of shelterin components such as TRF2 causes DNA damage response and premature senescence.[8] Cell senescence can also be induced by various stress situations such as irradiation, excess ROS, that mediated through DNA damage response that lead to cell senescence.

Under normal physiological condition, senescent cells are eliminated by our innate immune system, however in older people; this immune surveillance system has also become suppressed.

IDENTIFICATION OF SENESCENT CELLS

To identify senescent cells, ideally one would need specific surface markers, or intra-or extra cellular markers, in order to differentiate from neighboring healthy cells. Most of the plasma membrane proteins such as receptors, glycoproteins, and lipids on senescent cells are non-specific. The most frequently used biomarker for senescent cells is the increased activity of beta-galactosidase (B-gal) which reflects increased lysosomal mass.[9] In addition, there are markers for cell cycle genes, p16, p21, p53, and Rb can provide a mean to distinguish dividing cells from non-dividing cells. P53 and p16 tumor suppressor genes are strong markers in senescent cells, their expression induced permanent cell cycle arrest at G1 phase. Beside markers there are morphological changes of senescent cells with increased in size, sometimes more than twofold as compared to non-senescent cells,

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and its cell membrane became stiff. Activation of pRB tumor suppressor causes formation of senescence-associated heterochromatin foci (SAHF) that silence pro-replicative genes.[10] Senescent cells with persistent DNA damage repair signaling contain persistent nuclear foci called DNA segments with chromatin alterations reinforcing senescence or DNA-SCARS, which also includes dysfunctional telomeres, telomere dysfunction-induced foci or TIF[11] - a DNA damage foci at the telomere.

THERAPEUTIC INTERVENTION OF CELLULAR SENESCENCE

In general, there are two types of therapeutics. Senomorphics which reduces or suppress the fraction of senescent cells without killing the cells, where as senolytics specifically killed the senescent cells. Senomorphics also work to counter the deleterious effects of SASP.

Senomorphics

Examples are rapamycin, rapalog (synthetic analogues to rapamycin), NDGA (nordihydroguaiaretic acid), metformin, and NAD precursors, most of it work through the inhibition of mTOR pathway, that regulate cellular responses such as cell growth, proliferation, and apoptosis.

Senolytics

Most cancer drugs induced senescence of cancer cells. Paradoxically however, some cancer drugs are also senolytic. It is possible to find new senolytics by screening the cancer drugs. Senolytics can be classified according to senescent cell associated anti-apoptotic pathways.[12]

However, for practical purposes relevant to health-care providers, we propose the following categories of senolytics:

1. Natural compounds. This group includes polyphenols/flavonoid, such as:

- Quercetin, flavonoid found in many dietary foods such as apple, apricot, broccoli, Brussels sprout, cauliflower, etc. It induces senolysis on mouse senescent endothelial cells and senescent bone marrow stem cells, but not senescent preadipocytes, and embryonic fibroblasts.[3] However, when used in combination with other senolytics, such as dasatinib, the cell types covered expand.
- Fisetin, a flavonoid rich in strawberry, has also been shown to selectively induces apoptosis, however not in the proliferating IMR90 fibroblasts or in Human preadipocytes.[13] Fisetin also enhance cognitive function.
- Piperlongumine, a natural compound with many pharmacological effects, including preferentially killed human senescent WI-38 fibroblast.[14]
- Tocotrienols (T3), have 4 isomers: alpha, beta, gamma, and delta. Quercetin T3 also been showed to induce senescence and promote apoptosis in several cancer cell lines. Senolytic activity on T3 has not been tested, however since the metabolic and apoptotic pathways in T3 in cancer cells overlap with other compound such as quercetin that has been shown with senolytic activity, it suggest that rejuvenating effects of T3 may also be due to its possible senolytic activity.[15]
- Tocopherol or vitamin E is very similar in their effects and functions with T3. A PhD thesis studied to proof, if gamma tocopherol and gamma T3 are the cause of reduced incidence of prostate cancer in Mediterranean and South Asia. The study examined the NFkb (nuclear factor kappa beta) pathway known to inhibit apoptosis of cancer cells, and shown that gamma tocopherols and gamma T3 down regulate NFkb pathway, and up regulated caspase 8 suggesting induction of apoptosis.[16] Tocopherols however have not been tested for senolytic activity.
- Epigallocatechin-3-Gallate (EGCG), polyphenols in green tea. Studies have suggested EGCG as a senomorphic compound, however currently there is no evidence that it has senolytic activity.[17]
- Genistein, an isoflavonoid present in most bean, pea and lentil. Currently there is no information about whether it has senolytic activity or not, however its inhibitory effect on tyrosine kinase is similar to known senolytic dasatinib,[18] further investigation is warranted.
- Apegenin, a flavonoid found in certain fruits and vegetables, found to suppresses ASP of human fibroblasts strains induced by ionizing radiation,[19]

2. Inhibitors peptides and antibiotics

BCL-XL and BCL-2 inhibitors:

- BCL-XL (B cell lymphoma extra large) encoded by BCL2 like gene, its inhibitor 331852 and A1155463 are senolytic in human umbilical vein endothelial cells and in IMR90 cells (human lung fibroblasts), but not human preadipocytes.[20]
- Navitoclax an inhibitor of the BCL-2 family senolytic in cells types similar to BCL-XL inhibitors, but less specific and more hematological toxicity.[21]
- HSP90 inhibitors: These senolytics are antibiotics in nature, including geldanamycin, tanespimysin (17-AAG), both are ansamycin-derived heat shock protein- 90 inhibitors. HSPs are molecular chaperone important for protein stabilization and degradation.[22]

3. Forkhead box 'other' (FOXO)

FOXOs are a family of transcription factors with important roles in aging, longevity, growth, and stress signaling, regulate p21-cip1 a prominent factor in senescent growth arrest. They also inhibit the stemness regulator beta-catenin. As such they are ideal target to counter senescence.

Senescence cells carry type of DNA damage that should spur a protective protein p53 to induce apoptosis, but instead FOXO4 bind to p53 and prevent it from doing its duty. To counter this effect, a shorter version of FOXO4 called FOXO4-DRI (D-retro-inversion) was design to replace FOXO4 from p53 and induced apoptosis of the senescent cells.[23]

As FOXO4 binding to p53 is one of the hall marks of senescent cells that prevent the apoptosis of senescent cells. FOXO4-DRI can be a universal synolotic for all types of senescent cells.

4. Miscellaneous drugs and chemicals with effects on cellular senescence

Adiponectin, secreted by adipocytes has been shown to prevent diabetic premature senescent of endothelial progenitor cells by inhibiting ROS/p38 MAPK/p16-ink4a signaling.[24]

Hydrogen sulfide, member of gasotransmitter family, generated in our body by enzyme cystathionine gamma-lyase. H2S attenuate oxidative stress and delay cellular senescence.[25]

5. Viral senolysis

Using selectively-lytic viruses for cancer therapy is not new.[26] By applying the same strategy, senolytic viruses could be

developed against senescent cells. These are genetically modified viruses which replicate, lyse and kill cells in the presence or absence of specific gene product therefore allowed selective targeting.

6. Exercise induced senolysis.
   Not much research attention on the subject of exercise in this respect. With our limited search, we found only one publication[27] from Mayo Clinic team, that found in mouse model with metabolic dysfunction and increased markers of senescence induced by fast food, can be prevented by exercise.

CLINICAL TRANSLATION

All mentioned above are results of research studies on mouse models using genetically modify mice and wild type as control, or in-vitro study of culture human or mouse cell lines. There is a need to advance these 'proof of concept' results into clinical trial, after screening these senolytic for toxicity.

Recently a study[28] from John Hopkins on osteoarthritis induced in mice by cutting their anterior cruciate ligaments to mimic injury in knee joints. Then they use a specifically designed senolytic UBX0101 to selectively remove or lysis the senescent cells. UBX0101 significantly reduced the development of injury induced osteoarthritis.

Ideally, after toxicological screening, a pilot clinical study can be designed to test if this

senolytic also works in human osteoarthritis, which is a common disease in the elderly.

CONCLUSION

1. Potential viral senolytic is tremendous; when we consider manipulation of viruses for clinical applications over several decades have been relatively low risks and safe. These ranges from vaccine developments, attenuated viruses as vector in gene therapy, and oncolytic viruses for cancer therapy.
2. Exercise is another area which researches need to explore more intensively. We know exercise is good for our health in general, but how can it help older people with their age-related diseases. More evidence is needed to explain the benefit of exercise in delaying or preventing age-related diseases through senolysis.
3. Caloric restriction is classical for extending life span. Is there a link between caloric restriction and the development of senescent cells. Could an intermittent fasting also "senolytic" "or senomorphic"?
4. Natural compound such as quercetin and fisetin are available in the market as supplements. However, we are yet to see comparative to validate their beneficiary effects. The difficulty will be evaluation parameters; frailty and CRP (for degree of inflammation) might be some of the possible end-points to consider.

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