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Seno-suppressive molecules as new therapeutic perspectives in rheumatic diseases.

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Abstract

Over the past years, through *in vitro* studies and unique animal models, biologists and clinicians have demonstrated that cellular senescence is at the root of numerous age-related chronic diseases including osteoarthritis and osteoporosis. This non-proliferative cellular syndrome can modify other surrounding tissue-resident cells through the establishment of a deleterious catabolic and inflammatory microenvironment. Targeting these deleterious cells through local or systemic seno-therapeutic agent delivery in pre-clinical models improves dramatically clinical signs and extends health span. In this review, we will summarize the current knowledge on cellular senescence, list the different strategies for identifying seno-suppressive therapeutic agents and their translations to rheumatic diseases.

1. The concept of cellular senescence: an old story

Described and conceptualized by Hayflick and Moorhead in the 1960s, cellular senescence is a particular cellular response following either intrinsic signals that include telomeres attrition and mitochondrial deficiency, but also following inappropriate extrinsic stimuli such as free radicals, chronic exposure to inflammatory cytokines or trophic factors [1]. Senescence is dependent on a robust cell cycle arrest, associated with changes in cellular morphology, which becomes hypertrophic, altogether with metabolic adaptations, epigenetic modifications, and a senescence-associated heterochromatin foci (SAHF)-dependent transcriptomical signature (figure 1) [2]. Several cell cycle inhibitors such as the p16^{INK4a} or p21^{cdkn1a} proteins, which control cyclin-dependent kinase activities, participate to cell proliferation arrest. The senescence program will thus induce cellular functional changes such as apoptosis resistance, autophagy defects [1] and will allow attraction of immuno-clearance cells for instance macrophages and natural killer cells [3] while pushing the cells toward the establishment of a specific secretory phenotype called senescence-associated secretory phenotype (SASP) (Figure 1). Senescent cells could then influence surrounding tissue homeostasis by producing metalloproteinases, inflammatory, chemo-attractant and/or growth factors which favor senescence spreading [4]. Depending on the cell-types studied or the mode of induction, several subsets of cellular senescence can exist in organism [5-7]. Senescence should thus be considered more as a cellular syndrome rather than a single phenotype transposable to all senescent cells. Nevertheless, the expression of metabolic enzymatic activities such as acidic senescence-associated beta-galactosidase (SA-βGal) and an increase in cell cycle inhibitors are commonly accepted as universal senescence markers [2]. These features have allowed, in *in vitro* studies and in animal models, the detection of such cells in many mitotic and post-mitotic tissues [5].

2. Cellular senescence plays a role during development, tissue resilience and tissue functions.

Thus, based on such markers, a transient presence of cellular senescence has been found in placenta during embryo implantation [5]. Senescence onset also appears to coordinate limbs formation during mammalian embryogenesis [5] and limb regeneration in salamander [8]. Moreover, throughout life, by blocking aberrant cell proliferation, cellular senescence can be detected in primary neoplastic lesions therefore playing a central tumor suppressive role [1]. Furthermore, cellular senescence is also important in whole animal physiology by regulating the immune system and some terminal differentiation processes such as megakaryocytes [3]. Altogether, one believes that transient presence of senescence could coordinate tissue healing following injury by facilitating tissue remodeling and by regulating tissue immuno-clearance mechanisms during lifespan [3, 9].

3. Accumulating senescent cells links onset of age-related diseases and lifespan shortening in pre-clinic models.

Next to physiological roles, early studies revealed that the number of senescent cells progressively increases in numerous aging tissues [5]. This accumulation may be the result of intrinsic or extrinsic iterative signals that act continuously on tissue homeostasis through life. Thus, cumulative presence of senescent cells was detected within the cartilaginous, bone or muscle tissues in correlation with the emergence of their associated age-related degenerative diseases namely osteoarthritis (OA), osteoporosis (OP) or sarcopenia [10-12]. An increased number of senescent cells also appears in adipose tissue during age-related lipodystrophy, in arterial intima of patients suffering from atherosclerosis, in aging kidney or aging lung, related to renal and respiratory insufficiencies [2]. As mentioned, cellular senescence is dependent on a robust cell cycle arrest and the establishment of one specific tissue remodeling

secretory profile. By decreasing the renewal and repair capacities of the targeted organs, while creating chronic pro-catabolic and pro-inflammatory microenvironment, it was thus hypothesized that cumulative presence of senescent cells in aging tissues would be responsible for the emergence of various pathologies of the elderly. Although plausible, it was nevertheless necessary to demonstrate the veracity of such causal link between senescence and a progressive loss of tissue and organ functions with time. This demonstration came possible few years ago through the creation of transgenic animals expressing specific senescence biosensors. A first mouse model called p16-3MR (tri-modal reporter) consists, under the control of the promoter of the hallmark senescence marker p16^{INK4a}, in expressing the luciferase reporter gene fused to a truncated form of the herpes simplex virus 1 thymidine kinase (TK). Senescent cells expressing this TK-fusion protein become *in vitro* but also *in vivo* sensitive to the presence of ganciclovir, an antiviral drug [9]. The second model, called INK-ATTAC, consists, under the control of the same p16^{INK4a} promoter, in the expression of the luciferase reporter altogether with the caspase 8 suicide gene inducible by AP20187 molecule [13]. Both mouse models allow the detection of tissue-resident senescent cells, in the presence of luciferin and under CCD camera, but also their selective elimination through pharmacological modulation thus so-called senolysis. Through these models, the first demonstration of the beneficial effect for eliminating senescent cells was carried out on the premature aging mouse model expressing a hypomorphic form of the mitotic checkpoint serine/threonine kinase BubR1 (mouse BubR1^{H/H}). This kinase controls mitotic spindle assembly and deficient mice have a very short life expectancy by developing quickly after birth, cataract, sarcopenia and cardiac arrhythmias [13]. Remarkably, expression of INK-ATTAC construct followed by AP20187 administration, in this progeroid animal background, was sufficient to decrease senescent marker expression, to delay onset of numerous degenerative processes meanwhile increasing their lifespan [13]. All these findings were

translated to wild-type aging mice proving the universal efficiency of senescence removal on health and lifespan [14].

4. Cellular senescence hallmarks several osteoarticular diseases.

4.1. Articular senescent cells participate in both post-traumatic and spontaneous age-related osteoarthritis diseases onset and progression.

OA is the most common degenerative joint disease found in rheumatology: more than one-third of the population over 60 years old has radiographic evidence of osteoarthritis [15]. Prevalence increases with age [15] and in France, 4.7 % of men and 6.6 % of women are suffering from knee OA [16]. Each year, 4.6 million patients have a medical appointment for an OA-related symptom in France [17]. One of the major risk factors of developing OA is Chronological aging. But genetic predisposition, gender difference, some metabolic syndrome, cristal disease, or anatomic articular misalignments, and finally obesity-induced excessive joint loading could also play a role in OA onset [18]. To date, no curative medical treatment is dedicated to OA patients. The recently proposed new "slow-acting symptomatic drugs", such as glucosamine or hyaluronic acid joint injection, have not yet shown evidence of efficacy on long-term structural joint remodeling [19]. In recent meta-analysis, they proved efficacy only as pain-killer but had no effect on knee function and motility [20]. Data in humans are controversial for a structural effect on cartilage. Only symptomatic pain medications and innovative regenerative cell-based therapies remain the alternatives before orthopedic joint replacement. To develop innovative therapies, clinicians and biologists still need to better understand the full etiology of the multifaceted disease. We now know that several structures constituting the joint play a role in OA development: obviously the cartilage by expressing hypertrophic, catabolic, fibrotic markers but also the synovial membrane and

the sub-chondral bone [21]. The synovial membrane, which has a joint nourishing function, acquires during OA development an inflammatory and hypertrophic phenotype, thus contributing to the severity of the symptoms, to the formation of osteophytes and even to joint erosion [22]. Similarly, remodeling of the subchondral bone, located under the articular cartilage, is not only a consequence but also an active component of the disease, generating pain and loss of joint function [23]. To explain these OA-associated phenotypes, several cellular and molecular mechanisms have been proposed for the last 20 years (reviewed in [24]). But in early 2000, a role for cellular senescence as driving force in OA development was for the first time hypothesized by Martin *et al.* They indeed detected the presence of senescent p16^{Ink4a}-expressing chondrocytes harboring short telomeres within the joint of OA patients [25, 26]. Further studies demonstrate that *in vitro* p16^{ink4a} overexpression in 3D cartilage organoids could promote the production of hypertrophic catabolic enzymes matrix metalloproteinase (MMP)-1 and MMP-13 enzymes known as OA drivers [27]. The definitive causal link between the presence of articular senescent cells and OA pathologies came just recently with the use of the two previously described transgenic mouse models p16-3MR and INK-ATTAC [10]. An experimental post-traumatic OA (ptOA) through anterior cruciate ligament transection on one of the two knees was performed on two-month old p16-3MR mice. Intra-articular injection of luciferin in these animals revealed transient accumulation of senescent cells at 13 days post-surgery [10]. Through immuno-labeling, Elisseff J.H.'s team also detected senescent cells in the activated synovium and in the superficial areas of the cartilage [10]. To determine whether these senescent articular cells play an active role in the onset of induced ptOA, intra-articular injections of ganciclovir were done on the knee that had undergone surgery. Remarkably, the local elimination of these senescent cells was associated, at sacrifice of the animals, with less cartilage degradation, less articular expression of inflammatory markers such as interleukin (IL)-1 β and a decreased expression in hypertrophic

catabolic markers such as MMP-13 [10]. One of the main interests of this study was to find in the treated mice a higher expression level of two pro-chondrogenic markers, namely type II α 1 collagen and Aggrecan demonstrating the deleterious role of senescence on cartilage regeneration. To provide another convincing argument, the authors used the second INK-ATTAC transgenic model to study the spontaneous onset of OA in aged-mice [10]. Remarkably, treatment with AP20187, administered to these INK-ATTAC mice as early as 12 months until their natural death, maintains healthy cartilage, thereby reducing the occurrence of age-related signs of spontaneous OA [10].

4.2. Senescent osteocytes drive Osteoporosis

Osteoporosis (OP) altogether with osteopenia, is the second most frequent pathology touching the elderly population and accounted in rheumatologist waiting rooms. This skeletal disorder is characterized by a loss of bone mass and a disruption in bone micro-architecture favoring thus the risk of fracture. One third of postmenopausal women will be affected by OP and, in 40% of cases, osteoporotic lesions will be detected [28]. Osteoporosis is a disease characterized by reduced bone mass and deterioration of bone micro-architecture, leading to fragility and an increased fracture risk. Prevalence is high, ranging from 9% to 15% (based on total hip) and 16 to 38% (when spine data are included) for women in industrialized countries [29]. Men are also affected, with a global prevalence (when hip and spine were considered) between 3 and 8% respectively [29]. OP can also be a co-morbidity cause of death. Indeed, after a hip fracture in a woman over 65, the risk of death at 1 year increased to 20% [30]. Although widely studied and understood, the therapeutic management of OP remains today a problem. Inhibitors of bone resorption, such as bisphosphonate or denosumab, may be responsible for osteonecrosis of the jaw or atypical femoral fractures [31] [32]. In addition, if these drugs are effective enough to prevent vertebral fractures, their effectiveness is lower in the hip or wrist. Finally, the only commercially available bone-forming treatment, the

parathyroid hormone analog Teriparatide, suffers from stringent reimbursement criteria, which makes it difficult to prescribe in daily practice, and Strontium Ranelate, a combination agent, has recently been withdrawn from the market because of its cardiovascular side effects. These drawbacks justify further effort to identify new treatment strategies for osteoporosis. Osteocytes isolated from OP patients or from old mice express, similar to OA chondrocytes, several senescence markers [33]. Based on this finding, Far et al. used the INK-ATTAC transgenic mouse model to evaluate the impact of removing senescent cells on the bone architecture of old osteoporotic mice [11]. Following AP20187 oral administration, a decrease in number of senescent osteocytes but also in osteoclasts, the cells normally responsible for bone resorption, was detectable in treated old animals. Consequently, an increase in trabecular bone volume visualized by micro-Computed Tomography was associated with a greater number of bone trabeculae, and an improvement in bone biomechanical properties of treated old mouse. Altogether, these findings demonstrate the causal link between the presence of senescent cells and bone loss with aging.

4.3. Senescence marker detection in inflammatory rheumatic diseases

Next to OA in which aging is central, we found other rheumatic diseases that are more linked to systemic inflammatory situations and cartilage deterioration in all joints. Rheumatoid arthritis (RA) is the archetypical example for such disorders. With a frequency of 1-2% in the western population, RA is a multifactor autoimmune disease characterized by auto-antibodies and genetic susceptibility [34]. Interestingly, HLA-DRB1*4 subtype is one of the well-known RA susceptibility genes. This risk factor links accelerated telomere attrition with the high percentage of circulating senescent CD4⁺CD28^{null} T-lymphocytes cells in RA patients [35]. Indeed, an excessive telomere loss was detected in these patients in CD4⁺ T cells [35]. This accelerated telomere attrition during the first two decades of life would consequently reduce

homeostatic T cell proliferation during adulthood. Premature telomere loss also affected myeloid and lymphoid lineages in RA patients, suggesting that the hematopoietic stem cell is the primary target for premature senescence [35]. HLA-DRB1*4 alleles or other premature senescence-associated genes in linkage disequilibrium could then act on stem cell pool homeostasis and contribute to the accumulation of premature senescent and auto-reactive T cells in RA patients [36-38]. One proposed hypothesis is that these senescent T cells have altered receptor profiles. By acquiring these new sets of regulatory receptors, senescent CD4⁺ T cells become responsive to novel environmental cues and find ideal stimulatory conditions in the synovial microenvironment of RA patients [39]. Recently, links between cellular senescence and RA were further underlined. Flessler *et al.* published that senescent T-cells were able to promote osteoclastogenesis and therefore bone loss. Based on all these findings, it would be worth further assessing the consequence of depleting senescent CD4⁺ T cells in RA patients to offer innovative long-term treatments. Nevertheless, attempts to induce senescence through intra-articular p16^{INK4a} or p21^{cdkn1a} gene transfer in preclinical RA joint models gave therapeutic benefits. This unexpected finding probably highlights here the double-edged sword effect of senescence in tissue degeneration but also in tissue repair [40-42].

Of interest, T-cell senescence does not seem to be restricted to RA, but could also be found in other inflammatory rheumatic disease family members including axial spondyloarthritis [43] and the Juvenile Idiopathic Arthritis (JIA) [44]. JIA is the most frequent cause of inflammatory arthritis in childhood. Accumulation of CD8⁺CD28^{null} cells was originally described as a characteristic of JIA [44]. Down-regulation of CD28 is now considered as a marker of highly differentiated oligo-clonal lymphocytes [44, 45]. Remarkably, the naive T cell compartment of JIA patients also harbors shortened telomeres and reduced proliferative capacity. A recent study that analyzed CD8⁺ T cell subsets in JIA patients highlighted the over-representation of CD8⁺CD31⁺CD28^{null} cells that express the senescence marker p16^{INK4a},

accumulate DNA damage as revealed by γ -H2AX staining, and show reduced proliferative capacity, as expected for senescent cells [1, 44]. Independent of T cell receptor activation, PECAM-1 binding on these CD8⁺CD31⁺CD28^{null} cells can trigger the production of IFN- γ and IL-10, cytokines that are among SASP factors [44, 46]. Based on these sets of data it would be therefore essential to challenge the benefit of depleting senescent CD8⁺ T cells on JIA pre-clinical models as innovative therapies.

5. Targeting senescent cells through different seno-therapeutic strategies

The recent exploitation of transgenic mouse models in which one can detect and eliminate senescent cells, gave unique opportunities to link senescence state found in a given tissue and the emergence of degenerative pathologies (for review [2]). Corroborative associations have been demonstrated as described above in OA and OP but also in liver-associated steatosis, in chronic obstructive pulmonary disease, during kidney impairment or even brain aging [47]. All these studies showed that elimination of senescent cells delay the onset of numerous diseases and increase longevity in murine models (for review [2]). The resulting notion of so-called "senotherapeutic strategy" was proposed to *in vitro* and *in vivo* recapitulate specific senescent cell elimination as promoted by AP20187 or ganciclovir deliveries. This notion was also extended to any drug capable of reducing the deleterious effects on surrounding cells and tissues related to the presence of senescent cells. We will present here seno-therapeutic strategies currently developed and the list of available so-called seno-suppressive molecules with their potential clinical applications (Table 1).

5.1. Restoring sensitivity of senescent cells to cell death signals.

Through their resistance to apoptotic signals, senescent cells are long-lived cells characterized by hyper-activation of pro-survival signaling pathways. The cell cycle inhibitor p21^{cdkn1a}, the

anti-apoptotic protein Bcl-xL (B-cell extra-large lymphoma) [48], the heat shock protein HSP90 [49], the ERK/MEK pathway [50], the oxidative stress resistance protein (OR1) [51] and even the transcription factor Forkhead Box O4 (FOXO4) protein [52], are found among others contributors in protecting senescent cells against induced-cell death. Moreover, the production of SASP factors, including plasminogen activator inhibitor-1 (PAI1), CC pattern chemokine ligand 5 (CCL5) or ephrines EFNB-1 and 3, also favor apoptosis resistance [48, 50, 52, 53]. The obvious senolytic strategy proposed by scientists was therefore to target these pro-survival pathways in senescent cells in order to facilitate their elimination. Navitoclax (ABT263) was one of the first so-called "senolytic" compound tested. Originally developed to target tumor cells through its inhibitory activity of Bcl-2 family proteins, Navitoclax can also trigger an *in vitro* cell death on senescent fibroblasts [54]. Consequently, per os administration of this compound in irradiated mice allowed the rapid elimination of induced-senescent cells. As expected this treatment also decreased SASP production and, ultimately, reduced the clinical signs of several pathologies including pulmonary fibrosis [55]. Interestingly, the promising combination of Dasatinib and Quercetin was also proposed as seno-suppressive strategy. Dasatinib is a pan-inhibitor of tyrosine kinases used as a second-line treatment in onco-haematological conditions [56] through its ephrin-dependent survival signaling pathways inhibition [2]. Quercetin is a flavonoid used for its anti-oxidant, anti-inflammatory [48] as well as anti-serpin properties (for review [2]). Combination of Dasatinib and Quercetin shows synergistic effects for inducing cell death on senescent endothelial cells and preadipocytes [48] as well as extending lifespan and health span in mice [57]. Similarly, disrupting the interaction between FOXO4 and the p53 tumor suppressor by the use of an inhibitory peptide triggers *in vitro* senescent cell apoptosis and *in vivo* an improvement in renal function associated with senescence characterizing xeroderma pigmentosum group D (XPD) progeroid mouse model [52].

5.2. Modulating cGAS signaling pathways

Because senescent cells are not dividing cells, they show a specific reduction in the nuclear structure component Lamin B1 leading to an increase in cytosolic chromatin fragments [58]. These fragments will in turn activate the cyclic GMP-AMP Synthase (cGAS) signaling pathways promoting a cGAMP-dependent STING (STimulator of INterferons Genes) transcriptional activity for inflammatory genes [59]. Remarkably, mouse embryonic fibroblasts (MEF) deficient in cGAS protein show lower levels in SA-βGal staining, in expression of p16^{INK4a} and the inflammatory cytokine IL6, and a higher proliferation rate compared to wild-type mice [59]. Based on these data, small inhibitory molecules targeting STING have been developed and tested in a mouse model of auto-inflammatory diseases with promising results [60].

5.3. Restoring autophagy in senescent cells

Autophagy is a mechanism by which cells can recycle their dysfunctional components. Following cellular stress responses, autophagy and cellular senescence are tightly linked processes. On one hand, inhibiting autophagy delays HRAS-induced senescence and SASP production as autophagy process mobilized the reserves in amino acids and metabolites necessary for SASP factor production [61]. On the other hand, autophagy deficiencies trigger an increase in defective oxidized proteins that can drive senescence [62]. Autophagy has thus a dual role: showing a basal selective mode of action as anti-senescent by recycling at low level, impaired organelles (e.g.: non-functional mitochondria recycling), whereas impaired autophagy acts as a pro-senescent mechanism [63, 64]. Thus, restoring autophagy in senescent cells through pharmacological intervention can remove many senescence-associated features [53, 63]. Among them, the best characterized is Rapamycin. Indeed by inhibiting

mTOR pathway, Rapamycin regulates positively the Atg1/ULK complex involved in the autophagosome formation required for efficient autophagy [65]. As expected Rapamycin addition decreases SASP factors secretion in senescent ERCC1^{-/-} mouse fibroblasts [53] and can have pleiotropic positive effects on animal models [1]. Other compounds such as the sirtuins activator Metformin is also able to restore autophagy in senescent cells [66, 67] and therefore modulate SASP factor production [68]. Metformin has even been shown to extend nematodes and mice lifespan [69]. For these reasons, a clinical trial is ongoing to investigate whether this compound could delay age-related diseases (including cancer, cardio-vascular disorders and type 2 diabetes) that are associated with presence of senescence.

5.4. Taking advantage of metabolic adaptation induced by senescence syndrome

Next to the establishment of the SASP, cells that are undergoing senescence showed adaptive changes in their metabolism [70]. Schmitt's team was thus able to detect, upon chemotherapy, a glycolytic metabolic shift in induced senescent cancerous lymphoma blood cells. Administration of 2-deoxyglucose (2DG), an inhibitor of glycolysis, in combination with this chemotherapy, resulted in the complete elimination of circulating senescent cells and reduced dramatically the risk of cancer relapse in mouse model [71]. This finding paves the way for the development of new senolytic compounds that are taking advantage of these metabolic changes upon senescence onset.

5.5. Preventing senescence cell propagation by blocking SASP production

As mentioned above, senescence can be propagated to the surrounding cells through the deleterious paracrine and autocrine effects of the SASP. The stress-activated kinase p38^{MAPK}, the transcription factor NF- κ B, the Janus kinases (Jak1/2) or the chromatin modifying enzyme MLL1, are among others signaling pathways known to regulate senescent cell secretory

properties [2, 72]. Inhibition of such proteins through pharmacological interventions has been proposed as innovative seno-therapeutic strategy. Next to these approaches, we could also think of applying antibody-dependent immune-therapies to target SASP-associated circulating inflammatory factors. All these strategies will indeed reduce senescence spreading in *in vitro* studies [72] and could delay the onset of senescence-induced diseases in mouse models [11] [73] therefore demonstrating their therapeutic interests as seno-suppression.

6. Innovative seno-therapies as new perspectives for patients in rheumatology

6.1. Seno-therapies applied to OA

The discovery of the causal role for articular senescent cells in OA development prompts biologists and clinicians to test the potential efficacy of innovative senolytic drugs in OA models. The UBX0101 molecule developed by Unity Biotechnology was known to interfere with MDM2/p53 pro-senescent signaling pathway (www.unitybiotechnology.com). To test UBX0101 articular senolytic properties, they took advantage of the pre-clinical post-traumatic murine OA model, consisting in anterior cruciate ligament transection (ACTL), in which signs of articular deterioration appeared after 28 days post-surgery. An intra-articular injection in young animals of UBX0101, every 2 days, as soon as articular senescence was detectable after surgery significantly reduced cartilage degradation, OA-related pain, the expression of articular MMP-13, IL-6 and IL-1 β , while promoting the formation of neo-cartilage, with an increase in the production of type II collagen and aggrecan [10]. To translate these findings from murine models to OA patients, these authors offer additional evidence, by showing on human cartilage explants that *in vitro* UBX0101 addition reduces the expression of genes associated with senescence while increasing the proliferation rate of non-

senescent chondrocytes. By removing articular senescent cells, UBX0101 not only removes a causal actor in the OA process, but also restores a pro-chondrogenic environment. Thus, UBX0101 is a new promising seno-suppressive molecule in the medical arsenal for the management of ptOA. Its noteworthy that, years ago, without knowing its SASP inhibitory functions, the immuno-suppressive Rapamycin was also proven to have a local protective articular function in OA murine models [74, 75]. Finally, to further test the validity of such seno-therapeutic approach on spontaneous age-related OA, Jeon *et al* decided to treat old mice with UBX0101 either by local or by systemic administration. Interestingly, intraarticular UBX0101 injections showed no effect on cartilage degradation and did not improve chondrogenic gene expression unlike what was observed in young mice with ACLT. To conclude, intra-articular injection of senolytic compounds in spontaneous age-related OA patients are therefore unlikely to be sufficient. Systemic delivery should be then considered in order to eliminate other deleterious senescent cells found specifically in aged tissues and that could participate to progressive global loss of body functions with time [3]. The benefit / risk ratio of the systemic administration of such therapies needs to be carefully studied and accepted by the patient and care providers. Nevertheless, Jeon *et al* study led Unity Biotechnology to launch a phase I clinical trial to evaluate the safety of UBX0101 intra-articular injection in patients with painful OA knee (clinicaltrials.gov/ct2/show/NCT03513016).

6.2. Seno-therapies applied to OP

The elimination of senescent osteoblasts could restore bone structure in old transgenic mice. Based on these findings, biologists and clinicians therefore challenge the idea of targeting senescent cells as new therapeutic strategies for OP conditions. For instance, the intermittent oral administration of combining Dasatinib and Quercetin (1 gavage, once a month, for 4

months) to 20-month-old osteoporotic mice, shows as expected a decrease in senescent osteocytes, a decrease in p16^{Ink4a} protein expression as well as a significant improvement in the trabecular and cortical bone architecture [11]. Similarly, chronic treatment for 2 months of old mice with ruxolitinib, a JAK1 and JAK2 kinase inhibitor, at the origin of SASP, show a quite similar improvement in trabecular bone parameters and cortical while reducing the number of osteoclasts. Therefore, deleterious paracrine effects of senescent osteoblast cells in promoting osteo-clastogenesis could be blunt by Jak inhibitors [11]. Similar to its described intra-articular seno-suppressive beneficial effect, systemic delivery of Rapamycin, can *in vivo* restore bone loss in aged mice by reverting senescent onset in bone-marrow derived mesenchymal stem cells [76].

6.3. Seno-therapies applied to inflammatory rheumatic diseases

Numerous clinical applications have been developed in the last 15 years to target chronic inflammation and to reduce joint deterioration or pain in patients with autoimmune diseases. These applications could in fact be qualified as seno-suppressives. In particular, TNF inhibitors, which target one of the main SASP actors are classical biological agents commonly used to treat RA. Remarkably, recent evidence demonstrated that such compounds act through a reduction in T lymphocyte replicative senescence and enhanced their telomerase activities [77] demonstrating their seno-suppressor effects. Tocilizumab is an antibody against IL-6 receptor used in daily practice in patients with rheumatoid arthritis. This bio-molecule could thus be considered as seno-suppressive agent, as the specific blockade of IL-6 attenuated the inflammatory response associated with senescence [78]. Moreover, as mentioned above, Jak kinases are at the root of SASP establishment, their pharmacological inhibition by Tofacitinib or Baricitinib molecules are commonly given in some RA indications to circumvent chronic inflammation [79, 80]. Knowing the presence of senescent

cells in RA, the therapeutic efficiency of such inhibitors could be related to their anti-SASP roles with the exception for rituximab, a monoclonal anti-CD20 antibody that targets B-cells, which instead promotes cellular senescence [81]. Finally, although not routinely prescribed, we should also notice that the medical literature mentioned therapeutic efficacy of another seno-suppressive molecule namely Rapamycin in JIA patient [82].

7. A medical revolution is under way in rheumatology

In this review we have given strong evidences that establish a link between the cumulative presence of senescent cells within tissues, in particular osteo-articular tissues, and the emergence of organs-related pathologies. Seno-therapeutic interventions based on the elimination of senescence in a given tissue constitute a veritable medical revolution for elderly patients. Nevertheless, several open questions remain to be solved before getting these approaches generalized into clinics. First, as mentioned, cellular senescence is a physiological process that coordinates tissue repair and prevents tumor progression [1]. Long-term delivery of systemic seno-therapies will raise serious concerns on their safety for human health. By chance, cellular senescence exists under different “flavors” in the body depending on the concerned cell-type and the mode of induction. The discovery of universal seno-suppressive compound as fountain of youth that could target all senescent cells in one organism is not for tomorrow. As developed in cancer therapies, it would be therefore essential of clustering senescence sub-types found in different physio-pathological conditions. This will allow the development of seno-suppressive molecules having the lowest side effects and the best-adapted effects to well-defined diseases. These targeted seno-therapies would be applied in the next future to patients suffering from a variety of osteo-articular pathologies including OA, OP but also other chronic inflammatory diseases. The deliveries of such therapies, in the form of iterative treatments and even localized to a single organ as in OA joint, will leave the

possibility for other relative senescent cells, elsewhere in the body, to express their beneficial properties.

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Figure and table legend

Figure 1: Cellular characteristics induced by senescence syndrome.

List of abbreviation: cGAS: cyclic GMP-AMP synthase; SAHF: senescence-associated heterochromatin foci; SASP: senescence-associated secretory phenotype.

Table 1: List of seno-suppressive strategies and their associated molecules currently available.

Metformin is used in daily clinical practice in humans for melitus diabetes and metabolic syndrome. Tofactininb and baricitinib are drugs prescribed for patients with rheumatologic inflammatory diseases. Dasatanib, rapamycin and ruxolitinib are in current use for hematopoietic malignencies, solid organ transplantation and myeloproliferative disease respectively. Phase I, II or III trials in cancer have been published with navitoclax, trametinib, alvespimycin (17-DMAG) and 2-deoxyglucose. FOXO4-DRI peptide, piperlongumine, and H-151 are not used in patients. Quercetin is a flavonoid found in the alimentation.

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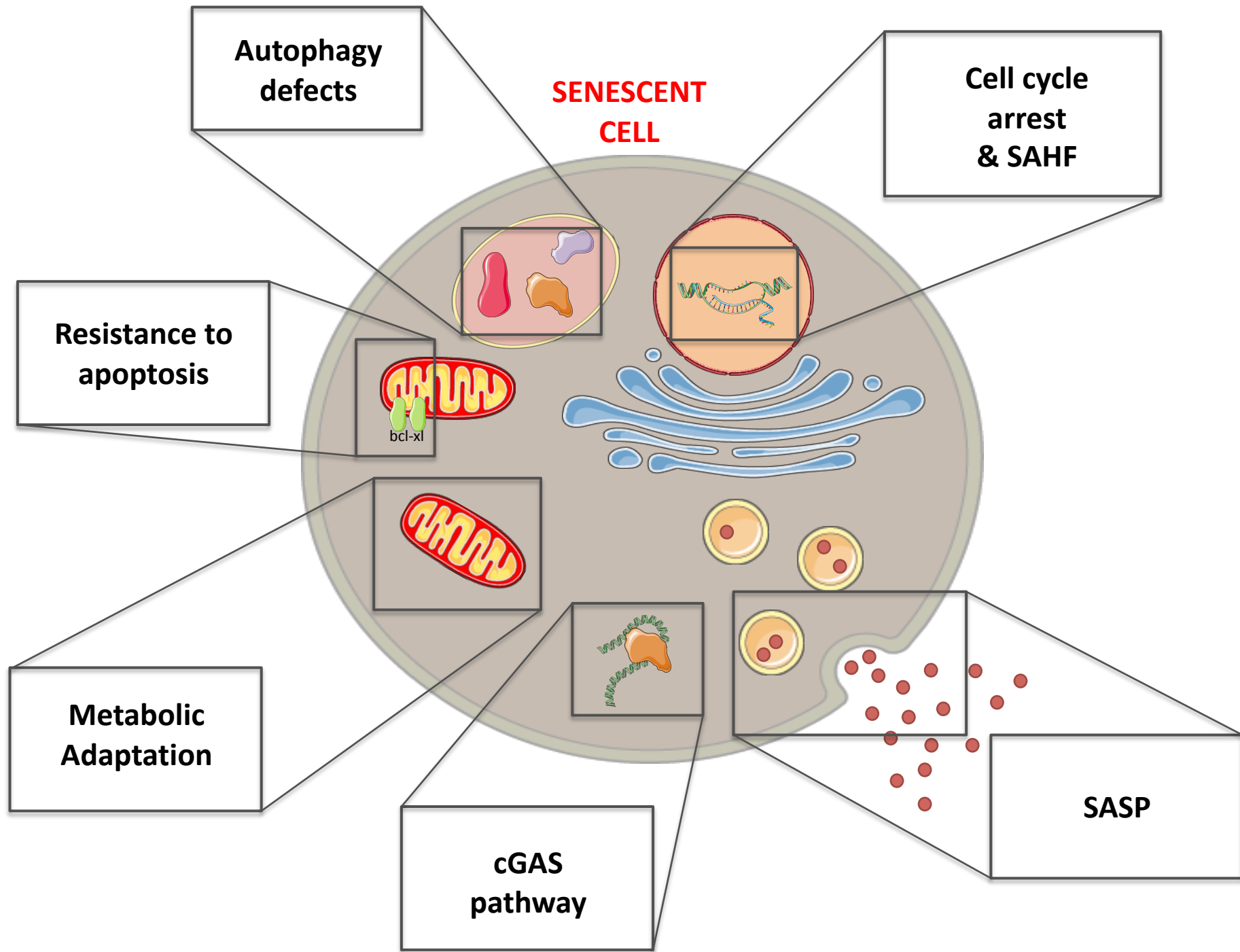
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Drugs	Action mechanisms (involved in senescent cells clearance)	Potential senescence linked- diseases treatment	Refs
Pharmaco-genetic approach			
AP20187	activate caspase 8 to induce apoptosis in INK-ATTAC mouse model	Osteoarthritis, osteoporosis, sarcopenia, cataract, heart failure, chronic kidney disease	9, 10, 12, 13
Ganciclovir	HSV1 tyrosine kinase inhibitor in p16-3MR mouse model	Osteoarthritis, osteoporosis, Parkinson disease	9, 10, 46
Restoring sensitivity of senescent cells to death signals			
UBX0101	MDM2-p53 interaction inhibitor	Osteoarthritis	9
Dasatinib + Quercetin	D : pan-inhibitor of tyrosine kinases, and ephrin-dependent survival signaling pathways inhibition ; Q : serpin inhibitor	Osteoporosis	10
Navitoclax	Bcl-xl inhibitor	Pulmonary fibrosis, atherosclerosis	2,54
FOXO4-DRI peptide	Inhibitor of FOXO4-p53 interaction	chronic kidney disease	51
piperlongumine	OR1 inhibitor	/	50
trametinib	MEK/ERK inhibitor	/	49
Alvespimycin (17-DMAG)	HSP90 inhibitor	/	48
Metabolic adaptation			
2-deoxyglucose (2DG)	Glycolysis inhibitor	Lymphoma	70
Restoring autophagy			
Rapamycin	mTOR inhibitor, autophagy inducer	Osteoarthritis, osteoporosis, JIA/sarcopenia	62, 73, 74, 75,79
Modulating cGAS-STING pathway			
H-151	STING covalent inhibitor	Autoinflammatory disease	59
SASP inhibitor			
Ruxolitinib	janus kinases 1 and 2 inhibitor	Osteoporosis	10
metformin	Sirtuin 1 activator, interferes with IKK/NF-κB activation	/	67, 68
Tofacitinib & Baricitinib	Janus kinases 1 & 3 (tofacitinib) and 1 &2 (baricitinib) inhibitor	Rheumatoid arthritis	77, 78

SENO-THERAPIES

