Chapter

Interventional Strategies to Delay Aging-Related Dysfunctions of the Musculoskeletal System

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Abstract

Aging affects bones, cartilage, muscles, and other connective tissue in the musculoskeletal system, leading to numerous age-related pathologies including osteoporosis, osteoarthritis, and sarcopenia. Understanding healthy aging may therefore open new therapeutic targets, thereby leading to the development of novel approaches to prevent several age-related orthopaedic diseases. It is well recognized that aging-related stem cell depletion and dysfunction leads to reduced regenerative capacity in various musculoskeletal tissues. However, more recent evidence suggests that dysregulated autophagy and cellular senescence might be fundamental mechanisms associated with aging-related musculoskeletal decline. The mammalian/mechanical target of Rapamycin (mTOR) is known to be an essential negative regulator of autophagy, and its inhibition has been demonstrated to promote longevity in numerous species. Besides, several reports demonstrate that selective elimination of senescent cells and their cognate Senescence-Associated Secretory Phenotype (SASP) can mitigate musculoskeletal tissue decline. Therefore, senolytic drugs/agents that can specifically target senescent cells, may offer a novel therapeutic strategy to treat a litany of age-related orthopaedic conditions. This chapter focuses on osteoarthritis and osteoporosis, very common debilitating orthopaedic conditions, and reviews current concepts highlighting new therapeutic strategies, including the mTOR inhibitors, senolytic agents, and mesenchymal stem cell (MSC)-based therapies.

Keywords: Stem cells, senescence, mTOR, Osteoarthritis, Osteoporosis, aging

1. Introduction

Aging is a process of progressive loss of physiological function and reserve, characterized by cellular senescence, stem cell exhaustion, DNA damage, telomere attrition, and deregulated nutrient sensing [1]. It is well known that aging-related stem cell depletion and dysfunction leads to reduced tissue regenerative capacity in various stem cell populations. Osteoarthritis (OA) and osteoporosis (OP) are two common aging-related skeletal diseases that influence the quality of life in the elderly.

Osteoarthritis is one of the most common chronic degenerative joint diseases that cause joint pain and dysfunction. Multiple factors, including mechanical, genetic, and aging-related factors, are involved in the development of OA [2]. At the cellular level, it is characterized by loss of tissue cellularity, and phenotypic changes to chondrocytes, and subsequent damage to the extracellular matrix (ECM) [3]. Chondrocytes are resident cells in cartilage tissue and are involved in both the synthesis and turnover of the ECM [4]; therefore, maintaining chondrocyte health is an essential factor in preventing articular cartilage degeneration. With age, articular cartilage ECM degrades and remodels with the fragmentation of the principal proteoglycan protein aggrecan, chondroitin sulfate, and relative increases in keratan sulfate and hyaluronan deposition [5, 6]. Chondrocytes also exhibit an autolytic phenotype causing the proteolytic cleavage of various collagen molecules [7]. Collectively, such ECM perturbations during aging lead to a concomitant decrease in water content, affecting tensile strength of the cartilage and transmission of load.

Osteoporosis is a systemic bone degenerative disease characterized by a progressive loss of bone mass accompanied by a significant reduction in the mechanical strength of the bones [8]. Bone loss during aging is also characterized by changes in bone shape, mineral content, adiposity, mineral turnover, and reduction in bone forming osteoblasts [9, 10]. Another bi-product of aging is a reduction of bone healing capacity exacerbated by chronic inflammation inherent with advancing age [11] that also disrupts homeostatic interactions between signaling factors and bone cells, resulting in a state of dysregulated remodeling and bone turnover. Thus, aging is one of the factors most closely associated with the development of OP [12]. Brittle bones, as a result of OP in the elderly have been known to cause fractures triggered by minor trauma, significantly worsening the quality of life and even reduce life expectancy [13].

Recent research is beginning to unravel the mechanisms of how aging makes bones and joints more susceptible to the development of these diseases. Understanding the underlying mechanisms may provide new treatments to delay or prevent the development of these aging-related orthopedic disorders. This chapter focuses on OA and OP among aging-related dysfunctions of the skeletal system and reviews current concepts on new therapeutic strategies, especially the mammalian/mechanistic target of Rapamycin (mTOR) inhibitors, senolytic agents, and mesenchymal stem cells (MSCs).

2. Autophagy as a therapeutic target in aging-related orthopedic diseases

2.1 Autophagy and mTOR in OA

Autophagy is an essential homeostatic process for the clearance of damaged intracellular components [14]. It is conserved evolutionarily across species and is commonly known to be activated under stress conditions, including nutrient-deficiency, to generate energy [15]. When cells face nutritional stress, the autophagy pathway is activated to degrade unnecessary intracellular components while maintaining only the minimum essential components to prevent energy loss. Recent evidence has linked autophagy in the pathophysiology of OA. Inhibition of autophagy has been reported to promote the expression of OA-like genes induced by interleukin-1β (IL-1β), whereas activation of autophagy reduces the intracellular reactive oxygen species (ROS) by removing damaged mitochondria, thereby protecting chondrocytes from OA-like changes [16]. During aging and especially in OA, autophagic flux is diminished, likely due to decreases in autophagy regulator genes ULK1, beclin-1, and LC [17], and is associated with increased chondrocyte

apoptosis and mitochondrial dysfunction [18]. These results suggested that increased autophagy is an adaptive response to protect chondrocytes from stress and that autophagy regulates OA-like gene expression changes via the modulation of apoptosis and ROS [19]. Thus, the modulators of autophagy, such as mTOR, may represent key targets to for the treatment of OA.

mTOR is a serine/threonine protein kinase that is a negative regulator of autophagy, integrates inputs from nutrients and growth factors, and regulates many basic cellular processes through two distinct protein complexes, mTORC1 and mTORC2. mTOR is an established longevity axis, and its inhibition either pharmacologically or genetically, has been demonstrated to extend lifespan in numerous species [20–22]. Recent studies suggest that mTOR plays an important role in cartilage growth and development, alters articular cartilage homeostasis, and contributes significantly to the cartilage degenerative process associated with OA [23] mTOR expression has been shown to be elevated in OA models and has been associated with increased chondrocyte apoptosis [24]. Given that mTOR is a negative regulator of autophagy, the deleterious effects of age-associated increases in mTOR activity can be linked to decreased autophagy in chondrocytes during OA. Pre-clinical experiments in rat OA models have also shown that suppressing the PI3K/AKT/mTOR signaling pathway promotes articular chondrocyte autophagy and alleviates inflammation [25]. Both pharmacological and genetic approaches for inhibiting mTOR signaling have been shown to reduce the severity of OA in pre-clinical animal models [24–27].

2.2 Pre-clinical studies targeting mTOR for the treatment of OA

Rapamycin is the oldest known natural mTOR inhibitor that has been traditionally used clinically as an immunosuppressive agent [28]. Rapamycin acts through binding of FK506-binding proteins and primarily destabilizes mTORC1, but to some extent can prevent the phosphorylation of downstream targets of mTOR1 [29–31]. Since its FDA approval in 1999, Rapamycin has been used by millions of patients. There is a litany of clinical evidence suggesting that Rapamycin is a safe and effective drug with few side effects for which all are reversible [31, 32].

Interestingly, recent studies have shown that Rapamycin acts to activate human chondrocyte autophagy *in vitro* primarily by inhibiting mTOR complex 1 (mTORC1) and suppressing the development of OA-like changes [19]. Furthermore, systemic administration of Rapamycin has been shown to reduce the severity of OA through activation of autophagy in a mouse model [26].

Our group has found that intra-articular injection of Rapamycin was a safe and effective therapeutic delivery method to protect articular cartilage from osteoarthritic changes in a mouse model of OA [27]. On the other hand, deletion of mTOR has been shown to up-regulate autophagy and protect mice from OA in inducible cartilage-specific mTOR knockout mice [24]. Torin 1, a selective ATP-competitive inhibitor of mTOR, which can cause induction of autophagy, is also regarded as a potent inhibitor of both mTORC1 and mTORC2. In a rabbit model of OA, intra-articular injection of Torin 1 was shown to reduce articular cartilage degeneration [33, 34]. Another agent with demonstrated ability to reduce mTOR activity is Metformin, which is FDA-approved for the treatment of type 2 diabetes [35]. Metformin has been shown to activate 5' AMP-activated protein kinase (AMPK), a negative regulator of mTOR. Recently, Metformin has also been shown to inhibit cartilage degeneration in OA mouse models by downregulating mTOR [36]. In another murine study, Metformin was shown to reduce OA structural worsening and reduce pain scores [37]. Therefore, multiple lines of evidence support the theory of mTOR signaling as a promising therapeutic target for OA, mainly through the modulation of autophagy.

2.3 Autophagy and mTOR in OP

Multiple proteins involved in the autophagic activity are essential for the survival, differentiation, and function of bone cells, including osteocytes, osteoblasts, and osteoclasts [38]. Autophagy is critical for the necessary crosstalk between bone resident cells and thus plays a critical role in the signaling dynamics for bone synthesis (osteoblasts) and degradation (osteoclasts). Dysregulation in the level of autophagic activity has been found to disrupt the balance between bone formation and bone resorption linked to the onset and progression of OP [38–42]. In addition, autophagy plays an important role in MSC function and lineage determination from adipogenesis to osteoblastogenesis, [43] and it has been linked to the increased adipogenic differentiation in bone MSCs [44]. Autophagic activity is known to decrease with age, especially in bone cells, [45] hence the regulation of autophagic activity is considered a promising strategy for the prevention and treatment of OP [14, 46].

Osteoblast dysfunction is a significant cause of aging-related bone loss, but the mechanisms underlying osteoblast dysfunction with aging are not fully elucidated. mTOR has been shown, through in-vitro studies, to regulate osteogenic genes (Runx2, Osterix), stemness genes (Oct3/4, Nanog), and mineralization through alkaline phosphatase production [47].

2.4 Pre-clinical studies targeting mTOR for the treatment of OP

Several studies have investigated the efficacy of targeting mTOR for the treatment of OP. Systemic delivery of autophagy modulators such as Rapamycin and its analogs have been tested in a number of animal models. In a study using 24-month-old rats, micro-CT showed that Rapamycin effectively inhibited aging-related bone loss in trabecular bone. In this study, Rapamycin treatment resulted in a significant decrease in the number of osteoclasts, as well as the induction of osteoclast autophagy and a decrease in osteocyte apoptosis compared to the control group [48]. Besides, mTORC2 signaling stimulates osteoblast differentiation and is involved in aging-related OP [49]. The expression of Rictor, a specific component of mTORC2, is decreased in osteoblasts during aging, which may contribute to aging-related bone loss, and deletion of Rictor in osteoblasts has been shown to accelerate aging-related bone loss in a mouse model [49]. The use of Everolimus, a Rapamycin analog and predominant mTORC1 inhibitor, has been shown to protect against OP onset in ovariectomized rats through the reduction of osteoclast formation and cathepsin K mediated matrix degradation [50]. Rapamycin has also been shown to reduce the severity of age-related bone conditions in trabecular bones of aged male rats by activating osteocyte autophagy [48]. Taken together, mTOR may play a critical role in aging-related OP and represents a promising therapeutic target.

3. Cellular senescence: a new therapeutic strategy for the treatment of aging-related musculoskeletal decline

3.1 Senescent cells

Senescent cells play a vital role in the aging process and promote degenerative diseases, geriatric syndromes, and potentially malignancy through the production of a Senescence Associated Secretory Phenotype (SASP) characterized by pro-inflammatory and catabolic anti-regenerative factors [51]. Senescence is a state

defined by replicative arrest and resistance to apoptosis with altered metabolic activity, and several factors, often related to cell or tissue damage, can induce senescence, including DNA lesions, mechanical/shear stress, reactive metabolites, proteotoxic stress, and inflammation [51]. When present, these factors can activate one or more pathways through the $p16^{Ink4a}$/retinoblastoma protein, $p53/p21^{Cip1}$, or other transcription factor cascades, resulting in cell cycle arrest, metabolic shifts, altered gene expression, and the production of deleterious SASP factors [51, 52].

Senescent cells accumulate in tissues throughout the lifespan and are normally removed by the immune system [53]. However, inefficient removal due to chronic stress or pathology may exceed the capacity of the immune system, especially during aging, where chronic inflammation disrupts the homeostatic immunologic clearance mechanisms [51, 53–55]. SASP factors from senescent cells are especially deleterious and include various cytokines, chemokines proteins, growth factors, and tissue degrading matrix metalloproteinases (MMPs) [51, 56, 57]. These factors stimulate inflammation, ECM degradation, fibrosis, and secondary senescence in surrounding cells [51, 54, 58]. SASP components can be cell-type specific, and senescence triggers are influenced by several factors, including hormones, stress, drugs, and pathogens [51]. A schema showing the association between senescence and OA is shown in **Figure 1**. With an increased number of senescent cells, a higher amount of secreted SASP components may cause an inflammatory, apoptotic, and cell- and tissue-destructing effect, eventually resulting in aging and chronic diseases. For these reasons, targeting senescent cells has garnered significant attention for the treatment of age-associated pathologies, especially musculoskeletal conditions [59].

According to the "geroscience hypothesis" [60], targeting senescent cells is appealing as they are a fundamental property of aging and may thus delay or reverse physiological consequences of the aging process or the development of aging-related diseases [51, 58]. However, there are no established markers

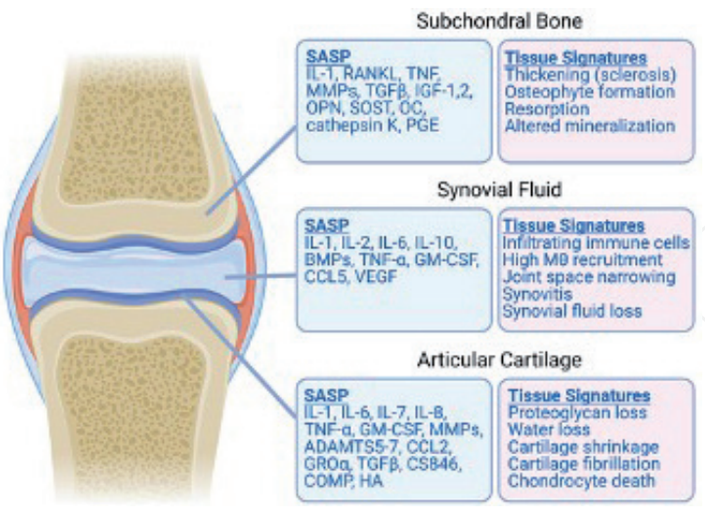

Figure 1.
Schema of the association between senescence and OA. Aging is accompanied by the secretion of the senescence-associated secretory phenotype (SASP), including various chemokines, cytokines, proteases, and growth factors, which act alone or together to cause degenerative changes in the subchondral bone, synovial fold, and articular cartilage, ultimately leading to OA. IL, interleukin; RANKL, Receptor activator of NF-κβ ligand; TNF, tumor necrosis factor; MMPs, Matrix metalloproteinases; TGFβ, Transforming Growth Factor-β; IGF, insulin-like growth factor; OPN, Osteopontin; SOST, Sclerostin; OC, Osteocalcin; PGE, Prostaglandin E; BMPs, Bone morphogenetic proteins; GM-CSF, Granulocyte Macrophage colony-stimulating Factor; CCL, C-C motif chemokine ligand; VEGF, vascular endothelial growth factor; GMCSF, Granulocyte-macrophage-colonystimulating factor; ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; GROα, Growth-related oncogene-α; CS-846, The 846 epitope of chondroitin sulfate; COMP, Cartilage oligomeric matrix protein; HA, Hyaluronic acid.

universally specific to senescent cells [51, 54]. Higher expression of $p16^{Ink4a}$ and $p21^{Cip1}$ usually occur in senescent cells [61, 62]. One of the prominent hallmarks, however, is resistance to apoptosis [63]. Zhu et al. reported that senescent cells anti apoptotic pathway (SCAP) networks are expressed at higher levels compared to non-senescent cells, playing an essential role in the resistance to apoptosis [64]. Of 39 transcripts targeted by small interfering RNAs (siRNAs), six transcripts (ephrin ligand (EFN) B1, EFNB3, $p21^{Cip1}$, plasminogen-activated inhibitor-2 (PAI-2), phosphatidylinositol-4,5-bisphosphate 3-kinase delta catalytic subunit (PI3KCD), and BCL-xL) were found to downregulate SCAPs and elicit apoptosis in senescent cells. Targeting several SCAP pathways may also be necessary for senescent cell removal and may further increase specificity for senescent cells while not affecting healthy non-senescent cells [51, 64].

3.2 Treatment of aging-related skeletal diseases with senolytic agents

The use of senolytic agents is a promising approach to delay aging and reduce the severity of chronic diseases through senescent cell depletion [58]. Various drugs have now been characterized that demonstrate the ability to eliminate senescent cells, namely through reduction in anti-apoptotic signaling [65]. For example, Fisetin is a recently characterized senolytic flavonoid phytonutrient found in fruits and vegetables that can extend health and lifespan in naturally aged and progeroid mice [66]. Yousefzadeh et al. tested ten flavonoids and found that Fisetin demonstrated the highest senolytic activity [66]. Fisetin has been found to target senescence associated pathways such as SIRT1, [67] BCL-2/BCL-X_L, [68, 69] HIF-1α, [70] p53/MDM2, [69, 71] and AKT, [69, 72] leading to elimination of senescent cells. However, it also has the ability to reduce oxidative stress via SIRT1/Nrf2, [73] decrease mitochondria-derived ROS via inhibition of GSK3β, [73] and exhibit anti-inflammatory effects. Another example, Metformin, is a widely prescribed anti-diabetic drug with lifespan extending effects in mice, [74] and exhibits senolytic activity via targeting some similar, but also several different senescence associated pathways from Fisetin. Metformin is known to activate AMPK, which indirectly inhibits mTOR, the most established nutrient sensing longevity regulating pathway to date [75]. In addition, Metformin reduces oxidative stress and SASP related inflammation through inhibition of NF-kB activity, [75, 76] and inhibits insulin and IGF-1 signaling, which are all hyperactivated during aging [75, 77]. Thus, many senolytic drugs have pleiotropic effects that mitigate age-related cellular and physiological dysfunction. Furthermore, senolytic agents can be administered intermittently since they do not interfere with a receptor or an enzyme, thereby reducing potential side effects [54]. Accordingly, intermittent treatment may maintain the positive effect of senescent cells on wound healing, cancer prevention, and homeostasis [78–81].

The list of agents with senolytic potential is growing. Zhu et al. tested 46 potential senolytic agents and found that Dasatinib and Quercetin demonstrated particularly good results in terms of reducing senescent cells [64]. Dasatinib is a tyrosine kinase inhibitor used in cancer treatment and is known to interfere with ephrin-dependent suppression of apoptosis [82, 83]. Quercetin is a naturally occurring flavonoid found in several fruits and vegetables known to inhibit PI3K, kinases, and serpines [64]. Noteworthy, Dasatinib, in combination with Quercetin was able to induce apoptosis more effectively in a broader range of senescent cell types than either one alone [84]. Navitoclax, a lymphoma drug, inhibits the anti-apoptotic proteins in the Bcl-2 family and has been used in cancer treatment [85]. It has demonstrated senolytic effects in several cell types *in vitro*. However, in aged mice,

it demonstrated reduced trabecular bone volume and impaired osteoblast function [86]. Additionally, Navitoclax is directed against a small number of SCAP network molecules and has shown poor specificity for senescent cells with increased risk of side effects, including thrombocytopenia due to its effect on platelets [87]. Thus, not all drugs with senolytic ability are clinically suitable due to a range of potential side effects or drug-to-drug interactions with existing medications. However, the ability to transiently dose and the existence of natural compounds with favorable safety profiles (ex. Fisetin, Quercetin, Metformin, Rapamycin, Geldanamycin etc.) offer the potential for continuous administration.

Some senolytic agents have been tested for their effects on the musculoskeletal system, demonstrating a potential to reduce aging-related diseases and conditions. Here we will focus on the use of senolytic agents in the setting of OA and OP.

3.2.1 Agents for the treatment of OA

3.2.1.1 Pre-clinical studies of senolytic agents for the treatment of OA

Senescent cells have long been associated with OA [88, 89]. Articular cartilage from patients with advanced OA have a significant number of senescent chondrocytes, [90, 91] with canonical hallmarks of senescence, including metabolic dysfunction, telomere attrition, and decreased autophagy [51, 52]. Aging-related oxidative stress, in addition to mechanical stress from cartilage loading, may contribute to chondrocyte senescence [88]. The cell's ability to compensate for stress reduces with aging, and an accumulation of senescent cells results in a loss of homeostasis with increased secretion of inflammatory mediators, reduced matrix synthesis, and impaired response to growth factor stimulation. This may result in the development of OA. The link between senescence and OA was demonstrated by Xu et al. [92]. Three months after injection of senescent cells into the knee joint of mice, they found changes suggestive of OA, including damage to cartilage and menisci, osteophytes, and changes of the subchondral bone. The mice also demonstrated pain and reduced function. These changes were not found in the control group, suggesting that high concentrations of senescent cells may act detrimentally on the articular cartilage homeostasis [92]. In addition, other groups have shown that local clearance of senescent cells genetically within the intra-articular space significantly reduced the development of injury-induced OA and promoted a pro-regenerative environment [93].

The role of the senescence marker $p16^{Ink4a}$ was studied by Diekman et al. [94]. The mRNA expression of $p16^{Ink4a}$ was significantly higher in aged mice than in young mice, which inhibited chondrocyte proliferation. Interestingly, SASP factors correlated to the expression of $p16^{Ink4a}$ regardless of age; however, inhibition of $p16^{Ink4a}$ did not affect the SASP production or prevent the development of age-induced or post-traumatic OA of the knee joint. In another study, Zheng et al. studied the effect of Fisetin *in vitro* on IL-1β stimulated human chondrocytes and *in vivo* in murine OA models [95]. In the IL-1β stimulated human chondrocytes, Fisetin increased the expression of the enzyme silent information regulator (SIRT) 1 and thereby inhibited the IL-1β induced increased levels of NO, PGE2, IL-6, TNF-α. Additionally, the mRNA expression and the protein levels of the IL-1β induced iNOs, COX-2, MMP-3, MMP-13, ADAMTS-5 were inhibited, and the induced downregulation of SOX-9, aggrecan, and collagen-II degradation was increased. Sirtinol, an inhibitor of SIRT1, reversed the effects of Fisetin on chondrocytes. In the murine OA models, Fisetin demonstrated findings of attenuated progression of OA, thus highlighting its potential as a senolytic treatment for OA [67].

SIRT1 has been reported to be important for cartilage homeostasis by promoting chondrocyte survival and ECM homeostasis [96]. However, data suggest that SIRT1 is proteolytically inactivated during OA [97]. Batshon et al. demonstrated that the NT/CT SIRT1 fragments were found in serum, and an elevated serum NT/CT SIRT1 ratio was associated with both post-traumatic and aging-related OA in mice [98]. A similar increase was found in humans with OA. Further analysis confirmed that the elevated NT/CT SIRT1 fragments are derived from chondrocytes. Senolytic treatment decreased the serum NT/CT SIRT1 ratio and enhanced the intracellular level of SIRT1 in chondrocytes, which correlated with the reduced severity of OA. Dai et al. showed that the combination of Dasatinib and Quercetin to remove senescent chondroprogenitor cells can inhibit SASP formation and thus effectively improve the results of distraction arthroplasty in vitro and in vivo [84]. Recently, it has also been found that intra-articular injection of Navitoclax in post-traumatic OA rats can reduce inflammation, remove senescent chondrocytes in OA, and promote chondrogenic phenotype [99]. Jeon et al. recently tested UBX0101, a senolytic that was found to selectively eliminate senescent cells, found that intra-articular administration of UBX0101 reduced the incidence of post-traumatic OA and associated pain resulting in the development of a prochondrogenic environment [93]. Faust et al. reported that IL-17 expression was increased in mice with post-traumatic OA and in aged mice, and that there was a correlation between senescent cells and IL-17. In their study, senescent fibroblasts increased the level of Th17 cells, when stimulated by IL-6, IL-1β, and TGF-β, and Th17 cells induced senescence in fibroblasts. Inhibition of senescent cells in mice reduced Th17 cells and IL-17; however, both local (UBX0101) and systemic (Navitoclax) senolytic treatment was necessary to reduce cartilage degeneration in aged mice [100]. These findings would provide markers for diagnostic screening and targets for senolytic agents in the treatment of OA.

3.2.1.2 Clinical studies of senolytic agents for the treatment of OA

Several Phase 1 and 2 clinical trials on senolytic agents for the treatment of OA are underway (ClinicalTrials.gov ID: NCT03513016, NCT04229225, NCT04129944, NCT04210986). At our clinic, we are carrying out multiple Phase I/II randomized controlled trials examining the efficacy of Fisetin in the setting of knee OA with (NCT04210986) or without co-treatment with bone marrow concentrate (BMC). In these studies, patients diagnosed with Kellgren-Lawrence grade II-IV knee OA and a numerical rating scale (NRS) pain score of 4–10 are included. Outcome measures include safety and tolerability of Fisetin administration for two days on and 28 days off (20 mg/kg), as well as patient report pain and function indices, OA and SASP related biomarkers, and magnetic resonance imaging (MRI) of cartilage. There have also been multiple other trials using the senolytic agent UBX0101 carried out by Unity Biotechnology (NCT04229225, NCT04129944, NCT03513016, NCT03100799). Many of these studies demonstrated the safety and tolerability of UBX0101 injected intra-articularly with different dosing regimens (single- vs. multi-dose, ascending doses) (https://doi.org/10.1016/j.joca.2020.02.752). However, none of the studies demonstrated a significant reduction in pain up to 12 weeks as assessed by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scoring system. A long-term outcome trial measuring safety and tolerability of UBX0101 at one year was also terminated, failing to meet primary or secondary objectives. (https://clinicaltrials.gov/ct2/show/NCT04349956) While many of these trials are underway, senolytic treatment for OA may nonetheless be a potentially groundbreaking novel treatment strategy to ameliorate the onset and/or progression of OA. More studies are needed to better understand therapeutic delivery (oral vs.

intra-articular), dosing, and senolytic drug of choice. It stands to reason that a combination of senolytic agents or alternative senolytics with higher potency to eliminate senescent cells of the joint may provide a more effective intervention.

3.2.2 Senolytic agents for the treatment of OP

3.2.2.1 Pre-clinical studies of senolytic agents for the treatment of OP

Several studies have located senescent cells in bone with aging [101, 102]. Farr et al. reported a higher expression of the senolytic markers $p16^{Ink4a}$, $p21^{Cip1}$, and p53, especially in osteocytes and myeloid cells, in aged mice compared to young mice [101]. Corresponding with the senescent osteocytes, the aged mice also demonstrated higher levels of SASP genes. Similar results were reported in bone biopsies from humans [103], suggesting that senescent osteocytes and SASPs may play an important role in aging-related OP. The role of senescent cells was further linked using a transgenic mouse line carrying a suicide transgene (INK-ATTAC) whereby $p16^{Ink4a}$ cells could be eliminated with the treatment of a drug (AP20187). The results showed that the AP20187 treated aged mice demonstrated clearance of senescent cells, lower osteoclast numbers, and improved trabecular bone of the spine and femur compared to the vehicle treated mice. In contrast, treatment with AP20187 in young mice did not change the bone quality. Further, this study showed similar results with oral senolytic treatment using Dasatinib and Quercetin, which led to significantly lower $p16^{Ink4a}$ mRNA expression and percentage of senescent osteocytes in bone compared to vehicle. They also found that the use of the JAK inhibitor Ruxolitinib reduced the SASP factors IL-6, IL-8, and PAI-1 with concomitant improved spine and femur bone microarchitecture [104]. These results suggest that targeting senescent cells or SASP from senescent cells may reduce bone resorption and maintain or enhance bone formation. Therefore, senolytic drugs may be a promising alternative for treating aging-related OP.

3.2.2.2 Clinical studies of senolytic agents for the treatment of OP

Osteoporosis is a debilitating disease that significantly increases the risk of fracture, costing an estimated 13.8 billion USD annually, and directly increases the mortality rate by more than 30% in elderly Americans [105]. Pharmacological treatment for OP consists of two main categories; antiresorptive (bisphosphonates, estrogen agonists, etc.) and anabolic drugs, all with the intent to reduce fracture incidence [106]. However, current therapies are limited given the widely known side effects of chronic use, including the functional decline of the gastrointestinal tract and kidneys, osteonecrosis, esophageal cancer, osteogenic sarcoma, atrial fibrillation, and venous thromboembolism [107]. Further, anti-resorptive therapies, the general first-line approach, are uniformly associated with a concomitant reduction in bone formation, which prevents optimal fracture healing [108]. Thus, disease modifying alternatives with a better safety profile (or that require less dosing) is certainly needed for the treatment of OP. Like OA, cellular senescence is thought to be a fundamental driver of age-associated decline in a bone [104]. Several clinical trials are investigating the use of senotherapeutic drugs in the setting of OP.

In one Phase 2 randomized controlled trial, the senolytic drugs Dasatinib plus Quercetin and Fisetin alone are being tested in healthy elderly women aged 70+, when bone density is known to be reduced (ClinicalTrials.gov ID: NCT04313634). Bone turnover serum markers CTX-I and P2NP are measured with and without senolytic treatment. There are also two other active trials examining the effects of Fisetin for the treatment of frailty syndrome (NCT03675724, NCT03430037), an

		Preclinical Animal Model			Clinical Trial						
Senolytic Agent	**Mechanism of Action**	**Species**	**Dose**	**Route**	**Condition**	**ID (Phase)**	**Dose**	**Route**	**Target Population**	**Primary Endpoint**	**References**
Dasatinib (D)	BCR-ABL, SRC, c-KIT, ephrin A receptor, P53 and PAI-2	rat	Mixed solution (1 ml) of D (500 nM) + Q (100 μM, weekly injection until 4 weeks post-op.	IA	OA	NCT04313634 Phase II	1. D + Q treatment group: Intermittent dosing of D (100 mg × 2 days) + Q (1000 mg × 3 consecutive days) every 28 days, repeated 5 times in total.	oral	elderly women	Percent changes in serum bone turnover markers C-terminal telopeptide of type I collagen and amino-terminal propeptide of type I collagen. (Time Frame: 20 weeks)	[59, 84, 109]
Quercetin (Q)	BCL-2/ BCL-X_L family, PI3K/ AKT, ROS, MDM2/p53/ p21/serpine (PAI-1&2), HIF-1α						2. F treatment group: Intermittent dosing of F (20 mg/kg/day for 3 consecutive days) every 28 days, repeated 5 times in total.	oral	elderly women		
Fisetin (F)	BCL-2/ BCL-X_L family, PI3K/ AKT, ROS, MDM2/p53/ p21/serpine (PAI-1&2), HIF-1α, SIRT1, IL-1β	mice	F: 20 mg/kg daily	oral	OA	NCT04210986 Phase I/II	F; 20 mg/kg for two consecutive days, followed by 28 days off, then 2 more consecutive days,	oral	Knee OA	Evaluation of liver and kidney toxicity and Tumor Lysis Syndrome by measuring peripheral blood chemistry. (Time Frame: 12 months)	[59, 95, 109]

		Preclinical Animal Model			Clinical Trial						
Senolytic Agent	**Mechanism of Action**	**Species**	**Dose**	**Route**	**Condition**	**ID (Phase)**	**Dose**	**Route**	**Target Population**	**Primary Endpoint**	**References**
Navitoclax (N) (ABT-263)	BCL-2/BCL-X_L family	rat	N: 0 25–5 μM, 4 injections within 4 to 6 weeks post-op.	IA	OA	N/A	N/A	N/A	N/A	N/A	[59, 99, 109]
UBX0101 (U)	MDM2/p53	mice	U: 1 mM, every other day starting from 2 weeks post-op. (5–6 shots)	IA	OA	NCT03513016 Phase I	U: 0.1–0 4 mg, single dose	IA	Knee OA	Safety and tolerability of a single dose of U. (Time Frame: 12 weeks)	[59, 93, 109]
						NCT04229225 Phase I	U: Single injection of 8.0 mg at week 0, or two doses of 4.0 mg (weeks 0 and 4).	IA	Knee OA	Safety and tolerability of a single and repeat dose of U. (Time Frame: 24 weeks)	
						NCT04129944 Phase II	U: Single dose of 0.5 mg, 2 mg, or 4 mg at week 0.	IA	Knee OA	Change in (WOMAC-A) Score from baseline to week 12. (Time Frame: 12 weeks)	

IA, intra-articular injection.

Table 1.
Senolytics as Potential Therapeutic Agents for OA and OP.

age-related condition characterized by sarcopenia and decreased bone density. In these studies, primary endpoints include serum inflammatory markers and mobility based on a 6-min walk test.

Another promising senolytic drug is Metformin. There are at least four active trials measuring the effects of Metformin on pre-frail to frail patients (NCT03451006, NCT02570672, NCT04221750, NCT02325245). Primary endpoints in these studies are mobility and motor skills functions, including gait speed, balance ability, and grip strength test, geriatric depression score, and weight loss. Similar to OA trials, many of these trials are not complete, but there is compelling evidence that senolytic agents might benefit a litany of age-related skeletal decline. Details of preclinical animal studies and clinical trials of major senolytics in OA and OP and their mechanisms are summarized in **Table 1** [59, 109].

4. Mesenchymal stem cell (MSC)-based therapy for the treatment of aging-related musculoskeletal decline

4.1 Biological mechanisms of MSCs

Mesenchymal stem cells (MSCs) are present in a variety of human tissues, including bone marrow, adipose tissue, synovial tissue, and cord blood [110–112]. However, there is currently a lack of conclusive evidence regarding the potential biological mechanisms of MSCs for the treatment of aging-related musculoskeletal diseases. Understanding the function of MSCs opens up the possibility of developing robust MSC-based therapies for musculoskeletal regenerative medicine. Until now, there are two theories on the mechanism of function: (1) Direct adherence and incorporation into the host tissue and (2) trophic effects resulting from the MSC-derived secretomes.

4.1.1 Direct adherence and incorporation of MSCs

One of the primary advantages of MSCs is their ability to interact with various chemokine receptors (such as CXCR4, which is involved in MSC migration), integrins, selectins, and vascular cell adhesion molecule-1, to home damaged tissues [113–119]. The original hypothesis regarding the tissue regeneration mechanism of MSCs was that implanted cells would migrate directly to injury sites, where they would differentiate into functional cells and eventually promote repair of damaged connective tissue [120]. In support of this hypothesis, it has been reported that injected MSCs have the potential to adhere to the injury site and repair the host cartilage through regeneration, and interestingly, MSCs may also migrate to the injury site for tissue regeneration [121, 122]. However, whether the introduced MSCs are actually taken up into the host tissue and act directly on the damaged tissue has yet to be verified.

4.1.2 Trophic effects of MSCs

With decades of research on the underlying functionality of MSCs, it has been found that there exists a discrepancy between the frequency and duration of transplants and the remarkable healing power of MSCs [120]. Numerous studies have been conducted to resolve this conundrum, presenting the concept that MSCs possess the ability to maintain the proliferation and survival of certain cell types by secreting trophic factors, and regulating certain aspects of the immune system, thereby ushering MSC-based therapies into a new phase [123].

Analysis of the MSC secretion and proteome has revealed various paracrine factors that can reduce apoptosis and inflammation and stimulate angiogenesis and self-renewal of progenitor cells [120]. Notably, MSCs are known to act as immunosuppressive cells that can alleviate inflammation and reduce monocyte activation by releasing anti-inflammatory factors, including interleukin-1 receptor antagonist (IL-1ra) [124]. Pro-inflammatory cytokines, such as interleukin-1β (IL-1β), are widely known to play an essential role in OA progression [95] and IL-1ra confers an overall inhibitory effect on IL-1ß mediated inflammation and matrix degradation. Taken together, MSCs should confer a therapeutic potential in OA patients.

4.2 Properties and benefits of each type of MSCs on aging-related musculoskeletal decline

4.2.1 Bone marrow-derived MSCs (BM-MSCs)

4.2.1.1 Characteristics and advantages of BM-MSCs

Bone marrow is generally considered to be the home of hematopoietic stem cells and is known to contain MSCs as part of the stromal fraction [125]. BM-MSCs possess a high potential for cartilage repair due to their ready availability [126]. BM-MSCs have also been widely studied as a treatment for OP due to their high ability of osteogenesis.

4.2.1.2 Pre-clinical studies of BM-MSCs for OA

The effect of BM-MSCs on OA has been verified in numerous animal studies. Chiang et al. investigated the effects of intra-articular injection of allogeneic BM-MSCs in an *in vivo* rabbit OA model. They observed that the BM-MSCs transplantation group had significantly better histological scores than the hyaluronic acid injection group [127]. Furthermore, Song et al. compared the efficacy of bone marrow mononuclear cells (BMMCs) and BM-MSCs in a sheep OA model and demonstrated that the BM-MSCs group had smaller lesions and a relatively smoother femoral condyle. They also reported that ICRS scores showed a greater improvement in the BM-MSCs group than the BMMCs and PBS (control) groups. They further stated that the results of histology showed fewer changes to cartilage and bone in the BM-MSCs group [128].

4.2.1.3 Pre-clinical studies of bm-MSCs for OP

Ichioka et al. demonstrated that direct injection of allogenic BM-MSCs into the bone marrow cavity of irradiated P6 sub-strain of senescence-accelerated mice (SAMP6), an osteoporotic mouse, resulted in inhibition of osteoblast and osteoclast formation in an age-dependent manner and promoted adipogenesis, increased trabecular bone and decreased bone mineral density [129]. Autologous BM-MSC transplantation has been reported to improve bone formation and strengthen osteoporotic bones in ovariectomy (OVX) -treated rabbits [130] and in estrogen-deficient goats, mimicking the postmenopausal OP that occurs in elderly women [110]. However, there is limited support for autologous BM-MSCs to treat OP in elderly patients because BM-MSCs isolated from the bone marrow of elderly patients have shown reduced proliferation and osteogenic capacity in vitro [131, 132].

4.2.2 Adipose-derived MSCs (A-MSCs)

4.2.2.1 Characteristics and advantages of A-MSCs

MSCs were first reported in adipose tissue in 2001 [133] and have been touted as an attractive source of MSCs. Although A-MSCs have the advantage of being easier to isolate than BM-MSCs, [134] BM-MSCs have been shown to be prone to chondrogenic differentiation, both *in vitro* and *in vivo* [135]. Interestingly, however, it has also been reported that the addition of paracrine or cytokine factors increases the cartilage capacity of A-MSCs to a level comparable to that of BM-MSCs [136]. It is worth noting that the yield of A-MSCs and their proliferation and differentiation ability is dependent on the site of tissue collection [137] and the age of the donor [138].

4.2.2.2 Pre-clinical studies of A-MSCs for OA

The effect of A-MSCs on OA has been investigated in numerous animal studies. Tang et al. compared the efficacy of three types of intra-articular injections, subcutaneous A-MSCs, visceral A-MSCs, and PBS (control), in a rat model of OA. Subcutaneous They found that A-MSCs injection decreased osteophyte and fibrous tissue formation compared to PBS or visceral A-MSCs. In addition, histologically, a smooth cartilage surface and distribution of lacunae and chondrocytes were observed in rats treated with subcutaneous A-MSCs [139]. Kuroda et al. verified the efficacy of A-MSCs for OA treatment using a rabbit model. They found that nearly normal cartilage was observed in the A-MSCs group, with less cartilage damage than in the control group. Further, the proportion of MMP-13-positive cells was significantly lower in sections of the A-MSCs group than in the control group [140].

4.2.2.3 Pre-clinical studies of A-MSCs for OP

The effects of A-MSCs have also been evaluated in OP animal models. Mirsaidi et al. performed the A-MSCs injection to SAMP6 mice and observed improvement of trabecular bone quality [141]. Additionally, Cho et al. studied the efficacy of human A-MSCs using OVX nude mice, showing that human A-MSCs could inhibit OVX-induced bone loss over eight weeks [142]. Furthermore, Ye et al. found that autologous A-MSCs enhanced bone regeneration in an OVX-induced rabbit model of OP, suggesting that this was due not only to autologous osteogenic differentiation but also to the promotion of osteogenesis and inhibition of adipogenesis through the activation of BMP-2 and BMPR-IB signaling pathways [143].

4.2.3 Synovium-derived MSCs (S-MSCs)

4.2.3.1 Characteristics and advantages of S-MSCs

In 2001, De Bari *et al*. isolated the first MSCs from the synovium of the human knee joint [144]. Since then, S-MSCs have attracted attention because they are more readily accessible, possess a higher growth rate, and are less immunogenic compared with MSCs from other origins [145]. Sakaguchi et al. compared the yield, expandability, differentiation potential, and epitope profiles of human MSCs derived from five different mesenchymal tissue sources: bone marrow, synovium, periosteum, adipose tissue, and muscle, and concluded that S-MSCs had the highest capacity for chondrogenesis [146].

4.2.3.2 Pre-clinical studies of S-MSCs for OA

The beneficial effects of S-MSCs in promoting cartilage regeneration have been reported in pig, [146] leporin, [135] and canine models [147]. In a recent systematic review of *in vivo* studies on synovium-derived mesenchymal stem cell transplantation for cartilage regeneration, To et al. showed, in 4 human and 16 animal articles, that S-MSCs possess overall good chondrogenic potential and positive effect for treating chondral lesions and preventing OA [148]. Ozeki et al. found that intra-articular injection of S-MSCs in a rat model of OA could inhibit the OA progression and attenuate synovitis when administered once a week instead of a single dose [149]. Accumulating evidence that S-MSCs possess a strong chondrogenic potential and the fact that MSCs derived from synovial tissue is specific to target joints have led to a growing interest in the application of S-MSCs for a stem cell therapy of knee OA.

4.2.3.3 Pre-clinical studies of S-MSCs for OP

As it pertains to osteogenic potential, Sakaguchi et al. showed that S-MSCs possessed a higher capacity than adipose tissue- and muscle-derived cells, comparable to bone marrow-, and periosteal-derived [150]. However, S-MSC-based therapy for OP has yet to be well investigated.

4.2.4 Muscle-derived stem cells (MDSCs)/muscle-derived stem progenitor cells (MDSPCs)

4.2.4.1 Characteristics and advantages of MDSCs/MDSPCs

MDSCs/MDSPCs are pluripotent cells isolated from postnatal skeletal muscle via established preplating techniques. They are characterized by multiple critical features such as long-term proliferation/self-renewing capacity, resistance to oxidative and inflammatory stress, and multilineage differentiation potential [151–153]. Recently, it has been shown that skeletal muscle-derived MSCs from OA patients exhibit superior biological properties compared to the bone-derived MSCs counterpart, making them a promising candidate for autologous stem cell therapy [154]. MDSPCs have been shown to improve the regenerative capacity of various tissues, including bone, cartilage, skeletal muscle, and cardiac muscle, by promoting angiogenesis [155–159].

Of note, several studies have investigated differences in the proliferation and differentiation ability of MDSCs by sex and age [158, 160, 161]. It has been shown that male murine MDSPCs displayed higher chondrogenic differentiation capacity and cartilage regeneration potential than female murine MDSPCs [160]. Similarly, Corsi et al. showed that the osteogenesis of male murine MDSPCs was superior to that of female MDSPCs [162]. Furthermore, our group has recently found that in human MDSPCs, male MDSPCs possess a greater ability to undergo chondrogenesis and osteogenesis than female MDSPCs [161].

Our group, on the other hand, have reported that not only donor but also host sex affects bone regeneration; male murine hosts showed a greater amount of MDSPC-mediated ectopic bone formation and cranial defect healing than did female hosts [163].

4.2.4.2 Pre-clinical studies of MDSCs/MDSPCs for OA

An interesting study by Kuroda et al. indicated that local delivery of BMP-4 by genetically engineered MDSCs promoted chondrogenesis with a significant

improvement of articular cartilage repair in rats [164]. This suggests that MDSCs are advantageous concerning chondrogenic differentiation potential. Furthermore, Matsumoto et al. demonstrated in a rat model that MDSCs therapy with sFlt-1 and BMP-4 promotes chondrogenesis in OA, and inhibits cartilage resorption by inhibiting angiogenesis, thus enabling sustained cartilage regeneration and repair [165].

4.2.4.3 Pre-clinical studies of MDSCs/MDSPCs for OP

Our group isolated young and old populations of gender-matched human muscle-derived stem cells (hMDSCs) to examine the effect of age on osteogenic differentiation using a critical-size calvarial bone defect mouse model. In addition, the effect of donor and host age on hMDSC-mediated bone regeneration was investigated. We showed that donor age did not impair hMDSC-mediated bone regeneration, while host age had the adverse effect. We also found that hMDSCs form functional bone regardless of the age of the donor or host, suggesting that these cells are a promising resource for bone regeneration [166].

4.3 Clinical studies of MSC-based therapy for the treatment of aging-related musculoskeletal decline

4.3.1 Clinical studies of MSC-based therapy for OA

More than 30 clinical trials on the administration of intra-articular MSCs for the treatment of OA have been completed to date, including randomized controlled trials, retrospective studies, and cohort studies (https://www.clinicaltrials.gov/), and most of the published results have shown clinical benefit [111, 112, 167–178]. Currently, B-MSCs and A-MSCs are the most commonly used cell sources in clinical trials for OA, [179] however, finding an optimal treatment with MSCs is challenging due to the great diversity of patient populations, delivery methods, cell numbers, culture expansion methodology, and follow-up periods.

Until now, no clinical trials on the benefit of intra-articular administration of S-MSCs in patients with OA have been published. Interestingly, however, Sekiya et al. found that arthroscopic S-MSCs transplantation improved the clinical outcome of knees with articular cartilage defects at a mean follow-up of 52 months in 10 patients based on MRI, histology, and clinical outcome score evaluation [180]. Furthermore, Shimomura et al. conducted the first human pilot study of implanting scaffold-free tissue-engineered constructs generated from S-MSCs to the injury site for five patients with symptomatic knee cartilage lesions, demonstrating that self-assessed clinical scores for pain, symptoms, activities of daily living, sports activities, and quality of life improved significantly at 24 months postoperatively, with no serious adverse events. In addition, second-look arthroscopy and MRI confirmed complete defect filling in all cases, and biopsy of the regenerated cartilage showed that the repair tissue consisted of hyaline cartilage [181]. These results showed that implantation of S-MSCs could repair articular cartilage damage and prevent its progression to OA, and therefore, future clinical applications in patients with OA may be promising. There is also evidence that, in a phase I/II study, repeated administration of umbilical cord-derived-MSCs improved safety and clinical outcomes for long-term pain in patients with knee OA [112].

4.3.2 Clinical studies of MSC-based therapy for OP

A recent Phase 1 clinical trial has been conducted using fucosylated BM-MSCs for patients with OP. In this study, autologous BM-MSCs were harvested 30 days

prior to the infusion and cultured under good manufacturing practice (GMP) conditions to purify and obtain mesenchymal cell established dose range. This study is ongoing, and the recruitment of participants is currently closed. (ClinicalTrials.gov ID: NCT02566655).

Although the use of MSC products for the treatment of aging-related skeletal disorders is becoming increasingly prevalent, well-designed studies are imperative to conclusively prove their clinical benefits and identify the optimal indications, cell sources, delivery methods, and doses.

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