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COMMENT

Treating osteoarthritis via gene therapy with rejuvenation factors

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Cellular senescence and stem cell exhaustion are drivers of various aging-associated disorders, including osteoarthritis, a leading cause of disability and one of the most common degenerative diseases, the incidence of which increases with age [1–4]. Arthroplasty and autologous chondrocyte transplantation are osteoarthritis treatments, among which autologous chondrocyte transplantation is currently the most effective [5]. However, autologous chondrocyte transplantation is limited by the source of healthy donor cartilage and the proliferative capacity of primary chondrocytes [6]. More traditional treatment options involve mainly oral and intra-articular drugs and physical therapy (including electrotherapy and acupuncture). Nonsteroidal anti-inflammatory drugs, such as selective cyclooxygenase 2 inhibitors, are widely used due to their anti-inflammatory effects but may have adverse effects on the gastric mucosa and renal function and increase the risk for cardiovascular diseases. The analgesics used to ease patient pain are largely oral opioids and intra-articular corticosteroids. In addition, symptomatic slow-acting drugs, including glucosamine sulfate, chondroitin sulfate, and diacerein, are used. Furthermore, platelet-rich plasma seems to be another therapeutic option for the alleviation of osteoarthritis [7]. Despite the development of these therapeutic options, there is still no cure for osteoarthritis with nonsurgical disease-modifying treatment. Therefore, there is an urgent need for more specific therapeutic strategies developed on the basis of an in-depth molecular understanding of this disease.

Osteoarthritis usually emerges from disruption of the superficial zone of cartilage where mesenchymal stem cells (MSCs) and chondrocyte progenitor cells reside. Therefore, MSCs residing in the joint cartilage may be a critical target for the prevention of osteoarthritis [8]. Nonetheless, the key regulators of MSC senescence and aging-associated genes that act as potential targets for the treatment of human osteoarthritis are largely unknown. A comprehensive understanding of the underlying mechanisms of MSC senescence may help identify novel therapeutic targets for osteoarthritis.

With the development of gene-editing techniques, including helper-dependent adenoviral vector, traditional meganucleases, ZFNs, TALENs, and CRISPR/Cas9, over the past decades, adding, removing, or altering the genetic material at specific locations in an organism's genome has become feasible [9–11]. Despite certain technical limitations, CRISPR/Cas9 is by far the most convenient gene-editing tool widely applied to human embryonic stem cells (hESCs) as well as their derivatives for both basic and clinical studies [12–14]. Using this method in combination with directed hESC differentiation techniques, researchers have identified a list of novel rejuvenation factors for the alleviation of mouse osteoarthritis via the intra-articular administration (Fig. 1), which demonstrates that rejuvenating senescent cells may represent a new avenue to treat aging-associated disorders, such as osteoarthritis.

Recently, Deng et al. generated DiGeorge syndrome critical region 8 (DGCR8)-deficient MSCs with an accelerated senescence phenotype. Mechanistically, DGCR8 maintains heterochromatin organization by interacting with

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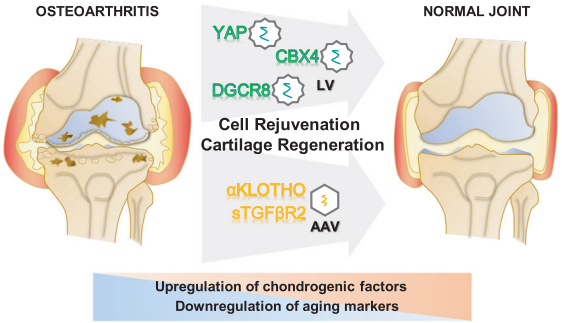

Fig. 1 Schematic of gene therapy using viral vectors encoding rejuvenation factors involved in the attenuation of osteoarthritis. LV lentivirus, AAV adeno-associated virus.

the nuclear envelope protein Lamin B1 and the heterochromatin-associated proteins KRAB-associated protein 1 (KAP1) and heterochromatin protein 1 (HP1), thus regulating human MSC (hMSC) senescence independent of its well-established function being a critical component of the canonical microprocessor complex for microRNA biogenesis [15]. Likewise, yes-associated protein (YAP) was first identified as a major effector of Hippo signaling that plays important roles in development and cell fate decision. Fu et al. generated YAP-deficient hMSCs with a premature cellular senescence phenotype and found that YAP cooperates with TEA domain transcriptional factor (TEAD) to activate the expression of forkhead box D1 (FOXD1), a geroprotective protein [16]. In addition, chromobox homolog 4 (CBX4), a component of polycomb repressive complex 1, was initially implicated in the maintenance of cell identity and organ development through epigenetic silencing. Ren et al. generated CBX4-deficient hMSCs and demonstrated that CBX4 counteracts hMSC aging via the maintenance of nucleolar homeostasis by recruiting nucleolar protein fibrillarin and heterochromatin component KAP1 to nucleolar rDNA for its stabilization, thus limiting excessive rRNA expression and cellular senescence [17]. Through these studies, a list of novel geroprotective factors, the absence of which result in accelerated hMSC senescence, have been individually identified.

On top of the identification of novel geroprotective factors, researchers have explored the possibility of expressing single or multiple geroprotective factors with viral vectors to enhance the regenerative capacity to restore the articular cartilage structure and function in osteoarthritis (Fig. 1). Accordingly, gene therapy with lentivirus-mediated ectopic expression of DGCR8 effectively attenuates hMSC senescence and alleviates the pathological phenotype of mouse osteoarthritis, as evidenced by the histological improvement and the downregulation of cellular senescence markers (p16 and p21) and inflammation factors [15]. Similarly, lentivirus-mediated gene transfer of YAP, FOXD1 or CBX4 each rejuvenates aged hMSCs and mitigates mouse osteoarthritis [16, 17]. In addition, soluble transforming growth factor-beta receptor 2 (sTGFβR2) and alpha KLOTHO (αKLOTHO) are two protein molecules that have been individually implicated in the maintenance of cartilage homeostasis. TGFβ1 is a reparative mediator that stimulates chondrocyte proliferation and inhibits chondrocyte hypertrophy [18] and is also involved in osteoarthritis development and progression, for which sTGFβR2 exhibits a high affinity. αKLOTHO, on the other hand, is an antiaging molecule that is downregulated in the cartilage and synovial membrane during aging and osteoarthritis [19]. Martinez-Redondo et al. conducted an experiment in rats with papain/cysteine-induced osteoarthritis via the intra-articular injection of AAV-DJ-αKLOTHO and AAV-DJ-sTGFβR2, and evaluated their combinatorial therapeutic effect on cartilage repair at 6 weeks after the viral injection [6]. The ectopic coexpression of αKLOTHO and sTGFβR2 improves the function of cartilage tissue and reverses the osteoarthritis phenotype via the downregulation of immune response and the promotion of joint tissue homeostasis. In addition, the treatment with αKLOTHO and sTGFβR2 recombinant proteins improves the expression of chondrocyte-specific markers and cell proliferation in human primary articular chondrocytes. While the detailed mechanisms of combined treatment with αKLOTHO and sTGFβR2 in cartilage repair remain to be understood, this study serves as a good example of the combined application of rejuvenation factors to osteoarthritis for synergistic and improved therapeutic effects [6]. However, the establishment of highly efficient screening systems to identify individual geroprotective factors as well as the most beneficial combinations remains a great challenge.

Through a series of studies, researchers have uncovered a list of rejuvenation factors that alleviate hMSC senescence and mouse osteoarthritis. These breakthroughs in the field of gene therapy for osteoarthritis indicate the feasibility of using "rejuvenation (de-senescence) factors" targeting senescent stem cells, shedding light on future clinical interventions against various aging-related diseases. However, it is of scientific and clinical interest to identify more rejuvenation factors and aging factors via approaches such as CRISPR/Cas9-based genome-wide screens, which will likely lead to the identification of new therapeutic targets for aging-associated diseases through both positive and negative regulations. More efficient and targeted gene-editing tools are yet to be developed and will be useful for precise genetic and epigenetic modulations, such as in vivo target gene activation or repression. In addition, it is important to keep in mind that virus-mediated gene therapy may have potential biosafety issues due to insertional mutagenesis. The effectiveness of viral vectors in gene therapy delivery should also be taken into careful consideration with regard

to their packaging capacity, infection efficiency and target specificity [20]. Despite the existing challenges, we believe that continuous efforts by the aging research community will lead to new mechanistic interpretations of rejuvenation factors and aging factors and even more finely tuned and efficient tools for gene editing, facilitating the development of therapeutic strategies for the treatment of aging-related diseases in humans.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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