Mini Review

Regulatory role of mesenchymal stem cells in osteoclast differentiation

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Mesenchymal stem cells (MSCs) are adult totipotent cells that can differentiate into osteoblasts and chondrocytes. Based on this property, they are theoretically useful for treatment of bone erosive diseases such as rheumatoid arthritis (RA) including joint repair. MSCs constitutively produce a variety of cytokines and growth factors, which explain their immunomodulatory effects on inflammatory cells. Recent clinical trials have shown their efficacy in graft versus host disease. However, whether MSCs can be used for treatment of RA remains unclear. Especially, there is a need to identify and characterize all soluble mediators, i.e., the "trophic effects" of MSCs on the differentiation of osteoclasts, which are involved in bone destruction in RA. We reported previously that human MSCs suppress osteoclast differentiation by constitutive production of osteoprotegerin, the decoy receptor of RANKL. Our results further highlighted the potential usefulness of MSCs for RA treatment by preventing the progression of bone damage by inhibiting osteoclast differentiation. The next step in the clinical application of MSCs includes identifying the best tissue source for these cells and refinement of RA treatment methodology. Recent studies have confirmed that MSCs are important for both bone and synovial tissue homeostasis acting as precursors of osteoblasts and chondrocytes and through their trophic effects. Taken together, MSCs are a hopeful tool to combat joint inflammation and enhance joint repair in RA, ensuring complete cure of this devastating disease.

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic synovitis, in which the presence of proinflammatory cells and cytokines leads to cartilage and bone damage, resulting in irreversible deformity of the joints and low quality of life. The recent introduction of biological agents targeting tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) to the treatment of RA has allowed marked reduction in disease activity and induction of remission[1, 2]. However, there is no satisfactory joint repair treatment at present. Therefore, development of a novel joint repair treatment for RA is desirable.

Mesenchymal stem cells (MSCs) have been widely studied for decades as a novel therapeutic tool for treatment of a variety of diseases, due to their totipotency and immunosuppressive properties linked to the secretion of soluble mediators, also known as the "trophic effect" of MSCs (Fig.1). We have shown previously that human MSCs inhibit osteoclast differentiation by constitutive production of osteoprotegerin (OPG)[3], adding support to the potential usefulness of MSCs for treatment of RA in addition to aforementioned properties. Moreover, MSCs also seem to play important roles in bone and cartilage homeostasis, based on their trophic effect. In this review, we discuss the utility of MSCs as a therapeutic tool for bone and cartilage destructive diseases including RA.

Trophic effects of human MSCs and suppression of osteoclast differentiation

MSCs are defined as the adult stem cell populations that possess both self-renewal properties and ability to differentiate into various mesenchymal lineages, including osteoblasts, chondrocytes, and adipocytes[4]. Human MSCs can be isolated from diverse mesenchymal tissues including bone marrow, adipose tissue, and the synovium, and are generally positive for CD73, CD90, and CD105 and negative for CD11b, CD14, CD34, and CD45. Their immunosuppressive and anti-inflammatory properties have been demonstrated previously. These functions mainly depend on the secretion of a number of soluble mediators such as anti-inflammatory cytokines[5], indoleamine 2,3-dioxygenase (IDO) that inhibit allogenic T cell response[6] and prostaglandin E_2 (PGE_2) that suppresses the maturation of T lymphocytes and dendritic cells (DC)[7, 8]. In addition, other MSC-secreted factors, such as TNF-α stimulated gene 6 protein (TSG-6), nitric oxide (NO), and human leukocyte antigen-G5 (HLA-G5), have been reported to mediate the regulation of proinflammatory cells[9-11]. As a matter of fact, MSCs are currently employed in clinical trials aimed at treating steroid-resistant acute graft-versus-host disease (GVHD)[12]. Likewise, MSCs has been proposed as a cell therapy tool for autoimmune diseases. However, it is important to clarify the effects of MSCs on bone metabolism before they are considered for treatment of RA. In compliance with this policy, we first assessed the effects of MSCs on differentiation of osteoclasts, which are known to play an important role in bone destruction. Co-culture of human MSCs with peripheral blood mononuclear cells (PBMCs) stimulated with macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL), in the absence of cell-cell contact, significantly inhibited osteoclast differentiation and reduced the expression of cathepsin K[3]. In addition, the conditioned medium obtained from MSCs cultures also reduced the number of osteoclasts and expression levels of cathepsin K and

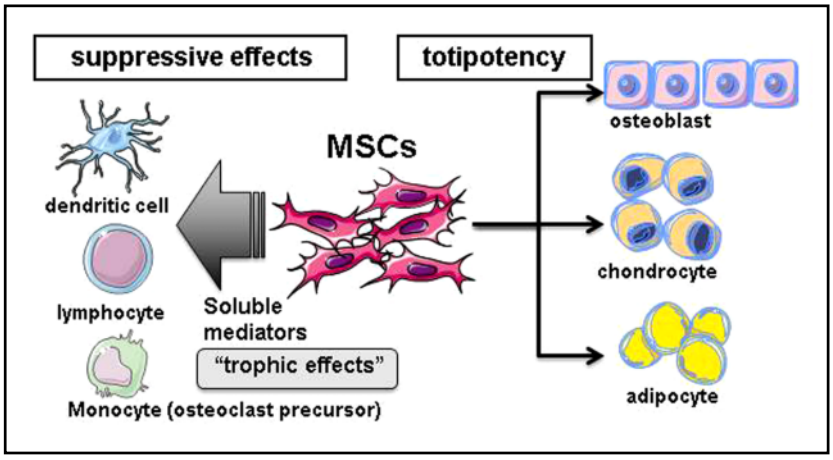

Fig.1 Characteristics of mesenchymal stem cells

Mesenchymal stem cells (MSCs) isolated from bone marrow or almost all mesodermal tissues are characterized by their self-renewal ability, totipotency to differentiate into various mesenchymal lineages, and immunosuppressive property due to their "trophic effects". The trophic effects likely affect not only immune cells but also other surrounding cells, including osteoclast precursors by paracrine effect.

NFATc1. To identify the factor(s) in the conditioned medium that caused inhibition of osteoclast differentiation, cytokine array was performed. The results showed that MSCs produced large amounts of OPG, a decoy receptor of RANKL. To confirm the role of OPG in MSCs-induced osteoclastogenesis, anti-OPG neutralizing antibody or gene silencing with OPG siRNA was added to the cultures. The results showed partial recovery of osteoclastogenesis from PBMCs. Although other molecules, such as IL-6, tissue inhibitor of metalloproteinase(TIMP)-1/2, monocyte chemotactic protein (MCP)-1, and angiogenin were also detected in the medium, OPG was the only one involved in osteoclastogenesis. On the other hand, IL-6 and TIMP-1/2 are known to play important roles in metabolism of cartilage matrix[13, 14]. Our results indicate that MSCs can maintain and repair bone and cartilage and regulate immune response through their trophic effects, adding further support to their usefulness as treatment tool for RA.

Role of MSCs in maintenance of bone and joint formation

MSCs are well known precursors of osteoblasts and chondrocytes and therefore important for joint formation[4]. They also regulate bone resorption based on their trophic effects as mentioned above. These characteristics indicate that MSCs play key roles in bone and cartilage metabolism and homeostasis. Although there is no direct evidence for a link between MSCs dysfunction and joint destruction in RA, recent data from a mouse model of RA suggests their role in joint formation. Bone marrow MSCs from IL-1Ra knockout mice, which exhibit RA-like symptoms, demonstrated poor capacity for self-renewal and osteoblast differentiation, compared to the wild type[13]. Based on this abnormality, histological analysis of these knockout mice showed significant reduction in the number of osteoblasts at the tibial endo-cortical surface and a tendency for increased numbers of osteoclasts. Moreover, IL-1 and TNF-α , which are both inflammatory cytokines known to play major roles in RA animal models, have been reported to modify osteoblast differentiation[16, 17]. On the other hand, recent reports have pointed out the presence of MSCs in the articular cartilage suggesting their potential role in cartilage homeostasis and repair[18-20]. Indeed, a high density of MSCs was found in the synovial fluid of patients with osteoarthritis (OA)[18, 19] and their numbers correlated negatively with the disease severity[20], whereas their number do not increase in RA synovial fluid regardless of the disease stage[18]. Moreover, synovial fluid of RA patients has less capacity for chondrocyte differentiation from subchondral cortico-spongious progenitor cells, which possess similar characteristics to MSCs, compared with OA and healthy individuals[21]. Presumably, IL-1 and TNF-α, which are also known as inflammatory cytokines known to be present at high levels in the joint of RA patients and inhibit chondrogenic differentiation[22, 23], contribute to this cartilage disorder. Examinations during structural remission, i.e., complete inhibition of joint destruction, in RA patients treated with TNF-inhibitors also indicate a certain link between TNF and cartilage disorder[24]. In this context, the migratory capacity of MSCs to the defective sites is reduced in synovial fluid of RA patients but not OA or healthy individuals[25]. These results suggest that disorders of either differentiation or migratory capacity of MSCs can cause incomplete repair leading to enhancement of joint destruction. Considered together, these results suggest that the trophic effects of human MSCs, including their inhibitory effects on osteoclast differentiation, are reduced in RA joints, leading to progressive joint damage. Thus, MSCs seem to play important roles in bone and cartilage maintenance and formation.

Clinical application of MSCs as a therapeutic tool for RA treatment

Several clinical trials are currently underway testing the potential benefits of human MSCs in the treatment of GVHD, multiple sclerosis, systemic lupus erythematosus, and type I diabetes (www.clinicaltrials.gov). Early results suggest that MSCs can prolong survival of patients with steroid-resistant severe acute GVHD, probably due to their immunosuppressive effects[12]. However, there are challenges to overcome before MSCs can be used in RA. Several reports have shown conflicting results about the efficacy of MSCs on synovial inflammation in type II collagen-induced arthritis (CIA) in mice[26, 27]. Augello et al.[26] reported that a single injection of allogeneic MSCs prevented the occurrence of irreversible damage in the bone and cartilage based on their immunomodulatory function by inducing regulatory T cells. In contrast, Chen et al.[27] demonstrated that Flk-1$^+$ MSCs aggravated arthritis in CIA mice by up-regulating IL-6 secretion, which favors Th17 differentiation. Thus, the utility of MSCs for RA treatment based on their anti-inflammatory properties remains to be demonstrated. Since MSCs are, to a certain degree, a heterogeneous population of cells, standard-

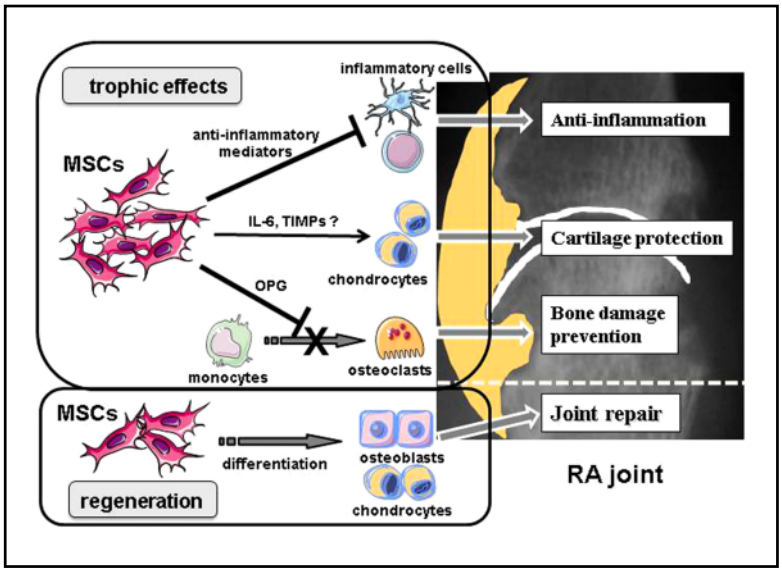

Fig.2 Strategy for novel RA treatment using MSCs

MSCs are a promising tool for complete cure of RA. First, based on their trophic effects, MSCs can act as immune cells, chondrocytes, and osteoclasts, thus enhancing immunosuppression, cartilage protection, and prevention of bone destruction, respectively. Second, MSCs are capable of RA joint repair due to their totipotency with capacity of differentiation into both osteoblasts and chondrocytes.

ization and optimization of the methods used for isolation and culture is important. There is no clear answer as to the best mesenchymal tissue source for MSCs most appropriate for RA treatment, and also the most efficacious treatment method (intra-articular or intravenous).

On the other hand, the important issue in RA treatment is to prevent progression of bone destruction related to excessive bone resorption caused by accumulation of activated osteoclasts. Osteoclast differentiation and activation in RA synovial tissue involves increased RANKL-expressing cells, including inflammatory synovial fibroblasts and activated T lymphocytes[28]. Therefore, MSCs cell therapy is an attractive strategy to prevent bone damage based on the constitutive production of OPG. Moreover, identification of dysfunctional resident MSCs in RA joint can be rectified by restocking MSCs capable of inducing complete recovery of bone metabolism. Taken together, MSCs are a promising tool for RA treatment to control both inflammation and bone destruction based on their trophic effects, as well as to normalize bone and cartilage homeostasis. Further studies are necessary to establish the best-case scenario to ascertain the concept of MSCs use in RA treatment.

Conclusion

In this review, we discussed the potential of clinical application of MSCs in RA, especially focusing on their trophic effects. As progenitor cells capable of differentiating into osteoblasts and chondrocytes, MSCs are a fascinating tool for regeneration of damaged joints of RA and other bone erosive diseases. Although appropriate strategy has not yet been established, various advanced technologies, such as tissue engineering, are expected to overcome methodological problems. On the other hand, the use of MSCs, unlike that of embryonic stem cells and induced pluripotent stem cells, does not create complex ethical issues and is not associated with the risk of tumorigenic transformation. Therefore, MSCs are a promising tool for the advancement of joint repair therapy. In addition, we also reviewed the physiological roles of MSCs, highlighting their importance in bone and cartilage homeostasis and also their potential dysfunction in RA synovial tissues. These evidences suggest that MSCs-restocking therapy could be an alternative therapy for joint repair.

In conclusion, since joint deformity leads to a significant decline in quality of life, development of novel treatments that combat bone destruction is desirable. We believe MSCs are a promising tool for bone erosive diseases including RA due to their anti-inflammatory properties, prevention of bone destruction, and regeneration of bone and cartilage (Fig.2).

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Disclosure of conflict of interests

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