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Mini Review

Mesenchymal stem cells: A new treatment tool for rheumatoid arthritis

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Rheumatoid arthritis (RA) is a common autoimmune inflammatory disease causing bone destruction. Although the etiology of RA remains unanswered, it is well known that inflammatory cytokines play key roles. Although biological agents targeting these inflammatory cytokines strongly suppress inflammation as well as bone damages, repair of destructed bone is still challenging. Mesenchymal stem cells (MSCs) are capable to differentiate into osteoblasts and chondrocytes and moreover, they have been reported to possess immunomodulative effects without severe adverse events in patients with graft versus host disease. Recently, we have reported that MSCs produce osteoprotegerin (OPG), a decoy receptor of the receptor activator of NFκB ligand (RANKL) resulting in reduced osteoclastogenesis. We herein demonstrate the promotive effect of inflammatory cytokines on osteoblast differentiation through Wingless-type MMTV integration site family (Wnt) 5a/ receptor tyrosine kinase-like orphan receptor (Ror) 2 signaling pathway. Our results suggest that MSCs is a powerful treatment tool for RA patients to aim suppression of inflammation and bone regeneration in parallel.

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Introduction

Rheumatoid arthritis (RA) is a common autoimmune inflammatory disease represented by chronic inflammation at the articular joints. Excessive inflammation leads not only to bone destruction by activating osteoclasts with suppressed osteoblast differentiation, but also is in connection with multiple organ involvement[1]. Although the cause of RA is still uncertain, it is well known that inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-17 (IL-17) play key roles in pathogenesis, and biological agents targeting these cytokines are highly effective in controlling disease activity. Approximately 30% of patients are able to achieve clinical remission with complete inhibition of progressive bone damages[2-4]. Although bone repair at the involved joints can be observed in rare cases[3],

in general joint repair cannot be achieved even though biologics are used. In the early phase of joint inflammation, bone destruction is observed at the bare-area where the synovium is directly attached to the bone. As a matter of fact, this anatomical aspect is considered as a cause of joint subluxation at the early phase of the disease. Mesenchymal stem cells (MSCs) are the stem cells that are known to be able to differentiate to mesenchymal tissues. However, these cells are also known to possess an immunosuppressive effect. Therefore, we have considered MSCs as a treatment tool aimed at both suppression of inflammation and bone regeneration in RA patients.

Mesenchymal stem cells

MSCs have been reported as a cell population able to differentiate into several different lineages such as osteoblasts, chondrocytes and adipocytes[5]. Recently, MSCs have been administered to patients with graft versus host disease GVHD and have shown clinical efficacy without severe adverse events, proving their potential for immunosuppressive potency[6]. Following this report, the efficacy of MSCs on a variety of diseases has been investigated intensively. Previously, human MSCs were reported to effectively suppress collagen-induced arthritis (CIA), an animal model of RA.

Regarding skeletal tissue engineering, Quarto et al. have shown *in vivo* osteogenesis by autologous transplantation of MSCs with macroporous hydroxyapatite scaffolds in patients with large bone defects[7]. In order to obtain efficient bone regeneration, transplantation of MSCs with partially demineralized bone matrix scaffolds[8] or gene transfection[9] has resulted in some success.

Recently, we have reported the production of osteoprotegerin (OPG), a decoy receptor for the receptor activator of NFκB ligand (RANKL) by MSCs, resulted in inhibition of osteoclast differentiation[10]. In addition to multipotency and immunosuppressive effects, our finding adds another basis for MSCs to be a suitable tool for cell-therapy aimed at joint repair in RA patients.

Efficacy of MSCs on collagen induced arthritis

Several groups have demonstrated conflicting results regarding treatment effects of MSCs on animal arthritis models. The first report by Djouad et al. demonstrated that administration of murine mesenchymal cell line C3H10T1/2 to CIA mice[11] worsened arthritis through IL-6 production. However, a report by Augello et al. has shown the inhibitory effect of a single intraperitoneal injection of murine allogenic bone marrow-derived MSCs (BM-MSCs) by modulating the abnormal T cell response, cytokine production and induction of $CD4^+CD25^+Foxp3^+$ T_{regs}[12]. On the other hand, other reports utilizing BM-MSCs showed no beneficial effects[13]. Under these conflicting results and limited effects of primary MSCs on established CIA, some modulation on MSCs has been performed with beneficial effects. For instance, IL-10-transduced MSCs revealed treatment effect on CIA after onset by suppressing IL-6 and increasing IL-4, while non-transduced BM-MSCs did not show any effects[14]. More recently, Park et al. succeeded in improving established arthritis and prevented bone destruction using TGF-β-transduced MSCs that modulated not only T cell response and Th17 induction but also osteoclast differentiation[15].

Although these data have shown the possibility of using modified MSCs as a useful treatment tool for established RA, its application to clinical practice is far in the future considering its genetic approach. Recently, Liu et al. successfully treated established CIA with human MSCs originated from umbilical cord (UC)[16]. In addition to previous reports, their results also intimate the safety and convenience of MSCs. In contrary to the results with mouse MSCs, human MSCs seem to be more efficacious and safety has been proven not only by the animal models but also with clinical trials on GVHD. We have recently observed that human MSCs possessed prophylactic effects on the incidence of CIA rats, whereas rat MSCs did not (Zhang et al., in preparation). According to these previous results and from a viewpoint of clinical application, human MSCs were utilized in our experiments to assess their possible use as a novel treatment tool aimed at joint repair in RA patients.

MSCs for bone regeneration

Previous reports have demonstrated the potential of MSCs as a treatment tool for large bone defects[7]. However, it is uncertain whether MSCs are able to differentiate into osteoblasts under inflammatory circumstances. In order to address this question, MSCs were cultured in osteoblast induction media in the presence of inflammatory cytokines and osteoblast differentiation was evaluated by expression of osteoblastic markers. Among the evalu-

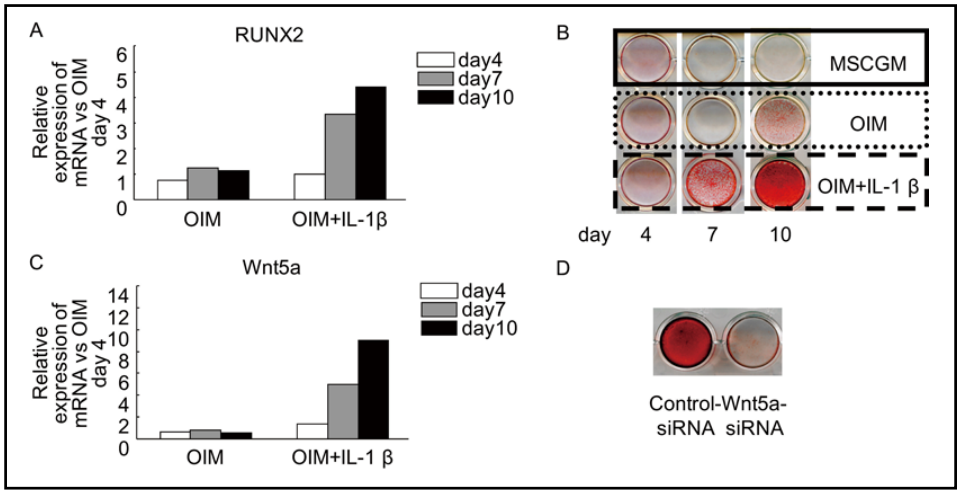

Fig.1 IL-1β enhances osteoblast differentiation

Human MSCs were cultured in osteogenic induction medium (OIM) with or without IL-1β. (A) IL-1β enhanced the mRNA expression of runt-related gene 2 (RUNX2), (B) mineralization detected by alizarin red S staining. (C) IL-1β up-regulated the gene expression of Wingless-type MMTV integration site (Wnt) 5a, and inhibition of Wnt5a resulted in suppression of mineralization (D).

ated inflammatory cytokines (TNF-α, IL-1β, IL-6 or IL-17), IL-1β prominently enhanced expression of RUNX2, a marker for osteoblasts. Moreover, enhanced mineralization was observed with the activation of the Wnt5a/Ror2 pathway (Fig.1). We also confirmed that OPG production by MSCs did not change even after osteoblast differentiation by osteogenic condition with IL-1β (data not shown). Our results indicate that MSCs are able to differentiate into osteoblasts even under inflammation and suggest the usefulness of MSCs on bone regeneration in patients with RA. However, we have also noticed that higher concentrations of cytokines as detected in severely inflamed sites inhibited osteoblast differentiation (data not shown). Thus, cell-therapy with MSCs should be considered as a subsidiary treatment to achieve bone repair after controlling the pre-existing synovitis.

In healthy individuals, osteoblasts are in fine tune with osteoclasts. This so called bone-coupling mediates bone turnover determining the bone density. Osteoblasts are associated with bone formation expressing RANKL, which simultaneously leads to differentiation of osteoclasts. Therefore, alteration of the bone-coupling can cause either loss or gain of bone tissue. In the RA joint, up-regulated expression of RANKL on synovial fibroblasts or auto-reactive lymphocytes induces differentiation of osteoclasts, resulting in inadequate bone resorption[17]. Thus, MSCs possessing anti-inflammatory effect and their multipotency is the most relevant tool to cell-therapy aimed at regeneration of destructed joints in RA patients. In previous clinical trials, intravenous or local infusion has been performed for treatment with MSCs. For our purpose, delivery of MSCs directly to the destructed lesion is a reasonable strategy and the most simplistic approach might be intra-articular injection. However, intra-articular injection of MSCs is known to result in diffusion into the articular tissue, which prevents the cells from appropriate delivery of the necessary number of cells to the targeted lesion.

In order to resolve these issues, we and others have considered utilizing tissue engineering techniques. Transplantation of MSCs in combination with scaffolds, such as β-tricalcium phosphate, poly-lactic-co-glycolic acid and hydroxyapatite[18], provide not only strength but also a three dimensional environment which seems to be an adequate circumstance for osteoblast or chondrocyte differentiation. Although a large number of studies with scaffolds have focused on the efficacy on bone defect animal models, adequate conditions in inflammatory circumstances should be investigated.

Source of MSCs

Tissue sources of MSCs are of interest. MSCs reside mainly in the bone marrow[5], adipose tissue[19] and synovial membrane[20]. No matter the tissue source, they possess the potency to differentiate into three different lineages,

although their differentiation potency seems to be quite different and depends on the tissue source. Sakaguchi Y. et al., reported that MSCs originating from bone marrow, synovium and periosteum are advantageous in bone regeneration[21]. On the other hand, donor of MSCs is also another issue providing some ideas to the pathology of the disease. Sun L.Y. et al. have demonstrated that MSCs from patients with systemic lupus erythematosus (SLE) have less ability for self expansion and concluded that MSCs play an important role in the pathogenesis[22]. MSCs from RA patients are not well understood. Based on the report by Sakaguchi Y. et al., utilizing MSCs from synovium rather than other tissues might be an ideal strategy for the purpose of joint repair. Morimoto D. et al. have reported the potency of hMSCs to differentiate to osteoblasts originating from different disease and found no difference between osteoarthritis and RA[23]. However, based on the previous reports pointing out the possible role of MSCs on SLE pathology, autologous MSCs may possess disadvantages in osteogenesis and immune suppression. Thus, even though autologous transplantation is an ideal treatment strategy, allogenic transplantation should also be considered to achieve appropriate regeneration of the joint tissue.

Conclusion

Biologics targeting inflammatory cytokines have altered the treatment goals in RA. Prominent and immediate suppression of arthritis can be achieved and joint destruction can be ceased with biologics. However, repair of the destructed joints is observed in rare cases and cannot be achieved intentionally. Therefore, we have defined our next treatment goal of RA treatment as bone repair. Based on these ideas, we have considered MSCs as valuable cell-therapy tools in the post-biologic era. Although there still are numbers of problems to be solved, we have shown here that inflammatory cytokines enhance osteoblastogenesis and are able to support bone regeneration even under the presence of inflammation (Fig.2).

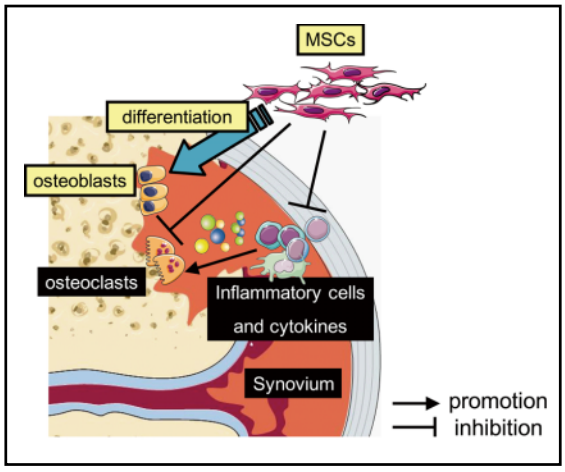

Fig.2 MSCs as a novel treatment tool for RA
MSCs suppress inflammation as well as osteoclast differentiation. Since MSCs are able to differentiate into osteoblasts, MSCs can be a novel treatment tool of RA from the viewpoint of both inflammation control and bone repair.

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Conflict of interests

Dr. Tanaka has received consulting fees, speaking fees, and/or honoraria from Mitsubishi-Tanabe, Chugai, Eisai, Takeda, Astellas, and Abbott and has received research grant support from Mitsubishi-Tanabe, Takeda, MSD, Pfizer, Astellas, Chugai, Abbott, and Eisai. The other authors declare no conflict of interest.

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