Inflammation and Regeneration Vol.32 No.5 NOVEMBER 2012

Mini Review

Functional change of synoviocytes and mesenchymal stromal cells through adipogenesis: A possible model of pannus and bone edema formation in rheumatoid arthritis

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The biology of fibroblast-like cells is important in understanding of pathogeneis of rheumatoid arthritis (RA). Fibroblast-like synovial cells (FLSs) is major component of pannus in inflamed joint, and mesenchymal stromal cells (MSCs) is thought to be in bone edema, a recently reported RA lesion in bone marrow that is detectable by MRI. It is interesting that MSCs share many characteristics with FLSs. Both types of cells can secrete cytokines, and differentiate into mesenchymal lineage cells such as osteoblasts, chondrocytes, and adipocytes. In this review, we discuss the possible contribution of the adipogenesis insufficency of FLSs or MSCs to the development of synovial hyperplasia and bone edema in RA.

Rec.12/28/2011, Acc.4/4/2012, pp202-207

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Key words adipogenesis, bone edema, cytokines, mesenchymal stromal cells, rheumatoid arthritis

Introduction

It is important to observe the pathological hallmarks of rheumatoid arthritis (RA) to understand the pathogenic mechanism of the disease. In addition to synovial hyperplasia in inflamed joints, which is a well-known characteristic of the disease, bone edema was recently identified as a pathological change in RA bone marrow detected by magnetic resonance imaging (MRI)[1, 2]. Even though the lesions are separated from each other by bone cortex, both lesions have similar cellular components, including monocytes, osteoclasts, and fibroblasts[3, 4]. It is also noteworthy that changes in fat tissue are evident in both lesions. Namely, synovial hyperplasia seems to invade surrounding fat tissue[5] and bone edema replaces fat tissue with non-fat tissue[4]. However, it is not understood how fat tissue is replaced with other cells in joint or bone marrow and how the aberrant replacement of fat tissues affect the clinical course of RA.

Fibroblasts in synovial hyperplasia are called fibroblast-like synovial cells (FLSs). FLSs are regarded as a thera-

peutic target of RA because of their ability to secrete cytokines and proteases[3]. Likewise, there are bone marrow resident fibroblast cells called mesenchymal stromal cells (MSCs)[6]. The pathogenic role of MSCs in the pathogenesis of RA is not fully understood[7-9]. However, it is interesting that MSCs share many characteristics with FLSs. MSCs have high potential to proliferate and secrete interleukin-6 (IL-6)[3, 10]. Besides this, both types of cells can differentiate into mesenchymal lineage cells such as osteoblasts, chondrocytes, and adipocytes[11-13].

In this review, we present the hypothesis that adipogenesis of FLSs and MSCs may be involved in the formation of synovial hyperplasia and bone edema, respectively. This hypothesis may give rise to a new therapeutic strategy to regulate FLSs and MSCs to reduce their joint-destructing ability to ameliorate joint inflammation.

Adipogenesis of FLSs and RA

In 2001, De Bari et al. reported that FLSs differentiate into mesenchymal lineage cells such as osteoblasts, chondrocytes, and adipocytes[12]. These discoveries paved the way to tissue engineering technology using FLSs to repair damaged joint structure in RA patients. Another application of the phenomena is to induce FLS differentiation *in vivo* to alter the cellular characteristics of FLSs to ameliorate RA. In this regard, we attempted to induce the adipogenesis of FLSs using troglitazone, a synthetic PPARγ ligand[13, 14]. Troglitazone induces adipogenesis of FLSs as well as the established adipogenesis induction method introduced by De Bari et al.

We examined whether the inflammatory milieu in rheumatoid synovial tissues affects the adipogenesis of FLSs. We tested the effects of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-1β on a troglitazone-mediated FLS adipogenesis system. Troglitazone-induced adipocyte differentiation of FLSs was effectively inhibited by TNF-α and IL-1β. This can be explained by the report by Suzawa et al., which stated that MSC adipogenesis can be blocked by a signal via nuclear factor-κB (NF-κB)-inducing kinase (NIK)--mediated NF-κB activation[15]. Interestingly, IFN-γ also inhibits the adipogenesis process of FLSs. These findings indicate that the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway also plays a role in inhibiting the adipogenesis of FLSs[13, 14].

One of the striking changes in FLS function is that the secretion of IL-6 is significantly reduced after the adipogenesis of the cell. In addition, the production of IL-8 and matrix metalloprotease-3 (MMP-3) in troglitazone-differentiated adipocyte-like FLSs is diminished[13, 14]. These functional changes in FLS can be explained by decreased NF-κB nuclear activity induced by TNF-α in troglitazone-differentiated adipocyte-like FLSs compared to non-treated ones. Since NF-κB is an important transcription factor for IL-6, IL-8, and MMP-3 expression, these transformations of FLSs are very favorable for the regression of RA. It is important that the differentiation of FLSs into adipocyte-like cells is not stable, because the production of IL-6, IL-8, and MMP-3 is restored 8 days after the withdrawal of troglitazone; this means that FLSs return to an undifferentiated or active state without differentiating stimulus[13, 14].

Adipogenesis of MSCs and RA

A recent histological examination of bone edema revealed that adipose tissue, a major cellular component of bone cavity, is replaced by inflammatory cells such as monocytes, fibroblasts, and osteoclasts[4]. More importantly, the extent of bone edema is related to the prognosis of RA[16, 17]. Therefore, it is expected that bone edema plays roles in inflammation and the destruction of joint in RA. These findings about bone edema also give rise to 2 questions: (1) how does bone edema emerge in bone marrow, and (2) how does the lesion contribute to the disease progression of RA?

MSCs are spindle-shaped adherent cells that can be enriched from bone marrow. The most remarkable characteristic of these cells is their ability to differentiate into mesenchymal lineage cells such as osteoblasts, adipocytes, and chondrocytes[11]. Besides this, MSCs can maintain their multipotency even after their expansion *in vitro*[18]. Interestingly, recent studies show that MSCs modulate the activities of T, natural killer, and dendritic cells[19]. This suggests the therapeutic potential of MSCs for treating RA[20]. However, it remains unclear whether MSCs are beneficial for RA therapy, because the cells are also implicated to have pathogenic roles in RA[21].

We examined whether cytokines block MSC adipogenesis, as is observed in FLSs. As a result, the adipogenesis of MSCs was dramatically reduced by culture with TNFα, IL-1β, IL-6, or transforming growth factor β (TGFβ). These data suggest that the inflammatory milieu inhibits the adipogenesis of MSCs as is observed in FLSs; a variety of

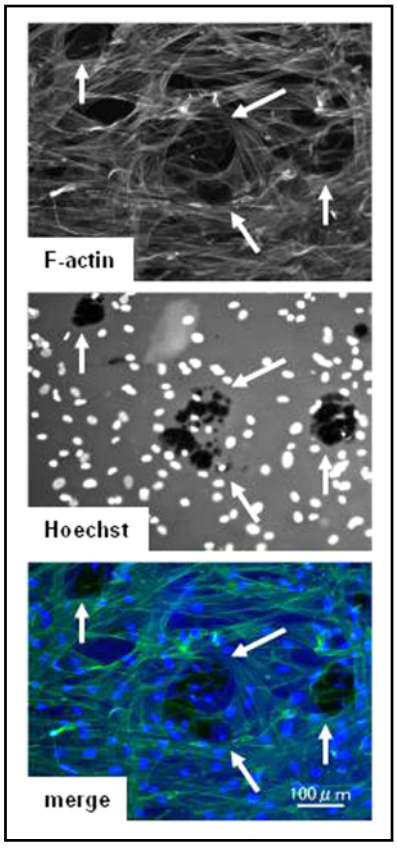

Fig.1 Stress fibers in MSCs were visualized by staining F-actin with fluorescent phalloidin after adipogenesis (top, and merge: green). Hoechst staining was used to visualize the nuclei (middle, and merge: blue)
The arrows indicate mesenchymal stem cells (MSCs) with lipid accumulation. Note that MSCs after adipogenesis do not exhibit stress fibers.

signaling pathway evoked by several cytokines mediates this[22]. These phenomena can explain how bone edema emerges in bone marrow under the inflammatory milieu.

Bone edema can be regarded as a lesion that must be treated to control RA, because the lesion contains inflammatory cells and osteoclasts, which are pivotal cells in chronic inflammation and bone destruction[4]. Given that bone edema is a joint-inflaming destructive lesion, we must determine whether MSCs are involved in the development of such lesions. To do so, we performed a comprehensive cytokine screening test in the supernatants collected from MSCs cultured with or without adipogenesis by using the Human Inflammation Antibody Array. It is known that MSCs secrete IL-6[10]. Interestingly, we confirmed a marked reduction of IL-6 in MSCs after adipogenesis[22]. This is consistent with the results of our previous study showing decreased IL-6 production by FLSs after adipogenesis[13, 14]. These results highlight MSCs as a source of IL-6 in bone edema that may support monocyte and osteoclast activation, aggravating RA.

Analyses of the intracellular structure of MSCs provide another evidence of the functional changes in MSCs via adipogenesis. These changes were initially noticed by a simple observation that adipogenesis-induced MSCs move less than undifferentiated MSCs in a migration assay[22]. Cell staining examination was used to identify the molecule responsible for causing the differences in the migration of undifferentiated MSCs compared to MSCs after adipogenesis. These analyses revealed that the expression patterns of F-actin and α-smooth muscle actin (α-SMA) are altered through adipogenesis[22]. As shown in Figure 1, the expression pattern of F-actin visualized by phalloidin in MSCs after adipogenesis is remarkably different from the so-called stress fiber pattern in undifferentiated MSCs[22]. Similar results were obtained by α-SMA immunostaining[22]. Therefore, these results suggest that the loss of functional α-SMA and F-actin in MSCs during adipogenesis causes the cells' loss of mobility.

The Impact of Adipogenesis Inhibition in RA

Inflammatory cytokines block the adipogenesis of both FLSs and MSCs. This finding implies that inflammation contributes to the replacement of adipose tissue with vigorously proliferating FLSs and MSCs, which may leads to the formation of synovial hyperplasia or bone edema. It is important that these pathological changes already appear at the early stage of RA. Our data suggest that the inflammation that triggers the onset of RA may also block the adipogenesis of FLSs and MSCs to form synovial hyperplasia or bone edema.

Interestingly, one arthritis model mouse exhibits a phenotype consistent with this hypothesis. MSCs obtained from IL-1 receptor antagonist knockout mice (IL-1ra$^{-/-}$), an arthritis model mouse, exhibit altered self-renewal and differentiation ability[23]. Interestingly, the population of MSCs with adipogenic potential decreases prior to the onset of arthritis. In addition, the adiposity of the IL-1ra$^{-/-}$ mouse decreases prior to arthritis. Thus, it is possible that inflam-

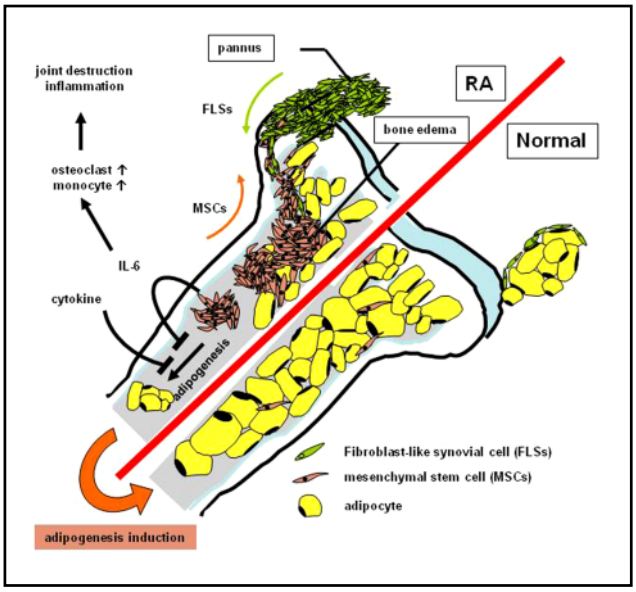

Fig.2 Due to their high ability to secrete IL-6, MSCs can be potent pathogenic cells similar to fibroblast-like synovial cells (FLSs) in the pannus of rheumatoid arthritis (RA) joints

IL-6 blocks the adipogenesis of MSCs and maintains the undifferentiated cell state. In turn, the undifferentiated MSCs secrete large amounts of IL-6. In this way, a vicious cycle involving IL-6 and undifferentiated MSCs contributes to bone edema as a joint-destroying inflammatory lesion. To treat such lesions, the adipogenesis induction by drugs such as synthetic PPARγ ligands may diminish bone edema and ameliorate the inflammatory lesions in bone marrow in RA.

matory cytokines such as IL-1β inhibit the adipogenesis of FLSs and MSCs, and bring about bone edema as well as synovial hyperplasia at an early stage of RA.

The IL-6 produced by FLSs and MSCs is remarkably reduced by adipogenesis. IL-6 is an important cytokine for the development of RA and is an evident therapeutic target in RA treatment[24]. Therefore, it is possible that the undifferentiated MSCs in bone edema intrinsically contribute to inflammation and bone destruction in RA, the same as FLSs in inflamed joint cavities. It is also important to note that IL-6 blocks the adipogenesis of MSCs. This implies that IL-6 can trigger bone edema at the early stage of RA. In this regard, it is plausible that the development of RA involves a vicious cycle involving IL-6 production and bone edema in bone marrow; that is, IL-6 prevents MSCs from differentiating into adipocytes, and the undifferentiated MSCs persist in secreting IL-6, which again contributes to the spreading of bone edema and chronic inflammation in RA.

The reduced migration capacity of MSCs after adipogenesis compared to undifferentiated MSCs is another important finding. It is thought that inflammatory cells and proliferating synovial cells in inflamed joints break cortical bone to generate bone edema[2]. We propose that MSCs also have the potential to initiate this cortical breaking from the bone cavity toward the joint cavity, because they can vigorously migrate and secrete IL-6. Taken together, it is possible that a bidirectional cortical break can occur in RA in which synovial cells stimulate MSCs and vice versa. In this scenario, treatments targeting RA lesions must explore bone edema. Our model is demonstrated in Figure 2.

The Potential of Adipogenesis Induction for RA Treatment

The marked induction of IL-8 in MSCs after adipogenesis compared to that observed in undifferentiated MSCs[22] completely contradicts the results described in our previous study using FLSs[14]. This can be explained by the difference in cells or the adipogenesis induction system used. We used adipogenesis induction medium containing indomethacin, IBMX, insulin, and dexamethasone for MSC adipogenesis. In the previous study on FLSs, we induced the adipogenesis of FLS using troglitazone, a synthetic PPARγ ligand. The synthetic PPARγ ligand, which is proven in arthritis model mouse to be a potent anti-rheumatic drug[25], may be a better choice for inducing the adipogenesis of FLS and MSC because it can inhibit both IL-8 and IL-6 production[14].

Conclusion

We discussed the possibility that MSCs may be a component of bone edema as well as a potent therapeutic target that deserves exploration. Accordingly, we propose "adipogenesis induction in FLSs and MSCs in the treatment of early-stage RA" as a future study. An existing group of drugs can induce MSCs adipogenesis; synthetic PPARγ ligands are now widely used to treat diabetes and can induce FLSs and MSCs adipogenesis. Using these compounds may diminish inflammatory lesions during the early stage of RA such as synovial hyperplasia and bone edema, blocking their progression to persistent and established joint-destructing lesions.

Further basic and clinical research is also desired to determine whether bone edema can be an indicator for an affirmative treatment in RA.

Acknowledgments

A Grant-in-Aid for Young Scientists (B) [21790949 to S.Y.] from the Ministry of Education, Science, Sports, and Culture supported this study.

Conflict of interests

None

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