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Review

Impact of immune regulation and differentiation dysfunction of mesenchymal stem cells on the disease process in ankylosing spondylitis and prospective analysis of stem cell transplantation therapy

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Abstract

Ankylosing spondylitis (AS) is a rheumatic bone and joint disease caused by inflammation, erosion, and pathological bone formation. The pathological features of chronic inflammation, bone destruction, and pathological ossification occur due to the disruption of the body's immune regulation and altered bone remodeling balance. Mesenchymal stem cells (MSCs) have multidirectional differentiation potential and immunomodulatory functions and play an important role in immune regulation and bone formation. The immune regulation and osteogenic capacity of MSCs in AS are altered by factors such as genetic background, internal environment, infection, and mechanical forces that drive disease development. This review further evaluates the role of MSCs dysfunction in inflammation and pathological bone formation by analyzing the effects of the above-mentioned factors on MSCs function and also looks forward to the prospects of MSCs in treating AS, providing some ideas for an in-depth study of inflammation and ectopic ossification.

Key messages

- The proliferation and differentiation, regulation of inflammation, and exocrine function of MSCs in AS patients are pathogenically altered under the influence of genetic background, internal environment, infection, and mechanical stress.
- ASMSCs not only have enhanced osteogenic ability, but also interfere with the differentiation and secretion function of various immune cells, resulting in dysfunction of immune system and imbalance of bone metabolism.
- Although stem cell transplantation therapy brings good curative effect to AS, it inevitably causes complications. Therefore, attention has gradually shifted to the transplantation therapy of more stable MSCs derivatives.

Keywords: ankylosing spondylitis, mesenchymal stem cells, dysfunction, inflammation, pathological ossification

Introduction

Ankylosing spondylitis (AS) is a chronic rheumatic bone and joint disease that affects ∼0.9%–2.0% of the world's population [1] and is twice as common in men as in women [2]. Inflammation is accompanied by bone erosion and new bone formation, often resulting in severe structural deformities of the bone and joints and functional impairment [3]. Disturbances in the balance between inflammation and bone remodeling are thought to contribute significantly to structural deformities of the sacroiliac and spinal joints [4]. Considering that AS is a disease characterized by the overproduction of pro-inflammatory cytokines, mainly tumor necrosis factor-α (TNF-α), anti-inflammatory agents, and TNF-α inhibitors (TNF-i) are widely used in clinical treatment; yet, they have been shown to fail in changing the prognosis of disability in a large number of patients [5]. Mesenchymal stem cells (MSCs) are important in the pathogenesis of AS, where their dysfunction promotes chronic inflammation and pathological bone formation; in healthy populations, they have been shown to exhibit therapeutic potential [6].

MSCs have the potential to differentiate in multiple directions and participate in the regulation of immune cell functions [7]. They are influenced by genetic background, internal environmental homeostasis, and inflammatory factors, exhibiting abnormal immune and bone remodeling homeostasis regulation in patients with AS. It was recently reported that MSCs derived from umbilical cord blood significantly alleviate symptoms in patients with AS [8], suggesting that the health status of MSCs determines the development and progression of AS to some extent.

Therefore, it is crucial to investigate the causes of abnormal MSCs function and their regulatory mechanisms in immune and

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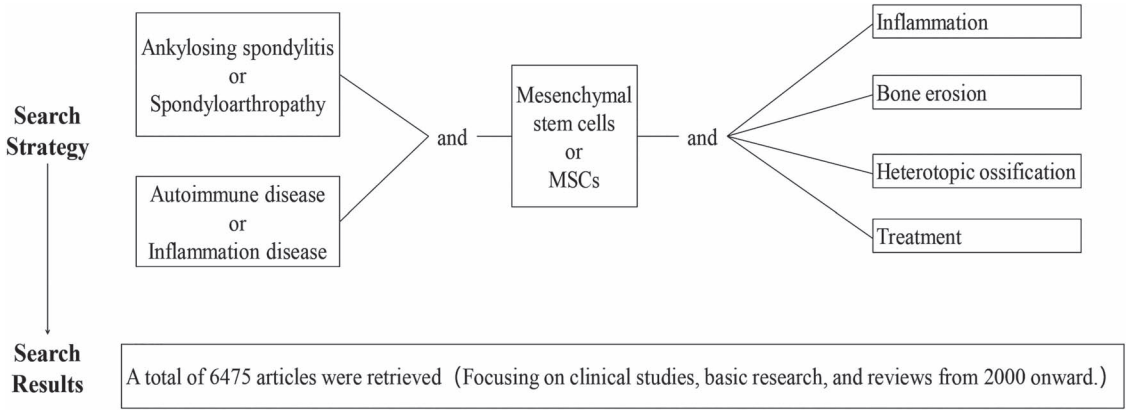

Figure 1. Flow diagram of search strategy.

Table 1. Pathogenic factors and characteristics of MSCs dysfunction in AS.

Pathogenic factors	Sites	Signal paths	Main metabolites	Function	Sources of information
HLA-B27 heavy chain HLA-B27 heavy chain monomers	Endoplamic reticulum	ER stress Unfolded protein response	TNAP Runx2	Promote osteogenesis	Chen et al. [10] Liu et al. [11]
Chronic inflammation				Increase the level of cellular oxidative stress	Christina et al. [12]
	ATP	ROS mediated oxidation reaction	p53 p21 p16	Promote cell senescence	Ye et al. [13]
IL-17A			Dkk-1	Promote osteogenesis	Daoussis et al. [14]
TGF-β		TGF-β/smad1	Wnt		Seo et al. [15]
IL-37		PI3K/AKT			Ye et al. [18]
miR-22-3p in Exosomes		PER2-mediated Wnt/β-catenin axis			Liu et al. [19]
TNF-α	TNFR	PI3K/AKT	Dkk-1 Wnt TNAP		Briolay et al. [24]
LPS		Smad1	TRAF4 Runx2		Li et al. [28, 29]
Mechanical force		Smad1/5/8 ERK			Zheng et al. [34]

bone remodeling homeostasis. We searched in Pubmed with keywords surrounding AS and MSCs, focusing on the role of MSCs in inflammation, bone erosion, ectopic ossification, and treatment of AS (Fig. 1). This article discusses the mechanisms of MSCs dysfunction and their regulatory role in immune and bone remodeling homeostasis and describes the value of MSCs and their derivatives in treating AS (Fig. 2).

Dysfunction of mesenchymal stem cells in ankylosing spondylitis

MSCs are pluripotent cells of mesodermal origin that can self-renew and differentiate into osteoblasts, chondrocytes, and adipocytes and have strong immunomodulatory effects [9]. Under the influence of genetic background, internal environment, infectious factors, and mechanical forces, MSCs in patients with AS exhibit varying degrees of functional abnormalities closely linked to the development of inflammatory response, bone erosion, and pathological ossification. (Table 1).

Genetic background

Human leukocyte antigen-B27 (HLA-B27) and endoplasmic reticulum aminopeptidase 1 (ERAP1) are considered critical genetic factors for AS development [10], leading to abnormal intracellular expression and accumulation of HLA-B27. Large amounts of disulfide bond-mediated HLA-B27 heavy-chain (HC) dimers (misfolded) and HLA-B27 HC monomers (unfolded) accumulate in MSCs, leading to endoplasmic reticulum (ER) stress and upregulation of the unfolded protein response, promoting inositol-requiring 1 phosphorylation and elevating X-box binding protein 1 transcriptional activity. It further promotes tissue non-specific alkaline phosphatase (TNAP) expression in combination with the retinoic acid receptor beta promoter, which enhances MSC differentiation activity through the non-Runt-related transcription factor 2 (Runx2) pathway. When knocking down the HLA-B gene in MSCs from patients with AS (ASMSCs), the investigators found a significant reduction in TNAP production [11], suggesting that HLA-B27 misfolding plays a vital role in the aberrant differentiation of MSCs.

Internal environment

Since AS is a chronic inflammatory disease, various cell proliferation, differentiation, metabolism, and apoptosis processes occur in the pathological inflammatory microenvironment. Christina *et al.* [12] suggested that chronic inflammation caused by autoimmune diseases causes prolonged oxidative stress states in

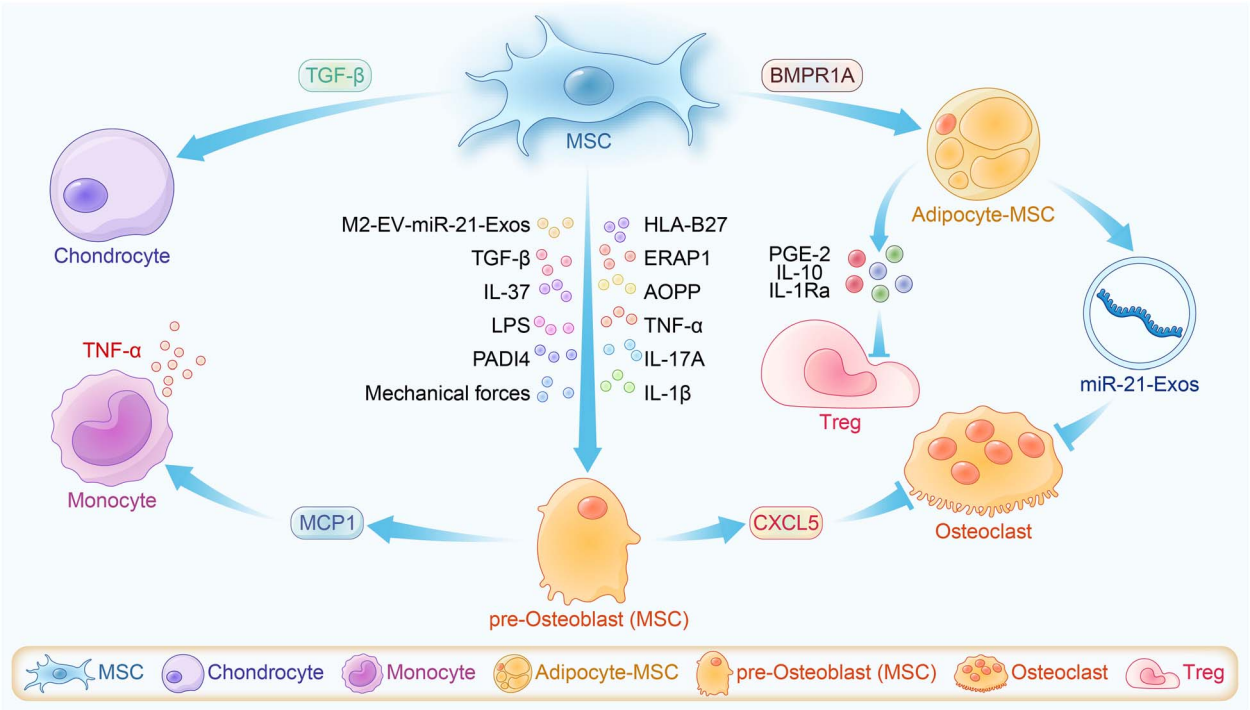

Figure 2. MSCs in AS tissue can differentiate into chondrocytes, adipose stem cells, fibroblasts, and osteoblasts; MSCs tend to differentiate into chondrocytes under the action of TGF-β, and tend to differentiate into adipose stem cells under the action of BMPR1A; BMPR1A inhibits osteoclast activity by releasing miR-21-containing exosomes and suppresses Treg function through PGE-2, IL-10, and IL-1Ra. MiR-21, IL-37, LPS, PADI4, AOPP, TNF-α, IL-17A, and IL-1β can regulate osteoblast differentiation; genetic and extrinsic factors such as HLA-B27 and ERAP1, and mechanical strength are also important triggers for osteogenic differentiation of MSCs.

cells. Researchers found that advanced oxidation protein products in AS serum led to decreased mitochondrial membrane potential, ATP production, and oxygen consumption in MSCs, resulting in elevated reactive oxygen species (ROS), which mediated the expression of cell cycle arrest-related proteins p53, p21, and p16 and promoted MSCs senescence, while the use of both serum and ROS inhibitors in healthy patients reversed the progression of MSCs senescence [13]. This observation suggests that prolonged exposure to an inflammatory environment leads to senescence and MSCs dysfunction.

Various inflammatory factors, such as TNF-α, interleukin (IL)-17A (IL-17A), and transforming growth factor beta (TGF-β), are thought to play essential roles in the development of AS. Compared to human dermis-derived MSCs (HDMSCs), Dickkopf-1 (Dkk-1) expression is significantly decreased in ASMSCs; thus, ASMSCs exhibit enhanced Wnt pathway activity [14]. IL-17A regulates the expression of Dkk-1 in ASMSCs [14], while Wnt protein expression is induced through the TGF-β-activated kinase 1 pathway [15], so IL-17A and TGF-β elevation further promotes the differentiation of ASMSCs into osteoblasts [16, 17]. Notably, the cytokine IL-37, which we overlooked, can also activate PI3K/AKT in ASMSCs and promote osteogenic differentiation [18]. It was even found that aberrantly expressed microRNA (miRNA), specifically miR-22-3p, within exosomes secreted by M2 macrophages in inflammatory endothelial cells, were phagocytosed by ASMSCs and demonstrated bone-producing functions, while M2-EV-miR-22-3p also exhibited functions promoting ossification in AS mouse models, suggesting that the formation of inflammatory endothelial cells is conducive to ossification formation [19].

While TNF-α binds to MSCs in various dysfunctional manifestations, two types of TNF receptors (TNFRs) are present on the surface of MSCs: TNFR-1 and TNFR-2 [20]. TNFR-1 has a pro-apoptotic effect, while TNFR-2 exerts an apoptosis-inhibiting function [21]. Therefore, it was found that TNF-α-induced ASMSCs showed significantly higher expression of p-PI3K/PI3K, p-AKT/AKT, and p-mTOR/mTOR proteins than HDMSCs, exhibiting inhibition of cellular autophagy [22]. However, Liu *et al.* [23] reported elevated expression of human TNF-related apoptosis-inducing ligand receptor 2 on the surface of ASMSCs stimulated by TNF-α, expressing enhanced apoptosis in ASMSCs. Interestingly, elevated peptidyl arginine deiminase type IV expression in ASMSCs enhanced TNF-α-mediated osteogenic differentiation of ASMSCs. TNF-α increased Dkk-1, Wnt10b, and Wnt5a levels in ASMSCs, elevating TNF-α promoted osteogenic differentiation through Wnt5a and TNAP expression in MSCs [24]. However, Lancel *et al.* [25] found that elevated TNF-α exhibited an inhibitory function in osteogenesis. This dichotomy may arise from the difference in the concentration of TNF-α, with ASMSCs predominantly expressing Wnt proteins in low concentrations of TNF-α and Dkk-1 transcripts in high concentrations of TNF-α [26]. Notably, much mystery remains regarding the regulation of ASMSCs by TNF-α. We are yet to explain individual differences in treating AS with TNF-i, perhaps a combination of concentration factors and TNFR.

Infectious factors

Gram-negative bacterial infections are considered important factors driving the pathogenesis and progression of AS, especially lipopolysaccharide (LPS), a pro-inflammatory substance derived from the outer membrane of Gram-negative bacteria, which can lead to MSC dysfunction [27]. TNFR-associated factor 4 (TRAF4) expression is significantly higher in LPS-stimulated ASMSCs than in HDMSCs [28], and it is mainly involved in embryonic development, cell polarity, cell proliferation, apoptosis, and

regulation of ROS production. Thus, the investigators found that elevated TRAF4 expression in ASMSCs increased Smad1-mediated Runx2, a major regulator of osteogenesis and transcription, by suppressing the expression of Smurf2, a classical regulatory factor that inhibits the osteogenic differentiation of MSCs [29]. They also found that TRAF4 inhibits MSC differentiation toward adiposity by activating pyruvate kinase M2 and promoting β-catenin production [30]; when TRAF4 expression was reduced, the osteogenic differentiation ability of MSCs was diminished, and the lipogenic differentiation ability was restored [29 30]. Further studies are required to elucidate LPS roles in the dysfunction of ASMSCs differentiation.

Mechanical forces

Nokhbatolfoghahaei *et al*. [31] systematically addressed the stress response of MSCs to various mechanical stresses and found that almost all mechanical stimuli induced activation of the Wnt signaling pathway and expression of Runx2. This could explain the increased osteogenic differentiation of MSCs in noninflammatory environments at ligament stops in AS [32]. Upon stimulation by mechanical forces, MSCs exhibit enhanced migratory capacity by upregulating the expression of ligand proteins to produce stronger connections with the stroma [33]. Compared with HDMSCs, ASMSCs showed stronger Smad1/5/8 and ERK signaling pathway activity in hydroxyapatite/tricalcium phosphate scaffold 3D biomimetic culture systems [34], indicating that ASMSCs are more sensitive to mechanical stimuli. Therefore, the researchers found that when the tail suspension and stretching training were used to treat a mouse model of AS, the reduction in mechanical forces on the axial joint slowed the progression of ossification [35, 36]. However, the regulatory effects of the matrix and internal forces generated by ASMSCs have not been specifically stated. We believe this will change the traditional understanding of the pathogenesis of AS and examine the development of ectopic ossification from the perspective of noninflammatory osteogenesis.

In conclusion, MSCs play a pivotal role in preserving the body's structural integrity and the dynamic equilibrium of the immune system under physiological conditions. In individuals with AS, MSCs experience aberrant signal transduction, triggered by HLA-B27, inflammatory factors, microbial infections, and extrinsic stress. This aberration leads to differentiation and secretion malfunctions, marking a critical component of AS's pathogenesis. Consequently, accurate detection of the B27 subtype is instrumental in hastening diagnosis and forecasting the prognosis of the disease. The judicious employment of inflammation inhibitors can ameliorate symptoms, with considerable advancements being made in this domain through the introduction of biological agents. Moreover, we can pinpoint the types of pathogenic microbes by analyzing lesion tissues using advanced technologies, such as second-generation sequencing. Ultimately, it is incumbent upon us to devise scientifically grounded exercise regimens and create appropriately designed assistive devices to offset the stress arising from the improper posture seen in AS patients.

Mesenchymal stem cells and inflammation

The function of MSCs is not limited to their potential for proliferation and differentiation; more importantly, they are involved in immune regulation, mediating the formation of a favorable immune microenvironment under physiological conditions and releasing growth factors that activate endogenous tissue repair [37]. However, in the immune microenvironment of AS, immune regulation is disturbed due to the dysfunction of MSCs, leading to intrinsic and adaptive immunity dysfunction, resulting in an inflammatory state dominated by abnormal secretion of TNF-α and IL-23 [38] (Table 2).

Mesenchymal stem cells and innate immune

Innate immune cells such as dendritic, macrophage, and natural killer cells are dysfunctional to some extent in response to classical cells in the study of AS [39]. It was found that during osteogenic differentiation, ASMSCs secrete more monocyte chemoattractant protein 1 than HDMSCs, which would cause increased local migration of monocytes and promote the ability of macrophages to polarize toward M1 and secrete TNF-α when the osteogenic capacity of ASMSCs was inhibited by specific short hairpin RNAs, the hyperpolarization of M1-type macrophages in the co-culture system was successfully reversed [40].

In addition to recruiting circulating monocytes, ASMSCs can influence macrophages' function at inflammation sites. Under physiological conditions, MSCs can transform macrophages from the inflammatory M1 to the anti-inflammatory M2 phenotype with immunosuppressive properties [41]. However, owing to the abnormal osteogenic differentiation of ASMSCs, their immunosuppressive properties are replaced by pro-inflammatory ones [40].

Mesenchymal stem cells and adaptive immunity

Adaptive immunity comprises two important branches: T cell-mediated cellular immunity and antibody-mediated humoral immunity. T/B cell dysfunction has been reported in all AS studies [42, 43], and an imbalance in the ratio of T cell subsets and dysfunctional secretion are considered to be important causes of AS pathogenesis [42].

ASMSCs also exhibit a stronger potential for lipogenic differentiation owing to the enhanced expression of bone morphogenetic protein (BMP) receptor 1A on the cell surface [44], and adipose-derived MSCs (ASCs) modulate adaptive immune cells, especially in the immune microenvironment of AS. It was found that although ASCs derived patients with AS (ASASCs) and healthy humans exhibited suppression of T cell proliferation, T cells co-cultured with ASASCs did not exhibit elevated IL-10 secretion; instead, two other factors inhibited cell proliferation-PGE-2 and IL-1Ra [45], implying that ASASCs play a weaker role in suppressing inflammation in the regulation of adaptive immunity. Significantly lower T-box expressed in T cells/GATA-binding protein 3 and retinoid-related orphan receptor c/forkhead box P3 in T cell subsets in a system co-cultured with ASCs and $CD4^+$ T cells imply reduced Treg production, but the investigators found increased production of interferon-gamma and IL-17 AF [46]. Although Toll-like receptor (TLR)-3 and TLR4 levels were decreased in ASMSCs, they still inhibited Treg production in the presence of increased IL-10 expression, a phenomenon that we could not explain and speculate might be due to a significant increase in TLR3 and TLR4 downstream pathway activity due to the abnormal function of ASMSCs.

In the discourse above, we discerned that the inflammatory microenvironment intensifies the senescence of MSCs, thereby undermining their immunomodulatory capacities and precipitating a disruption in immune system equilibrium. Thus, we propose a tripartite approach to inhibit the aging process of MSCs optimally. Initially, we can mitigate the oxidative stress within the internal milieu, thereby fostering more conducive survival conditions for MSCs. Subsequently, we can utilize biomaterials

Table 2. The regulatory effects of MSCs on immune cells.

Cell types	Secretory protein	Target cells	Key factors	Function	Sources of information
MSCs	Monocyte chemoattractant protein 1	Monocytes	TNF-α	• Migration (+) • M1 polarization (+) • TNF-α secretion (+)	Xie et al. [40]
	CXCL5	$CD14^+$ monocytes		Osteoclast differentiation (+)	Liu et al. [50]
Adipose-derived MSCs		T cells	PGE-2 IL-1Ra	Proliferation (–)	Kuca-Warnawin et al. [45, 46]
			• lower T-box • GATA-binding protein orphan receptor c • Forkhead box P	Treg differentiation (–)	

at the cellular plane to persistently target and administer age-ameliorating drugs to MSCs. Finally, in circumstances where the aging of MSCs proves challenging to reverse, we might contemplate transplantation therapies, an area that we will delve into in the succeeding chapters.

Mesenchymal stem cells and bone erosion

Imaging evidence suggests a period of bone erosion between the inflammatory response and heterotopic ossification [47], with bone erosion leading to cartilage destruction and subchondral bone [48]. This section describes how ASMSCs affect osteoclast function and bone erosion, contributing to disease progression.

Under physiological conditions, MSCs can regulate monocyte function through Indian hedgehog and nuclear factor kappa-B (NF-κB) ligand [49]. NF-κB is a nuclear factor important for regulating osteoclastogenesis, so the monocyte dysfunction caused by MSCs is not only limited to macrophage polarization but also manifests in abnormal osteoclast differentiation. Downregulation of miR-4284 in ASMSCs increased CXCL5 compared to that in HDMSCs, significantly inhibiting the osteoclast differentiation ability of $CD14^+$ monocytes in the co-culture system, and the inhibitory effect was lifted when the miR-4284 was upregulated [50]. This suggests that osteoclast function is strongly inhibited in regions where ASMSCs are active. A recent study found that elevated expression of Circ-0110634 in exosomes of ASMSCs degraded TNFR-associated factor 2 on the surface of monocytes that ingested exosomes, leading to the inactivation of the NF-κB pathway and inhibition of osteoclastogenesis [51].

Mesenchymal stem cells and heterotopic ossification

Ligamentous ectopic ossification in AS is divided into four processes typical of endochondral osteogenesis: inflammation, chondrogenesis, osteogenic activity, and pathological bone formation, which is typical of endochondral osteogenesis [52]. During the process of endochondral ossification, chondrocytes differentiate from MSCs to create cartilage templates. These templates aid in bone formation and also encourage the recruitment and proliferation of MSCs. These MSCs then differentiate into chondrocytes and osteoblasts, ultimately forming a mature bone matrix. This entire process drives endochondral bone osteogenesis-mediated ectopic ossification [53] (Fig. 3).

Mesenchymal stem cells and chondrogenesis

The process of osteogenesis within cartilage has been described as a 5-stage process: first, internal environmental factors stimulate MSCs to prepare for differentiation into chondrocytes; in the second stage, these chondrocytes aggregate to form cartilage; the third stage is characterized by chondrocyte hypertrophy and a 5–10-fold increase in cartilage volume; in the fourth stage, the matrix is mineralized by calcium phosphate, and osteoclasts begin to infiltrate; finally, in the fifth stage, vascular infiltration occurs, leading to chondrocyte apoptosis and osteogenic differentiation of MSCs [54].

Chondrocyte production in inflammatory-mediated ligaments has been studied by researchers [52]. Histological sequencing at the gene expression level revealed elevated expression of EDIL3, ANO6, HAPLN1, and ANTXR2 in ASMSCs, implying the enhanced activity of ASMSCs toward chondrocyte differentiation [55]. Cartilage formation was also supported by imaging and histopathological testing [52, 56]. Ma *et al.* [57] found active angiogenesis in the focal cartilage from patients with AS and SKG mouse models, further demonstrating the cartilage-vascular-osteogenic coupling process by inhibiting activating transcription factor 6, an important regulator of angiogenesis, using Chapin-A7; they found that osteogenic activity was significantly reduced.

Mesenchymal stem cells and osteogenic activity

Osteoclastic activity in cartilage recruits large numbers of $osterix^+$ osteoprogenitor cells. However, imaging evidence also shows cartilage destruction on articular cartilage surfaces [58]. TGF-β and TNF-α are key factors that promote targeted migration of ASMSCs into the ligamentous tissue [52, 59]; this may be due to the abnormal increase in engulfment and cell motility protein 1 (ELMO1) expression in ASMSCs, and the investigators found that ossification was effectively limited by inhibition of ELMO1 expression in the AS mouse model [59]. Cui *et al.* [60] found that high CXCL12 expression in AS bone lesions also induced the migration of ASMSCs to the lesion, and pathological new bone formation and migration of MSCs were significantly inhibited in AS mice after the conditional knockdown of CXCR4. This may explain why TNF-i are ineffective in stopping ossification progression, as TNF-α is not the only factor that promotes the migration of ASMSCs to the lesion during the osteogenic phase. This evidence also suggests sufficient cytokines in the osteogenic microenvironment to drive the migration of ASMSCs into the cartilage, promoting the activity of ASMSCs toward areas of bone

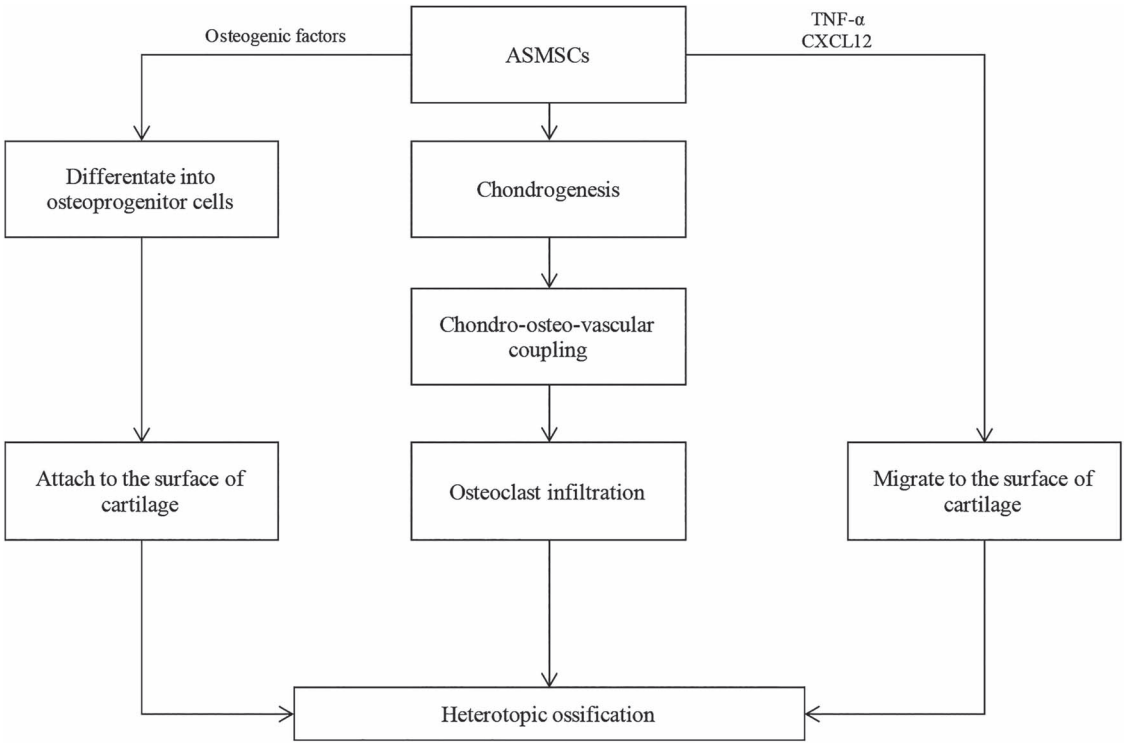

Figure 3. Pathways of MSCs in heterotopic ossification.

erosion within the cartilage and facilitating the progression of pathological ossification.

Mesenchymal stem cells and pathological bone formation

ASMSCs have an enhanced capacity for osteogenic differentiation, and MSCs migrating into the cartilage promote pathological ossification by differentiating into osteoblasts. The aberrant expression of HLA-B27 leads to the transcription of TNAP in ASMSCs [11]. In addition to ER stress, Navid *et al.* [61] found that HLA-B27 accelerates bone formation progression by binding to the BMP pathway receptor subunit, activin receptor-like kinase-2, which increases the sensitivity of the BMP-TGF pathway to TGF-β as well as TNAP expression. Mutations in the TNAP haplotypes rs3767155 (G), rs3738099 (G), and rs1780329 (T) are mainly associated with the development of bony ankylosis in AS [62]. Therefore, ossification of ASMSCs requires the synergistic effect of HLA-B27 and haplotype TNAP, which may explain why not all HLA-B27-positive patients develop osteogenic ankylosis.

In addition, inflammatory factor-mediated reduction of Dkk-1 in ASMSCs is a critical factor in pathological bone formation [14, 62]. Loss of Dkk-1 inhibited Wnt protein levels significantly in spinal ligament tissues, and activation of β-catenin and protein kinase C-δ enhanced NF-κB and JNK/AP-1 pro-transcriptional activity, which promotes osteoblast differentiation as well as bone matrix secretion and accelerates pathological bone formation [56, 62]. Interestingly, CXCR4 high expression promoted the migration of ASMSCs, decreased the level of β-catenin phosphorylation, and increased the Wnt pathway activity, which could arrest the development of the ossification process by blocking CXCR4 expression in isolated cells [60, 63].

In conclusion, cartilage formation emerges as a fundamental process in the ectopic ossification of AS [52]. Research in bone immunology reveals a close interconnection between the immune system and the skeletal system, with numerous shared cellular factors [64]. For instance, osteoprogenitor cells secrete chemokine ligand 12 (CXCL12) and stem cell factor, which stimulate the proliferation of myeloid stem cells. Additionally, osteocytes secrete sclerostin and granulocyte colony-stimulating factor, which regulate the differentiation of lymphoid and myeloid cells [65]. Therefore, comprehending the origin and functional characteristics of MSCs within lesions not only deepens our understanding of ectopic ossification but also enriches our comprehensive knowledge of the immune and skeletal systems. To achieve these goals, we propose several approaches. First, advanced techniques such as pathological examination and single-cell sequencing can be employed to identify the source of MSCs and elucidate the temporal relationship of their differentiation. Second, through cellular function experiments, we can identify the key molecules responsible for MSC hyper-ossification. These molecules can then serve as potential targets for biological treatments. Lastly, given the central role of cartilage formation in the ectopic ossification of AS, our future research will investigate interventions that can impede the differentiation of MSCs into cartilage.

Mesenchymal stem cells and treatment

Long-term anti-TNFα therapy with different anti-TNF-α drugs improved inflammation in patients with AS, but there was no trend toward reversal of structural damage [66, 67]. Pathological ossification and inflammation are subtle, and imaging evidence suggests that new bone formation is mostly located at sites of inflammation and that inflammatory factors also promote ossification [14, 62, 68]. Yet, imaging reveals bony lesions in non-inflammatory active areas in patients undergoing anti-inflammatory therapy [69]. Therefore, more appropriate treatment options for treating AS must be explored.

Human dermis-derived mesenchymal stem cells transplantation

MSCs can be obtained from bone marrows, adipose tissues, umbilical cords, mammary glands, molars, amniotic fluid, and peripheral blood, and transplantation of HDMSCs for treating AS

has gradually moved from conception to practical demonstration [70]. The earliest transplantation of HDMSCs for AS may have been a serendipitous discovery when the ligaments that had undergone pathological ossification were found to have partially regressed after the urgent HDMSCs transplantation in a patient with acute myeloid leukemia who also had AS [71]. In 2015, an *in vitro* study found that HDMSCs treated with all-trans retinoic acid co-cultured with peripheral blood mononuclear cells significantly reduced TNF-α, IL-17A, and interferon-γ expression in the cell supernatant and that HDMSCs showed therapeutic potential [72]. In 2017, investigators transplanting umbilical cord MSCs (uMSC) into patients with AS found decreased Bath AS disease activity and measures and increased Bath AS functional indices, with uMSC demonstrating excellent inflammatory modulation [8]. Although MSCs transplants do not bring about strong rejection reactions, some patients still exhibit transient fever [8]; this shortcoming will be revealed further as the study progresses.

Derivatives of human dermis-derived mesenchymal stem cells

Recipient rejection due to MSCs allografts and possible oncogenic effects due to the fusion of MSCs with target cells are concerns that should be addressed during MSCs transplantation [73]. In contrast, derivatives of HDMSCs are described as antigen-free alternatives to HDMSCs and are classified as extracellular vesicles or exosomes, depending on their size [73, 74]. Derivatives of HDMSCs are large delivery systems that wrap and protect regulatory proteins, DNA, RNA, and lipids through phospholipid bilayer structures and deliver these functional molecules to target cells for biological effects [74].

The study of extracellular vesicles and exosomes remains a gap in the AS research field. Only a few studies have elucidated the circRNA–miRNA–mRNA network and predicted its potential functions by exploring the expression of circRNAs in exosomes [75]. Notably, HDMSCs exosomes (HDMSCs Exo) with high expression of miRNA-21 could inhibit bone destruction significantly in AS mice [76]. This study highlights the role of HDMSCs Exo in treating AS because they have shown clear results in studies of tumor and injury repair [77, 78], and HDMSCs transplantation has seen preliminary results in AS [8]. Thus, we believe that HDMSCs Exo are of great value to be explored in the field of AS research as well as treatment.

Discussion

ASMSCs dysfunction promotes AS inflammation, bone erosion, and pathological ossification, suggesting that ASMSCs play a crucial role in AS pathogenesis. At the cellular level, MSCs exhibit a greater propensity to differentiate into osteoblasts in AS. Metabolically, MSCs undergo changes in their exocrine function, resulting in a dysregulation of inflammatory responses. Consequently, this leads to impaired osteoclast function and heightened osteogenic capabilities of MSCs themselves. These alterations manifest as persistent chronic inflammation, excessive bone formation, and suppressed bone resorption in local tissues.

AS has two primary features: chronic inflammation and pathological bone formation. Analyzing the interplay between the immune system and the skeletal system through the lens of bone immunology enhances our comprehension of AS. Given the capacity of MSCs to modulate both immune and bone metabolic equilibrium, understanding the functional state of MSCs assumes critical importance in understanding the pathogenesis of AS. In the ligaments affected by AS, MSCs demonstrate augmented osteogenic differentiation when exposed to inflammatory stimuli. Moreover, the cellular factors associated with the process of MSCs' osteogenic differentiation can reciprocally regulate immune cells, thereby exacerbating the functional impairment of MSCs.

From a clinical perspective, patients with AS exhibit distinctive radiological characteristics, including excessive ossification of ligaments and vertebral osteoporosis. Many patients require hospitalization due to spinal compression fractures. Consequently, AS cannot be simply regarded as a disease characterized solely by hyperactive bone formation. Based on the reviewed literature, we hypothesize that this phenomenon could be attributed to a shift in osteogenic activity from the vertebrae to the ligaments in AS patients. Under the influence of inflammation, MSCs gradually accumulate at the endpoints of ligaments. Simultaneously, the MSCs that aggregate within the ligaments form a cartilage plate, attracting stem cells with the potential for osteogenic differentiation to migrate to the local area. However, due to a decline in their own anti-inflammatory capacity, MSCs fail to effectively inhibit inflammation at the ligament endpoints. Consequently, under the influence of inflammation, MSCs, which should generally contribute to osteogenic processes within the vertebrae, migrate toward the ligaments. Not only do they fail to suppress inflammation adequately, but they also recruit additional MSCs by promoting cartilage formation. As a result, there is a depletion of MSCs within the vertebrae and an accumulation within the ligaments. Ultimately, this leads to reduced osteogenic activity within the vertebrae and ectopic ossification of the ligaments.

Under physiological conditions, HDMSCs play a role in suppressing inflammation, promoting tissue repair, and restoring the dynamic homeostasis of the body. ASMSC dysfunction, influenced by genetic background, internal environment, infectious factors, and mechanical forces, promotes disease progression. HDMSCs play a role in AS treatment; however, their therapeutic effect is uncertain, but rejection has been observed in patients, which led us to further focus on derivatives of HDMSCs. It remains uncertain whether the MSCs used for AS treatment are autologous or allogeneic, and the origin of these cells is worth exploring. Extracellular vesicles have a higher output efficiency than exosomes. Still, they lack a research base in the field of AS, and it is unclear whether the treatment should be applied in the early stages of the disease or otherwise. The route of administration, number of vesicles, and modification of the carriers should be chosen. Further studies are needed to gain insight into the role of MSCs in AS pathogenesis and their therapeutic prospects.

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Multiple choice questions

1. Ankylosing spondylitis is an autoimmune disease characterized by chronic inflammation and pathological osteogenesis; (true)
2. Mesenchymal stem cell dysfunction in patients with ankylosing spondylitis can lead to the development of chronic inflammation; (true)
3. Mesenchymal stem cell dysfunction in patients with ankylosing spondylitis can lead to an imbalance between local osteogenic activity and bone resorption activity; (true)
4. Abnormally active osteogenic differentiation of MSCs in patients with ankylosing spondylitis due to genetic background, inflammatory factors and mechanical factors leading to hyper-osteogenic activity; (true)
5. Transplantation of MSCs from normal population can effectively alleviate inflammation and ossification progression in patients with ankylosing spondylitis; (true).

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Authors' contributions

All authors drafted the article, revised it critically for important intellectual content, and approved the final version to be published.

Data availability

Data availability is not applicable to this article as no new data were created or analyzed in this study.

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