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Reconciling the effects of inflammatory cytokines on mesenchymal cell osteogenic differentiation

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Abstract

Therapies using mesenchymal stem cells are a popular current avenue for development and utilization, especially in the fields of *de novo* tissue engineering (Sanchez-Ramos J, Song S, Cardozo-Pelaez F, et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol* 2000;164:247.) or tissue regeneration after physical injury (Kitoh H, Kitakoji T, Tsuchiya H, et al. Transplantation of marrow-derived mesenchymal stem cells and platelet-rich plasma during distraction osteogenesis—a preliminary result of three cases. *Bone* 2004;35:892; Shumakov VI, Onishchenko NA, Rasulov MF, Krashenninnikov ME, Zaidenov VA. Mesenchymal bone marrow stem cells more effectively stimulate regeneration of deep burn wounds than embryonic fibroblasts. *Bull Exp Biol Med* 2003;136:192; Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. *J Cell Biochem* 1994;56:283.). The osteogenic potential of these cells is of particular interest, given their recent usage for the closure of critical-sized bone defects and other nonhealing bone scenarios such as a nonunion. Recent literature suggests that inflammatory cytokines can significantly impact the osteogenic potential of these cells. A review of relevant, recent literature is presented regarding the impact of the inflammatory cascade on the osteogenic differentiation of these cells and how this varies across species. Finally, we identify areas of conflicting or absent evidence regarding the behavior of mesenchymal stem cells in response to inflammatory cytokines.

Keywords

Mesenchymal cell; Inflammation; Adipose-derived stromal cell

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1. Introduction

Mesenchymal stem cells (MSCs) are being used in an exceedingly diverse and increasing number of applications in the field of regenerative medicine and across a large number of medical specialties [1]. Furthermore, the role of MSCs as a crucial player in vasculogenesis and osteogenesis after trauma has been the focus of numerous studies [2,3]. Specifically, MSCs have been shown to traffic to the site of injury and undergo vasculogenesis and osteogenesis [4,5]. Thus, understanding the effect of inflammation and inflammatory cytokines after trauma is crucial to harnessing their regenerative potential.

MSCs have been isolated from a wide variety of sources, including adipose, heart, dental pulp, peripheral blood, and cord blood [6]. Two cell types that have achieved particular prominence are bone marrow stromal cells (BMSCs) and adipose stromal cells (ASCs). MSCs have been shown to differentiate into osteogenic, adipogenic, myogenic, and chondrogenic lineages [7], as well as treat a number of soft-tissue [8] and bony defects [9]. Translation of this work to the bedside will potentially allow physicians to replenish local areas of trauma or cellular damage. In the future, such techniques would be of particular importance to a wide variety of specialties, such as trauma surgery.

Animal models have shown the potential for MSCs to treat inflammatory disease and insult, such as in the case of rheumatoid arthritis and burns [10–13], which are known to induce inflammatory cytokine cascades. Clinical trials using these cells have taken place for a number of pathologies, such as osteogenesis imperfecta [14–16], large bone defects [17], and cranioplasties [18]. However, utilization of these cells is not without danger; there has been at least one anecdotal report in the popular media of ASCs interacting with dermal filler to produce ectopic bone formation in a patient's eyelid [19].

An environmental factor that has shown great potential to regulate MSC osteogenesis is inflammation. It is known that inflammatory pathway signaling is a potent regulator of cell behavior, modulating apoptosis, cell adhesion, and cell permeability [6]. There are a vast number of inflammatory cytokines, many of which form complex inter-regulatory networks. Proinflammatory cytokines are small secreted proteins from monocytes and other cell types [20] that set off signaling cascades with systematic consequences. This is particularly true for MSC populations, in which cytokines have demonstrated the ability to influence mesenchymal cells to commit to an osteogenic lineage [21,22]. Yet, the interplay between cytokine expression and MSC behavior remains muddled.

This concise review article will explore recent literature regarding the influence of a number of inflammatory cytokines on BMSC and ASC osteogenesis. These cytokines include stromal derived factor 1 (SDF-1), tumor necrosis factor (TNF) alpha, interleukin (IL) 1, IL-6, IL-4, interferon (INF), and IL-10. We aim to (1) identify trends regarding the effect of inflammatory cytokines on MSC osteogenic differentiation, (2) disclose conflicting data of the effect of inflammation on mesenchymal cell osteogenesis in the existing literature, and (3) distinguish areas which require greater focus of investigation in the scientific canon.

1.1. Inflammatory cytokines that influence osteogenesis

The mechanisms that govern the osteogenic behavior of ASCs and BMSCs are complex and often contradictory. This is especially true for inflammatory cytokines, whose behavior can depend entirely on the location in which they are present. For example, IL-6 functions as both a proinflammatory cytokine in the tunica media [11] and an anti-inflammatory cytokine elsewhere [23], when interacting with inflammatory cytokines such as TNF- α . Although the mechanism of action for the other cytokines discussed herein appears to be more straightforward, it is entirely possible that similar conflicting natures exist (Table 1).

1.1.1. SDF-1—SDF-1 (or CXCL12) is an inflammatory cytokine (of the chemokine subfamily) that binds to chemokine receptors (CXCR4 and CXCR7). It is highly expressed by purified BMSCs; however, this expression is downregulated as the cells mature under osteogenic conditions [24]. It has previously been shown to be essential for the mobilization, migration, and homing of BMSCs in the bloodstream to sites where they are needed, such as sites of active fracture repair [21,22]. In murine fracture repair, SDF-1 is specifically expressed in the periosteum of the bone to assist in mesenchymal cellular recruitment [25]. In both *in vivo* and *in vitro* models of endochondral bone healing, SDF-1 is a critical chemoattractant for BMSCs to the wound-healing site. In SDF-1 knockout mice, bone formation at the wound site was significantly compromised [25].

SDF-1 is an important factor for the optimal function of bone morphogenetic protein 2 (BMP-2, an essential transcription factor in postnatal osteogenesis)–induced osteogenesis, and its disruption leads to diminished alkaline phosphatase (ALP) activity, osteocalcin synthesis, calcium deposition, runt-related transcription factor 2 (Runx2) and osterix (Osx) expression, and Smad and extracellular signal–regulated kinase phosphorylation in BMSCs [26]. Gelatin hydrogels concomitantly releasing SDF-1 and BMP-2 were shown to induce superior healing of critical-sized rat ulnar defects when compared with control or SDF-1 or BMP-2 only loaded hydrogels, further reinforcing the interplay of SDF-1 and osteogenesis [21]. Although BMSCs innately migrate to regions of high BMP-2 expression, the addition of SDF-1 potentiates this response, greatly increasing cellular recruitment in rats [27].

It appears that SDF-1 is an important but not exclusive promoter of osteogenesis. Its primary role is to recruit MSCs to sites where they are needed, at which point SDF-1 works in concert with other cytokines and transcription factors to promote osteogenesis.

1.1.2. TNF- α —TNF- α is a proinflammatory cytokine that has been reported to actively direct BMSCs away from an osteoblastic fate. However, the effects of this cytokine are dose dependent. Treatment of human derived BMSCs with 50 ng/mL of TNF- α transdifferentiates these cells to a neural fate. The same cells also demonstrate an upregulation of host of pro-osteogenic factor expression through the extracellular signal–regulated kinase 1/2 pathway such as BMP-2, CXCR4, and fibroblast growth factor 2 [28]. Furthermore, TNF- α treatment of BMSCs potentiated these cells to chemotaxis toward high concentrations of SDF-1 [28]. Another study [29] showed that stimulation of both human MSCs derived from bone and adipose tissue with TNF- α upregulates the osteogenic factors such as VEGF and insulin-like growth factor (IGF)–2 [30] as well as the antiosteogenic

factor hepatocyte growth factor [31]. This study used the same 50 ng/mL dose that previously specified a neural fate.

Although high doses of TNF- α can have both proneurogenic and pro-osteogenic effects, it appears that lower doses have a more straightforward effect. *In vitro*, at a lower dose of TNF- α (20 ng/mL *versus* 50 ng/mL), the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway is activated, upregulating BMP-2 and ALP—markers of early osteogenesis [32]. The authors of this study suggested that activation of NF- κ B assisted in directing multipotent BMSCs down an osteogenic lineage. In human BMSCs, cells transfected to constitutively express TNF- α repressed adipogenesis and preferentially differentiated into osteoblasts [33]. These contrasting studies imply a dose-dependent response in the presence of TNF- α , in which extremely high doses are neurogenic, and more moderate doses are pro-osteogenic.

A study by Lacey *et al.* [34] examined low concentrations of TNF- α and demonstrated that at 0.01–10 ng/mL, murine BMSCs cultured in lower-concentration media showed impaired osteogenesis as demonstrated through reduced mineralization, ALP staining, ALP expression, Runx2 expression, α 1(I) procollagen, Osx, osteonectin, and osteopontin (OPN). These data fit within the dose-dependent hypothesis, showing that below a threshold level of expression, TNF- α is antiosteogenic.

An *in vivo* study by Gotoh *et al.* [33] showed that subcutaneous implantation of 200 ng of TNF- α in a BMSC-treated critical-sized defect curbed BMSC regeneration of bone. It is difficult to compare dosages between *in vivo* and *in vitro* studies as physiological factors such as diffusion are not considerations in *in vitro* models; however, this study provides additional evidence that certain high dosages are, in fact, antiosteogenic to mesenchymal cells.

In an effort to elucidate this dose-dependent relationship between TNF- α and MSC osteogenesis, Huang *et al.* undertook a study wherein they treated *in vitro* cultures of murine BMSCs with varying levels of TNF- α after blocking intrinsic NF- κ B activity. They found that treating these cells with concentrations of up to 0.1 ng/mL stimulated ALP, Runx2, and Osx activity, whereas higher levels (10 ng and greater) were inhibitory to the same metrics. All doses above 0 ng were inhibitory to mineralization quantified by alizarin red staining, and extended treatment with TNF- α at any dose was inhibitory of mineralization [35].

Thus, the osteogenic effect of TNF- α appears to have two thresholds: first, a lower threshold before which the cytokine has any significant effect; second, an upper boundary after which its antiosteogenic effects outweigh its pro-osteogenic ones. Such behavior is not unusual; in fact, it is commonplace in the field of developmental biology, in which transcription factors at various concentrations along a diffusion gradient can have widely varying effects. In the case of TNF- α , it is possible that the activation of hepatocyte growth factor at sufficiently high doses can be sufficient to overwhelm the otherwise pro-osteogenic effects of the cytokine, yielding a net antiosteogenic effect and therefore specifying a neural fate.

1.1.3. IL-1—IL-1 was the first IL discovered and identified as such. It was originally named as lymphocyte-activating factor on its discovery in 1972, but was subsequently renamed because of the discovery of other family members. IL-1 is a heterodimer, which contains two subunits, IL-1 α and IL-1 β [36]. Many studies have examined the action of IL-1 and TNF- α jointly. In fact, the study by Gotoh *et al.* demonstrated that, in addition to TNF- α , IL-1 also represses peroxisome proliferator-activated receptor gamma, the protein essential to adipogenesis induction. Such peroxisome proliferator-activated receptor gamma inhibition would also favor osteoblastic differentiation and development [33].

Similar to TNF- α , IL-1 has been shown to have a wide range of action as well. The study by Lacey *et al.* also examined similar low doses of an IL-1 subunit (IL-1 β , 0.001–1 ng/mL). They found these low doses of IL-1 β to possess similar antiosteogenic effects to TNF- α , where murine BMSCs showed impaired mineralization, ALP staining, ALP expression, α 1(I) procollagen, Runx2, and Osx (but not the inhibition of osteonectin and OPN) [34]. However, IL-1 β demonstrated a decrease in cell proliferation, which was not observed with TNF- α . Unfortunately, few studies have been performed examining IL-1 at a variety of doses, so it is unknown whether IL-1 demonstrates the same dose-response effect that TNF- α appears to have.

Despite the prominence of IL-1 as a cytokine, relatively little work has been done specifically within the field of MSC osteogenesis. As the field stands, it appears that IL-1 is predominantly antiosteogenic at low doses. However, little work has been performed at high doses or specifically examining IL-1 α , which may have widely divergent effects.

1.1.4. IL-6—IL-6 is a widely studied multifunctional lymphokine that functions as both a pro- and anti-inflammatory cytokine. It is a central mediator of bone homeostasis, as it is secreted by osteoblasts to spur osteoclast development, and has been implicated in bone resorption [37]. In human BMSCs, IL-6 has been shown to positively influence the mitogen-activated protein kinase signaling cascade, which is essential for normal skeletogenesis and bone formation [38]. mitogen-activated protein kinase signaling upregulates Akt phosphorylation, which positively modulates mammalian target of rapamycin (mTOR). mTOR is responsible for osteoclastogenesis and osteoclast survival. Treatment of osteoclasts with rapamycin (an inhibitor of mTOR) and siRNA targeted to mTOR results in apoptosis [39]. These elements lie upstream of Runx2, the master regulator of osteoblast development and formation [40]. An IL-6 family member, leukemia inhibitory factor, is upregulated by TNF- α and respecifies human BMSCs to a neural fate and away from osteogenic lineage. However, in a mouse BMSC model, treatment of BMSCs with 200 ng/mL of IL-6 in osteogenic media led to no change in calcium deposition, demonstrated by alizarin red staining [41]. Human BMSCs, when cultured in osteogenic media, have been shown to decrease their expression of IL-6, reinforcing the idea that, in human MSCs, IL-6 is not pro-osteogenic [42,43]. However, this antiosteogenic behavior does not translate to proadipogenic behavior, as IL-6 (as well as other IL group members) inhibits preadipocyte differentiation *in vitro* [44].

Thus, it appears that the primary function of IL-6 is not antiosteogenic. Rather, the cytokine chiefly plays a pro-osteoclastogenic role, and its antiosteogenic effects are secondary in modulating bone homeostasis.

1.1.5. IL-4 and INF- γ —Murine studies have also tested IL-4 and INF- γ and demonstrated that the same dose (200 ng/mL) of each cytokine to be significantly antiosteogenic, as demonstrated by alizarin red staining [41]. When INF- γ was subcutaneously implanted (200 ng) at the site of a BMSC scaffold in a cranial defect, it reversed the ability for BMSCs to heal the defect, and injection of an INF- γ antibody rescued that ability. Furthermore, when BMSCs were treated with INF- γ and TNF- α , BMSCs underwent apoptosis, providing supplemental evidence for the antiosteogenic role of these cytokines [41].

1.1.6. IL-10—IL-10 is an IL and regulatory cytokine. It, too, functions to modulate osteogenesis. In mouse BMSC cultures, treatment with IL-10 does not impact cell proliferation. However, IL-10 stimulation does inhibit osteogenesis. Interestingly, this downregulation only occurs before ALP expression. When the cells progressed to stages of differentiation subsequent to ALP expression, this downregulation was not observed, indicating that IL-10 can only function to mediate early osteogenic differentiation but not late osteogenesis [45]. However, IL-10 knockout mice showed significantly compromised BMSC (the biologic osteoprogenitor cell) population. Furthermore, these cells showed an impaired ability to undergo osteoblastogenesis and had a lowered ALP activity when compared with normal BMSCs [46]. These data suggest that IL-10 plays a central role in the maintenance and generation of the pool of osteoprogenitor cells, and although high levels are detrimental to osteogenesis, a basal level is required for normal osteogenic behavior.

The inhibitory effect of IL-10 is thought to be caused by its inhibition of transforming growth factor β (TGF- β), as exogenous administration of IL-10 suppressed TGF- β synthesis, inhibited matrix mineralization, and downregulated ALP, collagen, and osteocalcin expression in murine BMSCs [47]. As concomitant exogenous administration of TGF- β rescued the ability of these cells to undergo normal osteogenic differentiation, the authors hypothesized that TGF- β is an intermediary of the effect of IL-10 on mesenchymal cell osteogenesis. This too is contradictory, as transforming growth factor beta 1 (a subset of TGF- β) has been shown to have an inhibitory effect on the osteogenic differentiation of ASCs, although it is possible that this is because of differential responses of ASCs *versus* BMSCs.

Somewhat perplexingly, recombination activation gene 1 (*RAG1*) knockout mice, which possessed an impaired adaptive immune system, saw measurable improvement in fracture healing, including acceleration of endochondral ossification and improved mineralization. *RAG1*^{-/-} mice were characterized by diminished production of proinflammatory cytokines (such as TNF- α , lymphotoxin β , INF- γ , IL-2, and IL-4) and increased production of anti-inflammatory cytokines, in particular, IL-10. As Toben *et al.* [48] described it, the rapid healing demonstrated by *RAG1*^{-/-} mice contained a strong anti-inflammatory component to the cytokine milieu.

IL-10 is a particularly interesting cytokine in the scope of MSC osteogenesis because of the number of conditions on its effects. It is antiosteogenic but only before ALP expression. It appears to have divergent effects on BMSCs and ASCs. However, *in vivo*, when IL-10 was coupled with diminished proinflammatory cytokine production, fracture healing was improved. As several of these proinflammatory cytokines were antiosteogenic in nature, it may be that increases in IL-10 production are not sufficient to counter the antiosteogenic effects of the other cytokines (Fig.).

1.1.7. Other signaling molecules—Inflammatory cytokines do not exert their effects singularly—rather, they work in close concert with myriad of other cytokines and signaling molecules, which are also influenced by inflammatory pathway. Adenosine triphosphate is known to be a mediator of mechanotransductive stress and is hypothesized to mediate inflammatory burn stress, playing a central role in osteogenesis because of heterotopic ossification (HO) [49]. OPN is a matrix-associated protein that plays a central role in osteogenesis and mediates inflammation-induced cytokine expression [50] and is negatively modulated by classic inflammatory cytokines such as IL-6, IL-1 β , INF- γ , and TNF- α . Erythropoietin (Epo), a hormone/cytokine most commonly known for inducing hematogenesis, also regulates osteoblastogenesis through the mTOR pathway, which, as previously mentioned, is an element of the IL-6 inflammatory response pathway [51]. Epo influences osteoclastogenesis in an mTOR-independent manner as well; cells concomitantly treated with Epo and receptor activator of NF- κ B ligand increased nuclear factor of activated T cells, cytoplasmic 1 (a regulator of osteoclast fusion) despite the presence of rapamycin, an mTOR inhibitor. IGF-1 is another inflammatory mediator, which too, activates the mTOR pathway used by BMP-2. IGF-1 stimulates osteogenesis in BMSCs, ASCs, and preosteoblasts as well, in which it has been shown that it exerts its action through the phosphatidylinositol 3 kinase/Akt pathway, the same pathway used by BMP-2 [52]. In this pathway, phosphorylated Akt2 prevents tuberous sclerosis complex 2 inhibition of mTOR. Activation of mTOR leads to downstream Runx2 activation and osteoblastogenesis (generation and differentiation of new osteoblasts; Table 2).

2. Current dilemmas

It is evident from these studies that a great deal of conflicting evidence exists on the mechanism of action of some of these inflammatory cytokines and their targets. Such variance in the scientific canon necessitates additional investigation to clarify the field of mechanistic action for these factors. However, this variance is potentially because of the pleiotropic nature of these signaling factors, inducing different phenotypes dependent on dose or effected cell type. For instance, TNF- α can be either proneurogenic or pro-osteogenic, dependent on dosage [28,29], and IL-10 appears to have differential effects on ASCs and BMSCs [45,47]. Given the diverse spectrum of pathways, which simultaneously use and regulate these cytokines, it is yet unclear whether precise clarity in the role of inflammatory cytokines is even possible or some discrepancy is unavoidable. Furthermore, studies into this area might allow for the identification of the cytokines, which allow for the regenerative benefits of vasculogenesis and osteogenesis which would be helpful in soft-tissue or skeletal trauma.

In addition to possessing a regenerative potential, these mechanisms can also be deregulated and cause destruction as the case in HO [53–56]. The debilitating condition referred to as HO describes the formation of bone in areas of soft tissue such as muscle, fascia, and joint spaces. This disease process afflicts >60% of blast and major burn injury patients making it an area of significant need of breakthroughs in its diagnosis and treatment. Current mechanisms posit that inflammation stimulates local osteopotent cells such as MSCs to undergo osteogenesis through BMP signaling. Key to its formation is this inflammatory insult [11]. Identifying and mitigating the cytokines, which stimulate the destructive processes such as HO would greatly improve the care of a large population of burn and blast patients.

The vast majority of literature examining inflammatory cytokines is done in BMSCs. Comparatively little work has been performed on examining the influence of inflammatory agents on adipose-derived cells. Both the cell type and the host species can have an immense impact on the effect of these factors in relation to osteogenesis [52]. For this reason, it is essential to validate this work (most of which has been done in human cells) in mouse, rat, and large animal MSCs, as research-based applications of these cells occasionally preclude human cell usage. For example, it is known that athymic rats have altered expressions of inflammatory cytokines [49], so surgical models exploring the use of MSCs could be negatively impacted by the use of xenograft animals.

In a biological and *in vivo* environment, some of these cytokines may not have as dramatic of an impact as some of these *in vitro* studies suggest. As previously discussed, Akt2 is an essential mediator of osteogenic gene expression [39]. In nonmesenchymal cells, it has been previously shown that this serine/threonine kinase is responsible for positive and negative regulation of NF- κ B as well as downregulation of IL-10 (an anti-inflammatory cytokine) and TNF- α (a central inflammatory cytokine). This implies that Akt2 may play a role in desensitizing these cells to more moderate doses of pro-and anti-inflammatory cytokines. Further research and *in vivo* correlates of cell-based studies must be undertaken to elucidate the precise physiological effect of such inflammatory cytokine cascades on MSC behavior.

Moreover, the body is an inordinately complex, interrelated, and inter-regulatory entity. As such, some *in vitro* experiments may lack the necessary components to sufficiently predict *in vivo* behavior. It is entirely possible that some of these cytokines have an antiosteogenic effect on MSCs in isolation but may in fact induce secretion of high levels of BMP-2 in adjacent epithelial tissue, thereby having a net pro-osteogenic effect. Unless the researchers exhaustively coculture MSCs with every possible cell and tissue type (an impractical task), such interactions may be missed. Therefore, it may be prudent to validate these results in *in vivo* models.

3. Discussion

Currently, a number of companies provide services or products aimed at facilitating the harvest and purification of these stem cells for clinical use. MSC osteogenesis is of particular clinical import, as it has been shown to assist in the healing of critical-sized bone defects in a variety of pathologic models [57–59]. Numerous signaling molecules (growth

factors, differentiation factors, hormones, and so forth) have been shown to influence the osteogenic differentiation of these cells, such as administration of parathyroid hormone, BMP-2, and human growth hormone [22,60,61]. Furthermore, the environment in which MSCs are situated has been shown to play a significant role in their behavior and differentiation [7]. Although these and other factors all play important roles in MSC osteogenesis, the focus of this review will be on inflammatory mediators.

This review illuminates the fact that the inflammatory cascade effect on MSC osteogenic differentiation is still a largely unexplored field, despite significant inroads. Additional research is required to answer questions regarding differences in xenogeneics and tissue type. Literature on certain inflammatory cytokines with regard to osteogenic differentiation—for example, IL-8—is scant. Furthermore, the action of these cytokines should be validated in *in vivo* models to couple the interaction of inflammatory cytokines on mesenchymal cell osteogenic differentiation with potential mediating effects of other secreted proteins from concomitantly located cells.

Nonetheless, a great deal of preliminary work has been accomplished demonstrating the both pro- and antiosteogenic effects of inflammatory cytokine—such as IL-4, INF- γ , and IL-6. In other fields, targeted therapies have been developed to regulate cytokine release and action. Similar therapeutics will doubtlessly be developed to harness and govern inflammatory cytokine action. As SDF-1 and TGF- β are the “true” (i.e., direct) promoters of osteogenesis in the current scientific canon, these may be excellent starting points for osteogenesis-related therapeutics. Potential application of this research will allow and prove useful for designing future strategies to improve treatment strategies for patients with bone-related injury and disease. For example, the clinician or surgeon of the future will be able to use this information to actively prevent conditions such as HO after burn or trauma, and conversely, the surgeon of the future will be able to use the body’s natural inflammatory processes to assist in bone healing and repair.

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Fig.

Overall mechanistic schematic representation of the effect of various classic inflammatory cytokines on osteogenic activity of MSC. As demonstrated, the inter-regulatory interplay between the various cytokines and their actions is complex and dependent on a number of factors. SDF-1 and TGF- β are the only true (direct) mediators of osteoblast activity. (Color version of figure is available online.)

Table 1

List of mechanistic elements discussed within this review article and their effects on osteogenesis.

Protein	Osteogenic effect	Comment
ALP	Pro	Early marker of osteogenesis [34].
α -1(I) Procollagen	Pro	Deficient α -1(I) procollagen can result in osteogenesis imperfecta [15].
BMP-2	Pro	BMP-2 is essential for postnatal osteogenesis but not for recruitment of osteogenic stem cells [22,26,31].
Erk	Pro	Erk phosphorylation determines adipogenesis or osteogenesis in human bone marrow-derived stem cells [26].
Erk 1/2	Pro	Can have both pro- and anti-osteogenic effects but predominantly pro- osteogenic in MSCs [28].
FGF-2	Pro	Exogenously applied FGF-2 accelerates osteogenesis in critical size bone defects and fracture sites [62].
HGF	Pro	HGF has been demonstrated to stimulate osteoblast proliferation [31].
Human growth hormone	Pro	Especially pro-osteogenic in adolescents [61].
mTOR	Pro	Member of PI3K-related kinase family. Reported to have both pro- and anti-osteogenic effects, predominantly pro-osteogenic in MSCs [51].
NF- κ B	Anti	Maintains bone homeostasis and osteoclastogenesis [32]. RANKL activates NF- κ B through receptor activator of nuclear factor κ B [63].
Nuclear factor of activated T cells, cytoplasmic 1	Anti	Dependent of RANKL [51].
Osteocalcin (BGLAP)	Pro	Early marker of osteogenesis [47].
Osx	Pro	Key transcription factor initiating and regulating the early osteogenesis and late mineralization of bone [34].
Osteonectin	Pro	Secreted by osteoblasts during bone formation [64].
OPN	Pro	Proliferating preosteoblasts express OPN and assists in response to mechanical stress [4,34].
Parathyroid hormone	Pro/anti	Pro-osteogenic when intermittent and anti-osteogenic when continuous [60].
PPAR- γ	Anti	PPAR- γ insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors [33].
PI3K	Pro	Promotes osteoblastic differentiation [65].
RANKL	Anti	TNF member that plays a pivotal role in osteoclast formation and activation [32,63].
Runx2	Pro	Essential for osteoblastic differentiation and key transcription factor initiating and regulating the early osteogenesis and late mineralization of bone [26,34].
Smad	Pro	Smads regulate expression of transcriptional factors and coactivators important in osteoblasts [26].

Erk = Extracellular signal-regulated kinase; FGF-2 = fibroblast growth factor 2; HGF = hepatocyte growth factor; PI3K = phosphatidylinositol 3 kinase; PPAR- γ = peroxisome proliferator-activated receptor gamma; RANKL = Receptor activator of NF- κ B ligand.

Table 2

Brief summary of other signaling molecules that impact mesenchymal cell osteogenesis.

Signaling molecule	Osteogenic effect	Comment
Adenosine triphosphate	Pro	Mechanotransductive pathway mediator
Epo	Pro	Osteoblastogenic + osteoclastogenic
IGF-1	Pro	Uses same pathway as BMP-2
OPN	Pro	Also has pro-osteoclastic effects