Histol Histopathol (2017) 32: 977-985

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Review

Microenvironmental factors that regulate mesenchymal stem cells: lessons learned from the study of heterotopic ossification

Chen Kan[1]*, Lijun Chen[1]*, Yangyang Hu[1]*, Haimei Lu[1], Yuyun Li[2], John A Kessler[3] and Lixin Kan[1,2,3]

[1]School of Basic Medical Sciences, Anhui Medical University, Hefei, [2]Department of Medical Laboratory Science, Bengbu Medical College, Bengbu, China and [3]Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

*Equal first authors

Summary. Bone marrow contains a non-hematopoietic, clonogenic, multipotent population of stromal cells that are later called mesenchymal stem cells (MSC). Similar cells that share many common features with MSC are also found in other organs, which are thought to contribute both to normal tissue regeneration and to pathological processes such as heterotopic ossification (HO), the formation of ectopic bone in soft tissue. Understanding the microenvironmental factors that regulate MSC *in vivo* is essential both for understanding the biology of the stem cells and for effective translational applications of MSC. Unfortunately, this important aspect has been largely underappreciated. This review tries to raise the attention and highlight this critical issue by updating the relevant literature along with discussions of the key issues in the area.

Key words: Mesenchymal stem cell (MSC), Microenvironmental Factors, Heterotopic ossification (HO), Tissue regeneration

Introduction

In the 1960s to 1970s, Friedenstein and colleagues found that bone marrow contains a non-hematopoietic clonogenic stromal cell population (Bianco et al., 2008; Fajardo-Orduna et al., 2015; Sacchetti et al., 2016), referred to as colony-forming unit-fibroblasts (CFU-F).

These cells were found to possess multipotent properties and thus were later called mesenchymal stem cells (MSC) (Roufosse et al., 2003; Minguell et al., 2001). Since then, similar cells that share common features with MSC are also found in most other organs. In fact, for a long period of time, most investigators called all these adherent fibroblastic cells MSCs, regardless of the tissue/organ origin, which created confusion. Therefore, it is appropriate to clarify our position on the controversial terminology before we move on to detailed discussion.

Many investigators believe the umbrella term "MSC" is inappropriate, since "MSC" has come to mean virtually any adherent fibroblastic population of cells, such as "skeletal stem cells", "marrow stromal cells" and "bone marrow stromal fibroblasts", whether or not they contain a subset of stem cells as defined by rigorous criteria. On top of that, it is becoming more and more clear that the so called MSCs from different tissues are not the same, since they have different differentiation capacities (Bianco et al., 2008; Sacchetti et al., 2016). Having said that, we still adopt the term MSC in most cases, throughout this article, not because we think the term is appropriate, but because of the nature of a review article. We believe that we simply do not have the right to change the terms used by the original authors.

Interestingly, MSC was originally thought to be only a type of supportive cell which provides signals for differentiation and proliferation of hematopoietic stem cells (HSCs) in the HSC niche (Fajardo-Orduna et al., 2015). Accumulating data, however, support the idea that MSCs reside in their own niche (MSC niche), and

Offprint requests to: Dr. Lixin Kan, School of Basic Medical Sciences, Anhui Medical University, Hefei 230032, China. e-mail: l-kan@northwestern.edu

DOI: 10.14670/HH-11-890

that other supportive cells and microenvironmental factors are needed to precisely regulate the homeostasis of MSC within the niche. Consequently, dysregulation of key microenvironmental factors may cause loss-of-function (LOF) and/or gain-of-function (GOF) changes of MSC. It is now clear that LOF of MSC might lead to deficiencies in tissue regeneration, whereas GOF of MSC might lead to ectopic overgrowth of mesenchymal tissues, such as heterotopic ossification (HO).

HO, which can be either acquired or hereditary, is increasingly recognized as a serious health concern. Acquired HO is a common complication of trauma, burns, neurologic injuries and/or various surgeries. Cardiovascular calcification and ossification of the posterior longitudinal ligament (OPLL) of spine are also thought to be atypical acquired HO (Kaplan et al., 2004; Bossche and Vanderstraeten, 2005; Forsberg et al., 2009). Hereditary HO, such as fibrodysplasia ossificans progressiva (FOP) and progressive osseous heteroplasia (POH), is rare but life-threatening. Even though many molecular pathways, such as BMP signaling, and cellular components, especially tissue resident mesenchymal progenitors/stem cells (MSCs), have been implicated in HO formation, the exact molecular and cellular mechanisms that lead to HO are largely unknown. Nevertheless, we reason that both acquired and hereditary HO can serve as models for understanding how GOF of MSC may lead to diseases.

Furthermore, MSCs are often cited as particularly promising donor cells for cell based regenerative therapies (Keating, 2012). However, to realize the translational potential of MSC, deep understanding of the microenvironmental factors that regulate cell behavior *in vivo* and *in vitro* is essential. Unfortunately, despite a plethora of studies of the therapeutic use of MSC, little attention has been paid to understand how the *in vivo* microenvironment regulates MSC fate and function, either in physiological or pathophysiological context, likely due to the technical limitations, such as lack of specific MSC marker, and the quiescent nature and low frequency of endogenous MSC.

One way to circumvent these problems is to study the GOF of MSC in pathophysiological context, such as HO, simply because the MSC in this context is activated, and the frequency is significantly increased. In addition, we also believe that understanding how dysregulation of microenvironmental factors leads to dysregulation of MSC and the downstream pathological process, will eventually reveal insights into how homeostasis of MSC is achieved in the physiological condition in the first place.

Based on this argument, this review will focus mainly on the regulatory roles of key microenvironmental factors that we have learned mainly from, but are not limited to, HO (GOF of MSC). It is our intention that this article will inspire more novel ideas about future basic research on homeostatic control of endogenous MSC, as well as broad clinical applications of MSC that could potentially target both the LOF of MSC (i.e., in degenerative diseases), and GOF of MSC (i.e., HO).

Microenvironmental factors that could regulate MSC behavior

A body of literature suggested that dysregulation of many different types of microenvironmental factors could dysregulate MSC *in vitro* and *in vivo*. These data indirectly support the idea that microenvironmental factors tightly regulate MSC homeostasis, i.e., maintenance of stemness and subsequent multi-lineage differentiation. To facilitate the discussion in a reasonable depth, we broadly categorize many different key candidate factors into a few groups, including:

Inflammatory factors

Injury induced inflammation plays a crucial role in HO, likely through dysregulation of tissue resident progenitors/MSC, but the exact roles of each individual factor is unclear so far. Furthermore, it is apparent that the effects of many cytokine/inflammatory factors are context-depend. For example, tumor necrosis factor alpha (TNF-α), a major pro-inflammatory cytokine, can either inhibit or promote osteogenic differentiation of MSC. Some studies have shown that TNF-α inhibits osteogenic differentiation of MSC and impairs skeletal bone formation (Riggs and Khosla, 2005; Wang et al., 2016). In a mouse model of estrogen-deficiency induced osteoporosis, TNF-α can inhibit semiphorin 3B via inhibiting wnt/β-catenin signaling, which in turn inhibits osteogenic differentiation of bone marrow derived MSC (Sang et al., 2016). Further, TNF-α inhibits osteogenic differentiation of human periodontal ligament tissue-derived MSC (PDLSCs) by decreasing miR-17 expression and increasing levels of Smurf1 (Liu et al., 2011).

In contrast, Yang et al. found that TNF-α promoted human MSC (hMSC) proliferation and osteogenic differentiation in the context of ankylosing spondylitis (AS), and that these effects were attenuated by the silencing of peptidyl arginine deaminase, type IV (PADI4) (Yang and Dai, 2015). PADI4 is a member of a gene family which encodes enzymes responsible for the conversion of arginine to citrulline residues. PADI4 plays a role in inflammation and immune response. Moreover, Ding et al. found that TNF-α and interleukin-1β (IL-1β) stimulated tissue-nonspecific alkaline phosphatase (TNAP) activity and mineralization in cultured human MSC from trabecular bone explants (Ding et al., 2009). TNF-α and lipopolysaccharide (LPS) also stimulate alkaline phosphatase (ALP) activity with subsequent matrix mineralization of human bone marrow MSC cultured in osteogenic medium with or without BMP-2 (Croes et al., 2015). Similarly, inflammatory cytokine-activated (TNF-α+interferon-γ (IFN-γ)) human bone marrow MSC promotes the capacities of osteoblast proliferation, migration, differentiation, and mineralization of these cells (Li et al., 2016a,b). Furthermore, inflammatory factors

including TNF-α have a pro-osteogenic effect on adipose-derived MSC from burn injured mice, in the context of burn injury induced HO (Peterson et al., 2015) .The seemingly divergent data about the effects of TNF-α may reflect both the heterogeneity of different populations of MSC, differences in the experimental context, or both.

Other inflammatory cytokines exert similar effects on MSC. For example, IL-1β enhances calcification of human bone marrow MSC *in vitro* despite having a suppressive effect on osteoblastic differentiation (Ferreira et al., 2013), and IL-17A and IL-17F, proinflammatory cytokines derived from T helper 17 cells, enhance osteogenic differentiation of human bone marrow MSC (Croes et al., 2016). Furthermore, inflammatory cytokines can also influence the stemness of MSC. For example, Wang et al. demonstrated that IFN-γ and TNF-α synergistically impair self-renewal and differentiation of MSC via nuclear factor ϰB (NF-ϰB) signaling pathway-mediated activation of mothers against decapentaplegic homolog 7 (SMAD7) in ovariectomized (OVX) mice (Wang et al., 2013a,b). However, Laschober et al. found that IFN-γ exerted no significant influence on self-renewal of human bone marrow MSC, although it regulated their differentiation potential (Laschober et al., 2011). Serum concentrations of IL-6 and levels of the neuroinflammatory molecule, substance p (SP), are increased in HO, suggesting an ongoing role for inflammation in the disease process, both in human and mice (Kan et al., 2011; Evans et al., 2012). Stromal cell-derived factor 1 (SDF-1), a chemokine that regulates cell trafficking and homing via the CXCR4 receptor, plays a major role in recruiting MSC to target tissue (Lau and Wang, 2011), but it is not necessary for MSC proliferation or osteogenic differentiation in a rat fracture bone model (Chen et al., 2016, 2017). Nevertheless, SDF-1 does regulate BMP2-induced osteogenic differentiation of primary human and mouse bone marrow MSCs (Hosogane et al., 2010).

To summarize, inflammatory factors, especially proinflammatory cytokines, play crucial roles in lineage commitment by MSC in a variety of different contexts, including ectopic osteogenic differentiation, and some cytokines may also help to regulate MSC self-renewal as well (Table 1).

Trophic factors

Many trophic factors, including growth factors, hormones and small molecules, regulate the stemness and osteogenic differentiation of MSC. For example, in trauma-induced HO patients, levels of many trophic factors including bone morphogenetic protein 1 (BMP1), growth and differentiation factor 11 (GDF11) and transforming growth factorβ1 (TGFβ1) are increased, whereas levels of others, such as BMP4, BMP5 and GDF10, are decreased (Jackson et al., 2011). Similarly, BMP-2, a potent osteoinductive protein (Sampath et al., 1990; Niedhart et al., 2003; Eyckmans et al., 2010), also plays an important role in HO (Maroulakou et al., 1999; Zhang et al., 2014;). Platelet derived growth factor (PDGF) acts as a chemoattractant for MSC after tissue injury (Hollinger et al., 2008; Caplan and Correa, 2011). Serum from patients with isolated trauma or polytrauma (associated with HO) induces proliferation of cultured MSC in a manner correlating with the concentration of PDGFs (Tan et al., 2015), and the number of MSC in bone marrow aspirates correlates with the serum concentration of PDGF-AA and –BB. Platelet-rich fibrin (PRF) slowly and continuously releases a variety of autologous growth factors (Lundquist et al., 2008), and Wang et al. found that PRF stimulated bone marrow MSC proliferation and osteogenesis *in vitro*, and MSC sheets co-injected with PRF into SCID (severe combined immunodeficiency) mice developed more and denser new ectopic bone compared with non-PRF controls (Wang et al., 2015).

Estrogen is known to maintain bone mass by maintaining the balance between osteoblast-mediated skeletal bone formation and osteoclast-mediated bone reabsorption (Raisz and Kream, 1983; Christian et al., 2002). Chen et al. found increased mRNA expression of the alpha estrogen receptor (ERα) and ALP, but reduced osteocalcin and IL-6, in estradiol treated bone marrow MSC from postmenopausal osteoporotic women; this suggests that estrogen may have a significant influence

Table 1. Summary of the effects of various inflammatory cytokines on MSCs.

	Enhance Osteogenesis?	Inhibit Osteogenesis?	Maintain Stemness?	Reference
TNF-α	yes	yes	-	Wang et al., 2016; Riggs and Koshla, 2005; Sang et al., 2016; Liu et al., 2011; Yang and Dai, 2011
IL-1β	no	yes	-	Ferreira et al., 2013
TNF-α+IL-1β	yes	no	-	Ding et al., 2009
TNF-α+LPS	yes	no	-	Croes et al.,2015
IL-17A/IL-17F	yes	no	-	Croes et al., 2016
IFN-γ	yes	yes	no	Laschober et af., 2011
TNF-α+IFN-γ	yes	yes	no	Li et al., 2016a,b; Wang et al., 2013a,b
SDF-1+BMP2	yes	no	-	Lau and Wang, 2011; Chen et al., 2016a,b; Hosogane et al., 2010

TNF-α: Tumor Necrosis Factor-α; LPS: Lipopolysaccharide; IFN-γ: Intereron-γ; SDF-l: Stromal Cells Derived Factors-l.

on osteogenic differentiation of MSC in osteoporotic women (Chen et al., 2013). Ogata et al. found that estrogen receptor polymorphisms are associated with ossification of the posterior longitudinal ligament (OPLL) of the spine (Ogata et al., 2002), and cultured spinal ligament cells obtained from OPLL patients responded to 3,17β-estradiol by accelerating production of bone Gla protein (BGP) (Wada, 1995), a specific marker for bone formation in postmenopausal osteoporosis.

Many trophic factors also help to maintain the stemness of MSC. For instance, basic-fibroblast growth factor (bFGF) is associated with the maintenance of a higher degree of "stemness" in mesenchymal progenitor cells from human bone marrow aspirates (Bianchi et al., 2003). Tasso et al. also found that stimulation of mouse bone marrow-derived MSC *in vitro* with bFGF influences their degree of "stemness". Furthermore, bFGF-treated MSC exhibit the ability to activate endogenous regenerative mechanisms by recruiting host cells when implanted *in vivo* (Tasso et al., 2012). Similarly, vascular endothelial growth factor (VEGF) plays a key role in human MSC-mediated myocardial regeneration (Zisa et al., 2009). Consistently, MSC cell lines, such as C3H/10T1/2, themselves secrete trophic factors, such as insulin-like growth factor-1 (IGF-1) (Chen et al., 2016), which may help to regulate tissue regeneration.

In contrast, it was also reported that bFGF reduces stemness of human stem cells/bone marrow stromal cells (SSCs/BMSCs) (Sacchetti et al., 2007). The plausible explanation is that bFGF could increase proliferation, but not self-renewal. In this case, SSCs/BMSCs expanded with bFGF formed bone without marrow *in vivo*, indicating that bFGF might also push the SSCs to an osteogenic state, which complicated the issue.

Overall, various trophic factors are associated with GOF of MSC, i.e., increased MSC self-renewal and proliferation, whereas others enhance osteogenic differentiation (Table 2).

Extracellular matrix (ECM)

ECM plays an important role in the retention of stemness of MSC. For example, Xiong et al. reported that decellularized ECM coating helps to preserve the stemness of cultured murine adipose-derived stem cells (Xiong et al., 2015). Rakian et al. compared colony-forming ability and differentiation of human primary MSC cultured on bone marrow derived Extracellular matrix (BM-ECM) with a commercial matrix and plastic (serum-free) and found that BM-ECM, but not other ECMs or plastic, provided a unique microenvironment that supports the colony-forming ability of MSC and preserves their stem cell properties (Rakian et al., 2015). In addition, human stromal-cell-derived ECM may promote human bone marrow MSC proliferation and preserve their capacity for differentiation (Antebi et al., 2015).

For the differentiation of human bone marrow derived MSC, Hoch et al. established a novel cell-secreted decellularized extracellular matrix (DMs), which includes glycoprotein motifs that support cellular adhesion with juxtaposed molecular cues in the form of chemokines and growth factors. This matrix likely directs cell fate through the presentation of a complex and physiologically relevant milieu which appears to better preserve the mineral-producing phenotype of MSC and stabilize the osteoblastic phenotype (Hoch et al., 2016). However, Gawlitta et al. found that unseeded decellularized cartilage-derived matrix (CDM) itself, as a scaffold, only minimally induces endochondral bone regeneration. In contrast, decellularized CDM with preseeded human bone marrow derived MSC in chondrogenic medium helps regain its osteoinductive potential at an ectopic location (Gawlitta et al., 2015). Cysteine-rich protein 61 (CCN1, also called Cyr61), a component of the extracellular matrix that is highly expressed in cancer cells, is found in the secretome of BM-derived MSC. CCN1 may mediate Wnt3A-induced osteoblastic differentiation of MSC cell line (C3H/10T1/2) (Si et al., 2006) and also can promote chondrogenesis of mouse primary MSC *in vivo* and *in vitro* (Wong et al., 1997).

Artificial biomaterials also can mimic some of the environmental effects of extracellular matrix. For example, hydroxyapatite foam made from calcium phosphate (Ca-P) can be used as a bone substitute.

Table 2. Summary of the effects of various trophic factors on MSCs.

	Recruit MSCs?	Stimulate Proliferation?	Enhance osteogenesis?	Maintain sternness?	Reference
IGF-1+BMP9	-	-	yes	-	Chen et al., 2016a,b
B-FGF	-	-	yes	yes	Bianchi et al., 2003; Tasso et al., 2012; Sacchetti et al., 2007
BMP-2	-	-	yes	-	Zhang et al., 2014; Maroulakou et al., 1999
PRF	-	yes	yes	-	Wang et al., 2015
PDGF	yes	yes	-	-	Caplan and Correa, 2011; Hollinger et al., 2008; Tan et al., 2015
Estrogen	-	-	yes	-	Chen et al., 2013

IGF-1: Insuling Growth Factors-I; B-FGF: Basic-Fibroblast Growth Factor; BMP2/9: Bone Morphogenetic protein 2/9; PRF: Platelet-rich Fibrin; PDGF: Platelet Derived Growth Factors.

Curtin et al. showed that nano-hydroxyapatite combined with MSC promotes osteogenesis both in 2D and 3D cultures (Curtin et al., 2012). Recently, Viti et al. used a gene expression microarray to investigate possible pathways involved in calcium-phosphate-driven differentiation of human bone marrow derived MSC *in vitro* and found increased expression of several genes related to osteogenic differentiation including osteopontin (SPP1), bone sialoprotein (BSP) and several bone morphogenetic proteins (BMPs) (Viti et al., 2016). There have been many attempts to construct artificial biomaterials that more fully mimic the biological effects of ECM either by coating material surfaces with recombinant ECM proteins and peptides or by employing decellularized allogenic or xenogenic tissue scaffolding. However, in general, the former fails to capture the complex composition and architectural characteristic of native ECM, and the latter suffers from concerns about reproducibility, availability, and immunologic responses.

Mechanical cues

To further understand the role of ECM, it is necessary to study the mechanical cues or biophysical aspect of ECM, since this aspect of the microenvironment also exerts important influence on stem cell behavior, especially the lineage-specific differentiation of MSC. Deep understanding of this area is also essential for developing new class of biomaterials for tissue engineering and regenerative medicine (Das and Zovani, 2014).

In vivo mechanical microenvironment of MSCs is known to be determined by the intrinsic stiffness, composition and configuration (topography/shape) of the local ECM, and extrinsic mechanical loading applied to this matrix (e.g. fluid flow, compression, hydrostatic pressure and tension) (Steward and Kelly, 2015). MSCs, on the other hand, have the ability to both sense and respond to their mechanical environment, with numerous membrane proteins and cytoskeletal components. Nevertheless, the exact *in vivo* mechanotransductive mechanisms are still poorly understood, and to our best knowledge, there is no report that specifically addresses the mechanotransductive mechanism of MSC in the context of HO, and there is no well-established *in vivo* system that can be used to systematically study the mechanobiology of MSCs either. For this reason, most studies so far exploring the mechanobiology of MSCs have been performed using 2D and/or 3D *in vitro* experiments. With these limitations in mind, we briefly update the consistent finding in this field.

In vitro experiments consistently demonstrated that matrix or substrate stiffness has been shown to play a key role in regulating the differentiation of MSCs towards specific lineages (Engler et al., 2006; Park et al., 2011). i.e., MSCs seeded onto soft substrates were shown to have a greater adipogenic and chondrogenic potential, while those on stiffer substrates had a stronger myogenic potential (Park et al., 2011). Such effects could be independent of chemical/biochemical inducers, and further studies suggested that traction-mediated forces and matrix stiffness-mediated integrin binding have direct roles on MSC lineage commitment (Huebsch et al., 2010). In contrast to stiffness, although cell shape has been shown to control adipogenic/osteogenic lineage commitment, uncertainty still exists in the literature.

Fluid flow, hydrostatic pressure (HP), compression and other extrinsic mechanical cues, can also influence cell behavior. For example, oscillatory fluid flow has been shown to regulate stem cell fate (Arnsdorf et al., 2009). Perfusion systems have consistently been found to promote osteogenesis of MSCs (Bancroft et al., 2002; Datta et al., 2006). HP, on the other hand, is a non-deforming mechanical stimulus. HP has been found to increase chondrogenic gene expression of human bone marrow stromal cells, while having no significant effect on osteogenic genes (Wagner et al., 2008). Interestingly, HP was also found to decrease calcification of bone marrow and infrapatellar fat pad derived MSCs in long-term agarose culture (Carroll and Buckley, 2014). Similar to HP, compression of rabbit bone-marrow derived MSCs has been found to be a strong pro-chondrogenic stimulus, and compression also increases TGF-β1 gene expression (Huang et al., 2004). Furthermore, compression increases chondrogenic gene expression in MSCs in the absence of exogenous growth factor stimulation, suggesting that compression alone is sufficient to induce chondrogenesis (Kupcsik et al., 2010).

Overall, even though there is some tentative evidence, further detailed research is needed to clearly elucidate how MSCs sense and respond to the complex sets of mechanical stimuli *in vivo*, especially following traumatic injuries.

Other microenvironmental factors

Numerous other factors are also implicated in the maintenance and differentiation of MSC populations. For example, *leptin*, an adipokine that plays a central role in maintaining energy homeostasis, is expressed in at least a subpopulation of mouse bone marrow MSC and plays important roles in peripheral regulation of MSC osteogenic differentiation (Han et al., 2010; Scheller et al., 2010). *Fetuin-A*, a molecule that is mainly synthesized in the liver, was originally discovered to be an inhibitor of vascular calcification in the early 1990s (Sage et al., 2010). Interestingly, fetuin-A was down-regulated in rats during HO after Achilles tenotomy (Wu et al., 2016). However, Tuylu et al. also found abundant fetuin-A in calcified tissues. These seemingly conflicting data suggest that Fetuin-A may be a part of a feedback loop in the process of HO in ankylosing spondylitis (Tuylu et al., 2014). *1,25-dihydroxyvitamin D* (*1,25OHD*), the most active metabolite of vitamin D, has been proposed to be a co-activator of osteogenic differentiation since it enhanced

the ability of hepatocyte growth factor (HGF) to induce hMSC osteogenic differentiation (Chen et al., 2012). *Neural epidermal growth factor-like* (*NEL*)*-like protein 1* (*NELL-1*), is highly specific to the osteochondral lineage and induces MSC osteogenic differentiation by regulating runt related transcription factor 2 (RUNX2) both *in vivo* and *in vitro* (Govoni et al., 2005; Zhang et al., 2010, 2011; Dalle et al., 2012). NELL-1 increases expression of chondrogenic genes, including aggrecan and collagen, in human perivascular stem cells (hPSCs), presumably a population of MSC, and greatly augments the effects of TGF-β3 + BMP-6 in accelerating the chondrogenic differentiation of hPSCs (Li et al., 2016b). NELL-1 has emerged as a possible therapy for osteoprotoic bone loss (James et al., 2015).

Inhibition of *cyclooxygenase-2* (*COX2*), with a consequent reduction in *prostaglandin E*(*2*) (PGE2) production, inhibited chondrocyte differentiation of mouse MSC (Zhang et al., 2002; Simon et al., 2002; Lin et al., 2010; Welting et al., 2011). Based on this observation, Wang et al. hypothesized that varying the n-3/n-6 polyunsaturated fatty acid ratio would reduce PGE2 production and either prevent HO or serve as an alternative treatment for the disorder (Wang et al., 2013).

Hypoxia inducible factor (*HIF-1α*), a master co-regulator of responses to hypoxia, plays a critical role in osteochondrogenic differentiation of mouse MSC. For example, Agarwal et al. found that inhibition of HIF-1α prevented both trauma-induced (burn/tenotomy) and genetic (*Nfatc1-cre*/*caACVR1*$^{fl/wt}$) HO. Similarly, pharmacologic inhibitors (PX-478 or rapamycin) of HIF-1α potently diminish ectopic bone formation in different models of HO (Agarwal et al., 2016). Recently, Zhou et al. also found that HIF-1α, combined with BMP2, increases the chondrogenic differentiation of MSC cell line (C3H/10T1/2). This has provided an enhanced method of maintaining cartilage phenotypes during cartilage tissue engineering (Zhou et al., 2015).

Conclusions and future directions

In summary, this article highlights the roles of selected microenvironmental factors that were mainly identified in the context of GOF of MSC, especially in HO. Based on this discussion, a few emerging ideas are apparent: 1) Based on current data, we can conclude that not all microenvironmental factors are created equal, i.e., some factors increase the capacity of MSC for osteogenic differentiation, others promote self-renewal of MSC, and some can do both. 2) Another salient feature is that many effects of microenvironmental factors are context-dependent. 3) A reasonable explanation for the context-dependent effects is that these factors likely cross-talk with each other locally to form a niche-like functional unit to orchestrate the homeostasis of MSC and the downstream differentiation. Nevertheless, more detailed study of GOF models of MSC, especially the HO model, is warranted.

The limitations of this article are: 1) even though it is beyond the scope of this article to cover all candidate factors at reasonable depth, ideally, it would be helpful to also explore the candidate microenvironmental factors they might play key roles in the context of LOF of MSC (i.e., degenerative diseases). 2) Because of the paucity of relevant literature, in many cases, we had to dependent on the reports that are not directly relevant to GOF of MSC or HO models, and in some cases, we extrapolated our conclusions based on the available data. 3) In most cases, the exact underlying mechanisms for the microenvironmental factors are largely unknown.

This discussion underscores that there are still great gaps in knowledge of the precise role of the microenvironment in regulating MSC function, and this points to the need for more detailed mechanistic studies in the future. Specifically, based on current data, we reason that, since there are many candidate microenvironmental factors that could be involved in the regulation of MSC in physiological or pathophysiological contexts, it will be hard to dissect the interrelationship. To circumvent this problem, we argue that a potentially promising approach is to study the microenvironmental factors as a local functional regulatory unit, which works together inter-dependently, similar to the concept of stem cell niche, to orchestrate the homeostasis of MSC and the downstream differentiation.

Acknowledgements. We appreciate the help from many members of the Kessler lab. LK is supported in part by national Nature Science Foundation of China (81472087) and Nature Science Foundation of Anhui Province, China (1508085MC45). This work was supported by NIH grant RO1 AR066539 (JAK).

Disclosure. The authors indicate no potential conflicts of interest

References

Agarwal S., Loder S., Brownley C., Cholok D., Mangiavini L., Li J., Breuler C., Sung HH., Li S., Ranganathan K., Peterson J., Tompkins R., Herndon D., Xiao W., Jumlongras D., Olsen B.R., Davis T.A., Mishina Y., Schipani E. and Levi B. (2016). Inhibition of Hif1α prevents both trauma-induced and genetic heterotopic ossification. Proc. Natl. Acad. Sci. USA 113, E338-E347.

Antebi B., Zhang Z., Wang Y., Lu Z., Chen X.D. and Ling J. (2015). Stromal-cell-derived extracellular matrix promotes the proliferation and retains the osteogenic differentiation capacity of mesenchymal stem cells on three-dimensional scaffolds. Tissue Eng. Part. C Methods. 21, 171-181.

Arnsdorf E.J., Tummala P., Kwon R.Y. and Jacobs C.R. (2009). Mechanically induced osteogenic differentiation--the role of RhoA, ROCKII and cytoskeletal dynamics. J. Cell Sci. 122, 546-553.

Bancroft G.N., Sikavitsas V.I., van den Dolder J., Sheffield T.L., Ambrose C.G., Jansen J.A. and Mikos A.G. (2002). Fluid flow increases mineralized matrix deposition in 3D perfusion culture of marrow stromal osteoblasts in a dose-dependent manner. Proc. Natl. Acad. Sci. USA 99, 12600-12605.

Bianchi G., Banfi A., Mastrogiacomo M., Notaro R., Luzzatto L.,

Cancedda R. and Quarto R. (2003). Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. Exp. Cell Res. 287, 98-105.

Bianco P., Robey P.G. and Simmons P.J. (2008). Mesenchymal stem cells: revisiting history, concepts, and assays. Cell Stem Cell 2, 313-319.

Bossche L.V. and Vanderstraeten G. (2005). Heterotopic ossification: a review. J. Rehabil. Med. 37, 129-136.

Caplan A. and Correa D. (2011). PDGF in bone formation and regeneration: new insights into a novel mechanism involving MSC. J. Orthop. Res. 29, 1795-1803.

Carroll S.F. and Buckley C.T. (2014). Cyclic hydrostatic pressure promotes a stable cartilage phenotype and enhances the functional development of cartilaginous grafts engineered using multipotent stromal cells isolated from bone marrow and infrapatellar fat pad. J. Biomech. 47, 2115-2121.

Chen K., Aenlle K.K., Curtis K.M., Roos B.A. and Howard G.A. (2012). Hepatocyte growth factor (HGF) and 1,25-dihydroxyvitamin D together stimulate human bone marrow-derived stem cells toward the osteogenic phenotype by HGF-induced up-regulation of VDR. Bone 51, 69-77.

Chen F.P., Hu C.H. and Wang K.C. (2013). Estrogen modulates osteogenic activity and estrogen receptor mRNA in mesenchymal stem cells of women. Climacteric. 16, 154-160.

Chen L., Zou X., Zhang R.X., Pi C.j., Wu N., Yin L.J. and Deng Z.L. (2016). IGF1 potentiates BMP9-induced osteogenic differentiation in mesenchymal stem cells through the enhancement of BMP/Smad signaling. BMB Rep. 49, 122-127.

Chen G., Fang T., Qi Y., Huang Z., Du S., Di T., Feng G., Lei Z., Zhang Y. and Yan W. (2017). Combined use of mesenchymal stromal cell sheet transplantation and local injection of SDF-1 for bone repair in a rat non-union model. Cell Transplantation (in press).

Christian J.G., Schneeberger C. and Johannes C.H. (2002). Production and actions of estrogens.. N. Engl. J. Med. 346, 340-352.

Croes M., Oner F.C., Kruyt M.C., Blokhuis T.J. Bastian O. Dhert W.J. and Alblas J. (2015). Proinflammatory mediators enhance the osteogenesis of human mesenchymal stem cells after lineage commitment. PLoS One. 10, e0132781.

Croes M., Öner F.C., Van neerven D., Sabir E., Kruyt M.C., Blokhuis T.J., Dhert W.J. and Alblas J. (2016). Proinflammatory T cells and IL-17 stimulate osteoblast differentiation. Bone 84, 262-270.

Curtin C.M, Cunniffe G.M., Lyons F.G., Bessho K., Dickson G.R., Duffy G.P. and O'Brien F.J. (2012). Innovative collagen nano-hydroxyapatite scaffolds offer a highly efficient non-viral gene delivery platform for stem cell-mediated bone formation. Adv. Mater. 24, 749-754.

Dalle carbonare L., Innamorati G. and Valenti M.T. (2012). Transcription factor Runx2 and its application to bone tissue engineering. Stem Cell Rev. 8, 891-897.

Das R.K. and Zouani O.F. (2014). A review of the effects of the cell environment physicochemical nanoarchitecture on stem cell commitment. Biomaterials 35, 5278-5293.

Datta N., Pham Q.P., Sharma U., Sikavitsas V.I., Jansen J.A. and Mikos A.G. (2006). *In vitro* generated extracellular matrix and fluid shear stress synergistically enhance 3D osteoblastic differentiation. Proc. Natl. Acad. Sci. USA 103, 2488-2493.

Ding J., Ghali O., Lencel P., Broux O., Chauveau C., Devedjian J.C., Hardouin P. and Magne D. (2009). TNF-alpha and IL-1beta inhibit RUNX2 and collagen expression but increase alkaline phosphatase activity and mineralization in human mesenchymal stem cells. Life Sci. 84, 499-504.

Engler A.J., Sen S., Sweeney H.L. and Discher D.E. (2006). Matrix elasticity directs stem cell lineage specification. Cell 126, 677-689.

Evans K.N., Forsberg J.A., Potter B.K., Hawksworth J.S., Brown T.S., Andersen R., Dunne J.R., Tadaki D. and Elster E.A. (2012). Inflammatory cytokine and chemokine expression is associated with heterotopic ossification in high-energy penetrating war injuries. J. Orthop. Trauma. 26, e204-e213.

Eyckmans J., Roberts S.J., Schrooten J. and Luyten F.P. (2010). A clinically relevant model of osteoinduction: a process requiring Calcium phosphate and BMP/Wnt signalling. J. Cell Mol. Med. 14, 1845-1856.

Fajardo-Orduna G.R., Mayani H. and Montesinos J.J. (2015). Hemato-poietic support capacity of mesenchymal stem cells: Biology and clinical potential. Arch. Med. Res. 46, 589-596.

Ferreira E., Porter R.M., Wehling N., O'Sullivan R.P., Liu F., Boskey A., Estok D.M., Harris M.B., Vrahas M.S., Evans C.H. and Wells J.W. (2013). Inflammatory cytokines induce a unique mineralizing phenotype in mesenchymal stem cells derived from human bone marrow. J. Biol. Chem. 288, 29494-29505.

Forsberg J.A., Pepek J.M., Wagner S., Wilson K., Flint J., Andersen R.C., Tadaki D., Gage F.A, Stojadinovic A. and Elster E.A. (2009). Heterotopic ossification in high-energy wartime extremity injuries: prevalence and risk factors. J. Bone Joint Surg. Am. 91, 1084-1091.

Gawlitta D., Benders K.E., Visser J., van der Sar A.S., Kempen D.H., Theyse L.F., Malda J. and Dhert W.J. (2015). Decellularized cartilage-derived matrix as substrate for endochondral bone regeneration. Tissue Eng. Part A 21, 694-703.

Govoni K., Baylink D.J. and Mohan S. (2005). The multi-functional role of insulin-like growth factor binding proteins in bone. Pediatr. Nephrol. 20, 261-268.

Han G., Jing Y., Zhang Y., Yue Z., Hu X., Wang L., Liang J. and Liu J. (2010). Osteogenic differentiation of bone marrow mesenchymal stem cells by adenovirus-mediated expression of leptin. Regul. Pept. 163, 107-112.

Hoch A.I., Mittal V., Mitra D., Vollmer N., Zikry C.A. and Leach J.K. (2016). Cell-secreted matrices perpetuate the bone-forming phenotype of differentiated mesenchymal stem cells. Biomaterials 74, 178-187.

Hollinger J.O., Hart C.E., Hirsch S.N., Lynch S. and Friedlaender G.E. (2008). Recombinant human platelet-derived growth factor: biology and clinical applications. J. Bone Joint Surg. Am. 90 (Suppl 1), 48-54.

Hosogane N., Huang Z., Rawlins B.A., Liu X., Boachie-Adjei O., Boskey A.L. and Zhu W. (2010). Stromal derived factor-1 regulates bone morphogenetic protein 2-induced osteogenic differentiation of primary mesenchymal stem cells. Int. J. Biochem Cell Biol. 42, 1132-1141.

Huang C.Y., Hagar K.L., Frost L.E., Sun Y. and Cheung H.S. (2004). Effects of cyclic compressive loading on chondrogenesis of rabbit bone-marrow derived mesenchymal stem cells. Stem Cells 22, 313-323.

Huebsch N., Arany P.R., Mao A.S., Shvartsman D., Ali O.A., Bencherif S.A., Rivera-Feliciano J. and Mooney D.J. (2010). Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat. Mater. 9, 518-526.

Jackson W.M., Aragon A.B., Onodera J., Koehler S.M., Ji Y., Bulken-Hoover J.D., Vogler J.A.,Tuan R.S. and Nesti L.J. (2011). Cytokine expression in muscle following traumatic injury. J Orthop Res. 29,

1613-1620.
James A.W., Shen J., Zhang X., Asatrian G., Goyal R., Kwak J.H., Jiang L., Bengs B., Culiat C.T., Turner A.S., Seim Iii H.B., Wu B.M., Lyons K., Adams J.S., Ting K. and Soo C. (2015). NELL-1 in the treatment of osteoporotic bone loss. Nat. Commun. 6, 7362.
Kan L., Lounev V.Y., Pignolo R.J., Duan L., Liu Y., Stock S.R., McGuire T.L., Lu B., Gerard N.P., Shore E.M., Kaplan F.S. and Kessler J.A. (2011). Substance P signaling mediates BMP-dependent heterotopic ossification. J. Cell Biochem. 112, 2759-2772.
Kaplan F.S., Glaser D.L., Hebela N. and Shore E.M. (2004). Heterotopic ossification. J. Am. Acad. Orthop. Surg. 12, 116-125.
Keating A. (2012). Mesenchymal stromal cells: new directions. Cell Stem Cell. 10, 709-716.
Kupcsik L., Stoddart M.J., Li Z., Benneker L.M. and Alini M. (2010). Improving chondrogenesis: potential and limitations of SOX9 gene transfer and mechanical stimulation for cartilage tissue engineering. Tissue Eng. Part A 16, 1845-1855.
Laschober G.T., Brunauer R., Jamnig A., Singh S., Hafen U., Fehrer C., Kloss F., Gassner R. and Lepperdinger G. (2011). Age-specific changes of mesenchymal stem cells are paralleled by upregulation of CD106 expression as a response to an inflammatory environment. Rejuvenation Res. 14, 119-131.
Lau T.T. and Wang D. (2011). Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine. Expert Opin. Biol. Ther. 11, 189-197.
Li C., Li G., Liu M., Zhou T. and Zhou H. (2016a). Paracrine effect of inflammatory cytokine-activated bone marrow mesenchymal stem cells and its role in osteoblast function. J. Biosci Bioeng. 121, 213-219.
Li C.S., Zhang X., Péault B., Jiang J., Ting K., Soo C. and Zhou Y.H. (2016b). Accelerated chondrogenic differentiation of human perivascular stem cells with NELL-1. Tissue Eng. Part A 22, 272-285.
Lin L., Shen Q., Xue T. and Yu C. (2010). Heterotopic ossification induced by Achilles tenotomy via endochondral bone formation: expression of bone and cartilage related genes. Bone 46, 425-431.
Liu Y., Liu W., Hu C., Xue Z., Wang G., Ding B., Luo H., Tang L., Kong X., Chen X., Liu N.,Ding Y. and Jin Y. (2011). MiR-17 modulates osteogenic differentiation through a coherent feed-forward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. Stem Cells 29, 1804-1816.
Lundquist R., Dziegiel M.H. and Agren M.S. (2008). Bioactivity and stability of endogenous fibrogenic factors in platelet-rich fibrin. Wound Repair Regen. 16, 356-363.
Maroulakou I.G., Shibata M.A., Anver M., Jorcyk C.L., Liu M.I., Roche N., Roberts A.B., Tsarfaty I., Reseau J., Ward J. and Green J.E. (1999). Heterotopic endochondrial ossification with mixed tumor formation in C3(1)/Tag transgenic mice is associated with elevated TGF-beta1 and BMP-2 expression. Oncogene 18, 5435-5447.
Minguell J.J., Erices A. and Conget P. (2001). Mesenchymal stem cells. Exp. Biol. Med. 226, 507-520.
Niedhart C., Maus U., Redmann E., Schmidt-Rohlfing B., Niethard F.U. and Siebert C.H. (2003). Stimulation of bone formation with an in situ setting tricalcium phosphate/rhBMP-2 composite in rats. J. Biomed Mater Res. A 65, 17-23.
Ogata N., Koshizuka Y., Miura T., Iwasaki M., Hosoi T., Shiraki M., Seichi A., Nakamura K. and Kawaguchi H. (2002). Association of bone metabolism regulatory factor gene polymorphisms with susceptibility to ossification of the posterior longitudinal ligament of the spine and its severity. Spine (Phila Pa 1976). 27, 1765-1771.
Park J.S., Chu J.S., Tsou A.D., Diop R., Tang Z., Wang A. and Li S. (2011). The effect of matrix stiffness on the differentiation of mesenchymal stem cells in response to TGF-β. Biomaterials 32, 3921-3930.
Peterson J.R., Eboda O.N., Brownley R.C., Cilwa K.E., Pratt L.E., De La Rosa S., Agarwal S., Buchman S.R., Cederna P.S., Morris M.D., Wang S.C. and Levi B. (2015). Effects of aging on osteogenic response and heterotopic ossification following burn injury in mice. Stem Cells Dev. 24, 205-213.
Raisz L.G. and Kream B.E. (1983). Regulation of bone formation (second of two parts). N. Engl. J. Med. 309, 83-89.
Rakian R., Block T.J., Johnson S.M., Marinkovic M., Wu J., Dai Q., Dean D.D. and Chen X.D. (2015). Native extracellular matrix preserves mesenchymal stem cell "stemness" and differentiation potential under serum-free culture conditions. Stem Cell Res. Ther. 6, 235.
Riggs B.L. and Khosla S. (2005). Pathophysiology of age-related bone loss and osteoporosis. Endocrinol. Metab. Clin. North Am. 34, 1015-1030.
Roufosse C.A., Direkze N.C., Otto W.R. and Wright N.A. (2003). Circulating mesenchymal stem cells. Int. J. Biochem. Cell Biol. 36, 585-597.
Sacchetti B., Funari A., Remoli C., Giannicola G., Kogler G., Liedtke S., Cossu G., Serafini M., Sampaolesi M., Tagliafico E., Tenedini E., Saggio I., Robey P.G., Riminucci M. and Bianco P. (2016). No identical "mesenchymal stem cells" at different times and sites: Human committed progenitors of distinct origin and differentiation potential are incorporated as adventitial cells in microvessels. Stem Cell Rep. 6, 897-913.
Sacchetti B., Funari A., Michienzi S., Di Cesare S., Piersanti S., Saggio I., Tagliafico E., Ferrari S., Robey P.G., Riminucci M. and Bianco P. (2007). Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131,324-336 .
Sage A.P., Tintut Y. and Demer L.L. (2010). Regulatory mechanisms in atherosclerotic calcification. Nat. Rev. Cardiol. 7, 528-536.
Sampath T.K., Coughlin J.E., Whetstone R.M., Banach D., Corbett C., Ridge R.J., Ozkaynak E., Oppermann H. and Rueger D.C. (1990). Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily. J. Biol. Chem. 265, 13198-13205.
Sang C., Zhang Y., Chen F., Huang P., Qi J., Wang P., Zhou Q., Kang H., Cao X. and Guo L. (2016). Tumor necrosis factor alpha suppresses osteogenic differentiation of MSC by inhibiting semaphorin 3B via Wnt/β-catenin signaling in estrogen-deficiency induced osteoporosis. Bone 84, 78-87.
Scheller E.L., Song J., Dishowitz M.I., Soki F.N., Hankenson K.D. and Krebsbach P.H. (2010). Leptin functions peripherally to regulate differentiation of mesenchymal progenitor cells. Stem Cells 28, 1071-1080.
Si W., Kang Q., Luu H.H., Park J.K., Luo Q., Song W.X., Jiang W., Luo X., Li X., Yin H., Montag A.G., Haydon R.C. and He T.C. (2006). CCN1/Cyr61 is regulated by the canonical Wnt signal and plays an important role in Wnt3A-induced osteoblast differentiation of mesenchymal stem cells. Mol. Cell Biol. 26, 2955-2964.
Simon A.M., Manigrasso M.B. and O'connor J.P. (2002). Cyclo-oxygenase 2 function is essential for bone fracture healing. J. Bone Miner. Res. 17, 963-976.
Steward A.J. and Kelly D.J. (2015). Mechanical regulation of

mesenchymal stem cell differentiation. J. Anat. 227, 717-731.

Tan H.B., Giannoudis P.V., Boxall S., McGonagle D. and Jones E. (2015). The systemic influence of platelet-derived growth factors on bone marrow mesenchymal stem cells in fracture patients. BMC Med. 13, 6.

Tasso R., Gaetani M., Molino E., Cattaneo A., Monticone M., Bachi A. and Cancedda R. (2012). The role of bFGF on the ability of MSC to activate endogenous regenerative mechanisms in an ectopic bone formation model. Biomaterials 33, 2086-2096.

Tuylu T., Sari I., Solmaz D., Kozaci D.L., Akar S., Gunay N., Onen F. and Akkoc N. (2014). Fetuin-A is related to syndesmophytes in patients with ankylosing spondylitis: a case control study. Clinics (Sao Paulo). 69, 688-693.

Viti F., Landini M., Mezzelani A., Petecchia L., Milanesi L. and Scaglione S. (2016). Osteogenic differentiation of MSC through Calcium signaling activation: transcriptomics and functional analysis. PLoS One. 11, 148-173.

Wada A. (1995). Affinity of estrogen binding in the cultured spinal ligament cells: an *in vitro* study using cells from spinal ligament ossification patients. Nihon Seikeigeka Gakkai Zasshi. 69, 440-449.

Wagner D.R., Lindsey D.P., Li K.W., Tummala P., Chandran S.E., Smith R.L., Longaker M.T., Carter D.R. and Beaupre G.S. (2008). Hydrostatic pressure enhances chondrogenic differentiation of human bone marrow stromal cells in osteochondrogenic medium. Ann. Biomed. Eng. 36, 813-820.

Wang L., Huang M.J., Liu B., Zhang Z.M., Zheng X.C., Yan B., Chen T.Y., Jin D.D. and Bai X.C. (2013a). Could heterotopic ossification be prevented by varying dietary n-3/n-6 polyunsaturated fatty acid ratio: a novel perspective to its treatment?. Med Hypotheses 80, 57-60.

Wang L., Zhao Y., Liu Y., Akiyama K., Chen C., Qu C., Jin Y. and Shi S. (2013b). IFN-γ and TNF-α synergistically induce mesenchymal stem cell impairment and tumorigenesis via NF-κB signaling. Stem Cells . 31, 1383-1395.

Wang Z., Weng Y., Lu S., Zong C., Qiu J., Liu Y. and Liu B. (2015). Osteoblastic mesenchymal stem cell sheet combined with Choukroun platelet-rich fibrin induces bone formation at an ectopic site. J. Biomed. Mater. Res. B Appl. Biomater. 103, 1204-1216.

Wang N., Zhou Z., Wu T., Liu W., Yin P., Pan C. and Yu X. (2016). TNF-α-induced NF-κB activation upregulates microRNA-150-3p and inhibits osteogenesis of mesenchymal stem cells by targeting b-catenin. Open Biol. 6: 150258.

Welting T.J., Caron M.M., Emans P.J., Janssen M.P., Sanen K., Coolsen M.M., Voss L., Surtel D.A., Cremers A., Voncken J.W. and van Rhijn L.W. (2011). Inhibition of cyclooxygenase-2 impacts chondrocyte hypertrophic differentiation during endochondral ossification. Eur. Cell Mater. 22, 420-436.

Wong M., Kireeva M.L., Kolesnikova T.V. and Lau L.F. (1997). Cyr61, product of a growth factor-inducible immediate-early gene, regulates chondrogenesis in mouse limb bud mesenchymal cells. Dev Biol. 192, 492-508.

Wu J., Xue F., Shi W.Z. and Xiao H.J. (2016). Fetuin a is down-regulated in RATS during heterotopic ossification after Achilles tenotomy. Biotech. Histochem. 91, 229-236.

Xiong Y., He J., Zhang W., Zhou G., Cao Y. and Liu W. (2015). Retention of the stemness of mouse adipose-derived stem cells by their expansion on human bone marrow stromal cell-derived extracellular matrix. Tissue Eng Part A 21, 1886-1894.

Yang Y. and Dai M. (2015). Expression of PADI4 in patients with ankylosing spondylitis and its role in mediating the effects of TNF-α on the proliferation and osteogenic differentiation of human mesenchymal stem cells. Int. J. Mol. Med. 36, 565-570.

Zhang X., Schwarz E.M., Young D.A., Puzas J.E., Rosier R.N. and O'Keefe R.J. (2002). Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J. Clin. Invest. 109, 1405-1415.

Zhang X., Zara J., Siu R.K., Ting K. and Soo C. (2010). The role of NELL-1, a growth factor associated with craniosynostosis, in promoting bone regeneration. J. Dent. Res. 89, 865-878.

Zhang X., Ting K., Bessette C.M., Culiat C.T., Sung S.J., Lee H., Chen F., Shen J., Wang J.J., Kuroda S. and Soo C. (2011). Nell-1, a key functional mediator of Runx2, partially rescues calvarial defects in Runx2(+/-) mice. J. Bone Miner. Res. 26, 777-791.

Zhang J., Zhao Y., Hou X., Chen B., Xiao Z., Han J., Shi C., Liu J., Miao Q. and Dai J. (2014). The inhibition effects of insulin on BMP2-induced muscle heterotopic ossification. Biomaterials 35, 9322-9331.

Zhou N., Hu N., Liao J.Y., Lin L.B., Zhao C., Si W.K., Yang Z., Yi S.X., Fan T.X., Bao W., Liang X., Wei X., Chen H., Chen C., Chen Q., Lin X. and Huang W. (2015). HIF-1α as a regulator of BMP2-Induced chondrogenic differentiation, osteogenic differentiation, and endochondral ossification in stem cells. Cell. Physiol. Biochem. 36, 44-60.

Zisa D., Shabbir A., Suzuki G. and Lee T. (2009). Vascular endothelial growth factor (VEGF) as a key therapeutic trophic factor in bone marrow mesenchymal stem cell-mediated cardiac repair. Biochem. Biophys. Res. Commun. 390, 834-838.

Accepted March 22, 2017