The role of catecholamines in mesenchymal stem cell fate

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Abstract Mesenchymal stem cells (MSCs) are multipotent stem cells found in many adult tissues, especially bone marrow (BM) and are capable of differentiation into various lineage cells such as osteoblasts, adipocytes, chondrocytes and myocytes. Moreover, MSCs can be mobilized from connective tissue into circulation and from there to damaged sites to contribute to regeneration processes. MSCs commitment and differentiation are controlled by complex activities involving signal transduction through cytokines and catecholamines. There has been an increasing interest in recent years in the neural system, functioning in the support of stem cells like MSCs. Recent efforts have indicated that the catecholamine released from neural and not neural cells could be affected characteristics of MSCs. However, there have not been review studies of most aspects involved in catecholamines-mediated functions of MSCs. Thus, in this review paper, we will try to describe the current state of catecholamines in MSCs destination and discuss strategies being used for catecholamines for migration of these cells to damaged tissues. Then, the role of the nervous system in the induction of osteogenesis, adipogenesis, chondrogenesis and myogenesis from MSCs is discussed. Recent progress in studies of signaling transduction of catecholamines in determination of the final fate of MSCs is highlighted. Hence, the knowledge of interaction between MSCs with the neural system could be applied towards the development of new diagnostic and treatment alternatives for human diseases.

Keywords Mesenchymal stem cell · Differentiation · Catecholamines · Adrenergic signaling · Migration

Abbreviations

ATF4 Activating transcription factor 4
BARK β-Adr kinase
BM Bone marrow
BMSCs Bone marrow stem cells
BMP4 Bone morphogenetic proteins
C/EBP CCAAT/enhancer-binding protein
DBH Dopamine-b-Hydroxylase
DEHP Bis (2-Ethylhexyl) phthalate
Ebf1 Early B-cell factor 1
EPAC Exchange protein activated by adenylyl cyclase
EPO Erythropoietin
FGF Fibroblast growth factor
GPC Growth plate chondrocytes
GPCRs G-protein-coupled receptors
GCs Glucocorticoids
HGF Hepatocyte growth factor
HSCs Hematopoietic stem cells
IGF-1 Insulin-like growth factor-1
IRFs Interferon regulatory factors
MAFbx Muscle atrophy F-box protein
MKP-1 Mitogen-activated protein kinase phosphatase

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MAFbx Muscle atrophy F-box protein
MKP-1 Mitogen-activated protein kinase phosphatase
MSCs  Mesenchymal stem cells
MuRF1  Muscle ring finger1
OGP  Osteogenic growth peptide
PB  Peripheral blood
PDEs  Phosphodiesterase proteins
PGE2  Prostaglandin E2
pRb  Retinoblastoma cell cycle-related proteins
PKA  Protein kinase A
ROS  Reactive oxygen species
SDF-1  Stromal-derived factor-1
SHH  Sonic Hedgehog protein
SNS  Sympathetic nervous system
SREBP  Sterol regulatory element binding proteins
S1P  Sphingosine1-phosphate
TBT  Tributyltin
TB4  Thymosin β
TF  Transcription factors
TH  Tyrosine hydroxylase
TGF-β  Transforming growth factor-β
TLR-9  Toll-like receptor 9
4EBP1  eIF4E binding protein1

Introduction

The isolation of mesenchymal stem cells (MSCs) from bone marrow (BM) compartments was originally reported in 1988 by Owen and Friedenstein (1998). Further studies have indicated the presence of multipotent cells known as adult stem cells in other tissues, including adipose tissue, muscle, cord blood, umbilical cord tissue, liver, spleen, BM and peripheral blood (PB) (Caplan and Bruder 2001). Phenotypically, these cells have no expression of the specific surface markers of hematopoietic lineage, including CD34, CD45, CD14, CD19, CD105, CD106, CD166 and Stro-1 surface markers (Pittenger et al. 1999). In addition, MSCs have reported to be CD80−, CD86−, CD18− and CD31− but positive for CD29, CD44, CD71, CD73, CD90, CD105, CD106, CD166 and Stro-1 surface markers (Pittenger et al. 1999; Zuk et al. 2002). MSCs of various tissues share some common characteristics; however, these cells represent specific differences in their specialized markers. For example, adipocyte tissue MSCs are CD49d+CD106− cells, while CD markers of circulating MSCs share most of their immunophenotype with BM-derived MSCs (Zuk et al. 2002). Indeed, despite the lack of tissue-specific characteristics in MSCs, these cells are phenotypically heterogeneous with varying differentiation potentials. Therefore, it is important to identify special characteristics and better definitions of various tissues-derived MSCs.

The most characteristic feature of MSCs is their capability of self-renewal and multi-lineage differentiation into osteoblasts, adipocytes and chondrocytes (Farshdousti Hagh et al. 2012). Additionally, under defined conditions, these cells can differentiate into neurons, skeletal myocytes and endothelial cells (Nadri et al. 2008; Pittenger et al. 1999; Soleimani et al. 2010). Among conditions, plastic adherence, expression of surface markers and differentiation capacity are considered as criteria of human MSCs (Dominici et al. 2006). The ability of differentiation and easy isolation have made MSCs cells suitable sources for studies linked to cell therapy for tissue regeneration and engineering (Khajeniaz et al. 2013; Yazdani et al. 2013). In this regard, one of the most important functions of these cells is supporting hematopoiesis by creating a suitable scaffold in BM through the expression of adhesion molecules, including stromal cell-derived factor-1 (SDF-1) and CXCR4 and secretion of useful soluble mediators (Ahmadbeigi et al. 2013; Saki et al. 2011). Moreover, MSCs can adopt HSCs in various situations, caused by different amounts of oxygen in BM compartments (Grayson et al. 2007). Hypoxia conditions in BM can mediate MSCs proliferation by hypoxia-inducible factor (HIF)-1 and, recently, the role of some miRNAs involved in the hypoxia pathway, known as hypoxamirs, has been determined in regulating proliferation of MSCs (Grayson et al. 2007; Minayi et al. 2014).

Several mechanisms are involved in regulating MSCs functions such as the nervous system (Havasi et al. 2013). It has been reported that migration and differentiation of MSCs are under neuronal control. First, BM compartments are highly innervated, especially in proximity to endosteum and endothelial cells, which are rich in stem cells, implying indirect control over BM cells like MSCs (Mignini et al. 2003). Second, a variety of neural receptors have been found on MSCs (Silva et al. 2003). Third, catecholamines derived from noradrenergic endings in damaged tissues could result in MSCs recruitment (Mignini et al. 2003). Finally, it has been demonstrated that tyrosine hydroxylase (TH), a specific enzyme of synthesis catecholamines, is expressed through immune cells that in combination with released cytokines can affect MSCs-derived tissue (Stanojević et al. 2013). To our knowledge, the functional link between catecholamines and MSCs is not well determined. Thus, here, updated information from recent research on these topics, especially the central role of catecholamines in osteogenesis, adipogenesis, chondrogenesis, myogenesis and migration of MSCs, will be discussed. We describe adrenergic signaling, the effect of catecholamines on MSCs differentiation and the role of adrenergic signaling in the mobilization of MSCs.

Adrenergic signaling pathways in MSCs

Several subtypes of neural adrenergic receptor are named α and β, which are further subdivided into α1, α2, β1, β2 and β3 (Cole and Sood 2012). There is an adrenalines ligand with
both α and β-adrenergic receptors (β-Adrs); however, catecholamine-mediated signaling is conducted more through subsets of β, due to α receptors being less sensitive to adrenalin (Cole and Sood 2012). The β-receptors belong to a large family of G-protein-coupled receptors (GPCRs). Ligation of β-receptors by catecholamines leads to Gαs-mediated stimulation of adenylate cyclase and increases cAMP intracellular levels with conversion of ATP into cAMP (Cole and Sood 2012; Rosenbaum et al. 2009). Intracellular cAMP activates protein kinase A (PKA), a major effector system, to phosphorylate multiple target proteins such as transcription factors, kinases and cell-surface receptors either concurrently or sequentially (Fig. 1) (Cole and Sood 2012). Furthermore, β-Adrs can activate Goi, which differentially mediates adenylyl cyclase. Phosphodiesterase proteins (PDEs) specifically suppress cAMP second messenger levels (Rosenbaum et al. 2009). Phosphorylation of β-Adrs by PKA and β-Adr kinase (BARK) activated by PKA leads to the recruitment of β-arrestin, which subsequently induces the Src/Ras/MAPK pathway (Fig. 1) (Cole and Sood 2012). Exchange protein activated by adenylyl cyclase (EPAC) is a second cAMP effector that activates other kinases such as P38 MAP and ERK1/2. Stimulation of ERK1/2 is limited through the concomitant activation of the mitogen-activated protein kinase phosphatase (MKP-1) pathways (Cole and Sood 2012). Although all three types of β adrenergic are associated with the same pathways, the timing and strength of the signaling can be different (Cole and Sood 2012). Generally, these signals can modulate the biological activity of catecholamines functions on the metabolism and mechanism involved in the proliferation and differentiation of MSCs (De Ugarte et al. 2003). Depending on the particular cell derived from MSCs, adrenergic signaling mediates different processes. Hence, a key question is how adrenergic signaling can perform different patterns with high specificity and reliability.

A brief overview of adrenergic signaling related to differentiation and mobilization of MSCs indicates that, downstream of these, signaling would involve genes allocation of each lineage

Fig. 1 Signaling of β-adrenergic receptors-coupled G-protein in MSCs. These receptors stimulate cAMP/PKA and MAP kinases
through the effect on regulatory transcription factors (TF) that can modify histones and methylation patterns. Recent findings on the MAPK pathway have shown that ERKs can regulate important enzymes of epigenetics, including DNA methyltransferase and acetyltransferase (Tsang and Cheng 2011). Furthermore, PKA-activated CREAB mediates transcription via regulating methylation patterns (Lee et al. 2013). In addition, adrenergic signals could serve different miRNAs of each cell derived from MSCs (Collino et al. 2011). The control of TF and epigenetics including miRNA by adrenergic signaling seem to regulate MSCs fate (Collino et al. 2011). Despite important epigenetic and various aspects of this process in MSCs fate, this mechanism is beyond the scope of this paper and we focus on the interaction between downstream adrenergic signals with genes involved in MSCs fate.

**Effect of catecholamines on mesenchyme-derived osteoblasts**

Nervous system involvement in the regulation of bone mass is in agreement with the significant variations of bone formation through neural agonists (Soleimani et al. 2012). Catecholamine-producing nerves in the vicinity of osteoblasts can regulate bone remodeling, so suppressing nervous system activity leads to increased bone formation (Mignini et al. 2003). The administration of isoproterenol, a non-selective β-Adrs agonist, reduces osteoblasts number and bone mineral density without affecting body weight (Elefteriou et al. 2005). Conversely, deficiency or blockade of β-Adrs in mice will increase bone mass and prevent bone loss followed by ovariectomy (Minkowitz et al. 1991). One of the most convincing pieces of evidence for the involvement of catecholamines in regulating bone formation is the interaction between the nervous system and leptin. Leptin inhibits osteoblasts through up-regulation of the catecholamines releasing/activity affecting the hypothalamus. So, infusion of β-Adrs antagonists suppresses the function of leptin on bone formation (Takeda et al. 2002). Moreover, it has been suggested that β-blockers could be considered as novel therapy for postmenopausal osteoporosis (Rejnmark et al. 2004).

Among all β-Adrs, β2-Adr is the main, if not the only, adrenergic receptor expressed in osteoblasts (Li et al. 2010a, b). Thus, lack of β2-Adr leads to a high bone mass phenotype (Li et al. 2010a, b). However, the role of β1-Adr in bone formation is unknown. Recently, studies have shown that a lack of both β1- and β2-Adrs in osteoblasts results in decreased rather than increased bone formation. Indeed, β1-Adr is required for high bone mass in β2−/−−Adr (Pierroz et al. 2006). The mechanism of the anti-osteogenic function of the nervous system seems to involve MSCs differentiation toward osteoblasts (Harada and Rodan 2003). Gene expression of adrenergic receptors has been detected in cell lines and primary osteoblasts, including α1-Adr in MC3T3-E1, β1-Adr in SaOS-2, OHS-4 and TE-85 and β2-Adr in primary osteoblasts, UMR106, MG63, ROS 17/2.8 and Saos2 (Suzuki et al. 1998). During MSC osteogenesis, the expression of the three β-Adrs is altered and β2-Adr is markedly increased. The alteration extent of β1-Adr is relatively low and expression of β3-Adr is up-regulated but is much less than β2-Adr (Li et al. 2010a, b). Generally, β2-Adr seems to be the only adrenergic receptor presenting at significant levels in osteoblasts (Elefteriou 2008; Takeda 2005). Regarding the pathway of adrenergic signaling, shown in Fig. 1, cAMP-induced adrenergic agonists has been considered as the key pattern of transcriptional response-dependent catecholamines to signaling of osteoblast differentiation (Li et al. 2010a, b). β-Adr-induced AP-1 provides the condition for osteoblast proliferation and leads to increased levels of ATF4, a CREB-related transcription factor that seems critical in the differentiation and function of osteoblasts (Xiao et al. 2005).

The effects of the agonist and antagonist of β-Adr on osteogenic differentiation of MSCs is unclear and there are conflicting data in this respect. Li et al. (2010a, b) showed that the β-agonist suppresses osteogenesis with a dose and time-dependent manner. Moreover, they proved that the inhibitory effect of isoproterenol on osteoblast differentiation from MSCs is through increased activity of cAMP and PKA in MSCs. So, propranolol, a potent PKA inhibitor, would abolish the effect of β adrenergic agonists on osteoblasts (Li et al. 2010a, b). In the same year, Uemura et al. (2010) insisted that neural agonists like epinephrine have anabolic effects on osteoblast differentiation on MSCs. In contrast to previous articles, they claimed that cAMP/PKA has an induced effect on the osteoblasts (Uemura et al. 2010). The CAMP pathway activated by G-proteins receptors such as β-Adrs can have a catabolic or anabolic role on bone formation in vitro. One of the possible explanations for this discrepancy may be due to using different doses of catecholamines and various in vitro conditions and methods. However, the effect of CAMP/PKA on osteogenesis should be clearly identified. This may suggest a role of several signaling TF and growth factors involved in differentiation of MSCs into osteoblasts by catecholamines, which could lead to down- or up-regulation of their expression (Table 1).

**Effect of catecholamines on mesenchyme-derived myogenesis**

Recently, Liu et al. (2011) found that myogenic progenitors are present in bone fractures (Liu et al. 2011). It has not yet been determined in this condition whether MSCs could differ from myogenic progenitors or would only provide appropriate stromals for myoblast cells. Furthermore, where do nervous systems stand in myogenic tissue? Very few studies on MSCs have been published showing that these cells could
represent a myoblastic lineage phenotype (Shiota et al. 2007). However, under appropriated induction conditions, MSCs would acquire the phenotypic properties of skeletal myoblasts and cardiomyocytes (Shiota et al. 2007; Toma et al. 2002). In addition, studies have demonstrated that MSCs injection into patients with skeletal muscle dystrophy and myocardial infarction leads to improvement of muscle compliances that are associated with differentiation into muscle cells, myocardial thickness and myotubes growth (Gang et al. 2004). Important roles of catecholamines have been demonstrated in myogenesis, regulating skeletal muscle regeneration and cardiomuscle formation (Lynch and Ryall 2008). New data suggest the important role of β-adrenoreceptors in myotoxic injury, including sarcopenia, muscular dystrophies and cancer cachexies (Beitzel et al. 2004; Koopman et al. 2009). Increasing muscle mass in neonatal mice by catecholamines injection has been found but it has not been identified whether or not differentiation of MSCs to muscle cells would be increased (Ryall et al. 2010). The direct effect of catecholamines is not yet been investigated on various stages of MSCs differentiation into myogenesis. However, it has been suggested that catecholamines play a pivotal role in the latter stages of myogenesis (Beitzel et al. 2004). For example, expression of β-Adrs is increased significantly 2 days post-differentiation of muscle precursors, which subsequently use p21 as a stimulator, while terminal differentiation is upregulated (Ryall et al. 2010). All three isoforms of β-Adrs express in skeletal and cardiac muscle cells but the β2 receptor contains the predominant population. In skeletal muscle, β agonists cause muscle mass through inducing PKA and PI3K rapamycin complex1 (mTORC1) and mediating phosphorylation of S6k and eIF4E binding protein1 (4EBP1) (Kline et al. 2007; Sneddon et al. 2001). Furthermore, decreasing key ubiquitin ligases of skeletal muscle, MAFbx (muscle atrophy F-box protein) and MuRF1 (muscle ring finger1), have been found followed by β-adrenoreceptor treatment (Ryall et al. 2010). Furthermore, β-Adrs can accelerate skeletal muscle regeneration and improve the structure and function of these muscles (Ryall and Lynch 2008). Concerning cardiac muscle exposed by catecholamines, β-agonists inhibit the differentiation of these cells through β1-Adr (Zaugg et al. 2000). Direct investigation of the effect of catecholamines on MSCs differentiation into muscle is necessary to determine the contribution of β-Adrs to myogenesis. In cardiac muscle based on receptor type, catecholamines have different effects and exhibit distinct outcomes on these cells (Shizukuda and

### Table 1 Signaling and signaling affecting on osteogenesis

<table>
<thead>
<tr>
<th>Signaling and growth factors</th>
<th>Linking pathway</th>
<th>Effect of catecholamine on factors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wnt signaling</td>
<td>Activate the non-canonical Wnt/JNK pathway</td>
<td>Up-regulation of β-catenin</td>
<td>Qiu et al. 2011</td>
</tr>
<tr>
<td>TGF-β signaling</td>
<td>Increased expression of mRNA involved in osteogenesis</td>
<td>Catecholamines and TGF-β1 have a positive feedback</td>
<td>Nogami et al. 1994; Zhao and Hantash 2011</td>
</tr>
<tr>
<td>SHH signaling</td>
<td>Interaction with Wnt and BMP signaling</td>
<td>Catecholamines and SHH have a positive feedback</td>
<td>Babashah et al. 2013; Charrier et al. 2001</td>
</tr>
<tr>
<td>FGF signaling</td>
<td>Phosphorylation of Runx2</td>
<td>Catecholamines and FGF have a positive feedback</td>
<td>Peng et al. 2002; Yu et al. 2003</td>
</tr>
<tr>
<td>Runx2</td>
<td>Regulation of genes involved in osteogenesis</td>
<td>Down-regulation of Runx2</td>
<td>Sato et al. 2007; Xiao et al. 2005</td>
</tr>
<tr>
<td>Osterix</td>
<td>Induction of preosteoblasts into fully functioning osteoblasts</td>
<td>Down-regulation of Osterix</td>
<td>Sato et al. 2007</td>
</tr>
<tr>
<td>TAZ</td>
<td>Induction of expression genes involved in osteogenesis</td>
<td>Not known</td>
<td>Xue et al. 2013</td>
</tr>
<tr>
<td>OGP</td>
<td>Stimulation of osteoblast-specific mRNA expression of core-binding factor 1 (cbfa1)</td>
<td>Not known</td>
<td>Chen et al. 2007</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Increased expression of transcriptional co-activator with PDZ-binding motif (TAZ)</td>
<td>Catecholamines and IGF-1 have a positive feedback</td>
<td>Sun and Ng 1998; Xue et al. 2013</td>
</tr>
<tr>
<td>Insulin</td>
<td>Stimulation of Akt-dependent pathway</td>
<td>Down-regulation of insulin; insulin increases secretion of catecholamines</td>
<td>Sauter et al. 1983; Srivastava et al. 2012</td>
</tr>
<tr>
<td>PGE2</td>
<td>Induction of the expression of ALPase and Osterix</td>
<td>Catecholamines and PGE2 have a positive feedback</td>
<td>Negishi and Ito 1992; Ninoiya et al. 2011</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Control osteogenesis positively and negatively.</td>
<td>Up-regulation of serotonin; serotonin leads to a down-regulation of catecholamine</td>
<td>Ducy and Karsenty 2010; Zweig and Axelrod 1969</td>
</tr>
</tbody>
</table>

Buttrick 2002). Several observations emphasize neural-induced programmed cell death of cardiac muscle by β1-Adr and antagonists of β2-ARs inhibit apoptosis (Xiao et al. 2006). Although it appears that catecholamines induce cardiac hypertrophy via α and β1-Adrs, selective overexpression of β2-Adr could restore ventricular function and hypertrophy (Morisco et al. 2001).

Effect of catecholamines on mesenchyme-derived adipocytes

The sympathetic nervous system (SNS) has a prominent role in differentiation and metabolism of adipocyte tissues and SNS innervation has been found in adipocyte tissues (Path et al. 2001; Zhu et al. 2003). β-Adr agonists take their place as the predominant regulators in catecholamines-induced lipolysis of stored triglyceride in both white and brown adipocytes of brain. Thus, β3 agonist may lead to increased energy expenditure and reduced fat stores (Gronning et al. 2006). Lack of the three known β-Adrs in mice decreases the metabolic rate and the extent of massive obesity due completely to the defeating of diet-induced thermogenesis (Li et al. 2010a, b). It has been suggested that 20–25 % of lipolysis is mediated through β-Adrs activation of ERK pathways (Collins 2011).

Moreover, the catecholamine-induced pathway of p38α MAPKs in adipocytes leads to the expression of transcription involved in the differentiation of brown adipocytes. However, the role of the p38 MAPK pathway in white adipocytes has not yet been reported (Collins 2011). The forkhead transcription factor FOXC2 affects genes involved in lipolysis via increased sensitivity of the β adrenergic-cAMP-PKA signaling pathways (Gronning et al. 2006). It has been well established that specific enzymes of neuronal cells such as dopamine-b-hydroxylase (DBH) and TH are expressed by adipocytes cells that are independent of nerve terminals, indicating the role of adipocyte tissue as a new source of catecholamine production (Barry and Murphy 2004). However, fat depots have different expression of neural enzymes and stress could stimulate the production of catecholamine in adipocytes (Barry and Murphy 2004; Vargovic et al. 2011). It can be suggested that there is an appropriate interaction between neural cells with adipocyte cells for the regulation of different effects on fat tissue.

Adrenergic receptors such as β1, β2 and β3 and α1 and α2 receptors on adipocyte surfaces have been demonstrated (Bengtsson et al. 2000). As mentioned above, adrenergic agonists lead to the activation of the cAMP/PKA signaling pathway. Hence, it has been noted that catecholamines control adipocyte tissue functions in a large part through signaling mediated by β-Adrs (Collins 2011). The expression alterations of particular β-Adr subtypes during adipogenic differentiation from MSCs indicate that β2 and β3 are the main receptors of β-adrenergics and up-regulate upon adipogenic induction (Bengtsson et al. 2000). β2-and β3-Adrs are expressed in early and late stages of differentiated adipocytes, respectively (Li et al. 2010a, b). β1-Adrs have a limited expression during the adipogenesis process of MSCs (Li et al. 2010a, b). In this line, studies on 3 T3-L1 fibroblasts demonstrated that, during the process of differentiation to the adipocyte, only up-regulated β2-Adr is observed and β1-Adr has an undetectable level (Guest et al. 1990). In other studies, in 3 T3-F442A cells, β1 and β3-Adr became detectable in early and late adipocytes, respectively (Feve et al. 1991). Nevertheless, it should be noted that different cell lines have exclusive characteristics. During brown adipocyte development, variations of the switch in the β-Adr subtypes have seen to be different from white adipocytes, so β1-Adr is expressed abundantly in preadipocytes, leading to increased proliferation, while β3-Adr is mainly detectable in later adipogenesis (Bronnikov et al. 1999). In human, most brown adipocytes are lost during the transition from newborn to adult. In vitro, the most frequent models of adipogenesis include using MSCs, preadipocytes and isolated adipocytes under suitable conditions (Hashemi et al. 2013; Yousefi et al. 2013). However, these cell populations have been reported to have the same differentiation potential into adipocytes (De Ugarte et al. 2003). Studies have shown that imipramine, a regulator of re-uptake of norepinephrine and serotonin, inhibits adipogenic differentiation in both 3 T3-L1 preadipocytes and mouse MSCs by stimulating β2- and b3-Adrs (Li et al. 2012). Moreover, β-Adr agonists, especially stimulators of β2 and β3, inhibit MSC adipogenesis with a trend of dose- and time-dependence, while antagonists have a positive effect on the development of adipocytes from MSCs (Li et al. 2010a, b). However, another study established that agonists adrenergics could lead to preadipocyte proliferation but inhibit its differentiation (Collins 2011). Finally, in brown-fat cells, a different behavior from catecholamines has been found, so that norepinephrine promotes the expression of factors involved in differentiation preadipocytes and also induces the proliferation of cultured brown-fat cells (Nedergaard et al. 1995; Rehnmark et al. 1990) (Table 2).

Effect of catecholamines on mesenchyme-derived chondrocytes

Under specific conditions, MSCs differentiate into chondroprogenitor cells as a result from condensation and subsequently chondrocytes arise from these progenitors (Kadivar et al. 2006). Chondrocytes can undergo proliferation and convert to endochondral ossification, which induces bone formation (temporary cartilage) or remain as resting cells to form the complex tissue of articular cartilage (permanent cartilage) (Kadivar et al. 2006). Indeed, the formation of a
cartilaginous model is well known as the physiological trigger for skeletal development.

As mentioned before, nerve endings of sympathetic origin innervate bone and could be involved in mediating endochondral ossification during skeletal growth (Grills et al. 1997; Harada and Rodan 2003). The effects of adrenergic activation on bone development could be regulated by the effects on chondrocytes (Lai and Mitchell 2008b). Expression of adrenergic receptors has been demonstrated in cell lines, for example in primary growth plate chondrocytes (GPC) and both proliferating and hypertrophic chondrocytes only the β2-Adr was detected and α- and other β-Adr were abundantly found in primary costal chondrocytes (Takarada et al. 2009). Epinephrine can lead to the suppression of chondrogenesis by stimulation of β2-Adr (Takarada et al. 2009). However, it has been found that epinephrine decreased apoptosis in chondrocytes (Takarada et al. 2009).

Infrequent evidence has been found for the functional role of catecholamines signaling in the mechanisms underlying differentiation stages of chondrogenics from MSCs (Lai and Mitchell 2008a). During the endochondral process, epinephrine inhibits SOX9, a transcription factor that is required in the early steps of chondrogenesis, through reducing SOX6 activation that is essential to form a functional heteromeric protein complex with Sox9 (Takarada et al. 2009). Furthermore, catecholamine regulates the function between β-catenin and SOX9 during chondrocyte differentiation (Takarada et al. 2009). Administration of a β-Adr antagonist would optimize tissue repair by promoting MSCs-altered chondrocytes, preventing catecholamines-reduced formation of hypertrophic chondrocytes and recruitment of osteocytes (Rejnmark et al. 2004). Moreover, recent studies suggest that signal transduction of β-Adrs coupled to stimulation of both MAP kinase and ERK1/2 induces the growth of chondrocytes and inhibits their differentiation (Lai and Mitchell 2008a). Previously, the inhibitory effect of PKA and ERK1/2 has been investigated on the role of chondrogenesis in the interaction between FGF receptor3 activation and parathyroid hormone-related peptide (Mabvuure et al. 2012). In general, β-ARs would inhibit Col II and SOX6 through PKA and ERK1/2 up-regulated AP-1 factor Jun-B (Ikegami et al. 2011). The neural control of articular chondroblast and chondrocyte differentiation has not yet been characterized (see Table 3).

### Effect of catecholamines on mobilization of MSCs

In steady-state conditions, isolation of MSCs from the PB with its phenotypic and functional criteria is difficult and controversial. However, studies have demonstrated that the numbers of MSCs

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**Table 2** Transcriptions and signaling affecting on adipogenesis

<table>
<thead>
<tr>
<th>Factors</th>
<th>Mechanism</th>
<th>Effect of catecholamines on factors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/EBPs</td>
<td>Binding to C/EBPs-response element of DNA</td>
<td>Down-regulation of C/EBPs</td>
<td>Li et al. 2010a, b</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Binding to PPARγ-response element of DNA</td>
<td>Down-regulation of PPARγ</td>
<td>Li et al. 2010a, b</td>
</tr>
<tr>
<td>E2F</td>
<td>Induction of E2F pathway</td>
<td>Not known</td>
<td>Porse et al. 2001</td>
</tr>
<tr>
<td>KLF</td>
<td>Induction of PPARγ and C/EBP expression</td>
<td>Not known; while Klf has a positive effect on synthesis of TH</td>
<td>Caiazzo et al. 2011</td>
</tr>
<tr>
<td>SREBP</td>
<td>Induction of PPARγ expression</td>
<td>Up-regulation of SREBP</td>
<td>Eberle et al. 2004;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Swierczynski 2006</td>
</tr>
<tr>
<td>IRFs</td>
<td>Stimulation of promoters of key adipocyte genes</td>
<td>Not known</td>
<td>Eguchi et al. 2008</td>
</tr>
<tr>
<td>Ebf1</td>
<td>Stimulation of differentiation by activating PPARg and C/EBPs transcription</td>
<td>Up-regulation of Ebf1</td>
<td>Jimenez et al. 2007;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lecka-Czernik et al. 2012</td>
</tr>
<tr>
<td>pRb</td>
<td>Increased transcription of C/EBPβ</td>
<td>Up-regulation of pRb</td>
<td>Fajas et al. 2002;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Masur et al. 2001</td>
</tr>
<tr>
<td>BMP4</td>
<td>Stimulation of the HMGA2 expression</td>
<td>Not known; but BMP4 has a positive effect on catecholamine production</td>
<td>Markowski et al. 2011;</td>
</tr>
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<td></td>
<td></td>
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<td>Varley and Maxwell 1996</td>
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<tr>
<td>DEHP</td>
<td>Induction of the peroxisome proliferator-activated receptors α and γ (PPARαγ)</td>
<td>Not known</td>
<td>Biemann et al. 2012</td>
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<tr>
<td>GCs</td>
<td>Up-regulation of the C/EBPs by increase in PPARγ2 and aP2 expression</td>
<td>Catecholamines and GCs have a positive feedback</td>
<td>Ito et al. 2007;</td>
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<td>Udelsman et al. 1987</td>
</tr>
<tr>
<td>ROS</td>
<td>Induction of genes involved in early stage of adipogenesis</td>
<td>Not known</td>
<td>Tormos et al. 2011</td>
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</table>

*C/EBP CCAAT/enhancer-binding protein, PPARγ peroxisome proliferator-activated receptor gamma, SREBP sterol regulatory element-binding proteins, IRFs interferon regulatory factors, Ebf1 early B-cell factor 1, pRb retinoblastoma cell cycle related proteins, BMP4 bone morphogenetic proteins, DEHP bis (2-ethylhexyl) phthalate, GCs glucocorticoids, ROS reactive oxygen species*
would considerably increase in circulating blood after treatment with stimuli agents such as G-CSF, which augments mobilization and transmigration of MSCs (Cashen et al. 2007). G-CSF is a potent inducer of the mobilization of bone marrow stem cells (BMSCs) to the circulation and is the principal cytokine using current stimuli for BM transplant (Lapid et al. 2008). Studies have demonstrated that G-CSF receptors are expressed on MSCs surface. So, this could be suggested to be a direct and indirect mechanism of G-CSF-induced mobilization of MSCs (Kim et al. 2005). One of the mechanisms of G-CSF-induced mobilization of BMSCs is dependent on CXCR4–SDF1 interaction (Semerad et al. 2005). Interestingly, both CXCR4 and SDF1 are expressed in MSCs that provide an appropriate microenvironment for hematopoietic stem cells (HSCs) in BM (Gheisari et al. 2012; Nadri et al. 2007). The density gradient between CXCR4 and SDF1 could determine the recruitment of BMSCs such as MSCs to BM or into the circulation (Petit et al. 2002). Therefore, G-CSF-increased CXCR4 expression in these cells leads to their mobilization to PB containing high levels of SDF-1 released from BM (Lapid et al. 2008; Petit et al. 2002). Indeed, G-CSF increases the secretion of SDF from producing cells (Katayama et al. 2006). However, it has not yet been determined that circulating MSCs have a high expression of CXCR4 or SDF1. Growing evidence has shown that nervous systems are the main regulator of the G-CSF-induced mechanism of MSCs mobilization (Saba et al. 2013). Severe neurological abnormalities lead to dramatically reduced mobilization of BMSCs into the circulation (Saba et al. 2013; Shome et al. 2012). Furthermore, a positive feedback between treatment of G-CSF and catecholamines has recently been described (Katayama et al. 2006). Catecholamines can induce the secretion of proteolytic enzymes in BM to cleave adhesion molecules, increasing bone absorption and the effect on expression genes involved in the mobilization of BMSCs (Dygai et al. 2012; Saba et al. 2013).

Stress has a positive effect on the migration of MSCs into PB or to damaged sites and tumor microenvironments (Kawada et al. 2004). Recently, epinephrine and nor-epinephrine-induced mobilization of MSCs to sites of tumors has been determined, which promote angiogenesis and protect tumor cells (Chakroborty et al. 2009). Dopamine as another catecholamine has been reported as remarkably reducing the migration of MSCs, acting through its D2 receptors (Saba et al. 2013; Shome et al. 2012). The release of various cytokines and growth factors in damaged tissue leads to increased mobilization of MSCs from BM to the circulation and finally from there to the wound bed (Hocking and Gibran 2010). It should be noted that the migration of MSCs is dependent upon the different cytokine/receptors and catecholamines having positive or negative effects in this scenario (Table 1). However, the signaling networks that orchestrate the processes of

<table>
<thead>
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<th>Factors</th>
<th>Mechanism of mobilization</th>
<th>Effect of catecholamines on the factors</th>
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<tr>
<td>SDF1</td>
<td>Recruitment of CXCR4 MSCs</td>
<td>Down-regulation of SDF1</td>
<td>Hu et al. 2013</td>
</tr>
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<td>CXCR4</td>
<td>Departure to sites of SDF1 rich</td>
<td>Up-regulation of CXCR4</td>
<td>Yu et al. 2012</td>
</tr>
<tr>
<td>CCL25(TECK)</td>
<td>The stimulation of CXCR2 ligands and LIF-receptor/gp130 ligand</td>
<td>Not known</td>
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<td>EPO</td>
<td>Induction of SDF-1 secretion in the microenvironments of injury</td>
<td>Up-regulation of EPO</td>
<td>Richter et al. 2013; Robertson et al. 1994</td>
</tr>
<tr>
<td>Tß4</td>
<td>(1) Increased IL-8 secretion, (2) enhanced the proliferation of MSCs</td>
<td>Up-regulation of Tß4</td>
<td>Hall et al. 1988; Jeon et al. 2013</td>
</tr>
<tr>
<td>S1P</td>
<td>Homing of MSCs into tissue via S1Preceptor</td>
<td>Up-regulation of S1P</td>
<td>Jang et al. 2011</td>
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<td>G-CSF</td>
<td>Increased bone resorption</td>
<td>Up-regulation of G-CSF; G-CSF also increases catecholamine expression</td>
<td>Brouard et al. 2010; Katayama et al. 2006</td>
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<td>IGF1</td>
<td>Increased Akt/Pi3K, MEK1/2-Erk1/2 and smad2/3</td>
<td>Not-known; while IGF-1 increases catecholamine expression</td>
<td>Hwang and Choi 1995; Kumar and Ponnazhagan 2012</td>
</tr>
<tr>
<td>HGF</td>
<td>Recruitment of MSCs into tissue via HGFR</td>
<td>Catecholamines and HGF have a positive feedback</td>
<td>Lindroos et al. 1991; Maina et al. 1998; Vogel et al. 2013</td>
</tr>
<tr>
<td>VEGF</td>
<td>Recruitment of MSCs into tissue via VEGFR</td>
<td>Up-regulation of VEGF</td>
<td>Yang et al. 2006; Zisa et al. 2009</td>
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<tr>
<td>PDGF-BB</td>
<td>Stimulation of MT1-MMP expression concert with ERK1/2 and PI3K/AKT activation</td>
<td>Up-regulation of PDGF</td>
<td>Sun et al. 2013; Vashish et al. 1992</td>
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<tr>
<td>TLR-9</td>
<td>Stimulation of MMP-13 synthesis in the CpG-activated MSCs</td>
<td>Not known</td>
<td>Nurmmenniemi et al. 2010</td>
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<tr>
<td>MMP-2</td>
<td>Degradation of major constituent basement membrane</td>
<td>Up-regulation of MMP</td>
<td>Song and Li 2011; Yang et al. 2006</td>
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SDF1 Stromal-derived factor1, EPO erythropoietin, Tß4 thymosin β4, S1P sphingosine1-phosphate, HGF hepatocyte growth factor, TLR-9 toll-like receptor 9
MSCs mobilizations are unknown and many questions remain unanswered. The stimulation of the sympathetic nervous system occurs in patients with severe injuries in whom plasma catecholamine levels are significantly higher than the steady-state condition (Jeschke 2013; Ostrowski et al. 2013). In addition, catecholamines secreted from immune system cells, such as lymphocytes, macrophages and neutrophils and epithelial keratinocytes in damaged tissue can subsequently lead to further release of inflammatory cytokines (Stanojević et al. 2013). Also, cytokines involved in damaged sites support catecholamine synthesis from immune cells. Such feedback between catecholamines and cytokines could increase the expression of SDF-1 tissue injury and recruit MSCs CXCR4+ to these environments (Pullar et al. 2008). Catecholamines can have complex behaviors for mobilization of MSCs to circulation and tissue injury. Agonists of adrenergic receptors in BM lead to mobilization of stem cells including MSCs into the circulation, suggesting high levels of migration of these cells to damaged microenvironments. Also, cytokines induced by catecholamines-derived tissues would differentiate MSCs to tissue lineage cells and promote tissue repair (see Fig. 2 for details) (Pullar et al. 2008). Moreover, injection of β blockers reduces MSCs migration to sites of damage, which could be having positive and negative effects on tissue repair (Baranski et al. 2011). These blockers would remain stem cells such as MSCs in BM compartments in patients with shock and severe damage tissue; therefore, MSCs recruitment to tissue repair would decrease (Baranski et al. 2011). It should more and more be considered that the neural system can be an effective factor for mobilization in both steady-state and trauma conditions.

From nervous system research to clinical application of neural drugs

The broad advances in nervous system research has promised to practitioners in the use of neural drugs in treating disease such as bone fracture, obesity and myocardial infarction that catecholamines play pivotal roles in these disorders (Collins 2011; Elefteriou 2005; Shizukuda and Buttrick 2002). In addition, catecholamines-induced appropriate and efficient MSCs mobilization could improve the conditions of tissue repair (Shome et al. 2012). Therapeutic modulation of the nervous system may involve more than tissue selectivity and leads to significant challenges in the whole body (Duncan et al. 1985). Expression of adrenergic receptors in most cells is another challenge, causing mechanism-based side effects and different effects. As an example, β agonists affect regeneration and formation of skeletal muscles but lead to cardiomyocyte failure (Ryall et al. 2010; Shizukuda and Buttrick 2002). Thus, exploiting the therapeutic purposes of these drugs, protein kinases, TF for example, could provide information to help face challenges that might be met. As mentioned above, the main objective of catecholamines is the stimulation of PKA and MAPK pathways through β- and α-Adrs, involving a variety of specific genes (Cole and Sood 2012). Hence, adrenergic drugs would involve these pathways and achieve their therapeutic approaches (Weiss et al. 2013). The adrenergic pathway is considered as a major pathway in this line and an attractive target for developing drugs to reduce skeletal, metabolism and cartilage diseases (Weiss et al. 2013). There are, however, examples in disorders of bone, muscle, adipocytes and chondrocytes where adrenergic drugs could be met (Collins 2011; Elefteriou 2008; Lai and Mitchell 2008b). Much has been reported about using propranolol, a β-blocker, as promising new therapies for preventing bone loss in postmenopausal women with the risk of bone fractures, in astronauts exposed to long-term microgravity and in bedridden elderly patients whose bone mass may reduce (Mano et al. 2010). Moreover, β-blockers have been found that attenuate cardiac hypertrophy and improve cartilage damage (Morisco et al. 2001). Interestingly, propranolol injections to patients with solid tumors decreases MSCs migration into the tumor environment and inhibits the

**Fig. 2** The effect of catecholamine on migration of MSCs to injury sites. BM catecholamines induce the mobilization of MSCs to peripheral blood through their effects on molecular adhesion and proteolytic enzymes. Positive feedback between catecholamines and cytokines leads to increased cytokines involved in MSCs differentiation to improve damaged tissues.
supportive role of MSCs in making appropriate stroma for cancer cells (Chakroborty et al. 2009). In addition, stimulating catecholamine release or blocking norepinephrine re-uptake through drugs such as phentermine and mazindol, respectively, have some inhibitor effects on adipocytes and increased lipolysis (Ranjit et al. 2011). Despite the supportive role of catecholamines in skeletal muscle injury, nervous excitation has some stimulant effects, including elevation of blood pressure and heart rate (Duncan et al. 1985). The central induction of MSCs mobilization via catecholamine stimulators has not been identified in patients and as with other therapies, exposing patients to neural drugs will be the main challenge.

**Conclusion**

The formation of adipocytes, skeletal, chondrocyte and myocyte cells during embryogenesis and migration of MSCs involves a network of signaling that has a pivotal role in MSCs differentiation toward the diverse lineages (Guzzo et al. 2013; Linsley et al. 2013; Xinaris et al. 2013). The functional interaction of catecholamines signals is the main method of regulating distinct sets of genes in MSCs. Catecholamines have direct effects on MSCs differentiation and cells derived from MSCs (see Fig. 3). In general, we can conclude that bone, adipocyte and cartilage tissue are suppressed via the nervous system and the direct effect of catecholamines on osteoblasts, adipocyte cells and chondrocytes increases remodeling bone, lipolysis adipocytes and delay in cartilage tissue repair, respectively (Fig. 3). Catecholamines have different effects on myogenesis and MSCs mobilization and lead to muscle hypertrophy and increased MSCs migration (Sarkar et al. 2013). Thus, emerging evidence addresses all downstream involved adrenergic signals but some important questions remain to be answered. For example, is there a role for adrenergic agonists in the control of MSCs physiology? The catecholamines are definitely important for the regulation of bone formation but do all the catecholamines such as epinephrine, nor-epinephrine and dopamine play a role in MSCs differentiation into bone? Adrenergic signals appear to control adipogenesis through modulating the differentiation of MSCs toward adipocytes; however, the direct role of adrenergics on the pathogenesis of disease with high adipocytes such as aplasia in BM has not been well described. We know that most adrenergic receptors are expressed in cartilage but the precise role of catecholamines in the induction of endochondral ossification for bone formation remains unclear. Finally, there is
considerable evidence to show that stress can cooperate with other pathways that promote cancer development through targeting of MSCs migration to tumor environments that can be therapeutic alternatives but the effects of feedback of cytokines and catecholamines on tumor progression induced by migration of MSCs are not understood (Sarkar et al. 2013). These and other questions will probably be discovered in the coming years.

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