

# An update of human mesenchymal stem cell biology and their clinical uses

Walid Zaher · Linda Harkness · Abbas Jafari ·  
Moustapha Kassem

Received: 5 March 2014 / Accepted: 18 March 2014 / Published online: 2 April 2014  
© Springer-Verlag Berlin Heidelberg 2014

**Abstract** In the past decade, an increasing urge to develop new and novel methods for the treatment of degenerative diseases where there is currently no effective therapy has led to the emerging of the cell therapy or cellular therapeutics approach for the management of those conditions where organ functions are restored through transplantation of healthy and functional cells. Stem cells, because of their nature, are currently considered among the most suitable cell types for cell therapy. There are an increasing number of studies that have tested the stromal stem cell functionality both *in vitro* and *in vivo*. Consequently, stromal (mesenchymal) stem cells (MSCs) are being introduced into many clinical trials due to their ease of isolation and efficacy in treating a number of disease conditions in animal preclinical disease models. The aim of this review is to revise MSC biology, their potential translation in therapy, and the challenges facing their adaptation in clinical practice.

**Keywords** Adipogenesis · Bone · Osteogenesis · Stromal stem cells · Regenerative medicine

## Introduction

Mesenchymal stem cells (MSCs) (also known as skeletal stem cells or bone marrow stromal stem cells) are plastic adherent, non-hematopoietic cells that reside in a perivascular niche in the bone marrow stroma, that possess self-renewal and multi-lineage differentiation capacity (Bianco et al. 2001, 2006, 2013). Friedenstein et al. was the first to demonstrate that within the stromal fraction of bone marrow, there exist stem cells with the ability to create heterotopic bone and bone marrow microenvironment upon *in vivo* transplantation in mice (Friedenstein et al. 1966). In a subsequent publication, they described “bone marrow osteogenic stem cells” as fibroblast colony-forming cells that serve as common precursors for bone and cartilage formation (Friedenstein et al. 1987). The widely used name of mesenchymal stem cell was coined by Caplan et al. to describe cells responsible for bone and cartilage formation, repair and turnover during embryonic development and adulthood (Caplan 1991). However, the accuracy of the term “mesenchymal” has been debated (Bianco et al. 2013), and alternative names for the same cell population have been proposed, e.g., skeletal stem cells (SSC) or stromal stem cells.

## Isolation and definition of MSC

Traditionally, MSCs have been isolated using plastic adherence (Kassem et al. 1993). However, this method leads to growth of a heterogeneous cell population with a mixture of true stem cells as well as their committed progenitors (Kuznetsov et al. 1997). Some recent studies have attempted to isolate an MSC population based on specific criteria (Houlihan et al. 2012; Mabuchi et al. 2013)

---

W. Zaher · L. Harkness · A. Jafari · M. Kassem (✉)  
Endocrine Research (KMEB), Department of Endocrinology,  
Odense University Hospital, University of Southern Denmark,  
5000 Odense C, Denmark  
e-mail: mkassem@health.sdu.dk

W. Zaher · M. Kassem  
Stem Cell Unit, Department of Anatomy, College of Medicine,  
King Saud University, Riyadh, Saudi Arabia

A. Jafari · M. Kassem  
Danish Stem Cell Center (DanStem), Panum Institute, University  
of Copenhagen, Copenhagen, Denmark

including surface markers (Tormin et al. 2011). We have employed DNA microarrays to define a set of non-canonical MSC markers predictive for their in vivo bone-forming capacity and stemness (Larsen et al. 2010).

Mesenchymal stem cells are defined as plastic adherent cells, expressing a variety of surface markers, e.g., CD44, CD63, CD105, CD146, with the capacity for in vitro differentiation into osteoblast, adipocyte and chondrocyte. Based on these criteria, isolation of MSC-like cells has been reported from different tissues including adipose tissue, umbilical cord, dental pulp, skeletal muscle, synovium, periodontal ligament and even brain (Bianco et al. 2001; Harkness et al. 2010; Kermani et al. 2008; Lian et al. 2010; Mahmood et al. 2010, 2012; Orbay et al. 2012; Paul et al. 2012). However, as shown by our group, these MSC-like populations are not identical and exhibit differences in their molecular phenotype and differentiation responses (Al-Nbaheen et al. 2013). Currently, only bone marrow-derived MSCs have documented evidence of stemness including the ability to form bone and bone marrow organ upon serial transplantation in vivo (Sacchetti et al. 2007), although direct demonstration of these “stemness characteristics” of MSC-like populations, isolated from other tissues, is still needed.

### Regulation of MSC differentiation

MSC has a great potential for use in cellular therapeutics targeting skeletal tissue regeneration. A prerequisite for their efficient use in therapy is to identify the molecular mechanisms controlling lineage-specific differentiation. MSC lineage specification is based on activation of lineage-specific transcription factors, e.g., Runx2, PPAR $\gamma$  and Sox9 for osteoblastic, adipocytic and chondrocytic lineages, respectively. The expression and activity of these transcription factors are regulated by micro-environmental conditions that include hormonal (e.g., PTH, vitamin D3, and estrogen), growth factors (e.g., BMPs, TGF $\beta$ s, IGF), and mechanical forces (Cook and Genever 2013). These micro-environmental factors induce a number of intracellular signaling pathways that involve protein kinases that on activation mediate the effects of different stimuli on transcription factors. In addition, there is an increasing interest in the role of non-coding RNAs (e.g., miRNAs) and epigenetic mechanisms in regulating the expression and function of transcription factors that determine the differentiation fate of MSC. Here, we will update on the biology of the main transcription factors that regulate differentiation of MSCs into osteoblasts, adipocytes, and chondrocytes.

#### Osteoblast differentiation

*Runt-related transcription factor-2* (Runx2, also known as Cbfa1) is the master regulator of osteogenesis which also

has a role in hypertrophic cartilage formation (Ducy et al. 1997; Hinoi et al. 2006). During skeletal development, expression of Runx2 starts at sites of mesenchymal condensation and its expression is detectable throughout different stages of bone formation (Franceschi et al. 2007). In addition to transcriptional regulation, the role of Runx2 is controlled by post-translational modification (such as phosphorylation, acetylation) and through interactions with other nuclear co-activators and co-repressors (Franceschi et al. 2003; Huang et al. 2007; Wang et al. 2013; Xiao et al. 2000). Runx2 has been shown to be necessary and sufficient to commit mesoderm-type cells into the osteogenic lineage (Franceschi et al. 2007; Marie 2008). In vivo over-expression of Runx2 in chondrocytes leads to skeletal malformation, due to ossification of permanent cartilage (Ueta et al. 2001). Mouse fetuses with loss of Runx2 function lack calcified bones and die at birth, due to respiratory failure (Franceschi et al. 2007). Haploinsufficiency of Runx2 in humans leads to the human disease of cleidocranial dysplasia characterized by hypoplastic clavicles, open cranial fontanelles, and decreased bone mass (Huang et al. 2007). The Runx2 consensus sequence (PuACCPuCA) is present in gene promoters of the majority of osteoblastic genes such as osteopontin (OPN), bone sialoprotein (BSP), type 1 collagen alpha 1 chain (Col1a1), and osteocalcin (OC) and thus acts as an activator of the osteoblast differentiation program (Marie 2008). In addition, Runx2 plays a vital role in regulating osteoblast proliferation and survival, through regulation of cell cycle and PI3 K-Akt signaling (Fujita et al. 2004; Pratap et al. 2003).

Expression of Runx2 is regulated by several transcription factors, such as beta-catenin ( $\beta$ -catenin), msh homeobox 2 (Msx2), and distal-less homeobox 5 (Dlx5). (Huang et al. 2007). Recently, small heterodimer partner-interacting leucine zipper protein (SMILE), an orphan nuclear receptor, has been reported to physically interact with and to negatively regulate Runx2 transcriptional activity (Jang et al. 2014). The physical interaction between Runx2 and the glucocorticoid receptor leads to inhibition of Runx2 function and impaired osteogenesis which is one of the possible mechanisms through which prolonged glucocorticoid treatment induces decreased bone formation (Koromila et al. 2014). ESET, a histone methyltransferase, has been shown to interact with Runx2 and negatively regulate its transcriptional activity (Lawson et al. 2013); jumonji domain-containing 3 (Jmjd3), a histone demethylase which specifically catalyzes the removal of trimethylation of histone H3 at lysine 27 (H3K27me3), is necessary for promoter activities of Runx2 and Osterix (Yang et al. 2013); and p300/CBP-associated factor (PCAF) directly binds to and acetylates Runx2, leading to an increased in its transcriptional activity and enhanced osteogenesis (Wang et al. 2013). Interestingly, Runx2 is target for several miRNAs

(miRs). miRNA-15b promotes osteoblast differentiation by targeting Smurf1 and protecting Runx2 from Smurf1-mediated proteasomal degradation (Vimalraj et al. 2014). Loss of function of miRNA-17-92 cluster is associated with impaired bone formation and reduced expression of Runx2 in bones in miR-17-92 +/Δ mice (Zhou et al. 2013). miR-3077-5p has been shown to be responsible for the reduced level of Runx2 protein and therefore reduced osteoblast differentiation of MSC isolated from osteoporotic patients (Liao et al. 2013).

*Osterix (Osx)* is another major transcription factor that regulates osteogenesis. *Osx* is also known as specificity protein 7 (Sp7). It is a zinc-finger osteoblast-specific transcription factor. *Osterix* induces the promoter activity of osteoblast differentiation genes such as OC, Col1a1, OPN, and ALP (Huang et al. 2007; Koga et al. 2005). The presence of the Runx2 responsive element in the *Osx* promoter (Nishio et al. 2006), and normal expression of Runx2 in *Osx*-deficient mice that exhibit severely defective bone formation (Nakashima et al. 2002), indicates that *Osx* functions downstream of Runx2. Expression of chondrocyte markers (e.g., Sox9 and Col2a1) by osteoprogenitor cells of *Osx*-deficient mice suggests that Runx2-expressing osteoprogenitor cells have the potential to differentiate into either osteoblasts or chondrocytes and that *Osx* functions downstream of Runx2 to induce the bipotential osteo-chondro-progenitors to differentiate toward the osteoblastic lineage (Nakashima et al. 2002).

The function of *Osterix* is modulated by post-translational modifications such as phosphorylation and ubiquitination (Li et al. 2013a; Ortuno et al. 2010; Peng et al. 2013). Phosphorylation of *Osx* by Erk1/2 and p38 MAP kinases, and Akt increase its transcriptional activity (Choi et al. 2011b, c; Ortuno et al. 2010). In addition, glycogen synthase kinase 3 alpha (GSK3α) and calmodulin-dependent kinase II (CaMKII) enhance transcriptional activity, protein levels, and protein stability of *Osx* (Choi et al. 2013; Li et al. 2013a). In addition, interaction of *Osx* with other transcription factors such as NFTA, TFII, p300, and Brg1 promotes *Osx* activity (Sinha and Zhou 2013). Similar to Runx2, miRNAs regulate *Osx* expression. Expression of *Osx* has been shown to be negatively regulated by miR-93, 125, 135, 138, 143, 145, 214, 322, and 637 (Eskildsen et al. 2011; Gamez et al. 2013; Goettsch et al. 2011; Jia et al. 2013; Li et al. 2014; Schaap-Oziemlak et al. 2010; Shi et al. 2013; Yang et al. 2012a; Zhang et al. 2011a). Moreover, epigenetic regulation of *Osx* transcription by histone demethylases Jmjd3 and NO66 has recently been reported (Sinha et al. 2013; Yang et al. 2013). Finally, it has been shown that *Osx* binding sites are present in the promoter region of *Osx* and auto-regulation is a major mechanism by which expression of *Osx* is controlled (Barbuto and Mitchell 2013).

## Other osteoblast-associated transcriptional factors

Activator protein-1 (AP-1), β-catenin, activating transcription factor 4 (ATF4), and members of Msx/Dlx family are transcription factors that have role in regulation of osteoblast differentiation and bone formation, but their expression is not limited to skeletal tissue (Cook and Genever 2013; Marie 2008).

## Adipocyte differentiation

*Peroxisome proliferator-activated receptor-γ (PPARγ)* is known as master regulator of adipogenesis. It is a nuclear hormone receptor transcriptional factor, which is sufficient and indispensable for adipogenic differentiation of MSC (Nuttall et al. 2014; Tontonoz et al. 1994). In vitro treatment of MSC with thiazolidinediones (TZD), which are ligand agonists of PPARγ, leads to enhanced adipogenesis and inhibition of osteoblastogenesis of MSC (Gimble et al. 1996). In vivo studies involving chronic exposure of rodents to TZDs demonstrated an increase in bone marrow fat content and decreased bone mass upon treatment with several but not all thiazolidinediones (Lazarenko et al. 2007; Tornvig et al. 2001).

Among the regulators of PPARγ that are relevant to MSC biology is the canonical Wnt-β-catenin pathway which inhibits the mRNA expression of PPARγ. Non-canonical Wnt signaling activates histone methyltransferase SETDB1 that represses PPARγ transactivation through methylation of histone H3K9 of the target genes (Takada et al. 2009b). TNF-α- or IL-1-induced TAK1/TAB1/NIK signaling cascade decreases PPARγ-mediated adipogenesis by inhibiting the binding of PPARγ to the DNA response element (Takada et al. 2009a). Nocturnin (NOC), which is a nutrient-responsive gene, binds to PPARγ and increases its nuclear translocation and transcriptional activity, thereby enhancing adipogenesis (Kawai et al. 2010). Snail, a transcription factor from the zinc-finger family, inhibits the transcriptional activity of the PPARγ gene by directly binding to the E-box motifs in the PPARγ promoter (Lee et al. 2013). Sterol regulatory binding element protein-1 (SREBP1) is a transcription factor that regulates adipocyte differentiation and cholesterol homeostasis. SREBP1 positively regulates the expression of PPARγ through interaction with E-box domains in the PPARγ promoter (Fajas et al. 1999). Lipin 1, a co-regulator of transcription factors that also has phosphatidate phosphatase activity, functions as a key regulator of PPARγ activity through its ability to release co-repressors and recruit co-activators (Kim et al. 2013). Both GATA2 and GATA3 negatively regulate adipogenesis through direct binding to PPARγ (Tong et al. 2000). In addition, a number of miRNAs such as miRNA-130b and miR-20a have been shown

to negatively regulate adipogenesis by targeting PPAR $\gamma$  (Pan et al. 2013; Zhang et al. 2011b).

CAAT/enhancer binding protein- $\alpha$  (C/EBP $\alpha$ ) is another key transcription factor that is involved in regulation of adipogenesis (Samuelsson et al. 1991). Overexpression of C/EBP $\alpha$  induces adipogenesis in fibroblasts, and loss of C/EBP $\alpha$  function inhibits adipogenesis (Freytag et al. 1994; Lin and Lane 1992). C/EBPs expression is regulated by a positive feedback loop that includes PPAR $\gamma$  expression (Park et al. 2012). There are C/EBP binding sites within the promoter of PPAR $\gamma$ , and expression of PPAR $\gamma$  is thought to activate C/EBP $\alpha$  (Park et al. 2012). C/EBP homologous proteins (CHOPs) negatively regulate adipogenesis through interactions with C/EBPs (Tang and Lane 2000). In addition, the negative regulatory role of GATA2 and 3 on adipogenesis is partly mediated through formation of protein complexes with C/EBP $\alpha$  or  $\beta$  (Tong et al. 2005). It has recently been demonstrated that post-translational modification and epigenetic mechanisms have a role in the regulation of C/EBP $\alpha$  expression and function (Borengasser et al. 2013; Li et al. 2013b; Pal et al. 2013). E6AP, an E3 ubiquitin ligase, inhibits adipogenesis through ubiquitination of C/EBP $\alpha$  and targets it to ubiquitin–proteasome pathways for degradation (Pal et al. 2013). In addition, increased propensity for adipogenesis in the male offspring of the overfeeding-induced obese rats is associated with increased in vivo expression of adipogenic regulators such as C/EBP $\alpha$  and alterations in DNA methylation of CpG sites and CGI shores of developmentally important genes, including key pro-adipogenic factors (Borengasser et al. 2013). Moreover, dexamethasone-induced osteoporosis characterized by decreased bone formation and increased marrow fat is associated with inhibition of C/EBP $\alpha$  promoter methylation leading to enhanced expression of C/EBP $\alpha$  and adipogenic differentiation of MSC (Li et al. 2013b).

#### Chondrocyte differentiation

*SRY-box containing gene 9* (*Sox9*), a high-mobility-group (HMG) box containing transcription factor, is known as the master regulator of chondrogenesis. Sox9 activates the expression of chondrocyte-specific genes such as Col2a1 and Agc1 and direct concomitant positive and negative transcriptional control by SOX9 ensures differentiation phase-specific gene expression in chondrocytes (Cook and Genever 2013; Leung et al. 2011; Yamashita et al. 2012). Moreover, Sox5 and Sox6 act in redundancy with each other to robustly enhance the functions of Sox9 (Lefebvre et al. 1998). Regulation of chondrogenesis, chondrocyte proliferation, and transition to a non-mitotic hypertrophic state by Sox9 is required for development of cartilage and endochondral bone (Leung et al. 2011). Heterozygous mutations in SOX9 cause campomelic dysplasia, a severe

skeletal dysmorphology syndrome in humans characterized by a generalized hypoplasia of endochondral bones (Oh et al. 2010).

Expression and function of Sox9 are regulated through recruitment of diverse transcriptional co-activators, histone-modifying enzymes, subunits of the mediator complex, and components of the general transcriptional machinery (e.g., Med12, Med25, and CBP/p300) to the transactivation domain of Sox9 (Akiyama and Lefebvre 2011). AT-rich interactive domain 5b (Arid5b), a transcriptional co-regulator of Sox9, physically interacts with Sox9 and synergistically induces chondrogenesis by facilitating the Phf2-mediated histone demethylation of Sox9-regulated chondrogenic gene promoters (Hata et al. 2013). Suppression of Sox9 transcriptional activity by Twist1 is the mechanism by which canonical Wnt signaling inhibits chondrogenesis (Gu et al. 2012). Notch signaling negatively regulates chondrogenesis by repressing Sox9 transcription through recruitment of the Rbpj/NICD transcription complex to the Rbpj-binding sites upstream of the Sox9 promoter (Chen et al. 2013). miR-145 has been shown to be a direct regulator of SOX9 in normal healthy human articular chondrocytes (Martinez-Sanchez et al. 2012). miR-101 has role in IL-1 $\beta$ -induced chondrocyte ECM degradation by targeting 3'UTR of Sox9 (Dai et al. 2012).

#### New source for MSC: generation of MSC-like cells from human pluripotent cells

The use of bone marrow-derived MSCs in therapeutic applications has been hampered by the limited ability to obtain a sufficient number of cells as the cells undergo replicative senescence during ex vivo culture expansion (Kassem and Marie 2011; Stenderup et al. 2003). Thus, alternative sources for generating MSC-like cells with increased proliferation potential have been studied. One of the most promising cell types are pluripotent stem cells (PSCs) either from embryonic (ESC) or induced (iPSCs) sources. These cells have an unlimited proliferation capability and ability to differentiate into all cells of the body including MSC-like cells (Harkness et al. 2011; Tremoleda et al. 2008). Differentiation of human PSC toward MSC-like cells has been performed through a number of different methods including recapitulation of gastrulation-like stages via embryoid body formation (EB) (Sottile et al. 2003; Tremoleda et al. 2008); direct addition of morphogens to PSC culture media (Boyd et al. 2009; Evseenko et al. 2010); co-culture of PSC with osteoprogenitors such as OP9 cells (Barberi et al. 2005; de Peppo et al. 2010; Inanc et al. 2007); or isolation of cells spontaneously differentiated at the edges of feeder-free colonies where an epithelial-to-mesenchymal transition (EMT) takes place

**Table 1** Cell sources of osteogenic cells used in cellular therapeutics

	Embryonic stem cells	Induced pluripotent cells	Osteoblastic cells	Stromal stem cells
Potency	Pluripotent cells	Pluripotent cells	Committed cells	Multipotent cells
Source	Blastocyst inner cell mass	Somatic cells by genetic modification using a number of methodologies	Biopsies taken from bone tissue	From almost all adult tissues, including bone marrow aspirates
Advantages	Pluripotent ability to differentiate to any cell type Ability to expand cultures indefinitely	Patient specific Pluripotent ability to differentiate to any cell type Ability to expand cultures indefinitely	Differentiated cells with no need to ex vivo differentiation	Ease of isolation Ability to modulate immune responses Secretion of useful factors that enhance regeneration Stimulation of resident cells Ease of genetic modification
Disadvantages	Teratoma formation Ethical concerns	Safety concerns about the method by which iPSCs are generated Teratoma formation	Limited to bone regeneration Present in very limited numbers in the bone tissue Osteoblast proliferation is slow and expansion is difficult	Present in low numbers in bone tissue Limited capability of differentiation

(Harkness et al. 2011; Olivier and Bouhassira 2011; Trivedi and Hematti 2008). The most commonly employed methods for differentiation are via EB formation combined with addition of growth factors during culture (Mahmood et al. 2010; Schuldiner et al. 2000) or addition of growth factors and morphogens directly to PSC monolayer cultures. The later method may lack the 3-D structure and microenvironment provided by EB formation (Matsumoto et al. 2011). When PSCs are induced into MSC-like cells through coculture with differentiated osteoblastic cells, the differentiated cells provide selective micro-environmental cues conducive for lineage specification (Fengming Yue et al. 2013).

Each of the methods mentioned has been reported in a number of publications (see (Abdallah Basem et al. 2011) for review). However, during the initial differentiation period, most methods (excluding cells undergoing EMT) demonstrate a degree of cellular heterogeneity. Repeated passaging (de Peppo et al. 2010; Karp et al. 2006), cell sorting (Brown et al. 2009; Lian et al. 2007), or selective isolation methods based on adhesion to specific extracellular matrix components (Harkness et al. 2011; Liu et al. 2012) have all been used to achieve a more homogeneous populations with MSC characteristics (Harkness et al. 2011). Nevertheless, the functional ability for these cells to regenerate bone and cartilage (in preclinical animal models) needs to be fully determined.

## From basic biology to clinical applications

### Stromal stem cells therapy

Cellular therapy is an emerging field in clinical medicine aimed at using cells (and in particular stem cells) for

treatment of chronic and degenerative diseases. As can be seen in Table 1, several cell types have been suggested in clinical applications based on their phenotype and functionality. Bone marrow-derived MSCs are among the most suitable candidates for cellular therapeutics because of their ease of isolation, differentiation potential into skeletal tissues, and their excellent safety record (Lepperdinger et al. 2008) as well as their immunomodulatory and regeneration promoting properties (Nauta and Fibbe 2007; Zhao et al. 2010). MSCs have been employed in an increasing number of clinical studies for enhancing tissue regeneration following injury of both skeletal damage, e.g., bone (Gangji and Hauzeur 2005; Le Blanc et al. 2005), cartilage (Wakitani et al. 2007), and non-skeletal diseases, e.g., type I diabetes mellitus (Bhansali et al. 2009; Estrada et al. 2008), Crohn's diseases (Duijvestein et al. 2010; Liang et al. 2012), and following myocardial infarction (Chen et al. 2004; Hare et al. 2009).

The clinical use of MSC in therapy has employed both local and systemic injections. Systemic infusion of MSC for tissue repair is a clinically attractive approach and is similar to route used for hematopoietic stem cell transplantation. However, the mechanisms that govern migration of MSCs to injured tissues are still poorly understood (Karp and Leng Teo 2009). A limited degree of MSC homing to damaged tissues has been described in many preclinical studies using animal models of brain injury (Ji et al. 2004), skeletal disorders (Devine et al. 2001; Shi et al. 2007), and acute radiation syndrome (Lange et al. 2011; Yang et al. 2012b). Although human MSCs do express several chemokine receptors and adhesion molecules (Sordi et al. 2005; Wu and Zhao 2012) known to mediate homing of leukocytes to inflamed tissues (Mohle et al. 1998; Quesenberry and Becker 1998), their precise role in MSCs homing is still under investigation.

Due to the limited homing capacity of MSC to injured tissues, the positive initial clinical effects of MSC therapy are thought to be due to “humoral” factors secreted by MSC that enhance tissue regeneration. MSCs are known to secrete a plethora of autocrine and paracrine chemokines and growth factors (TGFB, TSG6, PGE2) that stimulate endogenous/resident cells, exhibit anti-apoptotic and immuno-modulatory effects as well as enhance vasculogenesis (Gnecchi et al. 2008; Mirotsoy et al. 2011). While it was reported that systemically injected MSC may get entrapped in the lungs (Bentzon et al. 2005; Schrepfer et al. 2007), their paracrine effects ensure that MSCs still exercise a positive influence through secretion of factors that exert favorable actions on distant, damaged tissues (Choi et al. 2011a). Currently, more than 3,800 stem cell-based clinical trials are registered worldwide with the NIH (USA) clinical trials database (USA 62.4 %, Europe 20.15 %, China 7.5 %, Canada 4.3 %) (Database UNIOHNCtrrar In. 2014), and the initial results of many Phase I or Phase I-II trials are encouraging.

#### Examples of the use of MSC in clinical therapy

##### *Skeletal tissue regeneration*

Regeneration of bone tissue is needed in a growing number of skeletal diseases, e.g., local non-union bone defects following tumor removal or complicated fractures. Transplantation of stem cells that are capable of bone generation in vivo is therefore an attractive and alternative approach to bone autograft or allograft techniques.

The efficacy of use of BM MSC for repair of bone defects or complicated fractures has been tested in animal models and in some phase I/II clinical trials. Bone marrow MSCs over-expressing VEGF and BMP2 were systemically administered in mice with surgically induced tibial bone defects. In mice injected with overexpressing cells, enhanced bone formation was observed and was associated with enhanced tissue vascularity at fracture site when compared with controls (Kumar et al. 2010). Similarly, murine bone marrow-derived MSCs overexpressing *Osx* were implanted in mice calvarial critical size bone defects and resulted in efficient healing (Tu et al. 2007).

In the past decade, clinical studies have employed a variety of cell types, most commonly bone marrow-derived mononuclear cells (MNC) that contain MSC in addition to other hematopoietic cells. Hernigou et al. (2005) have demonstrated that injection of an autologous bone marrow aspirate-derived MNC into the site of bone non-union fractures in 60 patients did result in bone union in the 53 (88.3 %) of treated individuals. In the seven patients that exhibited failure of bone union, a low CFU-F (fibroblastic colony-forming unit) count was observed (which a surrogate measure of the

number of MSC in the injected cells), suggesting a role of MSC and progenitor cell numbers in determining the outcome of cell therapy (Hernigou et al. 2005). In a small case series, autologous bone marrow MSCs were extracted and cultured in platelet rich plasma (PRP) followed by transplantation to sites of bone defects in individuals with achondroplasia or limb hypoplasia undergoing distraction osteogenesis for limb lengthening. Healing was observed in the treated patients with new bone formation during femoral lengthening as a consequence of the cell transplant in these patients (Kitoh et al. 2004). Also, promising preliminary results for treatment of femoral head osteonecrosis have been reported (Gangji and Hauzeur 2005; Kawate et al. 2006).

Osteoarthritis is common degenerative joint disease and among the most frequent causes of joint pain and disability. In a recent pilot study, twelve osteoarthritic patients with chronic knee pain were treated with autologous bone marrow MSCs. Culture expanded MSCs ( $40 \times 10^6$  cells) were locally administered by intra-articular injection after which patients exhibited rapid and progressive improvement of functional recovery of the joint function with improvement of cartilage quality in most of the patients (Orozco et al. 2013). In another study, the effects of local injection with either autologous BM MSC or cultured chondrocytes on disease progression were evaluated in 72 patients suffering from osteoarthritis (OA). Patients in both groups showed significant improvement in “quality of life,” but no differences could be observed between both groups (Nejadnik et al. 2010).

##### *Myocardial regeneration*

The myocardium has a limited capacity for regeneration, thus, following myocardial infarction; myocardial repair is carried out by formation of scar tissue that has negative effects on the myocardial contractility and function. Cardiovascular diseases caused by such impairment of myocardium functions leading to heart failure are among the major causes of mortality worldwide (Fuster et al. 2011). MSCs have been tested for their ability to enhance myocardial regeneration following acute myocardial infarction (AMI) or chronic ischemic heart failure (CHF). Results from studies where undifferentiated MSCs were injected with aim of regenerating the myocardium have demonstrated that engraftment and/or differentiation of the injected cells into newly generated cardiomyocytes is very limited or non-existent despite observed beneficial effects (Noiseux et al. 2006; Perez-Ilzarbe et al. 2008). Thus, it is considered that the beneficial effects of MSC in cardiac regeneration are mediated by “humoral” factors secreted by MSC that enhance tissue regeneration (Mirotsoy et al. 2011) or stimulate and activate resident cardiac stem cells (CSCs) (Hatzistergos et al. 2010).

Modification of MSC to enhance production of cytokines or growth factors, known to enhance myocardial regeneration, has also been tested. For example, rat MSCs overexpressing IGF 1 were locally injected in a rat model of acute myocardial infarction. The injected cells led to increased local production of stromal derived factor-1 (SDF-1), reduction in infarct size, and increased ejection fraction (Haider et al. 2008). Chemokine receptor type 1 (CCR1), a member of the chemokine family, was overexpressed in mouse bone marrow MSC and injected locally in a mouse model of acute myocardial infarction and led to reduced apoptosis, increased vascularity, restoration of cardiac function, and reduction in the infarct size (Huang et al. 2010). In order to enhance survival of transplanted stem cells in the myocardium, overexpression of survival-enhancing factors, e.g., VEGF (Tao et al. 2011) and Akt (Shiojima and Walsh 2006), was tried and resulted in improved survival of injected rat MSC within the tissue. When transplanted in a rat model of AMI, a significant reduction in infarct size and improved left ventricular function were observed (Mangi et al. 2003; Shujia et al. 2008). In another study, rat MSCs overexpressing survival protein B cell lymphoma 2 (Bcl-2) were injected locally (intracardiac) into a rat model of AMI. The genetically modified cells exhibited long-term survival at the infarction site and resulted in 17 % reduction in infarct size (Li et al. 2007).

In the past decade, many clinical trials utilizing a number of bone marrow-derived cell preparations (including bone marrow-derived MNC) have been conducted (reviewed in (Jeevanantham et al. 2012; Zimmet et al. 2012)). A non-randomized study evaluating the effects of repeated intracoronary BMSC infusions in 32 patients with CHF (LV ejection fraction less than 40 %) demonstrated encouraging results. These patients received BMSC infusion at baseline and after four months. Follow-up consisted of serial echocardiograms (four, eight, and twelve months) after the first intervention, measurements of the ratio of transmitral flow (E) velocity to early mitral annulus (e') velocity (E/e'), left atrial (LA) volume, and plasma levels of N-terminal pro-brain natriuretic peptide (NT-pro-BNP). During the initial treatment phase, there were no changes in main outcome but after treatment with intracoronary BMSC, a significant decrease was observed in E/e' ratio, LA volume, and plasma NT-pro-BNP. The effect was greatest in patients who received the largest amount of CD34 (+) cells (Diederichsen et al. 2010). A placebo-controlled clinical study of intra-coronary injection of autologous MSC within twelve hours after the onset of acute myocardial infarction was conducted in 69 patients. No side effects or toxicity were reported during the six month follow-up. Positive effects of increased left ventricular ejection fraction and left ventricular end diastolic volume that improve contractility and enhance infarct viability were reported (Chen et al. 2004).

Hera et al. (2009) performed a double-blind, placebo-controlled, dose-ranging (0.5, 1.6 or  $5 \times 10^6$  cells/kg) safety trial of intravenous allogeneic MSC's in 53 patients with anterior myocardial infarction. Global symptom score and ejection fraction (an estimated of left ventricular function) were significantly improved in MSC-treated group compared with controls. In another study, 33 patients with dilated cardiomyopathy underwent intracoronary infusion of BMC using balloon catheter. After 3 months of cell administration, regional wall motion of the affected myocardium and global left ventricular ejection fraction were improved. The authors reported that the increase in regional contractile function was directly related to the functionality of the infused cells as measured by their colony-forming capacity (Fischer-Rasokat et al. 2009).

#### *Graft versus host disease*

Graft versus host disease (GvHD) is a potentially fatal disease that develops as a consequence of allogeneic hematopoietic stem cell transplantation. Human bone marrow derived and adipose tissue-derived MSCs (Fang et al. 2007) were tried out for treatment of GvHD with success based on their immunoregulatory characteristics as mentioned above. In a recent study, nineteen patients suffering from chronic GVHD were treated with MSCs ( $0.6 \times 10^6$  cells/kg). Fourteen (74 %) of these patients demonstrated partial or complete responses and five patients (25 %) discontinued immunosuppressive agents (Weng et al. 2010). The 2-year survival rate was 77.7 % in this study. Clinical improvement was accompanied by the increasing ratio of CD5+CD19+/CD5-CD19+ B cells and CD8+CD28-/CD8+CD28+ T cells. No patients reported side effects from the MSC therapy (Weng et al. 2010). The beneficial effects of MSC were also observed in a phase II clinical trial of 55 children and adult patients with acute severe and steroid resistant GvHD. Intravenous infusion of autologous MSC was safe and resulted in higher survival rates in patients with complete response and significantly lower transplantation-related mortality (Le et al. 2008). In another study, MSC was employed to treat nine patients (eight patients with steroid refractory acute GVHD and one patient with chronic GVHD). MSCs obtained from either identical siblings, haploidentical donors, and HLA-mismatched donors were systematically injected and caused clinical recovery in six out of the eight patients (Ringden et al. 2006). In a recent randomized clinical trial of 32 patients with grade II-IV GvHD that either received intravenous autologous MSC ( $2$  or  $8 \times 10^6$  cells/kg) or standard therapy, 77 % of patients that received MSC transplantation exhibited complete response and no MSC infusion-related toxicities were observed (Kebriaei et al. 2009).

### MSC as therapeutic vehicle

The ability and ease of genetic modification of MSCs have encouraged their use as a vehicle for gene transfer and/or secretion of ectopic proteins. Potential transplantation of modified MSC as vehicles for secretion of therapeutic factors has been suggested in a number of studies (Porada and Almeida-Porada 2010; Sarkar et al. 2010). In a recent study, mRNA transfection was utilized to generate MSCs that simultaneously expressed P-selectin glycoprotein ligand-1 (PSGL-1), Sialyl-Lewisx (SLeX) and secreted interleukin-10 (IL-10). Using membrane dyes, these cells were tracked in vivo following systemic injection, and a rapid homing of MSCs to the site of inflammation was observed with a higher anti-inflammatory effect which significantly decreased local inflammation (Levy et al. 2013).

Heile et al. (2009) tested the efficacy of human bone marrow-derived MSC transduced with the human telomerase reverse transcriptase gene (hTERT) (hMSC-TERT) (Simonsen et al. 2002) overexpressing GLP-1 (glucagon-like peptide 1), a protein known to enhance neuronal tissue regeneration, in a rat brain injury model. By assessment of MAP-2 and GFAP expression, implanted hMSC-TERT-GLP1 cells resulted in reduction in hippocampal cell loss as well as reduction in cortical and glial defects (Heile et al. 2009; Klinge et al. 2011). Based on these promising results, a phase I trial in patients with cerebral hemorrhage that required surgery was initiated where encapsulated GLP-1-overexpressing hMSC-TERT cells were transplanted within a retrievable mesh device (described as “tea-bag” approach) into the brain following removal of bleeding. Following a treatment period of 14 days, the “tea-bag” was removed (<http://www.biocompatibles.com/media/press-releases/first-ever-treatment-of-stroke-patient-with-stem-cell-therapy-product>). The feasibility and safety of this approach have been documented in this trial, and thus, the efficacy of treatment needs to be determined.

### Concerns regarding use of stem cells in therapy

The safety record of human MSC is excellent, and during ex vivo culture expansion, the cells exhibit a stable phenotype with no risk of spontaneous transformation (Stenderup et al. 2003; Wang et al. 2012). While PSCs are an attractive source for generating a large number of phenotypically stable cells suitable for therapy, there are a number of safety aspects relating to the use of human PSC and their derivatives in clinical applications that also apply to MSC. One of these is immune rejection. While autologous transplantation from MSC remains the safest method, allogeneic MSC

transplantation is also possible since MSC exhibit immune suppressive properties (De Miguel et al. 2012; Le Blanc and Ringden 2007). The recent development of patient specific iPSC allows the generation of cells compatible with the donor; and thus, generation and expansion prior to transplantation become less of an issue.

Another important concern is the risk for malignant transformation of the transplanted stem cells. As mentioned above, MSCs do not transform during in vitro culture and no history of development of cancer has been reported following administration of MSC in patients. Conversely, PSCs have a propensity for forming tumors when implanted as undifferentiated cells in vivo and thus the contamination of differentiated PSC with undifferentiated cells may pose a risk (Miura et al. 2009). However, it is currently unknown how many (or few) undifferentiated PSC would be acceptable within cells for clinical transplantation. Hentze et al. (2009) reported a detection limit of 1:4,000 for tumor formation when injecting single cells into immune compromised mice. However, the current ability to form tumors in vivo depends more on the strain of mice used (degree of immunocompromisation) (Quintana et al. 2008) than other factors such as site of injection (Cunningham et al. 2012), and thus, this method may not be predictive for the behavior of the transplanted cells in vivo.

Development of assays that screen stem cells for their safety prior to their transplantation is being developed. Demonstration of genetic alterations in cultured cell suggests a malignant transformation potential (Bentivegna et al. 2013; Nospikel 2013). Two routine procedures have been utilized to assess chromosomal and genetic abnormalities: karyotyping and identification of gross morphological changes (such as acquisition, deletion, or inversions) using G-banded karyotyping or the creation of a virtual karyotype using single nucleotide polymorphism (SNP) or comparative genomic hybridization (CGH), (Hagenkord et al. 2008). We have recently demonstrated the possible use of noninvasive Raman spectroscopy (Harkness et al. 2012) as a method for detecting transformed cells among cultured cells.

Several techniques have been proposed to eliminate undifferentiated PSC prior to transplantation. These include selective apoptosis of PSC (Bieberich et al. 2004), removal of PSC through flow cytometry (Schriebl et al. 2012) or through mechanical removal (Tang et al. 2012). It would be more efficient if the differentiation protocols utilized were robust enough to induce a homogeneous cell type without pluripotent cells remaining.

**Acknowledgments** The work was supported by a grant from the University hospital of Odense, Odense, Denmark and KACST, (Project Code: 10-BIO1308-02) (KSA). The funders had no role in preparation of the manuscript.



## References

- Abdallah Basem M, Harkness L, Mahmood A, Kassem M (2011) Direct differentiation of human embryonic stem cells toward osteoblasts and chondrocytes through an intermediate mesenchyme progenitor lineage. In: Atwood C (ed) *Embryonic stem cells: the hormonal regulation of pluripotency and embryogenesis*, InTech Europe, Rijeka, Croatia, p 607–618. <http://www.intechopen.com>
- Akiyama H, Lefebvre V (2011) Unraveling the transcriptional regulatory machinery in chondrogenesis. *J Bone Miner Metab* 29(4):390–395. doi:10.1007/s00774-011-0273-9
- Al-Nbaheen M, Vishnubalaji R, Ali D et al (2013) Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. *Stem Cell Rev* 9(1):32–43. doi:10.1007/s12015-012-9365-8
- Barberi T, Willis LM, Socci ND, Studer L (2005) Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS Med* 2(6):e161
- Barbuto R, Mitchell J (2013) Regulation of the osterix (Osx, Sp7) promoter by osterix and its inhibition by parathyroid hormone. *J Mol Endocrinol* 51(1):99–108. doi:10.1530/JME-12-0251
- Bentivegna AMM, Riva G, Foudah D, Butta V, Dalprà L, Tredici G (2013) DNA methylation changes during in vitro propagation of human mesenchymal stem cells: implications for their genomic stability? *Stem Cells Int* 2013:192425. doi:10.1155/2013/192425
- Bentzon JF, Stenderup K, Hansen FD et al (2005) Tissue distribution and engraftment of human mesenchymal stem cells immortalized by human telomerase reverse transcriptase gene. *Biochem Biophys Res Commun* 330(3):633–640. doi:10.1016/j.bbrc.2005.03.072
- Bhansali A, Upreti V, Khandelwal N et al (2009) Efficacy of autologous bone marrow-derived stem cell transplantation in patients with type 2 diabetes mellitus. *Stem Cells Dev* 18(10):1407–1416. doi:10.1089/scd.2009.0164
- Bianco P, Riminucci M, Gronthos S, Robey PG (2001) Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells* 19(3):180–192
- Bianco P, Kuznetsov SA, Riminucci M, Gehron RP (2006) Postnatal skeletal stem cells. *Methods Enzymol* 419:117–148
- Bianco P, Cao X, Frenette PS et al (2013) The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. *Nat Med* 19(1):35–42. doi:10.1038/nm.3028
- Bieberich E, Silva J, Wang G, Krishnamurthy K, Condie BG (2004) Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. *J Cell Biol* 167(4):723–734
- Borengasser SJ, Zhong Y, Kang P et al (2013) Maternal obesity enhances white adipose tissue differentiation and alters genome-scale DNA methylation in male rat offspring. *Endocrinology* 154(11):4113–4125. doi:10.1210/en.2012-2255
- Boyd NL, Robbins KR, Dhara SK, West FD, Stice SL (2009) Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells. *Tissue Eng Part A* 15(8):1897–1907
- Brown SE, Tong W, Krebsbach PH (2009) The derivation of mesenchymal stem cells from human embryonic stem cells. *Cells Tissues Organs* 189(1–4):256–260
- Caplan AI (1991) Mesenchymal stem cells. *J Orthop Res Off Publ Orthop Res Soc* 9(5):641–650. doi:10.1002/jor.1100090504
- Chen SL, Fang WW, Ye F et al (2004) Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 94(1):92–95
- Chen S, Tao J, Bae Y et al (2013) Notch gain of function inhibits chondrocyte differentiation via Rbpj-dependent suppression of Sox9. *J Bone Miner Res Off J Am Soc Bone Miner Res* 28(3):649–659. doi:10.1002/jbmr.1770
- Choi H, Lee RH, Bazhanov N, Oh JY, Prockop DJ (2011a) Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-kappaB signaling in resident macrophages. *Blood* 118(2):330–338. doi:10.1182/blood-2010-12-327353
- Choi YH, Gu YM, Oh JW, Lee KY (2011b) Osterix is regulated by Erk1/2 during osteoblast differentiation. *Biochem Biophys Res Commun* 415(3):472–478. doi:10.1016/j.bbrc.2011.10.097
- Choi YH, Jeong HM, Jin YH, Li H, Yeo CY, Lee KY (2011c) Akt phosphorylates and regulates the osteogenic activity of Osterix. *Biochem Biophys Res Commun* 411(3):637–641. doi:10.1016/j.bbrc.2011.07.009
- Choi YH, Choi JH, Oh JW, Lee KY (2013) Calmodulin-dependent kinase II regulates osteoblast differentiation through regulation of Osterix. *Biochem Biophys Res Commun* 432(2):248–255. doi:10.1016/j.bbrc.2013.02.005
- Cook D, Genever P (2013) Regulation of mesenchymal stem cell differentiation. *Adv Exp Med Biol* 786:213–229. doi:10.1007/978-94-007-6621-1\_12
- Cunningham JJ, Ulbright TM, Pera MF, Looijenga LHJ (2012) Lessons from human teratomas to guide development of safe stem cell therapies. *Nat Biotech* 30(9):849–857
- Dai L, Zhang X, Hu X, Zhou C, Ao Y (2012) Silencing of microRNA-101 prevents IL-1beta-induced extracellular matrix degradation in chondrocytes. *Arthritis Res Ther* 14(6):R268. doi:10.1186/ar4114
- Database UNIOHNCtrrar In. (2014) <http://clinicaltrials.gov/> Accessed 6 February 2014
- De Miguel MP, F-JS Blázquez-Martínez A, Pascual CY, Aller MA, Arias J, Arnalich-Montiel F (2012) Immunosuppressive properties of mesenchymal stem cells: advances and applications. *Curr Mol Med* 12(5):17. doi:10.2174/156652412800619950
- de Peppo GM, Sjoval P, Lennerås M, et al (2010) osteogenic potential of human mesenchymal stem cells and human embryonic stem cell-derived mesodermal progenitors: a tissue engineering perspective. *Tissue Eng Part A* 16(11). doi: 10.1089/ten.tea.2010.0052
- Devine SM, Bartholomew AM, Mahmud N et al (2001) Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Exp Hematol* 29(2):244–255
- Diederichsen AC, Moller JE, Thyssen P et al (2010) Changes in left ventricular filling patterns after repeated injection of autologous bone marrow cells in heart failure patients. *Scand Cardiovasc J* 44(3):139–145. doi:10.3109/14017430903556294
- Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G (1997) *Osf2/Cbfa1*: a transcriptional activator of osteoblast differentiation. *Cell* 89(5):747–754
- Duijvestein M, Vos AC, Roelofs H et al (2010) Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. *Gut* 59(12):1662–1669. doi:10.1136/gut.2010.215152
- Eskildsen T, Taipaleenmaki H, Stenvang J et al (2011) MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. *Proc Natl Acad Sci USA* 108(15):6139–6144. doi:10.1073/pnas.1016758108
- Estrada EJ, Valacchi F, Nicora E et al (2008) Combined treatment of intrapancreatic autologous bone marrow stem cells and hyperbaric oxygen in type 2 diabetes mellitus. *Cell Transpl* 17(12):1295–1304

- Evseenko D, Zhu Y, Schenke-Layland K et al (2010) Mapping the first stages of mesoderm commitment during differentiation of human embryonic stem cells. *Proc Natl Acad Sci* 107(31):13742–13747
- Fajas L, Schoonjans K, Gelman L et al (1999) Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol Cell Biol* 19(8):5495–5503
- Fang B, Song Y, Lin Q et al (2007) Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. *Pediatr Transpl* 11(7):814–817
- Fengming Yue SS, Ichikawa H, Yoshie S, Akimi Mogi SM, Nagai M, Yokohama T, Sasaki TDaK (2013) Induce differentiation of embryonic stem cells by co-culture system. In: Andrades PJA (ed) *Regenerative medicine and tissue engineering*. InTech Europe, Rijeka, Croatia, p 117–139. <http://www.intechopen.com>
- Fischer-Rasokat U, Assmus B, Seeger FH et al (2009) A pilot trial to assess potential effects of selective intracoronary bone marrow-derived progenitor cell infusion in patients with nonischemic dilated cardiomyopathy: final 1-year results of the transplantation of progenitor cells and functional regeneration enhancement pilot trial in patients with nonischemic dilated cardiomyopathy. *Circ Heart Fail* 2(5):417–423. doi:10.1161/CIRCHEARTFAILURE.109.855023
- Franceschi RT, Xiao G, Jiang D, Gopalakrishnan R, Yang S, Reith E (2003) Multiple signaling pathways converge on the Cbfa1/Runx2 transcription factor to regulate osteoblast differentiation. *Connect Tissue Res* 44(Suppl 1):109–116
- Franceschi RT, Ge C, Xiao G, Roca H, Jiang D (2007) Transcriptional regulation of osteoblasts. *Ann N Y Acad Sci* 1116:196–207. doi:10.1196/annals.1402.081
- Freytag SO, Paielli DL, Gilbert JD (1994) Ectopic expression of the CCAAT/enhancer-binding protein alpha promotes the adipogenic program in a variety of mouse fibroblastic cells. *Genes Dev* 8(14):1654–1663
- Friedenstein AJ, Piatetzky S II, Petrakova KV (1966) Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* 16(3):381–390
- Friedenstein AJ, Chailakhyan RK, Gerasimov UV (1987) Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. *Cell Tissue Kinet* 20(3):263–272
- Fujita T, Azuma Y, Fukuyama R et al (2004) Runx2 induces osteoblast and chondrocyte differentiation and enhances their migration by coupling with PI3 K-Akt signaling. *J Cell Biol* 166(1):85–95. doi:10.1083/jcb.200401138
- Fuster V, Kelly BB, Vedanthan R (2011) Global cardiovascular health: urgent need for an intersectoral approach. *J Am Coll Cardiol* 58(12):1208–1210
- Gamez B, Rodriguez-Carballo E, Bartrons R, Rosa JL, Ventura F (2013) MicroRNA-322 (miR-322) and its target protein Tob2 modulate Osterix (Osx) mRNA stability. *J Biol Chem* 288(20):14264–14275. doi:10.1074/jbc.M112.432104
- Gangji V, Hauzeur JP (2005) Treatment of osteonecrosis of the femoral head with implantation of autologous bone-marrow cells. Surgical technique. *J Bone Joint Surg Am* 87(Suppl 1 Pt 1):106–112. doi:10.2106/JBJS.D.02662
- Gimble JM, Robinson CE, Wu X et al (1996) Peroxisome proliferator-activated receptor-gamma activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. *Mol Pharmacol* 50(5):1087–1094
- Gnecchi M, Zhang Z, Ni A, Dzau VJ (2008) Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res* 103(11):1204–1219. doi:10.1161/CIRCRESAHA.108.176826
- Goettsch C, Rauner M, Pacyna N, Hempel U, Bornstein SR, Hofbauer LC (2011) miR-125b regulates calcification of vascular smooth muscle cells. *Am J Pathol* 179(4):1594–1600. doi:10.1016/j.ajpath.2011.06.016
- Gu S, Boyer TG, Naski MC (2012) Basic helix-loop-helix transcription factor Twist1 inhibits transactivator function of master chondrogenic regulator Sox9. *J Biol Chem* 287(25):21082–21092. doi:10.1074/jbc.M111.328567
- Hagenkord J, Parwani A, Lyons-Weiler M et al (2008) Virtual karyotyping with SNP microarrays reduces uncertainty in the diagnosis of renal epithelial tumors. *Diagn Pathol* 3(1):44
- Haider HK, Jiang S, Idris NM, Ashraf M (2008) IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. *Circ Res* 103(11):1300–1308
- Hare JM, Traverse JH, Henry TD et al (2009) A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol* 54(24):2277–2286
- Harkness L, Mahmood A, Ditzel N, Abdallah BM, Nygaard JV, Kassem M (2010) Selective isolation and differentiation of a stromal population of human embryonic stem cells with osteogenic potential. *Bone* 48(2):231–241. doi:10.1016/j.bone.2010.09.023
- Harkness L, Mahmood A, Ditzel N, Abdallah BM, Nygaard JV, Kassem M (2011) Selective isolation and differentiation of a stromal population of human embryonic stem cells with osteogenic potential. *Bone* 48(2):231–241
- Harkness LNS, Beermann J, Bozhevolnyi SI, Kassem M (2012) Identification of abnormal stem cells using Raman spectroscopy. *Stem Cells Dev* 21(12):8. doi:10.1089/scd.2011.0600
- Hata K, Takashima R, Amano K et al (2013) Arid5b facilitates chondrogenesis by recruiting the histone demethylase Phf2 to Sox9-regulated genes. *Nat Commun* 4:2850. doi:10.1038/ncomms3850
- Hatzistergos KE, Quevedo H, Oskoue BN et al (2010) Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res* 107(7):913–922. doi:10.1161/CIRCRESAHA.110.222703
- Heile AM, Wallrapp C, Klinge PM et al (2009) Cerebral transplantation of encapsulated mesenchymal stem cells improves cellular pathology after experimental traumatic brain injury. *Neurosci Lett* 463(3):176–181
- Hentze H, Soong PL, Wang ST, Phillips BW, Putti TC, Dunn NR (2009) Teratoma formation by human embryonic stem cells: evaluation of essential parameters for future safety studies. *Stem Cell Res* 2(3):198–210. doi:10.1016/j.scr.2009.02.002
- Hernigou P, Poignard A, Beaujean F, Rouard H (2005) Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *J Bone Joint Surg Am* 87(7):1430–1437
- Hinoi E, Bialek P, Chen YT et al (2006) Runx2 inhibits chondrocyte proliferation and hypertrophy through its expression in the perichondrium. *Genes Dev* 20(21):2937–2942. doi:10.1101/gad.1482906
- Houlihan DD, Mabuchi Y, Morikawa S et al (2012) Isolation of mouse mesenchymal stem cells on the basis of expression of Sca-1 and PDGFR- $\alpha$ . *Nat Protoc* 7(12):2103–2111
- Huang W, Yang S, Shao J, Li YP (2007) Signaling and transcriptional regulation in osteoblast commitment and differentiation. *Front Biosci* 12:3068–3092
- Huang J, Zhang Z, Guo J et al (2010) Genetic modification of mesenchymal stem cells overexpressing CCR1 increases cell viability, migration, engraftment, and capillary density in the injured myocardium. *Circ Res* 106(11):1753–1762
- Inanc B, Elcin AE, Elcin YM (2007) Effect of osteogenic induction on the in vitro differentiation of human embryonic stem cells

- cocultured with periodontal ligament fibroblasts. *Artif Organs* 31(11):792–800
- Jang H, Kim EJ, Park JK et al (2014) SMILE inhibits BMP-2-induced expression of osteocalcin by suppressing the activity of the RUNX2 transcription factor in MC3T3E1 cells. *Bone*. doi:[10.1016/j.bone.2013.12.028](https://doi.org/10.1016/j.bone.2013.12.028)
- Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B (2012) Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. *Circulation* 126(5):551–568. doi:[10.1161/CIRCULATIONAHA.111.086074](https://doi.org/10.1161/CIRCULATIONAHA.111.086074)
- Ji JF, He BP, Dheen ST, Tay SS (2004) Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. *Stem Cells* 22(3):415–427
- Jia J, Tian Q, Ling S, Liu Y, Yang S, Shao Z (2013) miR-145 suppresses osteogenic differentiation by targeting Sp7. *FEBS Lett* 587(18):3027–3031. doi:[10.1016/j.febslet.2013.07.030](https://doi.org/10.1016/j.febslet.2013.07.030)
- Karp JM, Leng Teo GS (2009) Mesenchymal stem cell homing: the devil is in the details. *Cell Stem Cell* 4(3):206–216
- Karp JM, Ferreira LS, Khademhosseini A, Kwon AH, Yeh J, Langer RS (2006) Cultivation of human embryonic stem cells without the embryoid body step enhances osteogenesis in vitro. *Stem Cells* 24(4):835–843
- Kassem M, Marie PJ (2011) Senescence-associated intrinsic mechanisms of osteoblast dysfunction. *Aging Cell* 10(2):191–197
- Kassem M, Mosekilde L, Eriksen EF (1993) 1,25-dihydroxyvitamin D3 potentiates fluoride-stimulated collagen type I production in cultures of human bone marrow stromal osteoblast-like cells. *J Bone Miner Res* 8(12):1453–1458
- Kawai M, Green CB, Lecka-Czernik B et al (2010) A circadian-regulated gene, Nocturnin, promotes adipogenesis by stimulating PPAR-gamma nuclear translocation. *Proc Natl Acad Sci USA* 107(23):10508–10513. doi:[10.1073/pnas.1000788107](https://doi.org/10.1073/pnas.1000788107)
- Kawate K, Yajima H, Ohgushi H et al (2006) Tissue-engineered approach for the treatment of steroid-induced osteonecrosis of the femoral head: transplantation of autologous mesenchymal stem cells cultured with beta-tricalcium phosphate ceramics and free vascularized fibula. *Artif Organs* 30(12):960–962
- Kebrlari P, Isola L, Bahceci E et al (2009) Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biol Blood Marrow Transpl* 15(7):804–811
- Kermani AJ, Fathi F, Mowla SJ (2008) Characterization and genetic manipulation of human umbilical cord vein mesenchymal stem cells: potential application in cell-based gene therapy. *Rejuvenation Res* 11(2):379–386. doi:[10.1089/rej.2008.0674](https://doi.org/10.1089/rej.2008.0674)
- Kim HE, Bae E, Jeong DY et al (2013) Lipin1 regulates PPARgamma transcriptional activity. *Biochem J* 453(1):49–60. doi:[10.1042/BJ20121598](https://doi.org/10.1042/BJ20121598)
- Kitoh H, Kitakoji T, Tsuchiya H et al (2004) Transplantation of marrow-derived mesenchymal stem cells and platelet-rich plasma during distraction osteogenesis—a preliminary result of three cases. *Bone* 35(4):892–898. doi:[10.1016/j.bone.2004.06.013](https://doi.org/10.1016/j.bone.2004.06.013)
- Klinge PM, Harmening K, Miller MC et al (2011) Encapsulated native and glucagon-like peptide-1 transfected human mesenchymal stem cells in a transgenic mouse model of Alzheimer's disease. *Neurosci Lett* 497(1):6–10
- Koga T, Matsui Y, Asagiri M et al (2005) NFAT and Osterix cooperatively regulate bone formation. *Nat Med* 11(8):880–885. doi:[10.1038/nm1270](https://doi.org/10.1038/nm1270)
- Koromila T, Baniwal SK, Song YS, Martin A, Xiong J, Frenkel B (2014) Glucocorticoids antagonize RUNX2 during osteoblast differentiation in cultures of ST2 pluripotent mesenchymal cells. *J Cell Biochem* 115(1):27–33. doi:[10.1002/jcb.24646](https://doi.org/10.1002/jcb.24646)
- Kumar S, Wan C, Ramaswamy G, Clemens TL, Ponnazhagan S (2010) Mesenchymal stem cells expressing osteogenic and angiogenic factors synergistically enhance bone formation in a mouse model of segmental bone defect. *Mol Ther* 18(5):1026–1034
- Kuznetsov SA, Krebsbach PH, Satomura K et al (1997) Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. *J Bone Miner Res Off J Am Soc Bone Miner Res* 12(9):1335–1347
- Lange C, Brunswig-Spickenheier B, Cappallo-Obermann H et al (2011) Radiation rescue: mesenchymal stromal cells protect from lethal irradiation. *PLoS One* 6(1):e14486
- Larsen KH, Frederiksen CM, Burns JS, Abdallah BM, Kassem M (2010) Identifying a molecular phenotype for bone marrow stromal cells with in vivo bone-forming capacity. *J Bone Miner Res* 25(4):796–808. doi:[10.1359/jbmr.091018](https://doi.org/10.1359/jbmr.091018)
- Lawson KA, Teteak CJ, Gao J et al (2013) ESET histone methyltransferase regulates osteoblastic differentiation of mesenchymal stem cells during postnatal bone development. *FEBS Lett* 587(24):3961–3967. doi:[10.1016/j.febslet.2013.10.028](https://doi.org/10.1016/j.febslet.2013.10.028)
- Lazarenko OP, Rzonca SO, Hogue WR, Swain FL, Lecka-Czernik B (2007) Rosiglitazone induces decreases in bone mass and strength that are reminiscent of aged bone. *Endocrinology* 148(6):2669–2680. doi:[10.1210/en.2006-1587](https://doi.org/10.1210/en.2006-1587)
- Le Blanc K, Ringden O (2007) Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med* 262(5):509–525
- Le Blanc K, Gotherstrom C, Ringden O et al (2005) Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 79(11):1607–1614
- Le BK, Frassoni F, Ball L et al (2008) Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 371(9624):1579–1586
- Lee YH, Kim SH, Lee YJ et al (2013) Transcription factor Snail is a novel regulator of adipocyte differentiation via inhibiting the expression of peroxisome proliferator-activated receptor gamma. *Cell Mol Life Sci* 70(20):3959–3971. doi:[10.1007/s00018-013-1363-8](https://doi.org/10.1007/s00018-013-1363-8)
- Lefebvre V, Li P, de Crombrugge B (1998) A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. *EMBO J* 17(19):5718–5733. doi:[10.1093/emboj/17.19.5718](https://doi.org/10.1093/emboj/17.19.5718)
- Lepperdinger G, Brunauer R, Jamnig A, Laschober G, Kassem M (2008) Controversial issue: is it safe to employ mesenchymal stem cells in cell-based therapies? *Exp Gerontol* 43(11):1018–1023
- Leung VY, Gao B, Leung KK et al (2011) SOX9 governs differentiation stage-specific gene expression in growth plate chondrocytes via direct concomitant transactivation and repression. *PLoS Genet* 7(11):e1002356. doi:[10.1371/journal.pgen.1002356](https://doi.org/10.1371/journal.pgen.1002356)
- Levy O, Zhao W, Mortensen LJ et al (2013) mRNA-engineered mesenchymal stem cells for targeted delivery of interleukin-10 to sites of inflammation. *Blood*. doi:[10.1182/blood-2013-04-495119](https://doi.org/10.1182/blood-2013-04-495119)
- Li W, Ma N, Ong LL et al (2007) Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. *Stem Cells* 25(8):2118–2127
- Li H, Jeong HM, Choi YH et al (2013a) Glycogen synthase kinase 3 alpha phosphorylates and regulates the osteogenic activity of Osterix. *Biochem Biophys Res Commun* 434(3):653–658. doi:[10.1016/j.bbrc.2013.03.137](https://doi.org/10.1016/j.bbrc.2013.03.137)
- Li J, Zhang N, Huang X et al (2013b) Dexamethasone shifts bone marrow stromal cells from osteoblasts to adipocytes by C/EBPalpha promoter methylation. *Cell Death Dis* 4:e832. doi:[10.1038/cddis.2013.348](https://doi.org/10.1038/cddis.2013.348)

- Li E, Zhang J, Yuan T, Ma B (2014) miR-143 suppresses osteogenic differentiation by targeting Osterix. *Mol Cell Biochem*. doi:[10.1007/s11010-013-1957-3](https://doi.org/10.1007/s11010-013-1957-3)
- Lian Q, Lye E, Suan Yeo K et al (2007) Derivation of clinically compliant MSCs from CD105 + , CD24 – differentiated human ESCs. *Stem Cells* 25(2):425–436
- Lian Q, Zhang Y, Zhang J et al (2010) Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. *Circulation* 121(9):1113–1123. doi:[10.1161/CIRCULATIONAHA.109.898312](https://doi.org/10.1161/CIRCULATIONAHA.109.898312)
- Liang J, Zhang H, Wang D et al (2012) Allogeneic mesenchymal stem cell transplantation in seven patients with refractory inflammatory bowel disease. *Gut* 61(3):468–469. doi:[10.1136/gut.jnl-2011-300083](https://doi.org/10.1136/gut.jnl-2011-300083)
- Liao L, Yang X, Su X et al (2013) Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow. *Cell Death Dis* 4:e600. doi:[10.1038/cddis.2013.130](https://doi.org/10.1038/cddis.2013.130)
- Lin FT, Lane MD (1992) Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. *Genes Dev* 6(4):533–544
- Liu Y, Goldberg AJ, Dennis JE, Gronowicz GA, Kuhn LT (2012) One-Step derivation of mesenchymal stem cell (MSC)-like cells from human pluripotent stem cells on a fibrillar collagen coating. *PLoS One* 7(3):e33225. doi:[10.1371/journal.pone.0033225](https://doi.org/10.1371/journal.pone.0033225)
- Mabuchi Y, Houlihan DD, Akazawa C, Okano H, Matsuzaki Y (2013) Prospective isolation of murine and human bone marrow mesenchymal stem cells based on surface markers. *Stem Cells Int* 2013:507301. doi:[10.1155/2013/507301](https://doi.org/10.1155/2013/507301)
- Mahmood A, Harkness L, Schroder HD, Abdallah BM, Kassem M (2010) Enhanced differentiation of human embryonic stem cells to mesenchymal progenitors by inhibition of TGF-beta/activin/nodal signaling using SB-431542. *J Bone Miner Res* 25(6):1216–1233. doi:[10.1002/jbmr.34](https://doi.org/10.1002/jbmr.34)
- Mahmood A, Harkness L, Abdallah BM et al (2012) Derivation of stromal (skeletal and mesenchymal) stem-like cells from human embryonic stem cells. *Stem Cells Dev* 21(17):3114–3124. doi:[10.1089/scd.2012.0035](https://doi.org/10.1089/scd.2012.0035)
- Mangi AA, Noiseux N, Kong D et al (2003) Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med* 9(9):1195–1201
- Marie PJ (2008) Transcription factors controlling osteoblastogenesis. *Arch Biochem Biophys* 473(2):98–105. doi:[10.1016/j.abb.2008.02.030](https://doi.org/10.1016/j.abb.2008.02.030)
- Martinez-Sanchez A, Dudek KA, Murphy CL (2012) Regulation of human chondrocyte function through direct inhibition of cartilage master regulator SOX9 by microRNA-145 (miRNA-145). *J Biol Chem* 287(2):916–924. doi:[10.1074/jbc.M111.302430](https://doi.org/10.1074/jbc.M111.302430)
- Matsumoto Y, Iwasaki H, Suda T (2011) Maintenance of adult stem cells: role of the stem cell niche. In: Phinney DG (ed) *Adult stem cells: biology and methods of analysis*. Stem cell biology and regenerative medicine. Springer, Berlin, pp 35–55
- Mirotsov M, Jayawardena TM, Schmeckpeper J, Gneccchi M, Dzau VJ (2011) Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *J Mol Cell Cardiol* 50(2):280–289. doi:[10.1016/j.yjmcc.2010.08.005](https://doi.org/10.1016/j.yjmcc.2010.08.005)
- Miura K, Okada Y, Aoi T, et al (2009) Variation in the safety of induced pluripotent stem cell lines. *Nat Biotech* 27(8):743–745 [http://www.nature.com/nbt/journal/v27/n8/suppinfo/nbt.1554\\_S1.html](http://www.nature.com/nbt/journal/v27/n8/suppinfo/nbt.1554_S1.html)
- Mohle R, Bautz F, Rafii S, Moore MA, Brugger W, Kanz L (1998) The chemokine receptor CXCR-4 is expressed on CD34 + hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. *Blood* 91(12):4523–4530
- Nakashima K, Zhou X, Kunkel G et al (2002) The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell* 108(1):17–29
- Nauta AJ, Fibbe WE (2007) Immunomodulatory properties of mesenchymal stromal cells. *Blood* 110(10):3499–3506. doi:[10.1182/blood-2007-02-069716](https://doi.org/10.1182/blood-2007-02-069716)
- Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH (2010) Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med* 38(6):1110–1116. doi:[10.1177/0363546509359067](https://doi.org/10.1177/0363546509359067)
- Nishio Y, Dong Y, Paris M, O'Keefe RJ, Schwarz EM, Drissi H (2006) Runx2-mediated regulation of the zinc finger Osterix/Sp7 gene. *Gene* 372:62–70. doi:[10.1016/j.gene.2005.12.022](https://doi.org/10.1016/j.gene.2005.12.022)
- Noiseux N, Gneccchi M, Lopez-Illasaca M et al (2006) Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol Ther J Am Soc Gene Ther* 14(6):840–850. doi:[10.1016/j.ymthe.2006.05.016](https://doi.org/10.1016/j.ymthe.2006.05.016)
- Nouspikel T (2013) Genetic instability in human embryonic stem cells: prospects and caveats. *Future Oncol* 9(6):867–877. doi:[10.2217/fon.13.22](https://doi.org/10.2217/fon.13.22)
- Nuttall ME, Shah F, Singh V, Thomas-Porch C, Frazier T, Gimble JM (2014) Adipocytes and the regulation of bone remodeling: a balancing act. *Calcif Tissue Int* 94(1):78–87. doi:[10.1007/s00223-013-9807-6](https://doi.org/10.1007/s00223-013-9807-6)
- Oh CD, Maity SN, Lu JF et al (2010) Identification of SOX9 interaction sites in the genome of chondrocytes. *PLoS One* 5(4):e10113. doi:[10.1371/journal.pone.0010113](https://doi.org/10.1371/journal.pone.0010113)
- Olivier E, Bouhassira E (2011) Differentiation of human embryonic stem cells into mesenchymal stem cells by the raclure method. In: Nieden NI (ed) *Embryonic stem cell therapy for Osteodegenerative diseases*. Methods in molecular biology, vol 690. Humana, Clifton, pp 183–193
- Orbay H, Tobita M, Mizuno H (2012) Mesenchymal stem cells isolated from adipose and other tissues: basic biological properties and clinical applications. *Stem cells Int* 461718. doi:[10.1155/2012/461718](https://doi.org/10.1155/2012/461718)
- Orozco L, Munar A, Soler R et al (2013) Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. *Transplantation* 95(12):1535–1541. doi:[10.1097/TP.0b013e318291a2da](https://doi.org/10.1097/TP.0b013e318291a2da)
- Ortuno MJ, Ruiz-Gaspa S, Rodriguez-Carballo E et al (2010) p38 regulates expression of osteoblast-specific genes by phosphorylation of osterix. *J Biol Chem* 285(42):31985–31994. doi:[10.1074/jbc.M110.123612](https://doi.org/10.1074/jbc.M110.123612)
- Pal P, Lochab S, Kanaujiya JK et al (2013) E3 ubiquitin ligase E6AP negatively regulates adipogenesis by downregulating proadipogenic factor C/EBPalpha. *PLoS One* 8(6):e65330. doi:[10.1371/journal.pone.0065330](https://doi.org/10.1371/journal.pone.0065330)
- Pan S, Yang X, Jia Y, Li R, Zhao R (2013) Microvesicle-shuttled miR-130b reduces fat deposition in recipient primary cultured porcine adipocytes by inhibiting PPAR-gamma expression. *J Cell Physiol*. doi:[10.1002/jcp.24486](https://doi.org/10.1002/jcp.24486)
- Park BO, Ahrends R, Teruel MN (2012) Consecutive positive feedback loops create a bistable switch that controls preadipocyte-to-adipocyte conversion. *Cell Rep* 2(4):976–990. doi:[10.1016/j.celrep.2012.08.038](https://doi.org/10.1016/j.celrep.2012.08.038)
- Paul G, Ozen I, Christophersen NS et al (2012) The adult human brain harbors multipotent perivascular mesenchymal stem cells. *PLoS One* 7(4):e35577. doi:[10.1371/journal.pone.0035577](https://doi.org/10.1371/journal.pone.0035577)
- Peng Y, Shi K, Wang L et al (2013) Characterization of Osterix protein stability and physiological role in osteoblast differentiation. *PLoS One* 8(2):e56451. doi:[10.1371/journal.pone.0056451](https://doi.org/10.1371/journal.pone.0056451)
- Perez-Illarbe M, Agbulut O, Pelacho B et al (2008) Characterization of the paracrine effects of human skeletal

- myoblasts transplanted in infarcted myocardium. *Eur J Heart Fail* 10(11):1065–1072. doi:[10.1016/j.ejheart.2008.08.002](https://doi.org/10.1016/j.ejheart.2008.08.002)
- Porada CD, Almeida-Porada G (2010) Mesenchymal stem cells as therapeutics and vehicles for gene and drug delivery. *Adv Drug Deliv Rev* 62(12):1156–1166. doi:[10.1016/j.addr.2010.08.010](https://doi.org/10.1016/j.addr.2010.08.010)
- Pratap J, Galindo M, Zaidi SK et al (2003) Cell growth regulatory role of Runx2 during proliferative expansion of preosteoblasts. *Cancer Res* 63(17):5357–5362
- Quesenberry PJ, Becker PS (1998) Stem cell homing: rolling, crawling, and nesting. *Proc Natl Acad Sci USA* 95(26):15155–15157
- Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ (2008) Efficient tumour formation by single human melanoma cells. *Nature* 456(7222):593–598
- Ringden O, Uzunel M, Rasmuson I et al (2006) Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation* 81(10):1390–1397
- Sacchetti B, Funari A, Michienzi S et al (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 131(2):324–336. doi:[10.1016/j.cell.2007.08.025](https://doi.org/10.1016/j.cell.2007.08.025)
- Samuelsson L, Stromberg K, Vikman K, Bjursell G, Enerback S (1991) The CCAAT/enhancer binding protein and its role in adipocyte differentiation: evidence for direct involvement in terminal adipocyte development. *EMBO J* 10(12):3787–3793
- Sarkar D, Vemula PK, Zhao W, Gupta A, Karnik R, Karp JM (2010) Engineered mesenchymal stem cells with self-assembled vesicles for systemic cell targeting. *Biomaterials* 31(19):5266–5274. doi:[10.1016/j.biomaterials.2010.03.006](https://doi.org/10.1016/j.biomaterials.2010.03.006)
- Schaap-Oziemlak AM, Raymakers RA, Bergevoet SM et al (2010) MicroRNA hsa-miR-135b regulates mineralization in osteogenic differentiation of human unrestricted somatic stem cells. *Stem Cells Dev* 19(6):877–885. doi:[10.1089/scd.2009.0112](https://doi.org/10.1089/scd.2009.0112)
- Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP (2007) Stem cell transplantation: the lung barrier. *Transpl Proc* 39(2):573–576. doi:[10.1016/j.transproc.2006.12.019](https://doi.org/10.1016/j.transproc.2006.12.019)
- Schriebl K, Satianegara G, Hwang A et al (2012) Selective removal of undifferentiated human embryonic stem cells using magnetic activated cell sorting followed by a cytotoxic antibody. *Tissue Eng Part A* 18(9–10):899–909
- Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N (2000) Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 97(21):11307–11312
- Shi M, Li J, Liao L et al (2007) Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. *Haematologica* 92(7):897–904
- Shi K, Lu J, Zhao Y et al (2013) MicroRNA-214 suppresses osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. *Bone* 55(2):487–494. doi:[10.1016/j.bone.2013.04.002](https://doi.org/10.1016/j.bone.2013.04.002)
- Shiojima I, Walsh K (2006) Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway. *Genes Dev* 20(24):3347–3365. doi:[10.1101/gad.1492806](https://doi.org/10.1101/gad.1492806)
- Shujia J, Haider HK, Idris NM, Lu G, Ashraf M (2008) Stable therapeutic effects of mesenchymal stem cell-based multiple gene delivery for cardiac repair. *Cardiovasc Res* 77(3):525–533
- Simonsen JL, Rosada C, Serakinci N et al (2002) Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. *Nat Biotechnol* 20(6):592–596
- Sinha KM, Zhou X (2013) Genetic and molecular control of osterix in skeletal formation. *J Cell Biochem* 114(5):975–984. doi:[10.1002/jcb.24439](https://doi.org/10.1002/jcb.24439)
- Sinha KM, Yasuda H, Zhou X, Decrombrugghe B (2013) Osterix and NO66 histone demethylase control the chromatin architecture of Osterix target genes during osteoblast differentiation. *J Bone Miner Res Off J Am Soc Bone Miner Res*. doi:[10.1002/jbmr.2103](https://doi.org/10.1002/jbmr.2103)
- Sordi V, Malosio ML, Marchesi F et al (2005) Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood* 106(2):419–427
- Sottile V, Thomson A, McWhir J (2003) In Vitro Osteogenic Differentiation of Human ES Cells. *Cloning Stem Cells* 5(2):149–155
- Stenderup K, Justesen J, Clausen C, Kassem M (2003) Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone* 33(6):919–926. doi:[10.1016/j.bone.2003.07.005](https://doi.org/10.1016/j.bone.2003.07.005)
- Takada I, Kouzmenko AP, Kato S (2009a) Molecular switching of osteoblastogenesis versus adipogenesis: implications for targeted therapies. *Expert Opin Ther Targets* 13(5):593–603. doi:[10.1517/14728220902915310](https://doi.org/10.1517/14728220902915310)
- Takada I, Kouzmenko AP, Kato S (2009b) Wnt and PPARgamma signaling in osteoblastogenesis and adipogenesis. *Nat Rev Rheumatol* 5(8):442–447. doi:[10.1038/nrrheum.2009.137](https://doi.org/10.1038/nrrheum.2009.137)
- Tang QQ, Lane MD (2000) Role of C/EBP homologous protein (CHOP-10) in the programmed activation of CCAAT/enhancer-binding protein-beta during adipogenesis. *Proc Natl Acad Sci USA* 97(23):12446–12450. doi:[10.1073/pnas.220425597](https://doi.org/10.1073/pnas.220425597)
- Tang C, Weissman IL, Drukker M (2012) The safety of embryonic stem cell therapy relies on teratoma removal. *Oncotarget* 3(1):7–8. PMID 22294556
- Tao Z, Chen B, Tan X et al (2011) Coexpression of VEGF and angiopoietin-1 promotes angiogenesis and cardiomyocyte proliferation reduces apoptosis in porcine myocardial infarction (MI) heart. *Proc Natl Acad Sci USA* 108(5):2064–2069. doi:[10.1073/pnas.1018925108](https://doi.org/10.1073/pnas.1018925108)
- Tong Q, Dalgin G, Xu H, Ting CN, Leiden JM, Hotamisligil GS (2000) Function of GATA transcription factors in preadipocyte-adipocyte transition. *Science* 290(5489):134–138
- Tong Q, Tsai J, Tan G, Dalgin G, Hotamisligil GS (2005) Interaction between GATA and the C/EBP family of transcription factors is critical in GATA-mediated suppression of adipocyte differentiation. *Mol Cell Biol* 25(2):706–715. doi:[10.1128/MCB.25.2.706-715.2005](https://doi.org/10.1128/MCB.25.2.706-715.2005)
- Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM (1994) mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 8(10):1224–1234
- Tormin A, Li O, Brune JC et al (2011) CD146 expression on primary nonhematopoietic bone marrow stem cells is correlated with in situ localization. *Blood* 117(19):5067–5077. doi:[10.1182/blood-2010-08-304287](https://doi.org/10.1182/blood-2010-08-304287)
- Tornvig L, Mosekilde LI, Justesen J, Falk E, Kassem M (2001) Troglitazone treatment increases bone marrow adipose tissue volume but does not affect trabecular bone volume in mice. *Calcif Tissue Int* 69(1):46–50. doi:[10.1007/s002230020018](https://doi.org/10.1007/s002230020018)
- Tremoleda JL, Forsyth NR, Khan NS et al (2008) Bone Tissue Formation from Human Embryonic Stem Cells In Vivo. *Cloning Stem Cells* 10(1):119–132
- Trivedi P, Hematti P (2008) Derivation and immunological characterization of mesenchymal stromal cells from human embryonic stem cells. *Exp Hematol* 36(3):350–359
- Tu Q, Valverde P, Li S, Zhang J, Yang P, Chen J (2007) Osterix overexpression in mesenchymal stem cells stimulates healing of critical-sized defects in murine calvarial bone. *Tissue Eng* 13(10):2431–2440
- Ueta C, Iwamoto M, Kanatani N et al (2001) Skeletal malformations caused by overexpression of Cbfa1 or its dominant negative form in chondrocytes. *J Cell Biol* 153(1):87–100
- Vimalraj S, Partridge NC, Selvamurugan N (2014) A positive role of microRNA-15b on regulation of osteoblast differentiation. *J Cell Physiol*. doi:[10.1002/jcp.24557](https://doi.org/10.1002/jcp.24557)

- Wakitani S, Nawata M, Tensho K, Okabe T, Machida H, Ohgushi H (2007) Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. *J Tissue Eng Regen Med* 1(1):74–79. doi:[10.1002/term.8](https://doi.org/10.1002/term.8)
- Wang YH, Han Z-B; Song Y-P; Han ZC (2012) Safety of mesenchymal stem cells for clinical application. *Stem Cells Int* 2012(Article ID 652034):4. doi: [10.1155/2012/652034](https://doi.org/10.1155/2012/652034)
- Wang CY, Yang SF, Wang Z et al (2013) PCAF acetylates Runx2 and promotes osteoblast differentiation. *J Bone Miner Metab* 31(4):381–389. doi:[10.1007/s00774-013-0428-y](https://doi.org/10.1007/s00774-013-0428-y)
- Weng JY, Du X, Geng SX et al (2010) Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. *Bone Marrow Transpl* 45(12):1732–1740. doi:[10.1038/bmt.2010.195](https://doi.org/10.1038/bmt.2010.195)
- Wu Y, Zhao RC (2012) The role of chemokines in mesenchymal stem cell homing to myocardium. *Stem Cell Rev* 8(1):243–250
- Xiao G, Jiang D, Thomas P et al (2000) MAPK pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbfa1. *J Biol Chem* 275(6):4453–4459
- Yamashita S, Miyaki S, Kato Y et al (2012) L-Sox5 and Sox6 proteins enhance chondrogenic miR-140 microRNA expression by strengthening dimeric Sox9 activity. *J Biol Chem* 287(26):22206–22215. doi:[10.1074/jbc.M112.343194](https://doi.org/10.1074/jbc.M112.343194)
- Yang L, Cheng P, Chen C et al (2012a) miR-93/Sp7 function loop mediates osteoblast mineralization. *J Bone Miner Res Off J Am Soc Bone Miner Res* 27(7):1598–1606. doi:[10.1002/jbmr.1621](https://doi.org/10.1002/jbmr.1621)
- Yang X, Balakrishnan I, Torok-Storb B, Pillai MM (2012b) Marrow stromal cell infusion rescues hematopoiesis in lethally irradiated mice despite rapid clearance after infusion. *Adv Hematol* 2012:142530
- Yang D, Okamura H, Nakashima Y, Haneji T (2013) Histone demethylase Jmjd3 regulates osteoblast differentiation via transcription factors Runx2 and osterix. *J Biol Chem* 288(47):33530–33541. doi:[10.1074/jbc.M113.497040](https://doi.org/10.1074/jbc.M113.497040)
- Zhang JF, Fu WM, He ML et al (2011a) MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix. *Mol Biol Cell* 22(21):3955–3961. doi:[10.1091/mbc.E11-04-0356](https://doi.org/10.1091/mbc.E11-04-0356)
- Zhang JF, Fu WM, He ML et al (2011b) MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. *RNA Biol* 8(5):829–838. doi:[10.4161/rna.8.5.16043](https://doi.org/10.4161/rna.8.5.16043)
- Zhao S, Wehner R, Bornhauser M, Wassmuth R, Bachmann M, Schmitz M (2010) Immunomodulatory properties of mesenchymal stromal cells and their therapeutic consequences for immune-mediated disorders. *Stem Cells Dev* 19(5):607–614. doi:[10.1089/scd.2009.0345](https://doi.org/10.1089/scd.2009.0345)
- Zhou M, Ma J, Chen S, Chen X, Yu X (2013) MicroRNA-17-92 cluster regulates osteoblast proliferation and differentiation. *Endocrine*. doi:[10.1007/s12020-013-9986-y](https://doi.org/10.1007/s12020-013-9986-y)
- Zimmet H, Porapakkham P, Sata Y et al (2012) Short- and long-term outcomes of intracoronary and endogenously mobilized bone marrow stem cells in the treatment of ST-segment elevation myocardial infarction: a meta-analysis of randomized control trials. *Eur J Heart Fail* 14(1):91–105. doi:[10.1093/eurjhf/hfr148](https://doi.org/10.1093/eurjhf/hfr148)