The International Journal of Biochemistry & Cell Biology 51 (2014) 75–78

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Medicine in focus

Bone defects: Molecular and cellular therapeutic targets

Vincenzo Desiderio, Virginia Tirino*, Gianpaolo Papaccio*, Francesca Paino

Dipartimento di Medicina Sperimentale, Sezione di Istologia e Biotecnologie (TERM Lab.), via L. Armanni, 5, Secondo Ateneo di Napoli, 80138 Napoli, Italy

ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form 24 March 2014
Accepted 25 March 2014
Available online 2 April 2014

Keywords:
Bone disease
Mesenchymal stem cells
Histone deacetylases
Wnt pathway

ABSTRACT

Bone defects are one of the most serious pathologies that need tissue regeneration therapies. Studies on mesenchymal stem cells are changing the way we treat bone diseases. MSCs have been used for the treatment of osteogenesis imperfecta, hypophosphatasia, osteonecrosis of the femoral head, osteoporosis, rheumatoid arthritis and osteoarthritis. In this context, it is becoming ever more clear that the future of therapies will be based on the use of stem cells. In this concise review, we highlight the importance of the use of MSCs in bone diseases, focusing on the role of histone deacetylases and Wnt pathways involved in osteogenesis. A better understanding of MSC biology and osteogenesis is needed in order to develop new and targeted therapeutic strategies for the treatment of bone diseases/disorders.

1. Introduction

Bone defects can be characterized by a lack of bone tissue due to a pathological process, such as osteomyelitis, osteoarthritis, osteoporosis, and cancer, or to injury. Bone defects can be treated by various surgical methods like decortication, excision and fixation, and cancellous bone grafting, which are all very invasive methods. Tissue engineering with the use of mesenchymal stem cells provides an attractive alternative to bone grafting.

Mesenchymal stem cells (MSCs) are multipotent cells able to differentiate into mesodermal cell types such as adipocytes, osteocytes and chondrocytes (Pittenger et al., 1999). MSCs are isolated from different tissues including bone marrow, dental pulp, adipose tissue, placenta, amniotic fluid and umbilical cord blood (Pittenger et al., 1999; d'Aquino et al., 2007; Paino et al., 2010; De Francesco et al., 2009; De Rosa et al., 2009; Zhang et al., 2004), and proliferate *in vitro* as adherent cells with a fibroblast-like morphology. The main role of MSCs is tissue repair (Giuliani et al., 2013). In this context, they can interact with cells of both the innate and adaptive immune systems, leading to the modulation of several effector functions. Following *in vivo* administration, MSCs induce peripheral tolerance and migrate to injured tissues, where they can inhibit the release of pro-inflammatory cytokines and promote the survival and the repair of damaged cells. Moreover, cell–cell contact-dependent mechanisms and soluble factors, including transforming growth factor-β, hepatocyte growth factor (HGF), nitric oxide and indoleamine 2,3-dioxygenase (IDO), promote the induction of MSC-mediated immune suppression by inhibiting the proliferation of T and natural killer lymphocytes, the functions of B lymphocytes, and the differentiation and activation of dendritic cells. Similarly, the migratory properties of MSCs are mediated by a series of chemokines and their receptors. The stromal cell-derived factor 1 (SDF-1)/CXC chemokine receptor type 4 (CXCR4) axis, the stem cell factor/c-kit axis, HGF/c-Met, vascular endothelial growth factor (VEGF)/VEGF receptor, platelet-derived growth factor (PDGF)/PDGF receptor, monocyte chemo-attractant protein-1 (MCP-1)/C-C chemokine receptor type 2, and high mobility group box 1/receptor for advanced glycation endproducts play important roles in activating the migration of MSCs. Therefore, MSCs repair, migrate, translocate, roll and immunosuppress. Although all these mechanisms are not fully understood, these characteristics make MSCs attractive therapeutic agents and suitable as delivery vehicles for targeted therapy. In this context, MSCs have been used for the treatment of bone diseases such as osteogenesis imperfecta, hypophosphatasia, osteonecrosis of the femoral head, osteoporosis, rheumatoid arthritis and osteoarthritis (Liu et al., 2014). In this review, we highlight the importance of mesenchymal stem cells in bone diseases, focusing on the role of histone deacetylases (HDACs), Wnt pathways and microRNA involved in osteogenesis.

2. Pathogenesis

Human bone pathologies, such as osteoporosis pseudoglioma syndrome, sclerosteosis and van Buchem's disease, have been associated with aberrant Wnt signalling (Monroe et al., 2012). The

* Corresponding authors. Tel.: +39 081 5667720; fax: +39 081 5666014.
E-mail addresses: virginia.tirino@unina2.it (V. Tirino), gianpaolo.papaccio@unina2.it (G. Papaccio).

http://dx.doi.org/10.1016/j.biocel.2014.03.025
1357-2725/

Wnt signalling pathway can be divided into the canonical and the non-canonical pathways. Canonical Wnt signalling is mediated by the stabilization of β-catenin in the cytoplasm. Without the Wnt ligands, β-catenin is phosphorylated by a degradation complex formed of glycogen synthase kinase 3β (GSK3β), axin and APC. GSK3β phosphorylates β-catenin, which is ubiquitinated and degraded (Rao and Kühl, 2010). Instead, in the presence of Wnt ligands, including Wnt 1, 3a and 8, the ligands bind to the frizzled (Fzd) receptors and one of the co-receptors, either low-density lipoprotein receptor-related protein (LRP)-5 or -6, phosphorylating the intracellular protein disheveled (Dvl). Phosphorylated Dvl blocks GSK3β, which does not phosphorylate β-catenin. As a result, β-catenin translocates to the nucleus and forms a transcriptional complex with T-cell factor (Tcf)/lymphoid enhancer-binding factor (Lef) to regulate the expression of target genes, including cyclin D1, axin2, c-Myc and peroxisome proliferator-activated receptor (PPARδ). The two main non-canonical Wnt pathways are the calcium-dependent and the planar cell polarity pathways. Regarding the former, Wnt ligands bind to their Fzd receptors with the activation of G protein, and a signal cascade releases intracellular calcium from the endoplasmic reticulum. The released calcium activates downstream mediators, including protein kinase C and calcium/calmodulin-dependent protein kinase II. These, in turn, activate transcription factors, such as nuclear factor κB, nuclear factor of activated T cells and cAMP response element binding protein (Rao and Kühl, 2010). Instead, the cell polarity pathway regulates the cytoskeletion by activating GTPases such as rac, rho and cdc42 through Fzd receptors and kinases such as c-jun NH2 kinase (Rao and Kühl, 2010). The Wnt signalling pathway plays an important role in promoting the osteogenic differentiation of MSCs. Case and Rubin showed that β-catenin promotes the differentiation of MSCs into osteoblasts while suppressing adipogenic and chondrogenic differentiation (Case and Rubin, 2010). This happens by inhibition of the expression of adipogenic inducers PPARγ and CCAAT/enhancer binding protein α, and upregulation of the osteogenic mediators Runx2, Dlx5 and Osterix. Moreover, also the non-canonical Wnt pathway suppress PPARγ to induce osteogenic differentiation. During the osteogenic differentiation of MSCs, the Wnt signalling pathway crosstalks with multiple signalling pathways, including BMP, Notch and Hedgehog pathways. Thus, because Wnt pathways play a key role in the osteogenic differentiation of MSCs, aberrations in these lead to failures of appropriate bone formation. The most notable case of altered Wnt signalling is osteoporosis pseudoglioma syndrome (OPPG), which is characterized by osteoporosis and low bone mineral density (BMD). It is caused by the inactivating mutation of LRP5 (Levasseur et al., 2005). A gain-of-function missense mutation of LRP5 with high bone density phenotypes in humans has also been identified (Boyden et al., 2002). Moreover, a missense mutation of LRP6 in humans leads to osteoporosis with features of metabolic syndrome, whereas polymorphisms of LRP6 are significantly associated with low BMD (Riancho et al., 2011) (Fig. 1). Equally, loss-of-function mutations in the SOST gene, coding for Sclerostin, lead to sclerosteosis characterized by a high index of bone density. Sclerostin is an inhibitor of LRP 5/6 function. A SOST-neutralizing antibody has been tested in phase 3 clinical trials, showing great promise as a therapeutic agent in osteoporosis by enhancing bone formation (Costa and Bilezikian, 2012). It is important also to consider loss of Wnt antagonists such as sfrp1, Dkk, Apc or Axin2 with increased bone density as well as intracellular mediators of the Wnt pathway. Functionality of β-catenin can directly affect the osteogenic differentiation of MSCs. Deletion of β-catenin in osteogenic progenitors prevents differentiation into osteoblasts and lead to chondrocyte differentiation (Day et al., 2005) (Fig. 1). On the contrary, β-catenin stabilization leads to high bone mass and premature ossification (Rodda and McMahon, 2006). In this context, it is interesting to evaluate microRNAs that

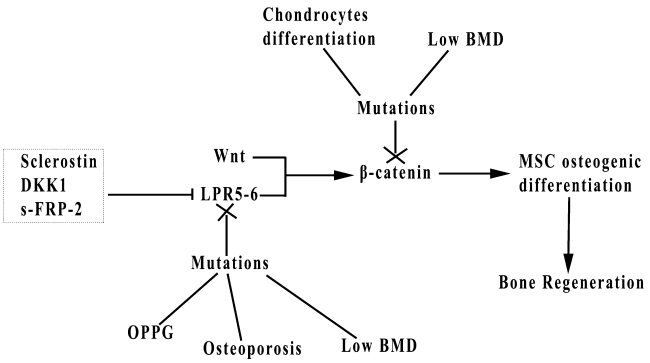

Fig. 1. Schematic of Wnt canonical pathway mutation related to bone defects.

act on the Wnt pathway. Recently, it has been shown that miR-218 directly promotes osteoblast differentiation by negative regulation of Wnt signalling inhibitors such as Sclerostin, Dickkopf2 (DKK2) and secreted frizzled-related protein (SFRP2) (Hassan et al., 2012). Similar mechanisms were described by Kapinas (Kapinas et al., 2010), in which Wnt signalling regulatory mechanism involving miR-29 were highlighted. miR-29 expression was induced by Wnt signalling and led to the suppression of Wnt inhibitors (Dkk1) and Kremen2 (sFRP2), enhancing bone differentiation. Moreover, miR-29 negatively regulates osteonectin expression during the matrix maturation and mineralization phases of late differentiation. miR-335-5p (Zhang et al., 2011) and miR-218 (Hassan et al., 2012) have been also described as Wnt signalling activators acting through the inhibition of Dkk1, confirming that the main mechanism of microRNAs in osteogenic enhancement is inhibition of Wnt suppressors.

In summary, Wnt signalling pathway alterations in progenitor cells causes bone disorders through by compromising osteogenic differentiation (Table 1). Thus, mediators of Wnt signalling may be attractive therapeutic targets to enhance proper bone formation.

3. Therapy

Stem cell-based therapy needs to be consolidated mainly because it is necessary to better understand the molecular mechanisms involved in stem cell survival and differentiation. The differentiation of adult stem cells involves extensive chromatin remodelling, mediated in part by histone deacetylase (HDAC) family members. Stem cell differentiation generally involves a decrease in HDAC gene expression. Many *in vitro* studies have shown that HDAC downregulation can reduce the features of stemness and upregulate differentiation markers (Fig. 2).

HDACs control transcriptional activity by removing the acetyl groups from lysine residues in histones, leading to a condensed chromatin and repression of gene expression. HDACs can also deacetylate non-histone proteins such as transcription factors.

Mammalian HDACs are divided into four classes: nuclear class I (HDAC1, 2, 3 and 8), nuclear/cytoplasmic class IIa (HDAC4, 5, 7 and 9) and cytoplasmic class IIb (HDAC6 and 10), class III (sirtuins, SIRT1-7), and class IV (HDAC11). Important distinctions exist not only between HDACs classes but also among individual HDACs within a class, exhibiting differential regulation through distinct expression patterns. Regarding the role of HDACs in bone differentiation, many studies using HDAC inhibitors or RNA interference have identified a series of enzymes acting as regulators of bone formation. HDAC3, HDAC6 and HDAC7 were found associated with Runx2, the essential transcription factor of bone formation, acting as transcriptional co-repressors of Runx2 activity in osteoblasts (Jensen et al., 2008). HDAC4 and HDAC5 also negatively regulated Runx2 activity by deacetylating lysines in the Runx2 protein and leading to ubiquitin-mediated proteolysis (Jeon et al., 2006).