ABSTRACT

Bone is a vital tissue and therefore, osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) are continuously at work to maintain bone quality and strength by replacing old and damaged tissue arising from physical assault, radiation exposure or genetic factors. Both osteoblasts and osteoclast arise from the mesenchymal and hematopoietic stem cells, respectively, resident in the bone marrow, and an imbalance in their activities is the cause of orthopedic disorders like osteoporosis and Paget’s disease. Although compounds like bisphosphonates, calcitocin, and estrogen receptor modulators are approved clinically, they work by inhibiting osteoclast activity rather than promoting bone formation. Thus there is a need to identify and develop newer approaches to stimulate osteoblast formation for treating natural/accidental/radiation-induced orthopedic defects.

The development of novel approaches will rely upon our understanding of the osteoblast differentiation process. Over the years, the major signaling pathways like Wnt, BMP/TGF-β, Notch and Ras-ERK have been identified to play a role in osteogenesis, in addition to discovery of several critical transcription factors like Runx2, Osx, Dlx5 and Atf4. Activation of canonical Wnt signaling (mediated via β-catenin) is essential for osteogenic differentiation as inactivating mutation in Wnt co-receptor LRP5 (low density lipoprotein-related protein 5) results in osteoporosis pseudoglioma syndrome whereas activating mutation leads to high bone mass. Thus, the Wnt cascade represents an amiable target for designing strategies to promote bone anabolism. However, information on the spatial-temporal conditions of activation of these pathways and their downstream genes remains unknown.

As osteoblasts arise from mesenchymal stem cells, the effect of stimulation of Wnt signaling on osteogenic differentiation was evaluated. Using lithium (5mM) as a Wnt mimetic, as it is clinically used for psychiatric disorders, treatment of mesenchymal stem cells resulted in an increase in alkaline phosphatase activity, an early osteogenic marker. Thus to gain a deeper understanding of the underlying mechanism, microarray-based approach was used to identify the changes induced in the transcriptome. Lithium was able to reprogram mesenchymal stem cells as adipogenic transcription factor CEBPA was
suppressed along with TWIST1 and PBX1 (negative regulators of osteogenesis), while osteogenic transcription factor ATF4 and extracellular matrix protein tetranectin (CLEC3B) were found to be induced. Also, down-regulation of osteoclastogenic factors and immunogenic cytokines like IL8, CCL20 and CXCL12 secreted by osteoblasts was observed, suggesting hypoinmunogenic character of lithium-primed mesenchymal stem cells. Further evaluation of gene expression by real time PCR upon recombinant Wnt3a-mediated activation of Wnt cascade led to the identification of ATF4 and CLEC3B as possible Wnt target genes. However, PBX1 expression was not altered by Wnt3a suggesting lithium-mediated suppression of its expression by a Wnt-independent mechanism.

To assess whether these primed mesenchymal stem cells possessed higher osteogenic potential, the cells were observed to synthesized greater amount of collagen-I, the major extracellular matrix protein, and deposited more calcium under osteogenic conditions. This enhancement of osteoblast differentiation was also supported by an increase in the gap junction protein connexin43 levels in osteoblastic SaOS2 cells upon lithium treatment, as assessed by western blotting. To determine if connexin protein was being regulated by glycogen synthase kinase-3β (GSK-3β), the kinase responsible for inhibiting Wnt signaling by phosphorylating β-catenin and a target of lithium's action, an alternative GSK-3β inhibitor, 6-bromoindirubin 3'-oxime (BIO), was used. BIO also led to an increase in connexin43 levels in SaOS2 cells but did not regulate connexin43 protein stability.

The study was extended further to decipher how lithium-mediated signaling interacts with osteogenic differentiation induced by Osterix, an osteoblastic transcription factor necessary for differentiation. For this purpose, doxycycline-inducible stable SaOS2 cells were generated to avoid effects of constitutive overexpression. Lithium did not alter collagen-I expression in SaOS2 cells, however, lithium treatment of doxycycline-induced Osterix expressing cells revealed enhancement of collagen-I expression and protein. All these findings highlight the potential of lithium as a 'priming' molecule capable of enhancing osteogenic differentiation of mesenchymal stem cells via Wnt-dependent and independent mechanisms. These 'primed' cells present immense therapeutic benefit for treating critical fractures, osteoporosis and radiation-induced bone damage.