

# Wnt3 and Wnt5 Proteins Mediate Shock Wave-Promoted Osteogenic Differentiation of Mesenchymal Stem Cells

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Osteogenesis has been implicated in recapitulation of embryonic skeletal development. The Wnt family of growth factors are important regulators of skeletogenesis. In animal studies, we have reported that shock waves (SW) can promote osteogenic differentiation of mesenchymal stem cells through TGF-beta-, BMP- and VEGF-mediated signal transduction. Here, we further found that SW promotion of osteogenic differentiation of bone marrow stromal cells mediated by the Wnt and beta-catenin-dependent pathway. SW treatment (0.16 mJ/mm<sup>2</sup>, 1 Hz, 500 impulses) promoted cell proliferation, alkaline phosphatase activity and mineralized nodule formation of primary human bone marrow stromal cells. Real-time PCR results showed that SW increased Wnt3a and Wnt5a, but not Wnt7a mRNA expression of cell cultures. Inhibition of Wnt3a and Wnt5a signalling by Wnt3a and Wnt5a neutralizing antibodies reduced the promoting effect of SW on osteogenic differentiation of stromal cells. Further studies demonstrated that SW significantly promoted cytosolic beta-catenin accumulation and nuclear osteogenic transcription factor Cbfa1/Runx2 activation. Wnt3a and Wnt5a neutralizing antibodies reduced SW-enhanced cytosolic beta-catenin and nuclear Runx2 activation. Mesenchymal cells responded to recombinant Wnt3a and Wnt5a protein by increasing cytosolic beta-catenin expression, nuclear Runx2 activation and bone nodule formation. Moreover, serum harvested from patients with non-union receiving SW treatment increased alkaline phosphatase activities and bone nodule formation of mesenchymal cells that were blocked by Wnt3a and Wnt5a neutralizing antibodies. Taken together, we have shown that SW increased Wnt3a and Wnt5a synthesis followed by cytosolic beta-catenin accumulation and nuclear Cbfa1/Runx2 activation, resulting in an increase of osteogenic differentiation of mesenchymal cells.