Wnt Signaling in Zebrafish Fin Regeneration: Chemical Biology Using GSK3b Inhibitors

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Bone growth can be impaired due to disease, such as osteoporosis, and Wnt signaling pathways regulate bone growth. The parathyroid hormone (PTH) is therapeutic for anabolic bone growth (bone building), which activates Wnt signaling, leading to bone growth. GSK3b (glycogen synthetase kinase 3 beta) protein inhibitors activate Wnt signaling, including in bone growth models. Our study utilized a zebrafish model system to study Wnt activated fin regeneration and bone growth. Wnt signaling is the first genetically identified step in fin regeneration, and bony rays are the main differentiated cell type in fins. Thus, zebrafish fin regeneration may be a useful model to study Wnt signaling mediated bone growth. Fin regeneration experiments were conducted using various concentrations of GSK3b inhibitor compound for different treatment periods and regenerative outgrowth was measured at 4 and 7 days post amputation. Experiments revealed continuous low concentration (5-6 nM) treatment to be most effective at increasing regeneration. Higher concentrations inhibited fin growth, perhaps by excessive stimulation of differentiation programs. In situ hybridization experiments were performed to examine effects of Gsk3b inhibitor on Wnt responsive gene expression. Initial experiments show temporal and spatial changes on individual gene markers following GSK3b inhibitor treatment. Additionally, confocal microscopy and immunofluorescence labeling data indicated that the Wnt signaling intracellular signal transducer, beta-catenin, accumulates throughout Gsk3b inhibitor treated tissues. Finally, experiments are underway to quantify phosphohistone-3 staining in regenerating tissue to measure effects of Gsk3b inhibitor on cell proliferation. Together, these data indicate that bone growth in zebrafish fin regeneration is improved by activating Wnt signaling. Zebrafish Wnt signaling experiments provide good model to study bone growth and bone repair mechanisms, and may provide an efficient drug discovery platform.

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