Effects of GSK3-β Inhibitors on Wnt Signaling in Zebrafish Fin Regeneration: Chemical Biology

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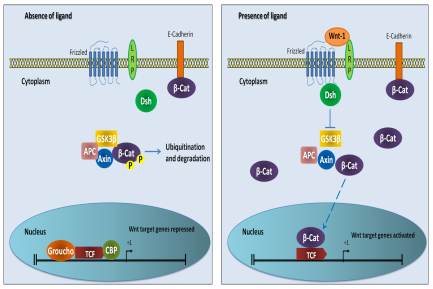
Abstract

In order to develop beneficial drugs for osteoporosis it is important to understand the molecular mechanisms of bone regeneration and define specific regulatory factors. Zebrafish can regenerate damaged tissues, and they prove to be a good model to study bone growth and repair. Previous research showed that GSK3β inhibitor compound at various concentrations and for different treatment periods effectively stimulated fin regeneration. Conducted experiments identified temporal and spatial fluctuations on individual gene markers after GSK3β inhibitor treatment at various concentrations. Recent analyzed data uses the Lilly Research Labs experimental compound LSN 2105786 at 3 nM and 5 nM to stimulate tissue regeneration to determine whether activating Wnt signaling produces cell proliferation and β-catenin translocation to the nucleus for zebrafish bone regeneration. This research has potential to identify mechanism of bone growth and repair, leading to more suitable drugs for patients suffering with osteoporosis.

Introduction

Recent studies indicate that the Wnt signaling pathway is an important component in bone formation, homeostasis, and repair. The common human genetic disease, osteoporosis-pseudoglioma syndrome is caused by mutations in LPR5/6, a part of the Wnt signaling pathway. Evidence of the role Wnt signaling plays in bone growth has increased interest and research of the modulation of the Wnt signaling pathway for therapeutic treatment of osteoporosis. Studying regenerative processes allows for a better understanding of cellular mechanisms in bone repair. Zebrafish fins are composed of segmented bony rays, which are covered by a single layer of osteoblasts. When the fin is amputated, regeneration occurs quickly. The bones that regrow post amputation are formed in the absence of a cartilage intermediate, which is similar to intramembranous bone formation. Zebrafish fin regeneration is classified by three stages: wound healing; formation of a blastema consisting of mesenchymal progenitors; and regenerative outgrowth/pattering of bony ray structure. It is known that Wnt/β-catenin signaling is essential during all stages of fin regeneration in order for growth to occur.



(Schematic diagram from: www.intechopen.com/books/current-topics-in-gastritis-2012/gastric-cancer-molecular-pathology-state)

•The Wnt signaling pathway is expressed during cell proliferation, specification, and migration. In the presence of the Wnt ligand, β-catenin translocates to the nucleus where it affects target genes. In the absence of the Wnt ligand, β-catenin is degraded. Glycogen synthase kinase-3β (GSK-3β) regulates the Wnt signal transduction pathway by phosphorylating β-catenin, leading to its destruction. See figure above.

•Presence of GSK-3β inhibitor: Phosphorylation of β-catenin does not occur, protecting it form degradation. Then, β-catenin accumulates and translocates to the nucleus where it activates transcription of target genes.

•Absence of the GSK-3β inhibitor: β-catenin phosphorylation by the GSK3-β enzyme causes β-catenin degradation.

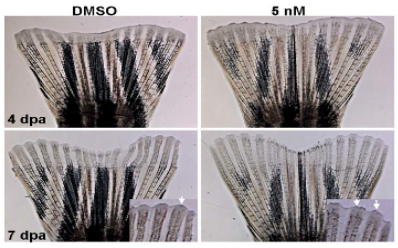
Methodology

Immunohistochemistry
Regenerating fin tissues were obtained at 1 and 2 dpa. Fin tissue was fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and immunostained with antibodies against phospho-histone H3 or β-catenin. Images were produced using confocal microscopy.

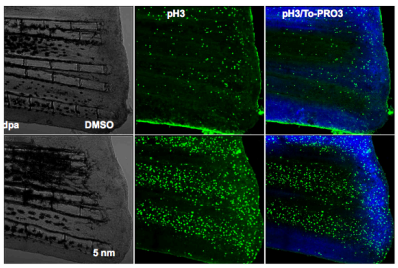
Zebrafish caudal fin amputation with GSK-3β inhibitor treatment
Adult zebrafish caudal fins were amputated, then treated with GSK-3β inhibitor compound or DMSO control and kept at 31ºC. On the 4th and 7th day post-amputation (dpa) fish were anesthetized and images were collected using a dissecting microscope. Regenerative outgrowth was measured to examine the effect of GSK-3β inhibitor on fin regeneration.

Whole-Mount In Situ Hybridization
Regenerating fin tissues were collected and fixed in 4% PFA in PBS. Fin tissues were probed for expression at 3 dpa using *shh* and *lef1* probes. Images were produced using a dissecting microscope.

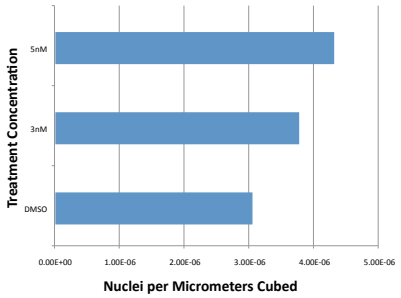
Results



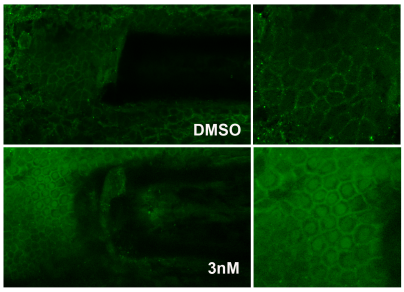
•Increased caudal fin regeneration observed at 4 and 7 dpa in fish treated continuously with GSK-3β inhibitor
•5 nM treatment was most effective at increasing regenerative outgrowth



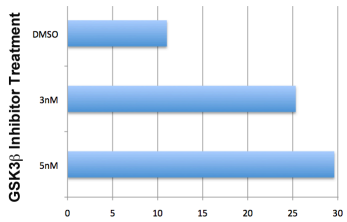
•Phospho-histone H3 staining revealed increased proliferation in fins treated with the GSK-3β inhibitor.



Results



β-catenin Intensity in Blastema Region



Treatment with GSK-3β inhibitor compound increased nuclear accumulation of β-catenin in the blastema at 2 dpa. Immunofluorescence staining of β-catenin was imaged using confocal microscopy, and images were analyzed using Image-J to specifically measure fluorescence intensity within the nucleus in growing blastema cells. Increased β-catenin staining was detected in 3 and 5 nM GSK-3β inhibitor treated fins as compared to control vehicle treated fins (DMSO). In situ hybridization, experiments were preformed, which showed stimulatory effects of GSK3β on Wnt responsive gene expression. Also, confocal microscopy and immunofluorescence labeling data indicated that the Wnt intracellular signal transducer, β-catenin, increases throughout GSK3β inhibitor treated tissue.

Conclusions

•Continuous low concentration GSK-3β inhibitor treatment increased caudal fin regeneration.

•Treatment with GSK-3β inhibitor compound increased nuclear accumulation of β-catenin in the blastema cells.

•Expression of the Wnt/β-catenin target genes (*lef1* and *shh*) was increased by treatment with GSK-3β inhibitor.

•GSK-3β inhibitor treatment increased cell proliferation in regenerating tissue.

•Ongoing research shows an increase of activity of the blastema region where GSK3β inhibition increases cell proliferation, expanding the regenerating fin tissue.

•GSK3β inhibition does stimulate proliferation and β-catenin nuclear localization, which improves bone growth during zebrafish regeneration.

Acknowledgements

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