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Growth hormone induces proliferation in the zebrafish inner ear

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Background

While acoustic trauma results in a loss of hair cells in the ears of fishes, they have the ability to recover their hair cells and hearing sensitivity within a few weeks [1]. Our previous microarray and RT-PCR analysis of sound-exposed zebrafish (*Danio rerio*) ears, showed that growth hormone (GH) was significantly upregulated during zebrafish auditory tissue cell proliferation and hair cell regeneration [2]. This upregulation was greatest two days following acoustic trauma, coincident with an increase in cell proliferation [3]. In order to better understand the role of GH in the regenerative abilities of the zebrafish ear, we performed two GH-injection experiments.

Materials and methods

In Experiment 1, treatment fish were injected intraperitoneally with salmon GH at 10 ug/1g body weight while controls were injected with buffer solution. Both groups were then allowed to recover for 24 h at 25 °C before BrdU injection. Four hours following BrdU injection, fish were sacrificed and their saccules prepared for immunohistochemistry using mouse monoclonal anti-BrdU antibody (Invitrogen, Carlsbad, CA) as the primary and Alexa Fluor 568–conjugated rabbit anti-mouse antibody as the secondary.

In Experiment 2, fish were exposed for 36 h to a 100 Hz tone at 179 dB re 1 μPa RMS in a 19-L sound exposure chamber at 25 °C, after which the fish were removed for immediate injection (GH or buffer control), and then moved to a recovery tank for a predetermined length of time. The effects of GH on hair cell proliferation after noise exposure were assessed by BrdU assay 48 h later. The role of GH in changing hair cell bundle density was examined by phalloidin staining 60 h post-sound exposure.

Results

GH injection resulted in increased cell proliferation in the zebrafish ear, particularly in the utricle (Fig. 1A). At

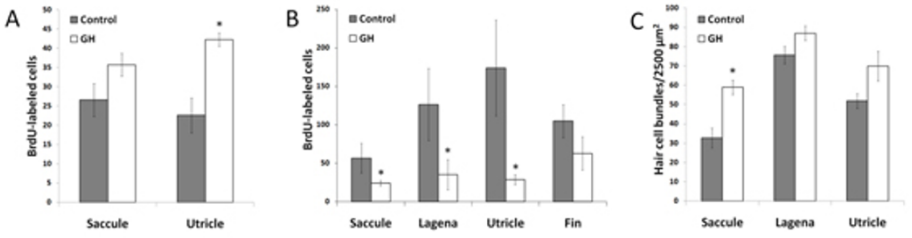

Figure 1 The effect of growth hormone injection on mean (±SE) number of BrdU-labeled cells (A and B) and hair cell bundle density (C) in zebrafish ear sensory tissues. Fish in (A) were not exposed to a sound stimulus, while fish in (B and C) were dissected 48 and 60 h post-sound exposure, respectively. N=6-12. * P<0.05.

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48 h post-sound exposure, the saccules, lagenae, and utricles of GH-injected fish had significantly reduced BrdU-labeled cells compared to controls (Fig. 1B). GH may have induced proliferation earlier so that few cells were mitotic at this time point. At 60 h post-sound exposure, mean hair cell bundle densities were greater in GH-injected fish compared to controls, particularly in the saccule (Fig. 1C).

Conclusion

GH plays an important role in auditory cell proliferation and hair cell regeneration in zebrafish. Future experiments will examine genes involved in this process and if GH is necessary for such regeneration.

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