FUNCTIONAL ANALYSIS OF CYTOPLASMIC y-ACTIN MUTATIONS CAUSING NON-SYNDROMIC, PROGRESSIVE AUTOSOMAL DOMINANT HEARING LOSS

By

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Mutations in cytoplasmic y-actin cause non-syndromic, post-lingual, autosomaldominant, progressive sensorineural hearing loss. LLC-PK1- CL4 cells provide a model system to study the distribution of actins and the role of y-actin and its mutations in repair of damaged structures like microvilli. Immunohistochemistry and confocal localization studies showed that (3-actin was found primarily at the periphery of cells while y-actin is abundant in the perinuclear space and cytoplasm of the cell. Exogenous expression of mutant y-actins showed distribution to all the actin structures in the cell; the periphery, stress fibers and perinuclear space. In response to exogenous espin, filamentous mutant actins co-localized with espin in the microvilli. Co-transfection of espin and mutant actin resulted in each of the mutants co-localizing with filamentous actin in the microvilli. Cytochalasin D treatments of WT y-actin and mutant y-actins showed no difference in the repair of the damaged microvilli. Measurements of the lengths of microvilli however indicated that the microvilli expressing mutant actins were -20-25% shorter than the WT y-actin microvilli. Quantitative FRAP assays heat shock promoter, were used to over-express the mutant actins in the zebrafish. Confocal images of hair cells of cristae and maculae from fish at 4-day post fertilization (dpf) showed that five out of six mutants are expressed in hair

did not reveal any differences in the recovery rates between WT and mutants actins. Our data suggest that mutations in y-actin exhibit subtle phenotypes and might interfere with basic actin assembly dynamics. To determine the physiological relevance of the cell culture data, a multi-site Gateway system based EGFP tagged WT and mutant y-actin constructs were made to create a transgenic zebrafish model for the y-actin mutants. These constructs, under a heat shock promoter, were used to over-express the mutant actins in the zebrafish. Confocal images of hair cells of cristae and maculae from fish at 4-day post fertilization (dpf) showed that five out of six mutants are expressed in hair cells and stereocilia. Fish harboring these mutations did not show any morphological defects and appeared healthy like the WT counterparts.