Cochlear Proteomics: Insights into the Auditory Pathophysiology

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Introduction: Hearing loss (HL) often results from permanent damage to the inner ear hair cells (HC), crucial for acoustic signal transduction. Unlike in lower vertebrates, mammalian cochlear HC does not regenerate spontaneously; however, recent evidence suggests a potential for regeneration from supporting cells (SC) in early postnatal mice. Understanding the intricate mechanisms underlying auditory pathophysiology requires a deep analysis of the molecular composition of the cochlea. Nevertheless, due to the challenges in accessing the human cochlea, animal models are invaluable tools for such investigations. Leveraging the advancements in mass spectrometry (MS) technology, proteomics emerges as a powerful approach for unraveling the Protein-Protein Interaction networks associated with HL and the regeneration of sensory epithelium. Aim: Characterize and compare the proteomic profile of the cochlea from hearing adults, deaf adults, and neonatal mice. Methods: Nano LC-MS/MS analysis of mice cochlea (P28) with normal-hearing (NH) and noise-overexposure-induced hearing loss (NIHL) and neonates (P2-7, NN) were conducted. In adult mice, Brainstem Auditory Evoked Potential evaluated hearing, and deafness. Protein identification and quantification utilized MaxQuant software, with statistical analyses performed using Perseus. Identified differentially expressed proteins (DEP) underwent in silico studies focusing on gene and phenotype ontology, biological pathways, deafness genes, or mapped loci without identified genes. Reuse of public transcriptomics data was carried out to determine the cochlear cell types in which the genes of these proteins are localized. Results: The analysis revealed 1572 proteins in NH, 1605 in NIHL, and 2113 in the NN profiles, totaling 2413 unique proteins. Among these, 271 (11.2%) proteins are encoded by genes already associated with syndromic or non-syndromic deafness in humans. Eighty-one differentially expressed proteins (DEPs) were identified in NH vs. NIHL: 23 upregulated and 27 downregulated in NIHL, 10 solely in NH, and 21 in NIHL. Gene ontology over-representation analyses significantly grouped proteins in specific biological processes (associated with oxidative stress) and cellular components (mitochondria). The study revealed that 20 proteins have their genes mapped to human deafness loci, without an identified gene to date, and 19 enriched in HC, SC, and/or neurons of the mouse cochlea. Oxidative stress is associated with NIHL, which is caused by a complex interaction involving HC damage and axonal retraction of spiral ganglion neurons. In NH vs. NN, we have 476 DEP (191 downregulated, and 285 upregulated in NN); 58 only to NH, and 197 to NN, totalizing 731. Among these DEPs, 35 are encoded by genes associated with phenotype alterations in the mammalian cochlea and three are predicted to be transcriptional regulators involved in the SC into HC transdifferentiation; 38 proteins were classified by enrichment analysis in the biological pathways of the mitotic cell cycle. Among these 476 DEPs, 232 of these protein transcripts are enriched in the HC, SC, and neurons of the mouse cochlea. There are 119 enriched transcripts in SC. Conclusion: This study underscores the utility of proteomics in advancing our understanding of cochlear pathophysiology. It identifies novel proteins potentially involved in auditory disorders and offers targets for future therapeutic strategies aimed at HL restoration and sensory epithelium regeneration. The robust proteomic dataset generated in this study offers profound insights into the intricate physiology of the cochlea.

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