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Research Report

Inner ear proteomics of mouse models for deafness, a discovery strategy☆

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ABSTRACT

Inner ear dysfunction is often associated with defective hair cells. Therefore, hair cells are the focus of study in many of the mouse mutants showing auditory and vestibular deficits. However, harvesting sufficient numbers of hair cells from the tiny bony mouse inner ear for proteomic analysis is challenging. New approaches that would take advantage of mouse mutants and avoid processing steps, such as decalcification or microdissetion, would be more suitable for proteomic analysis. Here, we propose a novel approach called SSUMM—Subtractive Strategy Using Mouse Mutants. SSUMM takes advantage of the differences between control and affected or mutant samples. We predict that SSUMM would be a useful method in proteomics, especially in those cases in which the investigator must work with small numbers of diverse cell types from a tiny organ. Here, we discuss the potential utility of SSUMM to unravel the protein expression profiles of hair cells using the *Pou4f3* mouse mutant as an example. *Pou4f3* mutant mice exhibit a total loss of inner and outer hair cells, but supporting cells remain relatively intact in the cochlea, thus providing an excellent model for identifying proteins and transcripts that are specific to the hair cell at all life stages. SSUMM would maximize the sensitivity of the analyses while obviating the need for tedious sessions of microdissection and collection of hair cells. By comparing the mutant to control ears at specific time points, it is possible to identify direct targets of a gene product of interest. Further, SSUMM could be used to identify and analyze inner ear development markers and other known genes/proteins that are coexpressed in the ear. In this short technical report, we also discuss protein-profiling approaches suitable for SSUMM and briefly discuss other approaches used in the field of proteomics.

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1. Introduction

The mouse models available for human hereditary hearing disorders offer extraordinary tools for molecular pathway studies and drug discovery (http://www.sanger.ac.uk/PostGenomics/mousemutants/deaf/). These single mutation mouse models having well-characterized phenotypes on genetically defined backgrounds enable us to use gene and protein expression profiling to identify molecular pathways involved in inner ear structure and function. Proteomic approaches have become increasingly successful for the study of complex biological problems relevant to the auditory system. The majority of previous investigations were performed on other species including humans (Henzl et al., 1997, 1998, 2001; Hurle et al., 2003; Ornitz et al., 1998; Sakaguchi et al., 1998; Thalmann, 2001; Thalmann et al., 1980, 1986, 1987, 1990, 1994, 1995a,b, 1997, 2001, 2003; Thalmann and Thalmann, 1987, 1999; Wang et al., 1998). Only a few protein-profiling studies have been carried out in mouse inner ears (Henzl et al., 1997; Hurle et al., 2003; Ornitz et al., 1998; Sakaguchi et al., 1998; Wang et al., 1998). One reason for the lack of such studies is the difficulty of accessing the small number of diverse cell types within the hard temporal bone that encases the inner ear. As the technology of proteomics moves from theory to practical reality, auditory scientists will have to determine the most appropriate strategies for this technology in order to overcome difficulties particular to this research, such as the limited sample amounts and heterogeneous cellular composition of the tiny mouse inner ears. In this short technical report, we describe a subtractive strategy which overcomes these difficulties, taking advantage of mouse mutant and genomic information resources accumulated in the last decade. For a more comprehensive review of the application of proteomics to auditory research, the reader is referred to Thalmann (this issue) and McGuire and Casado (2004).

1.1. *Genetics of deafness*

Hearing loss affects more than 28 million individuals in all age groups in the United States (NIDCD Health Information homepage, http://www.nidcd.nih.gov/health/statistics/hearing.asp). Approximately one-half of these cases are thought to be of hereditary origin. Noise-induced and age-related hearing loss (presbycusis) may have genetic components as predisposing factors as well. The fundamental processes involved in the development and physiology of hearing are controlled by hundreds of genes. To date, nearly 150 deafness-related loci have been mapped, and more than 50 genes have been identified (www.uia.ac.be/dnalab/hhh/). Gene identification is only the first step towards finding a cure for human deafness. The next critical step for understanding the pathophysiology of hearing disorders is to profile the expression and regulation of deafness genes at the proteome and transcriptome level. Experimental animal models such as the mouse can play a key role in these critical steps.

1.2. *Mouse models as tools to unravel the genetic basis for human deafness*

Mice have many well-known advantages over other species for the study of human disease. The mouse ear is remarkably similar in structure and function to the human ear, and both species have many similar hearing disorders (Alagramam et al., 2001a,b, 2005; Donahue et al., 2003; Ikeda et al., 1999, 2002; Johnson et al., 1997, 1998, 2000, 2001, 2003; Johnson and Zheng, 2002; Letts et al., 2000; Lorenz-Depiereux et al., 2004; Munroe et al., 2000; Noben-Trauth et al., 1997, 2003; Staecker et al., 2001; Zheng et al., 1998, 1999, 2004; Zheng and Johnson, 2001). Because mouse mutations can be maintained in a controlled genetic background, it is possible to analyze the effects of a mutant gene for same-sex littermates that differ only in a single mutated gene out of the whole genome (Silver, 1995). To date, nearly 50 human hearing disorders have been identified that have parallel mouse disorders due to mutations in orthologous genes (www.jax.org). Mouse models as tools have been very useful for unraveling the genetic basis of human deafness. These mouse mutants also provide extremely useful tools to elucidate genomic regulatory networks and represent a powerful dynamic proteomic model system to study human deafness. To our knowledge, only a few proteomic studies of the mouse inner ear have been conducted on mutant mice, such as tilted (*tlt*). *tlt* is characterized by vestibular dysfunction associated with specific otoconial agenesis. However, proteomic analysis of mouse mutants that are deaf due to defects in the neuroepithelia have yet to be undertaken (Hurle et al., 2003; Ornitz et al., 1998). The small structures of the inner ear are separated by relatively large fluid spaces within a hard bony shell, and these factors pose difficult challenges to ear researchers. For example, because the total number of hair cells (inner+outer) per mouse ear is only about 3300 (Ding et al., 2001), more than 300 ears would be required to obtain the 1,000,000 hair cells necessary to extract microgram quantities of protein. Because hair cells must be isolated from a large number of ears and because of their enclosure by bone, neither laser capture microdissection (Lee et al., 2003) nor fluorescence-activated cell sorting (FACS) (Yang et al., 2004) would be a simple approach to collect a sufficient number of cells without damage due to processing, including in the case of laser capture fixation, sectioning, and microdissection. An easier approach that takes advantage of mouse mutants lacking hair cells would be an important step to circumvent the problems discussed earlier.

1.3. *Gene expression at the mRNA level and why we need to study gene expression using a proteomic approach*

In the past few years, the development of array-based methods for the analysis of genome-wide expression at the mRNA level has helped to generate a more integrated view of the relationship between the genome, gene expression, and phenotypes (Cui and Churchill, 2003). Recently, differential gene expression microarray analysis has contributed significantly to the identification of a downstream gene that is controlled by the *Pou4f3* transcription factor (TF) in the ear (Hertzano et al., 2004). The array-based experiments permit the measurement, simultaneously and semiquantitatively, of changes in gene expression between two different biological states. However, interpretation of the connection between changes in the mRNA expression level and phenotype is often complicated for several reasons: (1) relative differences between mRNA and protein turnover, i.e., a protein can still