Meeting report

Complexity and integration in the control of inner-ear development

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A report on the Sixth Molecular Biology of Hearing and Deafness Conference, Hinxton, UK, 11-14 July 2007.

A meeting held this summer at the Sanger Centre outside Cambridge (UK) focused on the molecular biology of hearing and deafness. Although several proteins are uniquely expressed in the vertebrate inner ear, these are primarily found in the sensory hair cells, where they form key components of the mechanosensitive stereocilia (the 'hairs') of the organ of Corti in the cochlea. In general, inner-ear development is guided by the same suites of genes that control development in other neurogenic tissues, making the inner ear a good model system for studying the genetic regulation of development. In this light, we will focus here on discoveries relating to morphogenesis and cell-fate determination, the control of gene expression by microRNAs, identification of deafness-associated genes, and implications of developmental studies for the remediation of hearing loss.

Inner-ear development

Many studies have looked at the overlapping roles of fibroblast growth factors (FGFS) in inner-ear development. The work on chick presented by Raj Ladher (RIKEN, Kobe, Japan) was unusual in focusing on the morphogenetic response of the otic placode to FGF signaling. As might be predicted from morphogenesis in other tissues, a key component of the transformation from placode to otic vesicle is a change in cell shape. Ladher reported that FGF signaling polarizes the distribution of cytoskeletal proteins within the placodal cells, and that this polarization underlies the shape change. The implications of these results extend beyond otic placode invagination to gastrulation and neurulation, two of the most basic morphogenetic events in vertebrate development.

Another key event in ear development is the determination of which cells will become sensory hair cells and which will become non-sensory supporting cells. A study in chick by Angelika Dötzlhofer (House Ear Institute, Los Angeles, USA) showed that this cell-fate determination is somewhat more complex than just toggling the switch for the ubiquitous Notch signaling pathway, which is involved in determining neural cell fate in other situations. In particular, she showed that cell fates may be dependent on the expression of Hes/Hey transcription factors, which can be regulated by both Notch-dependent and independent mechanisms. As a consequence, sensitivity to disruption of the Notch signaling pathway may differ between cell types expressing Notch-independent or Notch-dependent Hes/Hey genes. This differential responsiveness to Notch signaling might be important to achieve the complex cytoarchitecture of the mature organ of Corti.

The most striking demonstration that different cell fates can result from differential expression of the same few genes was presented by Matthew Kelley (National Institute on Deafness and other Communication Disorders, NIH, Bethesda, USA). In this study, manipulation of FGF signaling in mice revealed interactions between developing inner hair cells and the adjacent supporting cells during development. More remarkable was the demonstration that a separate signal emanates from the Hensen's cells of the cochlea, creating a second signaling gradient in the opposite direction to the FGF gradient emanating from the developing inner hair cells. Thus, there are two cues for position relative to the cochlear axis, which could account for the radial orientation of stereocilia bundles and differences along the radial gradient. Previous work by Kelley and colleagues accounted

for the alternation of hair cells and supporting cells in the auditory epithelium; this new work addresses differentiation within these cell types and the radial organization and ordering of subtypes. The new findings could have implications for hair-cell regeneration. Supporting cells, which often survive traumas that kill sensory hair cells, may have encoded positional information and might respond accordingly when stimulated to transdifferentiate into hair cells. Alternatively, the signaling gradients might be constitutively expressed and cells are oriented by these signals during transdifferentiation.

MicroRNA and the inner ear

New at this year's meeting was a session on microRNAs (miRNAs), a highly conserved class of small noncoding RNAs that negatively regulate gene expression by an RNA interference (RNAi) mechanism. They are produced from primary RNA transcripts by ribonuclease III family members Drosha and Dicer. Although miRNAs are known to influence cellular proliferation and differentiation, little is known about their function in mammalian inner-ear development. Several talks examined the links between miRNA expression, ear development and deafness.

Garret Soukup (Creighton University, Omaha, USA) addressed the function of RNAi and miRNAs in mouse inner-ear development using a conditional *Dicer* knockout. Preliminary results indicate that disruption of the RNAi pathway results in severe morphological defects, suggesting a key role for miRNAs in early inner-ear development. Donna Fekete (Purdue University, West Lafayette, USA) discussed the treatment of zebrafish embryos with antisense morpholino oligonucleotides directed against the miRNAs miR-96, miR-182 and miR-183, which are expressed in the lateral line of zebrafish and inner-ear sensory epithelium of both mouse and zebrafish. Treatment with these morpholinos both separately and in combination led to significant reduction in numbers of hair cells in the anterior and posterior macular epithelium of the embryonic inner ear without apparent changes in the embryonic stage or size of the sensory organ. Fekete reported, however, that overexpression of a double-stranded miR-96 RNA causes an increase in numbers of posterior macular hair cells and the precocious appearance of hair cells in the sensory cristae of the semicircular canals that detect head rotation. She suggested that the miRNAs may downregulate the translation of pro-sensory gene transcripts to facilitate the transition from a pro-sensory state to a hair-cell state.

MicroRNAs can also be linked to known deafness-related genes. Using bioinformatics and microarray techniques, Karen Avraham (Tel Aviv University, Tel Aviv, Israel) and her colleagues screened the mouse genome for predicted miRNA genes located near deafness-related genes or loci. Assuming functional conservation during vertebrate evolution, they also looked for murine homologs of known zebrafish miRNAs with high levels of expression in the zebrafish ear and lateral line. Avraham reported that some of the candidate deafness-related miRNAs thus identified in the mouse are differentially expressed over time, or are expressed differently in the cochlea and the vestibular organs. In addition, they found that some miRNAs not previously linked to deafness are expressed in the sensory epithelia of the newborn (postnatal day 0) mouse inner ear. Mutation analysis reported by several groups at the meeting also implicates miRNAs as critical regulators of mammalian hair-cell development. It remains unclear what genes are regulated by the miRNAs.

Deafness-related genes in humans

In the past 15 years, hundreds of human genes associated with hereditary deafness have been identified, and complementary studies in the mouse and zebrafish provide an avenue for understanding the roles of these genes in ear development and function. Two new protein-coding genes implicated in human deafness were described at the meeting. Fatemeh Alasti (National Institute for Genetic Engineering and Biotechnology, Tehran, Iran, and University of Antwerp, Belgium) reported the identification of a novel homeobox gene responsible for syndromic microtia (small ear) in an Iranian family. Rob Collin (Radboud University, Nijmegen, The Netherlands) reported the identification of mutations in *ESRRB* (encoding estrogen-receptor related β protein) by genome-wide analysis of single-nucleotide polymorphisms (SNPs) in a Turkish family and subsequently in the original Pakistani DFNB35 family, both of which have heritable autosomal recessive non-syndromic deafness. As reported by several groups in the UK and Australia, mouse N-ethyl-N-nitrosourea (ENU) mutagenesis screenings are now focused on identification of mutations in a recessive trait to explore molecules and models for recessive genes of human deafness.

Most genes known to be associated with human deafness have been identified in geographically isolated populations or small families with monogenic mutations. The elucidation of genes contributing to complex-trait hearing impairment is in its infancy and is far behind the investigation of the genetics of other complex diseases. Gene-environment interactions and ethnicity, age, gender and exclusion criteria (whether related to other diseases, asymmetric hearing loss, and so on) are all important factors to be considered. Lut van Laer (University of Antwerp, Belgium) reported that studies of SNPs in candidate genes found that age-related hearing impairment was associated with SNPs in the gene for the transcription factor grainyhead-like 2 (*GRHL2/DFNA28*) in several populations. In addition to the group's previous findings of an association of the genes for a voltage-gated potassium channel (*KCNE1*) and a catalase (*CAT*) with noise-induced

hearing loss, Annelies Konings **(**University of Antwerp, Belgium) reported a new association of hearing loss with *HSP70* (heat shock protein 70) in two populations.

Hair-cell regeneration

All vertebrates have hair cells, but only in mammals are the hair cells and supporting cells of the auditory epithelium highly ordered, and only mammals lack the ability to replace hair cells killed by toxins or loud noise. Several talks addressed the question of how differences in ear development between mammals and other vertebrates may account for this lack of regenerative ability.

Bernd Fritzsch (Creighton University, Omaha, USA) pointed out that the decision of cochlear cells to become hair cells is first indicated by their exiting the cell cycle. He also argued that Atoh1, a transcription factor that has been the focus of several hair-cell regeneration studies, is not likely to play a role in that process because it is not expressed before cell-cycle exit. He also discussed the need for a comprehensive model of gene regulation during cochlear development in order to understand regeneration, highlighting the roles of three transcription factors known to be important in normal development of the chicken and mouse inner ear: Prox1 (innervation), Gata3 (cochlear elongation) and Lmx1a (cochlear histogenesis).

Michael Lovett (Washington University, St. Louis, USA) presented microarray data from chickens showing that elements of several signaling pathways known to be important during inner-ear development are also activated during regeneration after deafening, including the Notch, Wnt and TGFβ pathways. These results will serve a reference studies for investigations into whether alterations in these pathways account for the failure of regeneration after deafening in mature mammals.

After noise or ototoxic chemicals kill hair cells, it is the supporting cells that must react to the absence of hair cells by sending and responding appropriately to repair and regeneration signals. Several presentations discussed factors influencing the ability of supporting cells to respond to the signals they receive during repair and regeneration. Nicholas Daudet (University College London Ear Institute, London, UK) reported on the activation of the Notch pathway during hair-cell regeneration in chickens and speculated that it may preserve a pool of supporting cells for future repopulation of the depleted auditory epithelium. Jeffrey Corwin (University of Virginia, Charlottesville, USA) demonstrated that cultures of chick utricular supporting cells can be perpetuated if they can be induced to separate from the substrate. They will then form hollow spheres and become polarized, which is critical for the subsequent differentiation of hair cells in those cultures. Azel Zine (University of Montpellier, France) described how several types of cochlear supporting cells from the postnatal mouse could be induced to express stem-cell markers, divide and redifferentiate in culture.

These results all suggest that cochlear supporting cells can be made receptive to signals for hair-cell development, but does not explain why they are normally unresponsive. Yehoash Raphael (University of Michigan, Ann Arbor, USA) illustrated morphological differences between supporting cells in deafened guinea pigs exposed to different deafening agents, and also showed that expression of several cell-signaling markers varies with both deafening agent and time since exposure to the agent. These results raise the intriguing possibility that the receptiveness of supporting cells to such developmental signals might depend on the mechanism of deafening and the amount of time after deafening. The findings may indicate that the failure of supporting cells to regenerate hair cells in the mammalian ear has different causes under different circumstances.

Over the past decade we have witnessed a tidal wave of discovery of hundreds of deafness genes in human families and mouse models and have gained tremendous understanding of the roles of these genes in inner-ear development and function. This meeting signaled a new era of hearing research, highlighted by recent discoveries in the contribution of miRNA, polygenic variations and interactions, molecular signaling, and last, but not least, increasing hope for treatment for hearing loss and deafness.

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