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Deafness

Lack of regulation encourages hair cell growth

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Hearing loss and balance disorders affect millions of people worldwide. In most cases, degeneration of hair cells (HCs), the sensory cells of the cochlea and the vestibular end organs is the cause of these disorders. New auditory HCs do not develop after embryonic development, and once lost, these cells are not replaced. So it is truly exciting when experimental attempts to generate new HCs succeed. In a paper published in Science Express on January 13, Sage *et al*[1] show that conditional deletion in the inner ear of a gene involved in cell cycle regulation, the mouse retinoblastoma gene (Rb1), leads to the generation of a large number of new HCs in addition to the normal population of these cells. This finding points the way to a genetic approach for generating new HCs in the inner ear as a treatment for deafness.

Rb1 is a tumor suppressor gene, mutation of which causes loss of cell cycle regulation and tumors in many tissues.[2] To achieve disruption of Rb1 in the inner ear, the authors crossed floxed Rb1 mice with collagen1A1 (Col1A1)-Cre mice, and analyzed ears in the F1 embryos for changes in cellular phenotype and function. Mutant Col1A1-pRb$^{-/-}$ ears exhibited significantly increased numbers of HCs, both in auditory and vestibular epithelia.

From a gene therapy perspective, perhaps the most exciting aspect of these results is that gene transfer methods might help translate them into clinically relevant treatments. As reported in their paper, Sage *et al* used Cre-loxP technology to examine the role of Rb1 in the control of cell proliferation during embryonic development of the inner ear. Gene transfer approaches have the potential to accomplish similar genetic manipulations in mature living animals, and so induce the growth of new HCs in ears with few or no remaining HCs. The challenge for such gene therapy approaches is to develop vectors that can reliably and efficiently target inner ear cells and induce a similar effect to the one that the Cre-loxP approach shows, without substantial side effects.

Several other recent studies have also demonstrated promising approaches for the generation of new HCs. One attractive possibility for generating new HCs involves the introduction of exogenous cells into the cochlea.[3,4] Such cells might be derived from embryonic stem cells or ear-specific stem cells, and would need to integrate into the sensory epithelium, differentiate, and function as replacement HCs. An alternative approach is to genetically or pharmacologically influence endogenous cochlear cells to produce new HCs.[5] Manipulation of endogenous cells could be aimed either at sensory or nonsensory cells that remain in the deaf ear. The generation of new HCs from nonsensory inner ear cells would require a phenotypic conversion of nonsensory cells into new HCs.[6]

Genetic manipulation in order to enhance proliferation and generation of new HCs has already been reported. One recent example involves the Cip/Kip family member p27^{Kip1}, which plays a critical role in cell cycle arrest and in maintaining the differentiated phenotype of supporting cells.[7,8] Cells in the mouse organ of Corti typically cease proliferating around e14.5, after which time supporting cells and HCs start to differentiate and assume their final positions and functions in the cochlea. In p27$^{Kip1-/-}$ mice, organ of Corti cells continue to proliferate for more than 2 weeks after e14.5, demonstrating a role for p27^{Kip1} in suppressing mitosis in the cochlear epithelium.[7,8] Similarly, encouraging for advocates of gene therapies for deafness, are the recent results from the lab of one of us (YR), which show that such approaches (in this case overexpression of *Atoh1*) can generate new HCs and improve hearing thresholds in mature deaf mammals.[9] Collectively, these studies and the report by Sage and co-workers demonstrate the power and potential of genetic manipulations of cochlear cells, raising the hope that effective therapies for HC regeneration might someday be feasible.

Despite the excitement surrounding these findings, the study by Sage and co-workers has several limitations that will need to be addressed in future studies. As Col1A1-Rb$^{-/-}$ mice die perinatally (probably due to loss of Rb1 in other Cre-expressing tissues), functional analysis of the newly generated HCs in Col1A1-Rb$^{-/-}$ mice was limited to *in vitro* assays such as transduction currents and FM1-43 dye uptake to assess HC mechanotransduction. The authors determined that transduction currents in mutant HCs were $\sim$10% of normal values, which may or may not indicate diminished functionality in an animal. This result is consistent with a previous study of mice with disruption of the p27^{Kip1} gene: these mice had supernumerary HCs but never attained hearing function despite their survival into adulthood.[8] Sage and co-workers also conclude that newly generated HCs in Col1A1-Rb mutant mice arise from previously formed HCs. This conclusion needs further testing, because an equally plausible explanation is that supporting cells in the tissue undergo interkinetic nuclear migration and account for the mitotic figures identified in these cochleae.

Future research could productively focus on mating lineage specific mice that express Cre only in HCs or supporting cells to Rb floxed mice and analysing the offspring. These analyses might point the way to means for overcoming the early lethality of Rb1 mutations, and for defining the source of newly formed HCs. Such an approach would also potentially enable functional and behavioural analysis in live animals, and validate these exciting findings for future studies and clinical applications. In addition, vector-based approaches to further modulate HC biology (RNAi, etc) could be very helpful for targeting genes to the ear.

The paper by Sage *et al* is highly significant for its interesting and truly important observation that an increased number of HCs appear with loss of Rb1 function. This finding is crucial to the development of clinical treatments for generating new HCs. If, as the authors suggest, HCs themselves can indeed replicate, patients with some remaining HCs would be the direct beneficiaries. Clinically, it would be even more exciting if supporting cells could divide and some of the progeny differentiate into new HCs, because ears with profound deafness (or vestibular deficits) contain supporting cells but few or no surviving HCs. For gene therapy studies, the major challenge now is to test whether the mature inner ear can respond to targeted silencing of pRb and generate new, functional HCs, and whether pRb dysfunction predisposes the ear to other pathologies such as tumor formation. ■

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