FUTURE THERAPIES FOR INNER EAR REGENERATION

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One in ten (30 million) Americans has hearing loss
- Causes include heredity, aging, noise exposure, disease
- Number is expected to double by 2030

Hearing loss is the #1 birth defect in America
- 1 in 1000 newborns is born profoundly deaf
- 2-3/1000 will have partial/progressive hearing loss

Hearing loss prevalence increases with age
- 1 in 3 over 65 years has significant hearing loss
- Among seniors, hearing loss is the 3rd most prevalent condition

The inner ear

The hair cell

Sensory hairs vibrate, "tip-links" open ion channels into hair cell
Ions flow into hair cell, changing its electrical potential
The nerve fiber

Electric potential causes chemical neurotransmitter release from synapse.

Neurotransmitter diffuses to nerve fiber and excites electrical activity in the form of action potentials.

Sensorineural hearing loss:

Hair cells and nerve fibers

Cochlear implant can directly stimulate.

Sensory Cell Loss

Nerve Fiber Loss

Regeneration of hair cells in chick inner ear

Can stem cell-derived inner ear progenitors replace lost hair cells in vivo (and restore hearing)?

Li et al., TMM (2004)
Approaches to regenerating inner ear cells

I. Generation of inner ear cells by gene therapy

II. Generation of inner ear cells from stem cells
   - in vitro followed by cell grafting
   - from endogenous cells in vivo

Gene therapy

- New hair cells: transfer Atoh1 gene
- Cell division of existing hair cells: silence Rb gene

Transfection of Atoh1 leads to new hair cells

Overexpression of Atoh1 and formation of new hair cells

Zheng et al

Izumikawa et al
Gene transfer of Atoh1 during development leads to new hair cell formation

Silencing of gene that controls cell division leads to new hair cells

Generate new hair cells from stem cells

- **Approach 1:**
  - Generation of inner ear cell types from stem cells in a dish
  - Injection of hair cells or neurons into the inner ear

- **Approach 2:**
  - Generation of hair cells or neurons from stem cells in the animal

Generate new hair cells from stem cells

Approach 1: Cells from exogenous sources

- Generate hair cells and neurons from exogenous stem cells
- Transplant cells into the inner ear
Embryonic Stem Cells

- Pluripotent (numerous cell types)
- Renewable
- No political issues for research use
- Avoid rejection problem because of autologous cells (from the same person)

Adult Stem Cells
Aim 3: Complete restoration

Grafting into de-afferented organ of Corti explants

Generate new hair cells from stem cells
Approach 2: Activation of inner ear stem cells

Can we activate genes that will lead to new hair cell or neuron formation?
Rebuilding the Human Inner Ear

MEEI research team finds stem cells in mammalian ears

Stem cells from adult mouse and human inner ear

Spheres form from individual stem cells and self-renew with a renewal rate of 2-3

Differentiation into hair cells

Differentiation into neurons and glial cells

TuJ1
GFAP
Myosin7a
Math1

Red F-actin

Mixed single cell suspensions from ROSA26 mice and C57BL6 mice do not form mixed spheres
Identify compounds that increase hair cell formation

Strategy: Look for compounds that activate Atoh1

progenitor cell → hair cell
Luciferase under Atoh1 promoter
No signal → Positive for luciferase

Find a drug that increases the yield of hair cells

Gene switches in the pathway turned on or off by small molecules

Drug screening facility
- Facility is well equipped
- Protocol approved for use of libraries and equipment

Screening Atoh1 reporter cells
Compounds increase hair cell differentiation from inner ear derived stem cells

Myo7a Math1-GFP TOTO

0000489 0000540

Many newer agents will:
--- have undesirable systemic effects
--- be highly specific, unstable molecules needed to direct repair
--- require timed, sequenced delivery

Such agents will not lend themselves to systemic delivery.

Getting drugs to their targets: systemic vs. local delivery

For therapies based on these discoveries to become clinically useful, need to develop safe and reliable methods for delivery of complex compounds *directly* into the inner ear.

Challenges for drug delivery into the cochlea

Cochlea is protected from most drugs applied to the bloodstream.
Cochlear fluid space is very small and sensitive to changes in fluid volume.
Useful drugs may be unstable over long periods of time in solution.
Frequent drug refills may introduce bacterial contamination.

Better hearing through chemistry: inner ear drug delivery

*Blood-Cochlea Barrier:*

Prevents ready access to cochlea (good and bad)

Future therapies based on complex compounds will benefit from *direct* intracochlear delivery
Rebuilding the human inner ear

Working with MIT engineers at Draper Lab

Our Vision
- a small implantable device to deliver drugs to the ear for several years
- a reservoir of dried, concentrated, stabilized drug
- timed, sequenced release of multiple drugs under microprocessor control.