Otology seminar

Cell transplantation in hearing repair

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Introduction

- Pathogenesis of hearing loss:
  - A variety of environmental and genetic causes lead to **hair cell** loss
  - Degeneration of spiral ganglion **neurons** may occur as a secondary (":"hair cell loss) or primary event
- **1988** Corwin and Cotanche, and Ryals and Rubel

Hair cells could regenerate in the sensory epithelium of birds and amphibians

- **1993** Forge et al., Warchol et al., 2001Montcouquiol and Corwin

Hair cells could regenerate in the mammalian vestibular system

- **1997** Puel et al. and 2003 Sekiya et al.

The nerve endings regrow after damage by glutamate toxicity in guinea pigs

- **2005** Sugawara et al.

Experimental sectioning of the auditory nerve in mice leads to extensive regrowth of fibers into the cochlea
Anatomy of the Cochlea and Auditory Neurons

- The auditory nerve: a bundle of bipolar auditory neurons
- Cell bodies: in Rosenthal's canal in the temporal bone
<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>92%</td>
<td>10-20 type 1 neurons converge on each inner hair cell</td>
</tr>
<tr>
<td>Type 2</td>
<td>8%</td>
<td>Type 2 auditory neuron contacts 30-60 outer hair cells</td>
</tr>
</tbody>
</table>

**Inner hair cells:** the primary sensory receptors
<table>
<thead>
<tr>
<th></th>
<th>myelin sheaths</th>
<th>supporting tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>oligodendrocyte</td>
<td>astrocyte</td>
</tr>
<tr>
<td>PNS</td>
<td>Schwann cells</td>
<td></td>
</tr>
</tbody>
</table>

Cells coming from the central side can pass the TZ (Obersteiner-Redlich zone)
## Clinical Damage

<table>
<thead>
<tr>
<th>Auditory neuron</th>
<th>exposed during operation</th>
<th>vestibular schwannomas, for microvascular decompression procedures, hemifacial spasm and trigeminal neuralgia…</th>
</tr>
</thead>
<tbody>
<tr>
<td>hereditary sensorimotor neuropathy</td>
<td>Charcot-Marie-Tooth disease, Friedreich's ataxia, Mohr-Tranebjaerg syndrome, Refsum's disease, the mitochondrial disorders…</td>
<td></td>
</tr>
<tr>
<td>Hair cells</td>
<td>presbycusis, Ototoxic drugs, acoustic overstimulation</td>
<td>Outer hair cells: more sensitive than inner hair cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>high-frequency end of the cochlea: more sensitive</td>
</tr>
</tbody>
</table>
Molecular Embryology

- Hindbrain
  - → otic placode
  - → otocyst (=otic vesicle)

- Ventral epithelial-cell phenotypes: induced by **Six1** (*sine oculis homeobox homolog 1*) and **Shh** (*Sonic hedgehog*)

- Day E9: neuroblasts
  - → delaminate
  - → basement membrane, mesenchyme
  - → auditory and vestibular ganglia
Molecular Embryology

- Differentiation of **Auditory Neurons**
  - A. Ngn1, NeuroD, GATA binding protein 3, Tbx1
  - B. Neurotrophins and their receptors
  - C. Electrical activity

- Differentiation of **Hair Cells**
  - A. Atoh1, Hes1, Hes5, and Zic1
  - B. Notch signaling
  - C. Hair Cells from Supporting Cells
  - D. Cell Cycle (p27kip1, Ink4d, Rb1)
Molecular Embryology - Differentiation of Auditory Neurons

Otic progenitor cells

Notch signal

Atoh1

Zic1

Supporting cells
p27kip1, cytokeratins, α and β-tectorin, Jag1, Hes-1, Hes-5, Prox1, S100A1
Pillar cell - Fgf3, p75

Hair cells
Ink4d, BDNF, NT-3, Atoh1, Pou4f3, Myosin VIIA, espin, α9, 10AchRs, Parvalbumin 3, Jag2, Lfnf
Outer hair cell - Prestin

Vestibular ganglion cells
NeuroD(+), GATA3(−)
TrkB, TrkC

Auditory ganglion cells
GATA3(+), NeuroD(−)
TrkB, TrkC

Neuroblasts
NeuroD, Islet1/2, BDNF, tubulin, Foxg1, GATA3, FGF-10

Type 1 auditory neurons
Type 2 auditory neurons

Nervous system

Otic Epithelium
### A. Ngn1, NeuroD, GATA binding protein 3, Tbx1

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function/Effect</th>
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<tr>
<td>Ngn1</td>
<td>Differentiation of all inner-ear sensory neurons, differentiation of sensory hair cells and supporting cells</td>
</tr>
<tr>
<td>Tbx1</td>
<td>↓ Ngn1-positive neuronal precursors, ↓ auditory and vestibular ganglion</td>
</tr>
<tr>
<td>NeuroD</td>
<td>↑ Ngn1 (in the otocyst and in delaminating neuroblasts)</td>
</tr>
<tr>
<td>GATA3</td>
<td>Regulates NeuroD in auditory neuroblasts</td>
</tr>
</tbody>
</table>

<table>
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<th>NeuroD</th>
<th>GATA3</th>
</tr>
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<tbody>
<tr>
<td>neuroblasts</td>
<td>+</td>
</tr>
<tr>
<td>auditory ganglion neuron</td>
<td>-</td>
</tr>
<tr>
<td>vestibular ganglion neuron</td>
<td>+</td>
</tr>
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</table>
B. Neurotrophins and their receptors

- Hair cells provide much of the trophic support: brain-derived neurotrophic factor (BDNF), neurotrophic factor 3 (NT-3), tyrosine kinase receptors (trk) B and C

  - In Pou4f3-null mutant mice
    - hair cells incomplete differentiation → hair cell degeneration → auditory neurons degeneration (∵ loss trophic support)

- Other trophic factors: glial cell-derived neurotrophic factor, ciliary neurotrophic factor, and fibroblast growth factor (FGF)-1 and-2
C. Electrical activity

- Membrane depolarization
- $\uparrow$ voltage sensitive Ca2+ channels
- $\uparrow$ intracellular Ca2+
- $\uparrow$ prosurvival signaling pathways
  (such as Ca2+/calmodulin dependent kinases II and IV and protein kinase A)
Molecular Embryology-
Differentiation of Hair Cells
# A. Atoh1, Hes1, Hes5, and Zic1

| Atoh1 (Math1) | 1. Atoh1 knockout mice lack sensory hair cells  
        2. Transfection of Atoh1 into postnatal rat cochlear cultures → ectopic hair cells | absolutely required for hair-cell specification in mice |
|---------------|------------------------------------------------------------------------------------------|------------------------------------------------------|
| Hes genes     | knockout of Hes1 >> ↑ inner hair cells  
        knockout of Hes5 >> ↑ outer hair cells | negative regulators of hair cell differentiation |
| Zic genes     | The binding of Zic1 to the Math1-enhancer region inhibit Math1 expression in the chicken neural tube | regulate hair-cell development |
B. Notch signaling

- Balance expression of Atoh1, Hes1, and Hes5.
- Ligands for Notch receptors (such as Delta, Jagged, or Serrate...) mediate reciprocal interactions from hair cells and supporting cells

- High level Delta ligand in a hair cell
  - ↑ Notch receptors in the adjacent supporting cells
  - ↑ Hes genes
  - ↓ express Atoh1
  - ↑ supporting cells
C. Hair Cells from Supporting Cells

- In mice: supporting cells → new hair cells
  - The deafened auditory epithelium
    (4 days after ototoxic lesion)
    → adenoviral vector, Atoh1 → scala media → induced regeneration of hair cells → functional recovery
    (evaluated with ABR)

- Most cases, both hair cells and dendrites are lost
- Adenoviral vectors: cytotoxic, immune response.
- An ectopic production of hair cells
D. Cell Cycle (p27kip1, Ink4d, Rb1)

- Cell cycle inhibitors (such as p27kip1): reentry of supporting cells into the proliferation phase
- Ink4d and Rb1 are required for regulating the cell cycle in hair cells.
- Rb1 inactivation: proliferation of hair cells.
Candidates for Cell replacement-
Auditory Neurons

   - Day E10.5, mouse otocyst, auditory neuroblasts
     → express markers found in neuroblasts
     → FGF-1 or FGF-2 in vitro
     → bipolar cells (similar to auditory neuron)
     → partially crushed auditory nerve
     → migrated peripherally and centrally

2. Rask-Andersen et al.
   human auditory ganglion cells
   (from petroclival meningioma surgery)
   → neural progenitor/stem cells
   → graft material in cell therapy
Candidates for Cell Replacement - Hair Cells

- Hair cell replacement:
  ES cells, adult inner ear stem cells, neural stem cells....

1. Hair Cells from **ES Cells**

- Murine ES cells
  → epidermal growth factor (EGF), insulin-like growth factor-1, FGF-1
  → Hair-cell progenitors, in vitro
  → integrated into the developing chick otocyst
  → markers for hair cell phenotype
  → **hair bundles** (characteristic of mature hair cells)
ES cells → Neural Progenitors → Neurons

In vivo

- Quabain
- 64 days

Normal cochlea and cochlear nerve

Cell implantation after toxin damage to cochlear nerve

Neural processes from the differentiated neurons to the organ of Corti
2. Hair Cells from Adult Inner Ear Stem Cells

Adult murine vestibular sensory epithelia

Features of stem cells:

- (a) self-renewal
- (b) multipotency
- (c) form spheres express markers
  - hair cells (myosin VI1a and Pou4f3)
  - supporting cells (pancytokeratin and p27Kip1)
3. Hair Cells from **Neural stem cells**
   From forebrain
   → inner ear (survive at least 4 weeks)
   → integrate into inner ear structure
   - Grafted into the drug-injured mouse inner ear
     → survived for several weeks

4. Cells from the **newborn rat organ of Corti**
   → EGF or FGF2
   → otospheres (consisting 98% nestin-positive cells)
   - In the injured postnatal organ of Corti, 
     Nestin-positive cells: a source of newly generated 
     hair cells and supporting cells
Cell-Transplantation Techniques - Auditory Nerve (as route and host) Approach

- Approach: retromastoid craniectomy

- Embryonic stem (ES) cells
  → transplanted at CPA
  → auditory nerve
  → TZ
  → toward the cochlear side
  → extending neurites peripherally
toward the hair cells
• In small experimental animals: the cochlear wall is directly sighted in the tympanic bulla
• Human cochlea: embedded deep in the temporal bone
Cell-Transplantation Techniques – Cochlear Approach

• The initial damage is to the hair cells: presbycusis, aminoglycoside ototoxicity, or noise-induced hearing loss.

• An auditory nerve approach should be considered in addition to the cochlear approach.
Cell-Transplantation Techniques – Cochlear Approach

A.B. Endolymphatic injection  
C. Perilymphatic injection  
D. Auditory nerve trunk
### Disturbing the homeostasis of the inner ear fluid environment

<table>
<thead>
<tr>
<th>Injection Type</th>
<th>Delivery Pathology</th>
<th>Potential Complications</th>
</tr>
</thead>
</table>
| Endolymphatic injection | Lateral wall of the cochlea or vestibular aqueduct → scala media                  | 1. Endolymphatic structures injury  
2. The injected materials → vestibular portion of the membranous labyrinth |
| Perilymphatic injection | Cells, viral vectors, or pharmacological agents                                    | 1. Perilymphatic fluid fistula  
2. Injected materials → cochlear aqueduct → CSF → contralateral ear >> cause unintended side effects |
| Round window niche      | Placement site for diffusible materials                                             | 1. Doses of drugs infiltrating the perilymphatic space are difficult to evaluate  
2. Nondiffusible materials cannot be transferred into the membranous labyrinth |
| Auditory nerve trunk    | from the cochlear side                                                             | Risk of hearing loss                                                                  |
Clinical Translation and Future Trends

- The quality control in all experiments
- The surgical technique and delivery methods
- Selection and preparation of donor cells:
  possibility of teratoma, carcinogenesis
- In vitro: ES >> neurites >> hair cell (morphology)
  In vivo >> function?
Reference


