Gene therapy for the restoration of cochlear sensory cells after noise-induced hearing loss

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Action on Hearing Loss
Hearing loss

Loss of hair cells
Loss of supporting cells
Degeneration of auditory neurons

Main research aim: To protect or regenerate sensory cells after hearing loss
Gene therapy

Gene therapy into the scala media after hearing loss

Adenovirus

Targets the sensory epithelium of the cochlea

Targets the high frequency region of the cochlea

Delivers therapeutic genes to sensory cells (e.g. neurotrophins)
Gene therapy

Target cells in normal and degenerating epithelium

Neurotrophin genes protect neurons

Neurotrophin genes protect hair cells

Protection of sensory cells after hearing loss

What about regeneration?
Hair cell regeneration

Atoh1 gene: a transcription factor essential for hair cell development

Introduction of Atoh1 into the sensory epithelium induces supporting cells to move towards hair cell phenotype

Supporting cells must be present after deafness for hair cell conversion

• In *ototoxic deaf* model supporting cells die by day 4
• In noise-induced hearing loss model supporting cells are present for much longer

Noise-induced hearing loss results in hair cell loss in high frequency region of cochlea

Gene therapy targets high frequency region of the cochlea

→ Atoh1 gene therapy in a noise-induced hearing loss model
Aim

To regenerate hair cells from residual supporting cells after high frequency noise-induced hearing loss using Atoh1 gene therapy

Groups

- 2 wk deaf control guinea pigs (n=3)
- 5 wk deaf control guinea pigs (n=3)
- GFP control gene therapy guinea pigs (n=4)
- Atoh1 gene therapy guinea pigs (n=4)
Methods

T-1: ABR to check for normal hearing
T0: Closed-field noise exposure
T2: ABR then gene therapy (Ad-GFP or Ad-Atoh1)
T5: ABR then perfusion

T-1wk:
ABR

T0:
Expose to noise

T2wk:
ABR
Inject gene therapy vector

T5wk:
ABR
Perfuse

130 dB
11,12,13 kHz (Upper basal turn region)
2 hours

Adenovirus type 5 with CMV promoter
(1x10^{11} viral particles/ml)
Inject 2 μl into scala media
Hair cells and supporting cells

State of the organ of Corti at the time of gene therapy

2 week group

5 week group

White=Myosin (hair cells)
Red=NFH (neurons)
Blue=phalloidin (supporting cells)
Hair cells and supporting cells

5 week group

Ad-GFP group

Ad-Atoh1 group

Upper basal

IHC

Lower basal

White=Myosin (hair cells)
Red=NFH (neurons)
Blue=phalloidin (supporting cells) or DAPI (nuclei)
Green=transgene (GFP or Atoh1)
Hair cells

Hair cells counted in the region of noise-induced damage (basal turn)

** p<0.001 one-way ANOVA

Normal ~ 110 HCs/mm basilar membrane
Hair cell co-localisation with Atoh1

Supporting cell to hair cell conversion occurs when Atoh1 gene expression is within a cell or in an adjacent cell.

Red=Myosin (hair cells)  
Blue=NFH (neurons)  
Green=transgene (Atoh1)

HC co-localises with Atoh1 GFP  
HC has direct contact with Atoh1 GFP
Appearance of hair cells

Basal location of hair cell
Rounded morphology
Hearing

Left side click and tone-pip ABR

5 week group  Ad-GFP group  Ad-Atoh1 group

No restoration of hearing with Atoh1 gene therapy
- Not enough new hair cells
- Position within the sensory epithelium is too basal
- No connection with auditory neurons
- No stereocilia

From Sterling and Matthews 2005
Gene therapy to the scala media is an ideal drug delivery technology for sensory cell protection or regeneration as it:

- Targets the high frequency region of the cochlea affected by noise
- Targets the sensory epithelium
- Targets the part of the cochlea that receives stimuli from a cochlear implant

We can protect and regenerate sensory cells after hearing loss
Further work

• Improving the transduction efficiency of gene therapy
• Further characterisation of hair cells – stereocilia, other hair cell markers, functional analysis
• Determine if hair cells are protected or regenerated - pre-label existing hair cells with a dye
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Conversion of supporting cells

Converted supporting cell have a nucleus with a more basal location than an existing hair cell
Supporting cells are found in the plane below hair cells

White=Myosin (hair cells)
Red=NFH (neurons)
Blue=phalloidin (supporting cells)
Green=transgene (Atoh1)

Mitzutari et al 2013 Neuron 77;58
Connectivity

Ctbp2 – Part of the hair cell synapse with auditory neurons

From Gubbels et al 2008

From Sterling and Matthews 2005

Reduced or absent Ctbp2 in Atoh1 hair cells
Summary

- After noise exposure there were supporting cells present in the sensory epithelium – targets for gene therapy
- Atoh1 gene therapy induced new hair cell formation in the lower basal turn of the cochlea after noise-induced hearing loss
- Hearing was not restored