Virtual BACO International

Advances in Hair Cell Regeneration for Hearing Loss: A review of the literature

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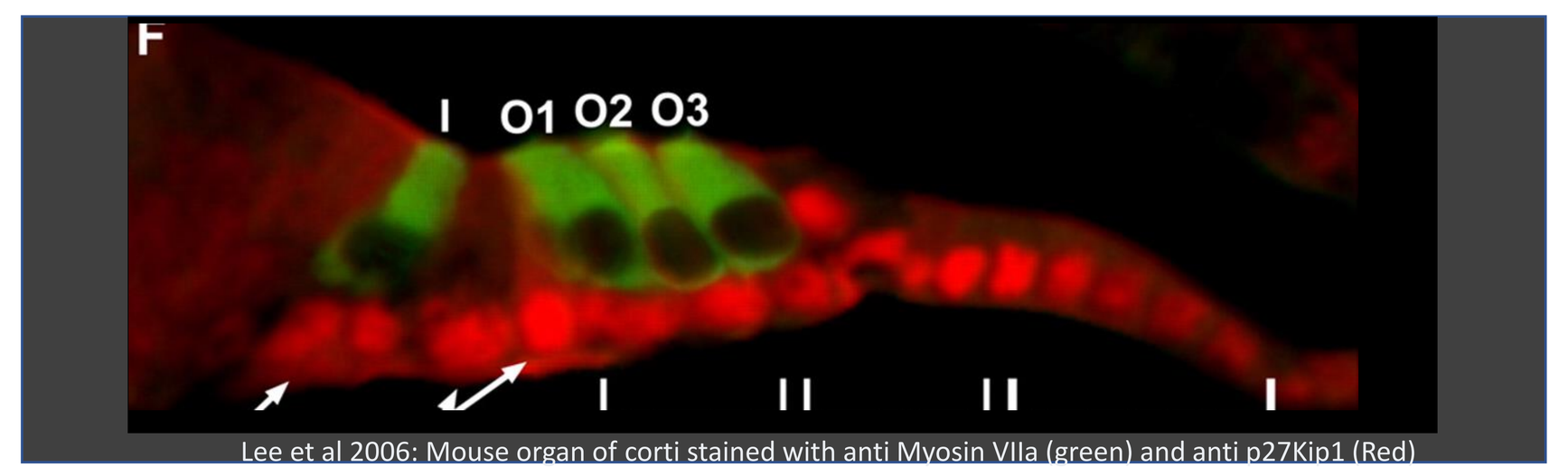
INTRODUCTION

Hearing loss affects 466 million people worldwide[1]. Sensorineural hearing loss accounts for 85% of cases, and damage to the hair cells of the human cochlea leads to irreversible hearing loss.

Advances in regenerative medicine and stem cell technologies have led to greater understanding of cochlear development and new approaches to treating sensorineural hearing loss. There is currently a limited number of human in vitro and in vivo studies



Lee et al 2006: Mouse organ of corti stained with anti Myosin VIIa (green) and anti p27Kip1 (Red)

BACKGROUND

It has long been accepted that the post-embryonic human cochlea does not regenerate.

However animal cochlea do show regenerative potential. Avian cochleae can fully recover after ototoxic injury[2] and more limited regeneration is seen in young rodents[3].

Current regenerative strategies include inducing Regeneration in situ, Stem cell transplant, and Gene therapies

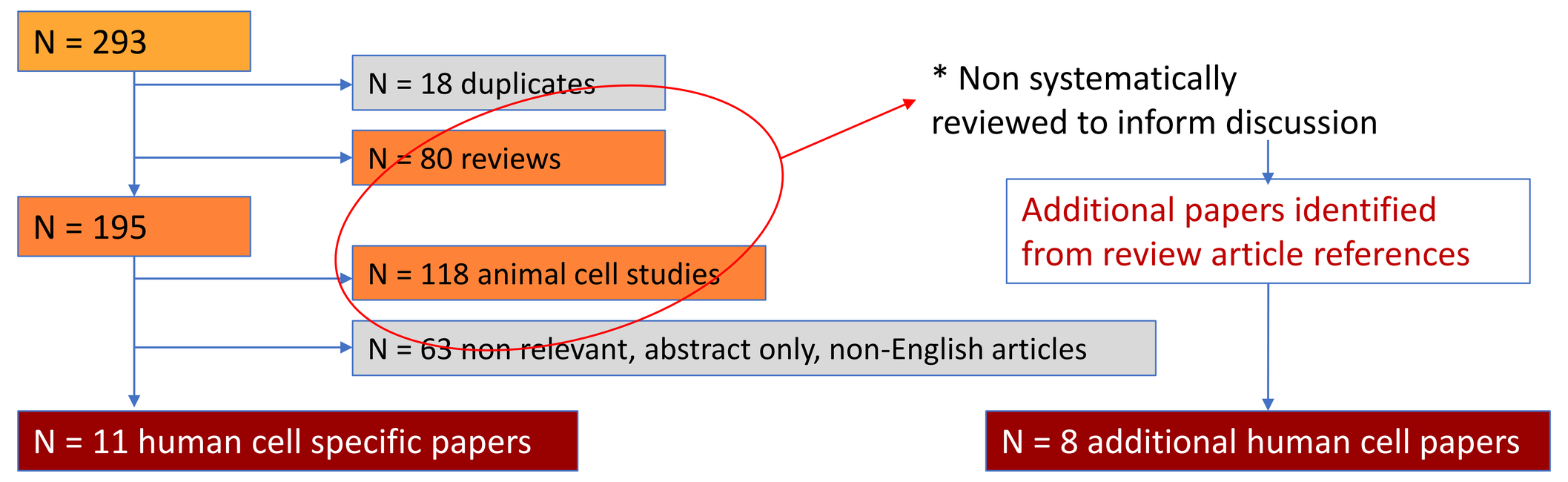
METHOD

Aims:

To systematically review the literature and evaluate the current state of progress in hair cell regenerative properties

Interrogation of Pubmed and Embase databases was performed on 4th Oct 2019 using the search terms: (hair cell[Title/Abstract]) AND ((((stem cell[Title/Abstract]) OR regenerate[Title/Abstract])) OR "regenerative medicine"[Title/Abstract])))

293 articles were returned, and duplicate articles, reviews, and non-english articles excluded. Titles and abstracts were screened to give 11 human cell specific relevant articles. Review articles and citation indexing provided 8 further relevant papers.



REGENERATION IN SITU

Do cochlear stem cells exist?

In rodents a proportion of cells from the organ of corti grown in suspension show stem cell characteristics, forming "spheroids" of self sustaining cells that can differentiate into both hair and supporting cells. Only three studies have tried to produce spheroids from human cochlear tissue.

- Chen et al 2009 - Human fetal (week 9-11) cochlear cells formed spheroids
- Massucci-Bissoli et al 2017 - Adult cochlear tissue formed spheroids expressing stem cell marker ABCG2
- Senn 2019 – unable to replicate results, their cochlear spheroids did not express stem cell or hair cell features, and appeared fibroblast-like

Stem Cell	Hair Cell
ABDG2	Prestin
Jagged1	Myosin VI
Notch1	Myosin VIIa
Musashi1	Math1
*markers observed in mouse studies	

Lgr5 signalling is thought to identify cochlear progenitor cells in rodents. However Lgr5 cells are a small population and no studies have been able to isolate Lgr5 expressing cells in the human cochlea.

Can hair cell regeneration be triggered by molecular signalling?

A number of growth factors have been shown to play a role in hair cell expansion and differentiation in normal development. These include WNT, β-catenin, Atoh1, EGF, IGF and FGF. In contrast p27kip and SOX2 appear to be inhibitory.

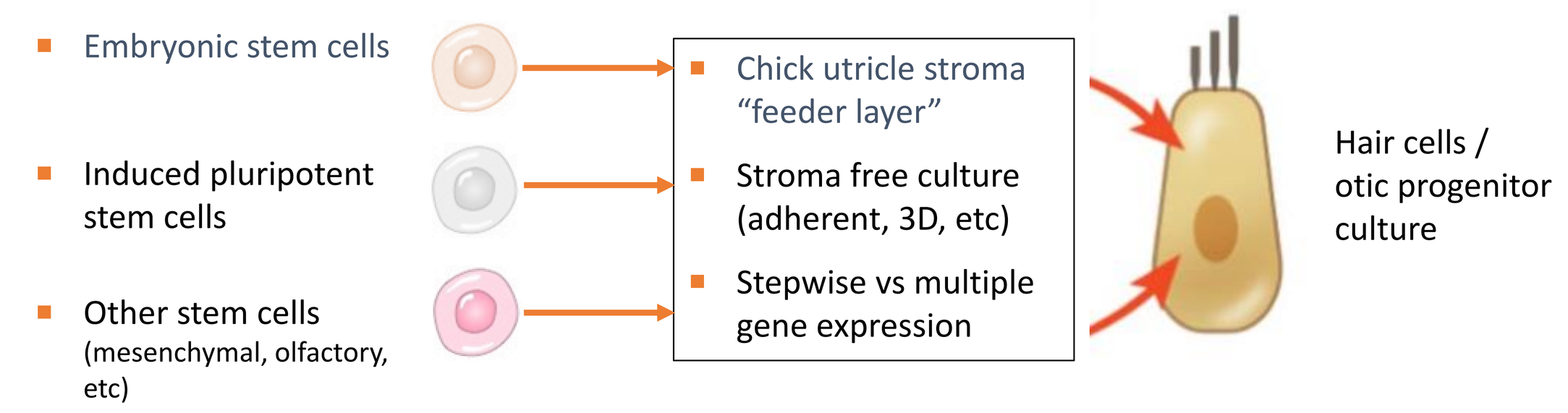
Only one study has trialled growth factor therapy in humans. Nakagawa et al (2010) applied IGF soaked hydrogels to the middle ear of 25 patients with sudden sensorineural hearing loss. 56% showed improvement at 24 weeks with no adverse events. However, the significance is questionable, as spontaneous improvement in SSNHL is reported in 33-63%.

STEM CELL TRANSPLANT (1)

Non cochlear stem cells

Inner ear progenitor cells are sparse, hard to identify, and difficult to expand in culture. The alternative is to use other sources of stem cells, control their differentiation into hair cells, and transplant these into the inner ear.

Embryonic stem cells, induced pluripotent stem cells, and other lineages such as mesenchymal and olfactory stem cells can all be made to differentiate into cells that display hair cell and otic progenitor properties in vitro. This can be achieved through applying specific growth factors, or by culturing cells in a stroma of utricle cells that promote differentiation into otic lineages.



Li et al 2003, Oshima et al 2010, Ouji 2017, Koehler et al 20013, Chao et al 2013, Costa et al 2015

STEM CELL TRANSPLANT (2)

Human stem cell culture in vitro

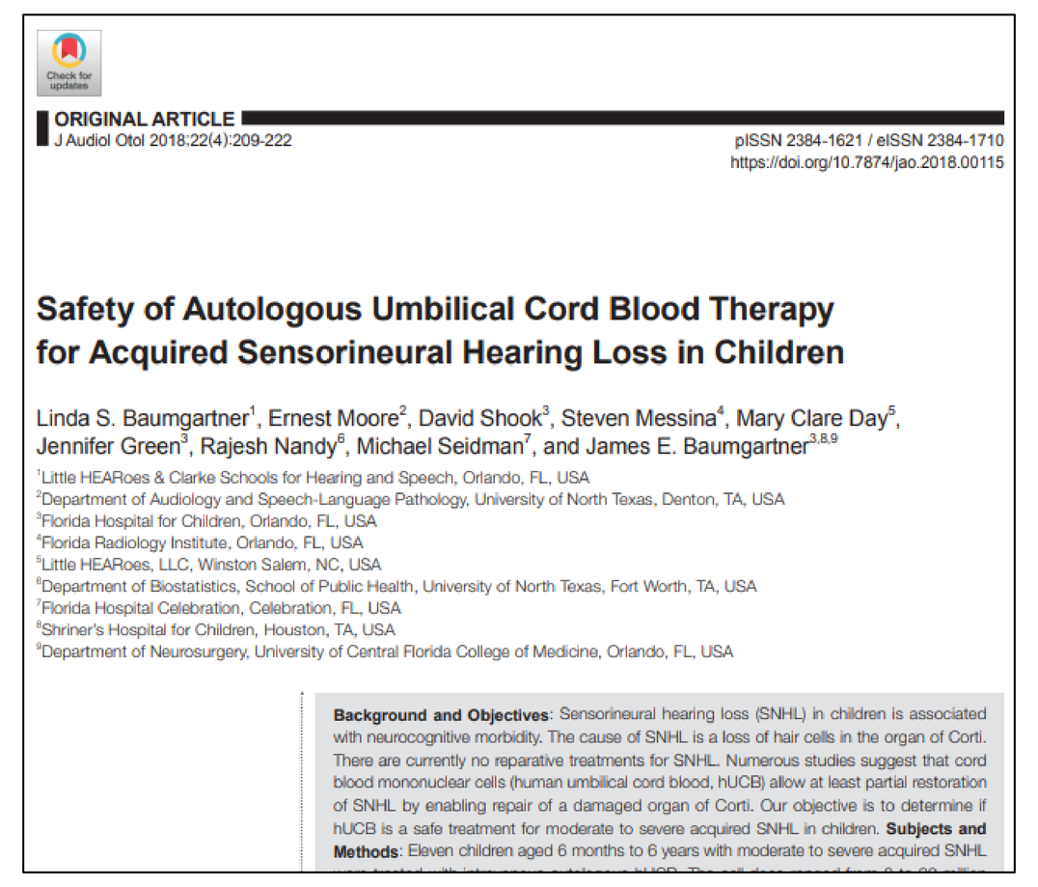
- Chen et al 2012: embryonic stem cells (ESC) cultured with FGF to form otic progenitors and hair-like cells
- Ronaghi et al 2014: ESC cultured in monolayer with FGF to sequentially differentiate toward hair cell fate
- Ohnishi et al 2015: induced pluripotent stem cells (iPSC) cultured with bFGF formed hair-like cells with electrophysiological signalling. These improved hearing when transplanted into deafened rodents
- Ding et al 2016: ESC cultured in conditioned medium (chick utricle stroma medium + EGF + Retinoic acid) formed cells with more hair cell features
- Koehler et al 2017: ESC cultured in 3D frame with FGF, FGF, BMP and Wnt generated "organoids" of organised sensory epithelia over 2 months
- Mattei et al 2019: iPSC cultured in rotary system with FGF & EGF formed hair cells and otoconia

Effect of stem cell transplant in vivo

Delivery of stem cells can be either through direct injection into the cochlea, or as intravenous systemic therapy. Several studies have shown that human hair cell progenitors injected into rodent cochleae appeared to localise to damaged hair cells, form synaptic connections, and improve hearing.

Human cord blood and mesenchymal stem cells administered systemically to deafened mice also appeared to improve hearing, possibly through paracrine effects. However large numbers of stem cells were found elsewhere, including in the lungs, raising concerns of tumour risk from these injected cells.

Human stem cell therapy trials


ORIGINAL ARTICLE
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Safety of Autologous Umbilical Cord Blood Therapy for Acquired Sensorineural Hearing Loss in Children

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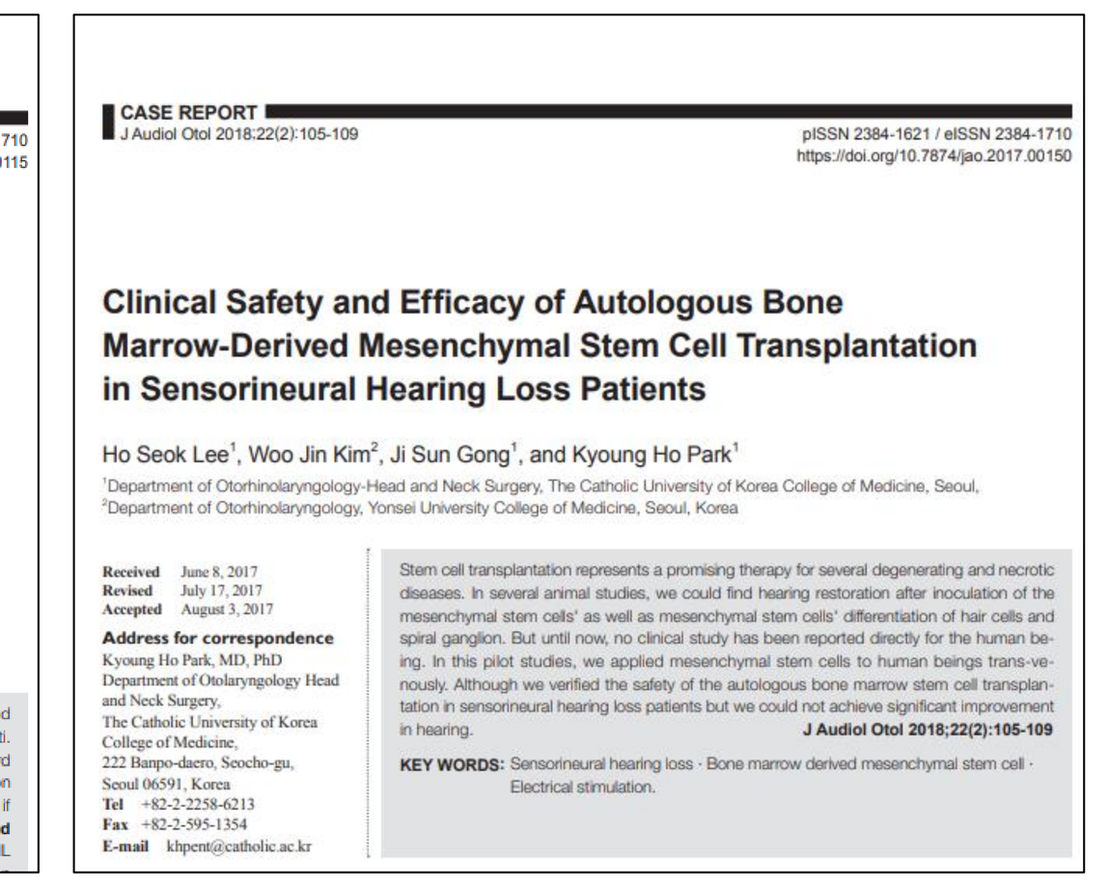
Background and Objectives: Sensorineural hearing loss (SNHL) in children is associated with neurocognitive morbidity. The cause of SNHL is a loss of hair cells in the organ of Corti. There are currently no reparative treatments for SNHL. Numerous studies suggest that cord blood mononuclear cells (human umbilical cord blood, hUCB) allow at least partial restoration of SNHL by enabling repair of a damaged organ of Corti. Our objective is to determine if hUCB is a safe treatment for moderate to severe acquired SNHL in children. **Subjects and Methods**: Eleven children aged 6 months to 6 years with moderate to severe acquired SNHL


CASE REPORT
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Clinical Safety and Efficacy of Autologous Bone Marrow-Derived Mesenchymal Stem Cell Transplantation in Sensorineural Hearing Loss Patients

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Stem cell transplantation represents a promising therapy for several degenerating and necrotic diseases. In several animal studies, we could find hearing restoration after inoculation of the mesenchymal stem cells' as well as mesenchymal stem cells' differentiation of hair cells and spiral ganglion. But until now, no clinical study has been reported directly for the human being. In this pilot studies, we applied mesenchymal stem cells to human beings trans-venously. Although we verified the safety of the autologous bone marrow stem cell transplantation in sensorineural hearing loss patients but we could not achieve significant improvement in hearing. J Audiol Otol 2018;22(2):105-109

KEY WORDS: Sensorineural hearing loss · Bone marrow derived mesenchymal stem cell · Electrical stimulation.

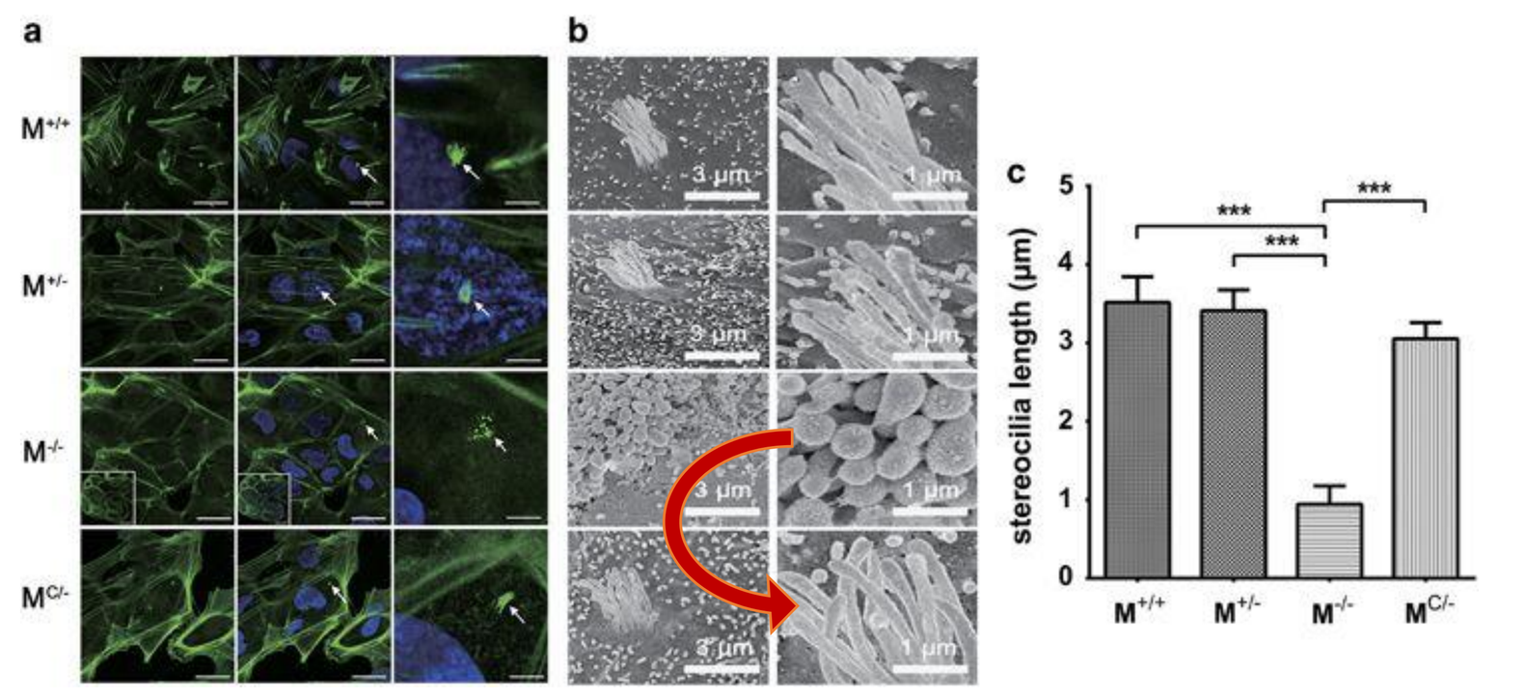
Two studies have attempted stem cell therapy in humans. Baumgartner et al (2014) carried out a registered phase 1/11 clinical trial where cord blood stem cells were administered intravenously to 11 patients aged 9months to 6 years, with <18 months of hearing loss. Auditory brainstem response thresholds improved in 5 patients, and there were no reported adverse effects. This can be considered a proof of concept and safety study, and further trials are awaited.

Lee et al published a case series in 2018 where undifferentiated stem cells were administered intravenously to a 67 year old female and 55 year old male with hearing loss of unspecified type. In both cases there was no impact on hearing. Considerable progress needs to be made to establish the safety and feasibility of this kind of treatment in humans

GENE THERAPIES

Gene therapies have the potential to correct some genetic causes of sensorineural hearing loss. Currently focus is on proving that genetic defects can be corrected in cultured stem cells in vitro, before potential transplant back into patients.

Chen et al and Tang et al (2016) have already used CRISPR-Cas9 technology to correct MYO7A and MYO15A mutations in induced Pluripotent Stem Cell colonies in vitro. These could then be differentiated into healthy hair with normal ciliary structures.



Hair cell cilia length in cells with MYO7 mutation vs CRISPR-Cas9 corrected cells

CONCLUSIONS

Hair cell regeneration is an important potential therapy for sensorineural hearing loss. Current regenerative strategies focus on regeneration in situ; stem cell transplantation, and gene therapies.

Stem cell transplant appears more achievable at present than direct stimulation of inner ear regeneration at present. There have also been successful application of gene therapies in vitro.

There remains a significant gap between animal studies and translational advances in therapy for humans. Nonetheless, promising avenues for further research have been identified,

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Appendix of review results

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