OTOSTEM

HUMAN STEM CELL APPLICATIONS FOR
THE TREATMENT OF HEARING LOSS

PUBLISHABLE SUMMARY

SECOND PERIODIC REPORT

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The lack of human otic cell models represents a significant roadblock hampering the development of drug-based or cell-based therapies. Hearing impairment is the most frequent human sensory deficit and is mainly caused by the irreversible loss of neurosensory cells in the cochlea. OTOSTEM addresses this urgent and unmet medical need for causal hearing loss therapies by focusing on human stem cell technology. We will generate human inner ear models as a platform for the development of novel therapies for sensorineural hearing loss. Purified otic progenitor cells from various human stem cell sources will be the core of this technology. This will provide the basis for preclinical and clinical development of drug and cell-based therapies.

Concept

Our ability to hear depends entirely on our auditory receptors – the sensory hair cells and their associated neurons that reside in the cochlear part of the inner ear (Figure 1). Mechanical signals (acoustic waves) are transformed in the organ of Corti into electric signals (action potentials). Hair cells activated by the movement of the cochlear fluid release chemical messengers, which stimulate the auditory nerve carrying the information to the brain for processing. The exquisite sensitivity of the inner ear comes with the risk for damage for example by noise trauma, ototoxic drugs, infections, age related degeneration and genetic causes. Once lost, the neurosensory cells of the ear are not replaced.

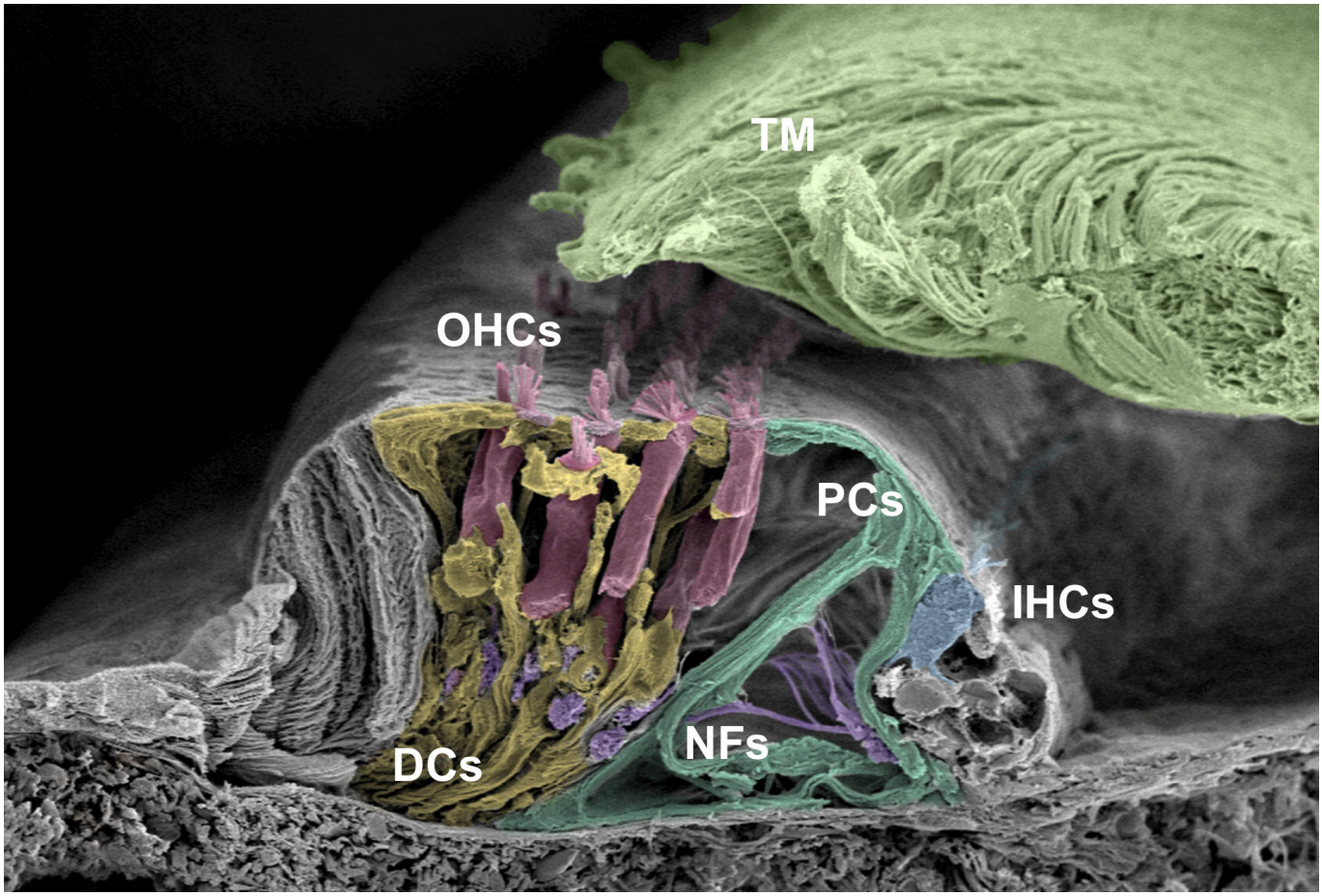

Figure 1: Pseudo-coloured scanning electron micrograph of the organ of Corti in a human cochlea. Outer hair cells (OHCs-red); inner hair cells (IHCs - blue); tectorial membrane (TM – green); Deiters cells (DCs – yellow); Pillar cells (PCs – turquoise); nerve fibers (NFs – purple). The image was kindly provided by Professor Helge Rask-Andersen (Uppsala Universitet).

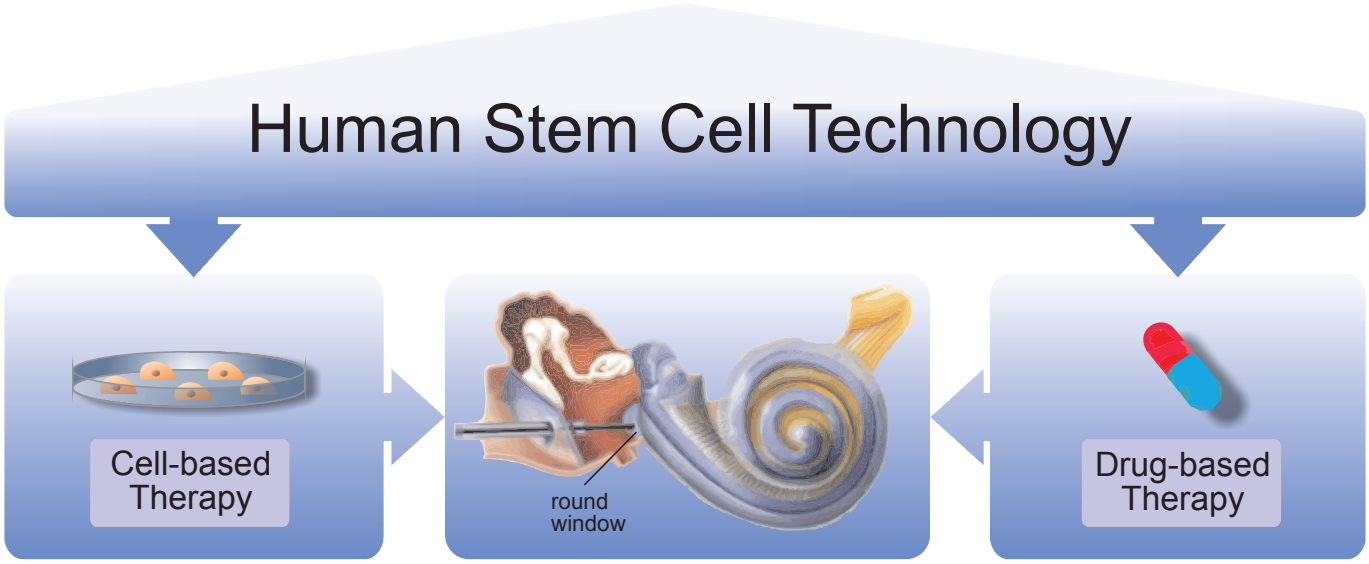

Figure 2: The two major applications for the therapeutic use of human stem cell technology for hearing loss are (i) direct cell based treatment by transplantation of human otic stem/progenitor cells into the cochlea and (ii) drug based treatment emanating from drug screening efforts for otoprotective and otoregenerative compounds. Both cells and drugs will be applied directly into the target organ – the cochlea –, which is self-contained and surgically accessible. An access route into the cochlear fluid space is provided through the round window membrane.

This, in turn results in chronic hearing impairment, a devastating and highly prevalent disorder of infancy and adulthood with widespread implications for the individual and society as a whole. Adult hearing loss alone ranks among the five leading causes of burden of disease in Europe, entailing enormous socio-economic costs. Prosthetic treatment with hearing aids and cochlear implants is limited and reaches only every fifth patient. Due to the cause of the hearing loss – neurosensory cell loss – hearing aid amplification often fail to improve language comprehension and hence perform unsatisfactory.

Towards cell-based therapies (Figure 2), OTOSTEM evaluates defined cell populations intended for therapeutic use for tumorigenic potential and validate their functional properties and biological potency in appropriate in vitro models.

Towards drug-based therapies (Figure 2), cellular otic models are developed to the level of artificial sensory epithelia or "Mini Ear" in vitro models mimicking the in vivo organ equivalent.

Otic cells have to be generated in sufficient numbers to allow screening in a high throughput/high content multi well plate format. In a subsequent step, these established and characterised cellular otic models are advanced into models of hearing loss. Exposure to ototoxic drugs allows for selective ablation of sensory hair cells and the establishment of "hearing loss in a dish" models for ototoxic drug screening.

One goal is to provide working assays for high throughput/content screens to the point that these assays can be used in the drug discovery setting represented by SMEs. Two therapeutic classes of drugs, one with otoprotective and the other one with otoregenerative effects are in the primary focus. Otoprotective effects aim to prevent cell death of human hair cells while otoregenerative effects aim at the replacement of lost human auditory neurons and hair cells.

Achievements

Controlling differentiation and proliferation in human otic stem cells

The consortium has made major progress in the purification and differentiation of neural and sensory otic cells from different human stem cells sources. Guidance protocols to differentiate stem cells into otic cells were further developed and published. Positive and negative surface markers of otic progenitor cells (OPCs) were identified, which may facilitate the purification of defined cell populations by flow cytometry. Several other methods that enrich for otic cells from heterogeneous cell populations were explored.

To characterize the generated cells in more detail, gene expression studies were performed on RNA and protein level. Given the fact that the consortium would still benefit from continuous improvement of these protocols, we will proceed our work to derive otic cells from human stem cells.

Otic progenitors cells suitable for cell transplantation

For transplantation therapies based on differentiated stem cells composed of heterogeneous cells, the ability to accurately purify the desired cell population prior to transplantation into a model organism is critical. To evaluate the suitability of the generated otic progenitor cells for cell-based therapies, the cells were sorted and their tumorigenic capacity was assessed to ensure safe transplantation trials.

Functional assays and electrophysiological measurement of otic neurosensory cells derived from stem cells were performed to validate the authenticity of these cells. Spontaneous and evoked action potentials were recorded from primary human otic neurons using Multiple Electrode Arrays. A whole organ in vitro system for exploring engraftment and functional integration of candidate otic populations was set up.

Hearing loss in a dish model, ototoxicity, and drug screening

Quantitative human cell-based assays to predict ototoxicity in drug safety studies are urgently needed. To address this unmet medical need, the consortium has developed an in vitro platform to test for cisplatin-induced ototoxicity based on differentiated human induced pluripotent stem cells. A library of known ototoxic drugs was employed to validate the screen. Further improvements will be implemented before the assay will be used for screening large compound libraries.

Drug screening aiming at otoprotection and otoregeneration

Bioassays similar to the hearing loss in a dish model were designed to identify compounds with otoprotective or otoregenerative activity and used for screening applications. Several compounds were identified and will be further investigated in vitro and in vivo.

In vivo investigations of stem cell and drug based therapies in hearing loss models

Four different in vivo rodent hearing loss models that cover a wide range of human hearing conditions were characterized and standardized. The partners involved in cell-based therapies exchanged protocols and thereby optimized surgical approaches. First trials of cell transplantations and drug-based therapies into the rodent inner ear were conducted.

Conclusion

In the second period of the OTOSTEM project synergistic collaborations within the consortium were continued with a vigorous exchange of material, knowledge and staff. Three Consortium Meetings were held. The effort on advertising and divulging results of the OTOSTEM project has been continued. The partners collectively presented parts of their contributions in more than 25 national and international events. Eight peer-reviewed publications have been published within the last 18 months.

The OTOSTEM Consortium

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Peer-reviewed publications in scientific journals

Distinct capacity for differentiation to inner ear cell types by progenitor cells of the cochlea and vestibular organs. Will J. McLean, Dalton T. McLean, Ruth Anne Eatock, and Albert S. B. Edge. *Development*. (2016): dev.139840. doi: 10.1242/dev.139840

Single-cell analysis delineates a trajectory toward the human early otic lineage. Megan Ealy, Daniel C. Ellwanger, Nina Kosaric, Andres P. Stapper, and Stefan Heller. *Proceedings of the National Academy of Sciences*. 113, no. 30 (2016): 8508-8513. doi: 10.1073/pnas.1605537113

The Severity of Infection Determines the Localization of Damage and Extent of Sensorineural Hearing Loss in Experimental Pneumococcal Meningitis. M. Perny, M. Roccio, D. Grandgirard, M. Solyga, P. Senn, and S. L. Leib. *Journal of Neuroscience*. 36, no. 29 (2016): 7740-7749. doi: 10.1523/JNEUROSCI.0554-16.2016

Scanning Electron Microscopic Examination of the Extracellular Matrix in the Decellularized Mouse and Human Cochlea. Peter A. Santi, Robair Aldaya, Alec Brown, Shane Johnson, Tyler Stromback, Sebahattin Cureoglu, and Helge Rask-Andersen. *JARO*. 17, no. 3 (2016): 159-171. doi: 10.1007/s10162-016-0562-z

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