Differentiation of human stem cell derived inner ear organoids.

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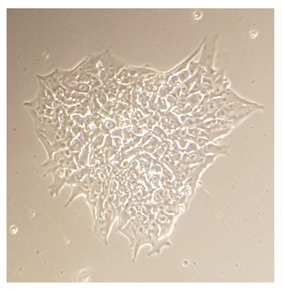
Introduction

I grew parts of the inner ear to help cure a deaf-blind disease. The most common inherited deaf-blinding disease is Usher syndrome, with an incidence of 15 per 100,000 live births. It is a disease that causes the permanent loss of both sight and hearing in affected individuals, which can be devastating to both them and their families. Hearing is possible because of tiny hairs in the inner ear that vibrate with the sound and transmit sound to the brain, and in Usher Syndrome it is these hairs that break down. Working at the UCL Institute of Ophthalmology, I aimed to grow the part of the ear that contains these hair cells from stem cells, which under the right conditions can make any type of cell in the body. If hair cells could be grown in the lab, they could either be transplanted into affected patients to allow hearing again, or used to study how the disease causes the breakdown of hearing.

Methods

We worked with 4 different lines of pluripotent stem cells, including induced pluripotent stem cell (iPSC) lines from Usher patient, one healthy iPSC line and one healthy embryonic cell line. We attempted to replicate the Koehler protocol *(K. R. Koehler et al. (2017). 'Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells'.)*, which lays out a process of three major steps along a 12 day timeline, before cell aggregates are allowed to self differentiate into otic vesicles and then sensory epithelia. We modulated BMP4, TGF-β, FGF-2 and Wnt signalling, with major focus on BMP4 concentrations at day 0, since at this point ectodermal cells must avoid becoming either epidermis, if BMP4 concentration is too high, or neuroectoderm, if BMP4 is too low. This step is made more difficult by the extremely precise concentration that needs to be met (with concentrations varying by as little as 0.25 ng.ml^{-1}) and the fact that individual cell lines release BMP4 intrinsically at varying concentrations.



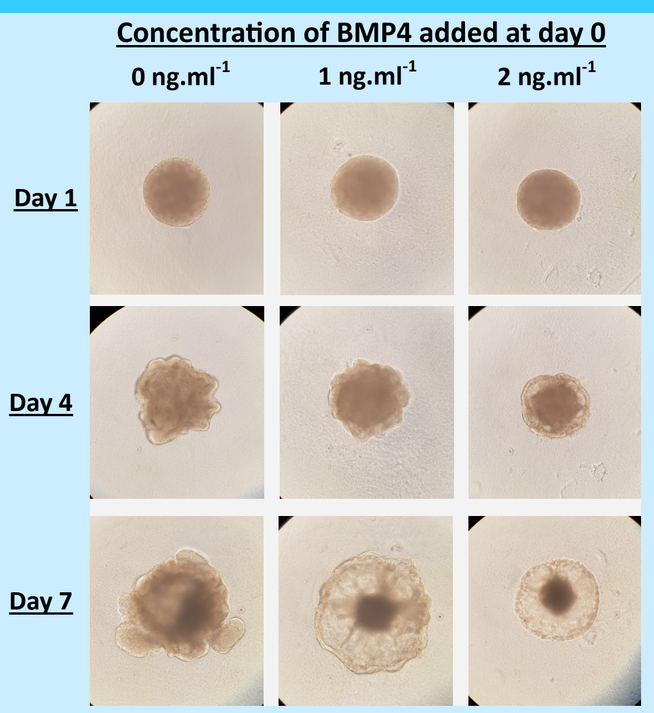
Induced pluripotent stem cells.

Path of differentiation of stem cells to otic-placode-like epithelium

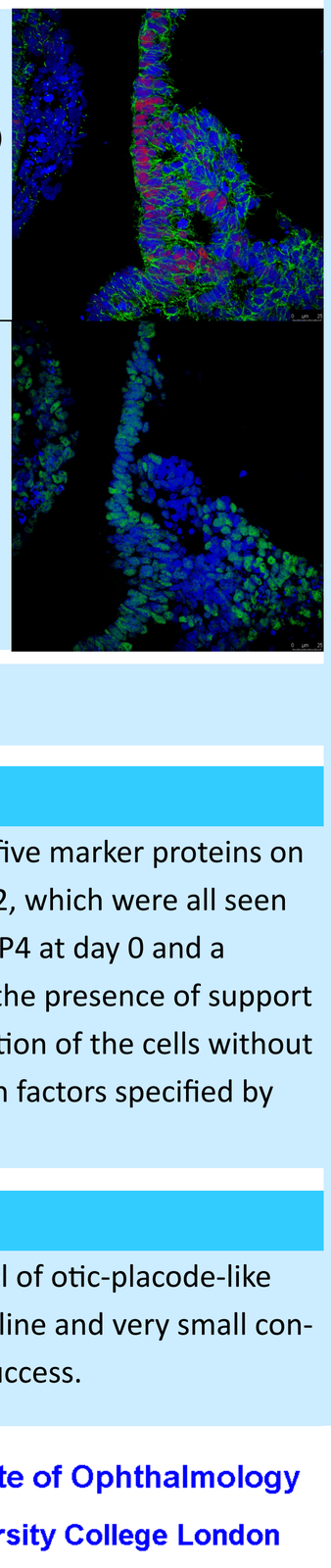
This figure shows the pathway of differentiation the stem cell lines must follow to successfully reach a point where they will self-differentiate, the growth factors that must be present or absent and the possible incorrect paths for the cells to take .

Morphological differences of aggregates throughout one week of differentiation at varying concentrations of BMP4

Significant morphological differences as the BMP4 concentration increased by only 1 ng.ml^{-1} shows the accuracy required to successfully differentiate stem cells into otic-placode-like epithelium. 0 ng.ml^{-1} BMP4 shows characteristics of neuroectoderm, yet 2 ng.ml^{-1} shows characteristics of epidermis. The middle aggregate was not successful in differentiating to otic-placode-like epithelium, meaning the correct concentration lies at least at an accuracy of 0.5 ng.ml^{-1}.



Day 12 aggregates:
0 ng.ml^{-1} BMP4 added @ day 0, 1.1% Matrigel. Imaged under confocal microscope. Blue signifies cell nuclei (DAPI).



Results

Immunohistochemistry was used to assess our differentiated aggregates. Using multiple colours and multiple markers, we are able see the presence of five marker proteins on the surface of one successfully differentiated otic placode. The five markers used to confirm our results were ECadherin, Pax8, NCadherin, Pax 2 and AP2, which were all seen on the outer surface of the aggregates at day 12, signifying presence of otic-placode-like epithelium. These results were obtained through use of no BMP4 at day 0 and a reduced concentration of Matrigel than what was used for other differentiations. Matrigel, when diluted in solutions allows stem cells to grow without the presence of support cells, however we believed that residual concentrations of BMP4 in the Matrigel as well as intrinsically produced BMP4 was enough to cause differentiation of the cells without having to add it to the solution. Concentrations of FGF-2, BMP4 inhibitor (LDN-193189), TGFβ inhibitor (SB-431542) and CHIR, as well as all other growth factors specified by the Koehler (2017) paper were kept the same.

Conclusion

This study shows that the translation of the Koehler et al. protocol (2017) to other cell lines is possible, with cell lines being differentiated up to the level of otic-placode-like epithelium. However, success was very rare, since the level of BMP4 required to reach the correct cell fate has to be determined for every different cell line and very small concentration differences can cause a differentiation to fail unpredictably. The large number of critical variables is most likely responsible for the rarity of success.

References

Koehler, K. R., Nie, J., Longworth-Mills, E., Liu, X., Lee, J., Holt, J. R., & Hashino, E. (2017). Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. *Nature Biotechnology, 35* (6), 583-589. doi:10.1038/nbt.3840

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