

**Inner ear hair cell-like cells from human embryonic stem cells.**

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**Public Summary:**

In mammals, the permanence of many forms of hearing loss is the result of the inner ear's inability to replace lost sensory hair cells. Here, we apply a differentiation strategy to guide human embryonic stem cells (hESCs) into cells of the otic lineage using chemically defined attached-substrate conditions. The generation of human otic progenitor cells was dependent on fibroblast growth factor (FGF) signaling, and protracted culture led to the upregulation of markers indicative of differentiated inner ear sensory epithelia. Using a transgenic ESC reporter line based on a murine *Atoh1* enhancer, we show that differentiated hair cell-like cells express multiple hair cell markers simultaneously. Hair cell-like cells displayed protrusions reminiscent of stereociliary bundles, but failed to fully mature into cells with typical hair cell cytoarchitecture. We conclude that optimized defined conditions can be used in vitro to attain otic progenitor specification and sensory cell differentiation.

**Scientific Abstract:**

In mammals, the permanence of many forms of hearing loss is the result of the inner ear's inability to replace lost sensory hair cells. Here, we apply a differentiation strategy to human embryonic stem cells into cells of the otic lineage using chemically-defined attached-substrate conditions. Generation of human otic progenitor cells was dependent on FGF signaling and protracted culture led to the upregulation of markers indicative of differentiated inner ear sensory epithelia. Using a transgenic embryonic stem cell reporter line based on a murine *Atoh1* enhancer, we show that differentiated hair cell-like cells express multiple hair cell markers simultaneously. Hair cell-like cells displayed protrusions reminiscent of stereociliary bundles, but failed to fully mature into cells with typical hair cell cytoarchitecture. We conclude that optimized defined conditions can be used in vitro to attain otic progenitor specification and sensory cell differentiation.

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