DIRECTED DIFFERENTIATION OF INNER EAR HAIR CELLS FROM MOUSE EMBRYONIC STEM CELLS (E14Tg2a)

Paul Mondragón Terán, Regenerative Medicine & Tissue Engineering Laboratory, Centro Médico Nacional "20 de Noviembre"–ISSSTE
p.mondragonteran@gmail.com
Miguel Ángel Juárez Mancera, Regenerative Medicine & Tissue Engineering Laboratory, Centro Médico Nacional "20 de Noviembre"–ISSSTE ; Escuela Superior de Medicina – IPN. Sección de Estudios de Posgrado
Juan Antonio Suárez-Cuenca, Regenerative Medicine & Tissue Engineering Laboratory, Centro Médico Nacional "20 de Noviembre"–ISSSTE
Rafael Manuel Navarro-Meneses, Dirección Médica del ISSSTE

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The hair cell plays an essential role in the transmission of the acoustic waves from the air to the auditory neurons in the brain. These cells are found inside the cochlea, a bone spirally duct fully of perilymph, a liquid with high concentrations of K^+. It has been reported around 25,000 hairs cells in the human ear. Deafness is a condition quite common; about 90% is due to neurosensorial condition and involves the loss of hair cells and their associated neurons (Rivolta, 2013; Chen et al., 2009). Age, genetic abnormalities and environmental factors (for example, noise and ototoxic drugs such aminoglycosides) are most common causes of deafness. Due to the lack of endogenous regeneration, and to the limitations of available therapies; the potential to develop a system based on the introduction of exogenous specialized cells offers new alternatives as deafness treatment. Embryonic stem cells are excellent candidates for biological implantation, as they have the potential to proliferate and differentiate (Rivolta, 2013). Protocols currently reported to differentiate hair cells from embryonic stem cells use "embryonic –bodies" and co-culture techniques or viral transfection. The use of these techniques results in spontaneous differentiation and low control over early differentiation. This work aims to establish a model of directed differentiation through monolayer culture, using chemically defined media, feeder-cell free, avoiding the use of embryoid bodies and the use of fetal bovine serum in order to obtain higher control over the whole differentiation process. Mouse Embryonic Stem Cells (E14Tg2a) were cultured in GMEM added with Leukemia Inhibitory Factor (LIF). Passage of mES was performed during the exponential phase. To start the differentiation process, 1×10^4 cells·cm^{-2} were inoculated and cultured for 16 hours in GMEM free of fetal bovine serum and LIF, in order to promote the adhesion. The proposed differentiation method was modified from Li et al. 2003 and involves three stages (all in chemically defined medium) as monolayer culture: 1) Generation of Otic Plate Precursors Cells, culture in GMEM added whit IGF-1 (50 ng·mL^{-1}), EGF (20 ng·mL^{-1}) and N2 supplement, renewing the media every 48 hours. The cells were maintained under these conditions for 240 hours; 2) Expansion of Otic Plate Precursors Cells, the media was replaced by GMEM added with IGF-1 (50 ng·mL^{-1}), EGF (20 ng·mL^{-1}), bFGF (10 ng·mL^{-1}) and N2 supplement, renewing the media every 48 hours during 192 hours. 3) Specialization of Otic Plate Precursors Cells into Hair Cells, all growth factors were removed from the media to promote the specialization, cultured in GMEM added only with N2 supplement during 240 hours. The evaluation of the pluripotent state and cell differentiation was assessed by flow cytometry and inmunocytochemistry. The expression of pluripotency markers for OCT3/4 was 96%, whereas for Nanog was 95% for mES maintenance. The expression of Pax2, Myosin VIIa and Math1 is essential for the development and maturation of hair cells, these markers were detected during the differentiation of embryonic stem cells as follows: In the generation of otic progenitors (168 hours) we observed Pax2 (64%), Myosin VIIa (86%) and Math1 (58%). Meanwhile the expression of these proteins during the specialization of hair cells (at 672 hours of culture) was Pax2 (46%), Myosin VIIa (53%), Math1 (38%). According to the proposed protocol, it is feasible to generate inner ear - hair cells through monolayer culture, feeder-cell free and using chemically defined medium. The expression in an early stage of Pax2 indicated the generation of Otic Precursors Cells, whereas coexpression of Myosin VIIa and Math1 it allowed us to determinate the presence of hair cells.

References

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