Neurite Outgrowth of Human Induced Pluripotent Stem Cell-derived Neurons – examination of the effect of different factors

Desislava Skerleva1, Hiroe Ohnishi1, Stefan Stoyanov2, Norio Yamamoto1, Juichi Ito3, Takayuki Nakagawa1
1Department of Otolaryngology, Head and Neck Surgery, Kyoto University, Kyoto, Japan; 2Medical Institute –Ministry of Interior, Sofia, Bulgaria

Abstract
The loss of spiral ganglion neurons lead to poor cochlear implant performance which makes hearing for profoundly deaf people challenging especially in noisy environment.

Human induced pluripotent stem cells allow us to confirm the results from animal experiments as it gives us the exciting opportunity to investigate their role in the field of regenerative medicine for inner ear diseases and to confirm the effects we observe in animal experiments in future translational studies.

The effect of BDNF, NT-3 and IGF-1 on neurite outgrowth of human induced pluripotent stem (hiPS) cell-derived neurons was examined. First, human iPSC cell line 20187-GFP was differentiated into neural progenitor cells. Neurospheres were prepared from neural progenitor cells by floating culture on U-bottom low adhesion plate. After that the neurospheres were transferred on Matrigel-coated plates and underwent neuronal differentiation for 7 days with or without additional neurotrophic or growth factors.

Immunocytochemistry showed βIII-tubulin and neurofilament positive cells in the neurospheres. The outgrowth was assessed by ImageJ plug-in NeuriteJ (1). No significant difference was observed between the different groups.

Since the effect of BDNF, NT-3 and IGF-1 on neurite outgrowth was confirmed by previous studies our result could be due to the genetic instability of iPSC cells.

Introduction
Cochlear implantation is the only available solution for patients with profound hearing loss. But the loss of spiral ganglion neurons is interfere with cochlear implants performance. Establishment of induced pluripotent stem (iPS) cells give us the exciting opportunity to investigate their role in the field of regenerative medicine for inner ear diseases and to confirm the effects we observe in animal experiments in future translational studies. Different studies demonstrate the role of IGF-1 and its related proteins in inner ear development (2-6). Also IGF-1 regulates neural outgrowth and neuronal migration, promotes formation of mature excitatory synapses and enhance cell proliferation and survival (7-10).

Aim
Our aim is to examine the effect of different neurotrophic and growth factors on neurite outgrowth of human iPSC cell-derived neurons and to establish a safe protocol for cell transplantation into the inner ear.

Materials & Methods
Neural stem cells (NSC) derived from line 20187 GFP human iPSC cells (11).- 1

Results
Characterization of neurospheres by RT-PCR analysis
RT-PCR analyses of 20187-GFP human iPS cell-derived neurospheres showed the expression of the neural stem cell marker genes NESTIN, SOX1, PAX6 and expression of the pluripotent marker gene OCT3/4; undifferentiated iPS cells were used as controls.

The effect of the growth factors
The neural outgrowth was assessed 7 days after administering the neurotrophic or growth factors by Neurite J, plug-in for ImageJ (1).

Discussion
✓ No significant dose-dependent effect was observed in all experiments.
✓ Characterization of neurospheres by RT-PCR and immunocytochemistry shows that the cells in the neurospheres are at different stage of differentiation.
✓ Since the effect of BDNF, NT-3 and IGF-1 on neurite outgrowth was confirmed by previous studies our result could be due to the genetic instability of iPSC cells.

Conclusion
✓ The range of the investigated growth and neurotrophic factors should be expanded.
✓ The effect on cell survival and migration should be examined.

References
8. Takeda H et al. SHANK2 is a neurotrophic factor with distinct roles in neuronal development and synaptic plasticity. PLoS One. 2013 Sep 25;8(9):e73530.