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Research Highlight

New march towards the regeneration of sensation and cognition: hear more, see more and learn more

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As many human sensory and cognitive diseases are caused by irreversible damage or loss of certain types of neurons, methodologies aimed at replacement of lost neurons are key to restore lost sensation. Recent advances in generation of ear-cell progenitors, optic-cup structures and cortical neurons from human embryonic stem cells and induced pluripotent stem cells provide versatile tools for modeling human diseases and developing cells for replacement therapies.

Deafness, the loss or severe impairment of hearing, is a pathological condition often resulting from the loss of specialized neural cells in the ear. An individual who has lost hearing due to the partial loss of sensory hair cells in the inner ear may still regain some hearing by using a hearing aid or cochlear implant. However, auditory neuropathy is a form of deafness caused primarily by the loss of auditory sensory neurons (spiral ganglion neurons), and currently no conventional treatment exists to compensate for this neuronal loss. Therefore, stem cell-based therapy to re-build the sensory circuitry is being considered to provide therapeutic cues. In a recent report from *Nature*, Chen et al. (2012) presented a new protocol to induce ear-cell differentiation from human embryonic stem cells (ESCs) adapting developmental signals involved in otic placode specification (as shown in Figure 1). They successfully generated two types of ear-cell progenitors that can further differentiate into hair-cell-like cells and auditory neurons which are involved in auditory response. Importantly, when transplanted into chemically damaged gerbil ears, otic neuroprogenitors can engraft, differentiate, and significantly improve auditory evoked response. These encouraging findings attested the functionality of stem cell-derived otic cells *in vivo* and pave the way for its potential clinical application. As a groundbreaking report, it suggests otic neuroprogenitor transplantation, in combination with cochlear implants, may offer a cell-based therapeutic solution for certain types of deafness.

As important as hearing, another essential human sensation is vision. The retina is the tissue which senses light and creates vision. It is a layered structure lining the inside of the back of the eye and made up of seven different cell types arranged into six distinct layers. The retina enables sight because it has special photoreceptor cells which respond to light and transmit electrical signals to the brain. Several pathological conditions such as retinitis pigmentosa (RP), one of the most common forms of inherited retinal degeneration, are caused by progressive loss of photoreceptor cells which eventually leads to blindness. Also, degeneration of the retinal pigment epithelium (RPE), which nourishes the retina visual cells, is believed to be the cause of photoreceptor loss in many sight-threatening diseases, including dry age-related macular degeneration (AMD) and Stargardt's macular dystrophy. Although these visual diseases lack efficient treatments at present, stem cell-based therapy is considered a promising therapeutic strategy to restore lost vision. In recent years, major progress has been made in setting up efficient protocols for the *in vitro* differentiation of RPE or neural retinal cells from human ESCs or induced pluripotent stem cells (iPSCs). Among all the efforts that have been made, two impressive breakthroughs have been accomplished recently.

The first milestone is the positive implication from perspective clinical trials transplanting human ESCs-derived RPE cells into two patients of AMD and Stargardt's macular dystrophy, respectively (Schwartz et al., 2012). Preliminary results reported by researchers at Advanced Cell Technology demonstrated the successful attachment of human ESCs-derived RPE and the survival of the grafts *in vivo*, while no signs of tumorigenicity, ectopic tissue formation, or transplant rejection were observed 4 months after transplantation. Although neither study was originally designed to test vision restoration (rather, they were testing the general safety of the procedure), the field is generally encouraged by the fact that two trial participants did not show further vision loss and the vision of one participant was even improved.

Another important breakthrough achieved was the generation of optic structures from human ESCs. In a recent study published in *Cell Stem Cell*, Nakano et al. (2012) reported the formation of a highly ordered 3D retina structure (optic cup) from human ESCs. Applying a modified procedure used to generate an optic cup from mouse ESCs (Eiraku et al., 2011), researchers from Sasai's group showed that the combination of the early Wnt inhibition, ECM addition, Hedgehog signaling activation, and FBS treatment strongly promoted the generation of retinal epithelium from human ESCs. Notably, the human ESCs-derived optic cups were able to form a

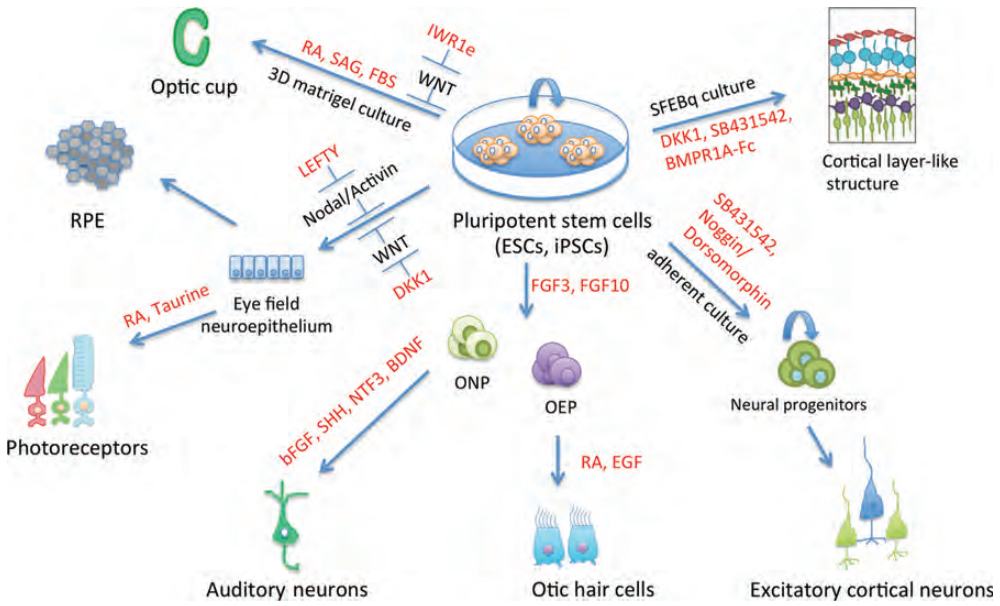

Figure 1 Directed differentiation of pluripotent stem cells towards optic, auditory, and cortical neural lineages. RPE, retinal pigment epithelium; ONP, otic neural progenitors; OEP, otic epithelial progenitors.

multi-layered structure, followed by proper spatial–temporal differentiation of retinal cells including both rod and cone photoreceptors. The significance of this study lies in its application potential for cell therapy. These artificial retinas may provide potential sources of stage-selected retinal cells for transplantation into patients affected by neurodegenerative diseases, such as RP or glaucoma. Of note, transplantation of rod-photoreceptor precursors has recently been shown to truly restore vision by integrating into a dysfunctional adult retina in a mouse model of rod degeneration (Pearson et al., 2012), thus paving the way for a similar therapeutic strategy after retinal degeneration in humans.

The human cerebral cortex is an immensely complex structure that plays a critical role in all types of human sensations. Signals from the physical world captured by the sensory system are transmitted to the realm of cerebral cortex where different senses are interpreted. Until recently, excitatory cortex neurons have not been efficiently produced and well characterized from human pluripotent cells by directed differentiation. Several protocols for generating cortical neurons with areal specificity have been reported. Shi et al. (2012a, b) developed a robust and efficient adherent culture system for differentiating human ESCs and iPSCs to all classes of cerebral neocortical neurons. Interestingly, the authors proposed that the *in vitro* process of differentiating human ESCs to cortex neurons recapitulates important stages in human cortical development and consists of several distinct steps: the directed differentiation of pluripotent stem cells to cortical stem and progenitor cells, followed by an extended period of cortical neurogenesis, neuronal terminal differentiation to acquire mature electrophysiological properties, and finally the functional excitatory synaptic network formation. Simultaneously to the adherent culture system, Mariani et al. (2012) reported the generation of neural 3D self-organized structures from human iPSCs using floating embryoid bodies. These 3D structures contain polarized radial glia, intermediate progenitors and a spectrum of layer-specific cortical neurons. More interestingly, their transcriptome closely recapitulates early dorsal telencephalic development in humans, which renders a possibility that combining with patient-specific iPSCs, this model may provide new insights into pathology of neural disorders with cortical dysfunction.

Efficient protocols to produce specific subtypes of neural cells or structures from human pluripotent stem cells may not only help us to better understand human neural development but also hold profound promise in the clinic. Though researchers cannot use them to study learning and memory in the way they do with animal models, these patient-derived cells provide a unique shortcut to uncover the underlying pathological mechanisms of human diseases at the cellular level, especially in cases when animal models fail to faithfully reflect the genetic complexity of humans. The generation of patient-specific iPSCs from Timothy syndrome (TS) patients, who suffer irregular heartbeat, hypoglycemia, and developmental delay, has been recently reported (Pasca et al., 2011). Efficient differentiation of TS-iPSCs to cortical neurons led to the identification of numerous pathologically significant defects, including decreased fraction of callosal projection neurons and an increased number of cells that project to subcortical structures. In addition, Pasca et al. (2011) found that TS neurons showed abnormal expression of tyrosine hydroxylase and increased production of norepinephrine and dopamine. Extended investigation of this finding has led to the identification of a drug which can reverse the cellular abnormalities. This study constitutes one of the first examples demonstrating the synergistic effects of coupling patient-specific iPSCs with directed differentiation in understanding human neurological disease.

Though exciting progress has been made, several important challenges remain unsolved. Firstly, the safety issue is always a major concern in stem cell-based therapies. As an innate feature of pluripotent cells, the risk of tumorigenicity should be stringently tested before any application in the clinic. Thus, efficient cell differentiation and purification methodologies are in high demand. Also, anti-tumor drugs could be combined to decrease the risk of excessive proliferation after cell transplantation.

Second, current protocols for neuronal differentiation need to be further optimized. Differentiation of human ESCs toward certain specific subtypes of neurons is an extremely time-consuming process, e.g. around 4 months are required for human photoreceptor differentiation. An alternative method, which may accelerate the differentiation process, could be direct reprogramming (Sancho-Martinez et al., 2012). Also, direct lineage conversion or transdifferentiation may provide an ideal way to minimize tumorigenesis risk by bypassing the pluripotent stage. Indeed, much progress has been achieved recently with respect to direct conversion of various types of somatic cells into neurons or neural stem cells (Karow et al., 2012; Liu et al., 2012a). Understanding the hierarchies of transcriptional circuitry for each neuronal subtype will be key to determine the optimal transcription factors or regulatory RNAs which facilitate neuronal fate determination.

Lastly, patient-specific iPSCs combined with gene editing technology and efficient neural differentiation protocols offer great hope for an unlimited supply of autologous cells for replacement therapy, as suggested in a recent report related to Parkinson's disease (Liu et al., 2012b). However, several questions remain to be answered. Are the different subtypes of neural cells derived from gene-corrected iPSCs fully functional? Are these naïve neurons or neural precursors generated *in vitro* capable of integrating into local neural circuitry *in vivo*? Can they survive and maintain physiological functions in an altered pathological environment *in vivo* and for how long? All these questions need to be carefully and thoroughly investigated in the future.

In summary, loss of sensation and impairment of cognition stems from a variety of neurological disorders in humans. Our understanding of the underlying mechanisms of these neurological diseases is profoundly hampered by lack of access to the affected neurons. The advancement of iPSCs technology and expanding knowledge of neuronal differentiation have provided great platforms to facilitate pathogenic studies as well as offer opportunities for developing novel cell therapies. Though many proof-of-principle studies have already been demonstrated, both human stem cell-based disease modeling and therapeutical research are still at an infant stage. What an exciting time for researchers all over the world to join this promising march towards the regeneration of sensation and cognition.

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