Stem Cell Therapies for Sensory Organ Disorders

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Abstract: Sensory organ disorders, such as visual impairment, hearing loss, and olfactory dysfunction, affect a significant percentage of the population. There are no effective therapies to restore cell damage and tissue function to these sensory organs. Human pluripotent stem cells (hPSCs) have the potential to expand out to an unlimited number of cells and differentiate into any cell type of the body, and therefore have high potential to restore tissue function in transplantation stem cell therapies for sensory organ disorders. This review elaborates on the specific sensory cells for the vision, auditory, and olfactory tissues that were generated from hPSCs. It then describes the effectiveness of using hPSC-derived sensory progenitors in animal models of disease and what needs to be done next in order to progress stem cell therapies to the clinic.

Keywords: stem cell therapies, sensory organ disorders, pluripotent stem cells

Sensory organ disorders

Sensory organs consist of specialized cells that detect stimuli in our environment and translate sensory information into signals for the nervous system to read. This review will focus on 3 major sensory organs: eyes, ears, and nose. The Global Burden of Disease (GBD) study reported a global estimation of 258 million people with mild vision impairment, 295 million people moderate/severe vision impairment, and 43 million people with complete blindness in the year 2020 [1]. Next, the GBD published that an estimated 1.57 billion people globally (20% of the population) had some level of hearing loss in 2019; 430 million of these people have disabling hearing loss [2]. Lastly, olfactory dysfunction is estimated to occur in 22% of the general population [3]. During the SARS-CoV-2 pandemic in 2020, loss of smell was one of the most common symptoms from the infection in which 59-86% of SARS-CoV-2 patients reported loss of smell [4]. Current treatments aide in the physical handicap of these disabilities, but there are no therapies to correct the underlying cause of these disorders.

Pluripotent stem cell model

Stem cells reside in essentially every tissue of the body, and their role is to replace damaged cells by producing new cells in the tissue [5]. If the stem cell produces one cell type, it is called unipotent; two cell types, bipotent; or three or more different cell types, multipotent. However, a stem cell that can produce many different cell types in the body is called pluripotent; these originate from an embryo [6]. The fertilized egg, or zygote, undergoes rapid cell division and morphs into a blastocyst which contains an inner cell mass that will develop into a fetus [7]. When the inner cell mass is isolated and placed into cell culture, it forms characteristic colonies known as embryonic stem cells (ESCs) or pluripotent stem cells (PSCs). These PSCs have the potential to differentiate into any cell type in the body [8-10] (Figure 1).

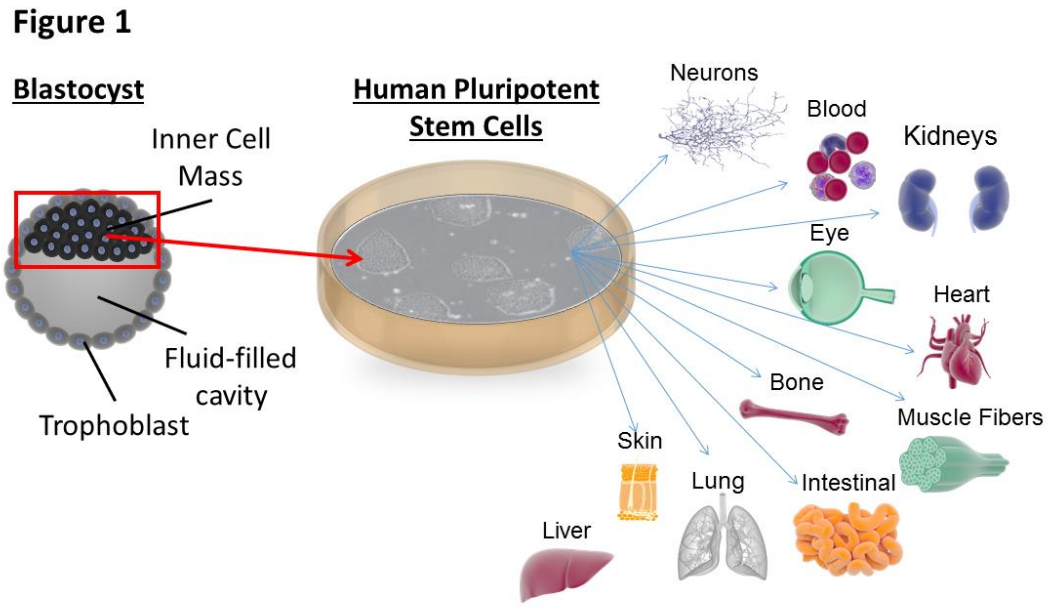

Figure 1: Pluripotent stem cells are derived from the inner cell mass of a blastocyst. Scientists add growth factors or small molecules to the culture to instruct the pluripotent stem cells to express or inhibit specific developmental pathways in order to differentiate into specialized cells.

Furthermore, patient-derived PSCs or induced PSCs are somatic cells that are collected from patients and reprogrammed into ESC-like cells by retroviral transfection of transcription factors that play roles in maintaining the pluripotent or embryonic state [11-13]. Stem cell scientists are now able to generate specific cell types from PSCs by adding growth factors or small molecules to the PSC culture that either activate or inhibit developmental pathways in an effort to recapitulate the natural embryonic development of the specialized cell type [14]. These PSC-derived tissue-specific cell progenitors are then transplanted into animal models of disease to determine their effectiveness in repairing damaged cells and restoring the function of the tissue [15,16]. This has the potential to be used in humans to restore tissue function in sensory organ disorders. Below is a review of the different types of sensory cells that were generated in human PSCs (hPSCs) and their effectiveness in preclinical studies.

Sensory organ development

A good understanding of embryonic developmental biology is essential to generate specific cells from hPSCs. Developmental biology is a complex process, and we are still learning about how specific cell types and tissues are produced. The use of hPSCs as a model allows us to understand development that is specific to humans as oppose to the other classic models, which include the frog, chicken embryo, zebrafish, and mice [14]. The sensory organs form from a collaboration of the 4 major regions of the ectodermal germ layer: non-neural ectoderm, neural ectoderm, pan-placode ectoderm, and neural crest cells [17]. Generally, the non-neural ectoderm develops into the skin, the neural ectoderm gives rise to the central nervous system, and the pan-placode ectoderm and neural crest cells produce cell types of the peripheral nervous system as well as a variety of other cell types [18]. These sensory organs contain specialized cells and peripheral sensory neurons that sense our environment and then connect with neurons of the central nervous system in order to communicate sensory information to the brain [17]. Specific cell types within the same tissue can be derived from different ectodermal regions, as will be described below in this review.

Generation of specific sensory cells derived from human pluripotent stem cells

Vision

The lens of the eye develops from the pan-placode ectoderm and is a biconcave, transparent structure that fine tunes and focuses light to the retina, which is located at the back of the eye [19]. There are multiple studies demonstrating the production of lens-like structures, known as lentoid bodies, from hPSCs [20-27]. The retina is derived from the neural ectoderm and contains photoreceptors and ganglion cells which capture light and transmit signals to the brain to perceive a visual picture [19]. Optical vesicles, photoreceptors, and ganglion cells were derived from hPSCs cells in a stage-wise manner [28-42]. The cornea is developed from the non-neural ectoderm and is a transparent tissue that covers the front of the eye; it works with the lens to focus light to the retina [19]. There are a number of studies that show the production of corneal endothelial cells from hPSCs [43-53]. These are well-established protocols for 3 different regions in the eye; however, they do not mimic the natural environment as they are created separately. Multiocular 3D models are being generated that contain all three cell types and better mimic natural development [54-56].

Auditory

Auditory sensory neurons and hair cells are two major cell types responsible for the detection of sounds, and damage to either one results in hearing loss [57]. These cells develop from the neural ectoderm and the otic placode, also known as the early developing inner ear [58]. In one study, scientists used a modified neural ectoderm induction protocol to differentiate hPSCs into auditory sensory neurons [59]. These neurons were co-cultured with cochlear explant cultures and were able to innervate the hair-cells indicating they are functional [60]. In a different study, hPSC-derived auditory sensory neurons were generated through a non-neural ectoderm and pan-placode ectoderm transition [61]. Next, inner ear sensory epithelial and hair-like cells were generated in hPSCs through a neural ectoderm transition in one study, [62] while they were produced from the pan-placode ectoderm and otic placode in another study [63]. Finally, 3D otic organoid cultures were establish to better mimic an *in vivo* environment to generate the different types of cells within the inner ear. The otic organoids produce functional vestibular and cochlear hairs cells [64-66], as well as, the otic supporting cells and sensory neurons [67]. More studies are needed to determine which cell types are derived from the specific ectodermal regions which is important in generating authentic and functional cells needed for transplantation in preclinical animal disease models.

Olfactory

Olfactory sensory neurons are the receptors in the nose that detect odors in the environment and relay odorant information to the olfactory bulb in the brain [68]. Olfactory sensory neurons originate from the early nasal cavity, or the olfactory placode, and migrate to the olfactory bulb of the brain [69]. The olfactory placode and olfactory sensory neurons were generated in hPSCs at a 20-30% differentiation efficiency; more research is needed to better understand the development and to increase the differentiation efficiency of the olfactory tissue [70]. Culture conditions were established for human nasal organoids [71] and for the expansion of primary human olfactory mucosa cells [72,73] which can be applied to future hPSC-differentiation and maintenance protocols. Generation of an induced hPSC line reprogrammed from human olfactory mucosa may be a potential cell line source for stem cell therapies, because this induced hPSC line may retain olfactory epigenetic signatures which may produce a more pure olfactory sensory neuron population [74]. Future differentiation studies are needed to produce other cell types in the olfactory tissue such as the horizontal and globose basal adult stem cells, the sustentacular supporting cells, and the olfactory ensheathing glial cells [75]. These other cell types may be important contributing cells that support olfactory repair in cell therapies for olfactory dysfunction.

Preclinical studies for human pluripotent stem cell derived sensory progenitors

Blindness / Vision Impairment

Cataracts occur when the lens epithelial cells become disorganized and cause a scattering of light which results in a cloudy appearance of the lens and visual problems [76]. Scientists are working on creating an *in vitro* model to recapitulate cataracts using patient-derived hPSCs in order to create novel drug targets and therapies [77,78]. Macular degeneration is a progressive atrophy of the retinal pigment epithelium and photoreceptors and results in central vision loss [79]. Transplantation of hPSC-derived retinal progenitors restored visual function in a rodent model of retinal degeneration [80-83], and

more studies are working on optimizing the procedure [84-89]. This therapy is now being tested in clinical trials for people with macular degeneration, and results demonstrated that the therapy is safe but has no or small improvements in vision function [90-92]. Keratoconus is a progressive thinning of the cornea causing blurring vision and sensitivity to light [93]. Transplantation of hPSC-derived corneal progenitors improved corneal integrity in animal models of corneal dysfunction [94,95]. The use of hPSC-derived progenitors for treatment of eye diseases is promising, but more research is needed to progress therapies to the clinic.

Deafness / Hearing Loss

Endogenous adult stem cells cannot regenerate auditory sensory neurons and hair cells, and therefore stem cell therapies may replace these damaged or loss cells to restore hearing loss [58]. Human PSC-derived auditory progenitors were transplanted into the cochlea of a rodent model of hearing loss; the results showed that some cells differentiated into hair and sensory neurons and responded to environmental cues [96-98]. Ablating hair cells and causing a lesion in the inner ear increased the engraftment of the transplanted progenitors [99,100]. More research is needed to improve the engraftment efficiency of the transplanted progenitors and to demonstrate that this therapy can restore hearing loss.

Anosmia / Olfactory dysfunction

Anosmia is the complete loss of the sense of smell [69]. Olfactory sensory neurons and their progenitors have the natural ability to migrate from the nasal cavity and connect into the brain [101]. In a proof of concept study, adult olfactory stem cells, labeled with a green fluorescent protein marker, were harvested from donor mice and transplanted into recipient mice with hyposmia (the reduced ability to smell) [102]. Results demonstrated that the transplanted stem cells differentiated into olfactory sensory neurons, migrated to the olfactory bulb, and restored loss of smell [102]. Additionally, researchers investigated the route that mesenchymal stem cells take to migrate from the nasal cavity to the brain and are considering this as a method to deliver stem cells to treat neurological disorders [103-105]. Olfactory progenitors derived from hPSCs may be an ideal cell therapy for anosmia and other neurological disorders as they can easily migrate to the brain [15,16]. Next steps for establishing a cell therapy for the treatment of anosmia would be to improve the differentiation and culture protocols and test the function of hPSC-derived olfactory progenitors in an animal model of hyposmia.

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