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Optogenetic approach improves neuroregenerative potential of stem cell-based therapies

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Genet. Mol. Res. 20 (6): gmr32452
Received May 27, 2021
Accepted June 10, 2021
Published June 17, 2021

ABSTRACT. Neural cells represent very limited regenerative capacity and cannot prevent the progression of central nervous system, and retinal, neurodegenerative diseases such as Alzheimer's, Parkinson's, Retinitis pigmentosa or age related macular degeneration diseases. Stem cell based regenerative strategies revealed successful preclinical transplantation outcomes, showing that exogenous stem cell derived-neurons can successfully engraft, differentiate, and integrate into the neural tissues. Stem cell-based therapies associate with important challenges including poor cell survival, limited differentiation, and reduced functional engraftment.

Key words: Oligodendrocytes; Optogenetics; Neurodegenerative cells

DESCRIPTION

Optogenetics stimulation represents a promising approach for regulating neural fate and improving therapeutic outcomes. Optogenetics integrate optical and genetic methodologies to allow precise spatial and temporal control of specific cell populations. Optogenetics stimulate neural differentiation *via* depolarization and $Ca2^{+}$ influx in stem cells [1,2].

In 2010, Weick et al. represented that, hESC-derived neurons are fully competent to excute functional neural networks *in vivo* [3]. Further studies represent that, optogenetics stimulation induces differentiation of stem cells into different neural cell lineages such as, oligodendrocytes, astrocytes, inhibitory neurons and excitatory neurons (Table 1).

Table 1. literatures focused on neural differentiation of stem cells by optogenetics stimulation.

Cell source	Optogenetics tools	Neural markers expression	*In vivo* study	Year	Reference
Embryonic stem cell (ESC).	Channelrhodopsin-2 (ChR2)	-Synapsin-1	Injection of cells to mice lateral ventricle	2011	[3]
Mouse ESC	ChR2	-Nestin -β-3-tubulin	Injection into the rat motor cortex	2012	[4]
C17.2 neural stem cell line	ChR2	-Nestin	-	2014	[5]
Glial progenitor cells	ChR2	-Galactocerebrosides (GalC) -Glial fibrillary acidic protein(GFAP)	Injection into mice dorsal column	2017	[6]
Human induced pluripotent stem cell (hiPSC)	ChR2	-beta-tubulin (Tuj1) -γ-aminobutyric acid B receptor 1(GABA-B-R1) -GFAP -vesicular glutamate transporter 1 (vGlut1)	-	2017	[7]
Schwann cells	ChR2	-Tuj1 -S100B	-	2019	[8]
ESC	ChR2	-Tuj1 -PDGFRα -GFAP -MAP2 -synaptophysin -vGAT -vGLUT1 -vGLUT2 -GAD1 -gamma aminobutyric acid (GABA) -oligodendrocyte transcription factor 2 (Olig2)	Injection into the rodent frontal neocortex	2019	[9]
Mouse ESC	ChR2	-vGlut -GAD-65/67 -NeuN	-	2020	[10]
Human dental pulp stem cells	ChR2	-Nestin -Doublecortin -MAP2	-	2020	[11]
Neural stem cell line	step-function opsin (SFO)	-NF200	-	2020	[12]
Rat spinal cords neural progenitor cell	ChR2	-Nestin -β-3-tubulin -Olig2 -GFAP	-	2021	[13]

In 2021, we reported that, cell membrane depolarization by optogenetics tools induced growth, proliferation and retinal neural differentiation in Opto-mGluR6 mouse retinal pigment epithelium cells (mRPE) and bone marrow mesenchymal stem cells (BMSCs). mRPE and BMSCs were selected for optogenetics study due to their capability of differentiation into retinal-specific neurons. This study revealed that, optical stimulation-induced inward currents and Ca^{2+} influx in Opto-mGluR6 engineered BMSCs and mRPE cells. Optogenetics stimulation-induced G1/S transition and cell-cycle progression in BMSCs culture. Differentiation into ganglion cells, horizontal cells, amacrine cells, photoreceptors, bipolar precursors, and Müller precursors were sensible in treated BMSCs cultures. While, mRPE cells revealed dominant differentiation into the Müller glial cells [14].

Eye formation in the human embryo begins at approximately the third week in embryonic development, and continues through the tenth week. So, it seems that, integral differentiation occurred during 40 days.

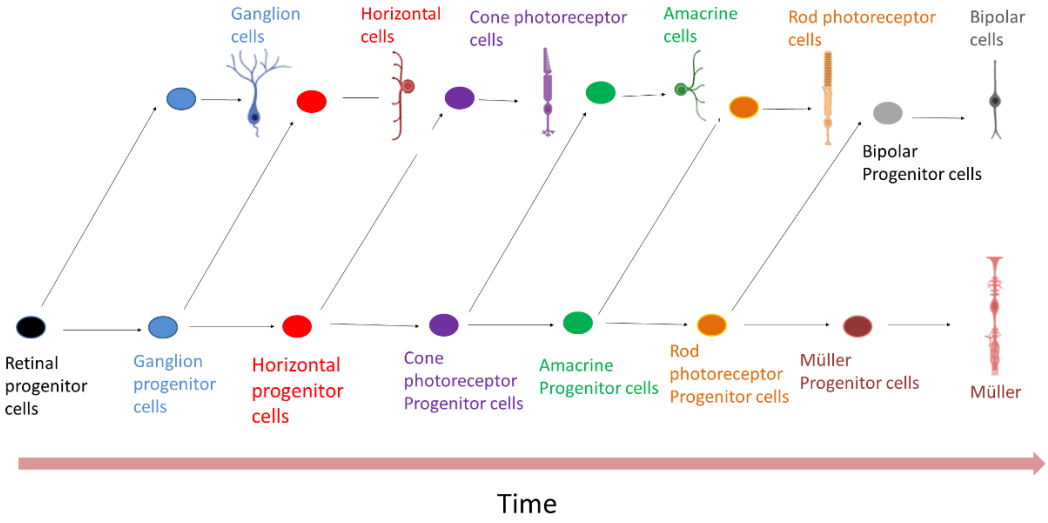

Figure 1. Retinal progenitor cells unidirectionally produce all the retinal cells. Retinal ganglion cells are the first cell type that emerge, horizontal cells and cones, amacrine cells, rods, bipolar cells and Müller glia are arose progressively later.

Retinal progenitor cells unidirectionally produce all types of retinal cells (Figure 1). Retinal ganglion cells are the first type of the retinal cells that arise. Horizontal cells, cones, amacrine cells, rods, bipolar cells and Müller glia are progressively generated later [15].

In our previous research, we surveyed for about 43 transcription factors that are crucial in differentiation pathway of progenitor cells into the retinal neurons (Figure 1). The data demonstrated that, in Opto-mGluR6 engineered BMSCs culture; ganglion cells', horizontal cells' and amacrine cells' specific markers were dominantly expressed. Accordingly, retinal differentiation seemed to be not occurred for all the neuroretinal cell types. It is suggested to pursue the optogenetics stimulation for at least 40 days to achieve to the more versatile terminal differentiation in treated cultures.

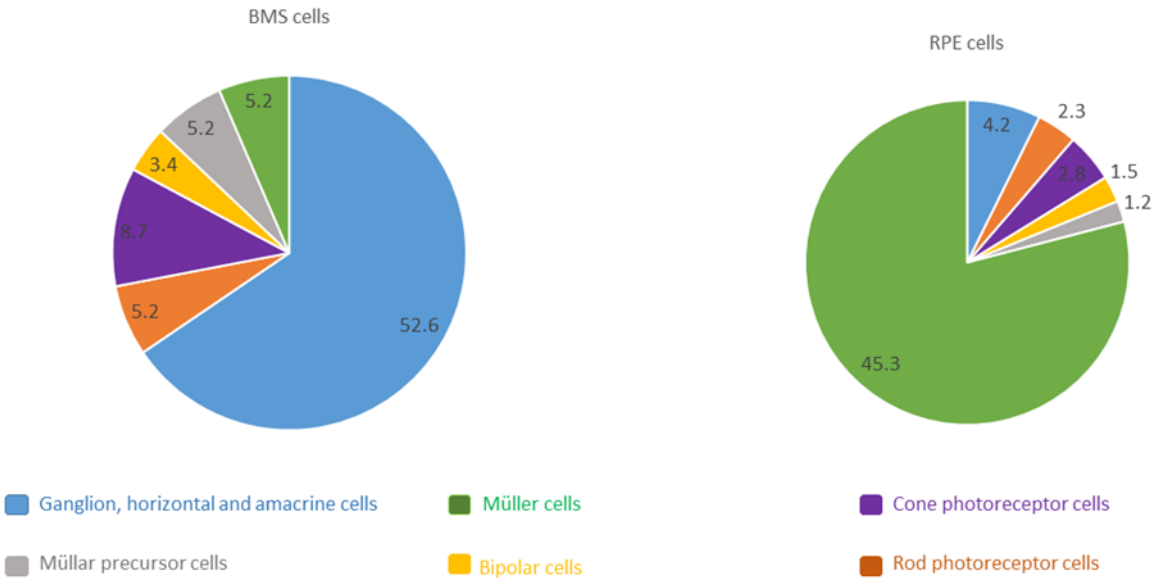

Figure 2. Gene expression pattern of optogenetically stimulated Opto-mGluR6 engineered RPE cells and BMSCs *in vitro*. Ganglion, horizontal and amacrine cells' specific genes were dominantly expressed in Opto-mGluR6 engineered BMSCs culture. While, Müller glial cells specific genes were dominantly expressed in Opto-mGluR6 engineered RPE cells' culture. BMSC, bone marrow mesenchymal stem cell; mRPE, mouse retinal pigment epithelium.

In Opto-mGluR6 engineered mRPE cell culture, Müller glial cells specific genes were dominantly expressed (Figure 2). It seems that, integral differentiation was occurred in the culture. To confirm this hypothesis, gene expression analysis should be pursued during 5 consecutive days.

In neuroregeneration, human pluripotent stem cells (hPSCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) hold great promise for regenerative medicine due to their capacity to differentiate to neuronal cells and integrating into the host retinal tissue [16]. Therefore, they hold promises in future applications of optogeneticS stimulation to develop efficient methods in retinal neural differentiation.

Human clinical trials represent that, cell replacement/transplantation can be used to prevent blindness and to restore eyesight in retinal disorders (NCT02320812, NCT02464436, NCT03073733). The results represent that; retinal cell translation is an effective therapeutic strategy to treat retinal degeneration. Optogenetically induced differentiated cells can be transplanted in to the subretinal space of murine mouse models to determine whether they can survive and integrate into the retinal layer and mediate light responses in the eye.

The retina contains highly organized networks of neuron cells arranged in three dimensions (3D). Furthermore, generation of retinal neurons in 3D requires bioactive scaffolds leading to a functional neural network that imitates those in the human eye. In order to overcome retinal degenerative disorders, generation of functional neuronal retinal cells in three-dimension (3D) in syntax with optogenetics targeting is a cutting-edge strategy [7]. This strategy not only aimed to retinal disorders, *via* tissue implantation, but also it would be a promise as an *in vitro* model for drug screening. So, f 3D culture microenvironment can be recruited to improve survival and differentiation potential of retinal cells *in vitro* or optogenetically engineered cells' transplantation *in vivo*. Bruch's membrane (BM) is a specialized basement membrane which transports nutrients to the retina. Substrate that are designed to mimic BM represent pivotal demand, regarding neural integration in the host retina. Phase 1/2a clinical study demonstrated that parylene membrane substrate which had been used for cell therapy of five patients with advanced AMD improved visual function reasonably (NCT02590692) [17]. Several other clinical trials represented that, PLGA (NCT04339764) polyethylene terephthalate (PET), collagen (UMIN000011929) scaffolds may be used in the near future for the treatment of AMD [18]. These scaffolds can be studied in directed research for transplantation of optogenetically engineered

cells in mice models of retinal degeneration to monitor their circuit-generating effects with physiological strategies.

CONCLUSION

Cell membrane depolarization by optogenetics tools may induce proliferation and neuron differentiation. Our previous research confirmed retinal neural differentiation of Opto-mGluR6 engineered BMSCs and mRPE cells by optogenetic stimulation. Studying optogenetics stimulation during different period of times, using different stem cell sources, generation of retinal neurons in 3D microenvironments, cell transplantion in to the subretinal space of murine mouse models may open new horizons in retinal regenerative researches.

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