===== REVIEWS =====

Reprogramming of Differentiated Mammalian and Human Retinal Pigment Epithelium: Current Achievements and Prospects

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Abstract—Impairment of the homeostatic and functional integrity of the retina and retinal pigment epithelium (RPE) is the main cause of some degenerative diseases of the human eye, which are accompanied by loss of eyesight. Despite the significant progress made over the past decades in the development of new methods for treatment for this pathology, there are still several complications when using surgical methods for correction of eyesight and so far insurmountable limitations in the applications of modern approaches, such as gene therapy and genetic engineering. One of the promising approaches to the treatment of degenerative diseases of the retina may be an approach based on the application of regenerative capacities of its endogenous cells with high plasticity, in particular, of RPE cells and Müller glia. Currently, vertebrate RPE cells are of great interest as a source of new photoreceptors and other neurons in the degrading retina in vivo. In this regard, the possibilities of their direct reprogramming by genetic, epigenetic, and chemical methods and their combination are being investigated. This review focuses on research in gene-directed reprogramming of vertebrate RPE cells into retinal neurons, with detailed analysis of the genes used as the main reprogramming factors, comparative analysis, and extrapolation of experimental data from animals to humans. Also, this review covers studies on the application of alternative approaches to gene-directed reprogramming, such as chemical-mediated reprogramming with the use of cocktails of therapeutic low-molecular-weight compounds and microRNAs. In general, the research results indicate the complexity of the process for direct reprogramming of human RPE cells into retinal neurons. However, taking into account the results of direct reprogramming of vertebrate cells and the accessibility of human RPE cells for various vectors that deliver a variety of molecules to cells, such as transcription factors, chimeric endonucleases, recombinant proteins, and low-weight molecular compounds, the most optimal combination of factors for the successful conversion of human RPE cells to retinal neurons can be suggested.

Keywords: retinal pigment epithelium, RPE, retinal regeneration, gene-directed reprogramming, genes, transcription factors, degenerative-dystrophic diseases of the eye

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INTRODUCTION. METHODOLOGICAL APPROACHES TO THE RESTORATION OF THE MAMMALIAN AND HUMAN RETINA

Retinal degeneration as a result of the death of photoreceptors, specialized cells that provide phototransduction, and retinal pigment epithelium (RPE) is the main cause of many degenerative and dystrophic diseases of the human eye that lead to the loss of eyesight (Fu et al., 2018). Currently, modern medicine is actively developing approaches for the correction of eyesight in this pathology aimed at preserving the original photoreceptors and RPE (Jiang et al., 2018), replacing cells by activating endogenous regeneration (Otteson, 2017), or by cell transplantation (Jiang et al., 2018; Léveillard and Klipfel, 2019). All this became possible because of the development of a number of cell and molecular biology technologies. Because of the usage of viral and nonviral vectors for delivery of functional genes to defective eye cells, the discovery of methods for the production of induced pluripotent stem cells (iPSCs) and the making of RPE from them, cells and retinal neurons for transplantation, and the creation of CRISPR/Cas9 technology, it is possible now to talk about a revolution that is taking place in ophthalmology (Jiang et al., 2018). Thus, thanks to the success of modern bioengineering, it has become possible to use the principles of gene therapy and genomic surgery for the treatment of inherited eye diseases (Chan et al., 2017). The main goal is to replace nonfunctional or defective genes with new, fully functional ones to return the level of genetic expression to normal. The first successful clinical example of gene therapy in ophthalmology was performed in patients with Leber congenital amaurosis caused by mutations in the *RPE65* gene (Bainbridge et al., 2008). Clinical tri-

als of gene therapy have also started for patients with Stargardt disease (clinicaltrials.gov NCT01367444), Usher syndrome (clinicaltrials.gov NCT01505062), and retinitis pigmentosa (clinicaltrials.gov NCT01482195) (Fu et al., 2018). Clinical trials of gene therapy aimed at treating retinal diseases have shown that it is safe and effective for people (Al-Saikhan, 2013; Öner, 2017; Fu et al., 2018; Jiang et al., 2018). However, gene therapy treatment is limited only to autosomal recessive diseases. In this case, a functionally defective gene remains in the cells, which must be blocked by making it nonfunctional or removed. The treatment of inherited eye diseases using genome surgery methods has been developing rapidly over the past few years. With the introduction of CRISPR/Cas gene-editing technology, which allows not only to block a defective gene but also to embed a working one, the attention of many researchers is directed now to the restoration of the retina and RPE (Burnight et al., 2017). In the near future, clinical trials of CRISPR/Cas9 on people with an ophthalmological disease, such as Leber type 10 congenital amaurosis, will begin (clinicaltrials.gov NCT03872479). Since gene therapy and genomic surgery have a high percentage of effectiveness only at the early stages of the development of degenerative retinal diseases, when photoreceptors and RPE are still preserved, much depends on the possibility of diagnosing diseases early. Also, these methods are not suitable for treating other types of retinal pathology.

Another approach in the treatment of some degenerative diseases of the retina, including age-related macular degeneration, is cell replacement therapy. Human embryonic stem cells (hESCs)- and iPSCs-derived RPE cells are already undergoing clinical trials and have great prospects both for the treatment of age-related macular dystrophy (Schwartz et al., 2012; Kharitonov et al., 2018; Luo and Chen, 2018; Kashani et al., 2018) and hereditary RPE-associated retinal dystrophies (Chichagova et al., 2018). Despite the highly elaborated effective protocols for obtaining RPE cells in sufficient numbers for transplantation, they remain labor- and time-consuming (Kharitonov et al., 2018; Artero-Castro et al., 2019). In addition to the positive effect, transplantation can cause some complications that can be triggered by surgical damage to the retina, leading to its detachment (Satarian et al., 2017), as well as by the cells themselves during long-term survival/integration, causing immunosuppression and tumor formation (Nguyen and Wong, 2017; Öner, 2018). In addition to scientific problems, moral and ethical questions about the usage of ESCs remain unresolved (Schwartz et al., 2015). Due to the increased interest in iPSCs and the possibility of producing RPE cells and retinal neurons from them for cell replacement therapy, the field of research into the regenerative abilities of their endogenous cells with high plasticities, such as RPE and Müller glia, in our opinion, has been unfairly overshadowed. It is obvious that direct reprogramming of endogenous cells into retinal neurons can be promising for the treatment of many degenerative diseases of the human retina, which allows us to avoid the above limitations and complications.

PREREQUISITES FOR DIRECT REPROGRAMMING OF RETINAL PIGMENT EPITHELIUM

RPE is a single-layer epithelium consisting of highly pigmented cells that perform vital functions in the physiology of the retina. RPE lies directly underneath the retina and forms an outer blood-retinal barrier. This anatomical arrangement provides a unique opportunity for direct reprogramming of RPE cells into photoreceptors and other neurons and regenerating the degenerating retina without surgery (Wang et al., 2010). During development, the RPE and retina originate from the same structure, the optic vesicle (Martínez-Morales et al., 2004). The neuroepithelial cells that form them have common molecular characteristics, are bi-potent, and can give rise to both RPE cells and retinal cells (Fuhrmann, 2010; Fuhrmann et al., 2014). During development, the optic vesicle invaginates to form a two-layer optic cup, creating an anatomical separation of the RPE (outer layer) and the retina (inner layer) (Martínez-Morales et al., 2004). RPE retains its simple, single-layer epithelial structure throughout life. The retina, however, is a highly ordered structure with five types of neurons, including ganglion, amacrine, bipolar, horizontal, and photoreceptor cells, and one type of glia, Müller cells (Zaghloul et al., 2005; Fuhrmann, 2010; Fuhrmann et al., 2014). Due to the common origin, RPE cells seem to retain molecular and cellular features that may contribute to switching their cellular fate during direct reprogramming. For example, recent studies (Dvoriantchikova et al., 2019) have shown that the RPE cells of the adult mouse eye are epigenetically very close to the phenotypes of retinal progenitor cells and photoreceptors. Based on data obtained using specific methods of DNA microarrays and methods based on chromatin immunoprecipitation and full-genome bisulfite sequencing (ChIP- and whole-genome bisulfite sequencing), the authors suggested the existence of at least two mechanisms that are required to trigger direct reprogramming of RPE cells into retinal neuronal cells. The first mechanism consists in remodeling of condensed chromatin, which contains the key genes of progenitor cells and mature retinal neurons by transcription pioneer factors. The second mechanism consists of the demethylation of regulatory elements of genes associated with photoreceptors (Dvoriantchikova et al., 2019). It can be assumed that these mechanisms are triggered in the cells of RPE in highly regenerating amphibians during retinal regeneration; they are probably suppressed or absent in mammals.

Table 1. Transcription factor genes used for direct reprogramming of vertebrate somatic cells into retinal cells.

Species	Source cells	Transcription factors	In vivo/in vitro/in situ	Characteristics of cells	I/II role	References
				Transcription factors of primary induction of retinal cells		
African clawed frog (lat. *Xenopus*)	Neural plate	*RX*	In vivo	Ectopic retina and RPE	I	(Mathers et al., 1997)
	Neural plate	*SIX3* or *SIX6*	In vivo	Retinal hyperplasia and the reduction of RPE. Ectopic optic-vesicular retinal-like structures in the midbrain and hindbrain. Disrupts the development of a normal eye	I	(Bernier et al., 2000b)
Japanese rice fish, medaka (lat. *Oryzias latipes*)	Neural plate	*SIX3*	In vivo	Retinal hyperplasia. Ectopic optic-vesicular retinal-like structures in the brain. Initiates, but does not implement, late stages of retinal development	I	(Loosli et al., 1999)
Mouse (lat. *Mus musculus*)	Brain cells	*SIX3* or *SIX6*	In vivo	Ectopic optic-vesicular retinal-like structures	I	(Lagutin et al., 2001)
	RPE	*SIX6*	In vivo	Neuronal morphology and markers expression	I	(Toy et al., 1998a)
	RPE	*PAX6*	In vivo	Fully formed ectopic retina	I	(Azuma et al., 2005)
Chicken (lat. *Gallus domesticus*)	RPE	*PAX6* *SIX 3* *SIX6* *CRX* *SIX9* *OPTX2*	In vitro	Unstructured ectopic retina	I	(Yan et al., 2010)
	RPE	*OTX2*	In vitro/in situ	Recovery of the RPE phenotype	II	(Kole et al., 2018)
Human (lat. *Homo sapiens*)	Retinal stem cells	*PAX6* *CHX10* *CHX10VP16* *SIX 3*	In vitro	Do not affect the direct reprogramming of cells into retinal photoreceptors	II	(Inoue et al., 2010)
		CRX *OTX2*	In vitro	Stimulate direct reprogramming of cells into retinal photoreceptors	I	

Table 1. (Contd.)

Species	Source cells	Transcription factors	In vivo/ in vitro/in situ	Characteristics of cells	I/II role	References
Human (lat. *Homo sapiens*)	Ciliary body	SIX3 PAX6 CRX CRX RX	In vitro	Do not affect the direct reprogramming of cells into retinal photoreceptors	II	(Seko et al., 2012)
	Müller glia cells	SIX3 PAX6 CRX CRX RX	In vitro	Do not affect the direct reprogramming of cells into retinal photoreceptors	II	(Seko et al., 2012)
	Iris	SIX3 PAX6 CRX CRX RX	In vitro	Do not affect the direct reprogramming of cells into retinal photoreceptors	II	(Seko et al., 2012)
Transcription factors of cellular specialization and differentiation of retinal cells						
Chicken (lat. *Gallus domesticus*)	RPE	NEUROD	In vivo/ in vitro/in situ	Morphologically, molecularly, and functionally fully developed photoreceptor cells, ganglion cell layer cells, and amacrine cells in the retina	I	(Yan and Wang, 1998; Liang et al., 2006, 2008)
		NGN1	In vivo/in vitro/ in situ	Morphologically, molecular, and functionally fully developed photoreceptor cells and other retinal neurons	I	(Liang et al., 2006; Li et al., 2010; Yan et al., 2010; Wang and Yan, 2014)
		NGN2	In vivo/ in vitro/ in situ	Morphologically, molecularly, and functionally fully developed photoreceptor cells, ganglion cell layer cells, and amacrine retinal cells	I	(Yan et al., 2001; Liang et al., 2006)
		NGN3	In vivo/ in vitro/ in situ	Morphologically, molecularly, and functionally fully developed photoreceptor cells, ganglion cell layer cells, and amacrine retinal cells	I	(Liang et al., 2006; Ma et al., 2009b; Li et al., 2010; Yan et al., 2010; Wang and Yan, 2014)

Table 1. (Contd.)

Species	Source cells	Transcription factors	In vivo/ in vitro/in situ	Characteristics of cells	I/II role	References
Chicken (lat. *Gallus domesticus*)		SOX2	In vivo/in vitro	Morphologically, molecularly, and functionally fully developed cells of the ganglion layer and amacrine cells of the retina	I	(Ma et al., 2009a)
		NSCL1	In vitro	Some markers of retinal ganglion layer cells	II	(Ma et al., 2004)
		NSCL2	In vivo	Degradation of retinal Müller glia cells	II	(Ma et al., 2009a)
		ASH1	In vivo/ in vitro	Molecular and morphological markers of retinal ganglion cells, amacrine and retinal horizontal cells	I	(Mao et al., 2008; Li et al., 2010)
		ATH3	In vivo/ in vitro	Does not contribute to the genesis of retinal bipolar cells	II	(Yan et al., 2010)
		ATH5	In vitro	Markers of retinal ganglion cell layer cells	I	(Ma et al., 2004)
	RPE	NGN1	In vivo/in vitro	In vitro, 30% of cells carry markers of retinal neural cells, 20% of cell demonstrate photoreceptor cell morphology, Ectopic retina	I	(Yan et al., 2013a)
		NGN3	In vitro/in situ	Ectopic retina	I	(Yan et al., 2013a, 2013b; Wang and Yan, 2014)
Mouse (lat. *Mus musculus*)	Astroglia of the cerebellum	ASH1, NGN2	In vitro	Neuronal morphology and markers, Synaptic maturation, Functional electrophysiology	I	(Chouchane et al., 2017)
	Müller glia cells	ASCL1	In vivo/in vitro/ in situ	Morphology and molecular markers of photoreceptor and amacrine cells with the dominance of markers of bipolar cells, Respond to various transmitters Integration into the retina	I	(Pollak et al., 2013; Ueki et al., 2015; Jorstad et al., 2017; Guimarães et al., 2018)
		NGN2	In vitro	Molecular, morphological, and physiological markers of photoreceptor cells, amacrine cells, and retinal ganglion cell layer cells	I	(Guimarães et al., 2018)
	Cone cells	NRL	In vivo	Transformation of cone cells into rod cells	I	(Montana et al., 2013)
Pig (lat. *Sus scrofa domesticus*)	RPE	NEUROD	In vitro	Morphological and molecular markers of photoreceptor cells	I	(Yan et al., 2013a)

Table 1. (Contd.)

Species	Source cells	Transcription factors	In vivo/ in vitro/in situ	Characteristics of cells	I/II role	References
Human (lat. *Homo sapiens*)	RPE	SOX2	In vitro	Elevation of expression of neural and glial markers, Absence of GABA-ergic neurons, Absence of synaptic mechanisms	I	(Hu et al., 2014; Ezati et al., 2018)
	hTERT-RPE1	NEUROD	In vitro	30% of cells with molecular and morphological markers of retinal photoreceptor cells	I	(Yan et al., 2013a)
		NGN1		50% of cells with molecular and morphological markers of retinal photoreceptor cells	I	
	ARPE19	NGN1	In vitro	10% of cells with molecular and morphological markers of retinal photoreceptor cells	I	(Yan et al., 2013a)
	Retinal stem cells	CHX10VP16, OTX2, CRX	In vitro	Induce direct reprogramming of retinal photoreceptor cells	I	(Inoue et al., 2010)
	Ciliary body	NEUROD, NRL	In vitro	Do not induce direct reprogramming into retinal photoreceptor cells	II	(Seko et al., 2012)
	Müller glia cells	NEUROD, NRL	In vitro	Do not induce direct reprogramming into retinal photoreceptor cells	II	(Seko et al., 2012)
	Iris	NEUROD, NRL	In vitro	Do not induce direct reprogramming into retinal photoreceptor cells	II	(Seko et al., 2012)
Combinations of different genes of transcription factors and other factors					I Factor	References
Chicken (lat. *Gallus domesticus*)	RPE	ATH5, bFGF	In vitro	Molecular, morphological, and physiological markers of retinal ganglion cells	ATH5	(Ma et al., 2004)
	RPE	ASH1, ATH3, CHX10	In vitro	Molecular, morphological, and physiological markers of retinal bipolar cells	ASCL1	(Yan et al., 2010)
Mouse (lat. *Mus musculus*)	Müller glia cells	ASCL1, TSA, Injury	In vivo	Molecular, morphological, and physiological markers of retinal bipolar and ganglion cell layer cells, with functional integration into the retina	ASCL1	(Jorstad et al., 2017)
	Müller glia cells	NGN2, FGF2, EGF	In vivo	Molecular, morphological, and physiological markers of photoreceptor cells, amacrine and ganglion cell layer cells	NGN2	(Guimarães et al., 2018)

Table 1. (Contd.)

Species	Source cells	Transcription factors	In vivo/in vitro/in situ	Characteristics of cells	I/II role	References
Mouse (lat. *Mus musculus*)	iPSCS	*MATH5* DAPT DKK1 NOGGIN	In vitro/in situ	Molecular, morphological, and physiological markers of retinal ganglion cell layer cells. When transplanted into the eye, the induced cells survived but did not integrate	*MATH5*	(Chen et al., 2010)
	Retinal stem cells	*CHX10VP16, OTX2* and *CRX*	In vitro/in situ	Functional retinal photoreceptor cells that can integrate into the recipient's tissue and establish synapses with bipolar cells	*OTX2* and *CRX*	(Inoue et al., 2010)
	Ciliary body	*SIX3, PAX6, CRX* and *RX*	In vitro	Photoreceptor cells without outer segments and transduction mechanisms	*CRX, RX*	(Seko et al., 2012)
	Müller glia cells	*SIX3, PAX6, CRX* and *RX*	In vitro	Photoreceptor cells without outer segments and transduction mechanisms	*CRX, RX*	(Seko et al., 2012)
	Iris	*SIX3, PAX6, CRX* and *RX*	In vitro	Photoreceptor cells without outer segments and transduction mechanisms	*CRX, RX*	(Seko et al., 2012)
		CRX, RX and *NEUROD1*	In vitro	Morphological and molecular markers of neuronal cells. Functional electrophysiology	*CRX, RX*	
Human (lat. *Homo sapiens*)	Fibroblasts	*ASCL1, BRN2 (POU3F2), MYT1L*	In vitro	Induced functional neurons	*ASCL1*	(Vierbuchen et al., 2010)
		NEUROD, ASCL1 and *MYT1L* miR-9/9, miR-124	In vitro	Neural cells with the main functional properties of neurons, including the formation of synapses	*ASCL1*	(Yoo et al., 2011)
		CRX, RAX, NEUROD1 and *OTX2*	In vitro	Molecular, morphological, and physiological markers of photoreceptor cells	*CRX, RX*	(Seko et al., 2014)
	iPSCs	*ATOH7 (MATH5)* DAPT DKK1 LEFTY A, NOGGIN	In vitro	Molecular, morphological, and physiological markers of retinal ganglion cell layer cells	*ATOH7 (MATH5)*	(Deng et al., 2016)

I—transcription pioneer factor; II—canonical transcription factor.

Classical experiments on animal models have demonstrated the capability of RPE cells for transdifferentiation, natural direct reprogramming into neural retinal cells. The process of transdifferentiation of RPE cells into neural cells in lower vertebrates is successfully reproduced in vivo. Thus, RPE cells in several amphibian species after retinal damage are reprogrammed into cells similar to neuroepithelial stem cells, descendants of which differentiate into all retinal neuronal cells, including photoreceptors, glia pigment epithelium, and completely restore retinal function (Chiba and Mitashov, 2008; Vergara and Del Rio-Tsonis, 2009; Grigoryan et al., 2013; Islam et al., 2014). In birds and mammals, RPE conversion occurs at the early stages of embryonic development only under the influence of the basic Fibroblast Growth Factor (bFGF) (Luz-Madrigal et al., 2014) and in adults after an increase or loss of function of genes involved in determining the cellular fate of RPE and the retina (Nguyen and Arnheiter, 2000; Bumsted and Barnstable, 2000; Martínez-Morales et al., 2003; Bäumer et al., 2003; Fujimura et al., 2009; Bassett et al., 2010; Bharti et al., 2012; Remez et al., 2017). In adult mammals, including humans, RPE may exhibit plasticity and proliferation. It has been shown that, in the mature rat eye, a small population of cells on the periphery of the RPE supports mitotic activity (Al-Hussaini et al., 2008). RPE cells that are normally at rest can reenter the cell cycle and proliferate under certain conditions, such as retinal detachment (Anderson et al., 1981), physical stimulation (Zhang et al., 1993), retinal damage, or degeneration (La Cour, 2008). A proliferative response can have two consequences: it can lead to the regeneration of RPE (Rabenlehner et al., 2008) or to proliferative retinopathy, in which RPE cells are transdifferentiated into fibroblast-like cells that cause retinal detachment (Tamiya and Kaplan, 2016).

Transdifferentiation of RPE cells in birds and mammals into retinal neurons can also be observed in vitro after the addition of morphogens and growth factors (Zhao et al., 1995; Engelhardt et al., 2005; Sakami et al., 2008; Salero et al., 2012). In cell culture, RPE cells lose their original features, such as pigmentation, significantly reduce the expression of specific markers RPE65, MITF, and CRALBP and acquire features of neural cells on markers MUSASHI1, NESTIN, βIII-TUBULIN, GFAP, DOUBLECORTIN, and NF 68 and 200 kDa. Our studies also demonstrate that RPE cells of the human embryo and adult in vitro in media with the addition of morphogens and growth factors lose their pigment granules, dedifferentiate, proliferate, and exhibit markers of several types of neural and glial cells (Milyushina et al., 2009, 2011, 2012; Kuznetsova et al., 2014; Kuznetsova et al., 2015, 2019). At the dedifferentiation stage, cells acquire stem/neuroepithelial cell traits by expressing *OCT4*, *NANOG*, *KLF4*, *OTX2*, *PAX6*, and *NESTIN* (Milyushina et al., 2009, 2011, 2012; Kuznetsova et al., 2014, 2015, 2019a, 2019b). Although mRNA expression of pluripotency

marker genes is very low compared to human iPSCs (Kuznetsova et al., 2019), the activity of these genes indicates that they can act as pioneer factors (Kuzmich et al., 2015). Human RPE cells in vitro acquire proneural properties while partially preserving the properties of RPE. The cell culture conditions clearly show the heterogeneity of the population of RPE cells. Thus, under 2D culture conditions, differences like cell growth and in the heterogeneity of the monolayer are revealed: cells are different in size, shape, degree of pigmentation, and number of nucleus as well as in the formation of colonies, cells form both densely packed epithelial colonies with different morphology and "loose" colonies with blurred borders, which reflects the ongoing clonal cell proliferation (Kuznetsova et al., 2011). Under 3D culture conditions (in a collagen gel, on a cell-free retinal framework), the heterogeneity of RPE cells is manifested in the division into two subpopulations of cells that are different in morphology and various in behavior: one subpopulation of cells migrates to the surface of a dense substrate, the other forms sphere-like structures of aggregated cells (Kuznetsova and Aleksandrova, 2017). The subpopulation that retains the ability to form a tight monolayer and to the redifferentiation of RPE might have beneficial effects for transplantation, whereas a second subpopulation, which forms cell aggregates, can exert a tractional effect on the surrounding tissues, which is unfavorable for transplantation. Such features of RPE cell heterogeneity should be taken into account when using it in tissue engineering.

Along with this, it is well known from pathomorphological studies that human eyes sometimes show cartilage and bone formations that develop from RPE cells (Frayer, 1966; Tso and Fine, 1979; Salero et al., 2012). In the cell culture conditions, RPE cells under the influence of specific inducers show signs not only of neural but also of smooth muscle, adipo-, chondro-, and osteogenic differentiation. According to some authors, RPE cells under certain conditions can become "multipotent stem cells" capable of producing cells of both neural and mesenchymal phenotypes (Milyushina et al., 2012; Salero et al., 2012). The capability for multiple differentiations emphasizes the interest in the extremely high plasticity of RPE cells and sets the task of searching for mechanisms that determine it and of factors for regulating directed differentiation.

The analysis of the presented papers shows that mammalian and human RPE cells, based on their anatomical, genetic, and epigenetic characteristics; origin; evolutionary inheritance; and the capacity for dedifferentiation, proliferation, and plasticity are of great interest as a source of new photoreceptors and other types of neurons in the degraded retina. However, the short-term nature of the manifestation of proneural properties by adult RPE cells in vitro initiated researches into searching for opportunities for direct reprogramming of RPE using genetic, epigene-
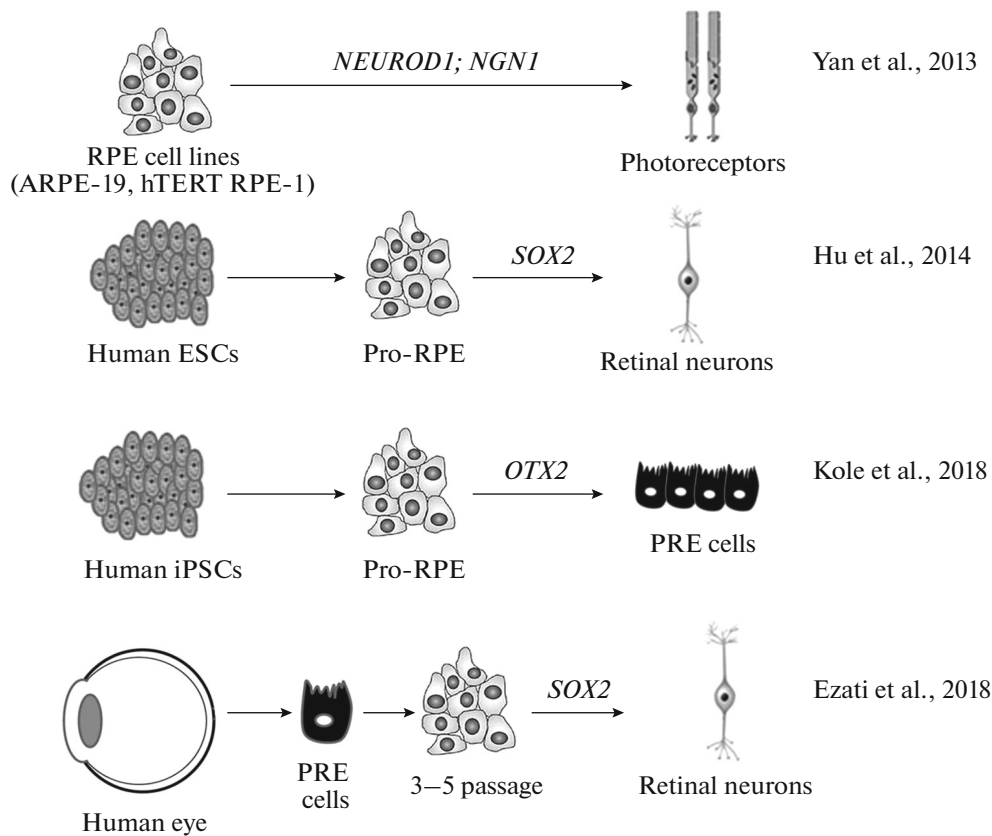
Fig. 1. Schematic representation for genetic direct reprogramming of human RPE cells obtained from various sources into retinal and RPE cells. RPE—retinal pigment epithelium; ESC—embryonic stem cells; iPSC—induced pluripotent stem cells.

tic, and chemical methods of influence, which will be discussed below.

GENETIC DIRECT REPROGRAMMING OF RETINAL PIGMENT EPITHELIUM

Genetic programs are the main driving forces of retinal development, which coordinate cell proliferation and exit from the cell cycle, determine cell fate, control the number of cells, and control cell maturation (Reese and Keeley, 2016). The differentiation or reprogramming of any cell is based on the recognition and activation of silent genes. These processes occur as a result of the combined action of the so-called primary, pioneer, or transcription pioneer factors with canonical transcription factors (Kuzmich et al., 2015; Mayran et al., 2019). When reprogramming cells, a combination of transcription factors is usually used, some of which are pioneer factors. Thus, when obtaining functional glutaminergic neurons from mouse fibroblasts using three transcription factors, ASCL1, BRN2, and MYT1L (Vierbuchen et al., 2010), it turned out that ASCL1 plays a central role in the initiation of direct reprogramming, since it alone is sufficient for the induction of fibroblasts into immature neural cells, while BRN2 and MYT1L are not suffi-

cient. Thus, ASCL1 is a transcriptional pioneer factor in neuronal direct reprogramming of fibroblasts (Iwafuchi-Doi and Zaret, 2014). When reprogramming fibroblasts into iPSCs, OCT3/4, SOX2, and/or KLF4 act as pioneer factors, in contrast to c-MYC (Iwafuchi-Doi and Zaret, 2014; Kuzmich et al., 2015). Therefore, for successful genetic direct reprogramming of human RPE cells into retinal neurons, we need to determine the pioneer factors without which this process is not possible and additional canonical factors that will promote cellular conversion after the initiation of the process.

Modern methods of genetic engineering, by enhancing or suppressing the function of genes involved in determining the cellular fate of RPE and the retina, have created the possibility of direct reprogramming of RPE cells in mammals. Researchers from the Department of Ophthalmology of the University of Alabama and the Faculty of Medicine at Birmingham, United States, as well as other groups of researchers, have shown that the cells of the RPE of birds, mice, and humans can be directly reprogrammed by the influence of various genes that are involved in the process of differentiation of the retina in vivo and in vitro (Mathers et al., 1997; Yan and Wang, 1998; Toy et al., 1998b; Loosli et al., 1999; Ber-

nier et al., 2000a; Lagutin et al., 2001; Yan et al., 2001, 2010, 2013a, 2013b, 2015; Azuma et al., 2005; Liang et al., 2008; Ma et al., 2009a; Li et al., 2010; Wang et al., 2010; Wang and Yan, 2014; Kole et al., 2018). Table 1 shows the genes necessary for the development of the eye and retina, which are used in experiments on genetic direct reprogramming of RPE cells and other vertebrate and human cells into retinal neurons. For this purpose, the studied genes were delivered to vertebrate and human cells in vivo and in vitro using viral vectors. Transcription factors involved in determining the cellular fate of the RPE and retina are homeodomain-containing transcription factors that are encoded by homeobox genes. They can be provisionally divided into factors of primary induction: factors of the eye field and factors of cellular specialization and differentiation, belonging mainly to the family with the basic structural motif basic helix-loop-helix, bHLH (Zagozewski et al., 2014).

The role of transcription factors of primary retinal induction in direct reprogramming. Transcription factors of primary induction of retinal cells PAX6, CHX10, RAX, SIX3, SIX6, OTX2, and CRX, which are regional factors of transcription of the eye field, are necessary for determining the fate of progenitor cells and for terminal differentiation of certain types of retinal cells (Zagozewski et al., 2014). The influence of these factors on the direct reprogramming of cells of neural origin into retinal neurons has been studied in several studies on vertebrates (Mathers et al., 1997; Toy et al., 1998b; Loosli et al., 1999; Bernier et al., 2000a; Lagutin et al., 2001; Azuma et al., 2005; Yan et al., 2010; Kole et al., 2018). Thus, in the work of Azuma and coworkers (Azuma et al., 2005), it was shown that a single *PAX6* gene is sufficient to cause direct reprogramming of chicken embryo RPE cells (lat. *Gallus domesticus*) into retinal neurons. The authors used a plasmid carrying human *PAX6* cDNA to induce direct reprogramming of avian RPE cells *in ovo* to form a complete ectopic retina. Despite this, other researchers (Yan et al., 2010) consider *PAX6* to be an "ineffective" gene for direct reprogramming of RPE cells into neural retinal cells since they did not observe it in their experiments.

Additional retina or retinal-like structures are also formed during ectopic expression of other eye field transcription factors. Thus, ectopic expression of *SIX3* or *SIX6* (also known as *OPTX2*) induced retinal hyperplasia and the formation of an ectopic visual vesicular or retina-like structure in the brains of Japanese rice fish (medaka, lat. *Oryzias latipes*), in clawed frogs (lat. *Xenopus*), and in murine embryos (lat. *Muridae*) (Loosli et al., 1999; Bernier et al., 2000a; Lagutin et al., 2001). The authors point out that *SIX3/SIX6* induces but does not fully accomplish later stages of retinal cell development. Ectopic expression of *SIX6* in embryonic or mature chicken RPE cells also converts them to neuronal morphology and expression of markers characteristic of developing retinal neurons

(Toy et al., 1998b). Clawed frog embryos injected with synthetic RNA *RX* but not *PAX6* and *OTX2* develop ectopic retinal tissue and RPE (Mathers et al., 1997). However, it was not possible to obtain a fully structured retina by directly reprogramming RPE with the *SIX3/SIX6* genes in these experiments.

One of the key homeobox-containing factors is OTX2, which regulates the initial specification of photoreceptors and RPE (Karali and Banfi, 2015). Thus, it was shown that the human retinal stem cells (hRSCs) are differentiated into photoreceptors in vitro after *OTX2* transfection (Inoue et al., 2010). However, Kole et al. (2018) showed that, in human iPSC-derived RPE cells, ectopic *OTX2* expression enhanced the activity of gene promoters that are suppressed during dedifferentiation of RPE cells in vitro, thereby contributing to the restoration and preservation of the original RPE phenotype, and did not induce the process of direct reprogramming of these cells into retinal photoreceptors (see Fig. 1). These results, according to the authors, as well as to Fisher and Ferrington (2018), have some significance since the maintenance of RPE cells in patients with retinopathies associated with functionally defective RPE, for example, with age-related macular dystrophy, will help to restore photoreceptor functions, since RPE and photoreceptors are a single functional unit in the retina (Fuhrmann, 2010; Fuhrmann et al., 2014).

Negative results were obtained in experiments with direct reprogramming of other human eye cells (iris pigment epithelium, Müller glia cells, ciliary body cells) into photoreceptors during viral transfection of *SIX3*, *PAX6*, *RX*, and *CRX* (Seko et al., 2012). The authors showed that none of the genes alone, when exogenously expressed, induce the formation of photoreceptor phenotypes in the studied cells in vitro (Seko et al., 2012). However, transfection of *OTX2* and *CRX* alone caused direct reprogramming of human RSCs into photoreceptors in vitro (Inoue et al., 2010). This difference in the effectiveness of direct reprogramming can be explained by the fact that more specialized cells (iris pigment epithelium, Müller glia, and ciliary body cells) are much more resistant to direct reprogramming than less specialized RSCs (Pasque et al., 2011).

Interestingly, that main tissue-specific homeobox genes, which are at the top of the gene regulatory network for eye and/or neural retinal development, showed little activity in direct reprogramming of vertebrate RPE into retinal neurons (Yan et al., 2010). Under the influence of these factors, a fully structured retina was not formed. Yan et al., (2010) considered these transcription factors as "ineffective" factors for direct reprogramming of RPE cells into retinal neurons. This "inefficiency" of the eye field genes in direct reprogramming of the eye cells can probably be explained by the common origin of the RPE and retina from progenitor cells with the same pattern of expres-

sion of the eye field genes. The initiation of their separation process occurs under the epigenetic influence of signal molecules synthesized by the tissues surrounding the optic vesicle, the integumentary ectoderm, and mesenchyme, which leads to the inclusion of highly specialized genes, for example, belonging to the bHLH family in the neural retinal progenitor cells and *MITF, TYR, TRP1, RPE65, CRBP, CRALBP,* and *PITX2* in the RPE (Fuhrmann, 2010; Fuhrmann et al., 2014; Zagozewski et al., 2014).

Thus, an analysis of the literature on direct reprogramming of amphibian, avian, and mammalian RPE cells into retinal neurons using ectopic expression of eye field genes showed the initiation of the cellular conversion process, which indicates their role as pioneer factors. Since eye field factors in humans do not induce direct reprogramming of RPE cells into retinal neurons, it can be assumed that they are either not required as pioneers in this process or they are passive primary factors that remodel chromatin, making it available for other transcription factors, but do not affect gene transcription themselves (Kuzmich et al., 2015). Also, taking into account the fact that some eye field genes, such as *PAX6, OTX2, RX,* and *LHX2*, are expressed in postnatal human RPE cells, (Milyushina et al., 2012; Salero et al., 2012), exogenous expression of these genes in direct reprogramming may not be required and, therefore, the expected result of direct reprogramming under the action of these genes is not observed (Masserdotti et al., 2016).

The role of factors of cellular specialization and differentiation of retinal cells in direct reprogramming. Factors of cellular specialization and differentiation are homeodomain-containing transcription factors belonging to the bHLH family and to the Forkhead box (FOX) family that work together to determine the fate of retinal cells (Zagozewski et al., 2014).

The effect of bHLH family genes on the ability of vertebrate RPE cells to be directly reprogrammed into retinal neurons has been investigated in many studies (Yan and Wang, 1998; Yan et al., 2001, 2010, 2013a, 2013b, 2015; Liang et al., 2008; Ma et al., 2009a; Li et al., 2010; Wang et al., 2010; Wang and Yan, 2014). The results of these studies show that transcription factors of the bHLH family, NEUROD, NGN1, NGN2, and NGN3, can directly reprogram differentiated chicken embryo RPE cells in dissociated cultures and explants into cells with the molecular, morphological, and physiological properties of young photoreceptor cells with small fractions of other types of retinal neurons effectively (Yan and Wang, 1998; Yan et al., 2001, 2009, 2010; Liang et al., 2008; Li et al., 2010; Wang et al., 2010; Wang and Yan, 2014). When transplanted into the eyes of developing chickens, RPE cells, which were converted in vitro, continue to develop in the photoreceptor direction. Some of the transplanted cells integrate into the outer nuclear layer of the retina and became embedded in the functional

host neuronal network (Liang et al., 2006). In addition, it has been shown that chicken RPE cells can be directly reprogrammed in situ (Li et al., 2010). In the eyes of chicken embryos, RPE cells modified with *NGN1, NGN2,* and *NGN3* showed molecular and morphological markers of photoreceptor, ganglion, and amacrine cells (Yan et al., 2001, 2010). In transgenic mice that express *NGN1* or *NGN3*, the appearance of ectopic retinal-like tissue in the vasculature, near the ciliary body, in the optic nerve, as well as in the subretinal space, is described (Yan et al., 2009; Wang and Yan, 2014). Interestingly, the cells of the additional ectopic outer nuclear layer had molecular and morphological features of photoreceptors, like normal retinal cells, but their spatial orientation relative to the RPE layer was disturbed. Ectopic retinal cells located in the inner nuclear layer expressed markers of amacrine and bipolar cells, while those located in the ganglion cell layer expressed markers of ganglion cells (Yan et al., 2013b). Such direct reprogramming occurs in the RPE cells of primary cultures of juvenile pigs, mice, and in the of immortalized human RPE cell lines (Yan et al., 2013a). Thus, using viral constructs that carry the *NEUROD* and *NGN1* genes, it was shown that approximately 30% of converted RPE cells show molecular and morphological markers of young photoreceptor cells during ectopic expression of *NEUROD* in the hTERT RPE cell line-1[1] and their number increases to 50% with ectopic expression of *NGN1* (Yan et al., 2013a). At the same time, the number of cells that were changed into photoreceptors reached only 10% in the ARPE-19 cell line[2] (Yan et al., 2013a). This difference in the effectiveness of direct reprogramming of human RPE cell lines can be explained by epigenetic memory, which remains from donor cells (Kim and Costello, 2017). Thus, the ARPE-19 line is represented by the most epigenetically differentiated cells (Dunn et al., 1996; Kim and Costello, 2017). However, the results obtained by Yan et al. (2013a) demonstrate that human RPE cells are capable to direct reprogramming under the influence of ectopic expression of the *NEUROD1* and *NGN1* genes. However, under the influence of ectopic expression of *NEUROD1* and *NGN2* cells of other human eye tissues (iris, Müller glia cells, ciliary body) did not form photoreceptor cells during direct reprogramming (Seko et al., 2012). It can be assumed that the transcription factors *NEUROD1* and *NGN1* for human RPE cells can act as master genes or pioneer factors. This may indicate the epigenetic availability of DNA of RPE cells for these factors, which arose during development and persists into adulthood under

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[1] The hTERT RPE-1 cell line was obtained by transfection of the RPE-340 cell line by a plasmid vector expressing the catalytic subunit of human telomerase (Rambhatla et al., 2002). RPE-340 cell line was obtained from a 1-year-old girl who died from injuries (Matsunaga et al., 1999).

[2] ARPE-19 was obtained by Aotaki-Keen in 1986 from a 19-year-old man who died of a head injury after an accident (Dunn et al., 1996).

the influence of endogenous expression of ocular field genes that act as passive pioneer factors.

Another highly plastic type of retinal cell, Müller glia cells, also has an intrinsic ability to be directly reprogrammed. Guimarães et al. (2018) have shown that retinal ganglion cells in mice can be obtained from postnatal Müller glia cells by overexpression of *NGN2*. Ectopic expression of *NGN2* contributed to the production of a pool of neurons with expression of photoreceptor genes, amacrine cells, and ganglion layer cells. The authors also showed that the presence of mitogenic factors, such as EGF or bFGF, which stimulate the proliferation of mouse Müller glia cells increased the effectiveness of direct reprogramming (Guimarães et al., 2018). Proliferation is one of the passive ways to remove the methylation of DNA, epigenetic memory, which increases the effectiveness of direct reprogramming (Masserdotti et al., 2016; Kim and Costello, 2017).

It was shown (Pollak et al., 2013; Ueki et al., 2015) that viral expression of *ASCL1*, another member of the bHLH transcription factor family, is sufficient for activation of the neurogenic program in mammalian (murine) Müller glia cells, both in dissociated cultures in vitro and in the intact retina in vivo. Ectopic expression of transcription factor *ASCL1* stimulates the process of direct reprogramming of Müller glia cells' descendants into photoreceptors, amacrine, and bipolar cells after damage in vivo (Ueki et al., 2015). This is consistent with the role of *ASCL1* in normal development, where *ASCL1* is known to be expressed in late progenitor cells that produce amacrine, bipolar cells, and photoreceptors, but not ganglion cells, and deletion of *ASCL1* in mice results in a decrease in the number of bipolar cells and photoreceptors (Brzezinski et al., 2011).

Another member of the bHLH family of transcription factors ASH1 (MASH1) is required in development for the production of late neurons, including photoreceptor rods and bipolar cells in mice (Tomita et al., 1996). Transgenic expression of *ASH1* initiates retinal neurogenesis in the RPE layer in mice in vivo (Lanning et al., 2005). In chickens, the temporal and spatial expression of *ASH1* corresponds to the genesis of amacrine cells (Jasoni et al., 1994), and overexpression of *ASH1* increases the population of these cells (Mao et al., 2009). Mao et al. (2008) show that *ASH1* can directly reprogram RPE cells towards retinal neurons in vitro at the molecular, morphological and physiological levels, and Li et al. (2010) demonstrated a similar effect in vivo. However, it was noted that direct reprogramming of cells into photoreceptors does not occur in case of infection of RPE of chicken embryos with RCAS-*ASH* (Li et al., 2010). Also, the same researchers believe that the joint ectopic expression of *ASH1, ATH3*, and *CHX10* contributes to a more efficient genesis of bipolar cells (Yan et al., 2010), where *ASH1* is responsible for narrow cell specialization.

However, not all bHLH family genes are expressed in the developing retina, and homologous to the proneural Drosophila genes can initiate direct reprogramming of RPE into retinal neurons. These "ineffective" genes include *NSCL1* and *NSCL2* (Wang and Yan, 2012).

Degeneration of the retinal ganglion layer cells is the main sign of glaucoma that affects the elderly; the restoration of ganglion cells is among the most important tasks, as is the restoration of photoreceptors (Guimarães et al., 2018). Ma et al. (2009a) showed the participation of the SOX2 transcription factor, belonging to the Sox family, in the induction of expression of markers of the ganglion and amacrine neurons in chicken embryonic RPE cells in vivo and in vitro and in inhibiting the expression of RPE-specific genes. Using the approach of Ma et al. (2009a) that was applied on chickens, other researchers (Hu et al., 2014) tried to reprogram the RPE cells obtained from human ESCs (see Fig. 1). As a result, they found increased expression of marker genes for neuronal and glial retinal cells and decreased expression of RPE-specific genes. However, in contrast to the results of Ma et al. (2009a), this group of researchers (Hu et al., 2014) did not see an expression of such ganglion cell markers as Islet 1/2 and Protein Kinase C nor vGAT expression, which indicated the absence of GABAergic neurons. In addition, converted human RPE cells did not utilize FM1-43C when stimulated with potassium, which, according to the opinion of Hu et al. (2014), indicates the absence of a synaptic transmission mechanism. The effect of *SOX2* overexpression in neonatal and adult RPE cells in vitro has also been evaluated in other studies (Ezati et al., 2018) (see Fig. 1). The results showed that *SOX2* induces direct reprogramming of human RPE cells in vitro with an increase in the expression of *PAX6, CHX10,* and *THY1* and the appearance of rhodopsin-positive cells, indicating the generation of neuronal terminally differentiated retinal cells (Ezati et al., 2018). SOX2 is a pioneer factor in direct reprogramming into retinal ganglion cells for both chicken and human RPE cells. In humans, however, a partial cellular conversion happens, without the formation of functional ganglion cells.

Thus, the analysis showed that transcription factors of cellular specialization and differentiation of retinal cells belonging to the bHLH and SOX family induce the process of direct reprogramming of vertebrate and human RPE cells into retinal neurons. The type of cells that were induced by direct reprogramming depends on which gene was used and on its intended role in retinal development. The specific genes for producing photoreceptor-like cells during direct reprogramming of RPE cells are *NEUROD1, NGN1*, and *NGN3* (Yan et al., 2013b; Wang and Yan, 2014); it is *ASH1* for producing bipolar cells (Li et al., 2010),

and it is *SOX2* for producing ganglion cells (Hu et al., 2014; Ezati et al., 2018). In addition, researches in direct reprogramming of various cell types indicated that the induced cell type depends on the original cell source. For example, *ASCL1* or *NGN2* can convert cerebellar and neocortical astroglia cells into brain neurons (Chouchane et al., 2017) and Müller glial cells into retinal neurons (Pollak et al., 2013). These data indicate the important role of the type and regional specification of the reprogrammed cell in determining the identity of induced neurons (Masserdotti et al., 2016). Also, it is obvious that the effectiveness of direct reprogramming strongly depends on the belonging of cells to a particular vertebrate species. Thus, chicken RPE cells under the influence of overexpression of certain genes transform into morphologically, molecularly, and physiologically fully complete specific retinal cells, whereas such a change in humans occurs only partially. A single genetic factor is sufficient for direct reprogramming of chicken RPE cells, while the transformation by a single genetic factor is inefficient for human RPE. However, factors of the bHLH and SOX families are capable of inducing direct reprogramming of human RPE cells into retinal neurons, which allows for using them as pioneer factors in direct reprogramming in contrast to transcription factors of the eye field.

The role of a combination of different transcription factors in direct reprogramming. The accrued data suggest that the most effective tool for direct reprogramming of adult human RPE cells should include not one factor but a specific combination of several transcription factors. The combination of transcription factors of the eye field and factors of cellular specialization from the bHLH family stimulates direct reprogramming of various human eye cells into retinal neurons (Inoue et al., 2010; Seko et al., 2012). In 2012, Seko et al. (2012), using viral transfection of the genes responsible for the formation and functioning of various retinal photoreceptors, such as *SIX3, PAX6, CRX, RX, NRL*, and *NEUROD1,* converted cells of the iris pigment epithelium, Müller glia, and the human ciliary body into photoreceptors and also converted fibroblasts into photoreceptors (Seko et al., 2014). As mentioned above, exogenous expression of one gene from this series alone does not induce the formation of photoreceptor phenotypes in the studied cells in vitro (Seko et al., 2012), while exogenous gene expression turns human iris cells into photoreceptors in various "effective" combinations, for example, of *CRX, RX,* and *NEUROD* (Seko et al., 2012). A combined transfection of the *OTX2* and *CRX* genes was used to obtain photoreceptors from human retinal stem cells in vitro (Inoue et al., 2010). The increased efficiency of direct reprogramming of RSCs in comparison with other eye cells can be explained, by analogy with neural stem cells, not only by epigenetic features but also by their gradual differentiation, in which alternating proliferation and intermediate states of differentiation occur,

which allows them to slowly acquire the most appropriate metabolism that is necessary for the acquisition and transformation of cell fate (Masserdotti et al., 2016).

Prospects for genetic direct reprogramming. Despite the pronounced success in direct reprogramming of human eye cells, the reprogramming is often not fully complete and, although the cells acquire many characteristics of neural retinal cells, they are not functional (Seko et al., 2012; Yan et al., 2013a; Hu et al., 2014). In contrast to chicken and mouse embryos, human RPE cells carry such multilevel blockages that even ectopic expression of specific genes does not fully convert them. This might be because terminally differentiated somatic cells are rich in epigenetic regulatory mechanisms that stabilize specific patterns of gene expression. There are works in which the preservation of epigenetic memory of the previous state in reprogrammable cells was shown. Thus, Hu et al. (2010) reprogrammed human fetal RPE cells into human iPSCs using lentiviral vector for expression of *OCT4, SOX2, LIN28,* and *NANOG*. The obtained iPSC lines showed morphology similar to human ESCs; they expressed stem cell markers and formed teratomas containing derivations of all three germ layers. However, some of these lines showed a clear preference for redifferentiation into the RPE. One of the possible mechanisms to preserve epigenetic memory is DNA methylation, which is capable to remain in cells for numerous cell cycles (Kim and Costello, 2017).

To summarize genetic direct reprogramming, we can say with complete confidence that the application of this method is ineffective for human RPE cells; it is necessary to continue searching for other reprogramming factors and conditions. In addition, the method of transfection of transcription factors has a low percentage of reprogramming efficiency (0.01−6%). This efficiency can be improved by using small molecules, noncoding RNA, growth factors, and other compounds that directly affect the molecular cascades involved in cellular reprogramming, signaling pathways, genetic transfection, cellular metabolism, proliferation, and cell death. It has been shown that protecting cells from oxidative stress destruction and death and reducing proliferation significantly improves the effectiveness of cellular direct reprogramming into neurons (Masserdotti et al., 2016). The method of consistently increasing the expression of different genes, which imitates the processes of induction and maturation of neurons, also increases the effectiveness of direct reprogramming. In addition, researchers use combinations of genetic transfection with epigenetic agents and growth factors. Yao et al. (2018) first stimulated the overexpression of β-catenin followed by overexpression of transcription factors *OTX2, CRX,* and *NRL* during direct reprogramming of Müller glial cells. Chen et al. (2010) showed in another study that overexpression of *MATH5* in a combination

with growth factors and small molecules DKK1, NOGGIN, and DAPT is necessary to produce cells of the ganglion layer from murine iPSCs of mice in vitro. Similar results were obtained in the research of Deng et al. (2016) in which overexpression of *ATOH7* (*MATH5*) in the presence of exogenous molecules, which are involved in retinal neurogenesis, such as DKK1, NOGGIN, and LEFTY A, stimulated the differentiation of human iPSC-derived retinal progenitor cells into cells of the retinal ganglion layer.

From the all above, we can assume that scrupulous selection of special conditions is necessary for successful direct reprogramming of human RPE cells into retinal cells. To achieve success, it is necessary to use a combination of epigenetic factors, in particular, of demethylating agents, and genetic factors, for example, by transfection of bHLH family genes, as well as cocktails of low-molecular compounds that directly affect chromatin remodeling, gene transcription, signaling pathways, the cell cycle (proliferation and apoptosis) and metabolism, and cell differentiation.

ALTERNATIVE METHODS FOR GENETIC DIRECT REPROGRAMMING

Chemical direct reprogramming is the direct reprogramming of cells by small molecules; the method is based on the modulation of signaling pathways that determine the cellular fate. Small-molecule inhibition of four signaling pathways, Notch, TGF-β, BMP, and GSK-3β, as was shown, is sufficient to activate neurogenesis in the mouse hippocampus in vivo (Yin et al., 2019). A typical low-molecular cocktail that is used for cell conversion includes the following components: epigenetic modulation molecules that suppress the initial properties of cells, compounds that induce the characteristics of the resulting cells, factors that contribute to the survival and functioning of reprogrammed cells in vitro (Xie et al., 2017). Thus, in the work of Zhu et al. (2010), the combination of chemical compounds and one transcription factor was sufficient for reprogramming somatic cells into iPSCs. Using a combination of *NGN2* with small molecules, forskolin, an activator of the PKA signaling pathway, and dorsomorphin, a BMP inhibitor, Liu et al. (2013) directly reprogrammed human fetal lung fibroblasts into cholinergic neurons with functional electrophysiological characteristics. Later, Hou et al. (2013) showed that a combination of only seven small molecules is sufficient for chemical reprogramming of somatic cells in iPSCs. Over the past few years, methods of application of low-weight molecules have achieved significant success in inducing pluripotent or functionally differentiated cells from somatic cell (Xie et al., 2017). Compared to other methods, low-molecular compounds have some unique advantages, such as the universality of the structure and simplicity of manipulation depending on time and concentration. Along with this, we should not forget about the problems of toxicity, methods of delivery of small molecules, and the fact that the process of selecting the required small molecules can be time-consuming and expensive. However, many researchers believe today that the development of technologies with the application of small molecules and the CRISPR/CAS9 system can form the basis of successful regenerative therapy for tissue repair.

MicroRNA direct reprogramming. MicroRNAs are known to play an important role in posttranscriptional regulation, neural differentiation, and morphological and phenotypic development (Ebert and Sharp, 2012). MicroRNAs regulate not only the expression of genes and proteins but also act as epigenetic factors. Many studies have shown that microRNAs have the property of directly affecting the subunits of chromatin remodeling complexes associated with ATP-dependent BRG/BRM factor (BAF), which is critical for neuron differentiation (Staahl et al., 2013; Abernathy et al., 2017; Lu and Yoo, 2018). MicroRNAs are used in combinations with various transcription factors in direct reprogramming of somatic cells into neurons (Yoo et al., 2011). Yoo et al. (2011) used neuronal-specific microRNAs miR-9/9* and miR-124 in combination with the *NEUROD1* transcription factor to convert human fibroblasts into neurons. However, the obtained cells did not always show a repetitive action potential, which indicates that the neurons were immature. To solve this problem, the same group of researchers used two other factors, *ASCL1* and *MYT1L*, and obtained cells with a higher degree of maturity (Yoo et al., 2011). Multiple studies indicate that the most effective way for obtaining neurons from other somatic cells is to use a combination of microRNAs and highly specialized transcription factors.

Although we have not found information in the available literature about the successful application of a combination of epigenetic, genetic, and low-molecular compounds for direct reprogramming of human RPE cells, the success achieved in direct reprogramming of other somatic cells into various subtypes of neurons gives hope for this possibility.

COMPUTATIONAL PREDICTION OF TRANSCRIPTION FACTORS

Designing specific software for predicting transcription factors that are required for cellular direct reprogramming is one of the recent advances in computational biology. The computational algorithms CellNet, Mogrify, BART, MAGICACT, and CellRouter analyze, classify, and predict the functions of transcription factors, thereby facilitating and speeding up the screening process (Morris et al., 2014; El Wazan et al., 2019). Another approach can be in designing a computer model of the cell that allows us to implement a simulation of events using certain factors of direct reprogramming, such as DeepNEU (Danter, 2019). Although these advanced algorithmic models are still

in their infancy, their further development will improve our understanding of the fundamental properties of cells and the molecular interaction of transcription factors required for direct reprogramming (El Wazan et al., 2019).

CONCLUSIONS

Existing studies show a high potential of vertebrate RPE cells for direct reprogramming into retinal neurons in vivo. Despite the small number of works on direct reprogramming of human RPE cells, we can say with confidence that human RPE cells are capable of responding to genetic engineering influences. RPE cells are available for different vectors that deliver a variety of molecules, such as transcription factor genes, chimeric endonucleases, the CRISPR/Cas system elements, recombinant proteins, and low-molecular-weight compounds. Cells respond to the presence of an exogenous genome in a manner that is predictable for researchers. Based on the results obtained by direct reprogramming of vertebrate cells into retinal cells and on the achievements of computational biology, we can assume the most optimal set of epigenetic, genetic, and chemical factors with the addition of neurotrophic factors and growth factors for the successful conversion of human RPE cells into retinal neurons.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest.

This paper does not contain any studies involving animals or human participants performed by the authors.

AUTHOR CONTRIBUTIONS

L.A. Rzhanova analyzed world-renowned publications and wrote the main part of the article. A.V. Kuznetsova participated in editing of the text and discussion about the content of this article, and M.A. Aleksandrova initiated the writing of the review, selected literature, and edited the text of the article.

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