Plasticity of Human RPE Cells

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The retina is a specialized light-sensitive tissue in the eye of mammals and humans that provides visual perception, and is actively studied at the cellular, molecular and genetic levels. Photoreceptor cells located in its outer part perform the function of converting light (phototransduction) into neurochemical signals, which are processed in the neurons of the retina and the brain and ultimately form our vision. Functional support for retinal neurons is provided by retinal pigment epithelium cells (RPE cells). The retinal pigment epithelium (RPE) is a single-row layer of pigmented, hexagonal, normally non-proliferating cells located between the choroid and the photoreceptor cells of the retina. The RPE performs many diverse functions to support the retina, including the transepithelial transport of substances, the phagocytosis of photoreceptor outer segments, and a number of processes in the visual cycle, as well as participation in the blood–retinal barrier and secretion of growth factors. The RPE plays an important role in regulating the redox homeostasis of retinal photoreceptors. A few cells have been isolated from the human RPE, which, according to strict clonal analysis and other stem cell criteria (self-renewal and the production of differential progeny), were classified as adult RPE stem cells (RPESCs). The number of mammalian RPESCs was determined in vitro experiments, from which it became clear that to 2% of cells are capable of proliferation, self-renewal, and the expression of specific genes characterizing stem cells. Depending on microenvironmental conditions, RPESCs can remain quiescent in a stemness state or exhibit multipotent differentiation. RPESCs can produce RPE cells and are capable of generating different types of photoreceptors and nerve cells, or mesenchymal cells.

retinal pigment epithelium RPE stem cells RPESCs differentiation proliferation plasticity

1. Proliferation of Retinal Pigment Epithelium Cells

Once the RPE cell differentiation is complete[1], the cells exit the cell cycle and persist throughout life, dividing rarely or not at all. Mammalian and human RPE cells are thought to be terminally differentiated, postmitotic, nondividing cells. However, there is evidence that in rodents, the RPE cells at the periphery of the layer are capable of proliferation [2]. It is also known that the peripheral RPE cells of macaques can incorporate 3H-thymidine, indicating their proliferation [3]. This information is complemented by data on the differential expression of genes associated with the cell cycle. Thus, it was shown that genes stimulating proliferation dominated in peripheral RPE cells [4]. In addition to exhibiting proliferative activity in situ, adult mammalian and human RPE cells can be activated to divide when placed into a cell culture, where their proliferation is facilitated by the loss of intercellular contacts and various growth factors in the culture medium.

In studies of the mechanism of entry of human RPE cells into the phase of DNA synthesis in vitro, it was found that mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) play a key role. In this signaling pathway, growth factor receptor activates Ras GTPase, leading to MAPK/ERK phosphorylation. The MAPK/ERK signaling pathway, in turn, regulates transcription factors, *c-myc, Pax6, klf4*, and *MITF,* the expression of which indicates a decrease in the level of RPE differentiation [5]. The ability to proliferate has been shown to be maintained through the persistent repression of cyclin-dependent kinase inhibitor (*CDKI*) genes, including *p16, Arf, p21*, and *p57*, which were expressed at very low levels in the RPE stem cells (RPESCs), compared with their parental RPE cells [6]. It is assumed that the proliferation of RPE cells in vitro occurs mainly due to a subpopulation of stem cells (SCs) in the RPE [5].

2. Expression of Stem Cell Markers and Differentiation Potential of the RPE Cells

The replacement of damaged or lost cells during regeneration occurs through various mechanisms, including dedifferentiation, transdifferentiation, or reprogramming [7]. All three mechanisms can be observed in the RPE layer of the vertebrate eye. Classic experiments on model animals have shown the ability of RPE cells to transdifferentiate/undergo reprogramming into neural cells of the retina, which is successfully reproduced in vivo in lower vertebrates. Dedifferentiation is the transition of terminally differentiated cells to a less differentiated state in which cells can divide and, within their cell lineage, locally replace lost cells, moving to their final differentiated state in some tailed amphibia [8]. During the transdifferentiation of RPE cells after retinal damage in these animals, the already-differentiated RPE cells change lineage and reprogram into neurogenic progenitors expressing *Klf4, Sox2, Pax6,* and *c-Myc* [8]. The descendants of these progenitor cells differentiate into all retinal cells, including photoreceptors, glia, and pigment epithelium, and then fully restore retinal function in amphibia [8]. In birds and mammals, RPE transdifferentiation into retinal neurons occurs during early embryonic development, only under the influence of basic fibroblast growth factor (bFGF) or lin-28 [9]. Damage to the retina in adult mammals and humans leads to the development of pathologies accompanied by the acquisition of a mesenchymal phenotype by RPE cells, which begin to actively proliferate and migrate [10]. The loss of intercellular contacts triggers the mechanisms of epithelial–mesenchymal transition (EMT). After the accumulation of the necessary pool of altered RPE cells, these cells begin differentiate into various types of cells, predominantly of mesenchymal origin. Some researchers draw a parallel between these processes in the retina and embryonic development, as well as the development of tumors [7]. In this regard, in adult mammals and humans, RPE transdifferentiation as a means of retinal regeneration is ineffective. However, some RPE cells in mammals may exhibit proliferation, plasticity, and conversion to neurons, detectable in vitro .

The expression of multipotency markers and stem cells in the RPE is an important criterion that allows us to classify some RPE cells as adult SCs. The some cells of native human RPE and cell culture have been characterized by the expression of stem cell markers, including *NANOG*, *OCT4*, *SOX2*, *SSEA-4*, *KLF4*, *C-MYC*, and *LIN-28* [11][12]. However, in order for cells to be classified as SCs, they must possess a number of characteristics, such as self-renewal and the generation of specialized differentiated progeny. Self-renewal is the

ability of SCs to proliferate symmetrically or asymmetrically and to produce a similar cell [13]. The proliferation of human RPESCs was stimulated by cultivating them in a free-floating state with the addition of growth factors, similar to neural stem cells (NSCs) [11][12]. Clonal analysis of adult RPE cells was used and it was shown that primary spheres from free-floating cells were formed after 4 days with a frequency of 1.5% of the number of initial seeded cells [12]. The RPE cells were cloned in adherent cultures to expand the number of RPESCs [14], using protocols for isolating and culturing RPESCs from previous studies [15][16]. In the adherent cultures, about 10.6% of the cells actively proliferated, although the vast majority did not divide or produced only limited progeny [12]. It was found that 10% of RPE cells proliferate once in culture, using the clonal plating. Among RPE cells, only 2% of cells proliferate very actively and can create up to 90% of the entire monolayer. These cells were classified as adult RPESCs [14]. The human fetal RPE (fRPE) was treated with vitamin C and valproic acid; the stemness properties of the resulting fetal RPESCs (fRPESCs) were studied, and a significant increase in the stem cell markers *SOX2, OCT4*, and *KLF4* was found [17]. As a result, the cells enter a retinal stem cell-like state. It was found that the combined effect of vitamin C and valproic acid activates the expression of the retinal progenitor markers *MITF, OTX2*, and *PAX6*, as well as the mesenchymal stromal markers CD133, CD73, CD105, and CD90 in fRPE cells. Researchers believe that a high expression of SOX2 in fRPESCs is a prerequisite for maintaining retinal stem cell properties and a multipotent differentiation potential [17]. In another study, RPESCs were activated by the influence of amniotic fluid factors, and the retinal progenitor cells obtained in this way were studied [18]. The mouse RPE cells were cultivated in the form of spheres to obtain induced iRPESCs. If mouse RPE cells are cultured through the sphere formation stage, approximately 0.003–0.013% of RPESCs can be activated [6]. These cells actively proliferated for more than ten passages, in contrast to other RPE cells, which showed limited proliferation and senescence after only five passages. From the above studies, it follows that in the adult human and mouse RPE cells are preserved that have signs of SCs that can be identified in vitro.

The search for and study of the stem properties of the RPE continues, using modern molecular genetic technologies. The RNA-Seq and scRNA-Seq studies of the functional heterogeneity of the native RPE cells of mice and humans were found subpopulations of cells expressing stem/progenitor cell genes [19][20]. Among the identified mouse RPE clusters, researchers paid special attention to cluster C1, containing only 1–2% of the total number of cells [21], and cluster 5, in which only 19 cells (0.59%) were found [22], which were cells with the characteristics of stem cells and/or progenitor cells. In these clusters, a high expression of stemness and stem cell maintenance genes was observed, against the background of a high expression of melanogenesis genes. This indicated that the cells were still in the process of differentiation. It was also found that there was a high correlation of gene expression between RPE cells of the C1 cluster and retinal cells, which possibly pointed to the ability of C1 cells to differentiate into different cell types of the neural retina [21]. In addition, it was demonstrated that the RPE cells in the adult mouse eye are epigenetically very similar to the phenotypes of retinal progenitor cells and photoreceptors [23]. Several growth factors, such as *Vegf, Tgf, Pdgf, Egf*, and *Ngf*; their receptors, *Vegfr, Egfr, Pdgfr*, and *Tlk4*; and two stem cell-associated signaling ligands, Kit-l and Lif, are involved in mouse RPE reprogramming into induced RPE stem cells (iRPESCs) and are highly expressed in iRPESCs. Mouse RPESCs were induced in vitro through the stage of sphere formation and it was discovered that the gene expression profile of iRPESCs was very different from the parental RPE. At the same time, changes in gene expression occurred immediately after the formation of

spheres. Mouse iRPESCs expressed increased levels of *c-Myc*, *Oct4*, *c-Kit*, and *Cd44* [6]. The expression of eight of the fifteen selected stemness genes, such as *Klf4*, *Alpl*, *Kit*, *Kitl*, and *Bmi1*, was significantly upregulated in RPE spheres, and the expression of *Abcg2*, *Bmi1*, *Cd44*, *Kitl*, and *c-Myc* was upregulated in iRPESCs, compared with the original RPE. Two DNA methylation genes (*Dnmt1* and *Dnmt3a*), four histone acetylation genes (*Hat1*, *P300*, *Myst2*, and *Myst3*), and seven deacetylation genes (*Sirt2*, *Sirt6*, *Hdac1*, *Hdac2*, *Hdac3*, *Hdac5*, and *Hdac6*) were highly expressed in the stem cells, reflecting epigenetic regulation that may promote the expression of major stemness genes, such as *Oct4* and *Klf4* in iRPESCs [6].

From the above studies, it follows that among the heterogeneous RPE cells of mice and humans, 0.003–2% of cells can be distinguished that are capable of proliferation, self-maintenance, and the expression of specific genes characterizing the state of stemness in cells. These cells may remain quiescent or exhibit multipotent differentiation into a mature cell type. Under expansion culture conditions in which RPESCs self-renew, they generate SC progeny, and, conversely, under differentiation culture conditions, RPESCs generate progeny of different cell types.

3. Differentiation of Stem/Progenitor Cells from the Retinal Pigment Epithelium into Muscle and Adipo-, Osteo-, and Chondrogenic Cells

The term RPESCs was first proposed after the ability of RPESCs for multipotent differentiation was shown in the neural and mesenchymal (osteogenic, adipogenic, and chondrogenic) directions [12] .

It was demonstrated that an adult RPE in vitro can generate cells expressing markers of cells of mesenchymal origin: muscle and adipo-, osteo-, and chondrogenic cells [12]. To exclude contamination and to prove the multipotency of the stem cells, the authors obtained RPESCs from a primary human RPE by cloning single cells. The cells were placed into different culture wells and exposed to adipocyte, chondrocyte, and bone differentiation media. This study was conducted on cells from a variety of sources, including human fetal RPE, RPE from various mammalian species, ARPE-19 cells, and RPE cells derived from induced pluripotent cells and SCs. The results of this study showed that mesenchymal differentiation is characteristic of the RPE. It was found that human fetal RPE is most resistant to acquiring mesenchymal fates. In recent research, stem cells from the fetal RPE were studied [17]. It was found that the combined effect of vitamin C and valproic acid activates the expression of the retinal progenitor markers MITF, OTX2, and PAX6, as well as the mesenchymal stromal markers CD133, CD73, CD105, and CD90 in fRPE cells. Cells of the fRPE and fRPESCs were placed into adipogenic, chondrogenic, and osteogenic differentiation media to test their mesenchymal differentiation capabilities. Staining cells with specific markers and quantitative real-time PCR showed that fRPESCs are more capable of differentiating into adipocytes, chondrocytes, and in the osteogenic direction than fRPE cells. The regulatory role of *SOX2* in the cellular conversion of fRPESCs was identified and it was shown that fRPESCs lose the ability for mesenchymal differentiation after the knockdown of *SOX2* [17] . Along with the differentiation of RPESCs in the mesenchymal direction in vitro, it is well known from many years of pathomorphological observations that the cartilaginous and bone formations that develop from RPE cells are sometimes found in human eyes [24]. These data indicate that RPESCs have a broad repertoire of differentiations, reminiscent of that of neural crest cells [25]. It can be

speculated that this multipotency of RPESCs may be somehow related to the very early release of neuroepithelial RPE progenitor cells during embryogenesis.

There is no information about the differentiation of RPESCs in the neural direction in mammalian and human eyes in vivo, while development in the mesenchymal direction, with the formation of epiretinal membranes in retinal pathologies, is a well-known medical fact.

4. Differentiation of Stem/Progenitor Cells from the Retinal Pigment Epithelium along the Neuronal Pathway and into Retinal Photoreceptor Cells

Subsequent studies of the behavior of adult and fetal RPE cells in vitro showed that cells in culture dedifferentiate and express stem cell and poorly differentiated cell markers *OCT4*, *NANOG*, *SOX2*, *PAX6*, and *PROX1*. Under in vitro differentiation conditions, the cells express neural and glial cell markers such as *NES, MSI1, TUBB3, NF-L/H, GFAP*. In addition, using immunostaining, markers of mature retinal and brain cells were detected: RCVRN (photoreceptor marker), dopaminergic neuron markers, tyrosine hydroxylase (TH) (retinal amacrine cell marker), GABAergic interneuron markers, nNOS (retinal amacrine or ganglion cell marker), as well as CNPase and O4 (oligodendrocyte markers) [26].

Other researchers have found photoreceptor markers in RPE cell lines and primary RPE cultures, such as Crx10 [27], *OPN3, OPN4*, *NRL, CRX*, *OPN1MW/1W SAG, NR2E3, RCVRN* [28]. The identification of the recoverin protein suggests that human RPE cells are capable of differentiating into retinal photoreceptor-like cells. In that study, the human RPE cells were cultured in a medium that promotes the differentiation of retinal neurons [12]. Using qPCR analysis, it was shown that the expression of the neural progenitor marker *NES* and the neuronal marker *TUBB3* was significantly activated in RPE cells (over 1000-fold and 90-fold, respectively). Interestingly, under these conditions, the cells increased the expression of the eye field marker genes *LHX2*, *OTX2*, and *RX*, which are characteristic of the early stages of eye development, while the levels of retinal progenitor markers *CHX10* and *RHO* did not change, and the expression of *PAX6* actually decreased. These culture conditions have been noted to promote the differentiation of RPESCs toward neural progenitors of the forebrain and retina [12].

The directed differentiation of human fRPESCs into photoreceptors using a special three-step protocol using different culture systems and media has been carried out [17]. During the process of differentiation, the fRPESCs changed their morphology, first forming a round shape and then extending several synapse-like structures to finally form a tubular rod-shaped structure resembling the outer segment of a photoreceptor. At the first stage of differentiation, retinal photoreceptor progenitor cells with the expression of markers *PAX6* and *VSX2* were obtained. At the second stage, the cells were differentiated into photoreceptor progenitors that significantly increased the expression of photoreceptor markers *NRL* and *CRX*. Finally, at the terminal stage of RPESC differentiation, rod photoreceptor cells expressing *REC*, *RHO*, *ARRESTIN*, and *GNAT1* were obtained [17]. Another study managed to differentiate fetal RPE cells into rod photoreceptors by chemical reprogramming [29]. Gene ontology analysis of RNA sequencing results from these cells revealed the upregulation of genes involved in

neuronal generation, neurotransmitter uptake, and photoreceptor cell differentiation, such as *SOX8*, *IGFN1*, *ASCL1*, *RXRG*, *THRB*, and *RORB*. On day 10 of reprogramming, the transcriptome profile showed a stable activation of genes specific to rod photoreceptors, but not to cones [29].

5. Differentiation of Stem/Progenitor Cells from the Retinal Pigment Epithelium along the RPE Pathway (Redifferentiation)

Mouse and human RPE stem cells are capable of redifferentiating to the original phenotype. The sphere-derived dedifferentiated RPESCs can differentiate back into RPE cells in vitro [17]. Monolayer cultures of RPE cells derived from human RPESCs (RPESC-RPE) have been described and characterized as cultures with the morphology and physiology characteristic of the native RPE [15][16]. The redifferentiation of RPESCs occurs within 8 weeks of cultivation. During this time, the cells change their morphology, proliferation rate, and polarization and also acquire the key phenotypic characteristics of the RPE. In cultured spindle-shaped RPESCs, a significant decrease in proliferation was observed after 2 weeks; after 4 weeks, the appearance of islets of cells with a cuboidal morphology was noted, and, by week 8, almost all cells acquired the morphology of a mature RPE. The secretion of vascular endothelial growth factor A (VEGF-A) through the apical and basement membranes increased from week 2 to week 4, while the secretion of pigment epithelium-derived factor (PEDF) appeared during week 6 of the cell differentiation[16]. In the cultured adult RPE, a similar but sometimes increased expression of RPE markers was observed, compared with the native tissue. Claudin-19 was present along the apical–lateral membrane along with the tight junction protein ZO-1, indicating the existence of a functional epithelial barrier. Ezrin, a membrane-associated protein involved in cytoskeletal organization, was found predominantly in the RPE microvilli. The visual cycle proteins, cellular retinaldehyde-binding protein (CRALBP) and RPE65, were localized, as expected, in the cytoplasm. Monocarboxylate transporter 1 (MCT1) was present on the apical surface of cells [14]. This indicates the establishment of the polarity characteristic of mature RPE cells. It is obvious that, in mouse and human RPEs, cells with stem properties are present, which also have the ability to redifferentiate to the original phenotype of RPE cells. Throughout the entire period of differentiation, the RPESC-RPE cells continuously expressed pigment epithelium cell fate determination factors OTX2 and MITF and did not express smooth muscle actin, an indicator of epithelial–mesenchymal transition. It has been shown that, during the differentiation period, transepithelial resistance increases and reaches the norm [15]. However, the phagocytic activity of RPESC-RPE cells decreased after 4 weeks of differentiation, apparently due to a lack of contact with photoreceptors, which are necessary for the full maturation of the RPE [16].

RNA-Seq and scRNA-Seq on differentiated RPE cells derived in vitro from human RPESCs identified 13 cell subpopulations with different functional specializations. The transcriptomic analysis showed that the subpopulation composition of the entire RPE cell population is subject to dynamics over time. These findings suggest that RPE subpopulations have overlapping but distinct functional profiles. Among these subpopulations, one subpopulation stands out in which a specific marker EZH2 (a transcription factor involved in histone methylation, self-renewal, and differentiation of SCs), has been isolated, indicating a stem or progenitor cell-like state.[30]. This study has

confirmed RPE heterogeneity under culture conditions, similar to the native tissue, and answered the previously raised question about the innate differences between RPE cells, and whether a trait can be accurately passed on to daughter cells in the reproducing population [31]. It is likely that the mosaicism of RPE cells is hereditary in nature, embedded in the genetic program of the cells themselves, and conditioned by epigenetic influence.

Based on these data, the human RPE may contain highly plastic cells that have (or acquire during the process of dedifferentiation) the SC phenotype with a dual potential. These cells may retain the potential to redifferentiate into RPE and to differentiate into retinal cells, under the influence of appropriate microenvironmental factors. RPESCs can produce RPE cells, and epithelial monolayers of RPE cells are easily obtained. In in vitro differentiated media that activate neural or mesenchymal differentiation, RPESCs can generate both neuronal and mesenchymal cells [12].

6. The Niche of Stem/Progenitor Cells from the Retinal Pigment Epithelium and Localization in Tissue RPE

It is obvious that RPESCs are not primitive cells but are differentiated cells, morphologically indistinguishable from the neighboring cells. The stem state of RPESCs maintains a well-structured niche microenvironment consisting of Bruch's basement membrane, intercellular lateral contacts in the RPE, and apical contacts with the outer segments of photoreceptor cells. Cell proliferation is controlled by the niche components in interaction with endogenous signals [32]. Like neural stem cells in the brain [33], RPE stem cells are quiescent and require changes in the microenvironment and signals in the niche for their activation and proliferation. The factors that activate the reprogramming of RPE cells and dormant stem cells are the same. This means the loss of intercellular contacts and contacts with surrounding tissues, the remodeling of the cell surface and cytoskeleton, the displacement of RPE cells from their stabilizing environment (niche), proliferation, migration, genome reprogramming, and, ultimately, the sequential acquisition of a different phenotype[34].

The RPE maintains tissue homeostasis through endogenous regulatory systems. Mature RPE can be compared with classical barrier epithelia, which are characterized by a constant cellular turnover throughout life, such as the corneal epithelium, skin epidermis, or intestinal epithelium [32]. To ensure the maintenance of tissue homeostasis, the rate of cell removal must match the production of new cells, which is achieved primarily through stem and progenitor cells. Mature RPE maintains tissue homeostasis mainly through long-term cell survival, although cell density decreases with age, especially in the peripheral RPE. The decrease in cell density is thought to be due to the continued growth of the eye during the juvenile period, and the gradual loss of senescent cells [35]. Maintaining the integrity of the RPE is achieved through hypertrophy without loss of contact with neighboring cells and/or through cellular replacement (migration from the periphery), which may explain the relative preservation of the middle part of the RPE, and a sharp decrease in the cell density in the periphery [36]. The long life of RPE cells does not exclude the possibility of the presence of a few stem/progenitor cells among them, although the question of their localization remains open. It is assumed that less differentiated RPE cells retain the ability to transdifferentiate into other cell types [32]. RPESCs should be located in a topographic zone with less differentiated cells, where there are less rigid cell contacts; this is the peripheral RPE [4], which is as close as possible to the

ciliary marginal zone. From an evolutionary point of view, it would seem that RPESCs can be preserved in the ciliary marginal zone of the eye. It was also suggested that the RPE subpopulation of the immediate periphery (zone P4) may contain a pool of RPE cells that retain the ability to proliferate [37]. In contrast to these assumptions, the scRNA-Seq results clearly showed that less differentiated cells are found in the central area of the RPE, in particular, in the macular region [19]. In the cells of this area, a high expression of stemness and stem cell maintenance genes was found, against the background of a high expression of melanogenesis genes, which indicates an ongoing process of cell development.

7. The Potential of Retinal Pigment Epithelium Cell Subpopulations for Transplantation

Retinal degeneration as a result of the death of photoreceptors and RPE is the main cause of many degenerative diseases of the human eye, leading to vision loss [38]. For vision correction during this kind of pathology, approaches are currently being actively developed in contemporary medicine, aimed at preserving the original photoreceptors and RPE [39] and/or striving to replace cells by activating endogenous regeneration [40] or through cell transplantation [39]. One approach to treating a number of degenerative retinal diseases, including age-related macular degeneration, is cell replacement therapy. RPE cells derived from human embryonic SCs and iPSCs are already undergoing clinical trials and have great promise for the treatment of both age-related macular degeneration [41] and hereditary RPE-associated retinal dystrophies [42]. Despite the highly effective protocols for obtaining RPE cells in sufficient quantities for transplantations that have been developed, they still remain labor- and time-consuming [43]. In addition, there is the problem of immunosuppression and tumorigenesis [44], and moral and ethical issues regarding the use of ESCs remain unresolved [45].

Unlike ESCs and iPSCs, adult human RPESCs, despite their limited proliferative potential, do not form tumors [12]. RPESCs are capable of producing RPE-RPESC progeny, which corresponds to the native RPE in its morphological and functional characteristics [15]. The preclinical transplantation of RPE derived from RPESCs has shown that an intermediate stage of RPE differentiation is more effective in restoring vision [16]. The successful transplantation of human fRPESCs has been demonstrated on animal models, where the cells differentiate into both RPE and photoreceptors [17].

The heterogeneity of RPE-RPESCs is manifested not only in the functional specialization of cells, but also in the ability of RPE-RPESCs to successfully transplant. Several subpopulations of RPE-RPESCs have been identified that may be potential candidates for effective transplantation [30]. These cells showed the enrichment of signaling pathways associated with cell differentiation and proliferation. Nevertheless, among them, only one cluster of EZH2-positive RPE-RPESCs was isolated, which is capable of integrating into the host RPE monolayer upon transplantation. A clear difference between the transcriptomes of transplant-effective and transplant-ineffective subpopulations of RPE-RPESCs was identified. In addition, molecular pathways associated with graft effectiveness were shown, and potential biomarkers for effective RPE cultures were established [30]. As a primary biomarker of transplantation success, the long noncoding RNA (lncRNA) Three Prime Repair Exonuclease 1 (TREX) was considered, which regulates various cellular processes, including migration and cell survival [30]. These data will

allow us to identify from the heterogeneous populations of RPESC-RPE, iPSC-RPE, ESC-RPE, and RPE obtained from other possible cell sources a separate subpopulation of cells that is most suitable for successful transplantation.

Currently, RPE derived from RPESCs represents a potentially unlimited source of HLA-compatible cells and an unlimited donor source with several qualities favorable for transplantation, including stability, ubiquity, and cost. In addition, active phase 1 clinical trials are being carried out in patients with dry AMD (NCT04627428) [46]. It is important to note that transplantation can cause a number of complications, which can be triggered by surgical damage to the retina, leading to its detachment [47]. In this regard, the stimulation of endogenous adult RPESCs present in the eye to produce a new autologous RPE in situ without surgery may be promising for the treatment of many degenerative-dystrophic diseases of the human retina and will help to avoid the above limitations and complications.

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