Retinal pigment epithelial cells as a therapeutic tool and target against retinopathies

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Retinal pigment epithelium (RPE) is a cell monolayer essential for photoreceptor function and forming the blood–retinal barrier. RPE and retinal neurons share the same origin and a polarized cytoarchitecture. Several factors determine the phagocytosis and permeability of RPE, influencing photoreceptor renewal and drug delivery, efficacy and toxicity. Adult human RPE expresses neuronal markers *in vitro*, indicating a potential transdifferentiation. Degeneration of the RPE leads to death of photoreceptors and retinal neurons, resulting in the vision loss of retinopathy. Here, we suggest tools for cell engineering to discover new ways for activating the endogenous regeneration of barrier functions and/or of the retinal precursors in RPE cells.

Introduction

The blood–retinal barrier (BRB) is an extension of the blood–brain barrier that separates the internal environment of the eye from the vascular system. The BRB is limited internally by tight junctions (TJ) between the vascular endothelial cells of the retinal vessels, whereas the outer side is formed by TJ between the cells of human retinal pigment epithelium (HRPE), a monolayer of epithelial cells separates the vascular choroidal system from the sensory retina [1]. Therefore, HRPE is a close and interactive partner to the photoreceptors as well as an interface with the endothelium of the choroid and thus with the systemic circulation. To fulfill these roles, the HRPE communicates with neighboring tissues, the layer of photoreceptors and the endothelium of the choroid, by the secretion of different factors, such as ATP, cytokines or metabolites, from the HRPE and by a large variety of transmembrane receptors on the surface of HRPE cells [2]. These include receptors of the renin-angiotensin system, complement receptors, purinergic receptors, growth-factor receptors, adrenergic and muscarinic receptors, receptors for immune modulators, such as phagocyte cell surface Mer tyrosine kinase receptors (MerTK) and Toll-like receptors, and neurotransmitter receptors (mainly for glutamate) [2]. In particular, dopamine receptors, MerTK, Toll-like receptor-4, α-adrenergic receptors and ATP receptors are located at the apical or retinal-facing side, whereas the angiotensin-2 receptor-1 is localized in the basolateral or blood-facing compartment [2]. Therefore, the highly polarized and multifunctional retinal pigment epithelium (RPE) is essential for maintaining photoreceptor function and forms the major component of the BRB [1].

The relationship between the BRB and photoreceptors is schematically represented in Fig. 1. Intercellular TJ prevent paracellular ion and water movement between the basal and the lateral side of endothelial and RPE cells. Transepithelial electrical resistance (TEER), which has been correlated with the amount, complexity and integrity of TJ between epithelial cells, is commonly accepted as an index of the epithelial barrier function. It must be taken into account that TEER is highly dependent on the types and characteristics of RPE cells (primary cell cultures, stabilized or immortalized cell lines) and on the specific culture conditions [3]. A gradual TEER increase following cell confluence has indeed been recognized to reflect the maturation of intercellular junction complexes in cell monolayers grown *in vitro* [4]. Physical (TJ, cell membranes) and dynamic (e.g., transporters) factors determine the barrier features of HRPE that influence drug delivery, efficacy and toxicity after intravitreal, subconjunctival, periocular and systemic administration [5]. Integrity of TJ is also fundamental for the correct polarized secretion of factors regulating the angiogenic process, such as the proangiogenic vascular endothelial growth factor

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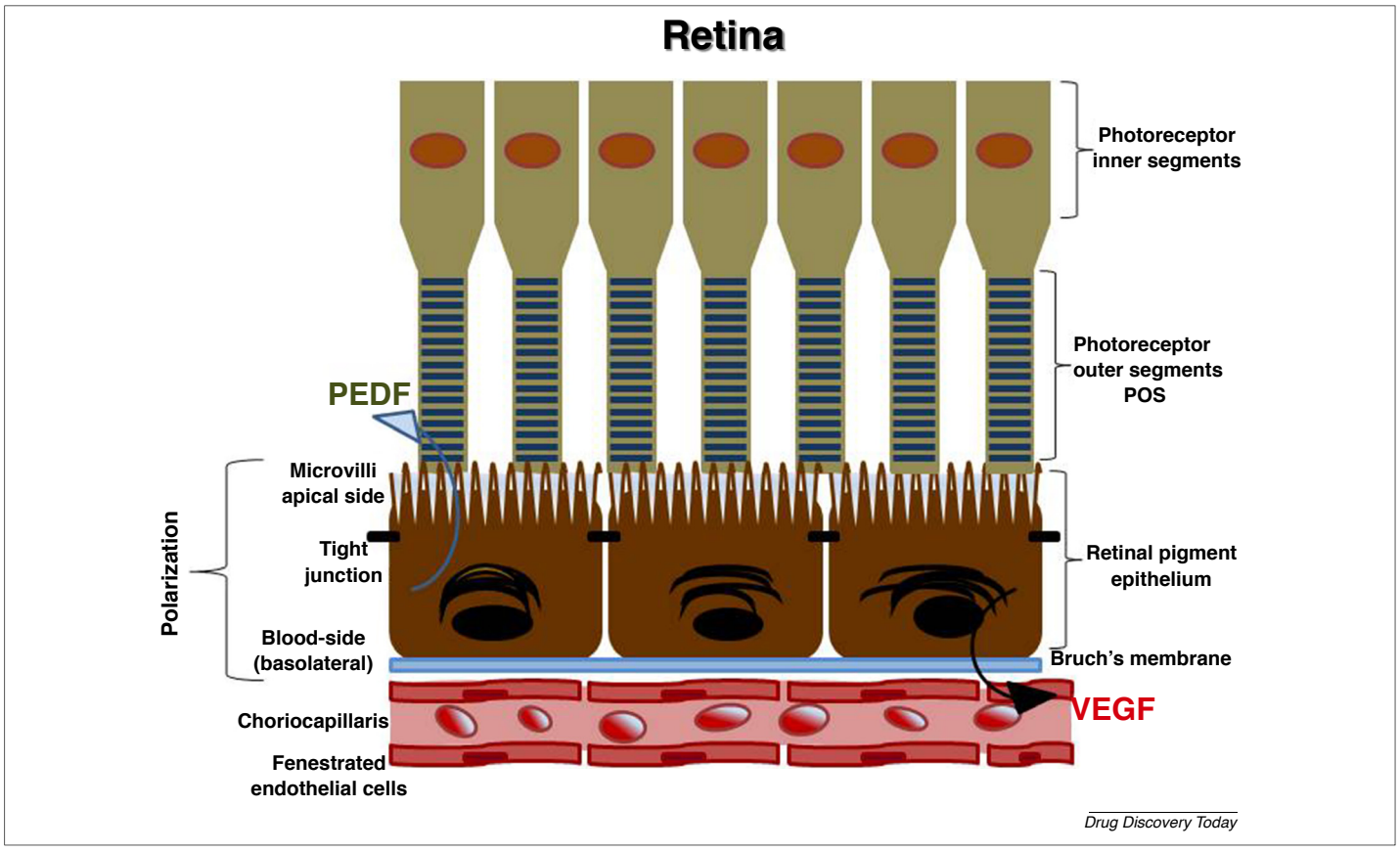

FIGURE 1

Schematic representation of the blood–retinal barrier (BRB), which separates the retina from the vascular system. Retinal pigment epithelium (RPE) is the main component of the BRB, and morphological and functional polarization is one of the most significant characteristics of RPE. Abbreviations: VEGF, vascular endothelial growth factor; PEDF, pigment epithelium derived factor.

(VEGF) and the antiangiogenic pigment epithelium derived factor (PEDF). The polarized secretion of VEGF on the basolateral side and PEDF on the apical side by the HRPE monolayer is required for the maintenance of the health and integrity of choroid and retina, respectively. Disruption of the equilibrium of secretion from apical and basolateral surfaces of the RPE monolayer is believed to promote a pathological microenvironment, contributing to various retinal diseases [3]. An unbalanced release of pro- and anti-angiogenic factors toward the proangiogenic ones is accompanied by an aberrant new vessel formation. Degeneration of the HRPE and neo-angiogenesis occur in age-related macular degeneration (AMD), in its exudative or neovascular ('wet') and nonexudative ('dry') forms. Degeneration of HRPE cells in the early and intermediate stages of AMD seems to begin with impaired clearance of cellular waste material [6]. This leads to a state of chronic inflammation in the eye, and eventually to the formation of abnormal yellowish subretinal deposits called drusen, which impair the function of RPE cells [6].

The more advanced dry form of AMD is characterized by age-dependent degeneration of the RPE and subsequently the overlying photoreceptors [7]. Patients with dry AMD frequently also develop wet AMD, suggesting a common pathomechanism. Wet AMD is characterized by infiltration of proliferating vessels from the underlying choroid into the subretinal space through the RPE, affecting the function of the overlying neurosensory retina by vascular leakage, hemorrhage and fibrosis with subsequent outer retinal degeneration and a final vision loss [7]. This choroidal neovascularization thus links proper RPE function with pathological neovascularization in wet AMD, because VEGF not only stimulates endothelial cell proliferation and migration but also impairs barrier function of the RPE [8]. Although neovascular AMD is the most damaging form of the disease, dry AMD accounts for ~90% of all cases [9]. Intravitreal injection of anti-VEGF agents has revolutionized the treatment and prognosis of neovascular AMD, although the intravitreal route remains invasive and accompanied by side effects. However, the administration of drugs via the systemic route would involve even more adverse effects and poor or lacking absorption by the retina. Finally, dry AMD treatment remains a challenge. However, there are currently no effective treatments to prevent progression of the underlying disease processes and advancement of dry AMD. Currently, the only approved treatment for dry AMD is the use of the Age-Related Eye Disease Study (AREDS)-based antioxidant formulation [10]. However, this multivitamin complex does not prevent AMD and its positive effects are modest because it only slows down the progression for patients at high risk of advanced AMD [11]. In the near future, it is likely that the treatment of dry AMD will be a combination of different drugs that will target the different pathways involved in the pathogenesis and progression of dry AMD [11,12].

RPE alteration is also involved in retinitis pigmentosa (RP), a genetically heterogeneous group of diseases characterized by

degeneration of the photoreceptors and RPE, which means night blindness followed by progressive loss of peripheral vision (most severely affecting rods). Cone degeneration and loss of central vision usually occurs after the death of most rods [13]. There are dominant, recessive and X-linked forms of inheritance in addition to rare mitochondrial and digenic forms of RP [13]. Retinal degeneration is advanced by metabolic changes, such as increased concentrations of γ-aminobutyric acid (GABA), the main inhibitory retinal neurotransmitter, in retinal glial Müller cells, which become hypertrophic and act as highways for neuronal translocation as well as pigment translocations into the inner retina from the RPE [13]. Indeed, an important partner of RPE cells to the photoreceptor development, function and survival are Müller cells, which release neurotrophic factors such as VEGF, PEDF, transforming growth factor-β (TGFβ), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) [14]. Release of these growth factors declines in many retinopathies, whereas restoration of their normal levels could enhance neuronal survival [14]. Although RPE cells have long been considered the principal mediators of several forms of retinal injury, Müller cell activation, migration, proliferation and transformation in retinal detachment and retinal injury have all been documented [15]. This review focuses on the RPE cells, because they form the first active interface to the systemic circulation, which reacts to systemic factors and/or pharmacological interventions.

Interestingly, recent studies showed that adult human RPE cultured cells can express neuronal markers such as β-III tubulin and Neurofilament 200, indicating an incipient potential to adopt different fates [16]. Furthermore, it has long been known that RPE cells derived from neonatal rats [17] and from fetal and adult humans express voltage-gated Na^+-channels and can produce action potentials – properties normally associated with neurons [18]. Therefore, in this review we aim to highlight the key features in HRPE cells aiding to develop drugs that can cross the BRB and specifically reach the retina by a systemic route. Again, they could help the discovery of new ways to activate the endogenous restoration of the barrier functions and the renewal of retinal dopaminergic precursors in RPE cells.

Engineering RPE cells with customized signaling behaviors

An interesting approach in retinal regeneration therapy is the performance of cellular and tissue-based products [19]. In fact, cell culture models are advantageous because they are defined systems in which experimental conditions can be controlled and manipulated. In addition, the results are usually more reproducible than those from animal models, many of which are available for AMD research but most do not recapitulate all aspects of the disease, hampering progress [20]. Some of the more recently described RPE-based models show promise for investigating the molecular mechanisms of AMD and for screening drug candidates [21], together with providing new insights into the emerging strategy of cell replacement therapy. Various types of dissociated RPE cells, such as cultured HRPE cell lines, immortalized adult RPE cell lines, human fetal RPE cells and human embryonic-stem-cell-derived RPE (hES-RPE) cells and induced pluripotent stem (iPS) cells, have been transplanted into the subretinal space of animal models with retinal degeneration caused by dysfunction of the RPE [22].

Many of these studies demonstrated protection of photoreceptors and improvement in visual function after transplantation. RPE cells were generally implanted early in the course of disease when most photoreceptors are still intact, showing protection of photoreceptors and improvement in visual function, but transplantation in late-stage disease produced loss of RPE cells and photoreceptors. Therefore, much needs to be learned about the ability of transplanted RPE to promote survival of existing photoreceptors and survival, differentiation and integration of transplanted retinal progenitor cells (RPCs) or photoreceptors [22]. In particular, the importance of functional polarization of hES-RPE cells linked to secretion of high levels of PEDF in improving RPC survival is known [22], providing an important feature in future cell therapy for atrophic AMD. Under these conditions, a combined useful prodrug strategy will also be impacted. Indeed, HRPE cells could be customized for tracking processes potentially related to retinal diseases, by engineering some of the features reported below, and prospective for optimal cell polarization; we had direct experience with some of them in our laboratory. First, we demonstrated a significant expression and activity of endogenous sodium-vitamin-C transporter type 2 (SVCT2) in HRPE cells [23]. Vitamin C is involved in the physiology of the nervous system, including the support and the structure of the neurons, as well as the processes of differentiation, maturation and neuronal survival, the synthesis of catecholamine and the modulation of neurotransmission.

SVCT is an active transporter for ascorbate (AA), the reduced form of vitamin C, and it is specific for the brain and the eye tissues, where this transporter has been shown as the principal route for sodium-dependent AA uptake that is affected with age [24]. AA is also known to serve as a co-factor for enhancing synthesis of catecholamines [25] that are involved in the regulation the RPE barrier properties and in the secretion of PEDF [26]. Therefore, an impairment of AA uptake can lead to a poor catecholaminergic activity with consequent disarrangement of the RPE barrier. Identification of the SVCT2 transporter system in HRPE cells had provided a new perspective for SVCT2-targeted prodrug delivery [23]. Hence, SVCT-targeted drug delivery has been proposed as a valuable strategy for enhancing ocular absorption of drugs administered by the systemic route against retinal diseases, at least concerning small molecules such as nipecotic acid.

Nipecotic acid is a competitive inhibitor of the transporter of GABA or a GABA agonist, depending on its concentration [27]. GABA dysregulation is a common phenomenon in retinopathies, such as RP disease, as reported above [13,27]. GABA uptake is linked to the metabolic support of photoreceptors and neurons, the defense against oxidative stress, the shaping and termination of the synaptic neurotransmitter action, the release of gliotransmitters and the detoxification of excess ammonia [28]. Thus, we hypothesize that nipecotic acid delivered selectively across SVCT2 in the RPE barrier as a prodrug with ascorbate could be suitable to reduce the GABA concentrations accumulating in retinal glial Müller cells in RP disease [13,27]. Therefore, the ascorbate–nipecotic-acid prodrug could be a prototype useful to design retinal neuroprotective drugs involving the interaction between the RPE and Müller cells. It should be noted that, other than in HRPE cells, expression and functionality of SVCT2 has also been characterized

in human corneal epithelial cells [24], which could suggest ocular drug delivery by drops.

Second, in HRPE cells we previously demonstrated that, unlike dopamine, the prodrug glucose–dopamine is a transportable substrate of glucose transporters (GLUTs) [29]. Indeed, we showed that dopamine and its prodrug permeate the cell, but only the uptake of the prodrug is inhibited by glucose, confirming that glucose transporters mediate the transport of the prodrug glucose–dopamine, but not of dopamine alone. Therefore, the GLUT-targeted prodrug approach can also be used as an attractive strategy to enhance the ocular absorption from the bloodstream of the glucose–dopamine conjugate. Indeed, dopamine is the main catecholamine found in the mammalian retina and, among its several important functions, a role in controlling photoreceptor disk shedding to the RPE is included. There is accumulating evidence of dopamine loss in retinal diseases with negative effects on neuronal survival. Thus, L-DOPA or levodopa and dopamine agonists can be used to restore visual and neuronal function in these diseases by recovery of the lost dopamine [14]. There is evidence that L-DOPA can stimulate G-protein-coupled receptors (GPCRs) like GPR143 in RPE that control secretion of PEDF, which could benefit diseases like AMD [14]. However, prolonged treatment with L-DOPA seems to require rising doses with consequent increasing significant side-effects that could be greatly reduced when treating with dopamine agonists or dopamine alone combined with an antioxidant instead of L-DOPA [14]. Regarding this, dopamine has been recently demonstrated to induce the release of ascorbate from retinal neurons by the reversion of the ascorbate transporter SVCT2 in response to glutamate triggering the inward sodium current [30].

Ascorbate released from retinal neurons is vital for the preservation of dopamine released into the extracellular space, increasing the efficiency of retinal dopaminergic neurotransmission. Dopamine increased ascorbate release, which might be important for modulating signaling events activated by dopamine, further substantiating that vitamin C homeostasis is pivotal for the physiology of the retinal tissue [30]. So, nipecotic-acid–ascorbate and glucose–dopamine prodrugs can be suggested as prototypic therapeutics via SVCT2 and GLUT transporters that can cross the RPE and reach the retina. Furthermore, human RPE cells have been reported to secret glial-cell-derived neurotrophic factor (GDNF) and BDNF together with the ability to synthesize dopamine [31], indicating HRPE cells as prominent candidates to be engineered for retinal dopaminergic neurons.

Two human adult RPE cell lines, young-derived ARPE-19 and old-derived H80HrPE cells, have been induced to transdifferentiate into neurons by treatment with medium containing all-trans retinoic acid, basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), expressing β-III tubulin as a neuronal lineage marker [32,33]. Therefore, the molecular tools for differentiation of HRPE cells into retinal dopaminergic neurons could be hypothesized to involve the retinoic acid pathway and neurotrophic factors. Indeed, neuronal trans-differentiation of RPE cells *in vitro* has been demonstrated to be enhanced by fenretinide, a synthetic retinoic acid derivative. HRPE cells treated with fenretinide express neurofilaments, calretinin and the neural cell adhesion molecule [34]. HRPE cells can also be stimulated in culture to generate multipotent cells: the HRPE stem cell [35].

The function of retinoic acid in neuronal differentiation could be driven by the activation of different RARs. In fact, in HRPE cells we characterized a selective expression of the subtype beta of the receptor for retinoic acid: RARβ, which is a senescence marker, and this expression increases with increasing passage of the cells, but it is not associated with decrease in telomere length [36]. As a result, RARβ could be a hypothetical therapeutic target for escape of HRPE cells from senescence and to transdifferentiate in retinal neurons or as marker to test HRPE performance of barrier features at the early and latest passage number in culture. Finally, in HRPE cells it has also been demonstrated that there is expression of active efflux transporters, such as the ATP-binding cassette (ABC) transporter family, the main function of which is to recognize different xenobiotics and to stop them from entering the vitreous humor through the BRB and obscuring vision [37]. Multidrug-resistance proteins MRP1, MRP4 and MRP5 are the main subtypes of efflux transporters in RPE cell lines [38]. MRP5 has a broad substrate and inhibitor specificity and it has also been linked to AMD development, and its expression decreases in senescent RPE cells. These efflux transporters could pose a significant barrier to delivery of several classes of drugs at the back of the eye from the bloodstream [39]. Recently, in HRPE cell monolayers showing epithelial barrier features we proposed a new prodrug strategy of antiviral drugs evidencing that the ester conjugation of AZT with ursodeoxycholic acid, a bile acid able to permeate the central nervous system, results in a prodrug (UDCA–AZT) that can elude the MRP transporters, for which AZT is a substrate [40]. In particular, this type of prodrug was not extruded from cell monolayers able to efflux AZT but, at the same time, the activity of the transporters was not inhibited by the prodrug itself [40]. These data suggest that the conjugation of antiviral drugs with bile acids could constitute a new strategy to avoid, without inhibiting, the ABC systems that normally preclude the entry of the antiretroviral drugs in HIV sanctuaries. ABC systems are also the main transporters for efflux for several neurotropic drugs. We have recently confirmed that UDCA–AZT is able to permeate and remain in murine macrophages with an efficiency 20-times higher than that of AZT [41]. This approach could therefore be functional to HIV infection in the retina and RPE, where the virus contributes to neural damage and BRB breakdown, preventing or reducing drug toxicity and HIV retinopathy secondary to invasion by HIV-infected macrophages [42].

It is also noteworthy that bile acid UDCA and its taurine-conjugated derivative tauroursodeoxycholic acid (TUDCA) are also recognized as powerful neuroprotective agents with multiple actions such as significant preservation of retinal function and photoreceptor structure and survival [14]. In particular, TUDCA provided benefit to the photoreceptors indirectly by enhancing phagocytosis of photoreceptor outer segments by activating MerTK receptor in the RPE cells [43]. In fact, RPE cells perform numerous processes to maintain and support photoreceptors, such as the continuous renewal of the light-sensitive outer segment portions of photoreceptors (POS), which are crucial for vision [44] (Fig. 1). The outer segments of rods and cones are dynamic structures that undergo constant renewal. Photoreceptors synthesize new outer segment components at a very high rate and form new outer segment disks, thereby gradually elongating outer segments. A process commonly termed disk shedding compensates

for this addition during which RPE cells and photoreceptors collaborate to remove the most distal tip of POS by phagocytosis [44]. Mutations in MerTK gene expression cause RP in human patients [44]. Therefore, HRPE cells could be a suitable model to study for devising optimal delivery of bile acids conjugated to drugs, which are substrates of efflux transporters, with the aim of obtaining prodrugs, acting at the same time as neuroprotective and efflux-transporter-evading compounds.

One of the challenges of translating neuroprotective strategies to the clinic for eye diseases is delivery of compounds to the eye without systemic administration to provide the optimal dose for the retina with the fewest systemic side-effects. However, intravitreal administration of drugs circumvents the BRB with high bioavailability and reduced systemic side-effects, but with a higher risk of local side-effects such as pain at the site of injection. Thus, a multidrug system involving prodrugs targeting specific transporters expressed in RPE cells would represent more-selective drug delivery reducing the systemic side-effects. All these insights are schematized in Fig. 2 and focus on a viable customized model of the BRB *in vitro* – essential for studying development of ocular diseases and establishing treatment strategies.

Epithelial and neuronal polarization and pigmentation

It is important to know that HRPE cells in culture undergo a spontaneous process of dedifferentiation into cell lines, characterized by a loss of pigmentation and a failure to regenerate a polarized epithelial cell shape. This innate capacity of the HRPE cells to dedifferentiate is also attested *in vivo* leading to a pathological condition called proliferative vitreoretinopathy (PVR), a common cause of visual loss [16]. PVR occurs in the eye when the monolayer of the RPE is disrupted, typically by detachment of the overlying neural retina [16]. Salero *et al.* [16] repeated this phenomenon *in vitro*, providing an important tool for identifying therapeutics that can inhibit this process. These authors identified a subpopulation of stem-like cells derived from human adult RPE cells that can be activated to self-renew and that exhibit multipotency, producing either stable RPE progeny or neural, osteo, chondro or adipo-lineage mesenchymal progeny.

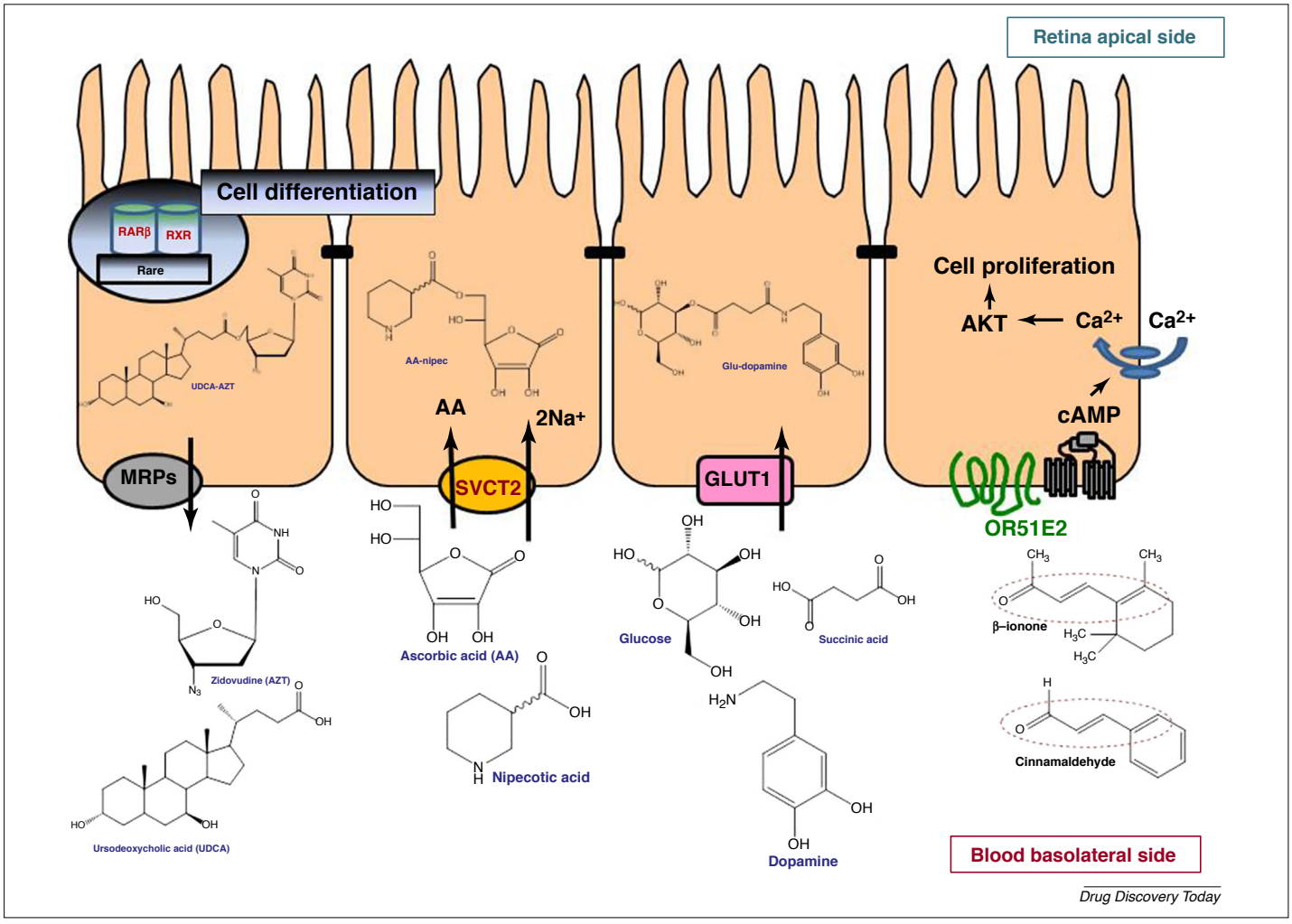

FIGURE 2

Drugs and prodrugs acting on receptors and transporters expressed in human retinal pigment epithelium (RPE) cells *in vitro*. Ascorbic acid (AA) forms useful prodrugs to transport drugs into the cell through sodium-vitamin-C transporter type 2 (SVCT2) as an AA-nipecotic acid conjugate. Multidrug-resistance proteins (MRPs) efflux zidovudine (AZT), which can be avoided, without inhibition, using a prodrug (UDCA–AZT) conjugating ursodeoxycholic acid (UDCA) and AZT. GLUTs transport the glucose–dopamine prodrug into the cells, but not dopamine alone. Retinoic acid receptor (RAR)β is a marker of senescence and a target for neural differentiation. β-ionone is an agonist of the olfactory receptor OR51E2 and activates, together with the putative agonist cinnamaldehyde, human RPE (HRPE) cell proliferation.

One of the strategies currently explored for retinal cell replacement and for reducing the possibility of transplant rejection involves transdifferentiation, also called direct conversion, the process of transforming an adult somatic cell into another adult somatic cell. Recent studies also presented new strategies, using criteria such as common cellular origin and developmental plasticity, to identify 'the best possible' cell for transdifferentiation. Equally, understanding how dedifferentiation and expansion of RPE cells is regulated could help in other diseases that involve RPE degeneration. A neuronal lineage derived from RPE multipotent stem cells would facilitate the development of transplantation therapies for retinopathies, drug testing and *in vitro* disease modeling. Our understanding of the development of the CNS, especially in the retina, would also be improved. The fact that upon neural differentiation stem-like RPE cells lost their microphthalmia transcription factor (MITF) marker, consistently with a fate change, and express features of anterior neural progenitor cells, suggests that their potential might be wide-ranging. It would also be valuable to investigate whether stem-like RPE cells can be advanced into other differentiated CNS phenotypes, such as striatal dopaminergic neurons, as already suggested [31,45].

In amphibians in which the RPE is stimulated to transdifferentiate into neural retina, pigment loss accompanies the transition to retinal cells [16]. Polarization development and melanization seem to be linked and both might be regulated by common signaling pathways [46]. In fact, the second messenger cAMP has been shown to promote differentiation and maturation in HRPE cells, hypothetically via proliferation-independent mechanisms, such as promoting melanosome and pigmentation-related pathways [47]. Moreover, after an appropriate treatment with high-glucose DMEM medium containing pyruvate, cultured HRPE cells have been shown to produce their pigment, acquiring dendritic morphology [48]. Otherwise, loss of pigmentation observed in several dedifferentiated cell lines could account for making these cells more competent to the transdifferentiation toward retina neuronal lineage [49]. Molecular and genetic changes in HRPE cells *in vitro* are generally similar to those observed in lower vertebrates *in vivo*, which reflects conservatism of the RPE reprogramming mechanisms. However, reprogramming of adult human RPE cells *in vitro* is a rapidly decaying process, which prompted the search for factors for its stimulation and maintenance [33]. bFGF has been shown as essential to reduce the degree of cell differentiation and trigger neuronal differentiation on ARPE-19 cells [33]. In this regard, human RPE cells could represent a stable and reproducible *in vitro* model, suitable and predictive to investigate which mechanisms drive epithelial and/or neuronal cell polarization and pigmentation disorders of RPE incoming before or during retinopathies.

A recent article by Khristov *et al*. [50] reported that polarized RPE showed distinct surface proteomes on the apical and basal plasma membranes, and a series of approaches are presented to identify and validate the polarization state of cultured primary human RPE cells using immunostaining for RPE apical and basolateral markers, polarized cytokine secretion, electrophysiology, fluid transport, phagocytosis and identification of plasma membrane proteins through cell surface capturing technology. Oxidative or other stresses causing injury to the RPE could impair the polarized secretion of VEGF and PEDF, for example by translocation from apical to basolateral membrane domains [3]. These drawbacks need to be taken into account in devising optimal prodrug delivery.

Development of drugs on cell-based models for retinal regeneration

GPCRs, other than MerTK, mediated phagocytosis of POS in RPE cells, because an increase in intracellular cAMP by stimulating β-adrenergic and A2 adenosine receptors shuts off POS phagocytosis [51]. Moreover, a recent transcriptome analysis revealed expression of several subtypes of other GPCRs, the olfactory receptors (ORs), in the human neural retina and human fetal RPE [52]. In particular, OR51E2 was demonstrated as the most highly expressed OR subtype in human adult and fetal RPE, whereas OR51E2 was not detectable in the human neural retina [53]. OR51E2 was also shown to be expressed in the vascular layer containing pigmented melanocytes: the choroid, suggesting that this receptor is particularly expressed in pigmented cells of the human eye [53]. This evidence concurs with our recent hypothesis that ORs might be related to the repair processes in pigmented epithelial and neuronal cells [54]. Furthermore, a genetic mutation of a specific OR: OR2W3, expressed in stem-cell-derived HRPE, was recently associated with the development of the autosomal-dominant RP disease [55]. Thus, an analysis of a hypothetical involvement of ORs in phagocytosis of POS would be of interest. Moreover, this finding could indicate the essential links between vision and olfaction, and strongly suggested an exchange in the importance of these two senses, a remarkable in-depth comparison is provided in the paper by Gilad *et al*. [56]. Therefore, several odorants binding to OR51E2 could be considered as druggable scaffolds for new therapeutics in RPE recovery. In particular, compounds characterized by the presence in their molecular structure of a carbonyl group conjugated to a butadiene system have been identified as strong OR51E2 agonists [57] (Fig. 2). Between them, cinnamaldehyde is not known as a ligand of OR51E2 but, according to its structure, its derivatives could be.

The OR51E2 agonist β-ionone is also known as a product from the oxidative cleavage of carotenoids, such as β-carotene and lycopene, and is catalyzed by b,b-carotene-9,10-dioxygenase 2 (BCDO2), and its key function is the conversion of provitamin A carotenoids to vitamin A. Vitamin A is crucial for physiological functions, such as vision, embryonic development and cell differentiation [58]. BCDO2 expression is detected in various human tissues, especially in the RPE, where β-ionone, as a cleavage product of the BCDO2 enzyme, was shown to activate a cAMP-mediated influx of extracellular Ca^{2+} that results in an activation of AKT-dependent signaling and finally in an increased proliferation rate [53]. As mentioned above, RPE cells undergo a terminal differentiation early, resulting in a minimal proliferation capacity throughout normal life. However, proliferation of RPE cells can be induced upon release from their niche, their highly structured microenvironment that normally maintains a quiescent state, such as *in vitro* cultures or in PVR and AMD human diseases [59]. The activation of proliferation by a variety of growth factors leads to a repair of failures in RPE monolayer cultures, providing useful models of wound healing by the RPE layer [59]. Conversely, in humans, RPE proliferation is uncommon and when it does occur it can lead to pathological conditions such as PVR, AMD

and RP [59]. As a consequence, based on these results, OR51E2 was suggested to act in a similar way to growth factor receptors in RPE cells and induce the proliferative and wound-healing responses, at least based on the behavior of *in vitro* cultured cells. OR51E2 was hence proposed as a promising therapeutic target protein for the treatment of proliferative RPE disorders, such as PVR [53]. In summary, we could propose HRPE cells as an *in vitro* model feasible to apply to the approach of polypharmacology, one of the major challenges to rationally design next-generation more-effective agents [60], combining two or more drug entities in a single molecule, capable of interacting simultaneously with multiple targets, directly or following their metabolism [60].

Concluding remarks

This review looks at the role that the RPE can take as an epithelial barrier and as a source of neural precursors against the development and progression of retinal diseases. These vision disorders can be triggered by dysfunctions, such as glucose instability, chronic inflammation and adrenergic overshoot, and can be linked to premature or physiologic aging processes. Each of these disorders can be reproduced in a single or combined manner in an *in vitro* human model of HRPE cells in 2D or 3D culture. Under these conditions, HRPE cells can assay the performance of the epithelial barrier, based on the expression of specific markers of aging, such as RARβ, or on the expression of specific transporters, such as SVCT2 or GLUT or MRP, to design drugs or prodrugs that could be successfully used for crossing the BRB or precluding the efflux of drugs administered by the systemic route.

The RPE barrier features, determining the directionality and selectivity of secretion of several growth factors and angiogenic molecules, need to be accounted for in devising optimal drug delivery. Odorant molecules such as β-ionone could also be a new scaffold for promising drugs acting on OR51E2 for the treatment of proliferative RPE disorders, such as PVR, or pigmentation disorders, such as RP. Finally, HRPE cells yield a model to study the potential differentiation of retinal neuronal precursors *in vitro* to design the revival of a resting endogenous repair *in vivo*. In conclusion, the resulting designed molecules could be successfully used as new multifunctional drugs.

Conflicts of interest

Authors declare that they have no conflicts of interest to disclose.

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