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Human Organoids for the Study of Retinal Development and Disease

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Abstract

Recent advances in stem cell engineering have led to an explosion in the use of organoids as model systems for studies in multiple biological disciplines. Together with breakthroughs in genome engineering and the various omics, organoid technology is making possible studies of human biology that were not previously feasible. For vision science, retinal organoids derived from human stem cells allow differentiating and mature human retinal cells to be studied in unprecedented detail. In this review, we examine the technologies employed to generate retinal organoids and how organoids are revolutionizing the fields of developmental and cellular biology as they pertain to the retina. Furthermore, we explore retinal organoids from a clinical standpoint, offering a new platform with which to study retinal diseases and degeneration, test prospective drugs and therapeutic strategies, and promote personalized medicine. Finally, we discuss the range of possibilities that organoids may bring to future retinal research and consider their ethical implications.

1. INTRODUCTION

We are in a revolutionary era of biological discovery. Together with the transforming breakthroughs of human pluripotent stem cells (hPSCs) (Takahashi et al. 2007, Thomson et al. 1998) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome editing (Cong et al. 2013, Jinek et al. 2012), advances in in vitro cell differentiation and organogenesis are changing the way biological and medical science is approached. Organoids, three-dimensional (3D) multicellular structures assembled in vitro that can recapitulate many aspects of in vivo tissue structure and function, are now being used not only to advance our understanding of developmental biology, but also to model and study a variety of diseases. Organoids developed from patient-derived hPSCs or from stem cells in which certain genetic variants are introduced can be used for defining tissue-wide disease etiology, as well as serving as a model for identifying biological pathways and chemical compounds that can modulate the disease state. This same system can now better define treatment strategies on an individualized basis, moving personalized medicine to an even more functional level. The future of integrating organoid systems (e.g., the retina, the optic nerve, and the brain), as well as generating a safe resource for transplantation approaches, is moving from a dream to a possible reality. In this review, we discuss these advances as they specifically relate to retinal organoids.

1.1. The Human Retina

The human retina, the layer of neural epithelium that lines the back of the eye, carries out phototransduction and initial signal processing and, through the optic nerve, transmits visual information from the eye to the brain (Demb & Singer 2015, Purves et al. 2001). Six classes of retinal neurons and one class of retinal glial cell, as well as potentially several classes of immune-related cells, account for the cells that comprise the developed human retina. In-depth studies of retinal development in various animal models suggest that multipotent retinal progenitor cells (RPCs) are capable of generating all of the retinal cell types, but as neurons differentiate, the potency of the progenitor cells is progressively restricted (Reese 2011) (**Figure 1**). The different neuronal cell types are organized into distinct layers, and these layers are structured with the aligned nuclei (nuclear layers) alternating with regions of organized synapses (plexiform layers). Müller glia cells span all of the layers and provide support to the neurons. Light is sensed by the rod and cone photoreceptor (PR) cells, which reside in the outermost layer of the retina and relay signals via bipolar cells to retinal ganglion cells (RGCs) in the innermost layer. Visual information is then transmitted to the brain via the axons of the RGCs that form the neural component of the optic nerve, which connects the retina to the lateral geniculate nucleus and related nuclei in the brain. The transmitted signal is modulated by interactions between PRs, bipolar cells, and RGCs, which are mediated by the horizontal cells and amacrine cells (Demb & Singer 2015, Masland 2012). Retinal organoids are a unique in vitro model system in that they structurally and functionally reflect many of the complexities of the human retina, with the variety of cell types self-organizing into the layers of the in vivo retina.

The development of the human retina occurs throughout gestation, with retinal cell markers expressed as early as four weeks postfertilization in human embryos (Mellough et al. 2019a). There are significant challenges to studying the initiation and early steps of retinal developmental pathways, as obtaining fetal tissue for research is difficult and ethically controversial. Although several studies have been done on human fetal retinal tissue (Hendrickson 2016, Hoshino et al. 2017, McLeod et al. 2006, O'Brien et al. 2004), our combined picture of human retinal development is still incomplete at best. Most of our understanding about retinal development comes from studies of animal models, and while the human retina shares many overarching characteristics and cell

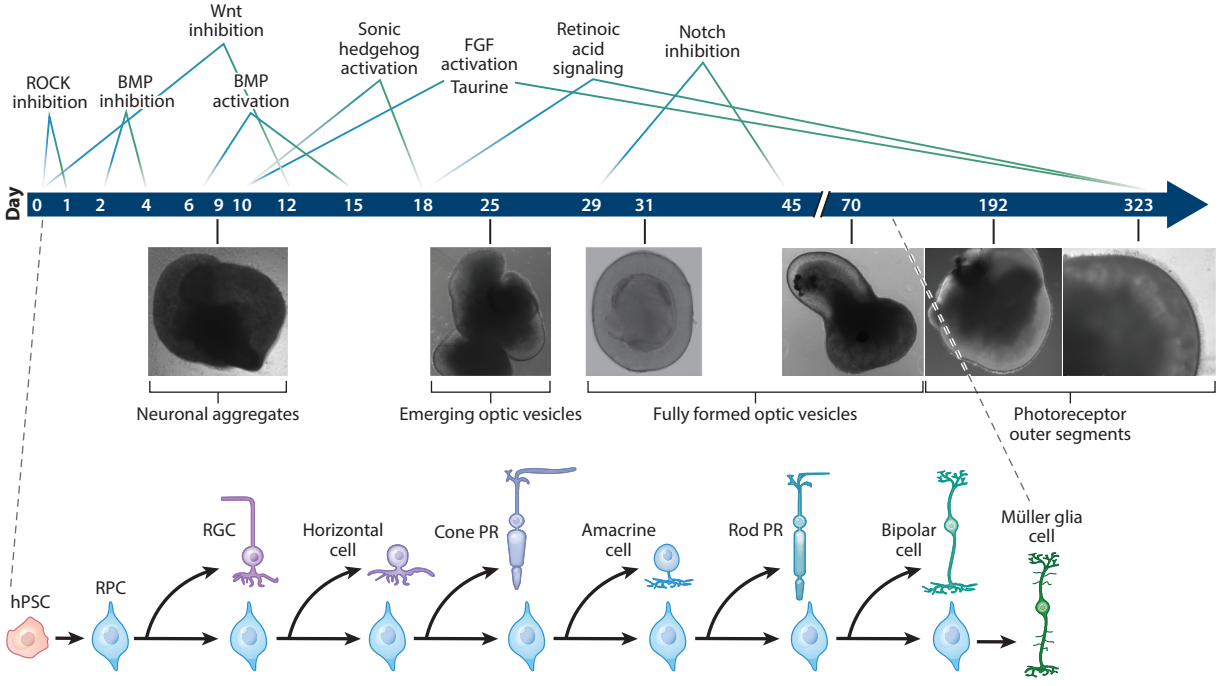

Figure 1

Organoid differentiation. Throughout the course of differentiation, the cellular pathways that are manipulated by small molecule treatment, and the ranges over which they are treated throughout the different protocols, are shown. The physical maturation of the retinal organoids is shown. The progressive development of retinal progenitor cells into the distinct types of terminally differentiated retinal cells occurs in the first 2–3 months of culturing the organoids. Abbreviations: BMP, bone morphogenic protein; FGF, fibroblast growth factor; hPSC, human pluripotent stem cell; PR, photoreceptor; RGC, retinal ganglion cell; ROCK, rho kinase; RPC, retinal progenitor cell.

types with those of common vertebrate model organisms, such as mice and rats, there are known differences in its cellular composition and function. Most mammals have two types of cone photoreceptors, which express S-opsin or M-opsin, while humans also have a third type of cone, which expresses L-opsin (Ortín-Martínez et al. 2014). Mice are also known to have a higher ratio of rods to cones than humans (Volland et al. 2015) and can have cones that simultaneously express both S-opsin and M-opsin (Masuda et al. 2014). Structurally, the human retina contains the macula and the fovea, central areas of the retina that are highly dense with cones and are responsible for high visual acuity (Peng et al. 2019, Provis et al. 2013). These differences, and how they are reflected in development and disease, further highlight the importance of a human model system, discussed in this review in the form of retinal organoids, to complement current animal models to move human retinal research forward.

1.2. Human Stem Cell–Derived Organoids

The establishment of human embryonic stem (ES) cell lines (Thomson et al. 1998) and the capacity to generate induced pluripotent stem cells (iPSCs) (Takahashi et al. 2007) laid the foundation for the explosive field of stem cell engineering over the past decade. The ability to propagate hPSCs and differentiate them into a range of cell types in vitro has yielded highly advantageous model systems for studying human development and disease mechanisms. Both ES cells and

iPSCs are capable of self-renewal and pluripotency, and each offer different benefits to the field. ES cells have the appeal of having never been differentiated into another cell type or been subjected to a lifetime of somatic mutations, but their use is accompanied with ethical controversies and challenges. iPSCs are obtained by reprogramming somatic cells and provide the opportunity for studying diseases and development in a patient-specific genetic background. It has also been hypothesized that researchers can take advantage of a cell's historical identity and choose the somatic cell type used for reprogramming from the same germ lineage as the target organ for differentiation, providing a more efficient differentiation process (Shrestha et al. 2019, Tucker et al. 2013).

While most of the initial stem cell differentiation protocols were focused on generating a single class of cells (Keller 2005; Osakada et al. 2008a,b; Sluch et al. 2015, 2017; Uygun et al. 2009; Zhou et al. 2015), there is now increasing interest in generating entire 3D mini-organs, or organoids, in a dish (Clevers 2016, Lancaster & Knoblich 2014). Organoids consist of multiple or all of the major cell classes of their in vivo human counterparts and provide an environment for cells to develop that is more representative of in vivo conditions, as cellular organization and inter- and intracellular signaling during development occurs in 3D space. For neuronal studies, this allows analysis of signaling, neuronal pathfinding, and plasticity as cells develop to be connected with their synaptic partners. For retinal disease, studying drivers of pathogenesis in a 3D structure is also advantageous, as it has been observed that retinal degeneration (RD) can develop in patches that influence the surrounding cells, suggesting that the local microenvironment can affect cell survival (Kedzierski et al. 1998).

Retinal organoids have now been successfully generated by several groups, discussed further in the next section. They closely follow the human retinal development timeline while generating all of the known classes of cells in self-organizing layers. Retinal organoids are already allowing the molecular mechanisms of human retinal development and disease to be studied in unprecedented detail and promise to continue to expand the field of vision science and complement what we have learned thus far from other vertebrate models.

2. RETINAL ORGANOID TECHNOLOGY

Since the initial human retinal organoid papers were published by Nakano et al. (2012) and Meyer et al. (2009, 2011), a number of additional protocols for generating retinal organoids from hPSCs have been established (Akhtar et al. 2019, Boucherie et al. 2012, Capowski et al. 2019, Chichagova et al. 2019, Cowan et al. 2019, DiStefano et al. 2018, Fligor et al. 2018, Gao et al. 2016, Gonzalez-Cordero et al. 2017, Kim et al. 2019b, Kuwahara et al. 2015, Lowe et al. 2016, Mellough et al. 2019b, Ohlemacher et al. 2015, Ovando-Roche et al. 2018, Parfitt et al. 2016, Reichman et al. 2014, Singh et al. 2015, Wahlin et al. 2017, Zhong et al. 2014). The general structure for these protocols involves aggregating cells into embryoid bodies (EBs) and then treating the EBs with various cocktails of small molecules at specific times through the course of differentiation (**Figure 1**); timing of treatment and composition of the small molecule cocktails were designed to mimic the cues received by cells throughout the course of in vivo retinal development. During human retinal development, optic vesicles form from evaginations of the diencephalon. This process involves an array of molecules from a set of signaling pathways that are well studied in other vertebrates (Adler & Canto-Soler 2007), including hedgehog (Hh) (Locker et al. 2006, Masai et al. 2005), Wnt (Liu et al. 2006, Meyers et al. 2012, Steinfeld et al. 2017), transforming growth factor beta (TGF-β) (Braunger et al. 2013, Walshe et al. 2011), bone morphogenic proteins (BMPs) (Murali et al. 2005, Steinfeld et al. 2017), and fibroblast growth factor (FGF) (Hochmann et al. 2012, Martinez-Morales et al. 2005). Taking their lead from these studies, groups developing

retinal organoid differentiation protocols treated EBs with these factors to mimic in vivo signaling during development. These factors (discussed in more detail below) can modulate developmental pathways to direct differentiation toward retinal cell fates (**Figure 1**). In vivo, the optic vesicles then invaginate to form optic cups, whose RPCs differentiate to eventually comprise fully formed human retinas. Further differentiation of optic-vesicle-like structures in vitro is promoted by the addition of a second wave of exogenous factors that simulate the signaling pathways that promote differentiation of the retinal progenitors. Modifications to the original retinal organoid protocols have for the most part been optimizations of the modulation of the signaling pathways that drive retinal progenitor development and maturation.

2.1. Retinal Organoid Methodology

The first step in differentiating pluripotent cells into retinal organoids is to form EBs, using one of several different approaches. (*a*) Mechanical EB formation is done by growing cells to confluency in a monolayer culture and then manually scraping cells into clumps, which are transferred to a suspension culture (Cowan et al. 2019, Kim et al. 2019b, Lowe et al. 2016, Meyer et al. 2011, Reichman et al. 2014). (*b*) Enzymatic generation of EBs is done by enzymatically detaching pluripotent cell colonies from the cell culture substrate and then culturing cells as aggregates in suspension (Boucherie et al. 2012, Capowski et al. 2019, Fligor et al. 2018, Meyer et al. 2009, Ohlemacher et al. 2015, Zhong et al. 2014). (*c*) EB formation by forced reaggregation of dissociated stem cells is done by seeding the single cell suspension into 96-well U- or V-bottom plates (Kuwahara et al. 2015, Nakano et al. 2012, Parfitt et al. 2016, Wahlin et al. 2017). The method used to generate EBs affects both the size of the retinal organoid and the efficiency of organoid generation and, based on a study that compared gene expression in organoids differentiated from these different methods (Mellough et al. 2019b), can ultimately affect the differentiation and maturation of retinal cells. While there were minimal differences in the gene expression of organoids differentiated from EBs formed by either mechanical, enzymatic, or forced aggregation after 5 weeks of differentiation, mechanically generated EBs produced retinal organoids most similar to actual human embryonic and fetal retinal tissue over the course of development.

Essentially all human retinal organoid differentiation protocols involve neuralizing hPSCs after aggregating them into EBs and then isolating the retinal structures that emerge, whether by selecting organoids with visibly more retinal material for further growth (Cowan et al. 2019; Fligor et al. 2018; Kim et al. 2019b; Kuwahara et al. 2015; Lowe et al. 2016; Meyer et al. 2009, 2011; Ohlemacher et al. 2015) or by microdissecting vesicles from organoids and continuing their culture (Capowski et al. 2019, Gonzalez-Cordero et al. 2017, Nakano et al. 2012, Ovando-Roche et al. 2018, Parfitt et al. 2016, Reichman et al. 2014, Wahlin et al. 2017, Zhong et al. 2014). hPSCs are directed toward an anterior rostral neural progenitor state by inhibition of the Wnt and BMP pathways with Dkk1 or IWR1 and noggin, respectively, and the addition of smoothened agonist (SAG) stimulates neural cell proliferation. Further supplementation by various small molecules may be used to specifically direct cells toward a retinal progenitor fate. Kuwahara et al. (2015) reported a dramatic increase in RPC marker expression with the introduction of BMP4 after a week of differentiation. Retinoic acid (RA), insulin-like growth factor 1 (IGF1), and basic FGF (bFGF) may be added to promote neuroretinal differentiation during early optic vesicle development. Additionally, it has been shown that using rho kinase (ROCK) inhibitors, such as blebbistatin, during the first 48 hours of differentiation not only prevents apoptosis during EB generation, but also results in earlier and more consistent formation of retinal neuroepithelium (Mellough et al. 2019b). However, ROCK inhibitors will disrupt in vitro optic vesicle organization if used too late during organoid development (Lowe et al. 2016).

Without additional factors, retinal organoids are able to produce cells that express PR-specific markers, but these cells do not make up as high a proportion of the retina as PRs do in the human retina, nor do they show the structural or functional features of mature human PRs (Boucherie et al. 2012, Nakano et al. 2012). Conditions have since been developed that promote differentiation of retinal organoids with a more stereotypical complement and robust population of PRs, capable of maturing several hundred days in culture. Since Notch1 was known to inhibit PR production in murine retina development (Jadhav et al. 2006), Nakano et al. (2012) reasoned that treatment with the Notch inhibitor DAPT would promote PR development. DAPT treatment at approximately four weeks into differentiation resulted in an increase of PRs from approximately 15% of total cells to approximately 60% of total cells, as determined by cells expressing the PR-enriched transcription factor, CRX. Boucherie et al. (2012) used a different set of factors to increase the PR population. They used a combination of taurine, which is critical for rod PR (Lombardini 1991) and RGC (Lake 1994) survival in mammals and promotes PR formation in vitro (Altshuler et al. 1993), and Sonic Hedgehog (SHH), which has been shown to both increase in vitro mouse RPC proliferation and enrich PRs later in development (Jensen & Wallace 1997, Levine et al. 1997). This resulted in a reported 60% of cells expressing *CRX*. Differentiation protocols modified by Zhong et al. (2014) and Wahlin et al. (2017) were designed to generate organoids with more mature PR outer-segment-like structures. Zhong et al. decreased the window of RA treatment with the hypothesis that prolonged exposure was hindering PR maturation, treating only from days 70–98, and were able to observe structures resembling outer-segment discs and measure photoresponsiveness in several cells. Wahlin et al. (2017) used DAPT similarly to Nakano et al. (2012) and also modified the RA treatment in the organoid differentiation protocol; they treated from day 20 (after retina formation) until day 120 (before PR maturation) to promote PR development but not hamper PR maturation. They were able to observe outer-segment-like structures after 130 days of differentiation and reported visual pigments compartmentalized to outer segments after 300 days.

Although differentiation conditions have been established that successfully generate organoids with mature PRs, it is interesting to note that the PRs in these organoids are not in direct contact with supporting retinal pigmented epithelial (RPE) cells. In the in vivo retina, the RPE abuts the PRs and is essential for normal PR development and function. The RPE is responsible for the visual cycle; for transport of blood-borne nutrients and metabolites to and from PRs; and for the daily clearance of shed, lipid-rich PR outer segments. With essentially all published retinal organoid protocols, the RPE forms as a separate clump in an area of the organoid separate from most of the PRs. Although the RPE in these organoids may be providing secreted factors that affect the PRs, the physical relationship between PRs and the RPE appears to be clearly different in organoids compared to the in vivo situation. Recently, Akhtar et al. (2019) cocultured developing retinal organoids with mouse primary RPE cells, which led to the RPE sheets adhering to the organoids, as well as the relative enrichment of PR precursors and more mature gene expression in the organoids. Other groups are also working on improving the PR–RPE interface in organoids (Achberger et al. 2019), and it will be interesting to see if such efforts lead to improved PR structure and function

Another challenge with human retinal organoids, which has been addressed by more recent protocols, is the issue of poor oxygen and nutrient exchange at the inside of the structures. It is believed that this increases cell necrosis and affects the function and survival of the early-differentiating RGCs that reside at the innermost part of the optic vesicles. It has been noted across multiple cell lines that, by the time PR outer segments develop in retinal organoids, significant loss and disorganization of inner retinal cells has occurred (Capowski et al. 2019). Several labs have begun differentiating organoids in shakers and bioreactors, or with intermittent high

oxygen, to help improve oxygen flow and nutrient distribution and minimize necrosis inside the organoids. These modified culture techniques have been shown to accelerate differentiation in early developmental cell populations (DiStefano et al. 2018), increase the number of PRs and lead to earlier development of outer segments (Ovando-Roche et al. 2018), improve the ratio of cone to rod PRs (Kim et al. 2019b), enhance RGC migration and maturation (Gao et al. 2016), and prevent adhesion of cells to culture plates (Reichman et al. 2014). However, a study by Mellough et al. (2019b) suggests that shaking conditions may increase the overall expression of retinal precursor markers but actually decrease levels of mature retinal cell markers, which could potentially hinder the use of organoids to study late-onset retinal conditions. Thus, although great strides have been made in optimization of retinal organoid differentiation, there is still room for further improvement. As the protocols evolve, the field needs to keep in mind that variations in protocols, even seemingly small ones, and variations in starting cell lines can lead to significant changes in the biology, cell populations, and behavior of the organoids (Capowski et al. 2019, Mellough et al. 2019b).

2.2. Retinal Organoid Morphology and Structure

As retinal organoids begin to differentiate, optic-vesicle-like structures emerge as a sheet of neuroepithelium and appear translucent and phase-bright via light microscopy (**Figure 1**). In in vivo human retinal development, the distal side of the optic vesicle invaginates to form a double-layered optic cup (OC), with the outer layer forming the RPE and the inner layer becoming the neural retina. While the RPE is seen to differentiate in hPSC-derived retinal organoids, as noted above, it typically forms as a mass of cells not adjacent to the PR layer. Both the in vivo neural retina and the in vitro retinal organoid form self-organized layers of cells based on intrinsic signals and local factors, with PRs in the most outer layer and RGCs in the most inner layer.

In addition to laminated epithelium, other ultrastructural features of the mature human retina have been observed in differentiated human retinal organoids. The external limiting membrane, a series of intercellular junctions between Müller glia and PR cells, has been detected via high-resolution microscopy in fully mature organoids (Capowski et al. 2019, Cowan et al. 2019, Lowe et al. 2016, Ovando-Roche et al. 2018, Wahlin et al. 2017, Zhong et al. 2014). PR outer- and inner-segment-like structures have been seen in mature retinal organoids (Capowski et al. 2019, Cowan et al. 2019, Gonzalez-Cordero et al. 2017, Kim et al. 2019b, Lowe et al. 2016, Mellough et al. 2019b, Ovando-Roche et al. 2018, Parfitt et al. 2016, Wahlin et al. 2017) (**Figure 1**) and are discussed further in the next section. Furthermore, a peripheral zone of neural retina progenitors, similar to the niche for ciliary margin stem cells in chicks, has been reported in mammalian fetal retinas and at the junction of neural retina and RPE in retinal organoids (Kuwahara et al. 2015).

Overall, the majority of retinal organoid differentiation protocols follow a timeline of retinal differentiation over three specific stages (Capowski et al. 2019) that can be compared to those of the human retina in utero. In vivo RPCs differentiate into the seven classes of retinal cells in a progressively restrictive manner, becoming less multipotent as differentiation proceeds. Birth-dating studies indicate that RGCs are the first terminally differentiated cells to emerge, followed by horizontal cells, cone PRs, amacrine cells, rod PRs, bipolar cells, and lastly Müller glia cells (Reese 2011, Stenkamp 2015). This same progression is seen in differentiating human retinal organoids, with retinal induction before day 30; RGCs and some amacrine cells seen at days 30–70; PR precursor cells, bipolar cells, and interneurons seen between days 70 and 120; and mature PRs and Müller glia by day 200 (Capowski et al. 2019, Cowan et al. 2019, Gonzalez-Cordero et al. 2017, Kim et al. 2019b, Mellough et al. 2019b, Nakano et al. 2012, Ovando-Roche et al. 2018, Reichman et al. 2014, Wahlin et al. 2017, Zhong et al. 2014). The incredible ability of retinal

organoids to match the cell types and temporal progression seen in human retinal development paves the way for significant developmental mechanisms to be learned by studying them.

3. HUMAN RETINAL DEVELOPMENT AND CELL TYPES IN ORGANOIDS

The predominant cues and events that occur during mammalian retinal development have been well studied in model organisms, mainly mice and rats (Carter-Dawson & Lavail 1979, Cayouette et al. 2006, Dyer & Cepko 2001, Hinds & Hinds 1979, Norden et al. 2009). These studies have given us an in-depth understanding of the timing, molecular mechanisms, and general cell types that emerge in retinal development, but they are not a perfect model for the in utero process of human retinal development. The developmental time period of the retina in humans, beginning four weeks post-conception and ending shortly after birth, makes studying these processes in humans both ethically and technically challenging. Additionally, in vivo human retina cells are unable to be engineered, preventing any studies perturbing normal developmental cellular function. As many genes identified to be involved in retinal disease have also been implicated in retinal development (Freund et al. 1996, Koenekoop 2004, Mansergh et al. 2015, Zagozewski et al. 2014), the ability to introduce genetic variability into hPSCs and study its effect on retinal development further highlights the benefits of using retinal organoids as a model system.

3.1. Single-Cell Transcriptomic Approaches

Single-cell transcriptomic techniques have rapidly advanced in the past decade. Bulk RNA-sequencing (RNA-seq) methods are able to analyze the average gene expression of a given population of cells and have certainly provided valuable information. However, single-cell RNA-seq (scRNA-seq) allows the gene expression pattern of individual cells within a complex population to be analyzed at any given point in time and has provided unprecedented detail on the transcriptomic and molecular signatures of cell classes and subtypes, as well as the subtle gene expression changes that occur during organ development and cell fate decisions. The majority of genetic markers for labeling and identifying developing retinal cells have been extrapolated from studies in other mammals, and the repertoire of retinal marker genes has been substantially increased by scRNA-seq studies in these animals (Clark et al. 2019, Laboissonniere et al. 2019, Macosko et al. 2015, Rheaume et al. 2018, Shekhar et al. 2016). Nonetheless, the human retina is significantly different from other retinas. This has been made abundantly clear by recent and ongoing scRNA-seq experiments on the nonhuman primate retina and on fetal human retinal tissue. Peng et al. (2019) performed scRNA-seq on over 100,000 cells from macaque fovea and peripheral retina and found that interneuron subtypes were well conserved between primates and mice, but that projection neuron subtypes had substantially differential gene expression. Major differences in cellular subtypes and functions in adult retinas between mice and primates would suggest that there are likely similar differences in the developmental programs of these cell types. The scRNA-seq that has been performed on fetal human retinal tissue (Hu et al. 2019, Lu et al. 2018) has already revealed valuable information about the transcriptional programs that direct human retinal differentiation, but these studies are limited in their time points and the number of available cells.

scRNA-seq experiments using retinal organoids overcome many of these challenges. Organoids may be collected and analyzed at multiple time points over the course of differentiation, and sample quantity is only limited by the number of organoids that a researcher is willing to generate. Several scRNA-seq studies have been done to compare the transcriptome of differentiating cells in an hPSC-derived retinal organoid to data obtained from fetal and adult human

tissue to assess the degree to which in vivo genetic programs for development and cellular diversity are recapitulated in the in vitro model system. A study by Cowan et al. (2019) utilized a machine learning approach to analyze published fetal retina transcriptomic data and was able predict the developmental age of retinal organoids based on their transcriptomes. Cowan et al. found that the rate of development in in vitro organoids closely mimicked that of the in vivo retina, with the gene expression and cell types of 38-week-old organoids closely resembling the newborn human retina. Additionally, developed organoid transcriptomes showed that organoid cell type compositions were strongly correlated with the adult peripheral retina, although cells with foveal gene expression patterns were also present.

Welby et al. (2017) used scRNA-seq of human fetal L/M-opsin cones to identify 93 genes that are differentially expressed over the course of cone development and analyzed the expression of these genes in bulk transcriptomes of cones differentiated in hPSC-derived organoids. Again, gene expression and developmental rates between the two sample types were strongly correlated. For example, cone markers *OPN1LW/MW* and *ARR3* were detected at 12–14 weeks in fetal tissue and 14 weeks in organoids, and again at 19–20 weeks in fetal tissue and 21 weeks in organoids (Welby et al. 2017). Kim et al. (2019b), focused less on correlating in vitro and in vivo development and instead compared gene expression profiles of fully matured PR and Müller glia cells. Based on single-cell profiles of over 11,000 expressed genes, 8-week-old retinal organoids and adult human macula yielded Pearson's correlation coefficients between the two sample types of $r = 0.98$, 0.91, and 0.86 for rods, cones, and Müller glia cells, respectively. These studies demonstrate the ability to use organoids to model retinal development in the context of both multiple and specific cell types, and as a function of time.

Single-cell approaches for studying developing cell populations and subtypes in hPSC-derived retinal organoids have already begun to identify new human-specific cellular markers and genetic programs. Fligor et al. (2018) used single-cell quantitative real-time PCR to assay expression of 34 guidance receptor genes in retinal organoid–derived RGCs and observed significant heterogeneity in the combinations and expression levels of receptors among cells. Collin et al. (2019) and Mao et al. (2019) both collected scRNA-seq data during organoid development and used pseudotime analysis, which arranges single cells along a spectrum based on their differentiated state, to investigate the transcriptomic signatures and networks associated with developmental stages and cell types. Mao et al. (2019) traced the single-cell transcriptomes of RPCs in organoids from proliferation through cell fate determination and reported that the transcriptomes of RPCs at day 28 were significantly different than RPCs after day 28, suggesting a change in RPC competence between the developmental time points. Pseudotime analysis over this transition showed positive regulation of Notch signaling promoting retinal neurogenesis, which is contrary to its normal role in lateral inhibition during neurogenesis and maintenance of progenitors (Mills & Goldman 2017). Expression of chromatin structure modifiers *HMGA1*, *BAZ2B*, and *MECOM* was also seen to fluctuate over this period, suggesting a chromatin remodeling program associated with RPC progression and human retinal development. Collin et al. (2019) further used scRNA-seq to transcriptomically identify the cell types and their relative abundances present in organoids over time and determine the pseudotemporal developmental relationships between cell clusters, generating a dual in vitro–in silico model of human retinal development. These studies show the vast potential of combining hPSC-derived organoids and single-cell technology for dissecting human retinal development in diverse and unparalleled detail.

3.2. Direct Observation of Development and Cell Types in Retinal Organoids

The large-scale ability to generate organoids and their ease of handling makes studying their structural and functional aspects relatively accessible with a range of live imaging techniques. David

Cobrinik's group was able to repeatedly analyze the same organoids via phase contrast microscopy, optical coherence tomography (OCT), fluorescence lifetime imaging microscopy, and hyperspectral imaging to observe retinal developmental stages in real time with negligible damage to the organoids, and compare structure and metabolic function to that observed in fixed organoids by staining (Browne et al. 2017). With these imaging techniques, they were able to see development of the nuclear layers of the retinal organoids, compare glycolytic activity throughout the organoids, and analyze retinol production, establishing the retinal organoids as a viable in vitro model system for investigating metabolic changes in human retinal tissue. Capowski et al. (2019) used OCT and 3D imaging as well as light microscopy, immunocytochemistry, metabolic imaging, and electron microscopy to define three distinct developmental stages across the same organoids from 16 different hPSC lines. They showed that at stage 1, organoids had RPCs and an inner layer formed by RGCs and amacrine cells. At stage 2, they showed initial deterioration of RGC cells and differentiation of PRs, horizontal cells, and amacrine cells. Stage 3 saw the development of PR outer segments and increased organization of the outer retinal layers with increased disorganization of the inner retinal layer. External limiting membrane and PR inner segments were also imaged, adding to the mature retinal features examined. These studies highlight the promising combination of various noninvasive imaging techniques and retinal organoids for studying developmental, structural, and metabolic features in hPCS-derived retinal tissue.

Given the genome engineering accessibility of cell lines, reporter constructs have already proven to be a powerful tool for studying cell-type-specific and whole-organoid temporal, structural, and organizational aspects of human retinal organoid development. Genomically engineered fluorescent constructs driven by promoters for pan–cell type markers allow for real-time observation of expression of markers of interest in differentiating cultures. One of the earliest groups to use reporter cell lines in differentiating human retinal organoids was Völkner et al. (2016). Their work used two *PAX6*-green fluorescence protein (GFP) reporter cell lines and observed GFP expression in neuroepithelium at day 41 of differentiation. As *PAX6* expression is involved in eye-field specification and retinal cell fate determination (Remez et al. 2017), the reporter construct was able to identify and track cells as they became multipotent retinal progenitors during the differentiation process. Fligor et al. (2018) used either an mCherry or tdTomato reporter driven by the endogenous *BRN3b* promoter (Sluch et al. 2015, 2017), an RGC-enriched gene that is expressed in most known RGC subtypes (Badea et al. 2009). This allowed them to observe RGCs developing and self-organizing in the inner layer of optic vesicles between 30 and 70 days of differentiation. Additionally, they were able to dissociate organoids and re-plate them in 2D culture and use the fluorescent reporters to study neurite outgrowth of the RGCs that were developed in a spatial and temporal method more reminiscent of in vivo conditions. To study PR cells, Gagliardi et al. (2018) created a reporter hPSC line for cone rod homeobox (*CRX*), with the *CRX* promoter sequence driving mCherry expression. Using this cell line, they generated and identified PR cells using a retinal organoid system and were able to characterize a cell surface antigen specific to *CRX*-positive cells. This enabled them to immunopurify the PR cells and assess the potential of mature purified cells for use in future PR transplantation studies. Additionally, Lam et al. (2019) created a triple reporter line, labeling multiple different cell types through retinal organoid development and allowing the study of their organization and interaction as they relate to each other in real time. In their reporter line, Cerulean, driven by the *VSX2* endogenous promoter, marks neural RPCs and differentiated bipolar cells, while *BRN3b*-eGFP and *RCVRN*-mCherry label RGCs and PRs, respectively. As single-cell studies continue to identify sub-type-specific genetic signatures in cells, more intricate reporter cell lines may be created for studying human retinal development in organoids.

Many aspects of retinal development that have been observed in other vertebrates have been hypothesized to hold true for human retinal development as well, but in fact only a small number of such findings have been experimentally tested with human tissue. For example, studies of mouse retinal development have shown that thyroid hormone receptor Thrβ2 plays a major role in determining the fate of S- versus M-cones, but the function of *Thrβ2* in human cone subtype specification was not known. Taking advantage of organoid technology, Eldred et al. (2018) demonstrated that the role of thyroid hormone signaling in the human retina is both similar to and distinct from that in the mouse. While murine *Thrβ2* knockout results in a loss of M-cone differentiation (Ng et al. 2001, Shibusawa et al. 2003), surprisingly, human retinal organoids from hPSCs that were null for *Thrβ2* had no significant difference in their S-to-L/M-cone ratio. Instead, Eldred et al. found that L/M-opsin expression was only lost if both isoforms of *Thrβ* (*Thrβ1* and *Thrβ2*) were knocked out, and they proceeded to unravel a human-specific thyroid hormone signaling pathway for regulating S- and L/M-cone identity during retinal development. The ability of organoids to recapitulate well-conserved aspects of vertebrate retinal development while also distinguishing human-specific mechanisms highlights their ability to address questions that previous animal research has left unanswered.

4. MODELING RETINAL DEGENERATION IN ORGANOIDS

Retinal organoids also present a powerful system for the study of human retinal disease. The retinal degenerations are a heterogeneous group of conditions that range from the Mendelian inherited orphan diseases, such as retinitis pigmentosa (RP), to the genetically complex disease age-related macular degeneration (AMD). RD is caused by irreversible dysfunction or death of retinal neurons and/or RPE cells, leading to vision loss and often blindness. While huge efforts have been put into RD research, besides the development of the highly effective anti-angiogenesis treatments for neovascular (wet) AMD (Al-Zamil & Yassin 2017) and the development of a gene therapy–based treatment for a form of the orphan disease Leber congenital amaurosis (LCA) (Russell et al. 2017), effective treatment of other RDs is limited. The use of retinal organoid technology will now not only allow researchers the ability to study the effects of genetic and environmental factors on the health of human retinal cells, but also to test potential protective measures against these damaging factors.

4.1. Mendelian Retinal Disease

Over 300 different genes and genetic loci have been associated with human retinal disease (Daiger et al. 1998), ranging from Mendelian inherited orphan diseases to genetically complex diseases attributed to multiple loci. Many of these disease-causing genes have been implicated in retinal development (Freund et al. 1996, Koenekoop 2004, Mansergh et al. 2015, Zagozewski et al. 2014), and as such, clinical symptoms present themselves as early onset during childhood or even at birth. Some of the most common monogenic and developmentally relevant retinal diseases are LCA, RP, and cone-rod dystrophy (CRD), which typically are caused by abnormal development or function of PR cells in the retina. LCA causes severe visual impairment at birth that may continue to deteriorate over time, with sensitivity to light and extreme hyperopia as additional visual defects. LCA is one of the most common causes of blindness in children, with an incidence of approximately 3 in every 100,000 infants. 14 different genes are known to cause LCA, but recessive mutations in the *GUCY2D*, *CEP290*, *CRB1*, or *RPE65* genes are most common. Mutations that affect PR development, phototransduction, or ciliary function have all been identified in LCA patients. RP patients experience a more gradual deterioration of PR cells, often beginning with night vision loss in childhood. As RP progresses, peripheral vision is lost, with central vision being affected

in adulthood. RP is a relatively common inherited retinal disease, affecting up to 1 in 3,500 people in the United States. Over 60 RP-causing genes have been identified, with one-third of those genes having an autosomal dominant effect. Mutations in the human *RHO* gene, encoding for the G-protein-coupled receptor rhodopsin normally expressed in rods and responsible for visual perception in low-light conditions, account for approximately one-quarter of autosomal dominant RP cases. In autosomal recessive inherited RP, mutations in genes responsible for phototransduction in rods, such as *PDE6A*, *PDE6B*, and *PDE6G*, are often responsible for the disease. CRD has a similar incidence rate to RP and is symptomatically similar except for the fact that deterioration of cones is seen before deterioration of rods. A decrease in visual acuity is seen in childhood, often accompanied by light sensitivity. Color vision and central vision are lost first, followed by peripheral vision loss and night blindness. Mutations in over 30 genes have been implicated in CRD, with the majority of them being inherited in an autosomal dominant manner. Mutations in *ABCA4* are the most common cause of autosomal recessive CRD, as PRs are unable to remove damaging byproducts of phototransduction. Autosomal dominant CRD is most often caused by mutations in *GUCY2D*, whose gene product is responsible for returning PRs to an unexcited state after light response, and *CRX*, whose expression is crucial for PR differentiation and maintenance. Many less common heritable retinopathies have been identified in patients as well, and retinal organoids provide a powerfully accessible and developmentally relevant model for exploring the molecular mechanisms behind these disease-causing mutations.

hPSC-derived retinal organoids provide two major advantages in studying heritable retinal diseases. First, stem cell lines may be genetically modified so that any desired genetic mutation can be introduced or corrected in cells. Since CRISPR/Cas9 was first discovered and then engineered into an affordable and accessible method for targeted genome editing (Cong et al. 2013, Jinek et al. 2012), researchers have been able to introduce mutations into stem cell line genomes with unprecedented efficiency (Wang et al. 2016). Cell lines engineered with retinal disease–associated mutations can then be differentiated into retinal organoids, and the effects that the mutation may have on human retinal cells and their development investigated. Even given the large number of genes implicated in heritable retinopathies, this technology makes possible a system to compare the mechanisms of various mutations in an isogenic background. The ease of genome editing also encourages the generation of model systems for orphan heritable retinal diseases that affect a limited number of people, as the time and expense to create a mouse model can be restrictive.

The ability to create retinal organoids from patient-derived iPSCs also allows scientists and clinicians to study the effects of retinal disease–associated mutations, but in the context of a patient's specific genomic landscape. These analyses would assess if and how the disease-implicated mutation(s) may interact with other modifying loci. Studying disease mechanisms in retinal organoids generated from cell lines created by these varying approaches (introducing a disease-associated mutation into a well characterized stem or iPSC line, using patient-derived iPSC lines, and repairing the disease-associated mutation in patient-derived iPSC lines) may help parse out disease-specific versus patient-specific clinical manifestations and improve personalized treatments in a clinical setting.

One of the earliest studies that used patient-derived cells to generate retinal organoids to study retinal disease was done by Tucker et al. (2013). Using keratinocytes from an RP patient with *USH2A* mutations, they generated an iPSC line with which they differentiated retinal organoids. *USH2A* codes for the protein Usherin, which is found in the basement membrane of the retina but has a largely unidentified function. *USH2A* mutations are the most common cause of autosomal recessive RP. *USH2A* knockout mice present mild and late-onset RD, which does not accurately model the phenotype seen in RP patients (Slijkerman et al. 2015), necessitating a better model system for studying *USH2A*'s role in maintaining retinal integrity. Exome sequencing for this

particular patient revealed over 400 mutations that were potentially disease causing. The patient had a known disease-causing coding variant in one copy of *USH2A*, but the mutation on the second allele could not be identified. iPSCs from the proband were differentiated into optic vesicles, and analysis of *USH2A* expression showed a mis-splicing event in the PRs. PRs from the *USH2A* cell–derived organoids also had a significant increase in the protein levels of two chaperone proteins, GRP78 and GRP94, compared to normal human retina cells and the PRs in organoids derived from normal control human iPSCs, suggesting an increased level of protein misfolding and ER stress in these cells. This study highlighted the use of patient-derived iPSCs and retinal organoid differentiation in associating disease phenotypes with patient-specific candidate disease mutations and illustrating phenotype–genotype relationships.

Using patient-derived iPSCs to create organoids for modeling retinal disease has been particularly useful for studying the effects of inherited retinal ciliopathies. The structure and function of the PR outer and inner segments, also known as the PR primary cilia, are critical for initiating phototransduction in the retina. Defects in genes encoding cilia-associated proteins, including proteins involved in ciliogenesis, have been implicated in many heritable retinal diseases, including LCA, RP, Bardet Biedl syndrome, and Senior Loken syndrome (Chen et al. 2019). PR ciliopathy has proven difficult to model in mice; affected cell types in the mouse models are often different than those affected in patients, as are the rates of RD (Slijkerman et al. 2015, Chen et al. 2019). Parfitt et al.'s (2016) group was one of the initial groups to use retinal organoids from iPSCs derived from an LCA patient's fibroblasts to study PR cilia. The patient line contained a homozygous mutation that causes mis-splicing of *CEP290*, a gene that encodes a protein crucial for primary ciliogenesis and that is the gene mutated in 20% of LCA patients. The patient-derived iPSCs were differentiated into retinal organoids, and compared to organoids from control patients, displayed defects in PR cilia. While, in the control PRs, CEP290 protein is localized to the base of connecting cilia, it was undetectable in the PRs of organoids generated from cells containing the *CEP290* mutation, and the absence of CEP290 correlated with a significant decrease in cilia number and cilia length. A *CEP290* mRNA isoform specific to differentiating PR cells was shown to be incorrectly spliced due to the patient's mutation, and an antisense oligo that blocked the *CEP290* splice variant, which included the aberrant exon, restored normal number and length of cilia when it was introduced to the organoids at week 13, a point in differentiation when ciliation was reduced and PRs still premature. This foundational study was the first of several (Megaw et al. 2017, Quinn et al. 2019) to highlight the great potential of hPSC-derived retinal organoids in monitoring both the progression and treatment of genetically based, tissue-specific RD.

One example of using retinal organoids to study the effect of a specific mutation is the work of Deng et al. (2018), who compared organoids generated from patient iPSCs with a disease-causing mutation with those generated from a line in which the mutation was corrected. iPSCs from three different RP patients, each with unique frameshift mutations in the *RPGR* gene, were differentiated into organoids. Organoids generated from each of these lines had altered PR morphology and localization in the retina, as well as deficiencies in PR cilium length, rhodopsin protein transfer, and electrophysiology (i.e., membrane potential and resistance). Using CRISPR/Cas9 genome editing, the *RPGR* mutation was corrected in one of the patient's iPSCs, and when this line was differentiated into organoids, essentially all of the physical and functional PR defects were rectified, suggesting that the *RPGR* correction rescued the disease phenotype. Buskin et al. (2018) also used CRISPR/Cas9 to correct a mutation in an RP patient-derived iPSC line in which the pre-mRNA processing factor *PRPF31* was mutated. When these edited cells were differentiated into organoids, splicing deficiencies and cilia-based deficiencies were alleviated, and the PRs developed normally and appeared to be normal. These studies offer a promising beginning to a larger effort in associating genotypes and phenotypes in retinal disease patients.

4.2. Complex and Late-Onset Retinal Disease

Studying genetically complex and later-onset retinal diseases, such as AMD and glaucoma, using in vitro systems is a challenge. Complex diseases involve a combination of genetic components potentially affecting the vulnerability of the patient and environmental factors that contribute to the disease. How to model individual susceptibility is very unclear. The role of the mutations that affect susceptibility is often unknown, making it difficult to determine what to assess in the model. One approach would be to subject organoids generated from patients with high risk–associated alleles to disease-associated stresses and determine whether the relevant cell types are more vulnerable to injury or death under certain conditions. If this was done in a high-throughput manner, it might be possible to generate a profile of paired susceptible genetic and environmental factors. Together with disease modeling and drug discovery strategies, this profile could be used as a predictor of potentially promising prophylactic strategies. While there are challenges with actually modeling these diseases, retinal organoids may still be a promising tool for anticipating patient risk and severity, as well as studying potential therapies. For example, Teotia et al. (2017) used patient-derived iPSCs with a *SIX6* risk allele to differentiate RGCs and found that the mutant cells were less efficient at generating RGCs and tended to generate more immature cells. Similarly, organoids can be used to model retinas from later-onset disease patients to determine if identifiable deficiencies or biomarkers (Daga et al. 2018, Nath et al. 2017) are present that may be used to identify future patients before their disease has actually manifested.

Modeling late-onset disease is also an unmet challenge. For these diseases, age is the major risk factor, and since differentiated organoids mimic the human timeframe, it is not feasible to model the natural time course of the disease in the lab. Organoids generated from iPSCs obtained from patients with late-onset disease could be used similarly to those described for the complex disease to determine whether there are increased susceptibility and/or biomarkers that are associated with these organoids compared to those generated from control patients (Daga et al. 2018, Nath et al. 2017). It may also be possible to accelerate the aging process in vitro. One approach that has been tried is to introduce the age-inducing protein progerin, a truncated version of the lamin A protein that has been shown to be involved in the pathology of Hutchinson–Gilford progeria syndrome (Ashapkin et al. 2019). Introduction of this protein into an iPSC line created from a Parkinson's patient induced hallmarks of Parkinson's, as well as age-related phenotypes (Miller et al. 2013). Another approach would be direct conversion of cells from an elderly donor. It has been shown that cells created from direct conversion retain the cellular hallmarks of age, while, when generating iPSCs, cells not only obtain pluripotency but also are rejuvenated, erasing age-associated factors (Frobel et al. 2014, Lapasset et al. 2011, Le et al. 2014). Since the most common retinal degenerative diseases are associated with aging, this is an important challenge worthy of attention.

5. FURTHER CONSIDERATIONS AND FUTURE PROSPECTS FOR RETINAL ORGANOIDS

5.1. Functional Screens

Drug development over the past several decades has focused on screens, usually in vitro, directed at identifying small molecules that inhibit or modulate the activity of a particular molecular target that has been shown by previous studies to be involved in the pathogenesis of a disease of interest. Another approach is to perform phenotypic, usually cell-based, screens to look for molecules that induce a desired phenotype, such as preservation of cell function and survival when cells are exposed to a disease-relevant stress, without necessarily having a priori knowledge of the underlying

biology. Although target-based screens are more common, of the 50 first-in-class small molecules discovered between 1998 and 2008 that resulted in US Food and Drug Administration approval, the majority were discovered using phenotypic approaches (Swinney & Anthony 2011).

If a robust, consistent, measurable phenotype can be discerned in modeling heritable diseases such as the examples described above, then it could be exploited as the basis for a drug discovery program. For example, if an assay could be developed to quantifiably measure the relative protein levels of GRP78 and GRP94 in PRs of organoids developed from patient lines containing the *USH2A* mutations discussed above, then it could be used to screen for small molecules, or genetic perturbations, that could restore expression of these proteins to wild-type levels. Such assays would require a reliable screening platform, and although there have been proof-of-principle and optimization studies published (Boretto et al. 2019, Kim et al. 2019a, Phan et al. 2019, Weeber et al. 2017), successful use of organoids in drug discovery screens has yet to be reported.

In addition to phenotypic outcomes due to a specific genetic variant, disease modeling can be built upon phenotypic outcomes of treating cells with disease-associated environmental stressors. Given that RDs have potentially hundreds of targets of interest, phenotypic screening for molecules that can help generally preserve retinal cell viability or function in the presence of stressors has great promise and could complement other already ongoing approaches (Sharma et al. 2017). The retina is exposed to several stresses that have been implicated in disease (Barrett 2005, Organisciak & Vaughan 2010, van Norren & Vos 2016), including light-induced damage, oxidative stress, and inherited mutations that can lead to protein misfolding. These stresses can be simulated in the lab, including via treatment with pharmacological agents that induce the oxidative or endoplasmic reticulum (ER) stress response [i.e., cigarette smoke extract, tunicamycin (an inhibitor of glycosylation), thapsigargin (an agent that promotes ER stress by depletion of lumenal calcium stores), and brefeldin A (a toxin that inhibits translocation of proteins from the ER to the Golgi)]. Perhaps the biggest current challenge to the use of organoids in large-scale screening platforms is the development of methodology to generate consistent organoids in large numbers.

5.2. Personalized Medicine

In addition to increasing our understanding of disease etiology on a patient-specific or mutation-specific level, organoids developed from patient-derived or engineered iPSC lines can help discriminate potential therapeutic strategies that work most efficiently. Just as patients' genetic backgrounds may affect the way disease manifests, these backgrounds may also affect the way that cells respond to a particular intervention; thus, retinal organoids could be used to identify the most productive treatment. For example, if we can use retinal organoids to create a profile for the panel of interventions and the phenotype that they are most efficient at correcting, then the phenotype of a patient-derived organoid can potentially be used for choosing the best strategy. The patient-specific genomes may provide broad information on the general pathways that are being affected by the disease and that may be targetable by treatment, and they may also provide information about a specific mutation. Specific interventions for a disease-causing mutation can be tested using the retinal organoid, for example, as in the case discussed above where a patient's disease is caused by a mutation in *CEP290*, which results in a splice variant that can be corrected with antisense oligos that are complimentary to the mutated DNA (Parfitt et al. 2016). These therapies may be directly tested in organoids with a patient's specific mutation and in the context of a patient's overall genetic background, determining efficacy at different stages of maturation and identifying unintended cellular side effects, before the therapy is actually administered to the patient. Finally, the possibility also exists to use patient-derived iPSCs in which the disease-causing mutation is corrected and generate transplantable retinal cells. The field of precision medicine promises to

decrease costs of clinical trials by identifying specific subsets of patients for whom a drug may be more efficacious, and organoids are a straightforward platform by which to screen and select those patients.

5.3. Cell Transplantation Studies

Retinal organoids have already begun to show promise as sources of patient-derived cells to be used for transplantation (Gagliardi et al. 2018, Lakowski et al. 2018). Organoids provide the added benefit of differentiating cells at a developmentally accurate pace, so that cells at various stages of precursor or mature identity may be isolated for transplantation. Researchers have published successful attempts at transplanting cells from hPSC-derived retinal organoids into multiple retinal degenerate mammalian models (Gagliardi et al. 2018, Gonzalez-Cordero et al. 2017, McLelland et al. 2018, Shao et al. 2017, Singh et al. 2019) and have measured some restoration of vision post-transplantation, as measured by electrophysiology and tracking eye movements (McLelland et al. 2018, Shao et al. 2017).

The combination of retinal organoids and genome engineering promises the ability to generate substantial amounts of rarer cell types for transplantation. The more that is learned about the gene expression changes that occur during retinal development, the more powerful the combination becomes. An example in which the expression of one gene seems to control the bifurcation of a progenitor cell is the case of bipolar cells, which are present in retinal organoids, but not as a large percentage, nor is there a published protocol for differentiating them in a 2D system. Expression of the transcription factor gene *PRDM1* has been shown to determine the bifurcation of progenitor cells into bipolar cells or PRs (Brzezinski et al. 2010, Katoh et al. 2010, Wang et al. 2014), where cells expressing the gene become PRs, while cells without its expression become bipolar cells. Potentially, knocking out *PRDM1* in hPSCs and differentiating those cells into organoids may yield retinal organoids with a major enrichment of bipolar cells, which could then be used for transplantation after validating that they are true bipolar cells.

5.4. Cell-Type Transdifferentiation

Transdifferentiation refers to a process in which one fully differentiated cell type transforms into another cell type. Transdifferentiation could potentially be used as a therapeutic strategy in which a subset of a certain retinal cell type can be induced to differentiate into a different cell type, potentially replenishing a depleted cell population. As all retinal cells share a close common cellular ancestor, the retina may be a promising organ in which to test transdifferentiation. In fact, non-mammalian vertebrates are capable of employing terminally differentiated cells for retinal regeneration (Bernardos et al. 2007, Fausett et al. 2008, Islam et al. 2014). Suggesting possible applicability to humans, a study by Pollak et al. (2013) demonstrated the ability to induce an RPC state in mouse cells by artificially overexpressing ASCL1 in Müller glia, and a study by Jorstad et al. (2017) showed that addition of a histone deacetylase inhibitor could lead to generation of new neurons in vivo. Human organoids may provide a suitable system to explore retinal transdifferentiation in human cells. Giannelli et al. (2011) isolated human Müller glia cells and were able to induce them into a PR cell state as determined by marker gene expression. The reprogrammed cells were even transplanted into mice retinas where they integrated into the outer nuclear layer, although no outer segments were identified. Advances in single-cell transcriptomics and lineage tracing provide a means for identifying the gene expression changes associated with cell fate decisions in retinal organoids. As scientists discover more of the specific genetic and temporal programs that are initiated in the differentiation of specific cell types and subtypes during retinal development,

those programs can be manipulated in mature cells to induce lineage reprogramming. As an example, PRDM1, which, as described above, is a regulatory factor that determines PR versus bipolar cell lineage, could potentially be modulated to induce the transdifferentiation of mature bipolar cells into photoreceptor cells. The National Eye Institute has established an Audacious Goals Initiative (AGI) to "demonstrate by 2025 the restoration of usable vision in humans through the regeneration of neurons and neural connections in the eye and visual system" (Gamm & Wong 2015). One of the focuses of the AGI is on transdifferentiation studies, particularly to replenish damaged PR populations, and it is likely that multiple groups will be using retinal organoids to investigate this approach in the coming years.

5.5. Caveats and Conclusions

Several caveats of organoid-based modeling of retinogenesis and retinal disease have been discussed. First, the inability of organoids to generate RPE that is in physical contact with PR outer segments does raise questions as to how accurate of a model system the organoids can serve as for studying PR function and the visual cycle; however, several groups, as mentioned above, are developing ways to culture RPE cells alongside retinal organoids to better mimic the in vivo cellular conditions. Second, it has been shown that the early-born cell populations located in the internal parts of organoids do suffer from poor oxygenation and nutrient accessibility, and deteriorate as a result. This again raises questions as to how the phototransduction pathway can be accurately modeled if the cell type at the receiving end of the process has been diminished. Organ-on-a-chip technology has become a fairly new field for modeling organ function and aims to culture cells on a microchip with perfectly controllable and tunable permeability, allowing precise control of the timing, location, and quantity of small molecule and factor delivery (Achberger et al. 2019, Radisic 2019, Wnorowski et al. 2019). Along with bioreactor technologies, several groups are innovating new ways to control the nutrient flow to and oxygenation of particular regions of cultured cells. Third, there is no discernable macula or fovea in current retinal organoids, which are regions of particular interest in studying PR degeneration. As discussed, cells with foveal gene expression patterns have been identified in organoids, suggesting that there may be potential to generate these regions in organoids, and several efforts to achieve this important goal are underway. Fourth, we discuss above the challenges in using organoids to model later-onset retinal diseases, which are not necessarily reflected in developmental phenotypes. However, with chemical and genetic modifications, we may be able to model aging in patient-derived cells and study the molecular mechanisms of these diseases in cells that are more reflective of a patient's condition.

Additional caveats that need to be considered when discussing retinal organoids are primarily focused on the difficulties in recreating the retina's surrounding conditions and influences in an in vitro system. Retinal organoids do not have vasculature, whereas in vivo, the retinal vasculature is crucial for transporting and consuming oxygen and nutrients and ensuring that the metabolic requirements for visual function are met. Again, with organ-on-a-chip technology, this limitation of retinal organoids is beginning to be addressed, but roles that the retinal vasculature plays in development and disease may prove difficult to identify and may confound observations seen in retinal organoids. Furthermore, the retinal organoid system is not subject to the external immune or hormonal signaling that in vivo retinas are. Immune cells that are normally integral to retinal function, such as microglia, are not present in organoid systems. While sex-related differences that are genetically based may be studied in retinal organoids, those that are based in hormone signaling cannot be as easily addressed. The first multi-organoid culture system was published earlier this year, showing the ability to model liver, pancreas, and biliary duct interactions in vitro (Koike et al. 2019), which promises the development of similar multi-organoid systems, possibly retinal ones,

in the near future that could overcome some of the challenges of modeling external environments and providing interactions between distinct organoids that are part of a larger system. However, as organoids become more complex, an additional and important challenge, although one that is unfortunately beyond the scope of this review, will be to more fully consider the wide array of bioethical issues related to the generation of, study of, and potential clinical use of human organoids (Bredenoord et al. 2017, Munsie et al. 2017, Sugarman & Bredenoord 2019).

Caveats aside, it is clear that the development of hPSC-derived retinal organoid technology has begun to revolutionize the field of retinal research and discovery, both biologically and clinically. Retinal organoids will enable us to study the impact that genetics, environments, and drugs have on human retinal cells at a level that was previously unattainable, and these findings will continue to broaden in their scope and impact as additions to retinal organoid technology evolve. The era of retinal organoid research has only just started and promises to bring us ever closer to understanding, intervening in, and preventing RD and vision loss in humans worldwide.

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LITERATURE CITED

Achberger K, Probst C, Haderspeck J, Bolz S, Rogal J, et al. 2019. Merging organoid and organ-on-a-chip technology to generate complex multi-layer tissue models in a human retina-on-a-chip platform. *eLife* 8:e46188

Adler R, Canto-Soler MV. 2007. Molecular mechanisms of optic vesicle development: complexities, ambiguities and controversies. *Dev. Biol.* 305(1):1–13

Akhtar T, Xie H, Khan MI, Zhao H, Bao J, et al. 2019. Accelerated photoreceptor differentiation of hiPSC-derived retinal organoids by contact co-culture with retinal pigment epithelium. *Stem Cell Res.* 39:101491

Altshuler D, Turco JJL, Rush J, Cepko C. 1993. Taurine promotes the differentiation of a vertebrate retinal cell type in vitro. *Development* 119(4):1317–28

Al-Zamil WM, Yassin SA. 2017. Recent developments in age-related macular degeneration: a review. *Clin. Interv. Aging* 12:1313–30

Ashapkin VV, Kutueva LI, Kurchashova SY, Kireev II. 2019. Are there common mechanisms between the Hutchinson-Gilford progeria syndrome and natural aging? *Front. Genet.* 10:455

Badea TC, Cahill H, Ecker J, Hattar S, Nathans J. 2009. Distinct roles of transcription factors Brn3a and Brn3b in controlling the development, morphology, and function of retinal ganglion cells. *Neuron* 61(6):852–64

Barrett JR. 2005. Focusing on vision through an environmental lens. *Environ. Health Perspect.* 113(12):A822–27

Bernardos RL, Barthel LK, Meyers JR, Raymond PA. 2007. Late-stage neuronal progenitors in the retina are radial Müller glia that function as retinal stem cells. *J. Neurosci.* 27(26):7028–40

Boretto M, Maenhoudt N, Luo X, Hennes A, Boeckx B, et al. 2019. Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. *Nat. Cell Biol.* 21(8):1041–51

Boucherie C, Mukherjee S, Henckaerts E, Thrasher AJ, Sowden JC, Ali RR. 2012. Brief report: Self-organizing neuroepithelium from human pluripotent stem cells facilitates derivation of photoreceptors. *Stem Cells* 31(2):408–14

Braunger BM, Pielmeier S, Demmer C, Landstorfer V, Kawall D, et al. 2013. TGF-β signaling protects retinal neurons from programmed cell death during the development of the mammalian eye. *J. Neurosci.* 33(35):14246–58

Bredenoord AL, Clevers H, Knoblich JA. 2017. Human tissues in a dish: the research and ethical implications of organoid technology. *Science* 355(6322):eaaf9414

Browne AW, Arnesano C, Harutyunyan N, Khuu T, Martinez JC, et al. 2017. Structural and functional characterization of human stem-cell-derived retinal organoids by live imaging. *Investig. Ophthalmol. Vis. Sci.* 58(9):3311–18

Brzezinski JA, Lamba DA, Reh TA. 2010. Blimp1 controls photoreceptor versus bipolar cell fate choice during retinal development. *Development* 137(4):619–29

Buskin A, Zhu L, Chichagova V, Basu B, Mozaffari-Jovin S, et al. 2018. Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. *Nat. Commun.* 9:4234

Capowski EE, Samimi K, Mayerl SJ, Phillips MJ, Pinilla I, et al. 2019. Reproducibility and staging of 3D human retinal organoids across multiple pluripotent stem cell lines. *Development* 146(1):dev171686

Carter-Dawson LD, Lavail MM. 1979. Rods and cones in the mouse retina. II. Autoradiographic analysis of cell generation using tritiated thymidine. *J. Comp. Neurol.* 188(2):263–72

Cayouette M, Poggi L, Harris WA. 2006. Lineage in the vertebrate retina. *Trends Neurosci.* 29(10):563–70

Chen HY, Welby E, Li T, Swaroop A. 2019. Retinal disease in ciliopathies: recent advances with a focus on stem cell-based therapies. *Transl. Sci. Rare Dis.* 4(1–2):97–115

Chichagova V, Dorgau B, Felemban M, Georgiou M, Armstrong L, Lako M. 2019. Differentiation of retinal organoids from human pluripotent stem cells. *Curr. Protoc. Stem Cell Biol.* 50(1):e95

Clark BS, Stein-O'Brien GL, Shiau F, Cannon GH, Davis-Marcisak E, et al. 2019. Single-cell RNA-seq analysis of retinal development identifies NFI factors as regulating mitotic exit and late-born cell specification. *Neuron* 102(6):1111–26.e5

Clevers H. 2016. Modeling development and disease with organoids. *Cell* 165(7):1586–97

Collin J, Queen R, Zerti D, Dorgau B, Hussain R, et al. 2019. Deconstructing retinal organoids: single cell RNA-seq reveals the cellular components of human pluripotent stem cell-derived retina. *Stem Cells* 37(5):593–98

Cong L, Ran FA, Cox D, Lin S, Barretto R, et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339(6121):819–23

Cowan CS, Renner M, Gross-Scherf B, Goldblum D, Munz M, et al. 2019. Cell types of the human retina and its organoids at single-cell resolution: developmental convergence, transcriptomic identity, and disease map. bioRxiv 703348. **https://doi.org/10.1101/703348**

Daga FB, Ogata NG, Medeiros FA, Moran R, Morris J, et al. 2018. Macular pigment and visual function in patients with glaucoma: the San Diego macular pigment study. *Investig. Ophthalmol. Vis. Sci.* 59(11):4471–76

Daiger SP, Rossiter BJF, Greenberg J, Christoffels A, Hide W. 1998. Data services and software for identifying genes and mutations causing retinal degeneration. *Investig. Ophthalmol. Vis. Sci.* 39:S295

Demb JB, Singer JH. 2015. Functional circuitry of the retina. *Annu. Rev. Vis. Sci.* 1:263–89

Deng WL, Gao ML, Lei XL, Lv JN, Zhao H, et al. 2018. Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. *Stem Cell Rep.* 10(4):1267–81

DiStefano T, Chen HY, Panebianco C, Kaya KD, Brooks MJ, et al. 2018. Accelerated and improved differentiation of retinal organoids from pluripotent stem cells in rotating-wall vessel bioreactors. *Stem Cell Rep.* 10(1):300–13

Dyer MA, Cepko CL. 2001. Regulating proliferation during retinal development. *Nat. Rev. Neurosci.* 2(5):333–42

Eldred KC, Hadyniak SE, Hussey KA, Brenerman B, Zhang P-W, et al. 2018. Thyroid hormone signaling specifies cone subtypes in human retinal organoids. *Science* 362(6411):eaau6348

Fausett BV, Gumerson JD, Goldman D. 2008. The proneural basic helix-loop-helix gene Ascl1a is required for retina regeneration. *J. Neurosci.* 28(5):1109–17

Fligor CM, Langer KB, Sridhar A, Ren Y, Shields PK, et al. 2018. Three-dimensional retinal organoids facilitate the investigation of retinal ganglion cell development, organization and neurite outgrowth from human pluripotent stem cells. *Sci. Rep.* 8(1):14520

Freund C, Horsford DJ, McInnes RR. 1996. Transcription factor genes and the developing eye: a genetic perspective. *Hum. Mol. Genet.* 5(Suppl. 1):1471–88

Frobel J, Hemeda H, Lenz M, Abagnale G, Joussen S, et al. 2014. Epigenetic rejuvenation of mesenchymal stromal cells derived from induced pluripotent stem cells. *Stem Cell Rep.* 3(3):414–22

Gagliardi G, Ben M'Barek K, Chaffiol A, Slembrouck-Brec A, Conart J-B, et al. 2018. Characterization and transplantation of CD73-positive photoreceptors isolated from human iPSC-derived retinal organoids. *Stem Cell Rep.* 11(3):665–80

Gamm DM, Wong R. 2015. Report on the National Eye Institute Audacious Goals Initiative: photoreceptor regeneration and integration workshop. *Trans. Vis. Sci. Tech.* 4(6):2

Gao L, Chen X, Zeng Y, Li Q, Zou T, et al. 2016. Intermittent high oxygen influences the formation of neural retinal tissue from human embryonic stem cells. *Sci. Rep.* 6:29944

Giannelli SG, Demontis GC, Pertile G, Rama P, Broccoli V. 2011. Adult human Müller glia cells are a highly efficient source of rod photoreceptors. *Stem Cells* 29(2):344–56

Gonzalez-Cordero A, Kruczek K, Naeem A, Fernando M, Kloc M, et al. 2017. Recapitulation of human retinal development from human pluripotent stem cells generates transplantable populations of cone photoreceptors. *Stem Cell Rep.* 9(3):820–37

Hendrickson A. 2016. Development of retinal layers in prenatal human retina. *Am. J. Ophthalmol.* 161:29–35.e1

Hinds JW, Hinds PL. 1979. Differentiation of photoreceptors and horizontal cells in the embryonic mouse retina: an electron microscopic, serial section analysis. *J. Comp. Neurol.* 187(3):495–511

Hochmann S, Kaslin J, Hans S, Weber A, Machate A, et al. 2012. Fgf signaling is required for photoreceptor maintenance in the adult zebrafish retina. *PLOS ONE* 7(1):e30365

Hoshino A, Ratnapriya R, Brooks MJ, Chaitankar V, Wilken MS, et al. 2017. Molecular anatomy of the developing human retina. *Dev. Cell.* 43(6):763–79.e4

Hu Y, Wang X, Hu B, Mao Y, Chen Y, et al. 2019. Dissecting the transcriptome landscape of the human fetal neural retina and retinal pigment epithelium by single-cell RNA-seq analysis. *PLOS Biol.* 17(7):e3000365

Islam MR, Nakamura K, Casco-Robles MM, Kunahong A, Inami W, et al. 2014. The newt reprograms mature RPE cells into a unique multipotent state for retinal regeneration. *Sci. Rep.* 4:6043

Jadhav AP, Mason HA, Cepko CL. 2006. Notch 1 inhibits photoreceptor production in the developing mammalian retina. *Development* 133(5):913–23

Jensen AM, Wallace VA. 1997. Expression of Sonic hedgehog and its putative role as a precursor cell mitogen in the developing mouse retina. *Development* 124(2):363–71

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337(6096):816–21

Jorstad NL, Wilken MS, Grimes WN, Wohl SG, VandenBosch LS, et al. 2017. Stimulation of functional neuronal regeneration from Müller glia in adult mice. *Nature* 548(7665):103–7

Katoh K, Omori Y, Onishi A, Sato S, Kondo M, Furukawa T. 2010. Blimp1 suppresses Chx10 expression in differentiating retinal photoreceptor precursors to ensure proper photoreceptor development. *J. Neurosci.* 30(19):6515–26

Kedzierski W, Bok D, Travis GH. 1998. Non-cell-autonomous photoreceptor degeneration in rds mutant mice mosaic for expression of a rescue transgene. *J. Neurosci.* 18(11):4076–82

Keller G. 2005. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev.* 19(10):1129–55

Kim M, Mun H, Sung CO, Cho EJ, Jeon H-J, et al. 2019a. Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. *Nat. Commun.* 10(1):3991

Kim S, Lowe A, Dharmat R, Lee S, Owen LA, et al. 2019b. Generation, transcriptome profiling, and functional validation of cone-rich human retinal organoids. *PNAS* 116(22):10824–33

Koenekoop RK. 2004. An overview of leber congenital amaurosis: a model to understand human retinal development. *Surv. Ophthalmol.* 49(4):379–98

Koike H, Iwasawa K, Ouchi R, Maezawa M, Giesbrecht K, et al. 2019. Modelling human hepato-biliary-pancreatic organogenesis from the foregut-midgut boundary. *Nature* 574(7776):112–16

Kuwahara A, Ozone C, Nakano T, Saito K, Eiraku M, Sasai Y. 2015. Generation of a ciliary margin-like stem cell niche from self-organizing human retinal tissue. *Nat. Commun.* 6:6286

Laboissonniere LA, Goetz JJ, Martin GM, Bi R, Lund TJS, et al. 2019. Molecular signatures of retinal ganglion cells revealed through single cell profiling. *Sci. Rep.* 9(1):15778

Lake N. 1994. Taurine and GABA in the rat retina during postnatal development. *Vis. Neurosci.* 11(2):253–60

Lakowski J, Welby E, Budinger D, Di Marco F, Di Foggia V, et al. 2018. Isolation of human photoreceptor precursors via a cell surface marker panel from stem cell-derived retinal organoids and fetal retinae. *Stem Cells* 36(5):709–22

Lam PT, Gutierrez C, Del Rio-Tsonis K, Robinson ML. 2019. Generation of a retina reporter hiPSC line to label progenitor, ganglion, and photoreceptor cell types. bioRxiv 658963. **https://doi.org/10.1101/658963**

Lancaster MA, Knoblich JA. 2014. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345(6194):1247125

Lapasset L, Milhavet O, Prieur A, Besnard E, Babled A, et al. 2011. Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev.* 25(21):2248–53

Le R, Kou Z, Jiang Y, Li M, Huang B, et al. 2014. Enhanced telomere rejuvenation in pluripotent cells reprogrammed via nuclear transfer relative to induced pluripotent stem cells. *Cell Stem Cell* 14(1):27–39

Levine EM, Roelink H, Turner J, Reh TA. 1997. Sonic hedgehog promotes rod photoreceptor differentiation in mammalian retinal cells in vitro. *J. Neurosci.* 17(16):6277–88

Liu H, Thurig S, Mohamed O, Dufort D, Wallace VA. 2006. Mapping canonical Wnt signaling in the developing and adult retina. *Investig. Ophthalmol. Vis. Sci.* 47(11):5088–97

Locker M, Agathocleous M, Amato MA, Parain K, Harris WA, Perron M. 2006. Hedgehog signaling and the retina: insights into the mechanisms controlling the proliferative properties of neural precursors. *Genes Dev.* 20(21):3036–48

Lombardini JB. 1991. Taurine: retinal function. *Brain Res. Rev.* 16:151–69

Lowe A, Harris R, Bhansali P, Cvekl A, Liu W. 2016. Intercellular adhesion-dependent cell survival and ROCK-regulated actomyosin-driven forces mediate self-formation of a retinal organoid. *Stem Cell Rep.* 6(5):743–56

Lu Y, Yi W, Wu Q, Zhong S, Zuo Z, et al. 2018. Single-cell RNA-seq analysis maps the development of human fetal retina. bioRxiv 423830. **https://doi.org/10.1101/423830**

Macosko EZ, Basu A, Satija R, Nemesh J, Shekhar K, et al. 2015. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* 161(5):1202–14

Mansergh FC, Carrigan M, Hokamp K, Farrar GJ. 2015. Gene expression changes during retinal development and rod specification. *Mol. Vis.* 21:61–87

Mao X, An Q, Xi H, Yang X-J, Zhang X, et al. 2019. Single-cell RNA sequencing of hESC-derived 3D retinal organoids reveals novel genes regulating RPC commitment in early human retinogenesis. *Stem Cell Rep.* 13(4):747–60

Martinez-Morales J-R, Del Bene F, Nica G, Hammerschmidt M, Bovolenta P, Wittbrodt J. 2005. Differentiation of the vertebrate retina is coordinated by an FGF signaling center. *Dev. Cell.* 8(4):565–74

Masai I, Yamaguchi M, Tonou-Fujimori N, Komori A, Okamoto H. 2005. The hedgehog-PKA pathway regulates two distinct steps of the differentiation of retinal ganglion cells: the cell-cycle exit of retinoblasts and their neuronal maturation. *Development* 132(7):1539–53

Masland RH. 2012. The neuronal organization of the retina. *Neuron* 76(2):266–80

Masuda T, Zhang X, Berlinicke C, Wan J, Yerrabelli A, et al. 2014. The transcription factor GTF2IRD1 regulates the topology and function of photoreceptors by modulating photoreceptor gene expression across the retina. *J. Neurosci.* 34(46):15356–68

McLelland BT, Lin B, Mathur A, Aramant RB, Thomas BB, et al. 2018. Transplanted hESC-derived retina organoid sheets differentiate, integrate, and improve visual function in retinal degenerate rats. *Investig. Ophthalmol. Vis. Sci.* 59(6):2586–603

McLeod DS, Hasegawa T, Prow T, Merges C, Lutty G. 2006. The initial fetal human retinal vasculature develops by vasculogenesis. *Dev. Dyn.* 235(12):3336–47

Megaw R, Abu-Arafeh H, Jungnickel M, Mellough C, Gurniak C, et al. 2017. Gelsolin dysfunction causes photoreceptor loss in induced pluripotent cell and animal retinitis pigmentosa models. *Nat. Commun.* 8(1):271

Mellough CB, Bauer R, Collin J, Dorgau B, Zerti D, et al. 2019a. An integrated transcriptional analysis of the developing human retina. *Development* 146(2):dev169474

Mellough CB, Collin J, Queen R, Hilgen G, Dorgau B, et al. 2019b. Systematic comparison of retinal organoid differentiation from human pluripotent stem cells reveals stage specific, cell line, and methodological differences. *Stem Cells Transl. Med.* 8(7):694–706

Meyer JS, Howden SE, Wallace KA, Verhoeven AD, Wright LS, et al. 2011. Optic vesicle-like structures derived from human pluripotent stem cells facilitate a customized approach to retinal disease treatment. *Stem Cells* 29(8):1206–18

Meyer JS, Shearer RL, Capowski EE, Wright LS, Wallace KA, et al. 2009. Modeling early retinal development with human embryonic and induced pluripotent stem cells. *PNAS* 106(39):16698–703

Meyers JR, Hu L, Moses A, Kaboli K, Papandrea A, Raymond PA. 2012. β-catenin/Wnt signaling controls progenitor fate in the developing and regenerating zebrafish retina. *Neural Dev.* 7:30

Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, et al. 2013. Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell* 13(6):691–705

Mills EA, Goldman D. 2017. The regulation of Notch signaling in retinal development and regeneration. *Curr. Pathobiol. Rep.* 5(4):323–31

Munsie M, Hyun I, Sugarman J. 2017. Ethical issues in human organoid and gastruloid research. *Development* 144(6):942–45

Murali D, Yoshikawa S, Corrigan RR, Plas DJ, Crair MC, et al. 2005. Distinct developmental programs require different levels of Bmp signaling during mouse retinal development. *Development* 132(5):913–23

Nakano T, Ando S, Takata N, Kawada M, Muguruma K, et al. 2012. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 10(6):771–85

Nath M, Halder N, Velpandian T. 2017. Circulating biomarkers in glaucoma, age-related macular degeneration, and diabetic retinopathy. *Indian J. Ophthalmol.* 65(3):191–97

Ng L, Hurley JB, Dierks B, Srinivas M, Saltó C, et al. 2001. A thyroid hormone receptor that is required for the development of green cone photoreceptors. *Nat. Genet.* 27(1):94–98

Norden C, Young S, Link BA, Harris WA. 2009. Actomyosin is the main driver of interkinetic nuclear migration in the retina. *Cell* 138(6):1195–208

O'Brien KMB, Cheng H, Jiang Y, Schulte D, Swaroop A, Hendrickson AE. 2004. Expression of photoreceptor-specific nuclear receptor NR2E3 in rod photoreceptors of fetal human retina. *Investig. Opthalmol. Vis. Sci.* 45(8):2807–12

Ohlemacher SK, Iglesias CL, Sridhar A, Gamm DM, Meyer JS. 2015. Generation of highly enriched populations of optic vesicle–like retinal cells from human pluripotent stem cells. *Curr. Protoc. Stem Cell Biol.* 32(1):1H.8.1–20

Organisciak DT, Vaughan DK. 2010. Retinal light damage: mechanisms and protection. *Prog. Retin. Eye Res.* 29(2):113–34

Ortín-Martínez A, Nadal-Nicolás FM, Jiménez-López M, Alburquerque-Béjar JJ, Nieto-López L, et al. 2014. Number and distribution of mouse retinal cone photoreceptors: differences between an albino (Swiss) and a pigmented (C57/BL6) strain. *PLOS ONE* 9(7):e102392

Osakada F, Ikeda H, Mandai M, Wataya T, Watanabe K, et al. 2008a. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nat. Biotechnol.* 26(2):215–24

Osakada F, Ikeda H, Watanabe K, Akaike A, Sasai Y, Takahashi M. 2008b. Differentiation of rod and cone photoreceptors from human embryonic stem cells under defined culture conditions. *Investig. Ophthalmol. Vis. Sci.* 49(13):3566

Ovando-Roche P, West EL, Branch MJ, Sampson RD, Fernando M, et al. 2018. Use of bioreactors for culturing human retinal organoids improves photoreceptor yields. *Stem Cell Res. Ther.* 9(1):156

Parfitt DA, Lane A, Ramsden CM, Carr A-JF, Munro PM, et al. 2016. Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. *Cell Stem Cell* 18(6):769–81

Peng Y-R, Shekhar K, Yan W, Herrmann D, Sappington A, et al. 2019. Molecular classification and comparative taxonomics of foveal and peripheral cells in primate retina. *Cell* 176(5):1222–37.e22

Phan N, Hong JJ, Tofig B, Mapua M, Elashoff D, et al. 2019. A simple high-throughput approach identifies actionable drug sensitivities in patient-derived tumor organoids. *Commun. Biol.* 2:78

Pollak J, Wilken MS, Ueki Y, Cox KE, Sullivan JM, et al. 2013. ASCL1 reprograms mouse Müller glia into neurogenic retinal progenitors. *Development* 140(12):2619–31

Provis JM, Dubis AM, Maddess T, Carroll J. 2013. Adaptation of the central retina for high acuity vision: cones, the fovea and the avascular zone. *Prog. Retin. Eye Res.* 35:63–81

Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia A-S, et al. 2001. The retina. In *Neuroscience*, ed. D Purves, GJ Augustine, D Fitzpatrick, LC Katz, A-S LaMantia, JO McNamara, SM Williams, art. 100. Sunderland, MA: Sinauer Assoc. 2nd ed.

Quinn PM, Buck TM, Mulder AA, Ohonin C, Alves CH, et al. 2019. Human iPSC-derived retinas recapitulate the fetal CRB1 CRB2 complex formation and demonstrate that photoreceptors and Müller glia are targets of AAV5. *Stem Cell Rep*. 12(5):906–19

Radisic M. 2019. Building a better model of the retina. *eLife* 8:e51183

Reese BE. 2011. Development of the retina and optic pathway. *Vis. Res.* 51(7):613–32

Reichman S, Terray A, Slembrouck A, Nanteau C, Orieux G, et al. 2014. From confluent human iPS cells to self-forming neural retina and retinal pigmented epithelium. *PNAS* 111(23):8518–23

Remez LA, Onishi A, Menuchin-Lasowski Y, Biran A, Blackshaw S, et al. 2017. Pax6 is essential for the generation of late-born retinal neurons and for inhibition of photoreceptor-fate during late stages of retinogenesis. *Dev. Biol.* 432(1):140–50

Rheaume BA, Jereen A, Bolisetty M, Sajid MS, Yang Y, et al. 2018. Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. *Nat. Commun.* 9(1):2759

Russell S, Bennett J, Wellman JA, Chung DC, Yu Z-F, et al. 2017. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet* 390(10097):849–60

Shao J, Zhou P-Y, Peng G-H. 2017. Experimental study of the biological properties of human embryonic stem cell-derived retinal progenitor cells. *Sci. Rep.* 7:42363

Sharma TP, Wiley LA, Whitmore SS, Anfinson KR, Cranston CM, et al. 2017. Patient-specific induced pluripotent stem cells to evaluate the pathophysiology of TRNT1-associated retinitis pigmentosa. *Stem Cell Res*. 21:58–70

Shekhar K, Lapan SW, Whitney IE, Tran NM, Macosko EZ, et al. 2016. Comprehensive classification of retinal bipolar neurons by single-cell transcriptomics. *Cell* 166(5):1308–23.e30

Shibusawa N, Hashimoto K, Nikrodhanond AA, Liberman MC, Applebury ML, et al. 2003. Thyroid hormone action in the absence of thyroid hormone receptor DNA-binding in vivo. *J. Clin. Investig.* 112(4):588–97

Shrestha R, Wen Y-T, Ding D-C, Tsai R-K. 2019. Aberrant hiPSCs-derived from human keratinocytes differentiates into 3D retinal organoids that acquire mature photoreceptors. *Cells* 8(1):E36

Singh RK, Mallela RK, Cornuet PK, Reifler AN, Chervenak AP, et al. 2015. Characterization of three-dimensional retinal tissue derived from human embryonic stem cells in adherent monolayer cultures. *Stem Cells Dev*. 24(23):2778–95

Singh RK, Occelli LM, Binette F, Petersen-Jones SM, Nasonkin IO. 2019. Transplantation of human embryonic stem cell-derived retinal tissue in the subretinal space of the cat eye. *Stem Cells Dev*. 28(17):1151–66

Slijkerman RWN, Song F, Astuti GDN, Huynen MA, van Wijk E, et al. 2015. The pros and cons of vertebrate animal models for functional and therapeutic research on inherited retinal dystrophies. *Prog. Retin. Eye Res.* 48:137–59

Sluch VM, Chamling X, Liu MM, Berlinicke CA, Cheng J, et al. 2017. Enhanced stem cell differentiation and immunopurification of genome engineered human retinal ganglion cells. *Stem Cells Transl. Med.* 6(11):1972–86

Sluch VM, Davis CO, Ranganathan V, Kerr JM, Krick K, et al. 2015. Differentiation of human ESCs to retinal ganglion cells using a CRISPR engineered reporter cell line. *Sci. Rep.* 5:16595

Steinfeld J, Steinfeld I, Bausch A, Coronato N, Hampel M-L, et al. 2017. BMP-induced reprogramming of the neural retina into retinal pigment epithelium requires Wnt signalling. *Biol. Open* 6(7):979–92
Stenkamp DL. 2015. Development of the vertebrate eye and retina. *Prog. Mol. Biol. Transl. Sci.* 134:397–414
Sugarman J, Bredenoord AL. 2019. Reflections on organoid ethics: Jeremy Sugarman and Annelien Bredenoord. *Cell Stem Cell* 24(6):849–51
Swinney DC, Anthony J. 2011. How were new medicines discovered? *Nat. Rev. Drug Discov.* 10(7):507–19
Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5):861–72
Teotia P, Van Hook MJ, Wichman CS, Allingham RR, Hauser MA, Ahmad I. 2017. Modeling glaucoma: retinal ganglion cells generated from induced pluripotent stem cells of patients with SIX6 risk allele show developmental abnormalities. *Stem Cells* 35(11):2239–52
Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, et al. 1998. Embryonic stem cell lines derived from human blastocysts. *Science* 282(5391):1145–47
Tucker BA, Mullins RF, Streb LM, Anfinson K, Eyestone ME, et al. 2013. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. *eLife* 2:e00824
Uygun BE, Sharma N, Yarmush M. 2009. Retinal pigment epithelium differentiation of stem cells: current status and challenges. *Crit. Rev. Biomed. Eng.* 37:355–75
van Norren D, Vos JJ. 2016. *Light Damage to the Retina: An Historical Approach*. Berlin: Nat. Publ. Group
Völkner M, Zschätzsch M, Rostovskaya M, Overall RW, Busskamp V, et al. 2016. Retinal organoids from pluripotent stem cells efficiently recapitulate retinogenesis. *Stem Cell Rep.* 6(4):525–38
Volland S, Esteve-Rudd J, Hoo J, Yee C, Williams DS. 2015. A comparison of some organizational characteristics of the mouse central retina and the human macula. *PLOS ONE* 10(4):e0125631
Wahlin KJ, Maruotti JA, Sripathi SR, Ball J, Angueyra JM, et al. 2017. Photoreceptor outer segment-like structures in long-term 3D retinas from human pluripotent stem cells. *Sci. Rep.* 7(1):766
Walshe TE, Leach LL, D'Amore PA. 2011. TGF-β signaling is required for maintenance of retinal ganglion cell differentiation and survival. *Neuroscience* 189:123–31
Wang H, La Russa M, Qi LS. 2016. CRISPR/Cas9 in genome editing and beyond. *Annu. Rev. Biochem.* 85:227–64
Wang S, Sengel C, Emerson MM, Cepko CL. 2014. A gene regulatory network controls the binary fate decision of rod and bipolar cells in the vertebrate retina. *Dev. Cell.* 30(5):513–27
Weeber F, Ooft SN, Dijkstra KK, Voest EE. 2017. Tumor organoids as a pre-clinical cancer model for drug discovery. *Cell Chem. Biol.* 24(9):1092–100
Welby E, Lakowski J, Di Foggia V, Budinger D, Gonzalez-Cordero A, et al. 2017. Isolation and comparative transcriptome analysis of human fetal and iPSC-derived cone photoreceptor cells. *Stem Cell Rep.* 9(6):1898–915
Wnorowski A, Yang H, Wu JC. 2019. Progress, obstacles, and limitations in the use of stem cells in organ-on-a-chip models. *Adv. Drug Deliv. Rev.* 140:3–11
Zagozewski JL, Zhang Q, Pinto VI, Wigle JT, Eisenstat DD. 2014. The role of homeobox genes in retinal development and disease. *Dev. Biol.* 393(2):195–208
Zhong X, Gutierrez C, Xue T, Hampton C, Vergara MN, et al. 2014. Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. *Nat. Commun.* 5:4047
Zhou S, Flamier A, Abdouh M, Tétreault N, Barabino A, et al. 2015. Differentiation of human embryonic stem cells into cone photoreceptors through simultaneous inhibition of BMP, TGFβ and Wnt signaling. *Development* 142(19):3294–306