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REVIEW **Open Access**

Patient iPSC-derived retinal organoids: Observable retinal diseases in-a-dish

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Summary. Induced pluripotent stem cells (iPSCs), reprogrammed from human somatic cells, hold the capacity to differentiate into most human body cells. iPSCs can be differentiated into retinal organoids, a three-dimensional structured retina containing various retinal cells. Patient-specific retinal organoids provide a powerful disease model to recapitulate the disease to study the pathogenesis of inherited retinal dystrophies, to screen or discover new drugs, and most importantly to supply an unlimited cell source for retinal regeneration.

Key words: Induced pluripotent stem cells, Retinal organoids, Inherited retinal dystrophy, Gene editing, Disease modeling

Introduction

Induced pluripotent stem cell (iPSC) technology, which allows the reprogramming of somatic cells into a pluripotent state, has the potential to produce unlimited tissue-specific progenitor cells or terminally differentiated cells (Jin et al., 2009; Avior et al., 2016). By comparing normal and patient-derived cells, this technology provides an ideal *in vitro* model to study the mechanism of a certain disease for disease modeling and therapeutic development.

Previously, iPSCs followed by two-dimensional (2D) differentiation has been a useful tool for simulating inherited retinal disease (Jin et al., 2011, 2012; Jin and Takahashi, 2012; Lukovic et al., 2015) and for studying cell replacement therapy (Jin et al., 2019), although the methods still lack a cellular network and a niche of a primordial retina. However, the pathogenesis of a retinal disease is usually associated with crosstalk or an interaction between diseased cells and surrounding cells. Alternatively, three-dimensional (3D) retinal organoids (ROs) break through the bottleneck of a 2D retinal differentiation system and provide a well-layered 3D retinal structure consisting of three orders of retinal neurons, which highly imitate the temporospatial arrangement of the retina (Eiraku et al., 2011; Zhong et al., 2014; Wahlin et al., 2017; Pan et al., 2020). As the self-formation of ROs mimics the natural development of the retina, it enables the investigation of the effects of a distinct mutation on retinogenesis (Tucker et al., 2013; Guo et al., 2019; Gao et al., 2020; Lukovic et al., 2020). In brief, the current ROs from pluripotent stem cells provide a well-structured and functioning retina-like organ *in vitro*.

CRISPR-based Cas (CRISPR/Cas) technology is currently the most commonly used and most efficient gene editing method, which includes the Cas nuclease and a guide RNA (gRNA). By cutting the DNA at a particular chromosomal position, the CRISPR/Cas system initiates two forms of DNA repair: homology-directed repair (HDR) and non-homologous end joining (NHEJ). As the more common repair method, the NHEJ mechanism introduces base deletion or insertion at the position of the cutting point, disrupting the open reading frame and resulting in the inactivation of the target gene (Burnight et al., 2017). It is mostly used to prevent the disease progression of autosomal dominant disease caused by dominant-negative or gain-of-function mutations (Bakondi et al., 2016; Burnight et al., 2017). Conversely, the HDR pathway relies on exogenous donor DNA to correct the disease-causing gene mutation, achieving a precise gene modification (Burnight et al., 2017; Zhang et al., 2017). The CRISPR/Cas system has been used to repair gene defects in retinal degeneration patient-derived iPSCs (Bassuk et al., 2016; Tang et al., 2016; Burnight et al., 2017). The gene correction of patient-derived iPSCs, followed by differentiation and transplantation of retinal cells, can minimize the possibility of immune rejection and provide an ideal method for treating retinal degeneration disease (Fig. 1).

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In this review, we mainly focus on the latest advances in patient iPSC-derived ROs and gene editing to examine and treat inherited retinal dystrophies (IRDs), a group of diseases characterized by progressive photoreceptor degeneration and vision loss, given their increasing application in this field.

Late-onset retinal diseases: Retinitis pigmentosa (RP)

RP is the most common IRD disease that displays exceptional genetic heterogeneity. Currently, more than 4,500 mutations in approximately 207 disease-causing genes have been identified (Ran et al., 2014) (http://retinogenetics.org/). *USH2A* is the most common pathogenic gene of non-syndromic RP. The early ROs derived from a patient with c.8559-2A>G mutation of the *USH2A* gene displayed obvious defects in morphology, expression of specific proteins, and expression of cilium-related genes (Guo et al., 2019). Gao et al. generated ROs from a RP patient-derived iPSCs harboring a homozygous c.694G>A mutation of the *PDE6B* gene. Transcriptome analysis revealed remarkabe changes in the expression level of genes regulating cGMP hydrolysis in the patient ROs at differentiation day 230, which might lead to impaired formation of synaptic connections and the connecting cilium in photoreceptor cells (Gao et al., 2020).

Despite the ongoing clinical trials for RP gene augmentation therapy (Ghazi et al., 2016; Zhang et al., 2021), the HDR mechanism triggered by CRISPR/Cas9 has successfully achieved gene repair in autosomal recessive RP (arRP) patient-derived iPSCs. To address the homozygous c.1513ins353 mutation in the *MAK* gene, Burnight et al co-delivered a sg1MAK-Cas9 plasmid and an HDR donor plasmid to patient-specific iPSCs (Burnight et al., 2017). Patient iPSCs were subsequently differentiated into photoreceptor precursor cells and RT-PCR was performed with primers flanking exon 9 of the *MAK* gene. Their results showed that the *MAK* transcript of the precursor cells restored the full length of exon 9. A similar strategy was applied to correct the c.992_993delCA mutation of the *MERTK* gene (Artero Castro et al., 2019). Different from previous studies, Cas9-crRNA-tracrRNA ribonucleoprotein (RNP) complexes and a homology-directed repair template were nucleofected into patient-derived iPSCs. DNA sequencing analysis confirmed the heterozygous and homozygous correction of *MERTK* mutation. Two clones were proved to have pluripotency and a normal karyotype. Moreover, Bassuk et al. transfected X-linked RP patient-derived iPSCs with a gRNA/hSpCas9 expression vector and a single strand oligodeoxynucleotide (ssODN) template and found that 13% of the sequencing reads contained a correction of the c.3070G>T mutation in the ORF15 of the *RPGR* gene (Bassuk et al., 2016). Deng et al. repaired the c.1685_1686delAT mutation of the *RPGR* gene in

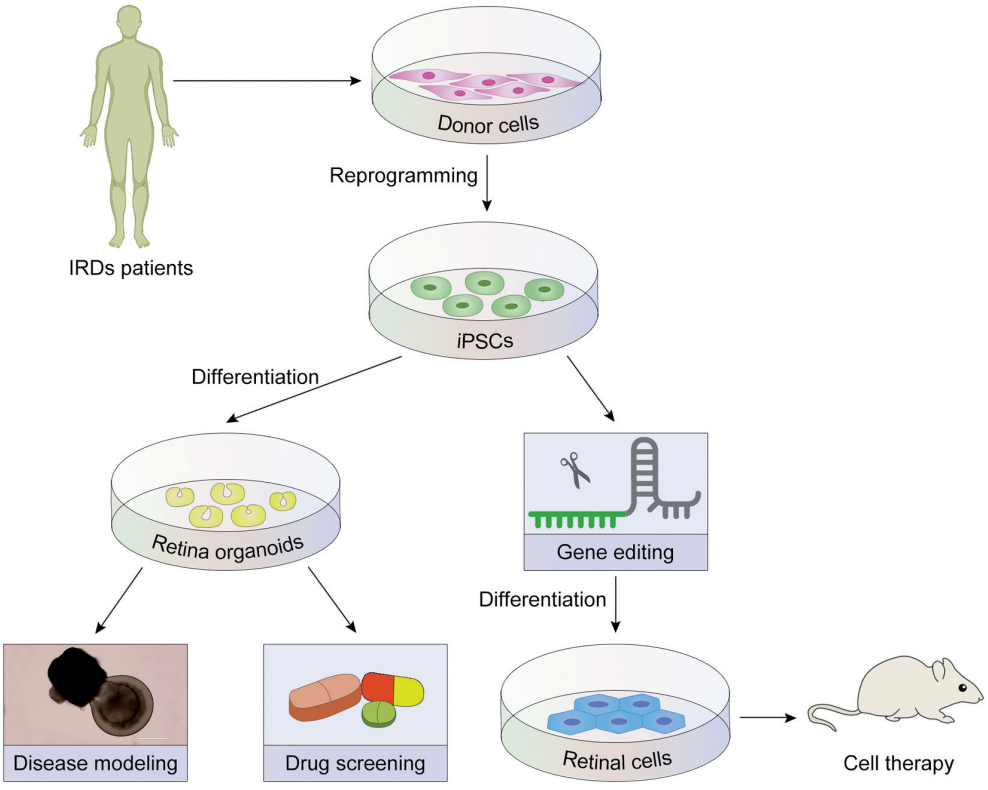

Fig. 1. Schematic diagram of patient-derived iPSCs and retinal organoids generation and their application for disease modeling and cell therapy.

patient-derived iPSCs using a gRNA/SpCas9 vector and a normal template and then differentiated the iPSCs into ROs (Deng et al., 2018). Gene-corrected ROs displayed rescued photoreceptor structure, reversed ciliopathy, and restored the expression level of retinal-related genes.

The CRISPR/Cas9 approach has also been used in autosomal dominant RP by means of silencing or correcting the gain-of-function gene mutations. CRISPR/Cas9-based gene editing combined with autologous iPSCs provides a promising research basis for a personalized transplantation strategy for RP. Burnight et al. delivered an expression vector including gRNA and SpCas9 cDNA sequence together with a donor HDR plasmid to patient-derived iPSCs to evaluate the correction of c.163C>A mutation in the *RHO* gene (Burnight et al., 2017). Gene correction was observed in five of nine clones. Using the NHEJ approach, they observed indel happened in clones with the mutant allele only. In addition, Buskin et al. generated iPSCs from a patient with the most severe clinical phenotype of RP11 due to the c.1115_1125del11 mutation in the *PRPF31* gene (Buskin et al., 2018). The CRISPR/Cas9 system in combination with ssODNs were applied to correct the *PRPF31* mutation in patient-derived iPSCs followed by ROs differentiation. The results proved that the mutation of the *PRFP31* gene affected ciliogenesis and cilia length that could be rescued by *CRISPR/Cas9* without causing off-target effects, demonstrating proof of concept that in situ gene editing is effective.

Early-onset retinal degeneration: Leber congenital amaurosis (LCA)

The incidence of LCA is estimated to be 1/80,000 live births, accounting for 5% of all IRD patients (Tsang and Sharma, 2018). At least 17 genes have been reported to cause LCA, with a more prevalent inheritance of autosomal recessive. More than half of LCA patients are attributable to *CEP290, GUCY2D,* or *RPE65* mutations that affect the function of photoreceptor cells or RPE. The optic cups derived from the iPSCs of *CEP290*-LCA patients exhibited less developed photoreceptor cilia (Shimada et al., 2017), demonstrating a molecular relationship between *CEP290* mutations and ciliary defects. Parfitt et al. differentiated ROs from iPSCs with a common intron mutation, c.2991+1665A>G, of the *CEP290* gene (Parfitt et al., 2016). Expression of CEP290 was not detectable at the connecting cilia of ROs. RT-PCR results revealed the presence of a cryptic exon between exon 26 and 27 of the *CEP290* gene. The aberrant exon expressed highest in photoreceptors and impaired ciliogenesis. Antisense oligonucleotide effectively blocked abnormal *CEP290* splicing and restored cilia-related protein function. Li et al. generated iPSCs from an LCA patient carrying p.L67R and p.Y144H mutations of the *RPE65* gene (Li et al., 2019). The RPE65-hiPSCs could generate well-layered ROs with all subtypes of photoreceptors. Compared with the healthy control, the RPE cells in patient-derived ROs had lower RPE65 expression, but had similar phagocytosis capacity and VEGF secretion level. Mutations in the *AIPL1* gene cause a severe phenotype, LCA4, which manifests as vision loss during the first year of life. Recently, Lukovic et al. generated iPSCs-ROs from an LCA4 patient carrying homozygous p.Cys89Arg mutation in the *AIPL1* gene (Lukovic et al., 2020). Their results showed a reduced level of mutant AIPL1 and effector PDE6 proteins in patient ROs, validating the findings in animal models. However, the patient-derived ROs maintained a normal retinal cytoarchitecture.

Syndromic retinal degeneration: Usher syndrome (USH)

USH is characterized by early-onset sensorineural hearing loss and late-onset vision loss. Mutations in the *USH2A* gene are a common cause of USH and arRP. Tucker et al. generated patient-derived iPSCs with *USH2A* mutations and differentiated the cells into optic cup-like structures with characteristics of retinal precursor cells (Tucker et al., 2013). An intronic splice site mutation of the *USH2A* gene resulted in the exonification of intron 40 and a premature stop codon. The elevated expressions of GRP78 and GRP94 suggested that the *USH2A* mutations result in protein misfolding and endoplasmic reticulum stress. Sanjurjo-Soriano et al. derived patient-specific iPSCs harboring two predominant *USH2A* mutations: Glu767Serfs*21 and Cys759Phe (Sanjurjo-Soriano et al., 2020). They then used enhanced specificity Cas9 to successfully correct the two mutations in the iPSCs of patients with USH or arRP. The expression analysis of iPSCs showed that the abnormal mRNA levels caused by the mutations were also reverted following gene correction. In the study of Tang et al., iPSCs were generated from a patient harboring compound heterozygous c.1184G>A and c.4118C>T mutations in the *MYO7A* gene, an asymptomatic parent, and a healthy donor (Tang et al., 2016). Plasmids expressing gRNA (aim at c.4118C>T mutation), SpCas9, and a 150-bp ssODN were electroporated into the iPSCs. The correction rate of one allele was 7% (3 of 45 clones). The corrected hair cell-like cells differentiated from patient-derived iPSCs were shown to recover the stereocilia-like protrusions and the electrophysiological characteristic.

Retinoblastoma (Rb)

Rb, a monogenic malignancy caused by biallelic inactivation of the *RB1* gene, a tumor-suppressor gene (Friend et al., 1986), is the most common ocular malignant tumor in infants and childhood, with the global mortality up to 70% (Dimaras et al., 2012). Most recently, we developed a sustainable human Rb model through mutagenesis of the *RB1* gene in PSCs followed by stepwise differentiation into ROs (Liu et al., 2020). Notably, the organoids at day 60 exhibited significant

tumorigenisis. The Rb organoids showed high similarity with the primary tumor in morphology, omics characteristic, and *in vivo* proliferation ability. We found that the maturing cone precursor is the cell of origin of Rb and inhibitors of spleen tyrosine kinase (SYK) could be considered therapeutic drugs. The Rb organoids model provides valuable insights into tumorigenesis and drug screening for ocular malignancies and other genetic cancers.

Perspectives

Human iPSC-derived ROs present a useful tool for studying retinal development, IRD modelling, drug screening, and cell therapy. However, a number of requirements should be considered. It is necessary to establish reliable and reproducible protocols to differentiate ROs from iPSCs more efficiently. Of particular importance is generating mature photoreceptors *in vitro*, which may require the co-culture of RPE cells or the use of biological scaffolds. A convincing disease modelling will not only benefit from RO differentiation with a reliable photoreceptor function but also from the establishment of iPSC lines with the same genetic background. However, reducing the variability of RO differentiation remains a major challenge. Generating family control iPSCs for patients may significantly reduce the genetic variation.

Patient-derived iPSCs may also become a choice for cell therapy in the future. Two important factors should be considered: the precision gene editing of the iPSCs with mutations and the ideal differentiation stage of iPSC-derived retinal cells to transplant. For clinical applications, the RNP CRISPR/Cas9 gene editing may be more advantageous because it shows a relatively high gene editing efficiency and a low off-targeting rate. Pausch et al. described a minimal functional CRISPR-Casϕ system comprising a 70 kd Cas protein, with an expanded target recognition capability (Pausch et al., 2020). The iPSC-derived retinal cells are strictly screened prior to transplantation. To ensure safety, karyotype analysis, tumorigenicity, and whole genome sequencing must be performed before clinical trials.

The major advantage of gene-corrected patient iPSC-derived cells for transplantation is reduced immunogenicity. However, the autologous iPSC-derived retinal cells for clinical use will be high-cost; therefore, other approaches are needed. Sugita et al. transplanted iPSC-derived RPE of non-human primate into the eyes of major histocompatibility complex (MHC)-matched donors (Sugita et al., 2016) and observed the allografts survived without signs of rejection. As a result, building of an iPSCs bank that includes homozygotes for common MHC types allows stem cell transplantation for patients with MHC matching, greatly reducing the differentiation cost and cell variability of iPSCs. In fact, phenotypic variation can be detected even in iPSCs from the same donor. If iPSCs are derived from the terminal differentiated cells of unrelated individuals, the phenotypic difference will be more significant (Burnight et al., 2017; Sanjurjo-Soriano et al., 2020). This encourages the finding of ways to minimize the differences between the iPSC lines.

Although ROs differentiated *in vitro* consist of a

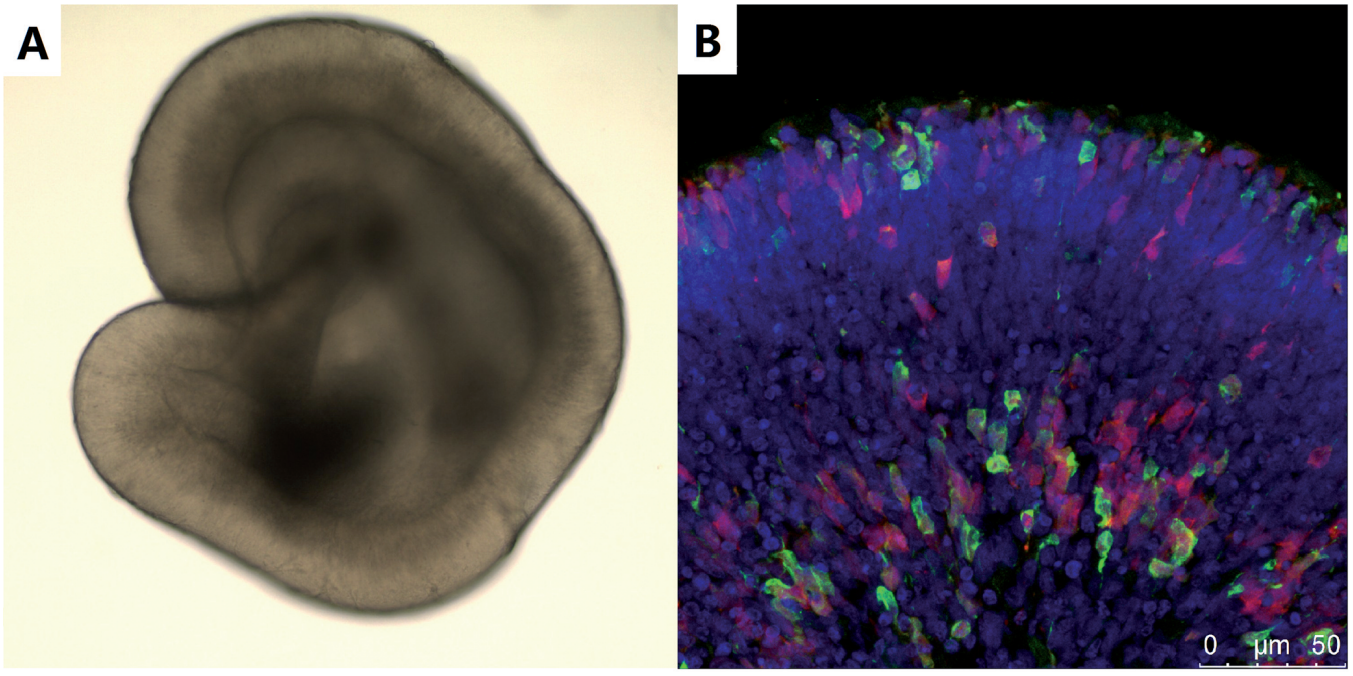

Fig. 2. Human iPSCs-derived retinal organoid **(A)** contains Recoverin+ (green)/ Crx+ (red) photoreceptor cells **(B)**.

variety of retinal cell types and electrophysiological characteristics of photoreceptors (Fig. 2), this 3D structure is still different from a far more complicated *in vivo* retina, such as the lack of vascularization and important physiological interactions (Achberger et al., 2019a,b). To simulate the *in vivo* niche, Achberger et al. developed a physiological model of the human retina (Achberger et al., 2019a,b), the retina-on-a-chip, which integrated more than seven retinal cell types and provided vasculature-like perfusion. The model increased the formation of outer segment-like structures on the ROs and allowed the establishment of a defined interaction site between the segment structures of the ROs and RPE. Furthermore, the interaction enhanced the function of outer segment phagocytosis and calcium dynamics.

In summary, the characteristics of patient-derived ROs make it the most reliable "diseases-in-a-dish" model, which is sufficient to identify the pathogenicity of suspected mutations, to simulate disease progress, to screen or discover new drugs, and to provide a source for autologous gene-corrected cell transplantation.

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References

Achberger K., Haderspeck J.C., Kleger A. and Liebau S. (2019a). Stem cell-based retina models. Adv. Drug Deliv. Rev. 140, 33-50.

Achberger K., Probst C., Haderspeck J., Bolz S., Rogal J., Chuchuy J., Nikolova M., Cora V., Antkowiak L., Haq W., Shen N., Schenke-Layland K., Ueffing M., Liebau S. and Loskill P. (2019b). 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.

Artero Castro A., Long K., Bassett A., Machuca C., León M., Ávila-Fernandez A., Cortón M., Vidal-Puig T., Ayuso C., Lukovic D. and Erceg S. (2019). Generation of gene-corrected human induced pluripotent stem cell lines derived from retinitis pigmentosa patient with Ser331Cysfs*5 mutation in Mertk. Stem Cell Res. 34, 101341.

Avior Y., Sagi I. and Benvenisty N. (2016). Pluripotent stem cells in disease modelling and drug discovery. Nat. Rev. Mol. Cell Biol. 17, 170-182.

Bakondi B., Lv W., Lu B., Jones M.K., Tsai Y., Kim K. J., Levy R., Akhtar A.A., Breunig J.J., Svendsen C.N. and Wang S. (2016). *In vivo* CRISPR/cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. Mol. Ther. 24, 556-563.

Bassuk A.G., Zheng A., Li Y., Tsang S.H. and Mahajan V.B. (2016). Precision medicine: genetic repair of retinitis pigmentosa in patient-derived stem cells. Sci. Rep. 6, 19969.

Burnight E.R., Gupta M., Wiley L.A., Anfinson K.R., Tran A., Triboulet R., Hoffmann J.M., Klaahsen D.L., Andorf J.L., Jiao C., Sohn E.H., Adur M.K., Ross J. W., Mullins R.F., Daley G.Q., Schlaeger T.M., Stone E.M. and Tucker B.A. (2017). Using CRISPR-cas9 to generate gene-corrected autologous iPSCs for the treatment of inherited retinal degeneration. Mol. Ther. 25, 1999-2013.

Buskin A., Zhu L., Chichagova V., Basu B., Mozaffari-Jovin S., Dolan D., Droop A., Collin J., Bronstein R., Mehrotra S., Farkas M., Hilgen G., White K., Pan K.T., Treumann A., Hallam D., Bialas K., Chung G., Mellough C., Ding Y., Krasnogor N., Przyborski S., Zwolinski S., Al-Aama J., Alharthi S., Xu Y., Wheway G., Szymanska K., McKibbin M., Inglehearn C.F., Elliott D.J., Lindsay S., Ali R.R., Steel D.H., Armstrong L., Sernagor E., Urlaub H., Pierce E., Lührmann R., Grellscheid S.N., Johnson C.A. and Lako M. (2018). Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. Nat. Commun. 9, 4234.

Deng W.L., Gao M.L., Lei X.L., Lv J.N., Zhao H., He K.W., Xia X.X., Li L.Y., Chen Y.C., Li Y.P., Pan D., Xue T. and Jin Z.B. (2018). Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. Stem Cell Rep. 10, 1267-1281.

Dimaras H., Kimani K., Dimba E.A., Gronsdahl P., White A., Chan H.S. and Gallie B.L. (2012). Retinoblastoma. Lancet 379, 1436-1446.

Eiraku M., Takata N., Ishibashi H., Kawada M., Sakakura E., Okuda S., Sekiguchi K., Adachi T. and Sasai Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 472, 51-56.

Friend S.H., Bernards R., Rogelj S., Weinberg R.A., Rapaport J.M., Albert D.M. and Dryja T.P. (1986). A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323, 643-646.

Gao M.L., Lei X.L., Han F., He K.W., Jin S.Q., Zhang Y.Y. and Jin Z.B. (2020). Patient-specific retinal organoids recapitulate disease features of late-onset retinitis pigmentosa. Front. Cell Dev. Biol. 8, 128.

Ghazi N.G., Abboud E.B., Nowilaty S.R., Alkuraya H., Alhommadi A., Cai H., Hou R., Deng W.T., Boye S.L., Almaghamsi A., Al Saikhan F., Al-Dhibi H., Birch D., Chung C., Colak D., LaVail M.M., Vollrath D., Erger K., Wang W., Conlon T., Zhang K., Hauswirth W. and Alkuraya F.S. (2016). Treatment of retinitis pigmentosa due to Mertk mutations by ocular subretinal injection of adeno-associated virus gene vector: results of a phase I trial. Hum. Genet. 135, 327-343.

Guo Y., Wang P., Ma J.H., Cui Z., Yu Q., Liu S., Xue Y., Zhu D., Cao J., Li Z., Tang S. and Chen J. (2019). Modeling retinitis pigmentosa: retinal organoids generated from the iPSCs of a patient with the USH2A mutation show early developmental abnormalities. Front. Cell. Neurosci. 13, 361.

Jin Z.B. and Takahashi M. (2012). Generation of retinal cells from pluripotent stem cells. Prog. Brain Res. 201, 171-181.

Jin Z.B., Okamoto S., Mandai M. and Takahashi M. (2009). Induced pluripotent stem cells for retinal degenerative diseases: a new perspective on the challenges. J. Genet. 88, 417-424.

Jin Z.B., Okamoto S., Osakada F., Homma K., Assawachananont J., Hirami Y., Iwata T. and Takahashi M. (2011). Modeling retinal degeneration using patient-specific induced pluripotent stem cells. PLoS One 6, e17084.

Jin Z.B., Okamoto S., Xiang P. and Takahashi M. (2012). Integration-free induced pluripotent stem cells derived from retinitis pigmentosa patient for disease modeling. Stem Cells Transl. Med. 1, 503-509.

Jin Z.B., Gao M.L., Deng W.L., Wu K.C., Sugita S., Mandai M. and Takahashi M. (2019). Stemming retinal regeneration with pluripotent stem cells. Prog. Retin. Eye Res. 69, 38-56.

Li G., Gao G., Wang P., Song X., Xu P., Xie B., Zhou T., Pan G., Peng

F., Zhang Q., Ge J. and Zhong X. (2019). Generation and characterization of induced pluripotent stem cells and retinal organoids from a Leber's Congenital Amaurosis patient with novel RPE65 mutations. Front. Mol. Neurosci. 12, 212.

Liu H., Zhang Y., Zhang Y.Y., Li Y.P., Hua Z.Q., Zhang C.J., Wu K.C., Yu F., Zhang Y., Su J. and Jin Z.B. (2020). Human embryonic stem cell-derived organoid retinoblastoma reveals a cancerous origin. Proc. Natl. Acad. Sci. USA 117, 33628-33638.

Lukovic D., Artero Castro A., Delgado A.B., Bernal Mde L., Luna Pelaez N., Díez Lloret A., Perez Espejo R., Kamenarova K., Fernández Sánchez L., Cuenca N., Cortón M., Avila Fernandez A., Sorkio A., Skottman H., Ayuso C., Erceg S. and Bhattacharya S.S. (2015). Human iPSC derived disease model of MERTK-associated retinitis pigmentosa. Sci. Rep. 5, 12910.

Lukovic D., Artero Castro A., Kaya K.D., Munezero D., Gieser L., Davó-Martínez C., Corton M., Cuenca N., Swaroop A., Ramamurthy V., Ayuso C. and Erceg S. (2020). Retinal organoids derived from hiPSCs of an AIPL1-LCA patient maintain cytoarchitecture despite reduced levels of mutant AIPL1. Sci. Rep. 10, 5426.

Pan D., Xia X.X., Zhou H., Jin S.Q., Lu Y.Y., Liu H., Gao M.L. and Jin Z.B. (2020). COCO enhances the efficiency of photoreceptor precursor differentiation in early human embryonic stem cell-derived retinal organoids. Stem Cell Res. Ther. 11, 366.

Parfitt D.A., Lane A., Ramsden C.M., Carr A.J., Munro P.M., Jovanovic K., Schwarz N., Kanuga N., Muthiah M.N., Hull S., Gallo J.M., da Cruz L., Moore A.T., Hardcastle A.J., Coffey P.J. and Cheetham M.E. (2016). Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. Cell Stem Cell 18, 769-781.

Pausch P., Al-Shayeb B., Bisom-Rapp E., Tsuchida C.A., Li Z., Cress B.F., Knott G. J., Jacobsen S.E., Banfield J.F. and Doudna J.A. (2020). CRISPR-CasΦ from huge phages is a hypercompact genome editor. Science 369, 333-337.

Ran X., Cai W.J., Huang X.F., Liu Q., Lu F., Qu J., Wu J.Y. and Jin Z.B. (2014). 'RetinoGenetics': a comprehensive mutation database for genes related to inherited retinal degeneration. Database. 2014, bau047.

Sanjurjo-Soriano C., Erkilic N., Baux D., Mamaeva D., Hamel C.P., Meunier I., Roux A.F. and Kalatzis V. (2020). Genome editing in patient iPSCs corrects the most prevalent USH2A mutations and reveals intriguing mutant mRNA expression profiles. Mol. Ther. Methods Clin. Dev. 17, 156-173.

Shimada H., Lu Q., Insinna-Kettenhofen C., Nagashima K., English M.A., Semler E.M., Mahgerefteh J., Cideciyan A.V., Li T., Brooks B.P., Gunay-Aygun M., Jacobson S.G., Cogliati T., Westlake C.J. and Swaroop A. (2017). *In vitro* modeling using ciliopathy-patient-derived cells reveals distinct cilia dysfunctions caused by CEP290 mutations. Cell Rep. 20, 384-396.

Sugita S., Iwasaki Y., Makabe K., Kamao H., Mandai M., Shiina T., Ogasawara K., Hirami Y., Kurimoto Y. and Takahashi M. (2016). Successful transplantation of retinal pigment epithelial cells from MHC homozygote iPSCs in MHC-matched models. Stem Cell Rep. 7, 635-648.

Tang Z.H., Chen J.R., Zheng J., Shi H.S., Ding J., Qian X.D., Zhang C., Chen J.L., Wang C.C., Li L., Chen J.Z., Yin S.K., Huang T.S., Chen P., Guan M.X. and Wang J.F. (2016). Genetic correction of induced pluripotent stem cells from a deaf patient with MYO7A mutation results in morphologic and functional recovery of the derived hair cell-like cells. Stem Cells Transl. Med. 5, 561-571.

Tsang S.H. and Sharma T. (2018). Leber congenital amaurosis. Adv. Exp. Med. Biol. 1085, 131-137.

Tucker B.A., Mullins R.F., Streb L.M., Anfinson K., Eyestone M.E., Kaalberg E., Riker M.J., Drack A.V., Braun T.A. and Stone E.M. (2013). Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. Elife 2, e00824.

Wahlin K.J., Maruotti J.A., Sripathi S.R., Ball J., Angueyra J.M., Kim C., Grebe R., Li W., Jones B.W. and Zack D.J. (2017). Photoreceptor outer segment-like structures in long-term 3D retinas from human pluripotent stem cells. Sci. Rep. 7, 766.

Zhang J.P., Li X.L., Li G.H., Chen W., Arakaki C., Botimer G.D., Baylink D., Zhang L., Wen W., Fu Y.W., Xu J., Chun N., Yuan W., Cheng T. and Zhang X.B. (2017). Efficient precise knockin with a double cut HDR donor after CRISPR/Cas9-mediated double-stranded DNA cleavage. Genome Biol. 18, 35.

Zhang C.J., Ma Y. and Jin Z.B. (2021). The road to restore vision with photoreceptor regeneration. Exp. Eye Res. 202, 108283.

Zhong X., Gutierrez C., Xue T., Hampton C., Vergara M.N., Cao L.H., Peters A., Park T.S., Zambidis E.T., Meyer J.S., Gamm D.M., Yau K.W. and Canto-Soler M.V. (2014). Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. Nat. Commun. 5, 4047.

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