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Minireview

RETINITIS PIGMENTOSA: AN UPDATE ON ANIMAL MODELS AND GENOME EDITING TECHNOLOGIES

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Abstract

Retinitis pigmentosa (RP) is a group of genetically heterogeneous diseases with mutations in more than 80 genes and different patterns of inheritance. Various factors make diagnosis of this blinding disease complex and lack of definitive cure increases the urgency to identify new drugs.

Animal models and innovative genome editing technologies are a powerful tools for the study of the molecular mechanisms leading to photoreceptor degeneration, feature of this disease.

Furthermore, they are the basis for therapeutic strategies aimed to retard or even stop disease progression.

In this review, the authors focus on animal models, describing their advantages and disadvantages, and CRISPR/Cas9 applications.

Keywords

Retinitis pigmentosa, retinal degeneration, eye, genome editing, gene therapy

Retinitis pigmentosa

Retinitis pigmentosa (RP, OMIM #600105) is a group of inherited vision disorders in which degeneration of rod photoreceptors, responsible for night vision, is more prominent than that of cone photoreceptors, which mediate day light and central vision. RP is an uncommon condition affecting about 1 in 4,000 people in the United States, and 1-5/10.000 in Italy [1]. The disease leading to progressive loss of the photoreceptors and retinal pigment epithelium, results in blindness usually after several decades, although in extreme cases a rapid development is observed.

Inheritance patterns

RP progression rate and age of onset depend on numerous factors, the principle factor being genetic transmission pattern [2]. The disorder may be inherited as an autosomal dominant (25%), autosomal recessive (39%), or X-linked recessive (4%) trait; maternal, mitochondrial inheritance, digenism and uniparental isodisomy

have also been described [3]. Today, mutations in more than 80 genes result associated to RP. Although the relative prevalence of each form varies between populations, a major proportion (40% or higher) of patients represents isolated or simplex cases (sRP) with unaffected carrier parents. Alternatively, it is estimated that de novo mutations in dominant inheritance genes are responsible for at least 1–2% of isolated cases.

Genetic analysis

Conventional methods for identification of both RP mutations and novel RP genes consists in: DNA extraction, exome sequencing by Next generation sequencing (NGS), detection and selection of candidate genes retrieved from the NCBI Gene database (http://www.ncbi.nlm.nih.gov/gene) and with the use of pathway analysis software like Cytoscape (http://www.cytoscape.org) and Ingenuity Pathway Analysis (http://www.ingenuity.com/products/ipa).
Subsequently mutations validation is processed by Sanger sequencing.
The final step is the use of animal models to investigate the role of specific gene mutations and the resulting cellular defects that finally lead to photoreceptor cell death.

Animal models

Several animal models of RP (mouse, rat, zebrafish, chicken and dog) [4-6] are available and their study allows a better understanding of the pathogenesis of the disease and to develope therapeutic strategies [7]. For example, mouse models carrying rhodopsin mutations mimicking autosomal dominant RP were generated [8,9].
More recently, use of Briard dog as model of congenital stationary night blindness has allowed interesting finding on cells the retinal pigment epithelium pigment (RPE) where the protein RPE65 is essential for vitamin A metabolism [10,11].
Although rodent models have proved to be important into visual sciences, they may not always be the ideal system for research on human vision [12,13]. Rodents have evolved to live a primarily nocturnal lifestyle, and hence utilize rods for their vision. They are also dichromatic and only possess short and medium wavelength cone PRs. Their vision therefore is very different from the trichromatic humans who follow a diurnal lifestyle. Rodents also lack calycal processes in photoreceptors which are found in human. Other animal models suchas dog, cat and pig are becoming increasing popular, not only to study disease mechanisms but also to assess the safety and efficacy of treatments in clinical trials. Among these, mutant dog models for PDE6β, PDE6α, RPE65, RPGR, RPGRIP1, RHO, RD3 and NPHP4 show strong genotypic and phenotypic correlations with human patients [14].
Transgenic pig is also emerging as an alternative animal model. In fact, in many aspects the pig eye more closely resembles the human eye compared to the mouse. Additionally, pig eyes are well suited for retinal injections for gene therapy. So far, pig models for autosomal dominant retinitis pigmentosa, expressing human rhodopsin mutations have been created [15].
Although there are several advantages to using large animal models, maintaining animal facilities is expensive and the phenotypes can be slow to develop.
For this reason, zebrafish (Danio Rerio) has become an attractive model of choice, given the ease with which genetic manipulations can be carried out. Zebrafish has a short generation time of 2–4 months, with a single mating pair producing large clutches of fertilized eggs (~100–200) at weekly intervals. Fertilization is ex utero, and the developing embryo is transparent, facilitating the visualization of early organogenesis and amenability to embryological manipulation.
Furthermore, seventy per cent of human genes have at least one zebrafish orthologue, with 84% of known human disease and number of chromosomes in zebrafish is similar to that of human (25 and 23, respectively). Zebrafish eyes are large relative to the overall size of the fish, making eye bud manipulation feasible during early embryogenesis. Zebrafish are visually

responsive by 72 h post fertilization (h.p.f.), by which time the retina resembles adult retinal morphology that is anatomically and functionally similar to humans [16,17]. The zebrafish retinal architecture presents photoreceptor subtypes arranged in a highly organized mosaic and, due to the diurnal nature of zebrafish, it is cone-rich similarly to the human macula.

Early studies in zebrafish used forward genetics screens and several retinal degeneration associated gene mutations were thus identified [18,19]. A zebrafish RP line carrying the truncation mutation Q344X originally identified in patients who suffered from autosomal dominant form of RP was created [20]. Another prominent model of RP is the *pde6c* zebrafish mutant [21,22]. It carries a splice-site mutation in *pde6c*, a subunit of cone-specific phosphodiesterase.

More recently, genetic manipulation techniques such as zinc finger nucleases (ZFNs) and transcription-activator–like effector nucleases (TALENs) have proven to be successful gene editing techniques but low efficiency and specificity limit their application.

A new genome editing technology, CRISPR/Cas9 has proven to be a powerful and versatile tool for genome engineering because can edit genome much more efficiently and specifically.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems have evolved in bacteria and archaea as a defense mechanism to silence foreign nucleic acids of viruses and plasmids.

These genome editing technologies are based on engineered endonucleases tha tenable the induction of targeted DNA double-strand breaks (DSBs) at specific sites. Once DNA DSBs occur, the cleaved DNA is repaired by non homologous end joining (NHEJ) or homology directed repair (HDR).

Engineered CRISPR systems contain two components [23]: a guide RNA (gRNA or sgRNA) and a CRISPR associated endonuclease (Casprotein). The gRNAis a short synthetic RNA composed of a scaffold sequence necessary for Cas-binding, that direct Cas9 to the genetic locus of interests gRNA.

CRISPR/Cas9 technology is a useful tool to generate RP animal models. Homozygous knock-in mice with the p.Leu135Pro variant, got with this technology, is able to mimic phenotypes of RP, as progressive photoreceptor degeneration and dysfunction of the rod photoreceptors [24]. Similarly CRISPR/Cas9 allowed to reveal causative mutation in a preclinical model of Retinitis pigmentosa[25].

Given its ability to edit target genome specifically and efficiently, CRISPR/Cas9 is becoming a powerful tool for gene therapeutic strategies in RP.

For example, *in vivo* editing of the human mutant Rhodopsin gene, which is a common cause of RP, by application of CRISPR/Cas9 system was reported [26].

More recently, a single subretinal injection of gRNA/Cas9 plasmid in combination with electroporation, was able to prevent retinal degeneration and improve visual function in the S334ter-3 rat model of autosomal dominant Retinitis pigmentosa.

Conclusions

In this review, studies on animal models of RP and novel genome editing technologies such as CRISPR/Cas9 are summarized.

As briefly reported, animal models play a fundamental role in the basic research for understanding the molecular mechanism leading to photoreceptors degeneration and for therapeutic approaches aimed at delaying or stopping the progression of the disease.

In particular, the authors focus on numerous advantages offered by Zebrafish model in eye-disease research compared to other animal models.

Finally, CRISPR/Cas9 application in generating animal models as well as gene therapy of Retinitis pigmentosa (RP) vas briefly described.

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