Commentary: CRISPR gene editing for inherited retinal dystrophies: Towards clinical translation

The clustered regularly interspaced short palindromic repeats (CRISPR) system won the Nobel Prize in 2020. What makes this technology intriguing is the ability of the CRISPR/Cas9 effector molecules to identify and bind to specific areas of the DNA, and make cuts or edits in those areas. While there were previous methods described to edit DNA (TALENs, Zinc Finger Nucleases), CRISPR has made the gene editing technology deliverable in terms of size and efficiency.

Inherited retinal dystrophies (IRDs) include conditions many of which do not have a definitive therapy yet. Multiple approaches, including cell-based therapy, gene augmentation therapy, bionic implants, hold the future of therapies for IRDs and have been described.[1] In this section, we dive deeper into CRISPR gene editing in IRDs.

The CRISPR effector molecules consist of the Cas9 enzyme, which is able to cleave the DNA, and a guide RNA, which is complementary to the targeted DNA mutation. Together, this complex is called the ribonucleoprotein (RNP) complex. Once the RNP binds to the target DNA, it causes double-stranded breaks (DSB) in the DNA, adjacent to areas of the DNA sequence called the protospacer adjacent motif (PAM). Subsequently, the inherent DNA repair mechanism of the cell, either homology directed repair (HDR) or non-homologous end joining (NHEJ), are able to repair the DNA, thereby correcting the mutation.[2]

However, gene editing using CRISPR is not without certain drawbacks. The most significant is the possibility of the CRISPR RNP complex to cause DNA editing at non-desired areas of the genome, called off-target effects. To mitigate this, advances and variations of the CRISPR gene editing have been developed: CRISPR base editing and prime editing.[3] CRISPR base editing consists of a catalytically deactivated Cas9 enzyme, which does not cause breaks in the DNA, but has a deaminase domain, which can convert an adenine base to guanine (adenine base editor or ABE) or cytosine to thymidine (cytosine base editor or CBE). Therefore, it is suitable to target transition mutations.

Smaller genes, that is, genes consisting of lesser base pairs (bps) are amenable to gene (augmentation) therapy because of packaging capacity of the viral vector adeno-associated virus (AAV) of 5 kbps. However, for genes having a larger size, packaging the entire wild type gene into the AAV vector may not always be possible. In addition, gene therapy is suitable for recessive mutations, wherein delivering a single wild type (or correct) copy of the gene may be sufficient for improvement in visual function. However, for dominant mutations, gene editing approach may be more desirable.[4]

Mutations affecting over 50 genes can cause retinitis pigmentosa itself, and within each gene, the mutations between affected individuals tend to be different. This makes targeted therapy toward a particular mutation a challenge, as well as, perhaps, the direction towards developing therapy.

Toward this effect, CRISPR-based gene editing entered clinical trials, the BRILLIANCE trial in 2019, for mutations affecting the *CEP290* gene which causes Leber congenital amaurosis-10 (LCA-10). The therapeutic agent EDIT-101 is injected into the subretinal space. It is able to edit the cryptic mutation in the intron 26 (IVS26 mutation), leading to an aberrant splice donor site which results in a truncated protein, thereby restoring the photoreceptor protein formation.[5,6]

CRISPR based therapy, to edit the *USH2A* gene, is also been researched. The therapy in development called EDIT -102, uses a gene editing approach to remove exon 13 which harbors the mutation and leads to an abnormal truncated protein. The edited protein, albeit shorter, is functional.[7]

Gene editing approach, similarly, is also being developed for the *RHO* gene, called EDIT-103. In this, the developers have used a "knockout and replace" strategy in which the mutant segment of the *RHO* gene is deleted using CRISPR, followed by gene augmentation by providing the wild type or normal gene.[8]

While targeting specific mutations can be overall challenging, a strategy is being developed to target another protein, neural retina-specific leucine zipper (NRL) protein, a rod fate determinant during photoreceptor development. Following *NRL* gene (coding for the above protein) disruption, rods gain partial features of cones. These rods show improved survival in the presence of mutations in rod-specific genes, consequently preventing secondary cone degeneration; this was shown in Rhodopsin knockout mice.[9]

The above are a few examples of CRISPR gene editing for IRDs. As the future for developing therapies towards IRDs looks bright, many more strategies are under development by different groups. In short, even while using CRISPR gene editing, various strategies are being employed to address a particular mutation, depending on the size of the gene, the inheritance pattern of the mutation, and the location of the mutation within the gene.

In conclusion, the type of therapy which is suitable for an individual affected by IRDs will significantly depend on the stage of presentation (or degree of vision loss, retinal changes), as well as the affected mutations. A genomic sequencing of all patients with IRDs is strongly warranted to address therapy options, prognosticate progression of IRD as well as from a family planning perspective. In cases with advanced IRD, significant vision and photoreceptor loss, regenerative cell-based therapies, and bionic eye implants are likely to play a role. All the same, gene therapy and gene editing approaches are likely more desirable at relatively early stages to prevent further progression of disease. Finally, since the spectrum of mutations affecting a population are likely to be affected by demographics and geography, it is important that we consider developing therapies suited to that region.

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