**Gene Therapy, CRISPR/Cas9’s Applications in the Medical Field**

Abstract

“CRISPR/Cas9-mediated glycolate oxidase disruption is an efﬁcacious and safe treatment for primary hyperoxaluria type I” (Barbiera et al. 2018), proposes, exactly as the title says, that the CRISPR/Cas9 system is a viable option for treating type 1 primary hyperoxaluria. It lays the groundwork for the proposal by mentioning several other genetic diseases that the CRISPR/Cas9 system has been used to treat, as well as the specifics of the gene defect that causes PH1, the AGXT gene. It states that en vivo correction is not a viable treatment option for the disease due to the lack of selective advantage for the modified cells and proposes a different approach. By targeting genes that express nonessential enzymes, the team believes that it can significantly reduces the oxalate buildup that causes the symptoms of the disease. This hypothesis is further confirmed by positive test results in mice. Human trials of GO silencing by this method were attempted in 2016, but there were some definite areas for improvement. While effective, the treatment currently needs to be administered several times to combat long term effects, and GO silencing using small molecules doesn’t block the enzyme completely, meaning that oxalate buildup still occurs, but a much slower rate, reducing patients’ risk of developing end stage renal disease (ESRD), an affliction of the kidneys that can be very fatal. The major questions for researchers hoping to take this topic further concern whether there is a method for more effectively inhibiting the glycolate oxidase enzyme while maintaining the standard of safety that the currently proposed procedure has.

Introduction

CRISPR/Cas9 or Clustered regularly interspaced short palindromic repeats/ CRISPR associated protein 9, is a system employed gene modification that uses a “programmable single guide RNA strand to guide Cas9 endonuclease to a specific locus on a DNA strand and induce Double Strand Breaks or DSBs.” The broken strands are repaired via homologous recombination or, if inducing mutations is the goal, through non-homologous end joining. Cas9 itself is a protein first found in *S. Pyrogenes*, as part of its immune system. CRISPR associated proteins are used in the bacterial and archaeal immune response to recognize and cleave viral DNA within cells, thus preventing expression of viral DNA that would be harmful to the cell’s normal functioning.

The CRISPR/Cas9 system has revolutionized the field of gene editing by making gene editing and gene therapy much more viable and efficient options for widespread use. With the advent of the CRISPR/Cas9 modification many new methods of treatment have arisen for various ailments. The system is being used to attempt to fight HIV. In addition to combating the spread of viruses, treatments that employ the CRISPR/Cas9 system are being developed to target several different genetic diseases such as hemophilia, Duchenne muscular dystrophy, and, as is the case with this article, primary hyperoxalurias. Primary hyperoxalurias are a group of genetic diseases characterized by a common cause, oxalate overproduction due to defects in the enzymes that control glyoxylate metabolism. While oxalate is normally present in the body, it is usually in much smaller amounts than in those affected by primary hyperoxaluria and is filtered harmlessly through the kidneys and out of the body through urination, in patients with PH however the buildup of oxalate in the renal system can cause a variety of health conditions culminating in end stage renal disease. There are three types of primary hyperoxaluria, named PH1, PH2, and PH3. The most prevalent type is PH1, a life-threatening affliction that affects the AGXT gene, which regulates the expression of alanine glyoxylate aminotransferase. Alanine glyoxylate aminotransferase’s function is to convert the relatively insoluble glyoxylate into glycine, a much less harmful compound for the kidneys. As the disease progresses it causes urolithiasis (presence of kidney stones in the urinary tract), nephrocalcinosis (buildup of calcium in the kidneys), ESRD (long-term kidney damage leading to kidney failure), and eventually death.

The research covered in the article proposes that CRISPR/Cas9 might be an efficient treatment method for the type 1 variant of this disease, Glycolate oxidase (GO) silencing. A method in which the CRISPR/Cas 9 system is used to silence the enzyme that oxidizes glycolate in to glyoxylate. This lessens the effects of Primary hyperoxaluria type 1 because glycolate is a much more soluble molecule than glyoxylate and thus can be filtered out through the urine without damaging the kidneys, much in the way glycine is in an individual unaffected by primary hyperoxaluria type 1. The research team calls this method substrate reduction therapy. Until a method that more effectively silences the GO gene, this method is the best hope for people with primary hyperoxaluria type 1 of living a longer, healthier life.

RECENT DEVELOPMENTS

Clinical trials on both mice and humans have been attempted. The human clinical trials are still ongoing, but the clinically tested GO silenced mice showed promising results. Glyoxylate buildup in the mice was dramatically decreased, going forward, this method of substrate reduction therapy, or the treatment of metabolic diseases by removing the chemical compounds that get converted into harmful substances within the body. According to the research team, during animal trials the mice showed no signs of nephrocalcinosis no reduced weight level in any of the mice, and a lack of toxicity and glyoxylate/oxalate buildup in urine samples (Barbiera et al. 2018).

Discussion

With the leaps and bounds made in the field of gene modification within the last decade, many new possibilities for treatment of genetic diseases have arisen. As our understanding of Genes, genetic disease, genetic modification, and the CRISPR/Cas 9 develops the possible implications of this technology will continue to provide newer, safer, and more powerful treatments. The animal tests have shown promising results, and the test overall has proven that, in mice that Glycolate oxidase silencing with the CRISPR/Cas9 System is an, as the team puts it, “efficacious and safe” treatment option for type 1 primary hyperoxaluria. While this study is promising and its results are valid, I would be interested in seeing the results of the human trials due to the major anatomical, biological, and more specifically genetic differences between the human body and that of a mouse. I’d also be interested in seeing the other possible genetic metabolic disorders this research could be applied to, perhaps primary hyperoxaluria type 2 or type 3?

References

CRISPR/Cas9-mediated glycolate oxidase disruption is an efﬁcacious and safe treatment for primary hyperoxaluria type I; December 21, 2018; Nature Communications Vol. 9; Pgs. 1,2,7; Barberia, Miren; Betancor, Isabel; Castro-Labrador, Laura; Gonzalez-Aseguinolaza, Gloria; Lara-Astiaso, David; Martin-Higueras, Cristina; Martinez-Turrillas, Rebeca; Olagüe, Cristina; Prosper, Felipe; Rodriguez, Saray; Rodriguez-Madoz, Juan R.; Salido, Eduardo; Torella, Laura; Vales, Africa; Zabaleta, Nerea; Zapata-Linares, Natalia