Kam Taghizadeh

Gene Editing for Correcting Pathogenic Gene Mutations that Give Rise to Heritable Diseases Such as Marfan Syndrome

Introduction

There are around 10,000 genetically related diseases that have been identified; of those, less than 6% have specific treatments (Zeng 2018). One such genetically related disease which affects 1 in 5000 people is Marfan Syndrome. This syndrome results in the malformation of one's connective tissue, which is extremely important in the development of the body. Some of the features that are included in this genetic disease include: aortic dilation, an abnormally curved spine, elongated/thin build, abnormally long arms, legs, and fingers (Table 1) (The Marfan Foundation 2014). To prevent the transmission of such mutant alleles, which lead to certain genetic diseases such as this one, they would need to get a Preimplantation genetic diagnosis (PGD). A PGD, however, is still prone to making diagnostic errors. There's strong evidence that gene editing will become a useful tool in the future once it is fully safe to utilize. In the previous years, researchers have used genome editing to manipulate an organism's DNA by using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9). This technique is performed by either inserting, deleting, or altering the genetic material in DNA (NIH 2020). In a study conducted by Zeng and colleagues (2018), researchers have successfully corrected the pathogenic FBN-1 mutation that results in Marfan Syndrome by using CRISPR/Cas 9 along with a base editing (BE) system.

Morphogenesis and Anatomy

Marfan Syndrome is a disease which alters the normal formation of connective tissue. During embryogenesis, mesenchymal cells which are loose and fluid will easily migrate and give rise to connective tissue. Undifferentiated mesenchymal cells are able to give rise to various forms of connective tissue, such as bones and cartilage (specialized connective tissue), along with tendons and ligaments (dense regular connective tissue) (Nassari 2017). Connective tissue of the head arises from the neural crest cells, while connective tissue of the trunk arises from somites, and connective tissue of the limbs originate from the lateral plate mesoderm (Nassari 2017). In specialized connective tissue it is observed that the precursors of the mesenchyme differentiate to either chondrocyte (cartilage) or osteoblast (bone) to initiate the formation of the skeleton (Nassari 2017). The transcription factors that are used to perform this function are Sox, which is integral for chondrocyte differentiation and Runx2, which is important for bone specification (Nassari 2017). It is important to note that fibrillin-1 is the backbone of microfibrils, which are structural elements that are vastly found within the connective tissues (Keene 1997). By using immunohistochemistry, Keene and colleagues (1997), studied the presence of FBN-1 early fetal development of connective tissue. At 10-11 weeks of gestation, FBN-1 is only found in loose connective tissue that is found around skeletal muscle and the tendons of developing limbs. At 16 weeks, FBN-1 is seen in a large amount in developing digits and limbs. In week 20, it is present as a loose network of fibers within the cartilage matrix. Up to

the point of early adolescence, fibrillin-1 is found in loose microfibrils within cartilage, while during late adolescence, fibrillin-1 is found in broad banded fibers surrounding cartilage (Keene 1997).

Molecular and Cell Biology

Fibrillin-1 provides tensile strength and elasticity to tissues. It does so by conducting mechanical forces across them. However, it also regulates the activity of nearby cells by interacting with integrin (transmembrane receptors that facilitate adhesion to the extracellular matrix) and syndecan receptors (transmembrane proteoglycan that serves as a co-receptor to growth factors) (Ramirez 2017). It also regulates the activity of local TGFβ (transforming growth factor-Beta: responsible for cell growth, differentiation, proliferation, and apoptosis) (Ramirez 2017). To suppress the activity of TGFβ, fibrillin-1 interacts with latent TGFβ proteins (LTBPs), which are composed within large latent complexes (LLCs), which contain TGFβ dimers that are non covalently bound to latent associated peptides (LAPs) (Ramirez 2017). The interaction of fibrillin-1 to TGFβ proteins and latent TGFβ proteins will signal adhesion of the large latent complex (LLC) to the extracellular matrix (ECM) (Ramirez 2017). The LLC's insertion into the ECM then signals correct concentrations of TGFβ to be readily available for cells or released when there is a need for tissue repair (Ramirez 2017). Mutation in fibrillin-1 would negatively affect the extracellular matrix and local TGFβ protein activity. The failure to bring together the LLC and its various components would result in the over activation of TGFβ proteins. Free TGFβ proteins would then interact with receptors at the cell surface and then phosphorylate smad proteins (which are the main receptors responsible for signal transduction of TGFβ receptors), which would result in their translocation from the cytoplasm to the nucleus, where TGFβ induced transcriptional responses would be initiated (Dietz 2007). This all results in genes that are downstream of TGFβ to result in the symptoms related to Marfan Syndrome. Gene editing will help edit out the mutated gene FBN-1 of fibrillin-1 that results in the over activation of TGFβ proteins (Figure 1). CRISPR/Cas9 are approaches to gene editing that have been used on many occasions (Zeng 2018). Small portions of RNA are created within the lab that act as a guiding sequence that binds to a sequence of interest within the DNA. The RNA, which is created to recognize the target DNA sequence, is also binded to the Cas9 enzyme (Zeng 2018). The cas9 enzyme that the RNA is bound to will then cut the DNA at the targeted sequence. Once the targeted DNA sequence is cut, scientists may use the repair machinery of the cell's own DNA to put the pieces back together, however, this is extremely error-prone. DSBs (double-strand breaks) are what result from DNA sequence cutting, along with the phenomenon of non homologous end joining (NHEJ) (Zeng 2018). This results in off-target mutagenesis and is unwanted. New base editing (BE) technology mitigates the problem of NHEJ and off target mutagenesis. BE is formed by fusing deaminase to Cas9 protein and is highly efficient in gene editing in that it converts bases from "C to T" or "G to A" through homologous recombination without the formation of DSBs (Zeng 2018). There have been several studies that prove the

efficacy of BE in the editing of human embryos. In a test conducted by Zeng and colleagues (2018), genetic correction of the FBN-1 mutation was achieved within heterozygous human embryos. A male with Marfan Syndrome who had symptoms such as flat foot and funnel chest and had a heterozygous mutation for the FBN-1 gene donated samples of his blood and sperm. The sequence around his mutation site was eligible for correction using the BE system that would convert C to T while using a single guiding RNA (sgRNA) that would target this DNA sequence, and they were successful in editing his DNA.

Clinical/Medical Ramifications

The idea of gene editing stems back to the 1950's with the discovery of the double stranded DNA. In the mid 20th century, scientists discovered that DNA sequences are passed from parent to offspring, and that miniscule changes within the nucleotides that are passed on could be the difference between a healthy and diseased individual. Fast forward to 2012, when CRISPR/Cas9 was discovered by Jennifer Doudna and colleagues, where these nucleotides could finally be edited out. Before Crispr/Cas9, other DNA breaking tools were used: ZFN's (zinc finger nucleases) and TALENs (transcription activator-like effector nucleases). ZFNs were used to recognize specific 3-6 nucleotide triplet sequences while TALENs were used to recognize just 1 nucleotide, and is less complex than ZFNs (Yeadon 2014). CRISPR/Cas9 has proven to be a simplified method in targeting the desired DNA sequence. Rather than relying on protein/DNA recognition, Crispr uses ribonucleotide complexes to target the sequences, and RNA's are much cheaper and simple to design. There have been countless gene editing on crop plants, farm animals, and model laboratory organisms such as mice, rats, and non-human primates. However, there has been less research on human embryos such as the correction of the mutated FBN-1 gene found in Marfan syndrome patients. In 2015, a restriction on the use of CRISPR for the editing of human embryos was imposed by a group of scientists including Jennifer Doudna until the safety and ethical implications have been fully considered. One lab in China, however, was the first to genetically edit twin baby girls using CRISPR in November 2018. It was He Jiankui's intention to make the twins more resistant to HIV. However, the edited genes may cause future adverse side effects. This caused international outrage as it was deemed unethical by multiple scientists (Begley 2019). Most scientists believe that it is too soon to be editing human embryos, since it is still unknown how safe CRISPR editing is. This prompted the International Commission on the Clinical Use of Human Germline Genome Editing to propose stricter guidelines for the DNA editing of human embryos.

Clinical research/clinical trials update

New US experiments are working towards making more advances within CRISPR in order to create DNA edited human embryos to prevent inherited diseases.
Dieti Egli, a researcher from Columbia University is conducting experiments for "research purposes only" in order to determine the efficacy and safety of using CRISPR for gene edited

embryos. He does this by stopping the development of modified embryos after just one day in order to study them (Stein 2019).

Impact on Society

The ability to edit pathogenic genes that results in heritable diseases is revolutionary for the world of genetic engineering (Fridovich-Keil 2019).To edit the mutated FBN-1 gene of prospective Marfan Syndrome parents and have children who are free of the mutation will relieve them greatly. Gene editing will alleviate the anxieties that surround patients with heritable diseases that would like to have children. However, this extreme advancement in gene editing has brought up multiple questions regarding ethical and societal implications. Many wonder whether it is ethical to use this tool to alter traits such as beauty, height, intelligence, weight, and many others. They also wonder if the advancements in gene editing of embryos will be available through insurance. Two professors at the Worcester Polytechnic Institute, Patricia Stapleton and Natalie Farny, say that "there could be a disproportionate disease burden where the lower class would be more susceptible" (Ishkanian 2017).

Opinion Piece

I believe that CRISPR should be used to edit out pathogenic genes that lead to heritable diseases once it is completely safe to do so. If it is possible for the 10,000 heritable diseases that have been discovered to be eradicated, then there will be a world of healthier people that are able to function and lead normal lives. This will not only result in the elevation of those individual's lives, but a benefit to society as well, as those who do not have underlying health conditions are able to contribute to their surroundings in a greater way. With this being said, gene editing of pathogenic genes should be under insurance, so there is not an even greater gap between the rich and the poor. I do not believe that CRISPR should be used in order to edit one's traits such as beauty, height, intelligence, etc. because it will create a world of superficiality, in which much attention will be put onto one's looks rather than their true being on the inside.

Conclusion

CRISPR/Cas9 editing tools are a great advancement to society for the prevention of heritable diseases. As seen in the editing of the FBN-1 mutated gene of fibrillin-1 by using Crispr and the BE system, it is a possibility for this type of gene editing to become a societal norm in the future. By looking at the normal development of connective tissue within an embryo and comparing that to the development of one who has Marfan Syndrome with less fibrillin-1 due to their mutation in FBN-1, we have seen the molecular and morphogenic applications of the syndrome, and to further that, we have seen the methods in which gene editing can be used to edit the mutation that causes less presence of fibrillin-1 and overactivation of TGFβ protein.

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Table/Figure/Graph

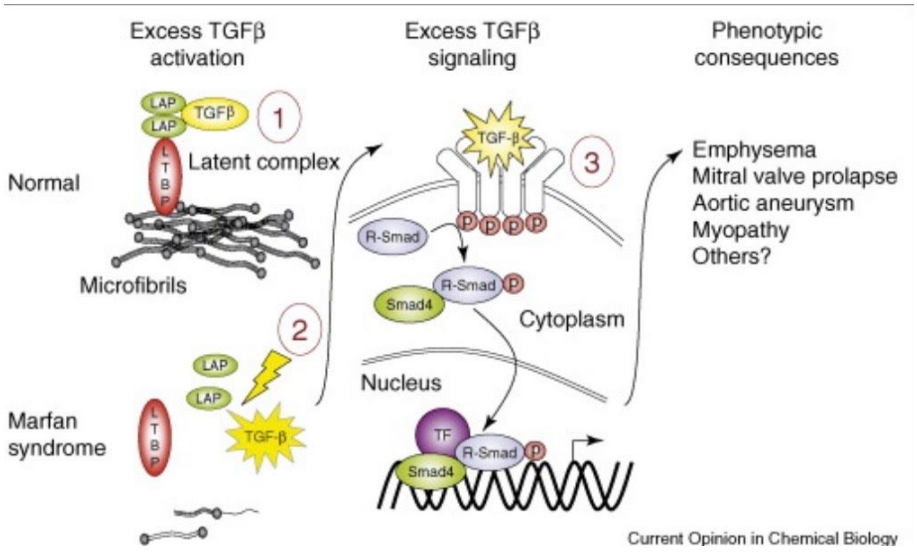

Figure 1: A depiction of normal TGFβ signaling in comparison to abnormal TGFβ signaling. It can be seen here that the latent complex is not formed in the one with Marfan syndrome, causing the over activation of TGFβ.

Zeng Y, Li J, Li G, Huang S, Yu W, Zhang Y, Chen D, Chen J, Liu, J, Huang X. 2018. Correction of the Marfan Syndrome pathogenic FBN1 mutation by base editing in human cells and heterozygous embryos. Mol. Therapy. 26(11):2631-2637.

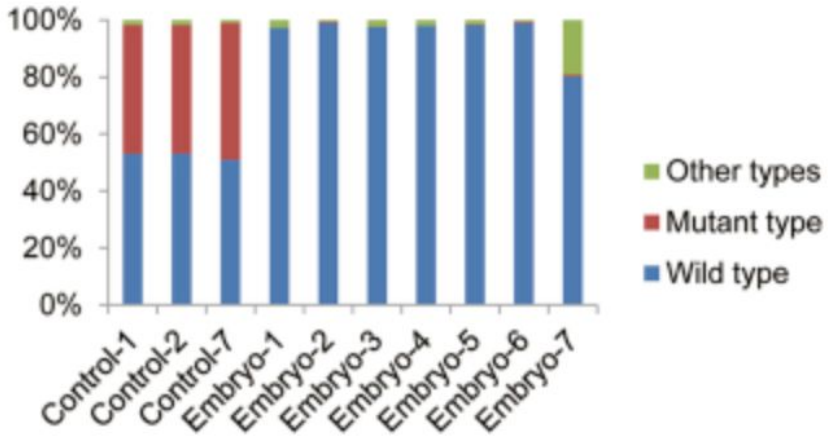

Figure 2: This is a graph that shows the analysis of 3 control DNA which are mutated, and 7 corrected DNA of heterozygous embryos. All of the DNA was extracted and amplified by PCR.

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Table 1: Diagnostic Criteria for Marfan Syndrome.

Stuart AG, Williams A. 2007. Marfan's syndrome and the heart. Archives of Disease in Childhood. 92(4):351-6.

System	Major criteria	Minor criteria
Family history	Independent diagnosis in parent, child or sibling	None
Genetics	Mutation FBN1	None
Cardiovascular	Aortic root dilatation, dissection of ascending aorta	Mitral valve prolapse, calcification of the mitral valve (<40 years), dilatation of the pulmonary artery, dilatation/dissection of descending aorta
Ocular	Ectopia lentis	2 needed of the following: flat cornea elongated globe myopia
Skeletal	At least 4 of the following: pectus excavatum needing surgery, pectus carinatum, pes planus, positive wrist or thumb sign, scoliosis >20° or spondylolisthesis, armspan-height ratio >1.05, protrusio acetabulae, diminished extension elbows (<170°)	For the skeletal system to be involved 2–3 major, or 1 major and 2 minor signs should be present: moderate pectus excavatum, high arched palate, typical facial features, joint hypermobility
Pulmonary		Spontaneous pneumothorax, apical bulla
Skin		Striae, recurrent or incisional herniae
Central nervous system	Lumbosacral dural ectasia	

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