Ind J Clin Biochem (Jan-Mar 2018) 33(1):1–4
https://doi.org/10.1007/s12291-017-0720-z

EDITORIAL

Gene Editing in Clinical Practice

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Received: 3 November 2017 / Accepted: 22 November 2017 / Published online: 20 December 2017

Progressive changes in biotechnology has led to invention of a most recent used technique that makes changes to specific DNA sequences in the genome of living organism. Here by means of engineered nucleases or molecular scissors the defective DNA is inserted, deleted or replaced. The advent of gene editing technique has generated more excitement in biology and medicine than any discovery since polymerase chain reaction. This technique has opened new avenues for multiple applications both in basic research such as cancer research, synthetic biology as well as assisted in gene therapy using induced pluripotent stem cells [1].

Amongst some genetic tools, the most talked about gene editing tool is CASPR-Cas9 which has made the headlines all over the world because of its potential to change the human race. Using this tool it has transformed the way DNA is manipulated and modified. First demonstrated in 2013 [2] it is based on a system bacterium use to defend themselves against viruses.

One would wonder what this amazing gene editing tool consists of and how does it function? CRISPR "spacer" sequences are transcribed into short RNA sequences ("CRISPR RNAs" or "crRNAs") capable of guiding the system to matching sequences of DNA. When the target DNA is found, Cas9—one of the enzymes produced by the CRISPR system—binds to the DNA and cuts it, shutting the targeted gene off. Also using the splendor of this technique, the defective part of the gene can be easily removed and the faulty part repaired. Using modified versions of Cas9, researchers can activate gene expression instead of cutting the DNA. Using this method, the defective genes are fixed naturally. Once a piece of DNA has been snipped out in a cell natural repair system kicks in repairing the damage. In more advance editing system there is yet another template that takes care of the repair mending the break making it possible to re-write the genetic code. There have been lot of trials ever since by various scientists. In this hot pursuit two American scientists claimed that they had fixed the faulty DNA in an embryo by removing the defective gene of inherited heart condition called Hypertrophic Cardiomyopathy. It is an inherited disease of heart muscle where the muscle wall of the heart becomes thickened. It is assumed to be one amongst thousands of inheritable diseases caused by an error in single gene. The frequency of this disease is 1 in 500 and is revealed only in adulthood. It can cause heart failure and sudden death of apparently healthy people. Recent progress exhibit (CRISPR)—Cas as clustered regularly interspaced short palindromic repeat (CASPR Cas-9), transcription activator—like effecter nucleases (TALENs)and zinc-finger nucleases, that have paved the way for gene editing into clinical practice [3]. This translation is a result of combining high nuclease activity with high specificity and successfully applying this technology in various preclinical disease models. Several clinical gene-editing trials, both ex vivo and in vivo, have been initiated in the past 2 years, including studies that aim to knockout genes as well as to add therapeutic transgenes. These programmable nucleases with high specificity has been translated applying this technology in various preclinical disease models, including infectious disease, primary immunodeficiencies, hemoglobinopathies, hemophilia and muscular dystrophy, Some studies in the

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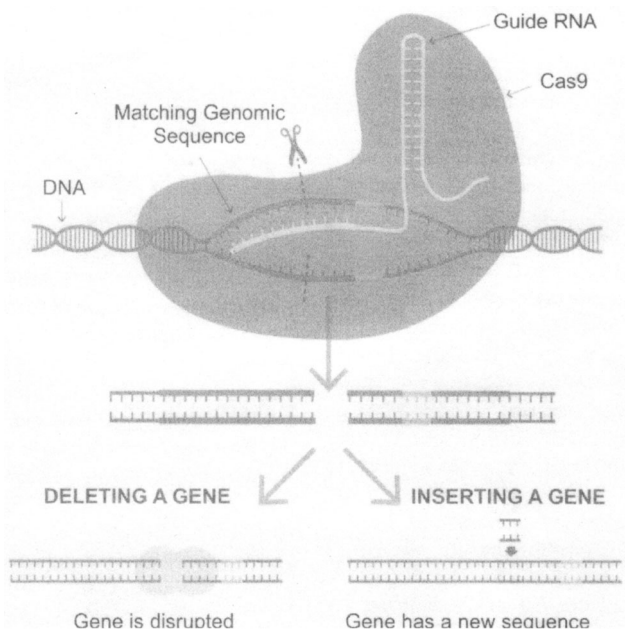
past 2 years have also been aimed to produce knockout genes as well as add therapeutic transgenes. The developing embryo then repaired itself inserting healthy genetic material into the gap. It is being hailed as the world's first technique to fix the faulty DNA in an embryo that could assumed to eradicate genetic 10,000 diseases that are caused by a single rogue gene in the egg or sperm.



A schematic image how CASPR Cas-9 works(courtesy Pros tar newsmagazine)

However growing debates and concerns over such experimentations have emerged like paving the way for designer babies which is highly unethical and according to several experts' state that it would be inappropriate for women to become pregnant with genetically altered embryo. Scientists at prestigious Stanford University have raised concerns over editing an embryo to fix a genetic disease could be portrayed as 'playing God' in an effort to create the best children possible thereby, damaging 'unconditional' love. Eight important organizations including Wellcome Genome campus in Britain have endorsed that at this time it is inappropriate to perform germline editing that culminates in human pregnancy. While Dr. Derek Scholes director of Science policy at the American Society of Human Genetics, said: 'While germ line genome could theoretically be used to prevent a child be born with a genetic disease, its potential use also raises a multitude of scientific, ethical and policy questions.

Targeted genome editing using engineered nucleases introduces new prospects for treating patients with life-threatening conditions. Although many technical hurdles related to nuclease activity and specificity have already been cleared, the delivery of gene-editing tools in vivo still presents a major challenge. The transduction of some organs, such as the liver and the eye, has been accomplished successfully with AAV vectors 28, 37, but much more work has to be invested to target other organs, in particular, muscle and the brain. CRISPR/Cas9 technology provides a powerful tool for targeted gene editing, and has already exhibited its strong potential in the therapeutic development of genetic diseases. Regardless of whether the technology is applied through in vivo administration such as In animal models or with ex vivo delivery such as in iPSCs editing, the use of CRISPR/Cas9 technology for correcting gene defects has shown outstanding progress, raising hopes for therapeutic gene editing in clinical settings Gene editing will herald a new era in the treatment of inherited and acquired diseases. Standardized assays for the evaluation of gene-editing products, including meaningful genotoxicity assays and protocols for testing the specificity and safety of engineered nucleases, will be a priority for further development of the field in the next few years and will, we hope, inspire investigators and regulatory authorities to collaborate to overcome this challenge. The other scientific uses CRISPR might have beyond genome editing is that it will allow scientists to quickly create cell and animal models, which researchers can use to accelerate research into diseases such as cancer and mental illness. In addition, CRISPR is now being developed as a rapid diagnostic. To help encourage this type of research worldwide, Feng Zhang and his team have trained thousands of researchers in the use of CRISPR genome editing technology through direct education and by sharing more than 40,000 CRISPR components with academic laboratories around the world.

Ethical Concerns and Implications of CRISPR–Cas9 Human Germline Editing

While Genome editing of somatic cells, which is at its various clinical stages, is a promising area of therapeutic development the use of CRISPR–Cas9 embryo genome editing that could completely eradicate genetic diseases, scientists have warned that it should be treated with caution. George Daley, a stem-cell biologist at Harvard Medical School in Boston, Massachusetts, stated that even though research reported is a landmark but has a cautionary tale that the technique is not yet ready for testing to eradicate genetic diseases [4]. Since germ line modification causes genetic changes to the embryos, changes that are heritable, this technique can have unpredictable effects to the future generations. Moreover, unethical uses of the technique could emerge from gene editing of the human embryos [4]. Genome editing in human embryos using

CRISPR–Cas9 could have unpredictable effects to the future generations. CRISPR–Cas9 technology could be used for non-therapeutic modifications [5].

Genome editing of the human embryo could hinder the ongoing research that involve gene editing of somatic cells that hold promise for therapeutic development. As rightly pointed out by Lanphier et al. [5], the public outcry about the ethical breach of human embryo genome editing could hinder the promising area of therapeutic development that are involved in making genetic changes in somatic cells and there should be an open discussion around the appropriate action should a compelling case arise for therapeutic benefit of germ line modification [5].

The nuclease may not be as efficient. The nuclease may not necessarily cleave both copies of the target gene or the cells may start dividing before the corrections are completed, resulting in genetic mosaic [5]. Mosaicism is the presence of the populations of somatic cells that are genetically distinct in an organism. Mosaicism is frequently masked. However, mosaicism can cause major phenotypic changes and reveal the expression of lethal genetic mutations [6]. Some of the genetic disorders that result from mosaicism include: Down syndrome, Klinefelter syndrome and Turner syndrome.

Another question that may arise regarding the embryo genome editing using CRISPR–Cas9 editing technology is the fate of the child produced by such technologies? While it is clear that people's informed consent is secured before genetically engineered somatic cells are used in clinical research, it is not clear what information would be needed from the prospective parents to adequately inform them about the risks involved in germ line modification [5]. The scientific community should engage in a dialogue to establish guidelines of research involving genetic modification of human germ cells. The discussions should involve stakeholders in different fields: the general public, scientists, bioethicists, public policy and legal experts. The discussion should make a clear distinction between genome editing in germ cells and in somatic cells. The significant progress being made in clinical development of approaches to cure deleterious diseases should not be impeded by concerns regarding the ethical implications of germline editing [4]. A voluntary moratorium should be called on genetic modification. According to Harris, the side effects of germ line editing should not be used as a justification to call a moratorium on genetic modification of human germ cells. It may be ethically justifiable to make the technique available in clinics. He argues that the genetic disease may be worse than the side effects because people with genetic disease will go on reproducing [7] and their progeny stand a higher chance of inheriting the defective gene responsible for a genetic disorder.

Conclusion

CRISPR/Cas9 is an effective and relatively inexpensive gene editing technique that shows promise as a novel treatment option for genetic disease where currently available treatment options are scarce. This system has been proven to correct mutations in vitro associated with diseases, such as thalassemia and cystic fibrosis, and is currently being used in vivo through phase I clinical trials for cancer therapy and the reduction of viral load in patients with HIV. While CRISPR–Cas9 genome editing technology holds promise to personalized medicine, human genetic modification and the development of new drugs, the technology has raised caution flags. Genome editing technology is a cautionary tale. One can easily get caught up in the glamour of scientific and technological advancement while at the same time oblivious to the ethical ramification of such scientific and technological advancement. Some scientists have expressed concern that human germ line editing has not only crossed the ethical redline; it is also laden with many challenges. The recent research by Chinese scientists using CRISPR–Cas9 to edit the embryo genome was not completely successful. So, it had to be abandoned at its preliminary stage. There were off-target mutations in the genome. These off-target mutations can be deleterious as they can cause cell death and transformation. Consequently, embryo germ line editing could be exploited in non-therapeutic research. For instance, it can be used to produce designer babies by eliminating undesired qualities and replacing them with desired ones. However, genome editing technology should not hinder the promising area of therapeutic development that are involved in making genetic changes in somatic cells. Due to the challenges and ethical concerns raised by CRISPRCas9 genome editing technology, a temporary moratorium should be called on the technology to allow the scientific community and other stakeholders to engage in a broad-based discussion to map the way forward for this technology.

Funding This was self-funded study.

Compliance with Ethical Standards

Conflict of interest Dr. Rama Devi Mittal declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by the author herself.

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