Review Paper

Bioethical Concerns of CRISPR: a Genome Editing Technology

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ABSTRACT

A recent and major scientific achievement in the field of biotechnology is the discovery of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). CRISPR has become one of the most modern and popular tools, mainly due to its low cost and efficiency, which could be used to edit the genome. As a result, this technology holds key to almost every dimension of biomedical and agricultural sciences, and has potential applications in the treatment of viral infections, hemophilia, cancer and inherited genetic anomalies. However, ethical issues could crop up when this technology for editing genes could be unfairly used to improve biological features, solely for the purpose of aesthetics or to gain advantage over others in the population. This would not only lead to societal discrimination and unrest, but also have the potential to change the course of evolution in living beings. In this regard, regulated implementation of the CRISPR technology, risk assessment, policies and procedures should be in place to prevent gross misuse of this technology.

Key words: bioethics, biotechnology, CRISPR, evolution, eugenics, gene editing

INTRODUCTION

The history of bioethics dates back to the twentieth century on the observation of cruelty to humans and animal, under the dictatorship rule of people who considered themselves as superiors, morally and ethically righteous, efficient and thus self-proclaimed decision makers. The term 'bioethics' was coined in 1970 by an American biochemist Van Rensselaer Potter and the field continues to remain as a fundamental platform for developing new strategies and interventions to regulate ethics in biological and medical research. The birth of bioethics also finds its root in Nuremberg trials (1945-1949) of Nazi doctors, who conducted heinous, immoral, distressing and miserable medical experiments during the World War II [1-2]**;** followed by unethical Tuskegee syphilis research [3-4], Sweden's forced sterilization [5-6] and so on. It was the 'Declaration of Helsinki (DoH)' [7-8] and Council for International Organizations of Medical Sciences- CIOMS [8-10] that shaped the major aspects of ethics with logical, thoughtful, humane decisions, moral justifications, law enforcements, awareness, strict adherence and strong endorsements of the principles of bioethics.

Bioethics, in general, concerns human values regarding religious beliefs, morality (both good and bad) and a host of such issues arising as a consequence of use of technology in biology and medicine [11]. Bioethical concerns got compounded with the idea of eugenics to selectively breed a character of interest, at the cost of eliminating or losing others. Such selective enrichment of life forms, including humans, will have a bearing on our evolutionary future [12]. If the population

becomes genetically homogeneous due to application of eugenics, then, biological variations, which are the raw materials for evolution, get eliminated. Such a situation casts a huge shadow on the ability of living beings to adapt and evolve in an unknown and unpredictable future. These bioethical concerns had already become grave with advances in recombinant DNA technology, animal cloning and *in vitro* fertilization. With the advent of new technologies like CRISPR and its consequent implications on our health and future, there is the need to not only accommodate and adapt to these technologies, but also evolve measures to regulate their misuse [13-14].

The biology of CRISPR

In history, twentieth century will be recognized as the century of biotechnology that has revolutionized both biological and biomedical world. Technologies like human cloning, stem cell therapy, gene therapy and RNA interference (RNAi) have proved to be promising technologies for eradicating infectious diseases and correcting genetic disorders. Among the recently discovered technologies, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein-9 nuclease (Cas-9) system is one of the revolutionary, easily accessible, cost-effective and efficient technology, which has the potential to edit the genome [13,15].

CRISPR, an adaptive immune system of bacteria, occurring in as many as ~50% of bacteria and in 99 % of archaea, is also found commonly in both pathogenic and commensal organisms.16-18 The mechanism of how CRISPR works is very simple; prokaryotes (e.g.: bacteria) retain some pieces of the virus and other invading DNAs. These pieces are clustered and regularly interspaced as short palindromic repeats for further recognition.18 The clustered repeats in CRISPR are based on sequence similarity that can be coupled into at least twelve major groups [19], and transcribed into small RNAs [20], as a result of which bacteria develop small RNA based acquired immunity [21-22]. On further invasion, this acquired immunity is remarkably efficient in cleaving any invading DNA [23-24] via a three-stage process namely, adaptation, expression and interference [17].

Four *CRISPR-associated* (*cas*) genes identified in CRISPR containing prokaryotes are suggested to be involved in DNA metabolism or gene expression [25]. Given their specificity to target a genomic site for disruption or repair of a specific gene [26], CRISPR/*Cas* genes are the most versatile tools of biological modulation [27]. However, this fascinating prokaryotic system shares no phylogenetic relationship with eukaryotes [22].

Though the CRISPR was discovered in prokaryotes, it can be engineered using recombinant DNA technology and introduced into any cell or the organism to edit the genome. When the technology is used to correct a genetic anomaly it might be beneficial. In contrast, it could turn out to be harmful when used to alter genes/genomes and create "designer" organisms. The latter could potentially upset the evolutionary landscape with detrimental consequences to our ability to adapt and evolve further, thus reaching an evolutionary dead-end. However, using this technology for limited tinkering of genes in an organism could be construed useful if, and only if, it is used to explore a gene's function, after ensuring proper safeguards. The situation could become complicated when proper regulations are not in place and the CRISPR technology is used for the purpose of eugenics.

Applications of CRISPR

CRISPR technology is a boon to biomedical world; as both somatic and gem cells can be modified, leading to corrected versions of disease-causing genes for repair and/or therapeutics [28-31]. In order to facilitate the study of pathology, immune function, cancer, cell therapy, drug screening and infectious diseases, gene editing could possibly be used to construct animal models of human diseases and also manufacture isogenic cell lines [32-36]. A major concern pertaining to this system is its specificity, in particular to edit duplicated genes or repetitive sequences in diploid or polyploidy species or cell lines [37-38]. These repetitive sequences could have a great impact on heredity and evolution of an organism [39-40]. It is quite evident and logical that a perfect matching of an assembled sequence is a must for the original genome [41] and thus it

becomes important and mandatory to minutely detect the off-target effects for translating and introducing CRISPR–Cas9 nucleases into human therapeutics [42].

The success rate of the technology has been immense since its birth in the year 2012, and has been applied in almost every field of biomedical and clinical fields. Research shows that CRISPR could be used to delineate the mechanisms underlying the development of human disorders and enable to precisely correct disease-causing mutations [43]. To name a few, CRISPR technology has shown the potential to treat modern day diseases such as cystic fibrosis [44], cataract [45], muscular dystrophy [46], β-thalassemia [34], type-1 diabetes [47], acquired immunodeficiency syndrome (AIDS) [48] and cancer [49]. In addition, CRISPR technology has been applied to raise the crop yield and food value [50-52] and also improve breeding technology [53].

Bioethical concerns in using CRISPR technology

The depth and vastness of bioethical concerns with the advent of newer technologies in biosciences/biotechnology seem to remain the same as before. However, such concerns take serious proportions and dimensions when technologies like CRISPR make engineering of the genome efficient and easy, that too with minimal investment and without proper regulations. Three of the major bioethical concerns evoked by the CRISPR related technology are:

1. Threat of CRISPR-engineered probiotic microorganisms evolving into new pathogens and hence could contribute to emergence of new diseases.
2. Disturbs the social harmony due to introduction of better genomes by the rich and powerful.
3. Selective enrichment of life forms creates genetically homogeneous population.

Such enrichment eliminates variations (the raw materials for evolution), casting a shadow on our ability to adapt and evolve. Though various safety levels are in place to handle chemicals or microorganisms, clear-cut procedures or legally binding regulations are not yet formulated to address any of the above serious implications. Hence, internationally acceptable and legally binding policies and procedures are needed to document and regulate technologies like CRISPR, and derivatives thereof, to monitor their misuse. Such regulations should not come in the way of therapeutics or basic research, but should strictly address three major concerns that are enlisted above.

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