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REVIEW ARTICLE

CRISPR-Cas and Future of Medical Research

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Abstract

To bring the change in genome has been the area of great scientific interest of all time for researchers. In this direction a recently discovered immune mechanism in bacteria and archea the CRISPR-Cas system led our way to make it possible with a great ease, using this natural phenomenon as required. In last five years many of its uses under experimental trials have been proved workable with much more efficiently as compared to the earlier used genome editing techniques like HR-mediated targeting, ZFN, TALEN and Cre-lox etc. The challenges in these techniques have been overcome using CRISPR-Cas system and is being taken as future technology for advancements in research and medicine because of its molecular level intervening. Here we have tried to explain some of its applications and future research perspective.

Keywords; CRISPR-Cas system, HR-mediated targeting, ZFN, TALEN and Cre-lox.

Introduction

All cellular entities from microbes to multicellular organisms face the threat of viral based infections, to tackle these infections almost every organism is equipped with some natural defense mechanisms. The bacteria and archaea have also evolved an immune mechanism against these invading viral genetic elements known as CRISPR-Cas System. The bacteria and archaea have to face a constant threat of invading exogenic mobile genetic elements like transposons, plasmids, genomic islands and phages (Wommack and Colwell, 2000). These invaders can have beneficial or even detrimental effects upon host. Such as a horizontal genetic element transfer can contribute in acquiring antibiotic resistance by environment of patho-genically treated other bacteria, along that the aquirance of virulent determinants can lead the bacterial strain toward toxigenicity (Weinbauer, 2004). So, bacteria and archaea are always threatening by viral predators. The phages are much more genetically diverse then bacteria, even their abundance exceeds then bacteria, according to an estimation there are about 1025 infections occurred every second (Hendrix, 2003; Wommack and Colwell, 2000).

Innate immunity against these invading phages can have mechanisms of mutation, abortive infection or DNA modification. Recently, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas (CRISPR-associated) has been discovered as an adaptive defense mechanism against phages (Bhaya et al, 2011; Deveau et al, 2010; Horvath and Barrangou, 2010; Marraffini and Sontheimer, 2010; Terns and Terns 2011; van der Oost et al, 2009; Wiedenheft et al, 2012. The CRISPR-Cas system is heritable and widely exist among bacteria and archaea and active in immunity

against various mobile genetic elements (Fineran and Charpentier, 2012).

Depending on the type of endonuclease, CRISPR/cas system is divided into two classes. Class-1 comprises types I, III and IV while class-2 contains types II, V and VI, where the differences lie in specific guide RNA molecule and endonucleases (Aqeel and Raza, 2017).

CRISPR-Cas system is basically a ribonucleoprotein complex mediated by RNA and Protein components functioning together. This system is consist of three basic phases first the adaptive phase in which the innate memory is generated, second the biogenesis phase where the guided RNA and other components are generated and the third phase is interference where the ribonucleoprotein complex cognate the invading phage and alter it (Fineran and Charpentier, 2012).

Applications of CRISPR-Cas Syatem

Although the CRISPR-Cas system was discovered in 1980's but in last five years lot of advances have been done in it. The tremendous success of the CRISPR-Cas genome editing tool is powered by the ease design principle of the guide RNA that targets Cas to the desired DNA locus, and by the high specificity and efficiency of CRISPR-Cas generated DNA breaks (Savic and Schwank, 2016). Several studies recently used CRISPR-Cas to successfully modulate disease-causing alleles in vivo in animal models and ex-vivo in somatic and induced pluripotent stem cells, raising hope for therapeutic genome editing in the clinics.

Recently the CRISPR-Cas mediated genome editing was made in mouse embryo for correction of a mutation in the gene *dystrophin*, responsible for inherited disease, X-linked Duchenne muscular dystrophy (DMD) (Long et al, 2014).

In another study, Liang et al, have also demonstrated the possibility of CRISPR-Cas mediated zygote editing in human embryos, (Liang et al, 2015) generating controversy among scientists and in the public (Cyranoski, 2015; Lanphier, 2015). The reported study used tripronuclear human zygotes to modify β-hemoglobin, the gene responsible for the blood disorder β-thalassemia. The researchers, however, found that the procedure led to a high number of additional unwanted modifications (off target effects), arguing against the use of the current technique for clinical applications. Moreover, because existing methods in prenatal diagnostics such as genetic profiling after in vitro fertilization already offer a less risky alternative for selecting against offspring with inborn diseases. It can also lead the parents to have offspring with desired non-natural traits. Because of these safeties and ethical concerns, we therefore do not support the legalization of CRISPR/Cas9-based genome editing studies in human zygotes (Savic and Schwank, 2016).

Hans Clevers recently demonstrated the possibility of CRISPR-Cas mediated genome editing in primary somatic stem cells. As a model, we used intestinal organoids, which allow infinite expansion of multipotent intestinal stem cells to correct a prevalent cystic fibrosis causing allele (Schwank et al, 2013).

Another ex-vivo CRISPR-Cas based gene editing study is focusing on combating human immunodeficiency virus (HIV) infection. During the life cycle of viroid it integrate into immune cells of host genome and serve as template for viral expression. At this stage, HIV infection can become transcriptionally silent, leading to a latent infection. Because the latent virus resides in long-lived cells such as memory T cells, the infection generally persists indefinitely even in the presence of potent antiretroviral drugs (Liao et al, 2015).

CRISPR-Cas mediated genome mutagenesis provide a tremendously precise approach to both coding and noncoding DNA sequences at their endogenous locations. Currently, with all these target nuclease experimental approaches, the entire mammalian genome can be investigated, in vivo, to detect and explain the role of coding and noncoding genome in physiology and pathology (Seruggia and Montoliu, 2016).

The fusion of two transactivation domains to CRISPR-Cas dramatically enhances gene activation upto the level that is needed to reprogram cell phenotype. Targeted activation of the endogenous *Myod1* gene using CRISPR-Cas system resulted into a stable and sustained reprogramming of mouse embryonic fibroblasts into skeletal myocytes. The levels of myogenic marker expression obtained by the activation of endogenous *Myod1* gene were comparable to that achieved by overexpression of *MYOD1* transcription factor (Chakraborty et al, 2014).

The simplicity of the CRISPR-Cas system of genome engineering has opened up the possibility of performing genome-wide targeted mutagenesis in cell lines, enabling screening for cellular phenotypes resulting from genetic aberrations. A genome-wide CRISPR library covering 13,501 genes, among which 8989 genes are targeted by just three or more independent single guide RNAs (sgRNAs) has been made of Drosophila melanogaster (Bassett et al, 2015).

Conclusion

The CRISPR-Cas system is being widely used in medical research and no doubt that the CRISPR will contribute to eradication of lethal infectious diseases in near future. But along with its positive usage it can also be used in wrong direction as well such as the non-natural changes in human embryo can lead to its implementation in non-ethical and even to the destructive way.

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