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Genome editing based on CRISPR/CAS systems: beginning of a new era of genetic manipulation and inheritance

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Introduction

Revolutionary scientific advances as a consequence of phenomenological observations have been systematically analyzed. The CRISPR/CAS system was initially observed in 1987 by Yoshizumi Ishino at Osaka University. During sequencing studies on *iap* gene of *E. coli* gene, Dr. Ishino found 29 repeats of nucleotide sequences with a 32 nucleotide spacing. However, the biological significance of these sequences are yet to be deciphered as sequence homologous to these have been found in others procaryotes.[1] In 1993, Francisco Mojica, at the University of Alicante in Spain, reviewed genome-sequence data from an extremophile archaea *Haloferax mediterranei* and noticed 14 unusual DNA sequences of 30 bases long. Mojica research focused on the repeat sequences and, in 2000, Mojica's team performed comparative analysis of prokaryote genomes and observed that the repeats were common among multiple archaea species and were called as short regularly spaced repeats (SRSRs).[2]

In 2002, Ruud Jansen of Utrecht University found a 21-37 bp interspaced short sequence repeats distinctly spaced among several bacterial species, such as, *Salmonella typhimurium* (21bp) and *Streptococcus pyogenes* (37bp). Jansen's team found that CRISPRs were unique to certain prokaryotes and not viruses and eukaryotes. Moreover, they identified a common sequence, GTT/AAC, at the ends and a long homologous sequence along the upstream without an open reading frame, indicating a conserved ncRNA segment. Their findings were similar to that of Ishino and Mojica, and they have referred the phenomenon as Clustered Regularly Interspaced Short Palindormic Repeats (CRISPR). The biological meaning of CRISPR remained obscure until 2005, when Pourcel, Mojica and Bolotin, independently, concluded that CRISPR were clearly derived from extrachromosomal DNA elements, with most being similar to bacteriophage and plasmids. Outstandingly, species containing CRISPR elements were protected against corresponding foreign invaders and had no residual prophage as evidence of prior infections.[4–6]

CRISPR loci are a part of microbial immune system of intriguing nature, with the spacers giving specificity to the system. In this model, the incorporation of new spacer into the CRISPR locus results in resistance to a new virus. Therefore, the content of the CRISPR locus can also serve as a record of past infections. Although the proposed hypothesis was very robust, only in 2007, Barrangou and colleagues showed a mechanistic proof that CRISPR served as a mechanism to defend against viruses. Rodolphe Barrangou and Philippe Horvath infected bacteria with viruses and looked for viral DNA in the CRISPR loci. They show that the viral DNA was contained in the immune cells and the removal of DNA sequences from the spacers has an impact and the immunity was lost.[7] Since then, the CRISPR biology became one the most exciting biological puzzles of the 21st century. By 2010, the CRISPR molecular biology research was explosing and in 2012.[8] Jennifer Doudna and Emmanuelle Charpentier from UC Berkeley University published their groundbreaking paper in 2012, revealing the CRISPR/Cas9 as a promising biotechnological tool for genome editing.[9] Doudna's group showed that is possible to program the endonuclease Cas activity to cut specific sequences on genome and the repairing mechanism could insert healthy gene copies. Doudna's lab, in fact, is engaged in a fierce patent battle with the Feng Zhang's group at MIT-affiliated Broad Institute, which claim the patent too. CRISPR technology has significant role in passing on the changes to the offspring in addition to genetic changes.

Scientists from Sichuan University were successful in using CRISPR/Cas technology to program T cells from the immune system of a human patient with lung cancer to kill tumor cell.[10] Researchers from UC Berkeley have used CRISPR/Cas9 gene editing toll to fix a single mutation in the β-globin, responsible for sickle cell anemia in stem cells.[11] Another successful therapeutic application of CRISPR/Cas technology was demonstrated by Kaminski and colleagues. They precisely removed the entire HIV-1 genome from latently infected human CD4+ T-cells.[12] Despite the promising results, presence of ambiguity cannot be denied. CRISPR/CAS is no exception and presents concern that should be carefully considered. The main concern is the off-target effect showed in the paper titled "Unexpected mutations after CRISPR–Cas9 editing *in vivo*". Published in 2017, this paper claims that the gene-editing tool caused widespread and unpredictable havoc in the genomes of edited mice, introducing hundreds of unintended errors.[13] In spite of ethical and biosafety concerns, their use in agriculture and basic research are still very enthusiastic because it allows scientists to quickly engineer cells and animal models to accelerate research into drug discovery, molecular genetics and complex diseases.

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None.

Conflicts of interest

Authors declare that there is no conflicts of interest.

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