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CRISPR Cas System: an efficient tool for cancer modelling

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Abstract

The Clustered Regularly Interspaced Short Palindromic Repeats–Cas-9 (CRISPR-Cas9) system has been a revolutionising tool in the field of molecular genetics, which provides a versatile range of editing potentials. Researchers can produce breaks or alter genomes with ease using the system. Cancer is one of the multi-gene diseases whose genes need to be studied in detail. The CRISPR-Cas9 technology may also provide a promising potential in the field of cancer genetics. The current narrative review comprised 50 research articles which were keenly analysed and the applications and outcomes of CRISPR-Cas9 system in cancer genetics were comprehensively and critically discussed. It was concluded that application of the system had great potential to help understand cancer biology of various types and could be used for its genetic modelling. However, much work is still needed to be done to apply the technology for understanding the mechanism of cancers and to help in the designing of appropriate therapies.

Key Words: CRISPR-Cas9, Cancer, Cancer modelling, Cancer genetics.

Introduction

Today, cancer is the most dangerous and serious threat to human health and life. A number of different types of therapies have been used and are still in practice for the cure/management of cancer and they include surgeries and therapies including radiation and chemotherapy, but the high rate of relapse and cancer's resistance to therapies contribute to the poor prognosis. Therefore, there is always a need for suggestion of new therapeutics and improvements in the present strategies for cancer therapies. It is known that in the genome of cancer cells there are many epigenetic and genetic factors contributing to the development of cancer. These changes include inactivation of tumour suppression genes, like PTEN (phosphatase and tensin homolog) and p53, activation of genes, like RAS (rat sarcoma) and ErbB (Erb-b2 receptor tyrosine kinase), chemo-resistance resulting from mutations in genes, like MDR1 (multi drug resistance 1), and mutations that occurs in epigenetic factors and their loci, like DNMT1 and others [1-5]. Therefore, genomes of cancer cells are needed to be corrected or disabled in faulty segments which may be one or more. The restoration of genes' function responsible for tumour suppression can provide a horizon for effective therapies of cancer [6]. In the modern era, many studies and researches have been carried out showing the therapeutic applications of genome engineering techniques that target the required defective segment of cancer genome precisely and effectively, resulting in knock-out and knock-in changes [7-8]. Molecular targets of cancer cells are repressed or activated for long term through these genetic techniques. Theoretically, these techniques need a tool that can specifically target the desired sequences and correct or disable them with low off-target actions so that their side effects are minimum.

For targeting genes and increasing its efficiency, double stranded breaks (DSBs) are needed to be produced in locus of genome of interest which are repaired by Non-Homologous End Joining (NHEJ) pathway and leads to Insertion-Deletion (Indels) [9]. When the deoxyribonucleic acid (DNA) template of external donor

is present, accurate modifications in DNA can be mediated by Homology Directed Repair (HDR) pathway while repairing DSBs. Genome engineering techniques are developed using endonucleases that are specific to the target site, like Zinc Finger Nucleases (ZFNs) and Transcription-Activator-Like Effector Nucleases (TALENs) [10] which have been used in a wide range of organisms and cell cultures [10]. They both have been used widely but high cost and complicated designing of the required specific endonuclease have limited made their application.

A simple and precise genome editing tool was discovered in 2012 [11-12] when two pivotal studies were released in the journals *Science* and *Proceedings of the National Academy of Sciences* (PNAS) of the United States, which helped transform bacterial Clustered Regularly Interspaced Short Palindromic Repeats–Cas-9 (CRISPR-Cas9) into a basic, programmable genome-editing tool, which has revolutionised genome editing techniques. CRISPR and CRISPR-associated 9 (Cas9) nuclease, together called CRISPR Cas-9 system, has been successfully applied in mammalian cells [13]. It is derived from the immune system of bacterial cell and consists of two components; endonuclease Cas9, which is guided by ribonucleic acid (RNA) and a chimeric single guide RNA (sgRNA). The combination of CRISPR RNA (cr(RNA) and trans-activating CRISPR RNA (tracr(RNA) [11] further streamlined the system by merging crRNA and tracrRNA to produce single guide RNA (gRNA)). The sgRNA binds and guides Cas9 to the specific target sequence based on complementarities [14]. The target sequence must be adjacent to the Protospacer Adjacent Motif (PAM) sequences which may be NAG or NGG for Cas9 derived from **Streptococcus** (S.) pyogenes. The sgRNA guides the Cas9 endonuclease to target sequence cleavage, producing DSBs which are then repaired by HDR or NHEJ. A study in 2014 reviewed the disruption of genes and its modification in different organisms and cell cultures through CRISPR and its repair by HDR and NHEJ [15], and found that

manipulation of DNA is a complex process and requires living organism, which have some common ethical concerns.

To study cancer biology, the common model organism used in studies are mice. As CRISPR technology is an advanced tool for gene editing, the ethical concerns associated with this technology are the same as that of the previous such tools.

The current narrative review was planned to review recent work done in cancer studies using CRISPR-Cas9 and to highlight its potential for effective solutions to the problems in cancer genetics.

Applications of CRISPR-Cas9 in Cancer StudiesCRISPR-Cas9 technology has been widely used in cancer studies which includes carrying out genetic modelling in rapid manner, mouse models being prepared rapidly and genomic engineering of somatic cells in different organisms and cell cultures as well [4,5,16,].

Carrying out genetic modelling in rapid manner

Large-scale efforts of sequencing genome in the current years have contributed largely to understanding the genetic changes that are present in tumours. Studying functional genetics at medium and large scales showing the role genes for tumour suppression and oncogenes in cell cultures, mouse models, allografts and xenografts are largely based on overexpression of complementary DNA (cDNA) and knock-down techniques by RNA interference (RNAi). All these techniques have led to several crucial discoveries in the field of cancer studies, but there are also some crucial hindrances in its way to effective strategy for cancer studies. Expression of genes is led to very high level in the systems based on cDNA expressions [17], which can cause artefactual and inverse effects on the biological process and signalling pathway of the cell. Inhibition stability and the degree to which gene is silenced is uncertain in case of inactivation based on RNAi. These may be useful and don't have any such limitation in some cases

and some experimental purposes, but, for consistency to be obtained, inactivation is required permanently and completely. Stable knockdown transfections using viral vectors and shorthair pin RNA (ShRNA) are also possible. Off-target actions also limit the techniques based on RNAi. These limitations can be overcome by using CRISPR-Cas9 system for specific target modification accurately and rapidly. It can also differentiate between passenger and driver mutations which effectively simplify the genetics of cancer genes. Single endogenous loci or multiple loci can be modified permanently through CRISPR system by delivering its component which may be stable or transient. Transient delivery of sgRNA and Cas9 coding plasmid has successfully edited genes of cells present in cell cultures [18] while some studies have reported successful results for transient delivery of sgRNA ribonucleoprotein (RNPs) [19]. On the other hand, for stable delivery of components of CRISPR, lentiviral vector or reteroviral vectors can be used [20-21]. Moreover, bionanoparticles have also been used recently for transient delivery of CRISPR components for cancer modelling [22-23]. Hit-and-run strategy is adapted by the CRISPR components in transient delivery so it has an advantage as it doesn't need constant supply or expression of CRISPR components for many endogenous editing processes and can lead to serial and unlimited editing. In vivo and cell-based assays can be used to test mutations in the cell lines in order to examine mutation's effect on phenotypes associated with cancer. Established primary or cancer cell lines of human or mouse origin can be subjected to this technique. Cancer genes can be studied well by generating models of animals through this approach as discussed below.

Generating mouse models with ease and speed

Different aspects of cancer that are fundamental for cancer development, like cancer progression, its initiation and maintenance, are better studied with the help of genetically-engineered mouse models (GEMMs) and non-germline GEMMs (nGEMMs) [24]. These models are also useful for testing range of

agents that play a critical role in anticancer therapy and drug resistance mechanism [25-26]. However, manipulation of embryonic stem (ES) cells or pronuclear injection is needed for GEMMs to be generated which is an expensive and slow process, along with extra caring of mouse that carry the gene of interest [24]. Multiplex genetic manipulations is a considerable limitation. CRISPR-Cas9 sets aside this limitation as it has the capability of modifying multiple genes at a time. Disruption of multiple alleles, up to eight, in a single step, two-gene deactivation in embryo of a single cell mouse and double knock-out animal production in one step have been done by CRISPR-Cas9 [27-28]. A study demonstrated that such mice can be generated that carry a small deletion with the help of sgRNA pairs [29]. Mice or ES cells can be generated through these methods that have quite a few loss of function (LoF) and gain of function (GoF), paving the way for precise, accurate and easy GEMM and nGEMM production. Studies provide many suggestions, like, while studying cancer through mouse models, it should be kept in mind that in most models, there are less induced mutations but CRISPR-Cas9 can induce many alleles responsible for cancer in mouse models [16]. Along with developing new models, CRISPR-Cas9 technology can also be used for advancing the existed models of mice for cancer studies.

Genome engineering of somatic cells

It is now possible to manipulate ES and germline cell-line at the genetic level through CRISPR-Cas9 with ease and speed, which also has potential of editing genome of somatic cells both in vivo and ex vivo.

In vivo cancer modelling from ex vivo CRISPR-mediated somatic genome editing

Many studies have demonstrated the use of CRISPR for ex vivo genome editing of somatic cells in order to study mutations and mouse models' generation

having haematopoietic cancers [20, 30-33]. In Arf−/−Eμ-Myc lymphomas, a tumour suppressor gene, Trp53, was edited ex vivo using CRISPR, which was then transfected into syngeneic mice. These cells, which lacked p53, showed substantial enrichment when treated with doxorubicin [26]. Another tumour suppresser gene, Mll3, was disrupted ex vivo by CRISPR in mouse primary hematopoietic stem and progenitor cells (HSPCs) with shf1;Trp53 -/- to study acute myeloid leukaemia (AML) tumour suppresser genes [30]. Another group [31] generated AML mouse models with ease and speed using CRISPR system by single and multiple genes ex vivo editing in mouse HSPCs mediated by lentivirus.

In vivo cancer modelling from in vivo CRISPR-mediated somatic genome editing

CRISPR system has also been useful in direct gene mutations in vivo in model animals. Two tumour suppresser genes, PTEN and Trp53, were successfully targeted by CRISPR-Cas9 for induction of liver tumour having exactly the same histopathology as observed in Ptenfl/fl Trp53fl/fl GEMMs. CRISPR components, along with DNA template having single strand that encoded β-catenin mutant form, were successfully transfected to wild type (WT) mice livers which resulted in low 0.5% hepatocytes generation with nuclear β-catenin, but had detectable frequency [34]. Apart from liver, three tumour suppresser genes of lung cancer were also mutated using crerecombinase and CRISPR components encoding lentiviral vector into mice models with KrasLSL−G12D/+Trp53fl/fl and KrasLSL−G12D/+ lung cancers which proved to have the required features of the targeted gene [35,36]. Furthermore, Indels were also harboured in target sites with no off-target activity being detected which paved the way for in vivo editing of somatic genome using targeted Cas9 activity. Rearrangement of large chromosomal oncogene was demonstrated in a similar study using CRISPR technology through adenoviral vector delivery that encoded echinoderm microtubule-associated protein-like 4–Anaplastic

lymphoma kinase (Eml4–Alk) targeting two sgRNAs and Cas9 nuclease to induce inversion [37,38]. Besides, CRISPR technology was also seen to have feasibility in these studies for modelling rearrangement of large genomic regions as Eml4 and Alk have about 11 mbp separation in between them. In a separate study, induction of in vivo rearrangement of chromosomes by using CRISPR technology was demonstrated [39]. Thus it is evident from these studies that mouse models can be generated more rapidly by using CRISPR-Cas9 system being delivered by viral vectors or plasmids. Some studies also suggested delivery of CRISPR components through nanoparticle –sgRNA complexes [40] and protein-RNA (Cas9-sgRNA) complexes [41]. In future, in addition to these methods, other delivery methods would also have to be developed.

Future Perspectives

CRISPR has been one of the hot topics since its discovery for its versatile nature and broad applications in almost all forms of life. Yet, CRISPR's role in translational and basic cancer research is still unfolding. CRISPR technology has been used in an effective way to generate experimental models for studying different types of cancers in both animal-based and cell-based cultures. It can also investigate the genome non-coding region, and will thus facilitate cancer genetics studies as it has been one of the poorly understood aspects of cancer. This system can be used as a person-specific system to study cancer by researchers and will aid researchers to study genome-specific traits and much more related to cancer genetics. If used in patients, it will allow researchers to identify potential resistance mechanisms, providing a roadmap to cancer genetics. Still, we are looking forward to seeing the results of pilot clinical trial [43] as these will change the whole history of gene therapy and surely will be great news for cancer patients and other genetically disordered people.

In future, CRISPR efficiency will depend on the effective delivery and limited or no off-target actions of CRISPR-Cas9. Though this technology still confers many limitations, there is no doubt that gene therapy for cancer patients using CRISPR technology is a prime opportunity for researchers to counter cancer. In short, CRISPR has no parallel so far in genome editing, and will greatly influence cancer research and studies.

Conclusion

Application of CRISPR-Cas9 system has great potential to help understand cancer biology of various types and could be used for its genetic modelling. However, much work is still needed to be done to apply the technology for understanding the mechanism of cancers and to help in the designing of appropriate therapies.

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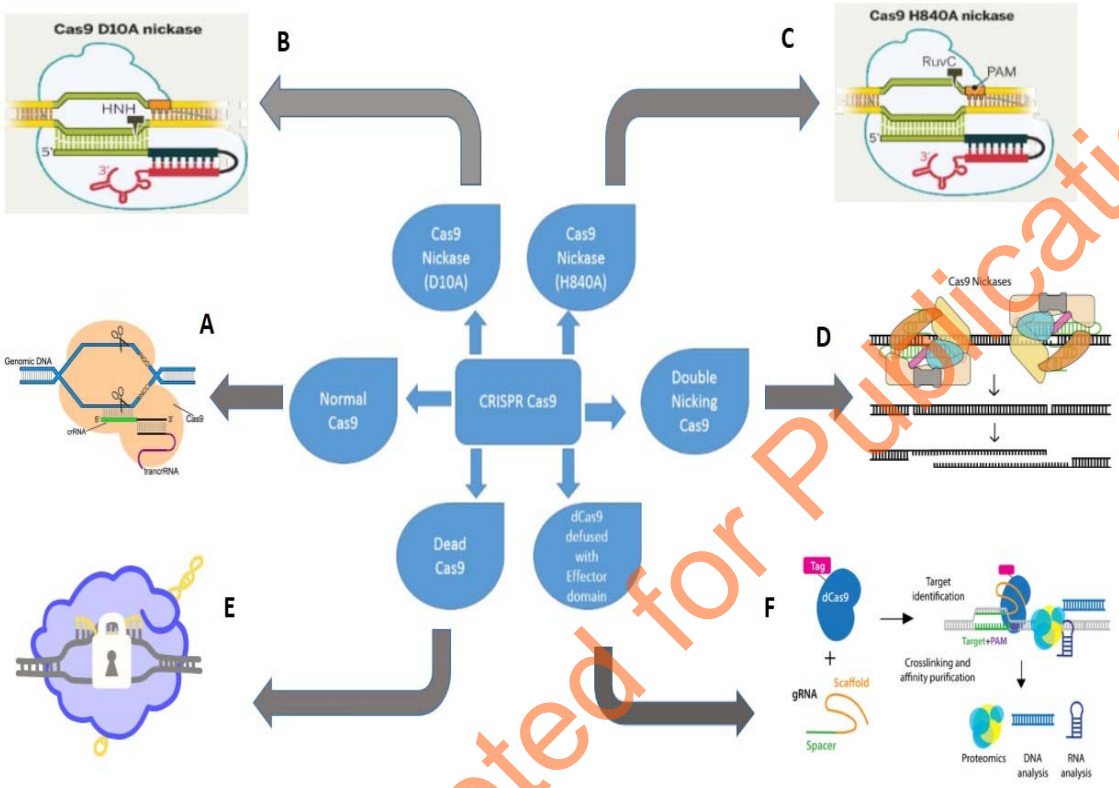

Figure 1; Different variants of Cas9 enzyme that can be used for different purposes. **A) Normal Cas9;** in this Cas9, both the domains are active and produces double stranded breaks (DSBs). These is the common type of Cas9 used for gene knockout and knock in. In this case, the DSBs are repaired either by Non-Homologous End Joining (NHEJ) method or Homology Directed Repair (HDR) method. **B) Cas9 Nickase D10A;** In this type, only one domain is working i.e. HNH while the RuvC domain is mutated (D10A) and not functional so it results in single stranded breaks. **C) Cas9 Nickase H840A;** it is also nicked i.e. one domain (RuvC) is functional while the other (HNH) is mutated (H840A). **D) Double Nicking Cas9;** In this type of Cas9 system, two nicked Cas9 are used (one D10A and one H840A nickase). They produce sticky ends and are very effective because of its less off target activity. It has been used to reduce off target action and has been fruitful. **E) Dead Cas9 (dCas9);** dCas9 has its both domains mutated and not functional. It binds to its target sequence but doesn't produce breaks. It has been used for gene silencing. **F) dCas9 with Effector Domains;** dCas9 has been tagged with many different effector domains according to required function and has been used for gene identification and transcription activation/inhibition.

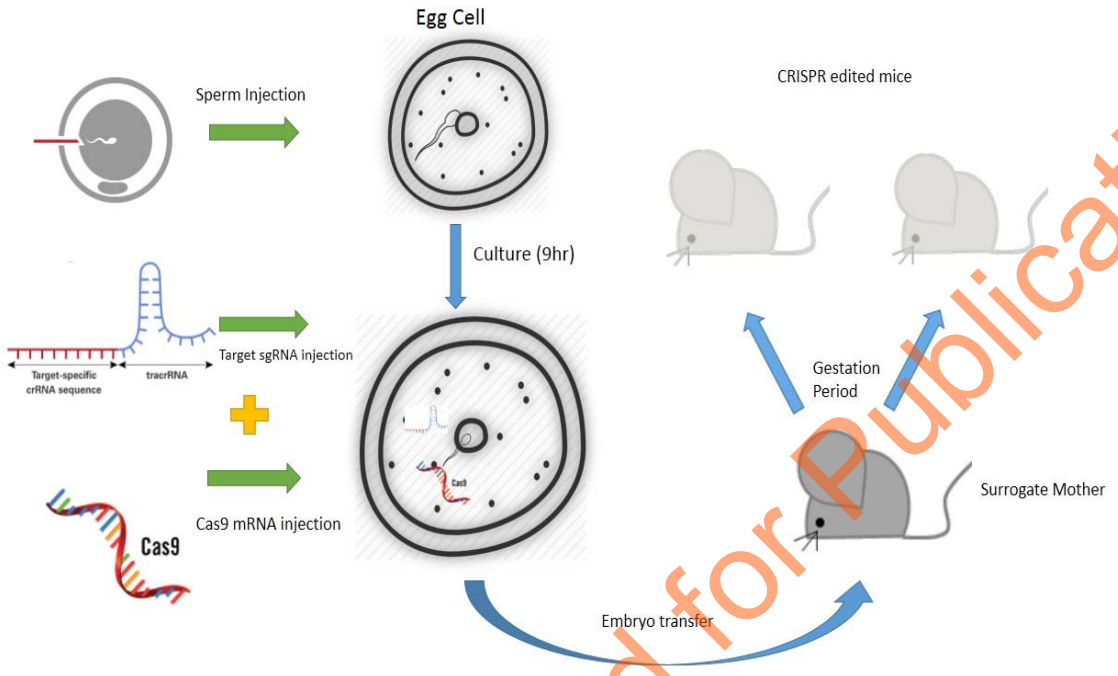

Figure 2: Mouse models generated with Clustered Regularly Interspaced Short Palindromic Repeats-Cas9 (CRISPR-Cas9). Mouse models with required genetic modifications was never as easy as it is now using CRISPR-Cas9 tools. It simply requires injection of sperm cell to egg cell followed by Cas9 messenger ribonucleic acid (mRNA) and target single guide RNA (sgRNA) injection. Then it is transferred to surrogate mother and will produce CRISPR edited mice with required characters.

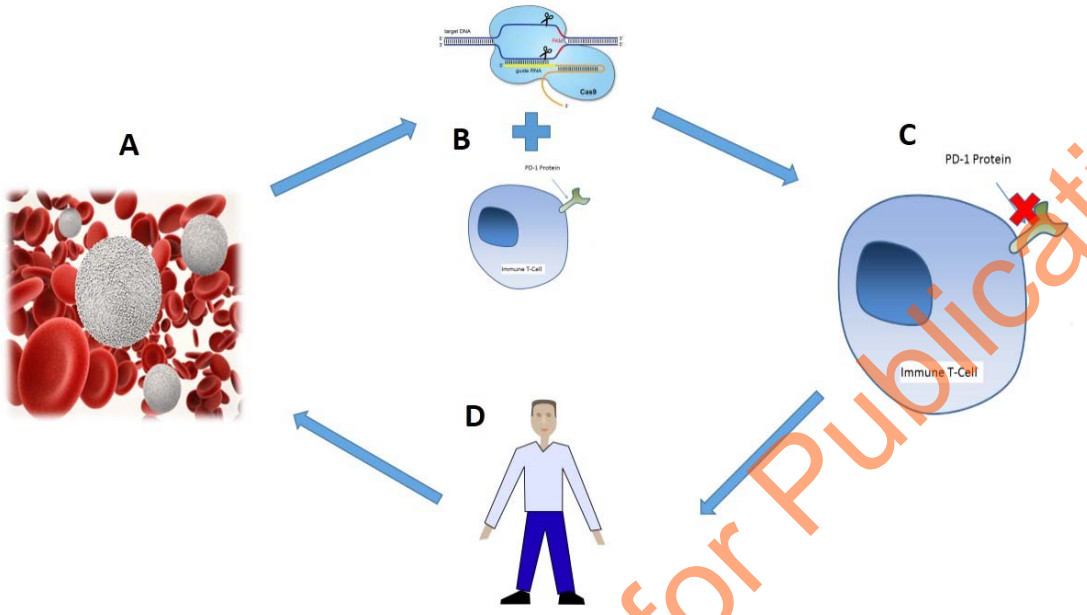

Figure3: Therapeutic applications of the Clustered Regularly Interspaced Short Palindromic Repeats-Cas9 (CRISPR-Cas9). This is the 1st phase of clinical trials. **A)** Blood Lymphocytes from patients with solid tumour have been taken. **B, C**) CRISPR-Cas9 mediated gene knockout of programmed cell death protein 1 (PD1) is performed. **D)** Then these cells are transferred back to the patients and are supposed to cause immunological response in patients [42].