



CRISPR-Cas genome editing tool: a narrow lane of cancer therapeutics with potential blockades

Devyani Bhatkar¹, Sachin C. Sarode², Gargi S. Sarode², Shankargouda Patil³, Nilesh Kumar Sharma¹

¹Cancer and Translational Research Lab, Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra 411033, India; ²Department of Oral and Maxillofacial Pathology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune 411018, Maharashtra, India; ³Department of Maxillofacial Surgery and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Jazan, Kingdom of Saudi Arabia

Contributions: (I) Conception and design: NK Sharma, D Bhatkar; (II) Administrative support: NK Sharma; (III) Provision of study materials or patients: NK Sharma, D Bhatkar; (IV) Collection and assembly of data: NK Sharma, D Bhatkar, SC Sarode, GS Sarode; (V) Data analysis and interpretation: NK Sharma, D Bhatkar; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Nilesh Kumar Sharma. Associate Professor, Cancer and Translational Research Lab, Department of Biotechnology, Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Pune, Dr. D. Y Patil Vidyapeeth Pune, Maharashtra 411033, India. Email: nilesh.sharma@dpu.edu.in.

Abstract: In recent, clustered regularly interspaced short palindromic repeats (CRISPR)-associated nucleases (Cas) system is emerging as a versatile genome editing tool with applications in basic science, preclinical and translational biology. This CRISPR-Cas genome editing tool is known as a precise and effective option to correct a part of the genome that may have implications in many human diseases including cancer associated genes such as oncogenes and onco-suppressors. Besides robust potential to edit target genes, CRISPR-Cas editing technology displays cellular alterations in the form of activation of DNA double strand break repair system and bringing genomic instability. As a consequence of repair of DNA double strand breaks, highly mitotically active cells may face hyper-DNA repair systems and there may be sometimes a situation leading to error prone mutations and unwanted genomic integrity. Additionally, the use of CRISPR-Cas editing technology in cancer therapy is limited in the backdrop of genotype and epigenomic heterogeneity in tumors. Therefore, a precaution should be considered to employ CRISPR-Cas technology in cancer therapy in view of tumor heterogeneity and environmental pressure.

Keywords: Neoplasms; transcriptional regulation; DNA; epigenetic; clustered regularly interspaced short palindromic repeats-associated nucleases (CRISPR-Cas)

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About CRISPR-Cas9 genome editing technology

From precellular evolution to cellular evolution, the contribution of RNA world theory is well appreciated. In recent decades, the discovery of a key components in adaptive immune systems of bacteria has revolutionized cellular and molecular biology (1,2). A discovery of clustered regularly interspaced short palindromic repeats (CRISPR)-associated nucleases (Cas) system in bacterial defense system that employs both RNA and proteins to achieve precision and efficient genome engineering (2-9).

In fact, such discovery strengthens the importance of RNA in cellular evolution and adaptations. To remove ambiguity among reported CRISPR-Cas systems, three major types are denoted with signature genes such as Cas3 in type I systems, Cas9 in type II, and Cas10 in type III systems (9-15). The common feature among these CRISPR-Cas systems is to be seen as one of RNA based adaptable defense system in bacteria. In essence, CRISPR-Cas9 system is elegantly shown to employ two RNAs as crRNA that complex with a tracrRNA to achieve the targeting of nuclease Cas9 to specific DNA sequences. Actually, fusion

of tracrRNA and crRNA is designated as single guide RNA (sgRNA) that directs CRISPR-Cas9 nuclease to position on target DNA sequence (12-15). In other way, Cas9 nuclease in complex with crRNA utilize tracrRNA as an accessory RNA to cleave target DNA sequence that is complimentary to crRNA (12-15).

Since the discovery of tracrRNA in human pathogen *Streptococcus pyogenes*, computation data reveal the location of tracrRNA on the opposite strand in the upstream from the Cas genes of a Type II-A CRISPR-Cas system (10-15). In fact, tracrRNA is known as a small RNA family that shows absence of sequence similarity within type II CRISPR-Cas loci. Furthermore, tracrRNA has anti-pre-crRNA repeat sequence (anti-repeat) and that enables to play a role in the form trans element (10-15). Due to the presence of inherent anti-repeat sequence in tracrRNA, existence of tracrRNA:pre-crRNA repeat duplexes with pre-crRNA repeats is suggested. Further, these complexes are cleaved by RNase III to activate Cas9 protein (10-15).

Among three types of CRISPR-Cas system, the type II prokaryotic CRISPR-Cas9, an adaptive immune component, shows RNA-guided site-specific DNA cleavage inside genomic loci in various target eukaryotic cells including human cells (1-5). CRISPR-Cas9 system can be integrated with multiple small guided RNAs and this combined are demonstrated to multiple targeting of genes location (6-19).

In a mechanistic landscape, CRISPR-Cas9 system achieves site-specific DNA recognition and cleavage by setting the association of Cas9 nuclease with a CRISPR RNA (crRNA) and additionally trans-activating crRNA (tracrRNA). Essentially, tracrRNA is discovered to possess complementarity to crRNA and tracrRNA helps in the maturation of crRNA that can generate multiple copies of pre-crRNAs (6-19).

To use these various types of CRISPR-Cas systems, literature cites potential delivery avenues such as lentiviral vector system, non-viral systems including electroporation, streptavidin-like molecules as a carrier, nanoscale biotechnology and exosome/extracellular vesicle mediated delivery will have future scope in both *in vitro* and *in vivo* delivery to achieve successful genome engineering (9-15).

CRISPR-Cas9 genome editing technology is actually an adapted form of powerful and inherent adaptive immune system conferred upon the microorganisms (19-27). This adaptive immune system in microorganisms is in the form of CRISPR-Cas9 that exists in three classes support these microorganisms to eliminate invading viruses. In essence, CRISPR-Cas genome editing technologies are known to

encompass various classes of nuclease enzyme such as Cas9, Cas1, Cas3, Cas4 and Cas12a (8-19). Interestingly, a recent discovery reports on a new class of gene editing enzyme as CasX that is claimed as more potent and efficient over Cas9 (15). The encoded nuclease Cas9 creates a complex structure with tracrRNA and crRNA and look for DNA sequences that match the spacer sequence present in CRISPR array loci. Type II systems are suggested as the most significant among three types of CRISPR systems and immensely contribute in genome editing technology (20-22,24-26). In recent, issues of off-target activity of CRISPR systems are reported. To remove these problems, attempts to modify CRISPR system are seen with the help of genomics and bioinformatics tools (8-19). Interestingly, the use of CRISPR editing tool is reported to generate programmable 3D nuclear organization that achieve specific set of gene expression (28).

Currently, CRISPR-Cas9 genome editing methodology is appreciably accepted various human cells lines, animal model system such as mice *Mus musculus*, Zebrafish *Danio Reio* and other model system such as fruit fly *Drosophila melanogaster*, insect model (10-15). To achieve successful application in human cell lines for the development of specific gene knockout, CRISPR-Cas9 editing technologies involves most important step to design sgDNA with ~20 nt with an approach of using either non-homologous end joining or homology-directed repair system. In such genome editing approach that starts with designing of target and sgDNA and modifications of genes may be achieved within one to two weeks (10-15). Apart from the designing and construction of sgDNA, delivery methods of sgRNAs to target cells can be through an expression cassette encompassing PCR amplicons or sgRNA-expressing plasmids. In case of insect model, the steps involved in the use of CRISPR-Cas9 based genome engineering involves the selection of stage of embryo injection, heteroduplex mobility assay as a screening stage, *in vivo* evaluation of sgRNA and G0 germline screening of edited insect (10-15).

In recent, CRISPR-Cas9 genome editing tool has received appreciable place in various aspects of science including development of genetic human disease models and gene therapy based treatment of human diseases such as cancer, heart disease, mental illness, single gene disorders and human immunodeficiency virus (HIV) infection (8-19). However, the use of CRISPR-Cas9 editing tool in cancer research and therapeutics is faced with issue of tremendous intra- and inter-tumor heterogeneity for a single type of cancer (20-27). Therefore, it is highly pertinent to know

the molecular, genomic and epigenomic landscape of cancer and then precise use of CRISPR-Cas9 genome editing tool to correct the aberrant cancer causing genes such as oncogenes.

Applications and limitations of CRISPR-Cas9 editing technology in cancer therapeutics

Human genome is gifted with 75% of viral and other pathogenic microorganism inserted genes and this conundrum justifies that probability of each individual to get cancer in their life time is up to 1/3 (1-14). Genetic engineering (or simply re-writing existing DNA sequences as choice based system, CRISPR-Cas9 has added a new dimensions to realize the degree and significance of genome editing of genomes from microorganisms to human cells (20-27). It appears to be novel and new tools for genetic and cellular engineers, but these steps are done by primitive microorganisms at their own cost and machinery. In essence, investigators do just by making copy of process adopted by simplest cells. In the process of targeted discovery of genome editing tools, investigators forgot to ask simple question, why nature has given these genome editing tools to simple microorganisms, but not human cells. Actually, it is a balance within the nature and ecosystem that simple organisms are gifted with powerful editing tools and surprisingly complex human cells are blessed with outstanding genomes, but devoid of genome editing tools. On the other hand, it is important to share that microorganisms are lacking strong human adaptive immune system as conferred to human system.

Now by the discovery of CRISPR-Cas9 genome editing tools, investigators intend to keep our complex genome and also the power CRISPR-Cas9 genome editing tools to create designer genome/genes for future survival, existence and therapeutics (20-28). It appears to be in contrast, because nature did not included the power of genome editing tools because our genomes are full of genes probably accumulated by similar type of natural genome editing during evolution of billions of years.

In an elegant paper (29), report that use of CRISPR-Cas9 genome editing tool in a normal and cancer cell can be linked to the status of p53 and ensuing DNA damage response. Hence, it would be good to see the status of p53 gene and their products and its implicated cellular response pathways such as DNA damage response. In short, CRISPR-Cas9 genome editing tool is having huge potentials, but caution and care should be taken to move

ahead with its therapeutic use.

Another key finding suggests the precise use of CRISPR-Cas9 editing technology in cancer stem cells over the normal stem cells, as wild type active p53 disrupts the action of editing technology in human pluripotent stem cells (29-35). While, cancer stem cells are known to harbor the mutation and gain of functions of mutant p53 and hence may be a good candidate to use CRISPR-Cas9 editing technology to restore the pro-tumor hallmarks by gene therapy (33). Additionally, emerging evidences point out those cancer stem cells a small pool of tumor microenvironment with p53 mutation and also gain of functions within mutant p53 show less prominent DNA damage response and safeguard mechanisms to genome. Therefore, plausible approach to use CRISPR-Cas9 editing tool in blocking the cancer stem cells by editing oncogenes or bringing onco-suppressors gene to quench its potential so serve as the origin for cancer drug resistance and relapse of cancer.

It is well appreciated idea that the use of CRISPR-Cas9 editing tool needs to be specific and selective based on the genome and epigenomic landscape. Therefore, applications of CRISPR-Cas9 editing tool should be centered upon the efficacy, minimal undesirable outcomes and long term impact in cellular landscape of targeted cells (21). Besides amelioration of oncogenes and tumor suppressors, there is an emerging view to tweak the oncogenic miRNAs by the use of CRISPR/Cas9 editing technology (30). The contribution of CRISPR-Cas9 editing tool is also extended in the cancer immunotherapy to create chimeric antigen receptor-T cells (CART) cells and other anti-cancer immune cells. In a recent attempt, authors claim the potential of CRISPR-Cas9 technology in combination with Cas9-low-molecular-weight protamine (LMWP) nano-carriers to edit the KRAS oncogene in lung cancer (36). To address the important aspects of off target impact of CRISPR-Cas9 editing tool (37) present an elegant study showing the absence of substantial off-target mutations in *in vivo* model of mouse liver. In essence, this study is a good attempt to remove concerns related to the use of CRISPR-Cas9 editing tool in therapeutic purpose. However, a precise study is the need of hour to encompass the issues of intra- and inter-heterogeneity and use of CRISPR-Cas9 editing tool in the removal of oncogenes and correction of tumor suppressor genes. In case of multi-drug resistance in osteosarcoma cells, disruption of *CD44* gene by CRISPR-Cas9 is reported to abrogate the problems of drug resistance (38). A recent paper reports on the rapid screening

of therapeutic targets including WRN helicase in several cancer types using CRISPR-Cas9 technology and further this study propose the use of CRISPR-Cas9 to identify genomic lethal targets to achieve cancer therapy success (14). Additionally, CRISPR-Cas9 technology is shown to attenuate elevated level of urokinase plasminogen activator receptor and this inhibition is suggested as option to bring down malignancies.

A recent report indicates on the use of CRISPR-Cas9 technology to bring down the *E6* and *E7* gene expression in case of high risk HPV mediated cervical cancer patients (39,40). A positive response on chimeric antigen receptor (CAR)-T cells mediated immunotherapeutic response in solid tumors is shown to target *PD-1* gene editing by CRISPR-Cas9 (41,42). A recent CRISPR-Cas system that utilizes liposome-encapsulated CRISPR/Cas9 genome editing technology is shown disrupt *PD-1* gene in T cells that may have applications in adoptive cancer immunotherapy (43). An *in vivo* study indicates on the use of lipid-based CRISPR/Cas9 delivery system to target HIF-1 α that employs plasmids encoding Cas9 and designed sgRNA targeted to HIF-1 α (44). Further, CRISPR/Cas9 system is shown to bring down the *HBsAg* gene and in turn block the proliferative potential of hepatocellular carcinoma cells (45). Taken together, several reports provide experimental data on the potentials of CRISPR-Cas system as anti-cancer therapeutic approach. However, limitations to use of CRISPR-Cas system are seen in the perspectives of genomic instability, genotypic/epigenomic heterogeneity and undesirable off target (secondary tumors).

Truly, investigators intend to disturb the genomic landscape of nature gifted cellular system and therefore, it may bring inevitable consequences of loss integrity of genome and drifting towards designer genome to destructive genome.

CRISPR-Cas9 and DNA repair landscape of cancer

It is logical to see the appreciable application of CRISPR-Cas9 editing tools in the correction of oncogenes in cancer types with high load of p53 mutation including lung cancer (30-35). Expanding data confirm that the tumor suppressor gene *TP53* is mutated in 50% of human cancers including breast, cervical and lung cancer and is referred as the indicator of poor prognosis (32). Other than p53 status amelioration, a clear role of CRISPR-Cas9 editing technology is elaborated in EGFR L858R-positive lung

cancer by precisely targeting point mutations as C > G, A > G, and T > G point mutations (46). Here, authors claim the specificity of CRISPR-Cas9 editing technology in cancer over normal cells by using protospacer adjacent motif to differentiate cancer single nucleotide mutation from normal cells. In support of link between CRISPR-Cas9 editing tool and DNA damage response (34), emphasize that use of DNA repair protein inhibitor SCR-7 may be a good option in combinatorial cancer therapeutic to avoid the undesirable consequences of editing technology. Therefore, a reasonable scope is warranted to bring back the wild type features of p53 in a specific type of cancer. Importantly, status of p53 within cancer cells, cancer stem cells and cancer associated immune and stromal cells is also a key features to find the success and failures of CRISPR-Cas9 editing technology.

Conclusions and future perspectives

In conclusion, CRISPR associated (Cas) proteins/enzymes, first discovered as Cas9 and then followed by Cas12a, Cas1, Cas4 are highly appreciated as genome editors that use small guided RNAs. In recent, these genome editor tools are faced with issues of off-target impact on the target genome and that leads to undesired mutation and DNA damage response. These unwanted DNA damage responses in the host genome may generate inevitable mutations and potentially a factor for the predisposition to tumor. A caution is also perceived in the use of CRISPR-Cas tools to edit oncogenes and eventually may also trigger the chance of secondary tumors in the host. In the face of these obstacles, Cas proteins/enzymes are continuously modified to create better acceptable tools including CasX, Cas-base editor, Cas-spacer, CasDrop. These Cas variant proteins/enzymes present prospects of CRISPR-Cas genome editor tool, beyond genome editing in case of tumors and other disease condition that include transcriptional and chromatin remodeling. The genome editing potential of CRISPR-Cas9 and other variants of Cas tools is garnering attention in preclinical and clinical success in many types of tumors including oral cancer. Further, the authors opine the use of CRISPR-Cas system in the understanding of basic pathway that contribute to OSCC formation and therapeutic avenues that may bring down the level of key oncogenic driver proteins. Besides these applications in OSCC, oral microbiome in the tumor niche may be targeted by CRISPR-Cas system that acts as pro-tumor agents. Finally, the use modified CRISPR-Cas system may be used in the understanding of transcriptional and chromatin remodeling

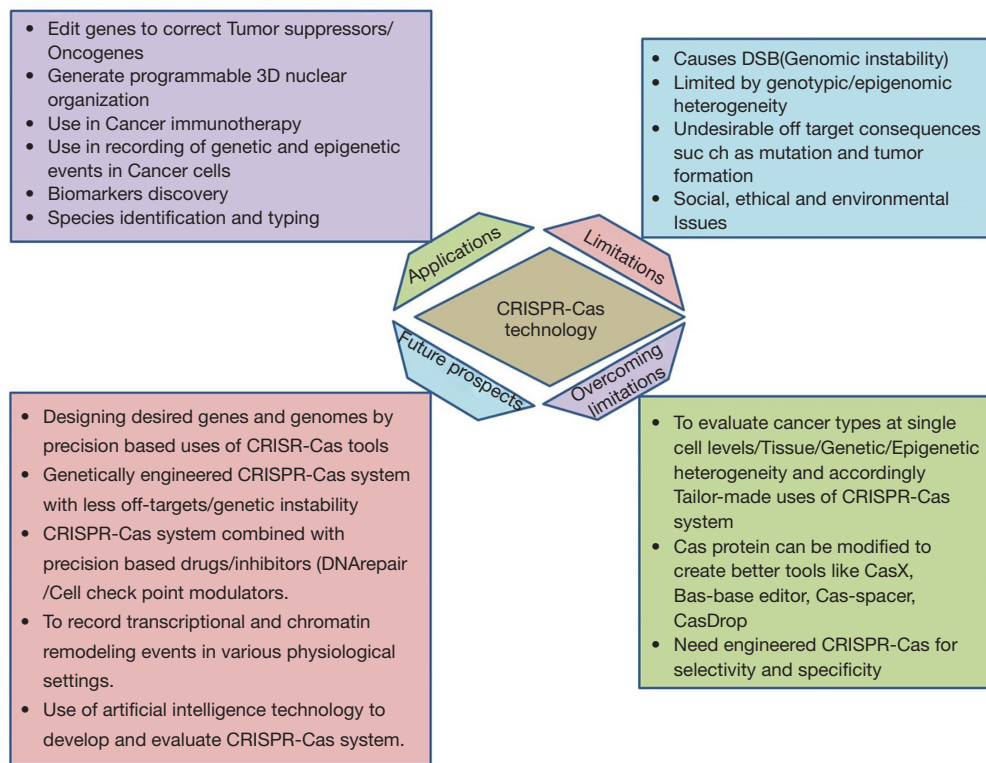


Figure 1 Applications, overcoming limitations and future alterations of CRISPR-Cas system. CRISPR-Cas, clustered regularly interspaced short palindromic repeats-associated nucleases.

events that drive the formation of precancerous lesions and initiation of OSCC. The revelation of these molecular, genetic and epigenetic pathways will set the platform for better new classes of therapeutics. A summary of scope, limitation and future modifications in the CRISPR-Cas system is illustrated in *Figure 1* (1-15).

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