MINI-REVIEW

CRISPR as a Versatile Technology for Gene Activation and Genome Editing

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ABSTRACT

CRISPR/Cas system, a microbial adaptive immune system, has rapidly transformed the ways researchers can interrogate the genome. CRISPR has many advantages over traditional methods such as Transcription activator-like effector nucleases (TALEN) and Zinc-finger nucleases (ZFN). Since CRISPR discovery as an adaptive immune system used by bacterial against viruses, it has been repurposed to help in many different genome-related studies such as gene knocking in and out, gene expression upregulation and downregulation. Also CRISPR holds vast therapeutic potential for the management of genetic disorders by straight modifying disease-causing mutations. Although the Cas9 protein has been revealed to attach and cleave DNA at off-target sites, the field of Cas9 specificity is quickly progressing, with marked modifying in guide RNA choice, protein and guide engineering, innovative enzymes, and off-target recognition methods. In current review we mostly focus on CRISPR unique ability in gene activation/upregulation, which has wide applications in different aspects such as gene studies, stem cell differentiation, and trans-differentiation. Compared to other gene activation methods such as viral gene overexpression, TALEN and ZFN, CRISPR offers many benefits such as easy designing and high precision.

Keywords: CRISPR-Cas9, Gene activation, Genome Editing

Introduction

One of the approaches to elucidate the function of a gene of interest is gene editing which includes insertion, deletion or upregulation.

Many different tools for genome editing have been using such as the conventional homologous recombination (HR), or more recent approaches such as Zinc Finger Nucleases (ZFNs),

Transcription Activator-like Effector Nucleases (TALENs). Most recently, Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Cas system has gain a lot of attentions and is considered as the biggest breakthrough in this century after PCR innovation [1-3]. CRISPR-Cas9 genome engineering technology has been constructed as a strong molecular instrument for multiple areas of biological study in which it is beneficial to target or modify particular DNA sequences [4].CRISPR-Cas9 has been used successfully in a broad range of organisms, from bacteria and yeast to plants and animals [5-7].

CRISPR has a significant advantages over its older encounters; it is technically easier to design because in spite of ZFNs or TALENs in which it is required to design a specific DNA binding domain for each DNA sequence, CRISPR uses guide RNAs which is more convenient to design and more precise to target sequence of interest. On top of it, it is more accessible and cost-effective [8].

Also, viral gene delivery has been widely used to over express genes in target cells, but most of these viral vectors carry strong promoters which result in uncontrolled and robust expression of the gene of interest[9]. Moreover, in ORF cloning one might lose the other splice variants and sometimes cloning of a longer gene could be problematic [10].CRISPR-Cas9 in its natural system consists of an endonuclease which is guided to a specific sequence of the genome by an RNA guide system made up of two RNA components: including a trans-activating crRNA (tracrRNA) and a precursor crRNA (pre-crRNA) [11]. In the synthetically reconstituted system, these two RNAs can be combined into a single guide RNA (sgRNA). sgRNA can easily base pairs to any particular DNA sequence (Fig1).

However, the targeted sequence needs to be right next to a specific DNA motif called protospacer adjacent motif (PAM) which is considered as a minor drawback in using CRISPR. Up to now, the Streptococcus pyogenes Cas9 (SpCas9) has been used largely to accomplish efficient genome editing in different species and cell types, comprising human cell lines, bacteria, yeast, zebrafish, fruit fly, mouse, rat, roundworm, common crops, monkey, and pig[12].SpCas9 is also intensely increasing the catalog of genetically manageable model organisms, for instance, by allowing the formation of multiplex mutations in cynomolgus monkeys [13]. SpCas9 is able to be targeted either with a set of crRNA and tracrRNA [14] or with a chimeric sgRNA [15], since the crRNA or sgRNA includes a 20 nt guide sequence that precisely matches the target site. The only necessity for the choice of Cas9 target sites is the adjacency of a PAM instantly downstream of the target site.

Meanwhile CRISPR-Associated (Cas) protein system of Streptococcus pyogenes was shown to function in cells and renders an easy and precise genome manipulating approach [2]. A mutant version of Cas9 with no endonuclease activity or nuclease-dead Cas9 (dCas9) has been developed and targeted to genes in bacteria, yeast, and human cells by sgRNAs to silence gene expression through steric hindrance [16].

However, CRISPR/dCas9 system gene activation might mimic more closely their natural expression system because the activation occurs "in context" (i.e. it happens through the gene's own promoters and transcription machinery). In some other studies, it has been used to activate the expression of endogenous genes by targeting a fusion protein comprised of dCas9 and a transactivation domain such as VP64 to gene promoters [17, 18].

By binding dCas9 to the FokI nuclease domain and then expressing binary guide RNAs, one can make a dimerization-dependent system that also recovers specificity[19]. CRISPR specificity must be considered in a wider sense to contain cell type and temporal and spatial specificity. Ultimately, the standardization of off-target analysis procedures and data reporting in investigations would advantage the field by authorizing comparison across studies and excellent algorithms for guide RNA design.

To study the function of a gene, one needs simply design a specific 20 nt sequence which can target the sequence of interest in the genome and guide the Cas9 to its target. Then the Cas9 enzyme can make a blunt double strand break (DSB) or make an Indel in DNA due to imperfect DNA repair system which can lead to a frameshift in targeted gene and loss of function [11]. Given the fact that CRISPR is a combination of RNA, DNA and protein interactions, it can act as a recruiter of many different gene modulators or probes to its binding site. The modulators could be an activator such as VP64. In other words, Cas9 can also be altered into a synthetic transcriptional activator by binding it to p65 or VP16/VP64 activation domains. Commonly, targeting Cas9 activators with a single sgRNA to a specific endogenous gene promoter cause moderate transcriptional upregulation[20, 21].

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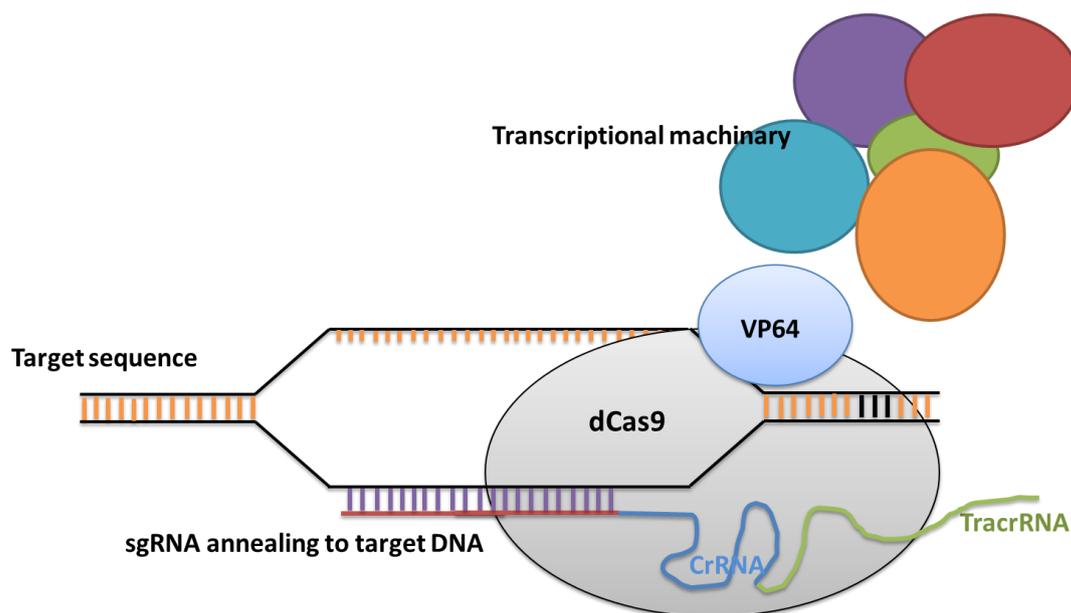
Figure 1: Schematic figure of CRISPR- dCAS9 activator system.

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On top of it, it is more accessible and cost- effective [8]. Also, viral gene delivery has been widely used to over express genes in target cells, but most of these viral vectors carry strong promoters which result in uncontrolled and robust expression of the gene of interest. Moreover, in ORF cloning one might lose the other splice variants and sometimes cloning of a longer gene could be problematic [10].

CRISPR- dCas9 as a gene activation tool

Upregulation of a gene is used widely to elucidate a gene function. It has been shown by different groups that binding of multiple transcriptional activators like VP64 increase the transcription efficiency [23]. Cas9 has been engineered to repurpose its endonuclease activity by introducing mutations into its two nuclease domains, RuvC and HNH, and disrupt its nuclease activity. This new mutated version of Cas9 is called deactivated Cas9 [6, 24]. After depriving Cas9 nuclease activity, it is now a good anchor, which can be fused to different gene activators and easily be guided to its site of effect. One type of effectors that can be fused to Cas9 is trans activators. Researchers have fused Cas9 to omega subunit of RNA polymerase in bacteria and it shows 3-fold higher expression [5].

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Although both P65 and VP64 have been tested as transactivators domains in eukaryotes, VP64 has shown a higher efficiency and has been used more frequently. It has been used in a couple of studies to activate/upregulate different genes or reporters [17, 18, 20, 23]. The activation in eukaryotic organisms is more moderate, and researches have shown that using multiple gRNA for a single promoter enhances its efficiency [25]. Therefore, studies to find ways for recruiting multiple activators on the same promoter has led to new strategies.

One strategy which is to enhance activation by transforming dCas9 into a scaffold capable of recruiting many copies of the activator of interest [23, 26]. This technique is called SunTag array in which dCas9 protein is fused with an array of scFv (single chain variable fragment) which can bind to multiple VP64 proteins and therefore at the same time many VP64 are recruited to the promoter. In spite of using single VP64 alone which typically leads to ~2-3 fold increase, SunTag array results to ~50 fold increase [26, 27]. This method has been used to activate CXCR4 gene expression in human erythroleukemia K562 cells and the high expression led to significantly increase migration capability. Chavez et al., have used another strategy in which they fused three different activators, VP64, P65, and Rta (VPR). These three activators were resulted in higher activation. It was able to increase the fold expression up to 300 folds compared to using single Cas9-Vp64 [28]. Synergistic Activation Mediator (SAM) system is another strategy developed by Konermann et al., they made more modifications in sgRNA in a way that they inserted two hairpins which can bind to MCB, a RNA binding protein. This MCB additionally fused to P65 and human heat shock protein. Therefore, four additional activators can be recruited to CRISPR site. Compared to single Cas9-VP64, SAM can increase the fold of expression up to 1000 folds [22, 29]. Recently, Shu Wei et al. have used CRISPR to enhance the expression of endogenous *Cdx2* and *Gata6* genes, thus mouse embryonic stem cells (ESCs) were directly converted into two extraembryonic lineages, i.e., typical trophoblast stem cells and extraembryonic endoderm cells [29].

Conclusion

CRISPR gives us the opportunity to manipulate almost any known gene or a combination of them. By using multiplex CRISPR, it is possible to manipulate many genes and elucidate their roles in different cell function [17, 22]. CRISPR has been used in a broad range of cells and species. The CRISPR-mediated activator system has been applied to activate specific endogenous genes and study differentiation of stem cells or trans-differentiation of one cell type to another [1, 25]. One hallmark of the natural CRISPR-Cas9 system is its intrinsic capability to efficiently cleave numerous diverse target sequences in parallel [30] by changing a pre-crRNA transcript comprising many spacers into distinct guide RNAs duplexes (mature crRNA and tracrRNA) through being hybrid with tracrRNA [31]. Morover, CRISPR-mediated activator system can also be used to manipulate the expression of endogenous non-coding RNAs that exist in untranslated regions and play important roles in cell fate regulation. Together, through the direct control of endogenous specific gene expression, CRISPR-mediated activator system provides a novel, low-cost and promising method for reprogramming cell lineage specification.

Acknowledgment

Conflict of Interest

The authors declare no conflict of interest.

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