CRISPR/Cas Derivatives

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The field of genome editing started with the discovery of meganucleases (e.g. the LAGLIDADG family of homing endonucleases) in yeast. After the discovery of transcription activator-like effector nucleases and zinc finger nucleases, the recently discovered CRISPR/Cas system has opened a new window of applications in the field of gene editing. Here, we review different Cas proteins and their corresponding features including advantages and disadvantages and we provide an overview of the different dCas derivatives. These dCas derivatives consist of an endonuclease-deficient Cas9 which can be fused to different effector domains to perform distinct *in vitro* applications such as tracking, transcriptional activation and repression, as well as base editing. Finally, we review the *in vivo* applications of these dCas derivatives and discuss their potential to perform gene activation and repression *in vivo,* as well as their potential future use in human therapy.

Keywords: CRISPR/Cas ; genome editing ; transcriptional regulation ; dCas9 derivatives

1. Introduction and History

In the last ten years, major breakthroughs have been made in the field of gene editing, which is the process where DNA is modified, deleted, inserted or replaced. The most recent discovery of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated proteins (Cas) system has opened up many novel opportunities as well as applications for gene editing both in vitro and in vivo. In this review, we provide an overview of the different gene editing techniques including the CRISPR/Cas system, which was discovered in 2012. After this finding, different Cas proteins were developed with unique features that allow for distinct gene editing approaches. Here, we summarize these different Cas proteins and detail their specific features. Besides the development of different Cas proteins, endonuclease-deficient Cas proteins (dCas) allow for additional applications of the CRISPR/Cas system. These endonuclease-deficient Cas proteins (dCas) can be fused to effector domains which exert additional functions such as transcriptional activation or repression, but also tracking and base-editing. These dCas effectors as well as their functions and in vivo applications will be the main focus of this perspective.

Safety and effectiveness are the two major concerns for delivering dCas9 derivatives into the affected cells, and thus influence treatment efficacy. Basically, three different formats can be used as delivery methods: gene expression plasmids, viral vectors and ribonuclear complexes. The selection of the delivery system ideally should provide: (1) tissue-specificity, (2) target cell entry ability and (3) without/low immunogenicity. Initially, as proof-of-concept, many of the therapeutic applications of the dCas9 derivatives were performed on animal models via the electroporation of plasmid DNA [1][2]. Electroporation can be highly toxic since it can harm the cell membrane. In some cases, this leads to permanent permeabilization of the membrane [3]. Viral vector-based gene delivery systems have been widely used in gene therapy and even entered clinical trials in some cases [4]. The most widely used are lentiviral and AAV vectors, each one having its own advantages and disadvantages. For example, a lentiviral vector has a large packaging capacity up to 8.5 kB, long-lasting transgene expression and is capable of transducing non-dividing cells [5]. However, a lentiviral vector is oncogenic, immunogenic and is likely to make a transgene insertion into the host genome, which largely constrains its applications [5]. On the other hand, AAV vectors have a large variety of target tissues with low immunogenicity and non-oncogenicity [6]. AAV vectors have a very small genome size however, and therefore a low packaging capacity, which makes it unable to deliver a large transgene within a virus [6]. Instead of delivering DNA into the cells, the dCas9 derivatives can also be first transcribed into mRNA and delivered into cells via ribonucleoproteins. The transient nature of this approach favors controlling the gene expression level and minimizes the window of immune activation [5]. Moreover, from a pharmaceutical point of view, ribonucleoproteins are much easier to scale up for clinical use and can be chemically functionalized with ligands to obtain target cell specificity [5].

2. CRISPR/Cas9 System

The discovery of the CRISPR/Cas9 system has revolutionized the field of epigenetic and genetic editing in the pre-clinic. This is a fast-growing field where different Cas9 proteins have been discovered with each their distinct features (e.g., length and recognition sites). The discovery of endonuclease-deficient Cas9 has led to the development of different CRISPR/dCas9 derivatives, which can be used to perform distinct functions such as tracking, transcriptional activation and repression, and base editing. Some of these dCas9-based derivatives have also been applied in vivo, where they contribute to transcriptional activation or repression which can lead to changes in disease phenotypes. This holds great promise for genetic screens to identify novel genes which are associated with a disease phenotype, and of course for potential therapies. Even though the discovery of high-fidelity Cas9 has significantly improved off-target effects, future research needs to be directed to improve methods to detect off-target effects (e.g., a recent study has demonstrated that engineering a hairpin secondary structure onto the spacer region of single-guided RNAs (hp-sgRNAs) can increase the specificity by several orders of magnitude) [7]. In addition, recent advancements in the unbiased detection of CRISPR off-target effects (DISCOVER-Seq) in patient-derived induced pluripotent stem cells directs us towards in situ off-target discovery within individual patient genotypes during therapeutic genome modulation [8]. Furthermore, future research needs to be directed at optimizing delivery methods for the clinical use of CRISPR/Cas (e.g., a recent study showed proof of concept of a self-deleting AAV-CRISPR system) [9]. Altogether, the CRISPR/Cas-based epigenetic and genetic editing is a fast-growing field with opportunities to develop novel applications altogether and increase the scale of already existing CRISPR/Cas-based applications.

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