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Applications of CRSIPR/Cas9 in Cancer Research

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Abstract

The technology based on clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) has been successfully applied to genome editing and has shown a promising future in gene functional studies. Human cancer is a complex disease due to multiple gene mutations, amplifications, deletions, up regulations or down regulations. It is a challenge to generate precise cell or animal cancer models in vitro and *in vivo* to investigate the complex process of cancer. The CRISPR/Cas9 technology provides a new opportunity to study human cancer by disrupting multiple genes or introducing point mutations at a specific locus of genome, and thus mimicking the features of human cancer in cell or animal models. Here we will review the current status of CRIPSR/Cas9 system and its potential application to cancer research.

Keywords: Cancer; CRISPR/Cas9; Application

Introduction

The clustered regularly interspaced short palindromic repeats (CRISPR/Cas) is an adaptive immune defense pathway in bacteria to maintain genome integrity by cleaving foreign plasmid or phage DNA [1,2]. There are three major types of CRISPR/Cas system in bacteria to interfere with the invading DNA through different cas proteins. In the type I and III systems multiple Cas proteins are required to cleave foreign DNA, but in the type II system only the Cas9 protein is required. The type II CRISPR/Cas9 system in bacteria has been harnessed and reprogramed for genome editing in mammalian cells [3-5]. The CRISPR/Cas9 system can be used to generate gene mutations (deletions or insertions) or introduce site specific mutations for gene correction, and also to transcriptionally activate or repress gene expression. Furthermore, human and mouse gRNA libraries are available to perform genome-wide studies for loss of function or transcriptional regulation [6,7]. Since it is fairly easy to perform and is highly efficient, CRIPSR/Cas9 has been widely used for gene functional studies *in vitro* and *in vivo*. Here we will review its potential application in cancer research.

CRISPR/Cas9 system

To target a specific gene using the CRISPR/Cas9 system, it is required to have a gRNA fused with a tracer RNA and the CRISPR-associated Cas9 protein. Specifically, a guide RNA (gRNA) forms a duplex with trans-activating crRNA (tracrRNA) and directs the CRISPR-associated protein Cas9 to a specific locus at the genome to induce double-stranded DNA breaks. Cas9 has two different nuclease domains (the HNH and RuvC-like domains) with each cleaving one strand of DNA. The double-stranded DNA breaks are repaired through non-homologous end-joining or homology directed repair, thereby leading to loss of gene function [5] (Figure 1).

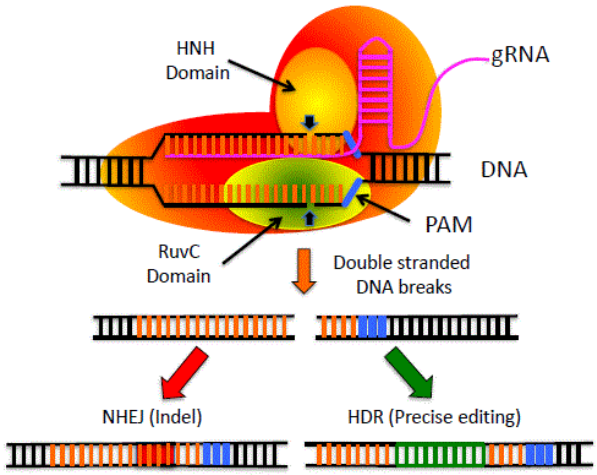

Figure 1: CRISPR/Cas9 mediated genome editing. A gRNA guides Cas9 to a specific locus of genome and introduces double-stranded DNA breaks. Cells repair the breaks through non-homologous end-joining and homology-directed recombination.

In addition, CRISPR/dCas9 mediated transcriptional activation or repression by targeting the promoter region using specific gRNAs and inactivated Cas9 through artificial transcriptional effectors has been employed to investigate the transcriptional regulation of target genes [7] (Figure 2).

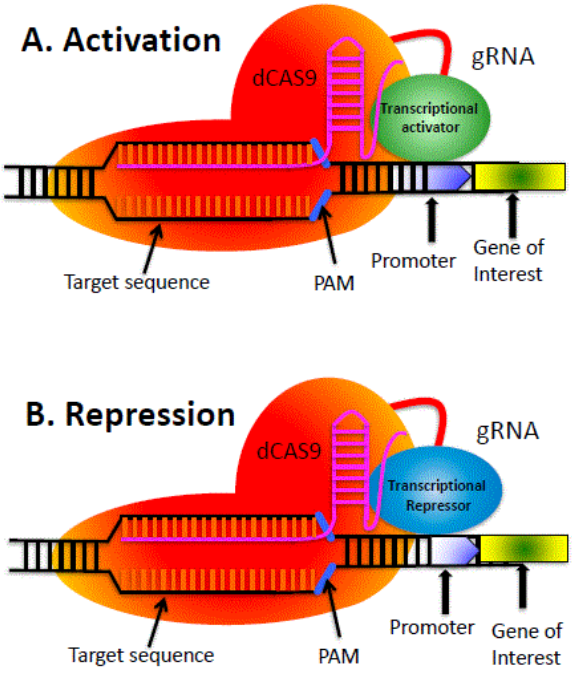

Figure 2: Lentiviral vector based CRISPR/Cas9 transcriptional activation or repression. Inactivated Cas9 is coexpressed with transcriptional effector or repressor and binds to the promoter region of a specific target genes guided by gRNAs, thus activating or repressing gene expression.

There are plasmid and viral vector systems, including lentiviral, adenoviral and adeno-associated viral vectors, to deliver gRNAs and Cas9 protein in cells *in vitro* or in animal models *in vivo*. The most widely used CRISPR/Cas9 expresses gRNAs with polymerase III promoter U6 or H1, while Cas9 is expressed in the same or different vector by polymerase II promoter, such as CMV, UBC or EF1α. However, wildtype Cas9 has been shown to induce off-target effects, which is a major concern in genome editing. There are several approaches available now to significantly reduce the off-target activity of CRISPR/Cas9 including the truncated gRNAs (16-18nt) [8], a mutant Cas9 (Cas9nickase) [9] and dimeric CRISPR RNA-guided FokI nuclease (RFN) through inactive Cas9 fused with FokI nuclease (CRISPR/RFN) [10]. Recently, Cas9 mutants were generated through site-directed mutagenesis based on structure and function. Several amino acid switch can significantly decrease off-target activity [11]. Therefore, CRISPR/Cas9 provides a novel approach to study gene functions *in vitro* and *in vivo* in a highly efficient way.

Potential application of CRISPR/Cas9 in cancer research

Since cancer is caused by multiple gene alterations, it is very difficult to mimic the oncogenic process *in vitro* and *in vivo*. However, CRISPR/Cas9 system provides a unique approach to investigate cancer initiation, progress and metastasis, as well as chemoresistance.

CRISPR/Cas9 mediated gene knockout

Knockout (KO) cell lines can be generated by simultaneously disrupting one or more oncogenes or tumor suppressors using a lentiviral vector based CRISPR/Cas9 system, and then loss of function studies can be performed. CRIPSR/Cas9 mediated knockout of Nanog and Nanogp8 in prostate cancer DU145 cells leads to significant reduction in the malignant potential, indicating that Nanog and Nanogp8 function as oncogenes in prostate cancer [12]. Knockout of Kras, p53 and Lkb1, while introducing a point mutation in $Kras^{G12D}$ at the genomic locus, significantly enhanced lung tumor growth in Cas9 transgenic mice using an AAV-9 vector [13]. Moreover, a gRNA library is available for genome-wide loss of function studies. A lentiviral vector based genome-scale library with 122,417 sgRNAs was constructed to target 19,052 human genes [14,15]. This library was used to screen genes associated with tumor growth and metastasis. 624 sgRNAs were screened to target the top-scoring genes that accelerated lung metastasis in a non-metastatic mouse cancer cell line [14].

CRISPR/Cas9 mediated transcriptional regulation

CRISPR/Cas9 mediated transcriptional activation and repression is novel approaches for cancer gene functional studies. Transcriptional activation and inhibition libraries are now available for genome-wide screening through targeting promoter region guided by gRNAs. A transcriptional activation library was constructed to target 23,430 coding isoforms with 70,290 gRNAs. In this lentiviral vector based gRNA library, the synergistic activation mediator (SAM), a single gene was targeted by three gRNAs and was used to co-transduce cells with transcriptional activators VP64, p65 and HSF1. Several drug resistant genes in melanoma were identified by screening this library [7]. Another genome wide transcriptional activation and repression library was constructed to identify genes essential for cell survival and differentiation [16]. In this library, each gene was targeted by 10 different gRNAs, and a total of 15,977 genes were targeted by 198,810 gRNAs. Therefore, CRISPR/Cas9 mediated gene mutations (deletion, insertion or point mutations) and transcriptional regulation provides strong platform to investigate cancer development.

CRISPR/Cas9 mediated chromosome translocation

In addition to generating multiple gene mutations in cells or animals, CRISPR/Cas9 can also be used to mimic chromosome translocation in various human cancers, such as the contribution of the pas3-foxo1 fusion gene in human alveolar rhabdomyosarcoma [17], the BCAM-AKT2 fusion gene in ovarian serous carcinoma [18] and the EML4-ALK fusion gene in lung cancer [19].

CRISPR/Cas9 is a powerful approach in defining drug resistance in cancer therapy-CRISPR/Cas9 not only provides a platform to mimic cancer in cells or animal models, but also to study cellular resistance to anticancer drugs. Genes associated with ispinesib and YM155 resistance was identified using the DrugTargetSeqR in combination of CRISPR/Cas9 approach [20]. Using a genome-wide CRISPR/Cas9 knockout library, vemurafenib resistant genes were identified, including two known genes (NF1 and MED12) as well as novel genes (NF2, CUL3, TADA2B, and TADA1) 6. CRISPR/Cas9 mediated disruption of HPRT1 or inducing ERCC3 point mutations leads to cell resistance to 6-thioguannie and triptolide [21]. CRISPR/Cas9 mediated C528S mutation of XPO1 gene confers resistance to selinexor, indicating that XPO1 is a drug resistant target in cancer chemotherapy

[22]. These studies suggest that CRIPSR/Cas9 provides a new tool to understand the molecular mechanisms in cancer chemoresistance.

CRISPR/Cas9 mediated somatic and germline cancer models

The CRISPR/Cas9 system also presents a new approach to generate somatic or germline animal models for cancer research. In particular, precision mouse models are urgently needed for drug screening and therapeutic studies in cancer research. It is a challenge to generate such animal models because multiple genes are mutated or abnormally expressed in a specific cancer type. However, CRISPR/Cas9 technology makes it possible to quickly generate precision mouse models by disrupting multiple genes or inducing point mutations at a specific genome locus simultaneously. For example, a Cre-dependent Cas9 knock in mouse was generated by inserting a Cas9 expression cassette into Rosa26 locus, in which Cas9 expression was dependent on the removal of LoxP-stop-LoxP cassette through Cre recombinase 13. This Cas9 knock in mouse can be used to generate constitutive or tissue specific knockout by breeding with a ubiquitous or tissue specific Cre mouse line, respectively. This mouse line has been used to induce tumor growth *in vivo* by knocking out several tumor suppressors and introducing Kras mutations 13. Introducing multiple mutations in the APC, SMAD4, TP53 tumor suppressor genes, and in the KRAS or PIK3CA oncogene using CRISPR/Cas9 in normal intestinal organoids leads to tumor formation after implanting such cells under the kidney capsule of mice [23]. Disruption of PTEN and P53 in mouse liver using CRIPSR/Cas9 leads to liver cancer [24]. Deletion of single (Ptch1) or multiple genes (Trp53, Pten, Nf1) in the mouse brain leads to the development of medulloblastoma and glioblastoma using CRISPR/Cas9 [25]. Taken together these studies demonstrate that CRISPR/Cas9 is a powerful tool to disrupt multiple genes in cell or animal models to study cancer development.

Conclusion

CRISPR/Cas9 is a powerful approach to generate or introduce mutations in cell line or animal models to mimic tumor development, and identify critical genes in tumorigenesis and drug resistance. Furthermore, this technology is highly efficient to generate precision cancer models by knocking out several tumor suppressors and introducing point mutations simultaneously as we summarized in (Figure 3).

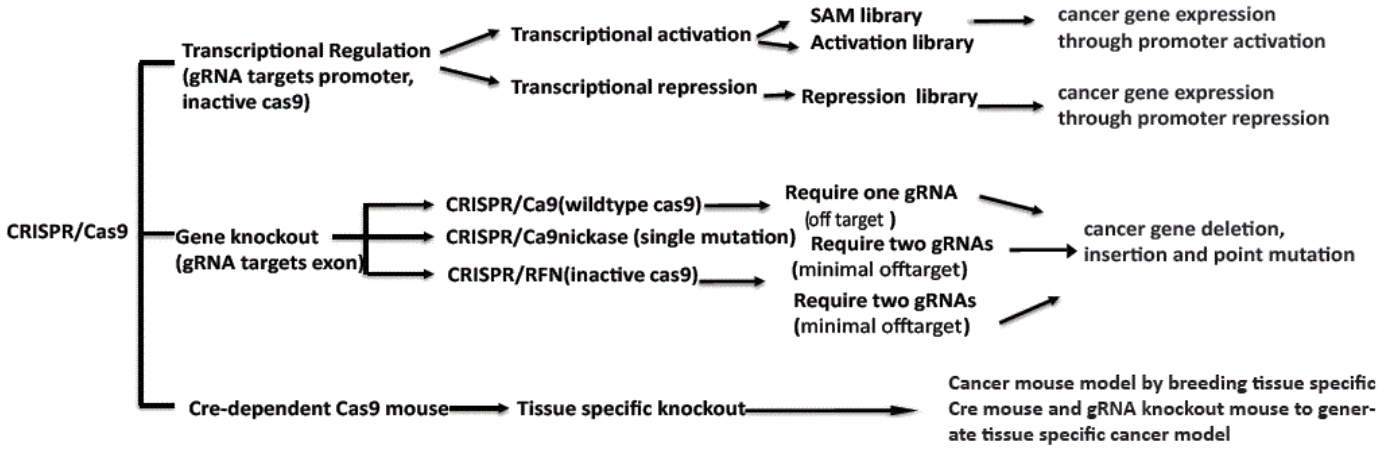

Figure 3: CRISPR/Cas9 mediated applications in cancer studies. CRIPSR/Cas9 mediated transcriptional regulation is to activate or repress endogenous cancer gene expression whereas the CRISPR/Cas9 is used to generate cancer gene deletion, insertion and point mutation. CRISPR/Cas9 nickase and CRISPR/RFN induce minimal offtargets whereas CRISPR/Cas9 has offtarget concern. A Cre dependent Cas9 knockin mouse was established to generate tissue specific knockout mouse model to study different type of human cancers by crossing tissue specific Cre transgenic mouse.

With the advent of gRNA libraries for gene knockout and transcriptional regulation, it is very convenient to perform genome-wide loss of function studies for screening or identifying genes related to tumor metastasis and drug resistance. Although CRISRP/Cas9 is a cutting-edge technology, fairly easy to perform and highly efficient in genome engineering, it is still a major concern and challenge to avoid the off-target effects caused by wildtype Cas9. Therefore, the potential off-target effects should be carefully considered in the analysis of data generated with CRISRP/Cas9. At least two different gRNAs should be considered by targeting the same gene from different locations of genome to validate the results of gene disruption when wildtype Cas9 was used for genome editing. If a more precise genome modification is expected, CRISPR/Cas9 nickase or CRISPR/RFN should be considered to target a specific gene in cancer cells. With the rapid improvement in diminishing off-target effects of CRISPR/Cas9, this technology will provide a novel approach in cancer therapy or drug screening by disrupting cancer genes or introducing point mutations and generating mouse cancer models.

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