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Controlling transcription in human pluripotent stem cells using CRISPR-effectors

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ABSTRACT

The ability to manipulate transcription in human pluripotent stem cells (hPSCs) is fundamental for the discovery of key genes and mechanisms governing cellular state and differentiation. Recently developed CRISPR-effector systems provide a systematic approach to rapidly test gene function in mammalian cells, including hPSCs. In this review, we discuss recent advances in CRISPR-effector technologies that have been employed to control transcription through gene activation, gene repression, and epigenome engineering. We describe an application of CRISPR-effector mediated transcriptional regulation in hPSCs by targeting a synthetic promoter driving a GFP transgene, demonstrating the ease and effectiveness of CRISPR-effector mediated transcriptional regulation in hPSCs.

1. Introduction/review

1.1. Human pluripotent stem cells

Deciphering human development and disease requires knowledge of the mechanisms regulating cellular differentiation and function. Human pluripotent stem cells (hPSCs) have the ability to self-renew *in vitro* and the potential to differentiate into any cell type of the body. These properties make hPSCs an effective model system to study factors and mechanisms governing the development of specific cell lineages and their impact on disease onset and progression. The ability to control transcription of specific genes in hPSCs allows for interrogation of the key factors necessary to maintain a given cellular state or drive cellular differentiation. Recently developed CRISPR (clustered regularly interspaced short palindromic repeats)-associated (Cas) systems have been adapted to regulate gene expression. These versatile systems allow rapid examination of gene function in human cells and have the potential to allow high-throughput analyses of either gene activation or repression in hPSCs and their differentiation intermediates.

1.2. CRISPR/Cas9-effector system

CRISPR systems are RNA-guided bacterial adaptive immune responses that detect and silence foreign DNA [1–4]. These systems involve recruitment and binding of a CRISPR-associated endonuclease to a specific target DNA sequence via a trans-activating CRISPR RNA (tracrRNA) and a CRISPR-derived RNA (crRNA). The protospacer, a sequence element of the crRNA, confers site-specificity through complementary base pairing, ensuring proper targeting to foreign DNA. The endonuclease then introduces double strand breaks in the target sequence [2,3,5].

The type II CRISPR system has been adapted as a tool for gene editing in mammalian systems. Combination of the tracrRNA and crRNA elements into one chimeric single guide RNA (sgRNA) can efficiently target Cas9 to specific DNA target sites [5–7]. This two component system (Cas9 and sgRNA) has been used to introduce double strand breaks in order to generate disease-relevant mutations or to insert donor sequences at target sites through homologous recombination [6,7]. In addition, CRISPR nucleases have successfully down-regulated target gene expression by introducing mutations or excising an enhancer [8,9]. However, nuclease-mediated modifications are irreversible, may require in depth knowledge of the element, can be inefficient because gene disruption relies on indel introduction caused by non-homologous end

Abbreviations: CAG, hybrid of cytomegalovirus immediate early enhancer, chicken β-actin promoter and intron, rabbit β-globin splice acceptor; Cas, CRISPR-associated; CRISPR, clustered regularly interspaced short palindromic repeats; crRNA, CRISPR-derived RNA; dCas9, nuclease-dead Cas9; hPSC, human pluripotent stem cell; KRAB, Krueppel repressor associated box; mESC, mouse embryonic stem cell; PAM, protospacer adjacent motif; sgRNA, single guide RNA; Sp, *Streptococcus pyogenes*; tracrRNA, trans-activating CRISPR RNA; TALE, transcription activator-like effector.

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joining repair errors, may disrupt local genomic architecture, and are not adaptable for gain-of-function approaches.

To adapt CRISPR/Cas9 for gene regulation studies, the nuclease activity of Cas9 has been inactivated through mutation of catalytic residues within its nuclease domains, resulting in a nuclease dead Cas9 (dCas9) [5,10]. Coupling dCas9 to effector domains converts the CRISPR/Cas9 technology into a site-specific programmable system, which through fused effector domains can regulate gene expression (Fig. 1) [11–19]. This versatile system of transcriptional control allows for characterization of the key factors and mechanisms governing a given cellular state. The ability to multiplex sgRNAs allows for synergistic effects on gene expression and permits targeting of multiple genes simultaneously [11,12,14,16,20]. In addition, the possibility of inducing dCas9-effector or sgRNA expression may allow for temporal and spatial manipulation of gene function. When applied to hPSCs, CRISPR/dCas9-effector systems could be used to influence cellular states of differentiation intermediates and can help to dissect the individual contribution of factors to development and disease.

1.3. dCas9-effector mediated transcriptional activation

dCas9/sgRNA complexes can be modified to activate gene expression in mammalian systems when targeted upstream of endogenous transcriptional start sites. Fusion of dCas9 to the p65 activation domain or to tandem repeats of the herpes simplex virus trans-activation domain VP16 (VP48, VP64, VP160, VP192) has been shown to activate endogenous gene expression [11,14]. In a subsequent study, fusion of dCas9 to the tripartite activator VP64-p65-Rta (VPR) resulted in higher levels of gene activation compared to VP64 fusion alone [21]. The CRISPR/dCas9 system has also been adapted in other ways for site-specific transcriptional activation. dCas9 fused to the single-chain antibody binding SunTag allowed for multimerization of antibody-fused VP64 domains, amplifying gene expression compared to dCas9-VP64 [19]. Tethering copies of the MS2 bacteriophage coat protein-binding RNA stem loop to the 3′-end of a sgRNA activated gene expression when coexpressed with MS2-VP64 fusion proteins and dCas9 [20]. More recently, using CRISP-Disp, RNA domains are combined with sgRNA scaffolds, enabling site-specific recruitment of functional RNA modules, including natural lncRNAs such as *HOTTIP*, which subsequently mediate gene regulation [22]. All of these adapted dCas9 systems can effectively increase expression of target genes highlighting their potential use in gain-of-function studies.

Recruitment of transcriptional activation domains to endogenous loci in hPSCs and differentiation intermediates may be used to interrogate the effect of gene expression on cellular state. dCas9-effector mediated transcriptional activation has been demonstrated in hPSC systems in order to induce expression of endogenous genes and alter cell fate. Targeting dCas9-VP64 to the promoter of the developmentally relevant transcription factor *SOX17* induced its RNA and protein expression in hPSCs [16]. Recruitment of dCas9-VPR to *NGN2* or *NEUROD1* induced neuronal differentiation, suggesting the potential use of the system to modify cell fate through gene activation [21]. The ability to activate expression of endogenous genes with CRISPR-effector systems may also prove beneficial for imposing cell fate through activation of transcription factor expression. Targeting of dCas9 fused to two VP64 domains to the *MyoD1* locus of mouse primary fibroblasts activated endogenous myogenic genes comparably to traditional *MyoD1* overexpression methods, resulting in the conversion of fibroblasts into skeletal myocytes [23]. dCas9-VP64 can replace exogenous *Oct4* (*Pou5f1*) during reprogramming of mouse embryonic fibroblasts to induced pluripotent stem cells (iPSCs) by targeting the *Oct4* distal enhancer albeit at lower frequency

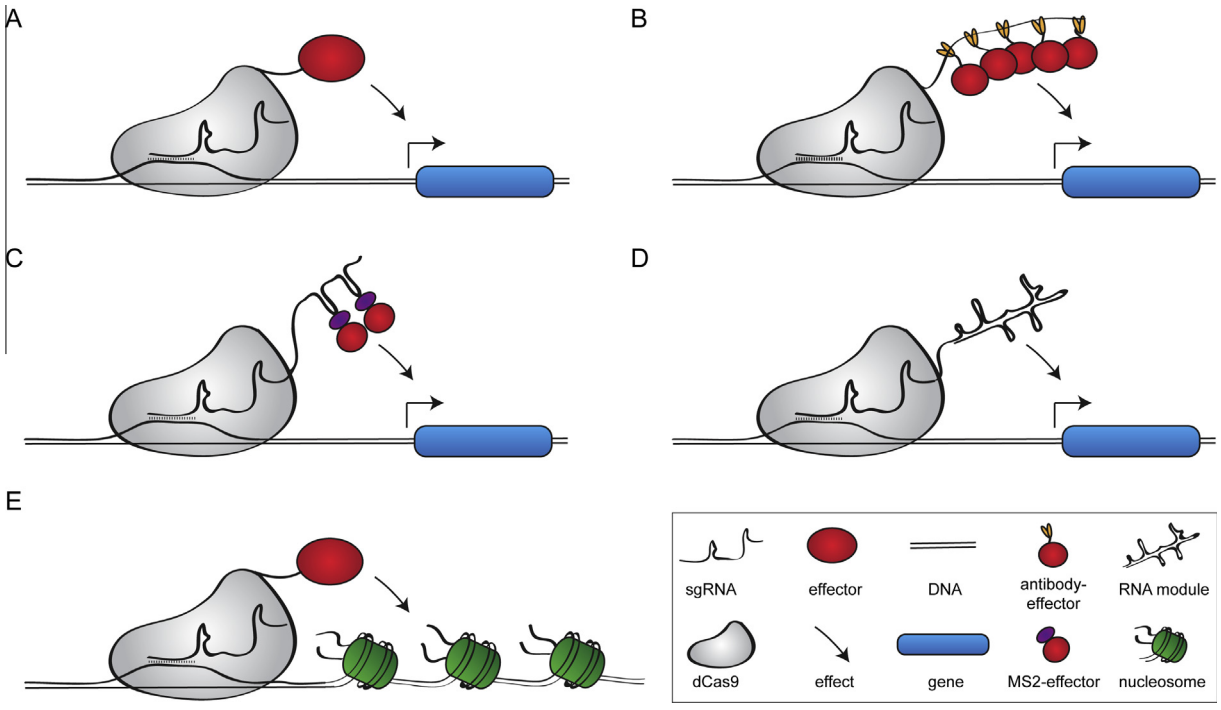

Fig. 1. CRISPR/Cas9 effector-mediated transcriptional control. (A) Schematic depicting dCas9-effector mediated transcriptional regulation through fusion of dCas9 to transcriptional activation or repression effector domains. (B) Schematic depicting dCas9-effector mediated transcriptional regulation through recruitment of multiple antibody-fused effector domains to a dCas9-fused epitope array. (C) Schematic depicting dCas9-effector mediated transcriptional regulation through recruitment of MS2-effector fusion proteins to tethered copies of the MS2 bacteriophage coat protein-binding RNA stem loop on the 3′-end of an sgRNA. (D) Schematic depicting dCas9-effector mediated transcriptional regulation through scaffolding of a functional RNA module (such as an aptamer or lncRNA) to an sgRNA. (E) Schematic depicting dCas9-effector mediated transcriptional regulation through fusion of dCas9 to epigenetic modifiers to alter local epigenetic marks.

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