GENETIC ENGINEERING

A CRISPR approach to elucidate the role of enhancers during development and disease

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Multicellular organisms are made of different cell types that express different set of genes. These distinct gene expression profiles are regulated by DNA regulatory regions, such as enhancers, which orchestrate a precise spatial and temporal control of gene transcription during development. Disruption of enhancers, and subsequent changes in gene expression, can lead to disease, which calls for further investigation of these elements. Several tools such as in vitro reporter assays and transgenesis are available to assess enhancers, but they do not recapitulate the native chromatin environment which might influence their activity. In a study published in *Nature Communications*, a team from University of Texas Southwestern Medical Center describes a new CRISPR-based epigenetic editing tool to modify enhancer activity in situ and in vivo. By allowing both gain and loss of function analyses in the native biological context, this approach provides new opportunities to uncover the role of enhancers in development and disease.

The investigators developed two systems: enCRISPRa to activate enhancers and enCRISPRi to inhibit them. In both cases, deactivated Cas9 (dCas9) was fused to epigenetic modifiers—activator VP64 or p300 in enCRISPRa; repressor KRAB or LSD1 in enCRISPRi—to modify the epigenetic state of the targeted enhancer and perturb its function. Previous studies using similar dCas9 complexes have shown that they can efficiently modulate transcription when targeting promoters found close to the target gene but that they are less powerful when targeting enhancers located more distantly. To improve the efficiency of the CRISPR system, the investigators modified the design of sgRNAs to allow the recruitment of an additional epigenetic modifier (dual effector).

The systems were first evaluated in vitro for their ability to modulate the activity of the HS2 enhancer at the human β -globin locus. Gene expression and ChIP seq analysis of HEK293T or K562 cells transduced with different CRISPR epigenetic editing systems revealed that dual effectors enCRISPRa and enCRISPRi led to more potent activation and inhibition of β -like globin genes than single-effector dCas9 systems, and induced more changes in histone marks at HS2.

enCRISPRa-mediated activation of *TAL1* super-enhancer promoted T-ALL growth in NSG mice xenografted with Jurkat cells transduced with sgGal4, sgMut2 or sgWT2, respectively. Reprinted with permission from Li et al. (2020) Springer Nature.

Next, the investigators examined whether they could use their systems to target disease-associated enhancers in vivo. They focused on an enhancer located upstream of the *TAL1* proto-oncogene, which is mutated in T-cell acute lymphoblastic leukemia (T-ALL) cell lines and patients; the mutations introduce binding motifs for the transcription factor MYB, leading to the formation of a super-enhancer (SE) involved in oncogenic transcription. Li and colleagues transduced T-ALL cells with enCRISPRa together with sgRNA targeting either the mutated allele or both wild-type and mutated allele, before xenotransplanting them into immunodeficient NSG mice. Four weeks after transplantation, the mice with cells expressing *TAL1* SE-targeting sgRNAs (sgMut and sgWT) showed greater tumor burden than control mice expressing control sgGal4. According to the investigators, these results not only establish the role of the *TAL* oncogenic SE in promoting T-ALL development, but also demonstrate the efficacy of their system for allele-specific perturbation of disease-associated enhancers in vivo.

Next, the team generated a knock-in dCas9-KRAB mouse model. The mice were used in bone marrow transplantation (BMT) experiments to evaluate the effect of enCRISPRi-mediated enhancer perturbations

during hematopoietic lineage differentiation in vivo. Hematopoietic stem and progenitor cells (HSPCs) from dCas9-KRAB mice were transduced with sgRNA targeting hematopoietic-specific enhancers and transplanted into CD45.1+ lethally irradiated recipient mice. Given that transplanted HSPCs are capable of reconstituting the entire blood system in the recipient mice, analysis of the different HSPC-derived cell lineages in the recipient mice can be used as “readout” for the effect of enhancer epigenetic editing during HSPC lineage differentiation. Analysis of the recipient mice 16 weeks after BMT demonstrated that the hematopoietic-specific enhancers targeted were required for HSPC differentiation into one or multiple hematopoietic lineages, and further confirmed the efficiency of the system in vivo.

In the discussion, the investigators emphasize that this tool could be useful for different fields of research: “Our studies not only identify candidate lineage-specific enhancers required for hematopoiesis, but also establish widely applicable platforms for unbiased analysis of noncoding regulatory genome which can be extended to other cell types and human diseases.”

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