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Review

Epigenome Editing: State of the Art, Concepts, and Perspectives

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Epigenome editing refers to the directed alteration of chromatin marks at specific genomic loci by using targeted EpiEffectors which comprise designed DNA recognition domains (zinc finger, TAL effector, or modified CRISPR/Cas9 complex) and catalytic domains from a chromatin-modifying enzyme. Epigenome editing is a promising approach for durable gene regulation, with many applications in basic research including the investigation of the regulatory functions and logic of chromatin modifications and cellular reprogramming. From a clinical point of view, targeted regulation of disease-related genes offers novel therapeutic avenues for many diseases. We review here the progress made in this field and discuss open questions in epigenetic regulation and its stability, methods to increase the specificity of epigenome editing, and improved delivery methods for targeted EpiEffectors. Future work will reveal if the approach of epigenome editing fulfills its great promise in basic research and clinical applications.

What is Epigenome Editing?

The genetic information of all cells in a multicellular organism is almost invariable. Despite this, cells have the potential to differentiate into hundreds of distinct cell types with unique cellular programs, morphologies, and functions. This outstanding feat is achieved by so-called **epigenetic** mechanisms (see Glossary), including **histone post-translational modifications** (PTMs), **DNA methylation** and **hydroxymethylation**, and non-coding RNAs (ncRNAs) [1], which in concert regulate the expression of genes and access to **chromatin**. The sum of this record of chemical changes set on the DNA and histone proteins is termed the **epigenome** and it is unique for each cell type in an organism. The epigenome can be understood as an additional regulatory layer imposed on the genome that is reversible but at the same time heritable, with indispensable roles in shaping and maintaining the cellular phenotype. Chromatin modifications are introduced by chromatin-modifying enzymes or enzyme complexes, and the dynamic modification state of a particular chromatin region depends on the relative activity of counteracting pairs of enzymatic systems at the target site, and the rates of DNA replication and histone turnover [2,3].

Numerous chromatin modifications have been profiled in hundreds of cell types, yielding thousands of global-scale so-called epigenomic maps [4–6]. Although these efforts have resulted in many pertinent general biological insights, such as the discovery of novel regulatory elements and chromatin states, their functional relevance has remained purely correlative in many instances. Moreover, in many cases it is unclear if particular modifications or combinations thereof have transient functions or if they are heritable and have epigenetic roles. The dissection of the functional roles of distinct modifications covering defined genomic regions has been kick-started only recently with the development of a new suite of experimental tools for targeted

Trends

Numerous studies have demonstrated that targeted deposition or removal of chromatin modifications (epigenome editing) is a powerful approach for functional studies of locus-specific chromatin modifications and their relation to gene expression and other processes.

Epigenome editing holds great potential as a therapeutic approach in the clinic for durable regulation of disease-related genes and in cellular reprogramming.

Before the full potential of epigenome editing can be realized, numerous questions related to the function, regulatory logic, and maintenance of chromatin modifications need to be answered.

The question of specificity of the DNA recognition domain needs to be addressed in a case-by-case manner. The activity of the EpiEffector (catalytic domain of a chromatin-modifying enzyme) needs to be tuned to achieve optimal chromatin modulation.

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Key Figure

Principles and Applications of Targeted Epigenome Editing

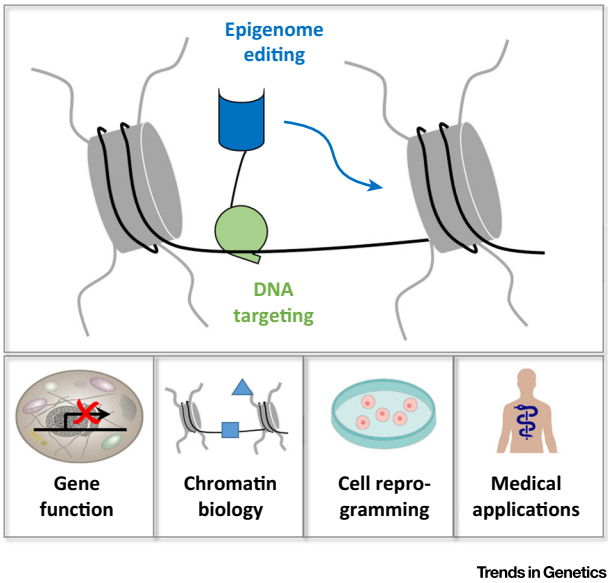

Figure 1. Epigenetic editing is based on fusion proteins comprising a designed DNA recognition domain which targets an attached enzymatic domain to defined genomic target sites. The applications of epigenome editing lie in basic research, such as gene function studies and the investigation of chromatin biology, cell reprogramming, and also in molecular medicine.

epigenome manipulation at defined loci. The core of this technology is based on the fusion of a DNA recognition domain with a catalytic domain of a chromatin-modifying enzyme to generate targeted EpiEffectors. The DNA recognition domain serves to bind a unique DNA sequence and deliver the annexed functional domain to defined target loci in the genome, where it can change the chromatin modification state and by this alter gene expression, cellular differentiation, or other biological processes (Figure 1, Key Figure). Epigenome editing is a very promising approach that can usher a new era of novel applications for basic research and molecular medicine. It was recently given an important boost with the discovery of the **CRISPR/Cas9** DNA-binding system, which facilitates the design of the DNA recognition domains needed for the application. Moreover, the continuous progress in our understanding of epigenetic mechanisms, including the discovery of novel effector domains such as those of the enzymes involved in DNA demethylation [7], has further powered our abilities for rational epigenome editing.

In this manuscript we describe current targeting modules together with the concepts of epigenome editing, and review the progress made so far. We further discuss the stability of the newly introduced chromatin states based on recent data. Finally, we set forth a vision for basic science and clinical applications of epigenome editing, and summarize open questions and directions for future work.

Overview of the Development of Specific DNA-Targeting Modules

The fundamental understanding of sequence-specific protein–DNA interactions dates back to the 1970s when the principles of the direct readout of a DNA sequence in the major groove by protein-mediated hydrogen bonds were first predicted [8]. However, it turned out that, for most

Glossary

Causative therapy: direct and targeted treatment of the major cause of a disease or phenotypic state.
Cellular reprogramming: the process of converting one cell type into another by changing the gene expression program of the cell.
Chromatin modification network: the structural and functional interplay and coexistence of histone and DNA modifications within chromatin.
Chromatin: nucleoprotein complex containing DNA, histones, non-histone proteins, and RNA. The basic structural unit of chromatin is the nucleosome, consisting of 147 bp of DNA wrapped around an octamer of histones H3, H4, H2A, and H2B.
CRISPR/Cas9: a prokaryotic immune system which protects bacterium against foreign DNA such as plasmids and phages. Mechanistically, in its simplest form, a nuclease (Cas9) binds to an appropriate small guide RNA molecule of the CRISPR class which targets the entire complex to its complementary target DNA sequence.
DNA hydroxylation: oxidation of the 5-methylcytosine to 5-hydroxymethylcytosine and higher oxidation states. This process is the first step in DNA demethylation and the modified bases function as a chromatin modification.
DNA methylation: addition of a methyl group on the C5 position of cytosine residues in DNA, typically in a CpG context, by enzymes termed DNA methyltransferases. DNA adenine-N6 and DNA cytosine-N4 methylation is not discussed here.
Epigenetics: scientific field studying mitotically and/or meiotically heritable changes in gene function that do not rely on changes in DNA sequences.
Epigenome: the sum of all chromatin modifications which may or may not be heritable (epigenetic).
Histone post-translation modifications (PTMs): enzymatically introduced covalent modification of histone proteins, including lysine acetylation, lysine and arginine methylation, lysine ubiquitination, serine or threonine phosphorylation, among others.
Imprinting: an epigenetic phenomenon where particular alleles are expressed in a parent-of-origin-dependent manner.