Reactivation of epigenetically-silenced genes by the CRISPR Technology

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The CRISPR technology has revolutionized the world of genetic editing, allowing the manipulation of the genomes of diverse organisms quickly and easily. The CRISPR (clustered regularly interspaced short palindromic repeats) methodology derives from a bacterial adaptive immune system that uses an RNA-guided nuclease (Cas9) to target and destroy invading DNA. The use of a catalytically inactive nuclease (dCas9) co-expressed with a short guide RNA (sgRNA) allows using CRISPR/dCas9 as a general platform for RNA-guided targeting of different effector proteins to specific genomic regions. Fusion of dCas9 to effector domains with epigenetic functions can be used for targeted transcriptional regulation in human cells.

To confirm that the CRISPR system allows targeted reactivation of epigenetically silenced genes, we fused dCas9 either to the transcriptional activation domain VP160, or to the catalytic core of the human p300 acetyltransferase. The targeted activity of both fusion proteins, co-expressed with different sgRNAs in human HEK293 cells, was tested on a luciferase reporter gene previously silenced by *in vitro* methylation.

Luciferase reporter assays and expression analysis by qRT-PCR showed that specific combinations of sgRNAs efficiently targeted dCas9-VP160 and dCas9-p300 for reactivation of the silenced luciferase gene. Bisulfite pyrosequencing revealed that such reactivation does not correlate with a decrease in methylation levels, suggesting that other epigenetic mechanisms are involved in the process.

Our results establish that the CRISPR system can be used as a modular and flexible DNA-binding platform for the recruitment of epigenetic effector proteins to a target DNA sequence, revealing the potential of CRISPR as a general tool for the precise regulation of gene expression in eukaryotic cells.