DEVELOPMENT OF A CRISPR-BASED SYSTEM TO REACTIVATE EPIGENETICALLY SILENCED GENES IN HUMAN CELLS

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DNA methylation is an epigenetic mark associated to gene silencing, and its targeted removal is a major goal of epigenetic editing. In human cells, DNA demethylation involves iterative 5-methylcytosine (5-mC) oxidation by TET enzymes followed by replication-dependent dilution and/or replication-independent DNA repair of its oxidized derivatives. In contrast, plants use specific DNA glycosylases that directly excise 5-mC and initiate its substitution for unmethylated C in a base excision repair process.

The CRISPR 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 epigenetics effector domains can be used for targeted transcriptional regulation in human cells.

In this work, we have fused dCas9 to the catalytic domain of Arabidopsis ROS1 5mC DNA glycosylase (ROS1_CD), and we have explored the possibility of directing ROS1_CD glycosylase activity to a specific target sequence in human cells. As control, TET1 human protein has been also fused to dCas9. 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 single or combined sgRNAs efficiently targeted dCas9-ROS1_CD for reactivation of the silenced luciferase gene. In contrast, no reactivation was detected when those same gRNAs were used to target dCas9-TET1. Bisulfite pyrosequencing revealed that reactivation induced by dCas9-ROS1_CD correlates with certain decrease in DNA methylation levels.

These findings suggest the use of plant 5mC DNA glycosylases for targeted active DNA demethylation and gene reactivation in human cells.