Identifying regulators of LINE-1 expression using FACS-based CRISPR Screens

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Abstract

Approximately one third of the human genome is composed of repetitive DNA elements derived from retrotransposons. Long interspersed element-1 (LINE-1) is the only active, protein-coding retrotransposon in humans, and its retrotransposition is normally repressed in somatic cells via several mechanisms, including epigenetic silencing. In contrast, LINE-1 overexpression and *de-novo* LINE-1 insertions are commonly detected in human cancers with *TP53* mutations. A retrotransposition competent copy of LINE-1 encodes an RNA binding protein (ORF1p), which is produced abundantly, and a protein with endonuclease (EN) and reverse transcriptase (RT) activities (ORF2p). Hence, the detection of ORF1p is ideal for detecting expression of retrotransposition competent LINE-1 copies in human cells. To identify suppressors of LINE-1 expression, we have performed genome-wide forward genetic CRIPSR screens using intracellular staining of endogenous ORF1p to detect LINE-1-expressing cells in knockout cell pools using fluorescence-activated cell sorting (FACS). Our screen revealed previously identified epigenetics suppressors of LINE-1 expression, including DNA methyltransferases and factors in the HUSH complex, as well as additional mechanisms of LINE-1 suppression. Together, these studies will thereby identify factors that regulate LINE-1, providing new insights into the regulation mechanisms of LINE-1 expression and its impact on human cancers.

Keywords: LINE1, Epigenetics, CRISPR Screens

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