LETTER

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Selective silencing of euchromatic L1s revealed by genome–wide screens for L1 regulators

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Transposable elements (TEs) are now recognized not only as parasitic DNA, whose spread in the genome must be controlled by the host, but also as major players in genome evolution and regulation[1–6]. Long INterspersed Element-1 (LINE-1 or L1), the only currently autonomous mobile transposon in humans, occupies 17% of the genome and continues to generate inter- and intra-individual genetic variation, in some cases resulting in disease[1–7]. Nonetheless, how L1 activity is controlled and what function L1s play in host gene regulation remain incompletely understood. Here, we use CRISPR/Cas9 screening strategies in two distinct human cell lines to provide the first genome-wide survey of genes involved in L1 retrotransposition control. We identified functionally diverse genes that either promote or restrict L1 retrotransposition. These genes, often associated with human diseases, control the L1 lifecycle at transcriptional or post-transcriptional levels and in a manner that can depend on the endogenous L1 sequence, underscoring the complexity of L1 regulation. We further investigated L1 restriction by MORC2 and human silencing hub (HUSH) complex subunits MPP8 and TASOR[8]. HUSH/MORC2 selectively bind evolutionarily young, full-length L1s located within transcriptionally permissive euchromatic environment, and promote H3K9me3 deposition for transcriptional silencing. Interestingly, these silencing events often occur within introns of transcriptionally active genes and lead to down-regulation of host gene expression in a HUSH/MORC2-dependent manner. Together, we provide a rich resource for studies of L1 retrotransposition, elucidate a novel L1 restriction pathway, and illustrate how epigenetic silencing of TEs rewires host gene expression programs.

Most of our knowledge about L1 retrotransposition control comes from studies examining individual candidate genes[2–6]. To systematically identify genes regulating L1 retrotransposition, we performed a genome-wide CRISPR/Cas9 screen in human chronic myeloid leukemia K562 cells using an L1-G418^R retrotransposition reporter[9] (Fig. 1a,b). Importantly, the L1-G418^R reporter was modified to be driven by a doxycycline (dox)-responsive promoter, as opposed to the native L1 5'UTR, to avoid leaky retrotransposition ahead of the functional screen (Extended Data Fig. 1a-c). The cells become G418^R antibiotic resistant only when the L1-G418^R reporter undergoes a successful retrotransposition event following dox-induction (Fig. 1b). For the screen, we transduced clonal L1-G418^R cells with a lentiviral genome-wide sgRNA library such that each cell expressed a single sgRNA[10]. We then dox-induced the cells to turn on the L1-G418^R reporter for retrotransposition, and split the cells into G418-selected conditions and unselected conditions, which served to eliminate cell growth bias in the screen analysis. The frequencies of sgRNAs in the two populations were measured by deep sequencing (Fig. 1a) and analyzed using Cas9 high-Throughput maximum Likelihood Estimator (CasTLE)[11]. Consequently, cells transduced with sgRNAs targeting L1 suppressors would have more retrotransposition events than negative control cells and would be enriched through the G418 selection; conversely, cells transduced with sgRNAs targeting L1 activators would be depleted.

Using the above strategy, we identified 25 putative L1 regulators at a 10% FDR cutoff, and 150 genes at a 30% FDR cutoff (Fig. 1c and Extended Data Fig. 1d; see Table S1 for full list). Despite low statistical confidence, many of the 30% FDR cutoff genes overlapped previously characterized L1 regulators (e.g. ALKBH1, SETDB1) and genes functioning in complexes with our top 10% FDR hits (e.g. Fanconi Anemia pathway, HUSH complex), suggesting that they likely encompassed biologically relevant hits. To increase statistical power in distinguishing bona fide L1 regulators among these, we performed a high-coverage secondary screen targeting the 30% FDR hits (150 genes) and an additional 100 genes that were either functionally related to our top hits or which were otherwise previously known to regulate L1 but fell outside of the 30% FDR cutoff threshold (See Table S2 for full list). This secondary screen validated 90 genes out of the top 150 genome-wide screen hits, a fraction close to expected with the 30% FDR cutoff (Fig. 1d and Extended Data Fig. 2a-c).

Altogether, our two-tier screening approach identified 142 human genes that either activate or repress L1 retrotransposition in K562 cells, encompassing over 20 previously known L1 regulators (Extended Data Fig. 2d). Novel candidates are involved in functionally diverse pathways, such as chromatin/transcriptional regulation, DNA damage/repair, and RNA processing (Extended Data Fig. 2e,f). While many DNA damage/repair factors, particularly the Fanconi Anemia (FA) factors, suppress L1 activity, genes implicated in the Non-Homologous End Joining (NHEJ) repair pathway promote L1 retrotransposition (Extended Data Fig. 2f). In agreement, mutations in some of the identified NHEJ factors were previously found to result in decreased retrotransposition frequencies[12]. Intriguingly, many hits uncovered by our screen (e.g. FA factors, MORC2 and SETX) are associated with human disorders[13–17].

To extend our survey of L1 regulators to another cell type, we performed both a genome-wide and a secondary screen in HeLa cells (Extended Data Fig. 1b, 1e) with the same sgRNA libraries used in the K562 screens. Importantly, top hits identified in the K562 genome-wide screen were recapitulated in the HeLa screen (e.g. MORC2, TASOR, SETX, MOV10) (Extended Data Fig. 3a). Furthermore, secondary screens in both K562 and HeLa cells showed concordant effects for groups of genes, for example, the suppressive effects of the FA complex genes, and activating effects of the NHEJ pathway genes (Extended Data Fig. 3b-e). Interestingly, however, a subset of genes showed cell-line selective effects (Extended Data Fig. 3c). At the same time, some of the previously known L1 regulators did not come up as hits in our

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