46. Enhancing Translation of LINE-1 encoded ORF2p

(Johns Hopkins University)

5TH KDDF GLOBAL C&D TECH FAIR

Asset Overview

Product Type	Small molecule
Disease Area	Oncology
Indication	Cancer
Current Stage	Lead Optimization
Target	ORF2p
MoA	ORF2p translation could activate by the bi-cistronic LINE-1 RNA
Brief Description	• A case of colon cancer with an aggressive tumor subclone that shut down LINE-1 expression concurrent with its accelerated growth. • LINE-1 triggers a tumor protein p53 (TP53)-mediated G1 arrest and an interferon response in nontransformed cells. • In TP53-deficient cells, inventor conducted a knockout screen to identify genes that affect the fitnesss of LINE-1+ cells. LINE-1+ cells rely on replication-coupled DNA-repair pathways, replication-stress signaling responses and replication-fork restart factors for growth. • LINE-1 expression activates the Fanconi anemia pathway, induces markers of replication stress and sensitizes cells to mitomycin C (MMC). Inventor proposes a model for LINE-1 toxicity wherein LINE-1 retrotransposition conflicts with DNA replication.
Intellectual Property	WO2021134040A2
Publication	LINE-1 ORF2p expression is nearly imperceptible in human cancers. Mob DNA, (2019) Cell fitness screens reveal a conflict between LINE-1 retrotransposition and DNA replication. Nat Struct Mol Biol, (2020)
Inventors	Kathleen H. Burns

Highlights

- The LINE-1– section derives from a LINE-1+ lineage, and loss of LINE-1 expression is associated with an enhanced growth rate.
- LINE-1 expression causes a p53–p21-dependent growth arrest.
- LINE-1 induces p53-mediated G1 arrest and an interferon response.
- LINE-1 inhibits cell growth in RPE by activating the p53-p21 pathway.
- LINE-1 activates a p53 and IFN response.
- High levels of ORF2p expression overwhelm the survival advantage conferred by TP53 deficiency.
- LINE-1 activity induces replication stress.
- LINE-1+ cells rely on FA-mediated DNA repair, replication-stress signaling and fork-restart pathways for growth.

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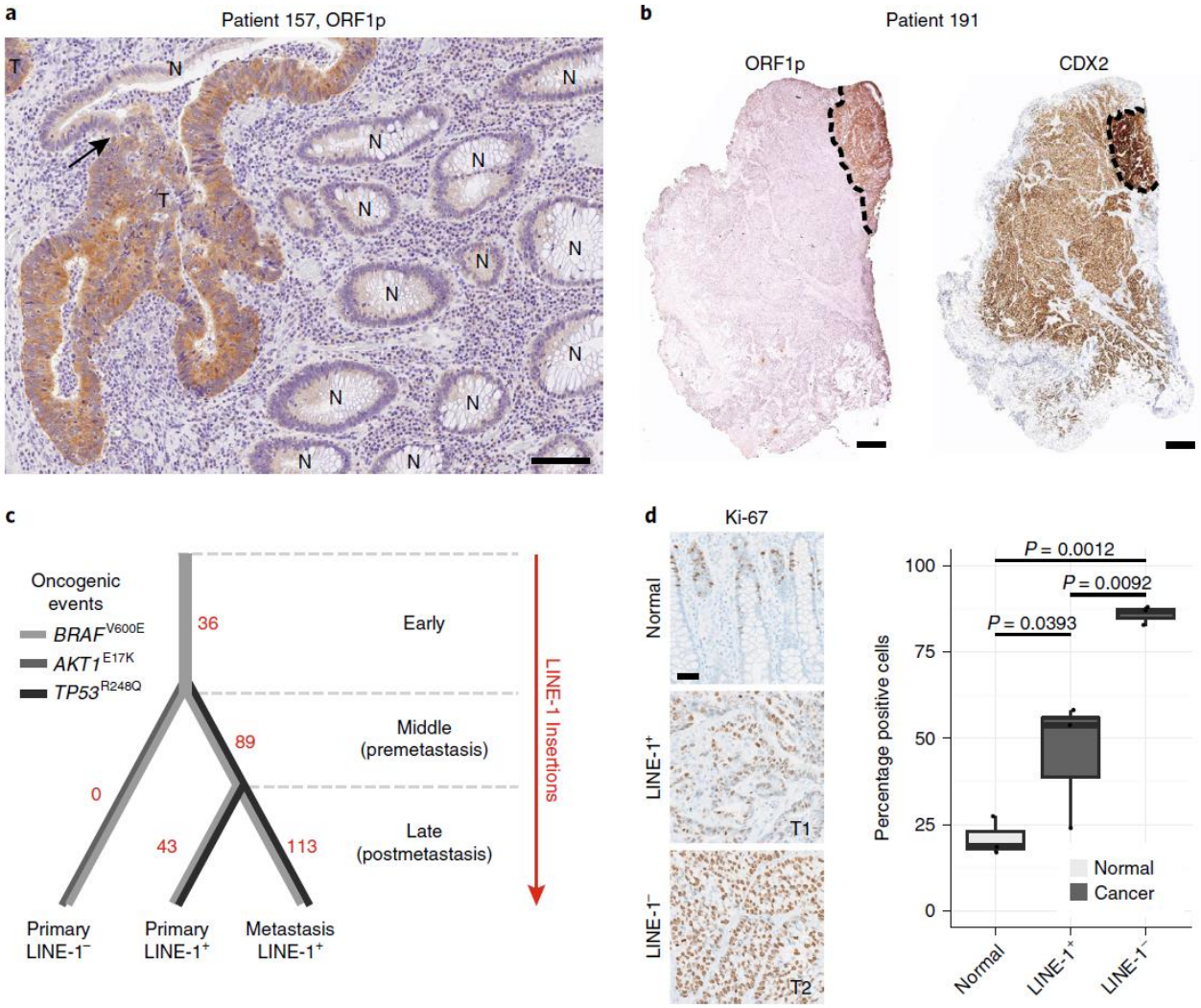
Key Data

Heterogenous LINE-1 expression in colon cancer



a, ORF1p immunohistochemistry (IHC) stain of formalin-fixed paraffin-embedded (FFPE) colon cancer tissue. LINE-1 immunostaining is seen in tumor (T) and not in normal colonic epithelium (N). The arrow indicates a transition from normal to tumor tissue within a gland. Scale bar, 50 μm. **b**, IHC stain of FFPE colon cancer tissue from patient 191. Left: low magnification of ORF1p intensely positive and negative tumor sectors. Right: low magnification of CDX2, a colon epithelium marker. LINE-1+ cells express higher CDX2 and form glands, whereas LINE-1– cells express lower CDX2 and do not form glands. Scale bars, 500 μm. **c**, Phylogenetic tree of the tumor subclones in patient 191, based on transposon insertion sequencing and known tumor-driver alleles. The number of de novo LINE insertions is indicated in red along the line edges. Using Sanger sequencing, we genotyped known tumor-driver alleles and found an *AKT1*E17K mutation in the CDX2dim cells and a *TP53*R248Q mutation in CDX2high cells (both primary and metastatic sites). All tumor specimens possessed a *BRAF*V600E allele regardless of LINE-1 expression status. The color of the lines indicates the presence or absence of known tumor-driver alleles. **d**, Ki-67 quantification of normal epithelium, LINE-1+ glandular cancer and LINE-1– solid cancer in patient 191. The percentage of positive cells was calculated as the number of Ki-67+ nuclei divided by the total number of epithelial cell nuclei. Three independent high-powered fields were counted per tissue morphology, and results were compared using ANOVA and two-sided *t*-tests. Scale bar, 100 μm.

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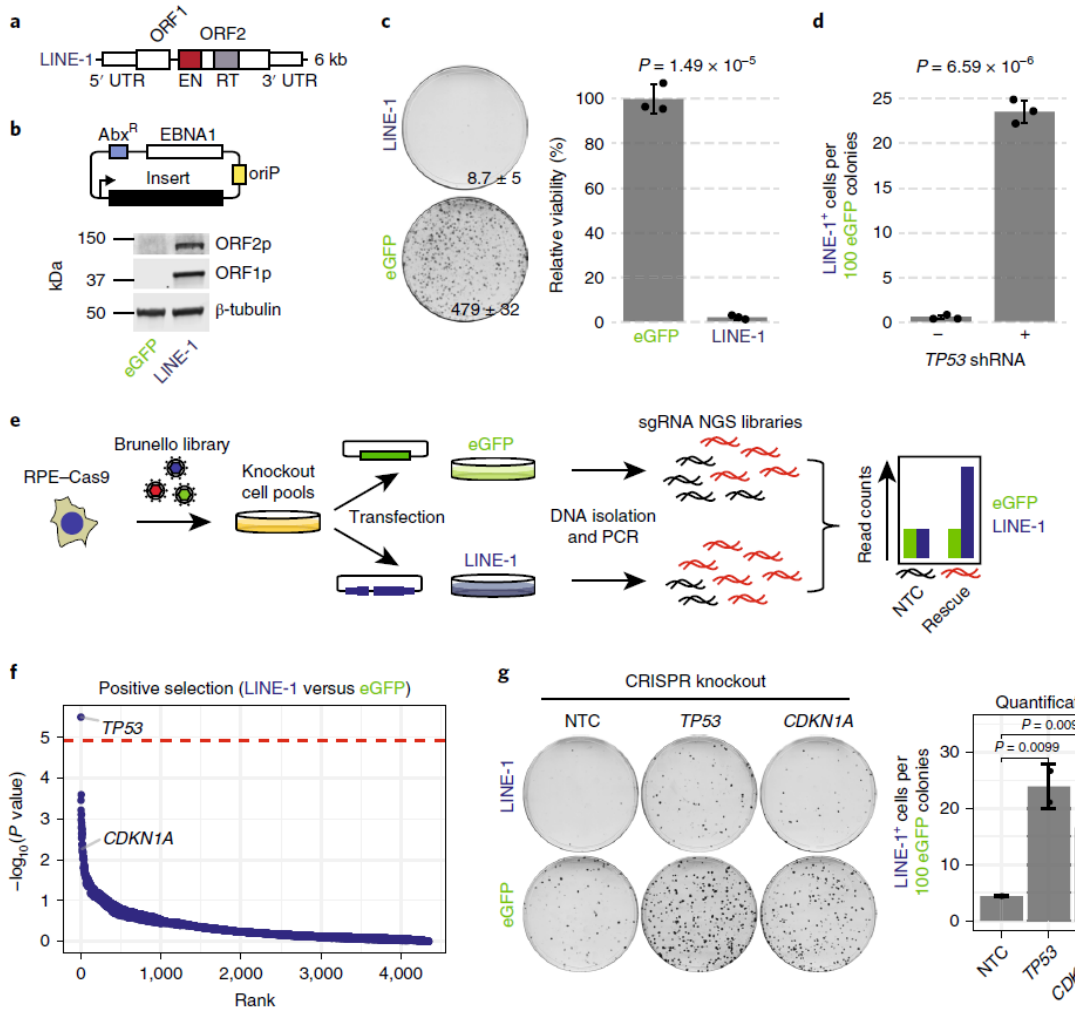
LINE-1 inhibits cell growth in RPE by activating the p53-21 pathway



a, LINE-1 sequence. The 5′ untranslated region (UTR) is a CpG-rich RNA polymerase II promoter. Open reading frame (ORF) 1 and ORF2 are separated by a 63-bp linker sequence. ORF2 has endonuclease (EN, red) and reverse transcriptase (RT, gray) domains. **b**, Top: episomal pCEP4 mammalian expression vector for eGFP (pDA083) or LINE-1 (pDA077). AbxR, antibiotic selection marker; EBNA1, Epstein–Barr nuclear antigen 1; oriP, EBNA-1 replication origin. Bottom: western blot of ORF1p and ORF2p from RPE cells transfected with each plasmid. Uncropped blot is shown in Supplementary Data 1. **c**, Clonogenic assay (day 12). Cells are transfected with eGFP or LINE-1. Representative plates with number of colonies indicated ± s.d. Right: quantification is normalized to eGFP-expressing cells set at 100%, from $n = 3$ independent experiments. P value calculated by two-sided unpaired t-test. d, Clonogenic assay (day 12). Cells are treated with lentivirus encoding *TP53* shRNA (+) or control vector (–). Data are presented as the number of LINE-1 colonies per 100 eGFP colonies ± s.e.m.; $n = 3$ independent experiments. P value obtained by unpaired two-sided t-test. **e**, Positive-selection CRISPR–Cas9 knockout screen workflow using the Brunello CRISPR knockout library. RPE–Cas9, RPE cells constitutively expressing Cas9 protein. KO, knockout. NGS , next-generation sequencing. **f**, Screen enrichment rank versus significance values of gene knockouts that rescue growth of LINE-1+ cells. The dashed red line is the family-wise error rate (FWER)-adjusted genome-wide significance level. Low ranks indicate rescue of LINE-1+ cells. **g**, Compared with non-targeting control (NTC), CRISPR knockout of *TP53* or *CDKN1A* significantly rescues clonogenic growth of RPE. Representative plates with all data presented as the number of LINE-1 colonies per 100 eGFP colonies ± s.e.m.; $n = 2$ biological replicates. P value was obtained by unpaired one-sided t-test.