



HHS Public Access

Author manuscript

FEBS J. Author manuscript; available in PMC 2024 November 20.

Published in final edited form as:

FEBS J. 2022 March ; 289(5): 1160–1179. doi:10.1111/febs.15722.

Transposable Element Regulation and Expression in Cancer

Erin E. Grundy^{1,2,3}, Noor Diab^{1,2}, Katherine B. Chiappinelli^{1,2}

¹Department of Microbiology, Immunology, & Tropical Medicine, The George Washington University, Ross Hall 2300 Eye Street NW, Suite 502, Washington DC 20037.

²The GW Cancer Center, The George Washington University, 800 22nd Street NW, Washington, DC 20052.

³The Institute for Biomedical Sciences at the George Washington University, Ross Hall 2300 Eye Street NW, Room 561, Washington DC 20037.

Abstract

Approximately 45% of the human genome is composed of transposable elements (TEs). Expression of these elements is tightly regulated during normal development. TEs may be expressed at high levels in embryonic stem cells but are epigenetically silenced in terminally differentiated cells. As part of the global “epigenetic dysregulation” that cells undergo during transformation from normal to cancer, TEs can lose epigenetic silencing and become transcribed, and, in some cases, active. Here, we summarize recent advances detailing the consequences of TE activation in cancer and describe how these understudied residents of our genome can both aid tumorigenesis and potentially be harnessed for anti-cancer therapies.

Keywords

Transposable elements; epigenetics; cancer; P53; LINES; SINES; LINE-1; ERVs; viral mimicry

Classes of transposable elements

Transposable elements (TEs), which compose approximately 45% of our genome, are globally dysregulated during cancer progression [1]. TEs are categorized into two general classes, the details of which have been extensively reviewed elsewhere [2–4]. Class I TEs, often referred to as retrotransposons, move throughout the genome via reverse-transcribed RNA intermediates while class II TEs, or DNA transposons, move autonomously throughout the genome as DNA segments (Figure 1a) [5]. Class I TEs include long-terminal repeat (LTR)-containing elements, such as endogenous retroviruses (ERVs). ERVs make up about 8% of the human genome and are the remnants of exogenous retroviruses that integrated into the germline millions of years ago. Like exogenous retroviruses, ERVs are composed of

Primary author contact information: Katherine Bakshian Chiappinelli, PhD | kchiapp1@gwu.edu.

Author Contributions

EEG, ND, and KBC performed the literature analysis and wrote the manuscript; EEG and KBC edited the manuscript.

Conflicts of Interest

KBC is a consultant for ROME Therapeutics, Inc.

three genes, *gag*, *pol*, and *env*, flanked by LTRs [6]. The majority (90%) of these germline integrated ERV sequences have accumulated mutations over time or seen recombination between two LTRs, excising the provirus and leaving behind a solo LTR [6]. Class I TEs also include non-LTR transposons, such as long interspersed elements (LINEs) and short interspersed nuclear elements (SINEs, including *Alu* elements), which together make up about 27% of the human genome [7]. LINE-1 elements (L1) are the only known autonomously active retrotransposons in the human genome. SINEs are non-autonomous and require L1 proteins to facilitate their retrotransposition, as do another class of non-LTRs, SINE-VNTR-Alu elements (SVAs) (Figure 1a) [8–9].

During normal development, transcriptional regulation and expression of TEs is tightly controlled. ERVs may be expressed at high levels in embryonic stem cells (ESCs) but are silenced by DNA methylation and repressive histone modifications in terminally differentiated cells [10]. LINE and SINE elements are similarly silenced by DNA methylation, histone modifications, and RNA-mediated silencing [11]. As part of the global epigenetic dysregulation that cells undergo during transformation from normal to cancer, both LTR and non-LTR elements can lose epigenetic silencing and become transcribed, and, in some cases, active [12]. Despite considerable data showing dysregulation and increased expression of these elements, it is currently unclear whether they are benign “passengers” of tumorigenesis or whether they could play a role in tumor initiation and evolution. Epigenetic regulation of these elements is key to understanding their role in tumor initiation and progression.

Epigenetic regulation of TEs in healthy cells

If left unchecked, the constant mobilization of TEs throughout the genome would lead to rapid genetic instability. TE mobilization can lead to genetic disruption in a myriad of ways, including insertional mutagenesis, modification of regulatory sequences, inappropriate activation of gene transcription, and exonization, among others (Figure 1b) [5, 13–21]. To prevent this potentially mutagenic mobilization, most TEs are epigenetically silenced in somatic cells. Although evidence does not completely rule out the exaptation hypothesis, which postulates that a subset of TEs have been co-opted, or exapted, into regulatory or coding elements by the host organism to increase its fitness [19, 22–24]. However, the vast majority of TEs are epigenetically silenced via several mechanisms, including DNA methylation, histone modifications, and RNA-mediated silencing.

DNA methylation

DNA methylation, a process carried out by DNA methyltransferase (DNMT) enzymes, is an epigenetic silencing mark that predominantly involves the covalent addition of a methyl group to a cytosine base that precedes a guanine, termed a CpG dinucleotide [25]. Non-CpG methylation – methylation of cytosine bases followed by an adenine, thymine, or another cytosine base – has also been reported in the human genome, mostly in ESCs and brain tissue [26–29]. DNA methylation silences gene transcription by recruiting methyl-binding domain proteins to prevent the transcription machinery from accessing a particular stretch of DNA [30].

The majority of methylated cytosines in the genome are located in TEs, mediating their suppression [31]. All catalytic DNMT enzymes contribute to TE silencing. In mouse embryos deficient in the maintenance methyltransferase DNMT1, LTR transcript levels were elevated 50–100-fold [32]. Upon double knockout of the catalytic *de novo* DNMTs DNMT3A and DNMT3B in murine ESCs, methylation at cytosine residues preceding an adenosine (CpA sites) was entirely absent [33]. While DNA methylation of TEs is fairly stable in somatic cells, the epigenetic reprogramming that occurs in germ cells and pre-implantation embryos is associated with high levels of TE activity [31]. In a study of non-CpG methylation, Guo *et al.* reported evidence for strand-specific non-CpG methylation in LINEs and SINEs, but not LTRs [34].

DNA methylation levels of TEs are inversely correlated with the age of certain TEs. Ohtani *et al.* has shown that DNA methylation is typically found on younger TEs, specifically human ERVs, which are more CpG-rich than older ERVs, due to the relationship between CpG methylation and mutation rate [35–36]. A methylated cytosine (5-methylcytosine) can undergo spontaneous deamination to a thymine base, irreversibly changing the DNA sequence [37–38]. The longer an ERV element has been integrated into the genome, the more cytosine to thymine mutations it will have accumulated, thus decreasing the CpG content of that particular ERV. Interestingly, a recent study by Zhou *et al.* hypothesized an interplay between genome expansion, TE insertion, and the mutation of methylated CpG sites over time [39]. Upon observing a negative correlation between genome size and methylation of CpG sites within TEs, this whole-genome sequencing analysis of 53 organisms suggests that the spontaneous deamination of 5-methylcytosine bases to thymines may render a once epigenetically silenced TE active, ultimately leading to genomic expansion. Overall, DNA methylation plays an important role in silencing several classes of TEs in both developing and terminally differentiated mammalian cells.

Histone modifications

In addition to methylation of DNA, the tails of histones--the positively charged proteins around which DNA is wrapped to package our genome-- can be covalently modified to render a particular region of DNA more or less accessible to the cell's transcription machinery. Unlike DNA methylation, histone methylation can be activating or suppressive depending on the location and degree to which a particular histone is methylated. Numerous other histone modifications exist, some of which include acetylation, phosphorylation, and ubiquitination [40]. Common repressive histone modifications, often associated with heterochromatin, are the trimethylation of lysines 9 and 27 on histone 3 (H3K9me3 and H3K27me3, respectively), which are frequently found on nucleosomes at TE loci [41–42]. Compared to younger, more recently acquired ERVs silenced by DNA methylation, the LTRs of some older ERVs are silenced by repressive histone modifications, including H3K9me2 and H3K9me3 [35]. In murine ESCs, LINE elements were enriched in dimethylation of arginine 3 on histone 4 (H4R3me2), marks associated with gene repression [43–44].

These silencing histone modifications are deposited by several groups of enzymes, often coming together to form multimeric complexes. Methylation of H3K9 is carried out by the

H3-specific histone methyltransferase SETDB1, also known as the H3K9 methyltransferase ERG-associated protein with SET domain (ESET) and KMT1E [45–47]. In murine ESCs, SETDB1 is required for ERV silencing through interaction with Krüppel associated box (KRAB) associated protein 1 (KAP1, also called TRIM28) via the bridging KRAB zinc-finger protein 809 (ZFP809) [48–51]. This sequence-specific KRAB-ZFP/KAP1/SETDB1 axis mediates *de novo* histone methylation of ERVs in murine and human ESCs [52–53]. The number of KRAB-ZFPs is strongly correlated with the number of LTRs, suggesting KRAB-ZFPs and TEs may have co-evolved [54]. Indeed, the relationship between TEs and KRAB-ZFPs is often referred to as an evolutionary “arms race” [55]. KAP1 has also been implicated in silencing LINE-1 (L1) elements—the only functional, protein-coding retrotransposon in humans. L1 elements were initially shown to be silenced via DNA methylation in normal tissues as well as in human ESCs [56–57]. Recent work implicates the Human Silencing Hub (HUSH) complex with TRIM28/KAP1 in silencing of L1 elements [58].

Another group of protein complexes involved in histone methylation, specifically of H3K27, are the chromatin-modifier polycomb-repressive complexes 1 and 2 (PRC1 and PRC2) [41, 59–60]. In murine ESCs, Leeb and colleagues demonstrated that PRC1 and PRC2 act redundantly to stably suppress ERVs via the addition of H3K27me3 marks [61]. However, PRC2 alone likely plays a minor role in suppression of ERVs [35].

Other chromatin complexes have been implicated in mediating TE silencing. In murine ESCs, a chromatin-associated complex composed of death-domain associated protein (DAXX) and α -thalassemia/mental retardation X-linked (ATRX) has been shown to mediate TE repression by recruiting the H3K9 histone methyltransferase SUV39H [62]. Additionally, the chromatin remodeler SWI/SNF2-related, matrix associated, actin-dependent regulator of chromatin, subfamily A, member 6 (SMARCA6)—also known as lymphoid specific helicase (LSH)—contributes to L1 silencing in primary murine embryonic fibroblasts [63–64]. In murine ESCs, the SWI/SNF-like remodeler SMARCA1 contributes to ERV silencing in an ATP-dependent manner [65–66]. Altogether, the silencing of TEs via a complex array of histone modifications deposited by several enzyme complexes illustrates the great lengths cells go to in order to silence these genomic elements.

RNA-mediated silencing

Although most TEs are epigenetically silenced by DNA methylation and histone modifications, RNA-mediating silencing of several TEs has been reported in mammals. Short (also referred to as small) interfering RNAs (siRNAs)—exogenously produced double stranded, non-coding RNAs complementary to a given mRNA sequence—can suppress L1 retrotransposition in human cell lines [67]. Depletion of DICER, a component of the RNA-induced silencing complex (RISC), results in a two-fold increase of L1 retrotransposition in these cell lines [67]. In murine ESCs harboring deletion of *Dnmt1*, TE repression was dependent upon DICER [68]. Micro-RNAs (miRNAs)—endogenously produced single-stranded non-coding RNAs that function similarly to siRNAs and are used to regulate normal cellular processes—have also been implicated in TE silencing. Hamdorf *et al.* identified miR-128 as a direct repressor of L1 retrotransposition in human cancer cells

and induced pluripotent stem cells [69]. Aside from the RNA interference mediators siRNAs and miRNAs, PIWI-interacting RNAs (piRNA)—single-stranded RNAs that guide PIWI proteins to genomic sites to be silenced—have been implicated in L1 repression. piRNAs can restrict L1 retrotransposition in male mouse germ cells as well as in human induced pluripotent stem cells [70–75].

Silencing of TE transcription can also occur via Spen, the repressor protein of the long noncoding RNA *Xist*, which is involved in X chromosome inactivation [76]. In haploid mouse ESCs, Spen knockout resulted in a marked increase in ERV transcripts, especially from ERV-K. Loss of Spen was associated with a decrease in suppressive H3K9me3 marks along with a gain in activating H3K27ac and H3K4me3 marks. The RNA-binding domain of Spen binds to RNA transcripts derived from ERV-K in V6.5 murine ESCs.

Additional mechanisms of TE silencing have been identified, such as the cytoplasmic retroviral sensor TRIM5 α , which restricts L1 retrotransposition via recognition of L1 ribonucleoprotein complexes [77]. L1 elements are also repressed by p53, a relationship discussed in detail below [78]. This extensive epigenetic silencing of TEs via DNA methylation, suppressive histone modifications, RNA-mediated silencing, and regulatory proteins prevents their mobilization, promoting the integrity and stability of the genome.

Epigenetic dysregulation of TEs in cancer

Due to the global epigenetic dysregulation that occurs during tumorigenesis, many TEs lose silencing and are transcribed in malignant cells. Cancer cells exhibit changes in the silencing DNA methylation mark, including global loss of methylation at regions that are silenced for genome stability, like TEs, and gain of methylation at the promoter regions of tumor suppressor genes [79]. A comparison of prostate cancer and normal cell lines found no significant differences in non-CpG methylation, suggesting that the hallmark methylation changes characteristic of cancer cells occur predominantly at CpG sites [80]. Histone modifications are likewise altered in cancer. Reduction of the repressive histone modification H4K20me3 is not only a hallmark of cancer but is also associated with hypomethylation of TEs [81].

One class of TEs that frequently escapes epigenetic silencing in cancer cells is ERVs. One potential explanation for ERV expression in malignancies was suggested in a recent RNA-seq analysis of 63 metastatic colorectal patients [82]. This study identified higher expression of the DNA demethylating enzymes TET2 and TET3 in tumors that had high levels of ERV expression, suggesting that demethylation may directly contribute to ERV transcription. These findings are supported by the numerous studies that have identified the expression of other human ERVs in cancers. ERV-K, a recently integrated ERV, is expressed in both hematologic and solid malignancies, including melanoma, breast cancer, ovarian cancer, pancreatic cancer, lymphoma, and prostate cancer [83–97]. In melanoma, expression of ERV-K may be due to increased transcriptional activity of the ERV-K promoter through demethylation of ERV-K LTRs [90]. ERV expression in ovarian cancer seems to be particularly common, as Wang-Johanning *et al.* have demonstrated the rare

concurrent expression of multiple ERV surface envelope proteins--ERV-E, ERV-K, and ERV3 [87].

ERV-W is also frequently expressed in malignant cells. An ERV-targeted microarray study by Gimenez *et al.* revealed that the LTRs of six ERV-W loci upregulated in testicular cancer had methylation levels ranging from 0 – 30% in tumoral samples compared to 82 – 100% in DNA from adjacent healthy tissues [98]. The ERV-W *env* gene (syncytin-1), which facilitates trophoblast fusion and the formation of the placenta, plays a conflicting role in the pathogenesis and prognostic impact of several cancers [99–100]. Unlike many TEs that have been co-opted into regulatory elements in our genome, syncytin-1 is a unique case in which an ERV element has been adapted into a functional gene and is present only in eutherians (placental mammals) [99, 101]. Syncytin-1 expression has been detected in several leukemia and lymphoma cell lines as well as in patients with cutaneous T-cell lymphoma [102–103]. Syncytin-1 can promote invasion and metastasis via activation of endothelial-to-mesenchymal transition in endometrial carcinoma [104]. Although syncytin-1 promotes breast cancer-endothelial cell fusions, thereby promoting metastasis, it was also shown to be a positive prognostic indicator of recurrence-free survival in patients with breast cancer [105–106]. Two years later, the same group found that expression of syncytin-1 was correlated with a *decrease* in overall survival in rectal but not colon cancer patients, concluding that the prognostic impact of Syncytin-1 may vary according to the specific type of malignancy [107].

Lastly, several codogenic ERVs are expressed at varying levels in endometrial carcinoma and several of its prestages [108]. These numerous instances of ERV re-expression in various malignancies illustrate the consequences of the widespread epigenetic dysregulation of TEs in cancer.

The effect of TE expression on oncogenic pathways

The expression of some TEs in cancer due to global epigenetic dysregulation has oncogenic potential. TE transcription can drive expression of neighboring oncogenes in multiple cancer types [109]. A recent study demonstrated just how widespread the process of onco-exaptation—epigenetically reactivated TEs that function as cryptic promoters—is in cancer [109]. This study identified 129 onco-exaptation events involving 106 oncogenes across 3,864 tumors. At least one onco-exaptation event was identified in 49.7% of all the tumors with a prevalence of onco-exaptation between 10–80% across different cancer types. In a survey of Hodgkin's Lymphoma cell lines, the expression of the transcription factor interferon regulatory factor 5 (IRF5) was driven by a normally dormant LTR located upstream of IRF5 [110]. Another Hodgkin's Lymphoma study revealed that transcription of the proto-oncogene colony-stimulating factor 1 receptor (*CSF1R*) is initiated by an aberrantly activated LTR *THE1B*, a member of the MaLR family [111]. Lock *et al.* analyzed RNA-seq data from 101 diffuse large B-cell lymphoma patients and identified 98 TE-gene chimeric transcripts where TEs appeared to be functioning as the promoter [112]. A similar finding was detailed in a recent study by Jiang and Upton, in which TEs were found to serve as a frequent source of transcription factor binding sites and promoters in breast cancer [113].

In addition to functioning as promoters, epigenetically reactivated TEs can act as enhancers of benign genes as well as oncogenes. In healthy cells, two phylogenetically conserved TEs in mammals are required for expression of the proopiomelanocortin (*Pomc*) gene in the hypothalamus of transgenic mice [114]. In cancer cells, TEs have been implicated in driving the expression of a novel isoform of IL-33 in colon cancer, an oncogenic truncated transcript of ERBB4 in anaplastic large-cell lymphoma, and an alternative transcript of the MET oncogene in bladder cancer [115–117]. Recently, TEs have been shown to act as enhancers in acute myeloid leukemia and chronic myeloid leukemia cell lines. Analysis of acute myeloid leukemia (AML) patients revealed TEs were more enriched for active transcription marks compared to normal tissues and a subset of these demonstrated enhancer activity [118]. Some ERVs contain STAT1 binding sites and act as interferon-inducible enhancers for a subset of interferon stimulated genes, important in promoting the inflammatory response, a process discussed in detail below [119–120].

At the protein level, TEs—particularly ERVs—have oncogenic potential. Inducible expression of ERV-K in healthy mice disrupts germ cell development, ultimately leading to carcinoma *in situ*, the precursor lesion of seminoma [121]. Other studies in several cancer types have demonstrated that knockdown of ERVs via short hairpin RNA (shRNA) or siRNA targeting reduces tumor growth in both cell lines and mouse xenograft models [88, 122–124]. In a syngeneic mouse model, renal cells expressing ERV-K *env* promoted the formation of pulmonary metastases [125]. Interestingly, ERV-K promotes intercellular fusion of melanoma cell lines, and syncytin (ERV-W *env*) is involved in the fusion of breast cancer cells to endothelial cells, allowing malignant cells to escape breast tissue and metastasize [126–128]. Similarly, syncytin-1 upregulation activates the epithelial-mesenchymal transition pathway in endometrial carcinoma patients [104]. Thus, TEs, specifically ERVs, can contribute to transformation and tumor growth by functioning as promoters, enhancers, and at the protein level [129].

Roles of LINE-1 (L1) in cancer

L1 retrotransposition in oncogenesis

As the only functional retrotransposon in humans, L1 plays a particularly important role in contributing to genomic instability in cancers and its expression is a hallmark of cancer [130]. L1 is transcribed as a bicistronic RNA that encodes an RNA binding protein, open reading frame 1 protein (ORF1p, also known as p40), and an endonuclease and reverse transcriptase (ORF2p) [5] (Figure 1a). L1 elements are usually epigenetically silenced in normal somatic tissues, but occasionally L1 promoters become active, resulting in L1 transcription and production of the ORF1p protein [5]. ORF1p is a nucleic acid-binding protein that is essential for retrotransposition of L1 elements in the genome. L1 elements can be amplified via the activity of ORF2p, which reverse transcribes L1 mRNA and inserts L1 DNA into a new position within the genome [131].

Retrotransposition of L1 elements has conflicting roles in oncogenesis. In some malignancies, L1 insertions are driver mutations, directly contributing to oncogenic transformation, while in others, they are benign passengers [132–133]. The first case in which a somatic L1 insertion was shown to be oncogenic was in a case of breast ductal

adenocarcinoma, in which an L1 sequence was identified in an intron of the oncogene *MYC* [134]. The second case involved an L1 insertion that inactivated the tumor suppressor gene adenomatous polyposis coli (*APC*) in a colorectal cancer patient [12]. In addition to causing familial adenomatous polyposis (FAP), a heritable predisposition of developing colorectal cancer, driver mutations in *APC* occur in a majority of sporadic colon cancers [135]. Since then, oncogenic L1 insertions in hepatocellular carcinoma have been shown to activate the oncogenic β -catenin/Wnt signaling pathway as well as the poorly characterized transcription factor suppression of tumorigenicity 18 (ST18) [136]. L1 insertions have also been shown to initiate transcript variants in esophageal adenocarcinoma, bladder, and breast carcinomas [117, 137–138]. The most comprehensive study of L1 retrotransposition in cancers, which analyzed just under 3,000 genomes of cancers from 38 histological subtypes, identified several oncogenic roles of L1 retrotransposition, including chromosomal deletions encompassing tumor-suppressor genes, structural variations, and promotion of breakage-fusion cycles that trigger oncogene amplification [139].

There are also reports of LINE reactivation in premalignancies, suggesting that early retrotransposon activation may be a causal factor in some cancers [140]. For example, L1 insertions are present in all gastrointestinal cancers and their metastases as well as in the cancer precursor colonic adenomas [141]. However, while the previously mentioned studies demonstrate the oncogenic potential of L1 insertions, only approximately 1% of L1 insertions are thought to be tumor-initiating events [133]. Several passenger mutations involving L1 insertions have been identified in colorectal, breast, lung, prostate, and ovarian cancers [142–145]. Thus L1 retrotransposition is common across cancer subtypes, providing potential areas for new therapeutic development.

Regulation of L1 by P53

The tumor suppressor P53 is one of the key regulators preventing cancer and the *TP53* gene that encodes this protein is the most commonly mutated gene in human cancers [146]. Around 90% of *TP53* mutations in human cancers, called “hotspot” mutations, cluster in the DNA binding domain of the gene [147]. Mutated P53 may prevent binding to canonical targets while also promoting oncogenic transcription by binding to other loci with different transcriptional binding partners [146–147]. P53 transcriptionally represses TEs in model organisms, including fruit flies and zebrafish [148], and has conserved binding sites in ERV and L1 elements in humans [149]. In fact, 30% of the predicted binding sites for P53 are in ERVs. As P53 is mutated in more than 50% of cancers [147], P53-deficient malignant cells are predisposed to elevated expression of TEs, which can alter the stability and composition of the genome.

There is contradictory evidence regarding the relationship between P53 and L1. An agonistic relationship has been reported in a germ cell line, in which a P53 binding site in a recently acquired L1 element can increase transcription of that element [148]. Another study found that wild-type P53 increased the transcription of L1 elements compared to mutant P53 [149]. As P53 is active in somatic cells and strongly responsive to double-stranded breaks in chromatin, it was proposed that activated L1 transcription leads to the production of ORF2p, which induces double stranded breaks and would cause increased P53 activity [149]. This

positive feedback loop created in response to DNA damage would ultimately cause the cell to undergo apoptosis (Figure 2a). In support of this proposed mechanism, Haoudi and colleagues showed that driving L1 expression induced apoptosis in the wild type P53 cell line G418R HCT-116, but not the mutant P53 G418R SW480 line, suggesting therapeutic potential for malignancies with wild-type P53 [150].

The majority of evidence, however, suggests an antagonistic relationship between p53 and L1. Ardeljan *et al.* have shown that L1 retrotransposition is limited by the P53 DNA damage response and interferon signaling [12]. In the context of DNA damage, P53 represses the transcription of Alu elements [151]. The most recent study to investigate the relationship between P53 and L1 showed that P53 directly represses L1 in human cell lines via binding to the 5' untranslated region (UTR) of L1 elements and promoting the addition of repressive histone modifications (Figure 2b) [152]. At the protein level, a comparison of ORF1p protein expression in wild-type and P53-mutant Wilms tumors revealed that L1 could be readily detected in P53 mutant tumors but not in P53 wild-type tumors [78]. Subsequent analysis of colon cancer samples in The Cancer Genome Atlas (TCGA) found that L1 transcripts were significantly elevated in tumors that harbored P53 loss [78]. A similar trend has been observed in *TP53*-deficient lung, pancreatic, and ovarian carcinoma patient samples [130].

Indirect evidence for the antagonistic relationship between P53 and L1 exists as well. In ovarian cancers, STIC lesions—the carcinoma *in situ* precursors to high grade serous ovarian cancer—are characterized by mutant *TP53* along with demethylation and increased expression of L1 elements, which may drive genomic instability [153]. L1 ORF1p expression is also detectable in pancreatic intraepithelial neoplasias—the precursors of pancreatic ductal adenocarcinomas [130, 140]. Thus, the “guardian of the genome”, P53, plays a complicated role in the regulation of TEs, having been shown to act as an activator *and* a repressor of TEs. The mutation of *TP53* in >50% of cancers may thus significantly affect TE transcription and retrotransposition, the effects of which are discussed below in the contexts of cell stress and tumorigenesis.

L1 retrotransposition induces cell stress via the DNA damage response

When L1 elements are active and able to retrotranspose, this can activate the DNA damage response and limit cell growth. Ardeljan *et al.* showed that overexpressing L1 in retinal pigment epithelium (RPE) cells (non-transformed) triggers a TP53-mediated G1 arrest and an interferon response [12]. L1 expression inhibited RPE clonogenic growth, which was rescued by knockdown of P53. However, when using a reporter assay to compare L1 insertion frequency in control and TP53-knockdown cells, no significant difference was found. Therefore, P53 restricts growth of these cells but not their retrotransposition potential. This study also demonstrated that L1 retrotransposition occurs in association with DNA replication; the DNA repair mechanisms that are coupled with replication reduce retrotransposon intermediates and it is the loss of these repair pathways that enhances L1 retrotransposition. This study utilized a knockout CRISPR screen in TP53-deficient cells and identified genes that influence the fitness of L1+ cells, including replication-coupled DNA repair pathways, replication stress signaling, and replication-fork restart factors. Due to the

apoptotic response facilitated by the induction of L1 retrotransposition in P53-competent cells, activation of human L1 could be used as a therapeutic strategy with intact P53. By activating L1 retrotransposition, genomic instability is induced, leading to DNA damage and eventually apoptosis (Figure 2a).

In addition to this genomic instability, cancer cell damage may be further compounded through the combined use of antineoplastic compounds currently used in clinical trials, such as the DNA synthesis inhibitor mitomycin C [154] or the Ataxia Telangiectasia and Rad3 related (ATR) inhibitor ceralasertib, which inhibits DNA repair [155]. Findings from a companion study reported that LINE-1 retrotransposition is restricted by double-stranded break (DSB) repair and Fanconi Anemia factors that are active in S/G₂ phase [156], suggesting a possible contribution of L1 retrotransposition to the pathogenesis of breast and ovarian cancers, a significant percentage of which are deficient in the DSB protein BRCA1.

L1 protein expression in human malignancies

Interestingly, pathologic analysis of L1 ORF1p expression in colon cancer points to a selective advantage for cells that lack L1 expression. In a heterogeneous L1 expression in colon cancer, Ardeljan *et al.* found that both L1+ and L1- parts of the primary tumor both shared a BRAF (V600E) mutation, as well as numerous somatically acquired L1 insertions prior to the cessation of retrotransposition in the L1- component [12]. The L1- section, which derived from a L1+ lineage, and the loss of L1 expression were associated with an increased growth rate.

Pathologic analysis of L1 expression in gynecologic malignancies supports the hypothesis that L1 expression requires disabled P53 signaling. Ovarian serous carcinoma, a type of tumor that arises mainly from the epithelial layer of cells in the ovaries, can be high-grade serous carcinoma (HGSC) or low-grade serous carcinoma (LGSC). HGSC contributes to the majority of deaths as it is the most common subtype of ovarian cancer, contributing to around 70% of all ovarian cancers [157–158]. About 90% of HGSCs have mutations in the TP53 gene while displaying a much lower mutational load in other genes [159–161]. HGSCs originate from precursor lesions of the distal fallopian tubes that likely evolved in a stepwise manner from P53 signature lesions [162–163]. During carcinogenesis, L1 promoter regions often become hypomethylated leading to the expression of the L1 proteins. Accumulation of ORF1p along with the formation of the precursor STIC lesions is followed by progression to HGSC, in which L1 expression persists [153].

Two recent pathologic studies detailed early and frequent L1 protein expression in gynecologic malignancies. L1 ORF1p overexpression is likely established during the evolution of ovarian cancer lesions, which begin with the emergence of a mutant P53 signature and progress toward the precursor STIC stage. L1 overexpression increased as the lesions progressed toward malignancy and upon seeding to the ovary, the level of L1 overexpression in HGSC-concomitant STIC lesions neared that of HGSC [153]. Expression of L1 was found to be prevalent in all histological subtypes of ovarian cancer, especially in HGSCs, which showed the highest L1 expression compared to the other subtypes that were tested [164]. These differences reflect the fact that virtually all HGSCs harbor TP53 mutations and therefore exhibit a higher level of genomic rearrangement. L1 ORF1p

expression was correlated with mutated P53 in HGSC and HGSCs had significantly higher expression of L1 compared to other ovarian cancer subtypes, which generally have wild type P53 [153, 164]. The overexpression of ORF1p in subtypes of ovarian carcinomas suggests the potential utility of ORF1p as biomarker for these malignancies [164].

Perturbation of TE epigenetic regulation for cancer therapy

The above examples serve as a direct illustration of the importance of epigenetic silencing of TEs in somatic cells, as their reactivation frequently leads to rapid genome instability via insertional mutagenesis, aberrant TE expression, and interruption of regulatory elements. However, recent work shows that therapeutic activation of TEs in cancers can actually initiate an anti-tumor immune response. Cancer cells resist the immune response in a process known as immune editing or immune evasion [165]. Initially, immune cells including natural killer cells and T-effector cells fight and kill cancer cells, but as cancers progress, malignant cells exhibit mechanisms of immune suppression. For example, tumors often express PD-L1, a ligand for the PD-1 protein on CD3 T-cells that inhibits killer T-cell action against tumor cells. T-regulatory cells that express CTLA-4 secrete cytokines to inhibit the action of T- and natural killer cells against tumors [165]. Recent advances utilizing therapies that reverse this evasion (specifically, inhibition of PD-1 and CTLA-4, termed “immune checkpoint blockade”) have shown durable responses for a proportion of patients with solid tumors [120]. Response often correlates with the amount of tumor infiltrating lymphocytes and the number of tumor neoantigens recognized by T-cells attacking the tumor [120].

Treating cancer cells with DNA methyltransferase inhibitors (DNMTi) “boosts” immune signaling from tumors through activation of type I and III interferon signaling induced by detection of double-stranded RNA (dsRNA) derived from TEs [167–169]. Low doses of the DNMTis Azacytidine (Aza) [170] and 5-aza-2'-deoxycytidine (Dac) [171] upregulate immune signaling, including the interferon response, cytokines, and antigen processing and presentation in breast, colon, lung, and ovarian cancer cell lines [167–169, 172]. DNMTis reduce global DNA methylation, which results in the upregulation of dsRNA, particularly dsRNA derived from inverted-repeat Alu elements [173]. The cytoplasmic dsRNA sensors TLR3 and MDA5 [168] detect these genetic elements and initiate a canonical interferon signaling pathway in a process termed “viral mimicry” (Figure 3). Studies have shown that the interferon response induced by DNMTi treatment was abrogated by inhibiting dsRNA sensors MDA5 and TLR3, proving that transcription of TE-derived dsRNA species caused the interferon response [168–169]. Subsequent work showed that the interferon response induced by DNMTi treatment can be increased by adding HDAC inhibitors (HDACi) [174], inhibitors of H3K9 methyltransferases [175], or Vitamin C, which is a cofactor for the TET DNA demethylases [176]. Treatment with DNMTis plus HDACis increased ERV expression in a mouse model of ovarian cancer, activating interferon signaling and recruiting CD8+ T cells to kill the tumors [177].

In addition to induction of type I and III interferon signaling, viral mimicry has also been shown to occur through the loss of the H3K9 methyltransferase SETDB1 and the H3K4me1/2 demethylase LSD1 (also known as KDM1A). SETDB1 is known to be

upregulated in several cancers and its loss in an AML cell line was shown to result in the transcription of dsRNA, promoting subsequent type I IFN signaling and apoptosis of AML cells [178]. Similarly, overexpression of LSD1 has been identified in multiple cancers [179–182]. Upon treatment of breast cancer cell lines with a catalytic inhibitor of LSD1, induction of viral mimicry was observed along with decreased expression of components of the dsRNA-degrading RISC complex [183–185]. This inhibition of LSD1 suppressed tumor growth and induced resistance to PD-1 blockade *in vivo*, further demonstrating the efficacy of using viral mimicry as a cancer therapy [184]. The HUSH complex has also been identified as a “gatekeeper” of type I IFN signaling via L1 repression [186]. Depletion of the HUSH complex in primary fibroblasts led to the induction of interferon stimulated genes (ISGs) via sensing of L1-derived dsRNA.

Pre-clinical and clinical studies testing epigenetic activation of TEs to induce immune signaling in cancer have shown promise. In mouse models, DNMTi treatment sensitized melanoma to subsequent anti-CTLA4 therapy [168, 187–188] and murine ovarian cancer to anti-PD-1 therapy [177]. The combination of Aza treatment with the depletion of the dsRNA-destabilizing enzyme ADAR1—which deaminates adenosine residues in RNA to inosines—has been shown to have synergistic anti-tumor effects in a xenograft mouse model of colorectal cancer [173]. A recent Phase Ib trial combining DNMTi treatment with anti-CTLA-4 in melanoma showed favorable results, including improved immune activation and anti-tumor activity [189]. This therapeutic exploitation of TEs to stimulate immune signaling is currently being tested in combination with checkpoint blockade therapy in clinical trials for melanoma, colorectal cancer, ovarian cancer, and kidney cancer, among others [189] ([NCT01928576](#), [NCT02961101](#), [NCT03019003](#), [NCT02811497](#), [NCT02546986](#), [NCT02397720](#), [NCT02530463](#)). Results from the METADUR trial-- a phase II trial that investigated the efficacy of CC-486, an oral form of Aza, with the T-cell survival promoter durvalumab (a monoclonal antibody against PD-1) in colorectal cancer, ovarian cancer, and breast cancer patients--were recently published [190]. It was found that viral mimicry was likely not induced as CC-486 did not penetrate the tumors, leading to no clinical responses from this trial. These data show the importance of developing epigenetic therapies with greater stability and improved pharmacokinetics to show efficacy in solid tumors.

Similarly, the cell cycle regulators cyclin-dependent kinases (CDKs) can also be therapeutically targeted to promote viral mimicry in cancer. As CDKs are integral for progression through the cell cycle, inhibitors of CDKs (CDKis) were initially developed to halt this process in rapidly dividing malignant cells [191–194]. Several studies have since demonstrated that inhibitors of CDK 4/6 also suppress the methyltransferase DNMT1 [195–197]. This inhibition of DNMT1 leads to hypomethylation of TEs, particularly ERVs, and can also be used to induce viral mimicry. While Cingöz and Goff have recently shown that CDKs are required for type I interferon production, the redundancy among CDK4/6 may be sufficient to overcome the post-transcriptional block of IFN β induced by the CDK1/2/4 inhibitor R547 [198–199]. As inhibitors of CDK4/6 will selectively target rapidly proliferating cancer cells, this is another therapeutic strategy that can be used to boost immune signaling in tumor cells while sparing more quiescent healthy cells.

TEs as potential cancer antigens

In addition to the type I and III interferon response induced by dsRNA activation, a subset of TEs may be translated into proteins that can be presented as peptides and recognized by immune cells as tumor associated antigens (TAAs)—proteins expressed by tumor cells but are absent from normal cells [120, 200]. As discussed above, ERVs are frequently expressed in several cancer types. However, their foreign nature as a viral element presents an additional, and potentially exploitable, degree of antigenicity. The aberrant expression of ERVs in cancers has led several groups to pursue the development of ERV-targeted adaptive immune strategies, an approach made all the more promising by studies demonstrating the antigenicity of expressed ERVs in renal cell carcinoma [201–202]. In order for a T-cell to recognize an antigen on a target cell, the antigen must first be processed into a peptide fragment inside the target cell. This peptide fragment is then transported to the surface of the target cell, where it is presented to the immune system in the context of a major histocompatibility complex (MHC) molecule. The peptide-MHC complex is then recognized by a highly specific receptor on the surface of a T cell, which simultaneously binds *both* the antigen and the MHC molecule on the target cell [203].

Studies have not only identified ERV-specific T-cells in cancer patients, but have also shown that the corresponding TE-derived peptides are presented in the context of MHC class I molecules on tumors, confirming the potential of TEs as tumor-associated antigens (TAAs). A recent analysis of thousands of tumors from The Cancer Genome Atlas (TCGA) shows that TE expression in cancer is associated with immune infiltration and increased antigenicity [204]. The antigenic potential of these TAAs can be taken one step further by combining them with epigenetic therapeutics. A multi-omic analysis of glioblastoma cell lines treated with the DNMTi Dac identified 16 peptides from several TE subclasses that were expressed only in Dac-treated glioblastoma cells²⁰⁴. A study of myeloid malignancies identified greater numbers of ERV-specific T-cells in these patients compared to healthy donors²⁰⁵. These studies directly illustrate the potential use of TEs, specifically ERVs, as neoantigens induced via epigenetic manipulation.

Humoral immunity has also been studied, albeit less thoroughly than cell-mediated immunity, as antibodies against ERVs have been identified in several cancers. Antibodies against ERV-K have been detected in ovarian cancer and melanoma patients as well as in teratocarcinoma cell lines [87, 206–207]. Antibodies against ERV-E and ERV3 have also been identified in ovarian cancer patients [87]. These autologous humoral and T-cell responses to TEs that have been identified in cancer patients serve as validation for the development of adaptive immune strategies targeting TEs as novel cancer therapies.

Conclusion

As 45% of the human genome is composed of TEs, proper epigenetic silencing of these elements is integral to maintaining genome stability. In terminally differentiated cells, TE expression is inhibited via DNA methylation, suppressive histone modifications, and RNA-mediated mechanisms. In cancer cells, global epigenetic dysregulation of TEs leads to their expression, which can be oncogenic in some contexts. While the regulation of TEs is fairly

well characterized, as is the expression of these elements in various malignancies, further exploration is needed in order to understand whether targeting TEs is a feasible therapeutic option. Recent work has shown that TE expression in cancer may be exploited via activation of the DNA damage response and innate immune signaling. Results from ongoing clinical trials combining epigenetic therapies that activate TEs with immune therapies will provide information on whether this combination can be effective in cancers. Further investigation is required in order to determine the feasibility of utilizing TEs as potential cancer antigens and/or biomarkers for specific cancers.

Acknowledgements

This manuscript was supported by the National Cancer Institute under Award R21CA227259 (to KBC), an award from The Mathers Foundation (to KBC), and The Marlene and Michael Berman Endowed Fund for Ovarian Cancer Research (to KBC). The authors would like to acknowledge the Institute for Biomedical Sciences at the George Washington University for graduate student support and training. Figures were created with BioRender.com. The funding sources above had no involvement in or influence on the writing or preparation of this manuscript.

Abbreviations

TE	transposable elements
ERV	endogenous retrovirus
LTR	long terminal repeat
LINE	long interspersed element
SINE	short interspersed nuclear element
SVA	SINE-VNTR-Alu
ESC	embryonic stem cell
CpG	cytosine-phosphate-guanine
DNMT	DNA methyltransferase
H3K9me2/3	histone 3 lysine 9 di/trimethylation
H3K27me3	histone 3 lysine 27 trimethylation
LINE-1 (L1)	long interspersed (nuclear) element-1
HUSH	human silencing hub
PRC1/2	polycomb repressive complex 1/2
siRNA	short/small interfering RNA
RISC	RNA-induced silencing complex
RISC	RNA-induced silencing complex
miRNA	microRNA

piRNA	PIWI-interacting RNA
shRNA	short hairpin RNA
AML	acute myeloid leukemia
UTR	untranslated region
TCGA	The Cancer Genome Atlas
STIC	serous tubal intraepithelial carcinoma
RPE	retinal pigment epithelium
DSB	double-stranded break
HGSC	high-grade serous carcinoma
LGSC	low-grade serous carcinoma
PD-1	programmed cell death protein-1
CTLA-4	cytotoxic T-lymphocyte associated protein-4
DNMTi	DNA methyltransferase inhibitor
dsRNA	double-stranded RNA
Aza	azacytidine
Dac	5-aza-2'-deoxycytidine
TLR3	toll-like receptor-3
MDA5	melanoma differentiation-associated protein-5
ISG	interferon-stimulated gene
CDK	cyclin dependent kinase
CDKi	cyclin dependent kinase inhibitor
MHC	major histocompatibility complex
TAA	tumor associated antigen

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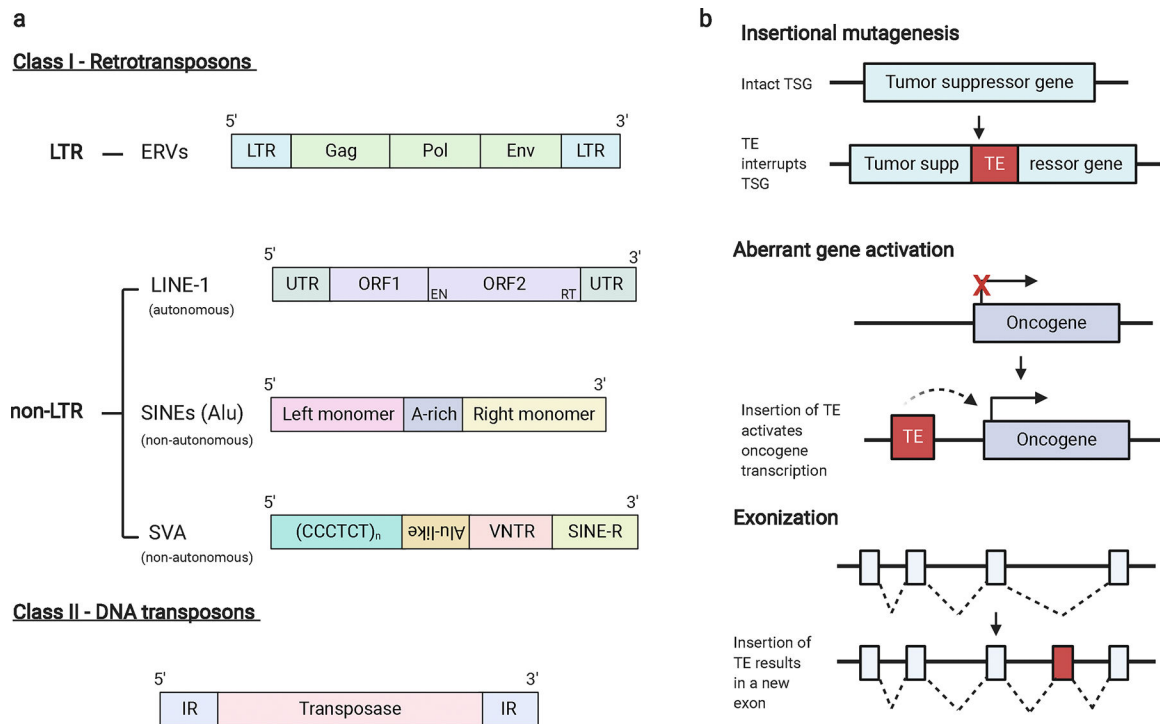


Figure 1. Mammalian TE classification and common mechanisms of mutagenesis.

a. TEs consist of 2 classes: class I (retrotransposons) and class II (DNA transposons). Class I TEs are further divided into those containing long-terminal repeats (LTRs) and those that do not (non-LTRs). ERVs are composed of *gag*, *pol*, and *env* genes flanked by LTRs. Non-LTRs consist of LINEs, SINEs, and SINE-VNTR-Alus (SVAs). LINE-1 elements consist of 2 open reading frames (ORFs) located between untranslated regions (UTRs). The second ORF encodes an endonuclease and reverse transcriptase. SINE elements, such as Alus, consist of a left-monomer and a right monomer separated by an A-rich region. SVAs contain a hexameric repeat of the sequence CCCTCT, an antisense Alu-like element, a variable number of tandem repeats (VNTR) region, and a SINE region (SINE-R). Class II transposons consist of a transposase flanked by inverted repeat (IR) regions. **b.** TE retrotransposition can promote oncogenesis via insertional mutagenesis (top), in which the insertion of a TE into a tumor suppressor gene (TSG) renders the gene inactive. Retrotransposed TEs can also act as promoters of oncogenes, rendering a normally silenced gene active (middle). TE mobilization can create new exons, a process called exonization, changing the resulting mRNA and protein composition (bottom).

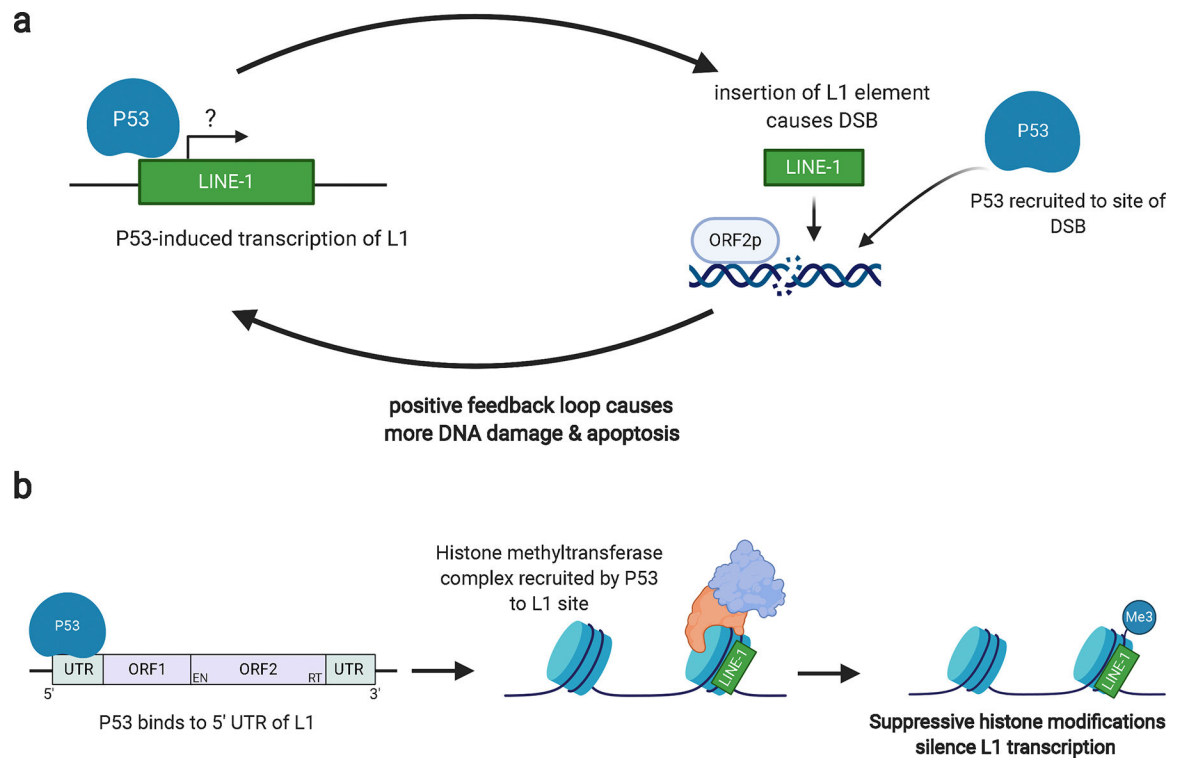


Figure 2.

Models of the conflicting relationship between P53 and L1.

a. In an agonistic relationship, activation of P53 may induce L1 transcription, leading to the production of ORF1p and ORF2p proteins. ORF2p induces DSBs in order to promote L1 insertion. P53 is recruited to sites of DSBs and a positive feedback loop may be induced. The accumulation of severe DNA damage from several DSBs may cause the cell to undergo apoptosis. In cells with defective DNA replication and repair machinery, this damage will become irreparable, further driving the cell towards apoptosis. **b.** In an antagonistic relationship, P53 binding to the 5' UTR of L1 elements recruits histone methyltransferase proteins. These epigenetic modifiers can then deposit suppressive histone modifications, silencing L1 transcription.

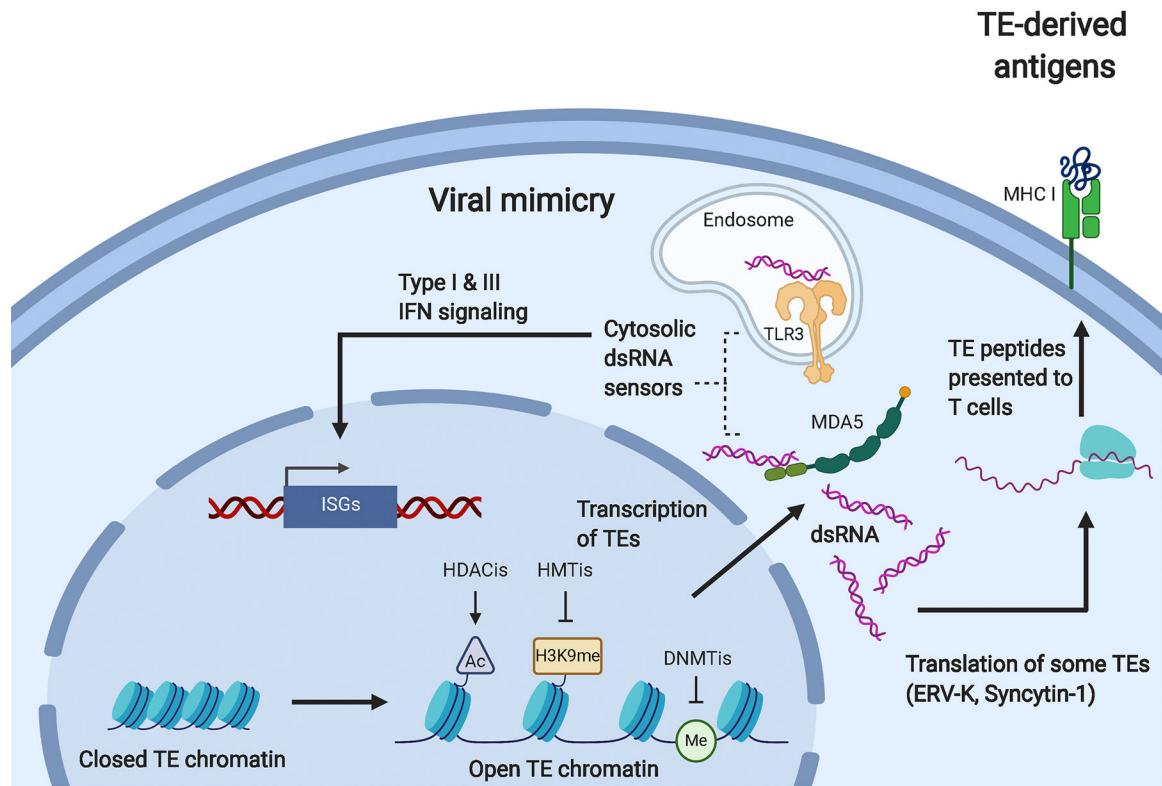


Figure 3.

Anti-cancer therapeutic strategies using viral mimicry and TE-derived antigens.

Treatment of tumor cells with DNMTis, HDACis, and HMTis induces transcription of TEs. The dsRNA produced from TE transcription can be detected by the cytosolic dsRNA sensors MDA5 and TLR3. This detection induces type I and III interferon signaling, which results in the transcription of interferon-stimulated genes (ISGs) and induction of an inflammatory response, a process termed “viral mimicry.” The TE-derived dsRNA can also be translated into peptides, as is the case for ERV-K and Syncytin-1. These peptides can bind to MHC I molecules on the surface of the tumor where they can be recognized by T cells. Upon recognition of these tumor associated antigens, the tumor cells can be targeted and killed by immune cells.