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EPIGENETIC DYSREGULATION OF TRANSPOSABLE ELEMENTS IN CANCER AND THEIR ROLES IN TUMOR PROGRESSION, IMMUNE REMODELING, AND THERAPEUTIC TARGETING

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ABSTRACT
Transposable elements (TEs) make up approximately half of the human genome and have recently been identified as key gene expression and tumorigenesis regulators. In healthy cells, TEs are silenced and repressed by diverse epigenetic mechanisms, such as DNA methylation, histone modifications, chromatin remodeling complexes, and non-coding RNA. In cancer, perturbations in regulation are induced by epigenetic instability, a tissue-wide destabilization of the epigenetic landscape of a genome, leading to a global loss of DNA methylation, heterochromatin loss, chromatin remodeling and TE activation. TE activity contributes to tumorigenesis by insertional mutagenesis, generating genomic instability, and modulating gene expression using TE-derived promoters and enhancers. TE-derived nucleic acids (e.g. double-stranded RNA) also trigger natural immune sensing pathways (such as cGAS-STING and MDA5-MAVS) to initiate antiviral-type interferon responses that can improve anti-tumor immunity. However, chronic TE RNA-induced interferon signaling may also promote immune escape through ADAR1-mediated RNA editing of TE-derived RNAs and immunosuppressive changes in the tumor microenvironment (TME). The involvement of TE activity in stromal reprogramming, immune dysregulation, and three-dimensional genome reorganization within the tumor ecosystem has been increasingly supported. TEs are now considered to play important roles in epigenetic dysregulation and immune response dysregulation and as potential therapeutic targets. Efforts to develop anti-TE-based therapeutics are being pursued using epigenetic drugs, immune checkpoint inhibitors, and CRISPR-based epigenome editing.

KEYWORDS: Transposable elements; cancer epigenetics; DNA methylation; tumor microenvironment; viral mimicry; immunotherapy

1. INTRODUCTION

Transposable elements (TEs) comprise nearly half the human genome, and, once derided as junk DNA, they are now known to be a dynamic class of repeat sequences that include RNA transposons, DNA transposons, and endogenous retroviruses. TEs shape the genome by providing regulatory innovation in evolution and in many human diseases [1,2]; however, the application of cancer genome sequencing demonstrated that TEs were not silent passengers. Hundreds of high confidence somatic retrotransposon insertions were discovered in a pan-cancer survey of 43 high-coverage whole genomes. They were present predominantly in epithelial cancers. Somatic LINE-1 (L1) insertions were nonrandomly improved in cancer-specific DNA-hypomethylated regions and in commonly mutated cancer genes [2]. In another 244 patients, 53% of tumors had somatic retrotranspositions, of which 24% were 3′ transductions. These 3′ transductions came from a small number of source L1s, and transposition activity seemed to be correlated with L1 promoter hypomethylation [3]. Accordingly, TEs are both a source of mutation, and potential biomarkers of the generalized epigenetic reprogramming that occurs with tumorigenesis [4].

The cancer epigenome is no longer perceived as cell-autonomous. Tumors are considered integrated ecosystems in which cancer cells coevolve with stroma, vasculature, and immune compartment [5]. In this context, DNA methylation, histone post-translational modifications, chromatin remodeling, and non-coding RNAs regulate the transcriptional programs of tumor cells and the differentiation, phenotypic plasticity, and tumor-promoting functions of cancer-associated fibroblasts, tumor-associated macrophages, and endothelial cells and infiltrating lymphocytes within the TME [6,7]. Importantly, although TME cells are usually not genetically instable, the pro-tumorigenic gene expression programs in TME cells are established and maintained in a largely epigenetic manner through the rewiring of the methylome landscape in a way that can become heritably fixed in chromatin [8]. Tumor-mediated signals (hypoxic stress, altered levels of extracellular ions, cytokines, or direct cell contact)

actively reshape the epigenomes of TME cells and convert an immunostimulatory response to a tumor-promoting one [6,8].

TE deregulation occurs at the juncture of these two models. TE silencing via heavy DNA and histone methylation in normal host development is uniformly reversed in cancer cells, where 5-methylcytosine is globally lost from all genes, without regard to TE sequence. This mechanism exposes TE-derived regulatory sequences in cancer cells [4,9]. Perhaps most considerably, genome-wide analysis has shown that onco-exaptation (i.e. the epigenetic reactivation of dormant TE promoters to transcribe oncogenes) operates in almost half of all cancers and implicates 106 oncogenes across 15 cancer types, including AluJb-mediated activation of LIN28B [9,10]. Second, in addition to mutagenic insertions, expressed TEs also produce double-stranded RNAs that activate a viral mimicry response, increasing tumor immunogenicity [11]. Downstream of this event, tumor interferon signaling and immune infiltration are activated [11]. Third, long-read chromatin conformation studies have revealed a previously unmapped insertion-independent mechanism whereby new LINE-1 transcripts create oncogenic 3D chromatin structures that modulate the expression of cancer-associated genes in trans [12]. Taken together, these findings position TE epigenetic deregulation as a convergent driver of genomic instability, transcriptional rewiring, and immune remodeling.

We will review here current evidence that transposon reactivation is not only a byproduct of cancer-related hypomethylation but an accessible mechanistic node linking cancer epigenetics, the tumor microenvironment and cancer treatment [7,13]. We will first describe the diverse molecular regulators, from DNMTs to histone modifiers, the HUSH complex and chromatin remodelers, which are required for TE silencing and, when disrupted, lead to TE derepression in cancer. Next, we review modes of TE reactivation that reprogram oncogenic transcription, including onco-exaptation, enhancer co-option, insertional mutagenesis and chromatin remodeling, and how TE-derived nucleic acids and the TME can communicate with each other, such as modulation of anti-tumor immunity via viral mimicry and epigenetic regulation of TEs by stromal cells [14]. Next, we discuss the development of new therapies targeting TE biology, including DNMT and histone-modifying inhibitors, ADAR1 antagonism, transposon-based gene therapy delivery systems, as well as TE-derived cancer vaccines and immune checkpoint inhibitors [13][14]. Finally, we discuss the implications of integrating TE biology from foundational to more recent studies, providing a conceptual framework for TE epigenetic deregulation in cancer and potentially a new model in precision oncology.

2. Landscape of transposable elements in Cancer

2.1 Classes of transposons: LINEs, SINEs, LTR/ERVs, DNA transposons

Transposable elements in the human genome are commonly categorized into two classes. Class I elements or retrotransposons propagate via an RNA intermediate through a copy and paste mechanism, mediated by a reverse transcriptase enzyme. Class I elements can be subdivided into long-terminal-repeat and non-LTR elements [15,16]. The latter can be subdivided into long interspersed nuclear elements (LINE-1/L1, LINE-2, LINE-3) and short interspersed nuclear elements (Alu and SVA). On the other hand, LTR elements include the endogenous retroviruses (HERV-I, HERV-K and HERV-L families) fixed in the germline from ancient retroviral infections [16,17]. Non-LTR retrotransposons occupy one-third of the human genome. LINE-1 (L1) sequences comprise ~17% of the human genome, with ~500,000 copies, of which ~100 full-length sequences are capable of retrotransposition (i.e., are retrotransposition-capable). In contrast to this rolling-circle mechanism, Class II DNA transposons use a cut and paste mechanism. Most DNA transposons are inactive, but RNA- and protein-based evidence suggests that transcriptional reactivation of some DNA transposon subfamilies occurs in many cancers [11,16].

In the human mobilome, retrotransposon age is a dominant predictor of cancer mutagenesis potential. The most active source of retrotransposon mutagenesis in the modern human genome are L1s, which have been continuously active in the human genome for close to 160 million years. In contrast, most HERVs and ancient DNA transposons have become fixed and domesticated as cis-regulatory elements [16,18]. This hierarchy of activity is responsible for the huge majority of all cancer retrotransposon detection, including the dominant L1 signal in tumor whole-genome sequencing [1,2].

2.2 Tissue-specific TE activity in solid tumors versus hematological malignancies

TE activity is not uniform across all tumor types. In a pan-cancer survey of 43 high-coverage whole genomes, 194 high-confidence somatic TE insertions were identified, almost all in epithelial tumors, with none in hematological cancers or brain malignancies [2]. Targeted L1-seq profiling of colorectal and non-small-cell lung cancer found similar levels of somatic retrotransposition in epithelial cancers, and these pan-cancer patterns were followed in a pan-cancer RNA-seq analysis of TCGA tumors, where the majority of stomach, bladder, liver, head and neck and lung squamous tumors overexpressed TEs, but breast, thyroid, kidney chromophobe and lung adenocarcinoma tumors were underexpressed relative to matched normals [19][20][11].

In contrast to the insertional pattern of carcinogenesis, hematological malignancies show an epigenetic pattern of TE deregulation. DNA hypomethylation reactivates endogenous retroviral transcripts in leukemia and lymphoma. This may interfere with the piRNA-mediated surveillance, although this does not seem to strongly require high rates of new retrotransposition. Tissue-specific enhancer activity appears to be a second dimension of TE activity. An integrative analysis of endodermal lineage cancer indicates that tumor types utilize different TE-derived enhancers bound by lineage-specific transcription factors to control gene expression specific to each tumor type

[22]. Gastrointestinal cancers are among the most affected, with common somatic L1 retrotransposition from the earliest stages of tumor evolution, with discrete patterns of L1 misincorporation consistent with an epithelial-specific window of L1 permissiveness different from that of testicular and brain tumors [2,23].

2.3 Somatic versus germline TE reactivation during tumor evolution

The cancer retrotransposon landscape is shaped by standing germline retrotransposon variation (two individuals of European ancestry differ by 500-800 retrotransposon insertions, 90 germline retrotransposon insertions are involved in humans with disease), and active somatic retrotransposition (tumors inherit a personalized TE substrate from the germline), as evidenced by the abundance of tumor-specific retrotransposon insertions in cancer genomes. Taken together, in a tissue-wide study across 244 epithelial tumors, 53% of tumors harbored somatic retrotranspositions, including 24% that were 3′ transductions, which are generated by a small number of highly active sources in tumors whose activity can fluctuate along the course of tumor evolution, and which correlate with L1 promoter hypomethylation [3]. In gastrointestinal cancers, hundreds of somatic L1 insertions can be detected even in precursors, suggesting that retrotransposition in apparently normal epithelium contributes to the earliest events in tumorigenesis [20,23].

Genomic footprints left by these two modes of TE reactivation differ. Germline polymorphic insertions are fixed, full-length or truncated, and found in matched normal tissue. Somatic insertions are clonally distributed, biased toward gene-body proximal or cancer-specific DNA-hypomethylated regions, biased towards genes that are frequently mutated in cancer, and frequently disrupt the expression of the targeted gene. Insertions of somatic L1 elements are largely clonal and may serve as phylogenetic markers [2,3]. L1 3′ transductions are an example of gene, exon, and regulatory region transfer to new genomic contexts, most commonly genomic heterochromatin, creating new TE-derived sequences that are not found in the germline sequence. Together, germline and somatic retrotransposition layers drive cancer genome remodeling across evolutionary timescales, from germline susceptibility through intra-tumor diversification.

2.4 TE burden as a hidden driver of genomic instability

In addition to single locus insertional mutagenesis, TE burden in the genome of a cancer is a major, but currently understudied, contributor to structural genomic instability. Human TEs have been estimated to comprise between 1/2 and 2/3 of the haploid human reference genome (over 4 million TEs annotated). Comparative genomic analysis suggests TEs contribute to around 10% of SVs >100 bp, with these being generated via ectopic DNA repair, using two homologous copies of a TE as the ectopic substrate [17,24]. They are likely responsible for the deletion of approximately 850 kbp of the human reference genome with respect to the chimpanzee draft genome and multiple cancer-predisposing syndromes [24].

The most direct evidence for TE-mediated oncogenesis is insertional mutagenesis. There are a few experimentally observed examples of cancer-causing TEs in humans, including an L1 insertion in APC in a colorectal carcinoma, the first somatic L1 insertion in a human cancer, and an L1 insertion in MYC in a breast carcinoma [19]. In contrast, TE-initiated DSBs can also lead to non-allelic homologous recombination and other rearrangements including large deletions, duplications, inversions and translocations, often not attributable to the single mobile element responsible for triggering them [18]. Moreover, cancer genome-wide hypomethylation of TE promoters and consequent increase in their expression has been observed in most types of tumors. The loss of pathways for DNA sensing and repair leads to a progressive increase in the deleterious effect of mobile elements on genome integrity along the path of tumorigenesis [1,18].

3. DNA methylation control of transposons

3.1 Promoter CpG methylation and TE silencing in normal cells

Under physiological conditions, DNA methylation of cytosines at the CpG dinucleotides of TE-derived promoter sequences provides the principal epigenetic barrier to the repression of TE movements and silencing of ectopic regulatory activity during all developmental stages, starting from early embryogenesis, when the de novo DNA methyltransferases DNMT3A and DNMT3B methylate across repetitive genome compartments (4); the maintenance methyltransferase DNMT1 transmits the marks to daughter cells after each mitotic division (15,25). The silencing effect of CpG methylation on TEs is carried out by two functions: it prevents transcription factors from binding to TE consensus promoter sites and it recruits methyl-CpG-binding domain proteins which recruit histone deacetylase and H3K9 methyltransferase to make heterochromatin, a state which is tightly bound and transcriptionally inactive [4,25]. This process generates a methylation landscape in somatic cells that maintains transcriptional silencing of the L1, Alu, and ERV families, with the exception of a few tissue-specific hypomethylated hot L1 loci that ease physiological regulatory innovation [2,15].

3.2 Global hypomethylation in Cancer and TE de-repression

In cancer, the opposite of global hypermethylation, global hypomethylation, occurs, as well as focal CpG island hypermethylation of tumor suppressor gene promoters. Despite 20 years of research stressing focal hypermethylation of tumor suppressor promoters, TE reactivation in cancer is instead directly caused by global repetitive element hypomethylation [4,25]. This hypomethylation frees TE promoters from silencing by chromatin-based mechanisms by blocking the chromatin silencing machinery, exposing more than four million annotated TEs across the genome to reactivation [1,9]. Reactivated TEs can provide substrates for continued

retrotransposition, generate dsRNA intermediates that activate natural immune sensing, and function as new cis-regulatory elements that are hijacked by the Cancer transcriptional programs [11,14].

3.3 DNMT1/DNMT3A/3B dysregulation found in transposons

Loss of function changes to the DNMT machinery are direct molecular drivers of TE de-repression in malignancy as demonstrated by the observation that DNMT1 deletion causes rapid increases in L1 transcript abundance and retrotransposition rates and the breakdown of the maintenance barrier that represses TEs in dividing cells [15,16]. De novo methylation is established by de novo methyltransferases DNMT3A and DNMT3B. Deletion or inactivation of these enzymes results in the formation of clusters of unmethylated repeat sequences, especially in heterochromatic regions of chromosomes [15,25]. Mutations in DNMT3A are a common feature of clonal hematopoiesis and acute myeloid leukemia (AML). As with loss of TET2, mutations that de-repress L1 and ERVs may be particularly consequential, as L1 sequences are recognized as endogenous and can form dsRNAs that are recognized by natural immune sensors [8,21]. This DNMT loss-of-function pattern is mechanistically linked to insertional mutagenesis and viral-mimicry-driven immune remodeling in Cancer.

3.4 LINE-1 hypomethylation as a biomarker of tumor aggressiveness

A clear example of the clinical utility of molecular findings is the use of L1 promoter hypomethylation for prognostic and predictive purposes. A meta-analysis of The Cancer Genome Atlas (TCGA) databases of 2,557 cancers representing 11 tumor types indicated that L1 promoter hypomethylation is a pan-cancer, recurrent epigenetic event that is common in high-stage epithelial tumors and is associated with poorly differentiated tumors, advanced tumor stage, and poor cancer prognosis [1,2]. The recent development of quantitative L1 methylation assays for circulating tumor (ct) DNA-based testing has shown that circulating tumor-derived ctDNA-L1 hypomethylation in ctDNA is correlated with tumor burden and treatment response in colorectal, breast and head and neck cancers [19,25]. L1 hypomethylation has also been increasingly used as a stratification biomarker for hypomethylating-agent treatments and drug combinations with viral mimicry, heralding the entry of epigenetic patient selection into the domain of precision oncology [13,14].

3.5 Epigenetic drift and TE reactivation during tumor progression

Likewise, as TE reactivation is a gradual epigenetic drift rather than a discrete on/off state aggravated by tumor evolution, single-cell methylome profiling of matched normal, adenoma, and carcinoma has shown that TE body demethylation accompanies histological progression, with the most prominent demethylation observed in metastases [5,23]. For gastrointestinal tumors, hundreds of somatic L1 insertions are detectable in very early tumors and L1 activity appears to continue to expand during neoplastic progression [20,23]. Hypoxia and inflammatory cytokines also drive this drift in response to the tumor microenvironment by destabilizing DNMT-mediated silencing in stroma and infiltrating immune cell lineages [7,12]. The coupling of repeated epigenetic erosion with TE mobilization has provided a coherent basis for the escalating characteristics of tumor evolution, including increasing genomic instability, increasing tumor-cell antigenicity and increasing therapeutic vulnerability to chromatin-directed therapies [5,10].

4. Histone modifications and chromatin architecture

4.1 H3K9me3 and HP1-mediated TE repression

The tri-methylation of histone H3 at lysine 9 (H3K9me3) is the canonical epigenetic signature for constitutive heterochromatin and the main barrier to transposable element activity in somatic cells [26]. The H3K9me3 mark is mediated by SETDB1, a member of the SET-domain containing protein methyltransferase family, while SUV39H1 and SUV39H2 mediate H3K9me2/H3K9me3. H3K9me1/H3K9me2 methylation (by GLP/G9a, EHMT1/EHMT2) bind the protein chromodomain of Heterochromatin Protein 1 (HP1, three mammalian isoforms), oligomerize on TE chromatin, recruit other repressing histone modification enzymes, and spread heterochromatin to neighborhoods of repetitive sequences. [26,27]. Loss of HP1 also results in chromosome segregation defects, deregulated centromeric and telomeric silencing, and is linked to tumorigenesis. The mouse HP1 triple-knockout restores retrotransposon expression in pre-cancerous liver, and accelerates development of hepatocellular carcinoma (HCC) [27,28]. H3K9me3 and DNA methylation are not fully redundant, since silencing of SETDB1 in hNPCs leads to preferential derepression of evolutionarily young L1s. Furthermore, establishment of H3K9me3 is necessary for maintenance of DNA methylation at L1 loci, as evidenced by a coordinated loss of CpG methylation at the L1 promoter following Setdb1 knockdown [4,29].

4.2 Polycomb (PRC1/PRC2) regulation of LTR elements

A second evolutionarily conserved mechanism of repression involves polycomb group proteins, which are thought to act mainly on CpG-rich sequences, including younger LTR retroelements [30][31]. The repressive mark H3K27me3 is deposited by PRC2 (EZH2, SUZ12, EED). PRC1 monoubiquitinates H2A and compacts chromatin, and the two complexes work together to stably repress chromatin throughout development and into differentiated adult tissues [30,32]. Genetic ablation of PRC1 and PRC2 in embryonic stem cells causes strong, cooperative derepression of the LTR-class retrotransposons, such as the murine leukemia virus, hinting at functional redundancy and suggesting rewiring in this layer of repetitive elements in Cancer [31]. EZH2 overexpression has also been observed as a negative prognostic indicator in metastatic castration-resistant prostate cancer (CRPC), although Polycomb-independent oncogenic activity of EZH2 has been demonstrated in CRPC cell lines [32,33]. However, only minor roles for PRC2 in silencing cancer-specific LTRs in cell lines were

inferred from an integrative RNA-seq analysis, except for when cytosine methylation was pharmacologically inhibited; in this case, EZH2 knockdown augmented a viral mimicry response resultant from the demethylating agent 5-aza-2′-deoxycytidine [34].

4.3 Loss of heterochromatin integrity in cancer cells

Indeed, cancer genomes always sustain large-scale loss of heterochromatin integrity, which drives TE derepression. Pan-cancer integrative chromatin studies showed that heterochromatin domains characterized by H3K9me3 and H4K20me3 are consistently reprogrammed in cancerous tissues, with repetitive sequences especially improved in H4K20me3-deficient and altered-looping chromatin states that favor illegitimate repair and inter-chromosomal translocations [35, 36]. Super-resolution imaging of pre-cancerous lesions during tumorigenesis demonstrated a progressive loss of higher-order chromatin folding consistent with spatial exposure of repetitive elements, linking heterochromatin collapse to the genomic instability required to acquire the hallmarks of cancer [35,37]. In line with this observation, genetic ablation of Suv39h1/Suv39h2 in double-knockout mice has been associated with loss of pericentric H3K9 methylation, aneuploidy and B-cell lymphoma formation. Moreover, G9a-deficient cells are more prone to genomic instability and give rise to aggressive tumors upon oncogenic challenge [27,37].

4.4 SETDB1, SUV39H1 and G9a contribute to TE silencing

The three main H3K9 methyltransferases have non-redundant functions in TE silencing and cancer-associated mis-regulation of these enzymes has emerged as a unifying mechanism coupling TE reactivation with immune evasion. The major histone methyltransferase of evolutionarily young L1s and class I/II/III ERVs is SETDB1, which is frequently increased in solid tumors. SETDB1 overexpression methylates tumor suppressor genes, retrotransposons and immune loci in cooperation with cofactors ATF7IP, TRIM28 and the human silencing hub (HUSH) effector complex [38-40]. In a genome-wide CRISPR-Cas9 screen in acute myeloid leukemia, SETDB1 was identified as a repressor of natural immunity, whose deletion de-repressed retrotransposons, produced endogenous dsRNAs and restored interferon signaling, which restricts leukemic proliferation [41]. Thereby, SETDB1 has been recast as a bona fide immune checkpoint in hematopoietic malignancies with stage- and lineage-specific roles in normal and malignant hematopoiesis [42]. The G9a/EHMT2 and SUV39H1 methyltransferases are responsible for silencing SINE, LINE and SVA elements. Thus, inhibition of SUV39H1 is sufficient to lower H3K9me3 marks at Alu elements and to improve RNA polymerase III recruitment and thus Pol III-driven transcription at these elements [29]. Loss of either of these enzymes or pharmacological inhibition thereof thus induces a multi-family TE reactivation program, leading to different outcomes for each tumor type with respect to immune exposure and genomic stability [9,15].

4.5 3D genome reorganization and TE accessibility in tumors

The 3D packaging of cancer genomes is now recognized to be a consequence and driver of TE reactivation. TEs have repeatedly provided the CTCF-binding motifs that delineate TADs, and species-specific CTCF sites are highly improved for SINE sequences. Repetitive elements have thus been co-opted as building blocks of genomic architecture throughout genome evolution in mammals [15,43,44]. In pluripotent stem cells, transcriptionally active HERV-H retrotransposons mark TAD boundaries with stage-specific boundary activity that is lost upon HERV-H deletion [44]. In Cancer, alterations to TAD hierarchy can occur via somatic structural variants or epigenetic reprogramming. This leads to LTR co-option as ectopic regulatory elements to activate oncogenes normally insulated by neighboring TAD domains [45,46]. Super-resolution microscopy and Hi-C data have also shown that misplaced CTCF-binding motifs (usually within newly acquired or derepressed TEs) are driving forces of chromatin loop reorganization in tumor evolution [12,43]. Taken together, these findings position TE-driven 3D genome reorganization as an integrative final step that converts epigenetic deregulation of transposons into durable and heritable changes in Cancer transcriptional programs, immune phenotype, and therapeutic vulnerability [14,40].

Figure 1: Epigenetic and architectural regulation of TEs in normal and cancer cells. DNA methylation and repressive histone marks in normal cells maintain closed chromatin and stable TAD structure, preventing transcription from TE regions. In cancer cells, global hypomethylation, chromatin remodeling, TE activation, TAD boundary disruption and genomic instability contribute to oncogenic reprogramming.

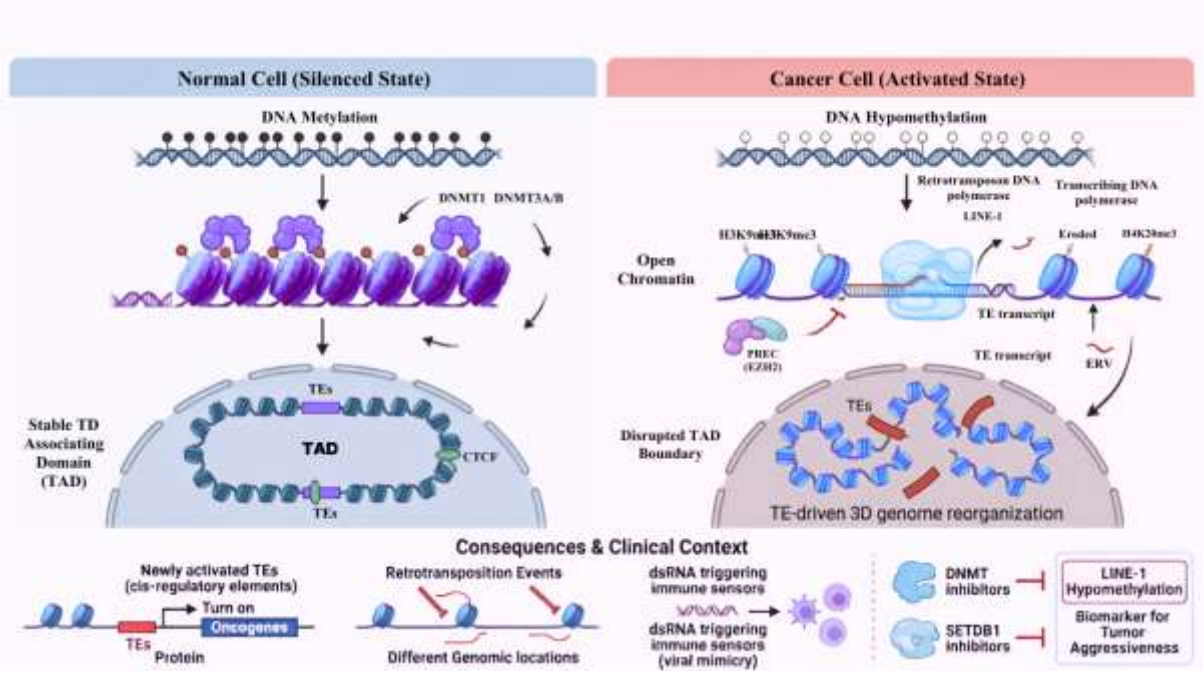

Figure 1. Epigenetic and structural regulation of transposable elements in cancer

5. Non-coding RNA networks governing transposons

5.1 piRNA pathway dysregulation in somatic cancer cells

PIWI-interacting RNAs (piRNAs) are a class of small single-stranded non-coding RNA associated with PIWI clade Argonautes that silence transposons, mainly in the germline. Piwi proteins act at transcriptional and post-transcriptional stages to prevent accumulation of transposable element transcripts by promoting heterochromatin formation and RNA degradation, respectively. [21] While the piRNA/PIWI axis was thought to be germline specific, PIWI proteins and piRNAs have been ectopically expressed in a variety of somatic cancers including seminomas, multiple myeloma, hepatocellular, breast, gastrointestinal, ovarian and endometrial cancers [21]. In general epigenetic reprogramming, PIWI/piRNAs were found in Cancer that correlated with poor clinical outcomes. Whether PIWI/piRNAs in cancer are drivers of tumorigenesis, or merely passengers of a more general epigenetic reprogramming of cancer cells, is not yet clear [21,47]. A driver role is plausible for germline-derived tumors such as seminoma in which the expression of PIWI proteins is typically high. In somatic tumors, PIWI proteins rapidly undergo positive selection in cancer genomes and are likely involved in the transcriptomic and epigenetic rewiring of lineages [21].

5.2. siRNA/Endo-siRNA systems in TE surveillance

Endo-siRNAs, which are Dicer-/Ago2-dependent and somatic cell-specific, form a second line of defense against retrotransposons, as a complement to the germline piRNA response [48, 49]. Deep-sequencing of small RNAs in human breast cancer cells has identified a population of endo-siRNAs, which directly target the LINE-1 retrotransposons. Endo-siRNAs are depleted in cancer cells when compared to normal breast cells, and their overexpression leads to silencing of endogenous L1 transcription by increasing DNA methylation of the L1 5′-UTR promoter, linking endo-siRNA expression, L1 silencing, and genome stability [50]. In another study, sense-antisense pairs between PPM1K and its transcribed pseudogene generated the endo-siRNAs downregulated in hepatocellular carcinoma cancers (the first detection of an endo-siRNA pathway in cancer) [48]. Endo-siRNAs thus powerfully silence TEs in a secondary layer of TE repression, the loss of which during Cancer development correlates well with loss of global microRNA expression, a hallmark of cancerous transformation [47,49].

5.3 lncRNA-mediated chromatin recruitment to TE loci

Recent studies reported lncRNAs as sequence-flexible scaffolds for chromatin modifiers to bind repetitive and transposon-derived chromatin. A chromatin-RIP-seq study of BT-549 cells using antibodies against H3K27me3 and EZH2 identified 276 lncRNAs improved in repressive chromatin. For example, the tumor suppressor lncRNA MEG3 forms RNA-DNA triplex complexes that recruit regulatory elements of the transforming growth factor beta (TGF-β) pathway genes to chromatin. HOTAIR was the first lncRNA shown to reprogram chromatin state in Cancer by binding to PRC2 mediating H3K27 trimethylation at hundreds of genome loci including metasterically relevant genes thereby establishing a functional dependency between a single lncRNA and PRC2 in promoting cancer invasion [52]. TUG1 is another p53-regulated lncRNA that epigenetically regulates HOXB7 expression by PRC2 recruitment in NSCLC. Conversely, LINC-PINT's tumor-suppressive role has been attributed to a highly conserved sequence that binds with PRC2 and represses invasion-promoting genes [53,54]. Biophysical analyzes suggest that PRC2 binds to numerous transcripts in a low-affinity and potentially promiscuous nature, and

lncRNAs are hypothesized to play a decoy or scanning role to maintain the repressive chromatin environment, particularly over genomic repeat sequences such as TE loci [51,55].

5.4 miRNA-TE interaction networks in tumor progression

The evolution and regulation of miRNAs and TEs are tightly intertwined: there are 409 miRNAs predicted to be derived from TEs in humans, in addition to other miRNAs predicted to contain TE-derived miRNA target sites in post-transcriptional regulatory networks [56,57]. For example, LINE-2 TEs have been experimentally shown to produce bona fide human miRNAs and target sites. Several miRNAs produced by LINE-2 TEs are up- or downregulated in malignant brain tumors and other human cancers [56]. In 33 TCGA carcinoma types, 34 TE-derived miRNAs are identified which stratify tumors by stage, status and survival. Examples include conserved miR-130a with renal and thyroid carcinomas, and human-/primate-only miRNA miR-625 with multiple carcinomas [58,59]. Since TEs have been a major source of both miRNA genes and miRNA target sites in mammalian evolution, dysregulation of TE-driven miRNA networks may be a methylation-linked specialized layer of tumor evolution distinct from the more common, bystander level of changes in global gene expression [56,59].

5.5 RNA editing and dsRNA derived from TEs

Another major post-transcriptional editor is adenosine deaminase acting on RNA 1 (ADAR1), which binds to dsRNA and deaminates adenosine to inosine, destabilizing potentially immune-detecting duplexes [60,61]. Most ADAR1 editing occurs in noncoding regions, such as the inverted-repeat Alu and LINEs, within introns and UTRs, creating long dsRNA stretches. The major motifs are the two inverted repeat pairs [60,61]. Most inverted-repeat TE A-to-I editing is carried out by the IFN-inducible cytoplasmic isoform of ADAR1, ADAR1p150, in a conserved manner from primates to less conserved rodents. Loss-of-function ADAR1 mutations are among the most prominent genetic factors driving TE-dsRNA-induced type I interferonopathies including AGS and SLE. Simultaneously, increased ADAR1 expression has been linked to cancer progression [15,63], with TE derived dsRNA editing by ADAR1 providing a potential agent of escape for cancer cells. By increasing the hyper-editing of dsRNAs generated from these reactivated TEs, the ADAR1-dependent quenching of the MDA5/MAVS and PKR sensors is disrupted, lowering the virally mimicking response and leading to tumor immune escape [13,14]. Therefore ADAR1 inhibitors seem promising to improve tumor antigenicity and to synergistically act with immune-checkpoint inhibitors [60,63]. Together with the above-mentioned layers of piRNA, endo-siRNA, lncRNA, and miRNA, ADAR1-dependent

As shown in Figure 2, cancer-relevant non-coding RNA networks regulate transposons using multiple interactive mechanisms like piRNA-PIWI, endo-siRNA, miRNA-TE, lncRNA-mediated chromatin recruitment, etc. These have been proposed to regulate transposons via various mechanisms such as DNA methylation, histone modification and post-transcriptional RNA degradation. TE activation and genome instability can be caused by dysregulation of these processes. RNA editing (ADAR1) changes the immune sensing of TE-derived dsRNA, promoting immune evasion in cancer.

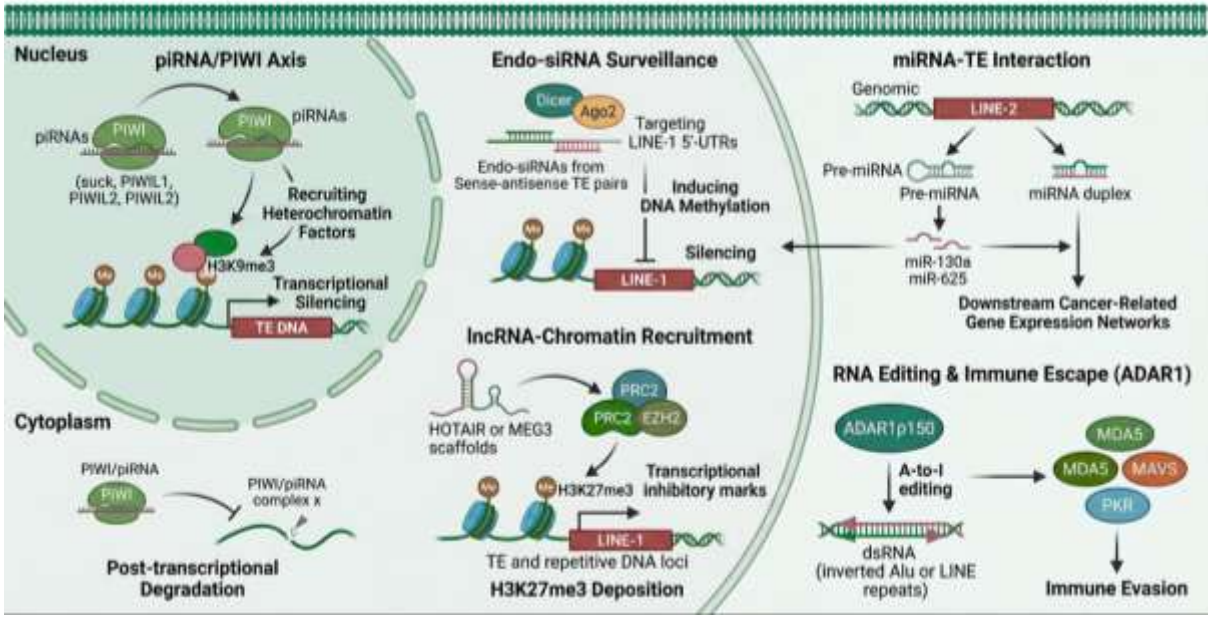

Figure 2. Non-coding RNA networks governing transposon regulation in cancer

6. Innate immune activation via transposons

6.1 Cytosolic DNA sensing: cGAS–STING pathway activation

Rich in nucleic acid intermediates, epigenetically derepressed TEs stimulate major natural immune pathways. This includes cytosolic DNA from endogenous retrotransposon replication intermediates, abortive L1 reverse-transcription products, and damaged mtDNA, all of which signal through the cGAS-STING axis connecting DNA

sensing to type I interferon and NF-κB pathways. For a full review of the cGAS-STING activation by nucleic acid intermediates, see [6]. Nonetheless, in cancer, cytosolic reverse-transcribed LINE-1 DNA is degraded by the three-prime repair exonuclease 1 (TREX1), but nucleic-acid-stimulated cGAS-STING activation is restored by chromatin fragmentation, mitochondrial outer-membrane permeabilization, and TE-mediated chromosomal fragmentation, each leading to the inundation of the cytosol with TE-derived DNA. This explains the clinical rationale for the co-administration of DNMT/SETDB1 inhibitors with STING agonists or with DNA-damaging agents currently in clinical trials [13,14]. Other TE-intrinsic properties further mark TE-derived nucleic acids as strong natural immune stimulators, including nuclear export of reverse-transcribed cDNA to the cytosol, foreign CpG-rich sequence context, and binding to novel pattern-recognition receptors [1,9].

6.2 Endogenous retrovirus-derived dsRNA and viral mimicry

ERV-derived dsRNA is the canonical viral mimicry trigger in Cancer. Upon epigenetic reactivation of ERV families by DNA hypomethylation drugs, histone methyltransferase inhibitors, or genetic deletion of SETDB1, convergent-sense and antisense ERV transcripts hybridize to form long dsRNA duplexes that are recognized by cytosolic MDA5 and RIG-I sensors [14,39]. In this case, MAVS-mediated IRF3/IRF7 signaling is triggered, leading to type I IFN and ISG production, and the upregulation of antigen presentation machinery (ie, MHC class I) (13). However, the abundance of dsRNA in tumor cells often results in higher immunogenic dsRNA levels than needed for the induction of PKR-mediated translational suppression, resulting in a switch from tumor cell intrinsic viral mimicry to paracrine effects in the tumor stroma and immune contexture (6,14). Viral mimicry has been experimentally induced by DNMT inhibition in evolutionarily young ERVs like the ERVFRD-1 in human embryonal carcinoma and the ERV3-1 in melanoma cells, supporting generality across tumor types [8,13].

6.3 IFN-I response triggered by TE de-repression

The resultant IFN-I response upon TE de-repression is thought to drive immunogenicity of the tumor. Pan-cancer TCGA analysis shows that high TE expression correlates considerably with higher tumor-infiltrating immune cell density, higher IFN-γ signatures, and higher tumor antigenicity scores in gastric, bladder, head-and-neck, and lung squamous tumors [11]. In AML, CRISPR-Cas9 screening identified SETDB1 as a negative regulator of the response to HERV-derived dsRNA. Loss of SETDB1 reactivates HERV-derived dsRNA, restores IFN signaling, and restricts leukemia growth in an MDA5/MAVS-dependent way [41]. Cutaneous ablation of EZH2 cooperated with 5-aza-2′-deoxycytidine to unleash ERV de-repression and viral mimicry in the epidermal keratinocytes consistent with Polycomb reinforcement of IFN suppression at repeat loci in response to demethylation [34,40]. In turn, the IFN response promotes the presentation of tumor antigens, mobilizing Batf3-dependent dendritic cells and priming $CD8^+$ T cells to target the tumor [6,14].

6.4 Immune escape mechanisms resulting from chronic TE activation

However, this chronic TE activation in established cancers is likely to further promote immune escape rather than immune activation. Ongoing accumulation of dsRNA may select against functional nucleic-acid sensors (such as MAVS, IRF3, MDA5), the IFN-α/β receptor (IFNAR1/IFNAR2), and other downstream JAK-STAT mediators, allowing tolerable TE burden but stripping away the capacity for antigen presentation [1,13]. Hyper-editing of TE-derived dsRNAs by ADAR1 is a common escape mechanism that destabilizes A-to-I-edited dsRNA duplexes, abrogates MDA5 or PKR activation, and interrupts IFN production. Constitutive, low-level expression of ADAR1 has been observed in solid tumor-derived cell lines. ADAR1 inhibition is able to reverse immune evasion when combined with checkpoint inhibitors in preclinical models [60,62,63]. Pharmacological ADAR1 blockade has also been shown to synergize with checkpoint blockade in preclinical studies [9,15,60]. Alternatively, transcriptional escape is mediated by expression of SETDB1, restoration of H3K9me3 heterochromatin, or remethylation of ERV loci by DNMT1, which has been observed in breast, endometrial, prostate, and hematological malignancies [4,38-40,42]. These mechanisms potentially explain how extreme TE expression can co-occur with low immune visibility in the most immunologically cold advanced tumors [1,8].

6.5 Inflammatory reprogramming of the tumor microenvironment

The main systemic effects of natural TE activation are inflammatory reprogramming through TE-derived cytokines, chemokines, and growth factors. The release of TE nucleic acids over prolonged periods into the TME occurs via exosomes and tumor-derived extracellular vesicles or apoptotic bodies. Importantly, uptake of these nucleic acids by stromal and immune cells elicits heterologous IFN and NF-κB signaling networks to mold the local cytokine milieu [7,8]. In cancer-associated fibroblasts, prolonged exposure to type I and II IFNs sets in motion the epigenetic reprogramming of immune response pathways and senescence-associated secretory phenotypes that paradoxically foster tumor growth and angiogenesis [6]. Persistent, dsRNA-induced epigenetic reprogramming of tumor-associated macrophages and dendritic cells downstream of H3K27ac and H3K4me3 remodeling, in conjunction with downregulation of antigen-presentation processes, shapes the imprinted immunosuppressive phenotypes of these APCs. This results in a pro-inflammatory TME rich in IL-6, IL-10, TGF-β, and CXCL12, which promotes tumor invasion, neoangiogenesis, and recruitment of myeloid-derived suppressor cells (MDSCs). This effectively links epigenetic de-repression of TEs in the tumor cell to immunosuppression in the TME across the body. Such strategies that aim to maintain viral mimicry, yet simultaneously block these adaptive immune-escape pathways, therefore represent an interesting translational potential [8,13,40].

Figure 3 summarizes that TEs regulate natural immunity through activation of the cGAS-STING pathway and the MDA5 and RIG-I pathways through TE-derived nucleotides, leading to the subsequent Type I IFN signaling and ISG expression to augment anti-tumor immunity. Nevertheless, cancer cells counter these potential threats by employing TREX1-mediated DNA degradation, ADAR1-dependent RNA editing, and immunosuppressive cytokines within the tumor microenvironment.

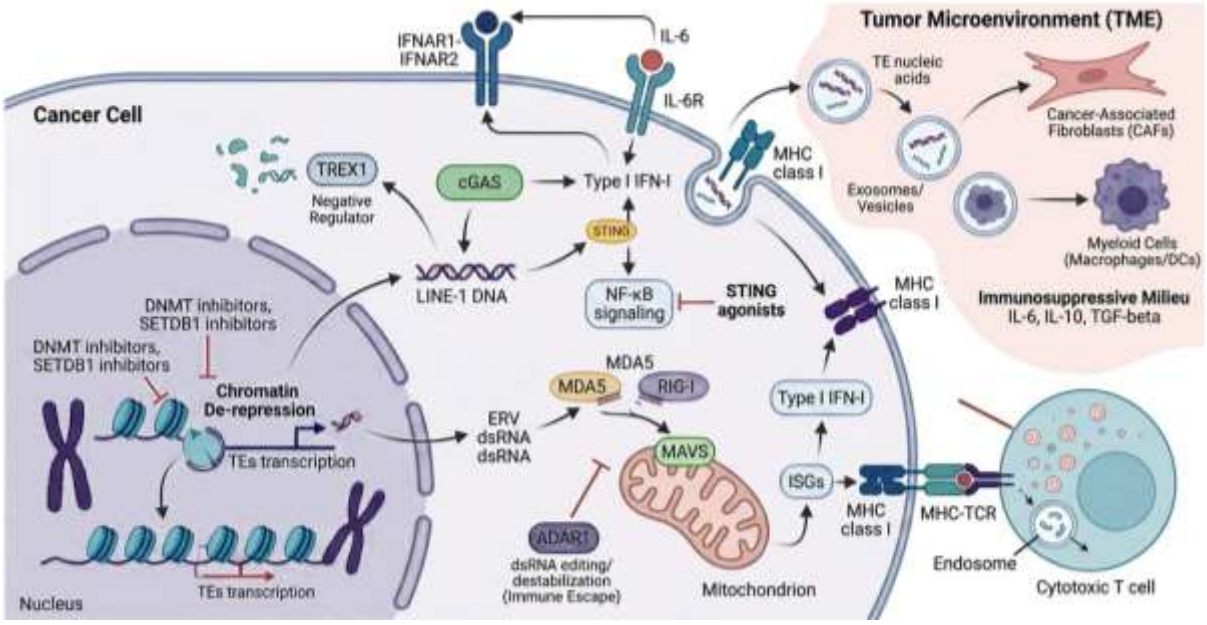

Figure 3. Innate immune activation and immune escape via transposable elements in cancer cells

7. Therapeutic targeting of transposon epigenetics

7.1 DNMT inhibitors and viral mimicry

The prototypic epigenetic drugs hypomethylating agents 5-azacitidine and decitabine mainly function to sequester DNMTs from the DNA at the replication fork. On each cell division, passive replication dilutes out 5-methylcytosine from the DNA and erases the silencing of tumor-suppressor genes. Low-dose DNMT inhibition with hypomethylating agents (HMAs) also derepresses ERV and LINE-1 promoters, producing cytoplasmic dsRNAs that induce viral mimicry and the release of immunogenic tumor-derived antigens by MDA5/MAVS signaling in a type I IFN-dependent manner. Supporting the relevance of this mechanism, decitabine and azacitidine, both FDA-approved for treatment of myeloid malignancies, have been shown to derepress ERV transcripts and induce type I IFN in myeloid neoplasms (the induction of both is quantifiable; see above [8, 13, 34]). The dependence of the HERV silencing process on evolutionary age and mechanistic studies that have shown DNA methylation control of HERV expression in evolutionary young elements, and H3K9me3/HMGA1/HMGB2 for older families, present a theoretically pharmacological model where DNMT inhibitors would preferentially release young ERVs known to be the most immunogenic ones [14,34].

7.2 HDAC and BET inhibitors in TE reactivation therapy

A second pathway to induce TE reactivation in DNMTi-resistant tumors is the use of HDAC and BET inhibitors, which globally hyperacetylate histones and maintain an open chromatin structure conducive to transcription in TE bodies. DNMT and HDAC inhibition have also been shown to synergistically induce viral mimicry signaling and immune infiltration in solid tumor models [13,14]. Inhibition of BRD4 using the BD2-selective degrader ABBV-744 or the first-generation BET inhibitor JQ1 can derepress TE promoters by removing BRD4 from repressive chromatin complexes. In enzalutamide-resistant prostate cancer xenografts, BET inhibition induces type I IFN signaling and class I MHC in a TE-dependent manner [8,13]. Likewise, dual inhibition of both BETs and TRIM28 or SETDB1 increases dsRNA production and might be leveraged to ameliorate ADAR1-mediated immune evasion in viral mimicry-refractory tumors with combination treatment of a BET inhibitor and another epigenetic modulator [13,38].

7.3 Targeting the SETDB1/KRAB-ZFP Axis in Cancer

The SETDB1/KRAB-ZFP/TRIM28 axis is a central node of cancer-relevant heterochromatin, and the first core heterochromatin component to be described as an actionable target for TE reactivation therapy. In AML, CRISPR-Cas9 dropout screens have revealed SETDB1 as a targeted immune checkpoint. SETDB1 depletion derepresses HERV transcripts, activating an MDA5/MAVS-dependent IFN response that inhibits leukemia engraftment in vivo [40,41]. In non-small cell lung carcinoma, melanoma and glioma, SETDB1 upregulation correlates with poor patient prognosis. Inhibition of selective SETDB1 inhibitors (R)-PFI-90 and its derivatives reduces L1 and HERVs H3K9me3 expression and increases cytosolic dsRNA levels [38,40]. Companion TRIM28 degraders, KAP1 inhibitors, and KZFP-targeting CRISPRa platforms could specifically derepress certain TE families and spare normal cells [39,42]. In stage- and lineage-specific hematological cancers, the SETDB1/STAT5 axis

represents a highly specific vulnerability that supports the combination of SETDB1 inhibitors with conventional chemotherapy or hypomethylating agents in leukemia [40,42].

7.4 Combination immunotherapy: TE activation + immune checkpoint blockade

One of the lessons learned from viral mimicry is that TE reactivation is unlikely to fully abolish tumor immune tolerance on its own, but can be combined with immune checkpoint blockade to do so. DNMT inhibitors combined with anti-PD-1, anti-CTLA-4 or anti-LAG-3 antibodies have shown durable anti-tumor efficacy in mouse models of melanoma, NSCLC and colorectal cancer. Additional mechanistic intermediaries include ERV de-repression, stimulation of IFN signaling and presentation of tumor-antigenic MHC-I products in the TME [11,13,14]. CRISPR-deconvolution screens, however, reveal ADAR1 loss as a synthetic-lethal enhancer of checkpoint blockade: ADAR1 loss on the viral-mimicry background of DNMTi-based therapy restores natural sensing of unmethylated TE-derived dsRNAs, leads to IFN-induced apoptosis and prevents standard monotherapy therapy exhaustion phenotypes [60,63]. In studies using preclinical models, checkpoint blockade of ADAR1-knockout or ADAR1-inhibitor tumors showed accelerated tumor regression and clonal expansion of $CD8^+$ T-cells compared to monotherapy, which provides a strong rationale for the combination of TE activation, ADAR1 antagonism and checkpoint blockade as a multi-pronged therapeutic strategy [14,60,63].

7.5 CRISPR-based epigenome editing of TE loci

CRISPR-based epigenome editing is the most precise approach to target individual TE loci. dCas9-SunTag, dCas9-KRAB, and dCas9-DNMT3A fusions have been used to deposit H3K9me3 or methylate CPGs at oncogenic ERV-derived enhancers, re-establishing H3K9me3-mediated silencing specifically at cancer-associated LTRs without affecting transcription of neighboring genes or other TEs [12,39]. Systems like CRISPRoff and other epigenome editing systems can restore heritable repression across TEs. Single-component systems can methylate thousands of CpGs at a locus in a scalable manner, and can be used to re-impose silencing on onco-exaptation events that underlie oncogene expression in many malignancies [12,15]. Alternatively, CRISPRa dCas9-SAM or dCas9-VPR systems can be used to reactivate the immunogenic TE promoters in engineered, tumor antigen-releasing cancer cells to render them sensitive to checkpoint blockade [13,38]. Recent long read chromatin-conformation capture studies also found that LINE-1 transcripts could nucleate oncogenic chromatin structure, which could reverse using dCas9-based chromatin remodeling on the L1 transcript loci, suggesting an additional potential druggable application of CRISPR-based epigenome editing [8,12].

Therapeutic strategies targeting the dysregulation of TEs in cancer include epigenetic drugs (DNMT, HDAC, BET, SETDB1 inhibitors), ADAR1 inhibitors, combinatorial cancer immunotherapy, and CRISPR-based epigenome editing (Table 1).

Table 1. Therapeutic strategies targeting transposable elements in Cancer and tumor microenvironment

Therapeutic strategy	Agents/targets	Mechanism of action on transposable elements	Immune / tumor microenvironment outcome	Clinical/preclinical impact
DNMT inhibition (hypomethylating agents)	5-Azacitidine, Decitabine [14]	Traps DNMT1/3A/3B at replication forks lacking 5-methylcytosine, leading to progressive passive loss of DNA methylation at TE loci during S-phase [4]	Reactivation of young ERVs and LINE-1 elements induces cytosolic dsRNA sensing via MDA5/MAVS, triggering type I interferon signaling and upregulation of MHC-I, CXCL10, and CCL5 [13]	FDA-approved in MDS/AML; active Phase I/II trials in combination with immune checkpoint blockade in NSCLC, melanoma, and CRC [8]
HDAC inhibition	Vorinostat, Panobinostat, Tucidinostat [14]	Maintains histone hyperacetylation at TE-rich regions, preventing heterochromatin re-establishment and sustaining TE chromatin accessibility [9]	Accumulation of ERV/LINE-1 transcripts enhances viral mimicry and potentiates interferon signaling; synergizes with DNMT inhibitors in CAFs and TAMs [13]	Preclinical synergy with DNMT inhibitors; ongoing clinical evaluation as epigenetic sensitizers with immunotherapy [14]
BET bromodomain inhibition	JQ1, ABBV-744, ZEN-3694 [13]	Disrupts BRD4 binding at TE-associated	Restores type I IFN signaling and MHC-I expression;	Preclinical efficacy in CRPC xenografts; ongoing

		regulatory regions and interferes with BRD4–TRIM28–SETDB1 repressive complex [14]	enhances $CD8^+$ T-cell infiltration, notably in therapy-resistant prostate cancer models [13]	combination trials with immune checkpoint inhibitors [8]
SETDB1 / SUV39H1 inhibition	(R)-PFI-90, SETDB1/SUV39H1 modulators [40]	Reduces H3K9me3 deposition at L1, HERV, and SVA elements, resulting in loss of heterochromatin-mediated TE silencing [38]	Reactivation of MDA5/MAVS signaling restores interferon responses, enhances antigen presentation, and suppresses leukemic clonogenicity [42]	Preclinical efficacy in NSCLC, melanoma, and glioma; context-dependent effects in hematologic malignancies [40,41]
ADAR1 inhibition	ADAR1 knockout, inducible depletion, small-molecule inhibitors [60]	Prevents A-to-I RNA editing of TE-derived dsRNA (Alu/LINE-1), preserving immunostimulatory RNA duplex structures [63]	Enhances MDA5/PKR activation, restores innate immune sensing, and reverses tumor immune evasion in viral mimicry-resistant cancers [14]	Strong preclinical synergy with immune checkpoint blockade; prevents immune exhaustion in AML and CTCL models [60]
Combination immunotherapy (TE activation + ICB)	DNMTi + anti-PD-1 / anti-CTLA-4 / anti-LAG-3; ADAR1 inhibition + anti-PD-1 [13]	Sequential TE de-repression followed by dsRNA-driven interferon amplification; blockade of ADAR1-mediated RNA immune escape [14]	Converts immunologically "cold" tumors into "hot" TME with increased $CD8^+$ T-cell infiltration, reduced exhaustion, and enhanced antigen presentation [60]	Multiple Phase I/II trials ongoing; strong tumor regression observed in melanoma and colorectal cancer models [13]
CRISPR-based epigenome editing of TE loci	dCas9-KRAB, CRISPRoff (dCas9-DNMT3A), dCas9-SunTag-SETDB1 [39]	Locus-specific re-establishment of DNA methylation or H3K9me3 at oncogenic TE-derived enhancers (LTR/ERV-driven regulatory elements) [15]	Selective silencing of oncogenic TE exaptation events (e.g., MACROD2, RASA3 enhancers) without global immune activation or toxicity [12]	Preclinical proof-of-concept in CRC and breast cancer organoids; emerging TE-based cancer vaccine strategies [14]

8. Emerging technologies and future perspectives

8.1 Single-cell epigenomics of transposon activity in TME

Integrative pan-cancer single-cell epigenomic analyzes further show that TE accessibility landscapes can stratify tumor/stroma/immune compartments, for example, decondensed chromatin over L1 and HERV enhancers in tumor-proximal cancer-associated fibroblast subpopulations [7,36]. Single-cell ATAC-seq, scBS-seq, and snmC-seq atlases reveal TE-on versus TE-off cell states on tumor evolution trajectories and the quantitative rather than binary nature of TE silencing disruption [4,5].

8.2 Spatial transcriptomics for TE mapping in tumor niches

Spatial transcriptomics platforms such as 10x Visium, Slide-seq and Stereo-seq are generating the first spatially-resolved TE expression maps of tumor niches. HERV and L1 transcripts are now being mapped to tumor microenvironments such as the intrusive front, hypoxic cores, and tertiary lymphoid structures, revealing spatial heterogeneity of TE reactivation. In comparison, tumor-core cells exhibit ADAR1-dominated immune evasion programs [13,21], transforming TE biology from merely a single-cell phenomenon to a spatially organized feature of tumor development and directly linking TE reactivation to immune geography [7,15].

8.3 Multi-omics integration: Epigenome-transcriptome-immunome axis

Multi-omic platforms allow the simultaneous capture of chromatin accessibility, methylation, transcriptomes and surface immunophenotypes to dissect the TE biology in Cancer [5,21]. Integrative workflows highlighted that the

enhancer activity of HERV elements correlates with the upregulation of TGFB1 and CXCL12 in CAFs, while single-cell multi-omic profiling revealed rare PIWI/piRNA-positive leukocyte and stroma subsets with altered L1 chromatin landscapes [6,36]. These observations imply that TE reactivation rewires the epigenome-transcriptome-immunome axis in these tissues, showing that these layers are not independently regulated [4,7].

8.4 Transposons as biomarkers for prognosis and treatment response

TE-derived n.a. sequences are promising as non-intrusive cancer biomarkers. Pan-cancer sequencing identified hundreds of tumor-type specific 3'-transduction sequences that act as personalized barcodes, with potential to detect minimal residual disease in liquid biopsies [3,19]. For example, HERV-H expression can stratify pluripotent states and the hypomethylation of L1 promoter exposed by ctDNA reflects the tumor burden and treatment response in colorectal, breast, and head and neck cancers [2,25]. Composite TE-abundance, methylation, and EV-packaged RNA quantification values of clinical samples are emerging as integrated risk and treatment response scores for hypomethylating-agent therapies, immune-checkpoint inhibitors, and immunotherapies [24,44].

8.5 Future therapeutic landscape: Precision epigenetic reprogramming

Next-generation TE-targeted therapies could thus utilize more targeted approaches, including CRISPRoff-based targeting of cancer-specific onco-exaptation events at LTR-driven oncogene promoters, dCas9-KRAB-based re-establishment of H3K9me3 at HERV loci derepressed in cancer, and inhibitor combinations targeting the SETDB1/ADAR1 pathway to selectively restore H3K9me3-mediated silencing at loci without inducing massive HERV reactivation. [14,38,40,60] Long-read identification of L1 transcript-driven chromatin-architectural drivers provides another targetable class, and transposon-derived cancer vaccines and TE antigen-checkpoint-combination therapeutic approaches are advancing closer to the clinic [9,12,64]. Collectively, these approaches portend a future in which transposons will be clinically actionable, spatially resolved, and therapeutically reprogrammable features of the cancer epigenome [13,15].

9. CONCLUSION

Activity of TEs has been increasingly implicated in the etiology of cancer, both in genome-wide instability and in power over signaling pathways. While silenced in normal cells through regulated epigenetic repression, dysfunctional repression is characteristic of most cancers, with de-repression being common. TE reactivation also eases tumor evolution through TE-dependent processes, including genomic and structural instability, and gene expression deregulation via TE sequences. TEs also impact tumor-immune interactions by stimulating the natural immune response via nucleic acid production, which can in turn drive a concerted type I interferon response. Although these factors may improve the immunogenicity of tumors, prolonged expression may be followed by the emergence of adaptive resistance mechanisms that promote immune escape and continued tumor growth, suggesting that TEs can serve as context-dependent tumor suppressor or promoter factors. Thus, targeting TE-associated epigenetic pathways is a promising approach to restore TE silencing and genomic stability, or to reactivate TEs to improve anti-tumor immunity in combination with immunotherapy.

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