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Reactivation of Endogenous Retroelements in Cancer Development and Therapy

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Keywords

viral mimicry, retrotransposons, repetitive elements, onco-exaptation, immunotherapy

Abstract

Domesticated retroelements contribute extensively as regulatory elements within host gene networks. Upon germline integration, retroelement mobilization is restricted through epigenetic silencing, mutational degradation, and innate immune defenses described as the viral mimicry response. Recent discoveries reveal how early events in tumorigenesis reactivate retroelements to facilitate onco-exaptation, replication stress, retrotransposition, mitotic errors, and sterile inflammation, which collectively disrupt genome integrity. The characterization of altered epigenetic homeostasis at retroelements in cancer cells also reveals new epigenetic targets whose inactivation can bolster responses to cancer therapies. Recent discoveries reviewed here frame reactivated retroelements as both drivers of tumorigenesis and therapy responses, where their reactivation by emerging epigenetic therapies can potentiate immune checkpoint blockade, cancer vaccines, and other standard therapies.

INTRODUCTION

Current models of the molecular genetics that underlie cancer development and therapeutic response are predominantly based upon characterizations of the 1–2% of protein-coding DNA sequences in the human genome and the interactions of their protein products. In contrast, contributions of repetitive DNA sequences that comprise nearly half of the human genome (Lander et al. 2001) remain poorly understood and largely unaccounted for within these models. Improved sequencing and mapping capabilities have facilitated increased understanding and consideration of repetitive sequences within models of cancer development and therapy.

Potential links between repetitive sequences and cancer have been considered since Barbara McClintock's discovery of controlling elements that transpose and regulate overt phenotypes (McClintock 1950). Mechanisms of transposition later emerged following the observation that serologic tests against viral gag proteins of the avian leukosis virus yielded positive signals in uninfected individuals (Dougherty & Di Stefano 1966, Dougherty et al. 1967). This suggested that RNA viruses could integrate into the host germline. The discovery of reverse transcriptase explained how RNA tumor viruses could endogenize as DNA proviruses within the host germline (Baltimore 1970, Temin & Mizutani 1970). It also explained the presence of the Rous sarcoma virus *src* viral oncogene (*v-src*) within genomes of uninfected chickens (Stehelin et al. 1976). Thus, concepts of mobile endogenous retroviruses (ERVs) that could be inherited through the germline emerged.

Since these early characterizations of ERVs, numerous classes of repeating DNA sequences have been discovered to comprise the majority of all eukaryotic genomes (Weiss 2006). Through mutagenesis and epigenetic silencing, host genomes domesticate and employ repetitive elements as regulators of host gene transcription and 3D genome organization. Cancers usurp domesticated repeats to deregulate transcriptional networks and promote genome instability, and their repression may establish therapy-resistant populations. Therefore, repeat silencing mechanisms reveal therapeutic strategies to acutely activate repeats to increase tumor immunogenicity and decrease cancer cell fitness (Ishak et al. 2018, Jones et al. 2019). Today, these repetitive sequences are the targets of multiple cancer immunotherapy strategies that include immune checkpoint blockade and cancer vaccines. This review expands upon recent investigations to identify emerging concepts and considerations that implicate repeat regulation in the development of malignancies and treatment responses in human cancers.

RETROELEMENT ENDOGENIZATION AND EXPANSION

Structural features and propagation mechanisms distinguish host domestication strategies of different repetitive elements. Interspersed repeats that expand through variations of transposition are alternatively termed transposable elements. Type II transposable elements mobilize through a DNA intermediate in a copy-neutral manner, while type I transposable elements undergo reverse transcription during duplicative retrotransposition and are termed retrotransposons (Boeke et al. 1985, Garfinkel et al. 1985, Kazazian & Moran 1998). Subclassifications of type I elements are based on the presence or absence of long terminal repeat (LTR) sequences (Mager & Stoye 2015).

The LTR class of retrotransposons encompasses families of ERVs that comprise ~8% of the human genome and derive in part from ancient retroviruses that infected germ cells or germ cell progenitors (Treangen & Salzberg 2012). Full-length ERVs possess LTRs that flank a 6–9-kb region of open reading frames (ORFs) that may encode Gag, Pro, Pol, and Env proteins to facilitate autonomous retrotransposition. Mutational degradation renders all ERVs in the human germline incapable of autonomous retrotransposition, and approximately 90% of all ERVs exist as solo LTRs missing all ORFs (Thompson et al. 2016).

Among non-LTR retrotransposon classes, long interspersed nuclear elements (LINEs) comprise ~21% of the human genome and largely reside as inactive fragments due to 5' end promoter truncations (Dombroski et al. 1991, Kazazian et al. 1988, Rogan et al. 1987, Treangen & Salzberg 2012). However, approximately 80–100 young LINEs that encode machinery within 2–3 ORFs to facilitate autonomous retrotransposition remain active in the human genome (Beck et al. 2011, Kazazian & Moran 1998, Levin & Moran 2011). Short interspersed nuclear elements (SINEs) comprise ~13% of the mammalian genome, are derived from transfer RNAs or 7S or 5S ribosomal RNAs, and contain 5' internal RNA polymerase III promoters. The nonautonomous SINE retrotransposons utilize LINE-encoded proteins for their retrotransposition. SINEs also contribute to SINE-VNTR-Alu retrotransposons that can contain LTR sequences (Slotkin & Martienssen 2007).

EPIGENETIC AND IMMUNE DEFENSES SILENCE REPETITIVE ELEMENTS

Domesticated repetitive elements are silenced early in development to prevent mobilization among other potential genomic insults. DNA methylation together with H3K9me3 and H4K20me3 at hypoacetylated histones characterize the constitutive heterochromatin that silences interspersed transposons and tandem repeats enriched at centromeric, pericentric, and telomeric chromosome ends (Allis & Jenuwein 2016).

Mechanisms of repetitive element heterochromatinization exhibit redundancy and compensatory potential, as exemplified by maintenance of transposon silencing during developmental periods of DNA hypomethylation (Rowe & Trono 2011). These trends are recapitulated in mouse embryonic stem cells with individual knockouts of any DNA methyltransferase (DNMT) or histone methyltransferase (HMT) that trimethylates either H3K9 or H4K20 (Bourc'his & Bestor 2004, Colum et al. 1998, Hata et al. 2002, Li et al. 1992). Maintenance of repeat silencing upon disruption of constitutive heterochromatin in embryonic stem cells (ESCs) is largely due to the compensatory effects of facultative heterochromatin characterized by H3K27me3 (Cooper et al. 2014, Karimi et al. 2011, Saksouk et al. 2014, Walter et al. 2016). The compensatory potential of repeat silencing in postmitotic mammalian tissues remains poorly understood and only characterized in a few select instances, such as murine splenocytes (Ishak et al. 2016). Elucidating epigenetic silencing in normal tissues is required to prospectively model and understand how cancers constitutively activate repeats.

Disruption of repetitive element silencing activates posttranscriptional defenses to restrict repeat activity. Host restriction factors target repetitive RNA for degradation or disrupt repeat RNA processing (Kassiotis & Stoye 2016). Transcripts derived from repetitive elements can assemble as double-stranded RNAs (dsRNAs) that stimulate interferon (IFN) and antiviral signaling cascades, collectively described as the viral mimicry response (Chiappinelli et al. 2015, Roulois et al. 2015). These cascades begin with the detection of dsRNAs by cytosolic RIG-I-like receptors or endosomal Toll-like receptors (TLRs) in a sequence-independent manner through associations with the RNA backbone and minor groove (Hur 2019). Identities of repeats that form immunogenic dsRNAs that stimulate RIG-I-like receptors or TLRs remain difficult to predict and are characterized in only a few select instances (Ahmad et al. 2018).

Upon dsRNA binding, RIG-I and MDA5 activate MAVS to promote phosphorylation and translocation of STAT and IRF transcription factors into the nucleus. Subsequent activation of IFN-stimulated genes (ISGs) increases tumor cell antigen presentation to increase visibility of cancer cells to cytolytic T cells and to induce immunogenic cell death, a process named viral mimicry (Roulois et al. 2015). Whether the subsequent IFN response is type I or type III appears

to be cell type specific (Chiappinelli et al. 2015, Roulois et al. 2015). Recent studies described below reveal that repetitive elements contribute extensively as regulatory elements to enable activation of viral mimicry responses against repeat RNA in cancer cells.

EXAPTATED REPETITIVE SEQUENCES REGULATE VIRAL MIMICRY RESPONSES

Repetitive elements evade complete negative selection from the host germline in part by providing utility toward host gene regulation. Replication errors and frequent substitutions at repeats underlie the evolutionary expansion and diversity of KZFPs (Krüppel-associated box domain-containing zinc finger proteins) required to establish repressive DNA methylation and H3K9me3 at repeats throughout the human genome (Jacobs et al. 2014, Najafabadi et al. 2015). Gradually, acquired mutations result in the accumulation of fragmented and dormant repetitive sequences that the host co-opts, or exapts, as novel genes or transcriptional regulatory elements.

Exapted repetitive sequences are an indispensable component of mammalian gene regulatory networks and are essential for proper mammalian placental development and embryogenesis (Blaise et al. 2003, Percharde et al. 2018). Beyond development, exapted repetitive elements are utilized to establish host immune cell lineages or regulate immune responses. Exaptation of a transposase from the *Transib* family of DNA transposons gave rise to the RAG1-RAG2 recombinase (Kapitonov & Jurka 2005). This domesticated enzyme utilizes arrays of V, D, and J gene segments in developing B and T lymphocytes to assemble the diverse repertoire of immunoglobulin and T cell receptor genes that are indispensable for host adaptive immunity (Zhang et al. 2019a). With respect to viral mimicry responses, exaptation of repeats as regulatory elements is more common than exaptation of ORFs.

Exaptation of repetitive elements is most frequently employed by co-opting regulatory sequences to regulate host genes. Intact ERVs are particularly favorable for exaptation since recombination between 5' and 3' LTRs removes internal ORFs but preserves a residual solo LTR to serve as a promoter or enhancer. LTRs account for up to 30% of all binding sites for certain transcription factors and confer tissue-specific control over developmental networks that are active within the mammalian placenta, the developing embryo, germ cells, and erythroid cells (Chuong et al. 2017).

An emerging body of literature increasingly implicates exapted LTRs in the regulation of host IFN pathways utilized in viral mimicry responses. Upon IFN- γ exposure, H3K27ac deposition permits IRF1 and STAT1 to bind LTR enhancers adjacent to ISGs in macrophages and cancer cells. Deletion of an upstream MER41A LTR reduces ISG expression, demonstrating that exapted LTRs are required for ISG induction (Chuong et al. 2016).

LTRs are also exapted at the 3' end of ISGs to enhance expression through feedforward loops. Approximately 15 ISGs with STAT1 motifs in their promoters contain ERVs embedded in the antisense orientation within their 3'UTRs, termed stimulated 3 prime antisense retroviral coding sequences (SPARCS) (Cañadas et al. 2018). Transfection of small-cell lung cancer (SCLC) cells with synthetic dsRNA or treatment with IFN- α , - β , or - γ promotes bidirectional transcription from both the ISG promoter and the 5' LTR of the antisense ERV. The resulting dsRNA amplifies the IFN response in a MAVS-dependent manner (Cañadas et al. 2018).

Exapted LTRs also facilitate long-range chromatin interactions to regulate the IFN response. In HeLa cells, MER41A and MER41B LTRs form extensive long-range interactions with ISG promoters characterized by STAT1 binding and open chromatin. Deletion of a MER41B LTR ~20 kb downstream of *IFI6* reduces IFN- γ -induced *IFI6* activation nearly twofold (Raviram et al. 2018).

It is tempting to speculate that a major purpose of LTR exaptation by the IFN pathway is to amplify dsRNA-induced IFN responses. However, ISGs are also utilized to establish immune cell lineages. T helper cell 2 (Th2) identity is maintained by silencing ISGs involved in establishing Th1 cell identity. These ISGs are most enriched for STAT1 binding motifs among other IRF and STAT transcription factor binding sites. Repression is established by SETDB1-dependent H3K9 trimethylation of ERVs, which serve as Th1 enhancers or as nucleation points for spreading H3K9me3 to silence proximal enhancers (Adoue et al. 2019). Collectively, these observations illustrate how exapted LTRs regulate viral mimicry responses and establish the integrity of immune cell identity.

PREMALIGNANT LESIONS ACTIVATE REPETITIVE ELEMENTS

Despite extensive epigenetic and immune defenses dedicated toward restricting repetitive elements, nearly every human cancer exhibits active repeat expression (Rodic et al. 2014, Rooney et al. 2015). Reports of LINE reactivation in premalignancy suggest that early retrotransposon activation may be a causal factor in cancer. Several types of premalignant lesions exhibit the activation of young, replication-competent LINEs. De novo LINE insertions have been identified in precancerous colonic adenomas (Ewing et al. 2015) that give rise to gastrointestinal cancers and in Barrett's esophagus (Doucet-O'Hare et al. 2015), which is a precursor of esophageal adenocarcinoma. Expression of the LINE ORF1 RNA binding protein is readily detectable in serous tubal intraepithelial carcinoma lesions (Pisanic et al. 2018) that give rise to high-grade serous ovarian cancers and in a subset of pancreatic intraepithelial neoplasias (Rodic et al. 2014) that give rise to pancreatic ductal adenocarcinomas. Notably, these studies report evidence of reverse-transcribed LINEs, suggesting that targeting reverse transcription may serve as a potential strategy for cancer interception. Extensive cancer-wide analysis of retrotransposon transcriptional activation in premalignancy remains unreported.

Reports of active LINEs in precancerous lesions suggest that retrotransposon reactivation is caused by the earliest events in tumorigenesis. These observations are resolved by the discovery that many cancer-initiating mutations target chromatin regulatory protein expression, function, or recruitment. Misregulation of repeats preceding onset of tumorigenesis has been prospectively modeled in mice deficient for DNMTs or HMTs. For example, mice hypomorphic for *Dnmt1* expression, the *Dnmt1*^{Chip/-} mouse, develop T cell lymphomas characterized by activation of repetitive elements and mouse mammary tumor virus integrations (Gaudet et al. 2003, Howard et al. 2008). Likewise, double-knockouts of the H3K9 methyltransferases Suv39h1/2 promote aberrant activation of repetitive elements in association with increased susceptibility to spontaneous B cell lymphoma development in mice (Peters et al. 2001).

Perhaps the most intriguing development linking repeat misregulation to early tumorigenesis is the discovery that functional inactivation of tumor suppressor proteins is associated with aberrant reactivation of repetitive sequences. Examples of such tumor suppressors include pRB (retinoblastoma protein) (Ishak et al. 2016), p53 (Wylie et al. 2015), VHL (Cherkasova et al. 2011), BRCA1 (Zhu et al. 2011), and ATRX (He et al. 2015). This likely explains how repeats become dysregulated in numerous premalignant lesions, although evidence remains largely derived from biochemical characterizations and mouse studies rather than direct characterization of human lesions. In some instances, tumor suppressors appear to promote silencing through recruitment of chromatin regulatory complexes that heterochromatinize repeats (He et al. 2015, Ishak et al. 2016, Zhu et al. 2011). However, the mechanisms by which tumor suppressors regulate repeat expression remain poorly understood, as is the broader question as to what role silencing of repeats plays in tumor suppression compared to the effect these tumor suppressors have on cell cycle control

and DNA damage response. We recently suggested that repeat silencing may represent an epigenetic checkpoint, where repeat-initiated viral mimicry responses result in culling of populations susceptible to perturbed epigenetic homeostasis (Ishak et al. 2018). In such a scenario, genetic inactivation of *TP53*, for example, would reactivate retrotransposons and initiate viral mimicry that would cull p53-deficient populations and preserve tissue homeostasis to prevent disease.

ABERRANT REPETITIVE ELEMENT ACTIVATION THREATENS GENOME INTEGRITY

Observations of repeat activation in premalignant lesions suggest that active repetitive elements may contribute to the acquisition of cancer hallmarks. Indeed, associations between activation of repetitive elements and genomic instability are well established (Belancio et al. 2010, Dombroski et al. 1991, Ionov et al. 1993, Kazazian et al. 1988, Miki et al. 1992, Strand et al. 1993). Due to the potential for mobilization, mechanistic links between repeats and genome instability have largely focused on L1 (LINE1) retrotransposition mapping in tumors (Lee et al. 2012, Rodic et al. 2015). It is now clear that the means by which repeats promote genome instability are multifaceted and differ depending upon cell cycle stage (Figure 1).

Repeats Facilitate Onco-Exaptation Post-G1 Phase

As the cell cycle proceeds beyond the G1 restriction point, coordinated programs of transcriptional activation commence to ensure proper DNA replication, chromosome segregation, and cell cycle exit (Bertoli et al. 2013). In cancer, aberrant transcriptional control of repetitive sequences during this period can misregulate host genes in a process described as onco-exaptation. Cancers reactivate repetitive sequences to serve as cryptic promoters to enhance oncogene expression or generate chimeric transcripts translated into fusion proteins with oncogenic properties, as observed for the *MET* oncogene in bladder cancers (Wolff et al. 2010). LTRs exapted by immune networks are subject to onco-exaptation. Examples of onco-exaptation in Hodgkin's lymphoma include the oncogenic *LTR-IRF5* chimeric fusion transcript (Babaian et al. 2015) or *cis*-activation of the *CSF1R* proto-oncogene by *MaLR* (mammalian apparent LTR retrotransposon) (Lamprecht et al. 2010). Likewise, *IL33* (interleukin 33) is targeted to generate an *LTR-IL33* fusion in human colorectal cancer (Lock et al. 2017).

Onco-exaptation is common across multiple cancer types. Analysis of TCGA (The Cancer Genome Atlas) RNA-seq data across 15 cancer types detects onco-exaptation events in every cancer type surveyed. Onco-exaptations are most enriched for LTRs; however, LINEs, SINEs, and DNA transposons are also exapted in cancer. Importantly, onco-exaptations recur across multiple cancers, with the *L1PA2-SYTI* chimeric transcript detected most frequently in ~10% of all tumors assessed. While many onco-exaptations occur within proximal promoters, a portion of recurrent onco-exaptations occur over long distances, in one case up to 57 kb upstream of the next exon. Acquisition of functional transcription factor binding motifs through somatic mutation contributes toward positive selection for onco-exaptation. In one instance, a recurrent *Alu7b-Lin28* onco-exaptation was translated into a functional fusion protein with oncogenic properties specifically in cancer cells (Jang et al. 2019). This work suggests that onco-exaptation occurs simultaneously with epigenetic remodeling during tumorigenesis. Further biochemical work is needed to validate functions of onco-exaptation protein products.

Repeats Promote Replication Stress and Transpose During S Phase

During S phase, repetitive RNA can promote replication stress through the formation of RNA:DNA hybrids (R-loops) that obstruct replication machinery progression to cause

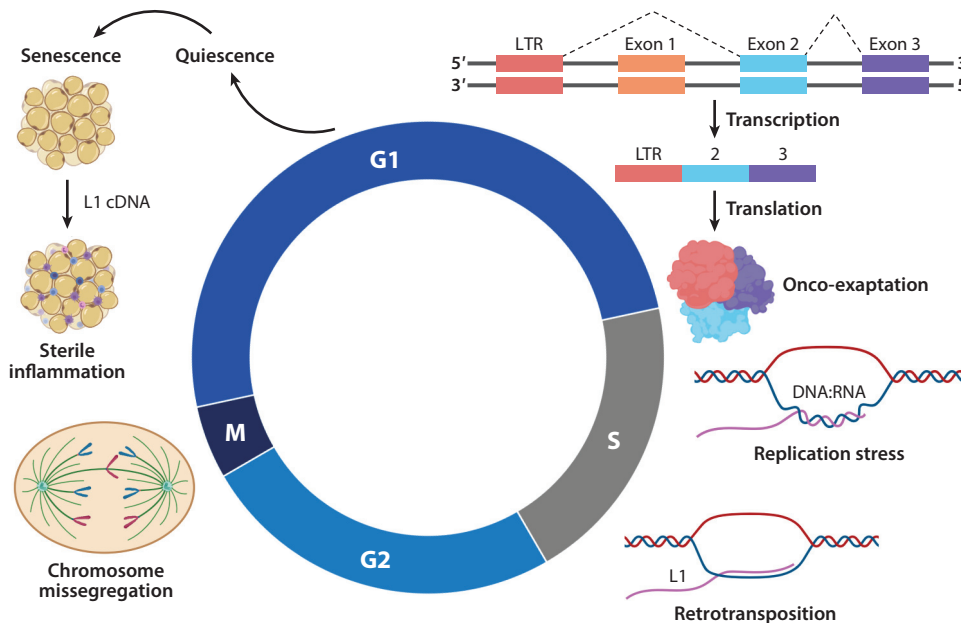


Figure 1

Repetitive element activation threatens genome integrity. (*Clockwise from top*) During the G1-S phase transition, increases in total transcription are initiated to commence DNA replication. During this period, onco-exapted repeats can serve as alternate regulatory elements to elevate oncogene expression or facilitate alternative splicing to form chimeric transcripts that are translated into proteins with oncogenic properties. As DNA replication progresses during S phase, activated satellite RNAs promote replication stress by displacing replication factors away from replication forks and forming DNA:RNA hybrids (R-loops) that impede fork progression. During S phase, expressed L1 elements incorporate into advancing replication forks to generate de novo integrations and increase copy number. Following DNA replication, elevated satellite RNA promotes chromosome missegregation, mitotic spindle defects, and chromosomal instability through mechanisms that remain poorly understood. As cells initiate permanent cell cycle arrest, elevated L1 cDNA in late senescence promotes sterile inflammation often observed in aging tissues. Collectively, the means by which repeats threaten genome integrity are multifaceted and differ depending on cell cycle phase. Figure adapted from image created with BioRender. Abbreviations: cDNA, complementary DNA; L1, long interspersed nuclear element 1; LTR, long terminal repeat.

replication fork collapse and dsDNA breaks (Aguilera & García-Muse 2012). In addition, repetitive satellite RNA commonly expressed in tumors (Ting et al. 2011) can directly bind and sequester replication factors away from replication forks. Direct targets of satellite RNA include prereplication complex subunits, such as MCM3, MCM4, and PCNA, and replication factors Ku70 and Lamin B1. Reduced fork association causes fork stalling and subsequently impaired DNA replication and restart. This results in the accumulation of satellite RNA:DNA hybrids that further impede fork progression and promote dsDNA breaks genome-wide (Zhu et al. 2018). Further work is required to determine whether repetitive RNA from LTRs or other retrotransposons can generate replication stress.

S phase also serves as a period of vulnerability during which LINEs can integrate throughout the genome. The 80–100 replication-competent young L1HS (L1 human-specific) LINEs in the human genome can utilize the L1 endonuclease and reverse transcriptase encoded by L1 ORF2 to integrate into DNA through target site–primed reverse transcription (Brouha et al. 2003). Screens using an engineered L1 reporter cross-referenced with OK-seq (Okazaki fragment sequencing) profiles in HeLa cells reveal a preference for L1 integration into advancing replication forks,

rather than replication origins or termination sites, independent of chromatin state (Flasch et al. 2019, Sultana et al. 2019). These reports reveal that DNA replication forks are major targets for repetitive sequences to establish replication stress or de novo L1 integrations.

Repeats Cause Mitotic Errors and Aneuploidy During M Phase

Upon entry into mitosis, proper chromosome condensation followed by proper segregation is required to maintain genome integrity. Missegregation establishes aneuploid cells, micronuclei, and chromosomal instability, which characterize an overall state of genome instability (Tasselli et al. 2016). Host machinery, such as condensin complexes, binds pericentric and centromeric satellite repeats to coordinate chromosome condensation and segregation (Hirano 2016). Increased satellite RNA expression compromises centromere structure and induces mitotic spindle defects and chromosome missegregation during mitosis (Coschi et al. 2014, Ishak et al. 2017). Mechanisms underlying how repetitive sequences promote mitotic errors remain poorly understood. However, rescue of mitotic errors upon knockdown of satellite transcripts in cancer cells (Tasselli et al. 2016) suggests that satellite RNA is indeed a causative agent of mitotic errors in cancer.

Repeats Promote Sterile Inflammation upon Cell Cycle Exit

Repetitive elements can also threaten genome integrity during cell cycle arrest. Disruption of heterochromatinization activates repetitive sequences in quiescent cells and postmitotic tissues (Ishak et al. 2016). Recent work demonstrates that even during cellular senescence, repetitive RNA can threaten genome integrity. Aging tissues undergo senescence-associated inflammation, or sterile inflammation, that increases susceptibility to age-associated diseases such as cancer. Recent work suggests that elevated retrotransposons are directly involved in establishing a later stage in senescence characterized by L1-induced chronic inflammation (De Cecco et al. 2019, Simon et al. 2019).

During late senescence, L1 elements become transcriptionally derepressed and stimulate a constitutive type I IFN response to establish and maintain the senescence-associated secretory phenotype (SASP) (De Cecco et al. 2019, Simon et al. 2019). Mechanistically, L1 accumulation coincides with reduced H3K9me3 and H3K27me3 due to reduced pRB occupancy of L1 5'UTRs. Loss of pRB occurs in tandem with L1 activation by the FOXA1 pioneer transcription factor and diminished TREX1 expression, resulting in reduced turnover of L1 complementary DNA. L1 elements activate type I IFN responses through stimulation of cytosolic DNA sensors to establish a mature SASP response and promote age-associated chronic inflammation in multiple tissues (De Cecco et al. 2019). Collectively, these reports emphasize that compromised genome integrity as a result of repetitive element activation is multifaceted and depends upon cell cycle phase.

REPETITIVE ELEMENTS ESTABLISH THERAPY-RESISTANT CANCER CELLS

Exapted repetitive elements regulate gene networks that maintain cell identity. HERV-H elements function as enhancers for pluripotency transcription factors to maintain human ESC identity (Lu et al. 2014) and establish a stem-like cellular state during cardiomyocyte development through the formation of topologically associating domains (Zhang et al. 2019b). Cancers exploit repetitive elements as mediators of cell state to establish intratumoral heterogeneity that contributes to therapy resistance and disease resurgence.

Repetitive elements maintain therapy-resistant SCLC populations. Chemotherapy promotes the transition of a subset of SCLC cells into a mesenchymal state characterized by reduced EZH2

expression. IFN- γ exposure of mesenchymal SCLC cells induces transcription of ISGs and antisense SPARCS within the 3'UTR of those same ISGs to generate dsRNAs. In normal cells, EZH2 silences SPARCS to prevent dsRNA formation. Reversion from the mesenchymal state upon *MAVS* deletion directly attributes epithelial-to-mesenchymal transition (EMT) to constitutive dsRNA signaling in SCLC. This EMT state underlies resistance to chemotherapy and immunotherapy (Cañadas et al. 2018). Direct characterization of DNA and histone modifications at SPARCS will provide further insight into their function in other contexts.

Lung cancer cells also establish therapy resistance through selective retention of silencing marks on retrotransposons. Treatment of *EGFR*-mutant non-small-cell lung cancer cells with erlotinib selects for drug-tolerant persister (DTP) cells characterized by H3K9me3 gains at centromeric and telomeric repeats, along with young primate-specific LINEs. Heterochromatin gains appear to be loci specific, as DTPs express other classes of repetitive elements. Disruption of heterochromatin at young LINEs decreases DTP cellular fitness in a partially RIG-I-dependent manner, suggesting that silencing of dsRNAs from young LINEs is required for DTP survival (Guler et al. 2017). These reports demonstrate that selective heterochromatin at retrotransposons maintains therapy-resistant populations of lung cancer cells. Whether repeats establish therapy resistance in other cancers requires direct investigation.

THERAPEUTIC EXPLOITATION OF REPETITIVE ELEMENTS THROUGH VIRAL MIMICRY

Tumors maintain constitutive expression of repetitive RNA and simultaneously evade innate immune responses against active repeats. Evasion from endogenous viral mimicry is likely achieved in part by increasing repeat RNA tolerance thresholds beyond those typical of somatic cells toward thresholds more commonly observed in ESCs during bursts of transposon activation (Ishak et al. 2018). Using drugs that perturb epigenetic silencing, acute derepression of repeats can surpass the tolerance thresholds to engage viral mimicry responses and increase cancer cell visibility to the host immune system. Viral mimicry induction has been most extensively characterized using DNA-hypomethylating agents and histone deacetylase (HDAC) inhibitors in cancer cells (Jones et al. 2019). Since our original discovery and characterization of viral mimicry induced by DNA-hypomethylating agents (Roulois et al. 2015), the repertoire of viral mimicry-inducing drugs has rapidly increased and now extends beyond direct epigenetic modulators (**Figure 2**). Further epigenetic characterization of repeats, biomarker discovery, and combination immunotherapies holds the potential to increase the efficacy of viral mimicry responses in cancer.

Leveraging Viral Mimicry Through Epigenetic Therapy

Current-generation DNMT inhibitors achieve 50% response rates as first-line therapies for myelodysplastic syndrome and acute myeloid leukemia (AML) (Loo Yau et al. 2019). Dividing cancer cells take up these agents more rapidly than postmitotic somatic cells, resulting in genome-wide DNA hypomethylation, resurrection of retrotransposons, and dsRNA-induced viral mimicry responses that promote antitumor cytolytic T cell activity (Chiappinelli et al. 2015, Roulois et al. 2015). However, current generation DNA-hypomethylating agents exhibit a short half-life due to rapid deamination. This short half-life may underlie poor efficacy in solid tumors as a monotherapy (Loo Yau et al. 2019).

Second-generation cytosine analogs such as guadecitabine (SGI-110) have been generated in an attempt to increase drug half-life and are in clinical phases of testing (Liu et al. 2018b). Additional approaches combine passive DNA demethylation through the use of

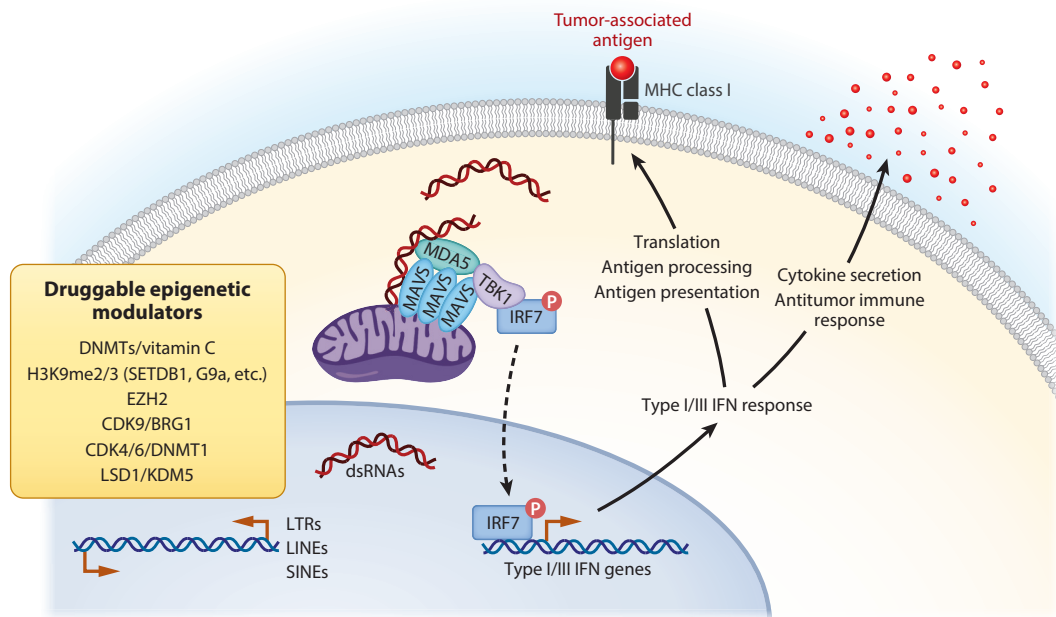


Figure 2

Viral mimicry induction in cancer. An expanding list of epigenetic targets can be inhibited by an increasing repertoire of small molecules. Inhibition perturbs repressive heterochromatin to increase accessibility and activation of repetitive elements that include families of LTRs, LINEs, and SINEs. Sense and antisense RNAs transcribed from retrotransposons assemble as dsRNAs that stimulate cytosolic dsRNA sensors. Activated dsRNA sensors, such as MDA-5, promote MAVS aggregation at the mitochondrial membrane to initiate phosphorylation and translocation of IRF and STAT transcription factors into the nucleus. Activated IRFs and STATs induce type I or type III IFN signaling in a cell-type-specific manner to culminate in the release of cytokines that promote antitumor immune responses and immunogenic cell death. In parallel, peptides translated from repeat-derived RNAs comprise the majority of tumor-associated antigens processed and presented to increase tumor immunogenicity upon induction of the viral mimicry response. Figure adapted from image created with BioRender. Abbreviations: DNMTs, DNA methyltransferases; dsRNA, double-stranded RNA; IFN, interferon; LINE, long interspersed nuclear element; LTR, long terminal repeat; SINE, short interspersed nuclear element.

DNA-hypomethylating agents with strategies to promote active DNA demethylation. Vitamin C serves as a cofactor for TET (ten-eleven translocation) DNA demethylases. When combined with the DNA-hypomethylating agent 5-aza-2'-deoxycytidine (5-aza-CdR), vitamin C can bolster dsRNA expression and efficacy of viral mimicry responses in cancer cell lines derived from breast carcinoma, hepatocellular carcinoma, and colorectal cancers (Liu et al. 2016).

Epigenetic therapies that induce viral mimicry are not limited to those that induce DNA hypomethylation. HDACs catalyze the removal of acetyl groups from histone tails resulting in compaction of chromatin (Taunton et al. 1996). In cancer cells, DNMT and HDAC inhibitors promote global increases in transcription from cryptic transcriptional start sites, of which 80% overlap with transposons predominantly comprised of LTR12C elements (Brocks et al. 2017). While both DNA-hypomethylating agents and HDAC inhibitors derepress LTRs, HDAC inhibition alone does not activate immune and viral defense genes induced by 5-aza-CdR (Brocks et al. 2017). Thus, while HDAC inhibitors exhibit some efficacy in cutaneous T cell lymphomas, clinical induction of viral mimicry through HDAC inhibition may be most effective if combined with DNMT inhibition (Jones et al. 2019).

More recently, histone demethylases that remove permissive histone marks have emerged as druggable targets for viral mimicry activation. LSD1 inhibition in cancer cell lines stabilizes

H3K4me1/2 to permit increased expression of ERVs that form dsRNA. The elevated levels of dsRNAs induced by LSD1 inhibitors stimulate MDA5 and TLR3 to activate IFN responses, increase T cell infiltration, and enhance efficacy of PD1 blockade in murine models of melanoma (Sheng et al. 2018). Inhibition of the KDM5 histone demethylase increases H3K4me3 levels and bolsters viral mimicry induction when combined with DNMT-hypomethylating agents in breast cancer cells (Leadem et al. 2018).

A decrease in repressive histone methylation can also promote dsRNA formation. Disruption of the H3K9 methyltransferase SETDB1 in THP-1 AML cancer cells diminishes H3K9me3, resulting in upregulation of LINEs, ERVs, and antiviral responses (Cuellar et al. 2017). Inhibition of another H3K9 methyltransferase, G9a/EHMT2, diminishes H3K9me2 within ERVs, resulting in a bolstered viral mimicry response in ovarian cancer cells (Liu et al. 2018a). Disruption of facultative heterochromatin may be especially appealing for viral mimicry induction since loss of DNA methylation can induce an epigenetic switch where the decreased DNA methylation is compensated for by increased repressive H3K27me3 (Gal-Yam et al. 2008), and it has been shown that EZH2 inhibitors promote dsRNA formation in SCLC cells (Cañadas et al. 2018). Identification of nonredundant retrotransposon silencing by H3K27me3 in somatic cells suggests that EZH2 inhibition may induce viral mimicry across a diverse spectrum of cancers (Ishak et al. 2016).

Leveraging Viral Mimicry Through Cyclin-Dependent Kinase Inhibition

In addition to a role for direct epigenetic modulators in the control of repeat element expression, recent studies have revealed an unexpected effect of cyclin-dependent kinase (CDK) inhibition on tumor cell antiviral signaling. CDKs regulate cell cycle progression or transcription (Otto & Sicinski 2017). CDK4/6 inhibitors were developed to inhibit G1 CDKs to promote cytostasis (Asghar et al. 2015). Recent investigations have suggested that the clinical efficacy of FDA (US Food and Drug Administration)-approved CDK4/6 inhibitors in estrogen receptor-positive breast cancer may be related to the activation of the dsRNA response in addition to cytostasis (O’Leary et al. 2016).

In human and murine mammary carcinomas, CDK4/6 inhibitors abemaciclib and palbociclib activate ERVs and a type III IFN response that can be attenuated upon DNMT1 overexpression. These effects occur in tandem with increased tumor cell antigen presentation that stimulates cytotoxic T cells. The authors attributed the enhanced immunogenicity induced by CDK4/6 inhibitors to pRB-dependent silencing of *DNMT1* and subsequent DNA hypomethylation of ERVs (Goel et al. 2017).

Further studies have also suggested that inhibition of a non-cell cycle CDK can also increase tumor immunogenicity. CDK9 is best characterized as a positive regulator of transcription through its ability to phosphorylate RNA polymerase II to facilitate transcriptional elongation. However, recent work has also identified a role for CDK9 as a negative regulator of transcription through its ability to phosphorylate BRG1 and promote its release from chromatin. CDK9 inhibition permits unmitigated chromatin remodeling by BRG1 that increases chromatin accessibility and ERV expression associated with increased antigen presentation and antitumor effects in syngeneic mouse models of ovarian cancer (Zhang et al. 2018). Roles of other CDKs in increasing tumor immunogenicity remain to be explored.

Leveraging Viral Mimicry Through Tumor-Associated Antigens

Viral mimicry responses increase processing and presentation of tumor-associated antigens (TAAs). Recent work suggests that the majority of these TAAs are likely derived from repetitive

elements (Laumont et al. 2018). Observations of antibodies against HERV-K *gag*- and *env*-derived peptides in the sera of cancer patients with seminomas, teratocarcinomas, testicular tumors, or lymphomas were reported over two decades ago (Boller et al. 1997). Repeat-derived TAAs harbor clinical potential because they promote antitumor immune responses. Indeed, following hematopoietic stem cell transplantation, the CT-RCC-1 TAA derived from a hypomethylated HERV-E element promotes cytotoxic T cell responses that underlie sustained tumor regression in patients with advanced metastatic renal cell carcinoma (Takahashi et al. 2008). Importantly, the CT-RCC-1 antigen is upregulated in response to inhibition of DNMTs or HDACs (Cherkasova et al. 2011).

Immunogenic retrotransposon-derived TAAs have since been identified across a diverse spectrum of cancers (Ishak et al. 2018). TAAs identified from *env* peptides of HERV-K, HERV-E, and HERV-H elements and *gag* peptides of HERV-K elements are now the targets of multiple immunotherapy strategies. In preclinical studies, chimeric antigen receptor (CAR) T cells exhibit efficacy against TAAs derived from HERV-K *env* peptides prevalent in melanomas and breast cancer (Krishnamurthy et al. 2015, Zhou et al. 2015). Beyond CAR T cells, vaccinations against retrotransposon-derived antigens produce no major adverse effects; thus, retrotransposon-derived TAAs are being tested as cancer vaccines (Kraus et al. 2013, Sacha et al. 2012).

Vaccinations against TAAs from HERV-E *env* or *gag* peptides restrict tumor growth in mouse models of renal cell carcinoma (Kraus et al. 2013, Kraus et al. 2014). Vaccination against a HERV-K *env* TAA restricts breast cancer cell growth in mice (Wang-Johanning et al. 2012). Potential development of vaccines against retrotransposon-derived TAAs in humans has been accelerated by improved proteogenomic pipelines for identification of TAAs from noncoding DNA (Laumont et al. 2016, 2018). Application of this pipeline to murine syngeneic tumor models yields TAAs predominantly from repeat-rich DNA. Dendritic cells primed against these TAAs confer immunity that underlies prolonged survival of mice challenged with T cell lymphoma cells.

Application of this proteogenomic pipeline to human primary tumors yields a repertoire of putative repeat-derived TAAs that require validation for immunogenicity (Laumont et al. 2018). Future work should explore junction sites of translated chimeric onco-exaptations as tumor-specific neoantigens for CAR T cell and cancer vaccines (Jang et al. 2019). Collectively, these studies demonstrate that repetitive elements serve as a rich source of TAAs that are more abundant and ubiquitous than neoantigens derived from the 1–2% of coding sequences in the genome. Thus, repetitive elements are positioned to serve as robust targets for multiple cancer immunotherapy strategies.

SUMMARY AND FUTURE DIRECTIONS

Some endogenized repetitive elements have evolved from genomic parasites into regulatory elements utilized to induce host immune responses against foreign pathogens (Chuong et al. 2017). This dichotomous host-parasite relationship extends to tumors, as repeats can promote genome instability and decrease cancer cell fitness through acute viral mimicry responses. As principles underlying these relationships emerge, so too do questions that should direct future studies regarding reactivation of retroelements in cancer.

There is a need to understand how repeats are silenced in cancer-cell-of-origin populations. These studies can reveal how epigenetic redundancy is thwarted to permit repeat activation in premalignant lesions and how active repeats promote acquisition of cancer hallmarks through mechanisms that may differ by cell cycle phase. This work can reveal therapeutic vulnerabilities of therapy-resistant cancer cells established by active and silenced repeats. Cancer-cell-of-origin

studies also hold the potential to uncover biomarkers for appropriate selection of effective epigenetic therapies to induce viral mimicry.

There is still much to learn about the underlying mechanisms of viral mimicry responses. For instance, it is not clear which repeats can engage pattern recognition receptors and whether these immunogenic repeats are conserved during evolution. Contexts in which cytosolic versus endosomal dsRNA receptors are engaged to induce viral mimicry require clarification. Therapeutic viral mimicry studies largely attribute ISG activation to stimulation of the MDA5-MAVS-IRF7 axis, despite a lack of published IRF7 cistromes detecting IRF7 at ISGs. Moreover, RIG-I-like receptors are not ubiquitously sufficient to restrict ERV activity in some TLR-deficient tissues (Yu et al. 2012). Determining contexts in which viral mimicry induction through TLRs cannot be compensated for through RIG-I-like receptors is essential for identifying when and how to apply viral mimicry as an anticancer strategy.

Induction of viral mimicry provides an opportunity to bolster the efficacy of emerging cancer immunotherapies. Epigenetic modulators or CDK4/6 inhibitors can upregulate immune checkpoints (Jin et al. 2019); therefore, combinations with anti-PD(L)1 blockade prove most effective (Stone et al. 2017). Beyond immune checkpoint blockade, repeat-derived TAAs and junction points of translated chimeric onco-exaptations induced with epigenetic therapies represent exciting future avenues for immunotherapy. Genotype associations with recurrent repeat-derived TAAs may accelerate current efforts to develop off-the-shelf tumor-infiltrating lymphocytes, CAR T cells, or cancer vaccines (Deniger et al. 2018, Malekzadeh et al. 2019).

In summary, once-parasitic sequences have been exapted to the extent that they are now indispensable for proper mammalian development. When temporal silencing mechanisms fail, the host employs viral mimicry responses by using exapted repeats in IFN signaling pathways. When these epigenetic and immune defenses are circumvented, repeats disrupt genome stability and establish subsets of therapy-resistant cancer cell populations. Understanding how repeats are epigenetically silenced reveals strategies to acutely bolster dsRNA levels to reactivate IFN responses and increase tumor cell visibility. Current efforts are now focused upon identifying and targeting repeat-derived TAAs that hold promise as abundant and robust cancer immunotherapy targets.

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