



Published in final edited form as:

Semin Cancer Biol. 2010 August ; 20(4): 200–210. doi:10.1016/j.semcancer.2010.06.001.

All y'all need to know 'bout retroelements in cancer

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Abstract

Genetic instability is one of the principal hallmarks and causative factors in cancer. Human transposable elements (TE) have been reported to cause human diseases, including several types of cancer through insertional mutagenesis of genes critical for preventing or driving malignant transformation. In addition to retrotransposition-associated mutagenesis, TEs have been found to contribute even more genomic rearrangements through non-allelic homologous recombination. TEs also have the potential to generate a wide range of mutations derivation of which is difficult to directly trace to mobile elements, including double-strand breaks that may trigger mutagenic genomic rearrangements. Genome-wide hypomethylation of TE promoters and significantly elevated TE expression in almost all human cancers often accompanied by the loss of critical DNA sensing and repair pathways suggests that the negative impact of mobile elements on genome stability should increase as human tumors evolve. The biological consequences of elevated retroelement expression, such as the rate of their amplification, in human cancers remain obscure, particularly, how this increase translates into disease-relevant mutations. This review is focused on the cellular mechanisms that control human TE-associated mutagenesis in cancer and summarizes the current understanding of TE contribution to genetic instability in human malignancies.

Keywords

retroelements; transposable elements; cancer; genetic instability; recombination

Introduction

Genetic instability is one of the key features associated with cancer causation and progression [1-5]. A variety of factors can contribute to this instability, including exposure to DNA-damaging mutagens, as well as heritable or somatic genetic defects in the DNA repair response. Mobile elements can contribute to the assortment of mutagenic events commonly identified in human cancers. Despite their massive influence on the evolution of mammalian genome (reviewed in [6]), and numerous sporadic examples of mobile elements contributing to cancer, the full impact of TEs on the transformation process has been difficult to determine.

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Human transposable elements include members of both DNA and RNA families of transposons, although there is no evidence of current activity of the DNA elements [7]. The RNA transposons, also referred to as retroelements, are further subdivided into long-terminal repeat (LTR)-containing or non-LTR groups (for the non-LTR group members see Figure 1; reviewed in [6,8]). Human endogenous retroviruses (HERVs) are the most well-known representatives of the LTR family in the human genome, but there is no documentation of their current activity. Non-LTR retrotransposons are the predominant source of TE-related mutagenesis in the human genome [reviewed in [8-10]]. This is in contrast to our rodent relatives who retain high levels of LTR-element activity [reviewed in [11]]. Endogenous retroviruses have been reported to influence tumor progression in mice [12,13] and *Xiphophorus* hybrids [14], however, no such claims have been made for human cancers.

The active, human non-LTR group includes Long Interspersed element-1 (LINE-1 or L1), short interspersed elements (SINE) represented by Alu, and the more recently characterized SVA elements [15-18] (Figure 1A). L1 elements have two open reading frames, ORF1 and ORF2, both of which are essential for L1 retrotransposition [19]. L1 ORF2 protein encodes endonuclease and reverse transcriptase enzymatic activities [20-22] that are responsible for host DNA nicking and L1 cDNA synthesis (see Figure 1B), respectively. While LINE-1 retrotransposons encode proteins necessary for their mobilization [19], Alu and SVA elements increase their copy number by parasitizing the L1 machinery [23,24]. The overall mobilization of retroelements declined significantly over the past tens of millions of years [7]. However, their extreme success in populating host genomes has allowed them to occupy about a third of the human genome resulting in a total of 500,000 copies of L1, 1,000,000 copies of Alu, and about 3,000 copies of SVA elements [7,15,17].

A. Transposable elements expression in normal and cancer cells

Early studies of L1 expression in mice put too strong an emphasis on primarily germ line expression of these elements [25,26]. These findings suggested that retroelement expression in normal human cells is completely suppressed by a variety of mechanisms, the number of which is still growing (see below), because of the potential harmful impact of these elements on genome stability. Most recent studies, however, report co-expression of L1 ORF1 and ORF2 proteins in human somatic cells, such as Leydig, Sertoli, and vascular endothelial cells [27], the presence of hypomethylated (*i.e.* likely transcriptionally active) L1 loci in normal human brain and skin [28], and various levels of L1 mRNA expression in a variety of human somatic tissues [29]. A low level of L1 RNA was detected in adult tissues of transgenic mice expressing an L1 element driven by its endogenous promoter and L1 retrotransposition during embryogenesis created somatic and germ line mosaicism in these animals [30]. Recovery and analysis of expressed sequence tags from immortalized human lymphoblastoid cell lines identified low expression of multiple L1 loci with noticeable variation among different individuals [31]. These observations support a concept that human somatic cells experience ongoing expression of endogenous L1 elements. Little is known about somatic expression of human SINE and SVA elements. However, similar to L1, Alu expression is strongly controlled by promoter methylation [32,33] that exhibits tissue specificity with significant hypomethylation reported in the male germ line [34]. It is, therefore, likely that human SINEs may also be expressed at low-levels in normal tissues.

In contrast to normal cells, the majority of human cancers, and cancer-derived cell lines, support variable, but typically much higher endogenous full-length L1 mRNA expression [35-38]. One of the major controlling mechanisms of this upregulation is the loss of promoter methylation [36,39,40]. Aberrant DNA methylation, which would be expected to increase L1 expression, is reported to occur very early in the step-wise transformation process [41-43]. Silencing of retrotransposons by methylation is at least in part controlled by the argonaute family of proteins

[44,45]. Changes in the L1 and Alu promoter methylation status between normal and cancer cells with the tendency toward hypomethylation even in the early stages of malignant transformation [41] have been extensively reported. Among the most informative are studies that assess methylation status and activity of retroelements in the same cellular environment [28] and studies that detail retroelement promoter methylation condition at different stages during cancer progression, such as progressive hypomethylation of the Alu promoter [46] or human-specific L1 loci [47]. Once the promoter suppression is released, production of retrotranspositionally competent L1 mRNA is further controlled by mRNA processing through extensive splicing and polyadenylation [48-50]. L1 expression may be additionally regulated by siRNA activity [51]. Elevated expression of Alu elements in human cancers has also been reported [33,52] and it can be further upregulated by stress and translational inhibitors [53, 54]. These observations, combined with the reported augmentation of mutation rates in the majority of human cancers, suggest that retroelement expression and presumably activity could be one of the forces driving genetic instability that may accelerate tumor evolution. Indeed, an involvement of L1-encoded ORF2 endonuclease in nuclear receptor-dependent tumor translocations that are associated with aggressive prostate cancer phenotype has been reported [55]. This observation establishes a direct connection between L1 endonuclease function and the progression of prostate cancer. Even though the association of retroelement expression with genetic instability in cancer remains largely correlative [56-58], it is serendipitous that human cancers vary drastically both in the levels of endogenous L1 expression and the amount and type of genomic instability [59,60].

B. Polymorphism of retroelement activity

In addition to the regulation of transposable element expression and insertion, variation in the combined damage resulting from TE activity in any given genome is influenced by the presence of polymorphic elements [61], as well as by variation in the retrotransposition activity of the same TE locus in different individuals [62,63]. For example, the potential for L1 activity is estimated to vary as much as 300-fold between individuals within a population [63]. No similar studies evaluating combined Alu activity have been reported. However, polymorphism of Alu elements in the human genome and the profound effect of cis-acting sequences within and adjacent to Alu on its ability to retrotranspose [64-67] strongly suggest that total Alu activity within any given genome is likely to fluctuate extensively.

An additional level of TE activity regulation arises from the recently discovered involvement of various cellular processes in the modulation of L1 and Alu retrotransposition. Examples of proteins involved in such regulation include members of the APOBEC family of proteins and Nucleotide Excision and Non-Homologous End Joining DNA repair pathways (NER and NHEJ) (for more detail see DNA repair pathways affecting TE activity section) [68-73].

Even though the number of the L1- and Alu-associated diseases including cancer, continues to grow, there is no clear understanding of the extent of the contribution of these elements to the origin and progression of the specific human cancers. Nevertheless, retroelements polymorphism, substantial variation in their activity in any given genome, and the diverse types of damage that they can cause (see below) raises a possibility for significant variation in the potential TE-associated contribution to cancer in the population.

C. Genetic instability associated with transposable elements in human cancers

Insertional mutagenesis

Human retroelements (L1, Alu, and SVA) amplify in the host genome by generating new integration events using an RNA intermediate, which also leaves the parental copy intact

(Figure 1B). This genome-wide, relatively random spread of transposable elements often inserts them in genomic regions with crucial biological functions resulting in insertional mutagenesis (a summary of TE insertion-mediated diseases is in [8] and relative retrotransposition is shown in Figure 2). Among the examples of human cancers caused by Alu- and L1-mediated insertional mutagenesis are disruptions of BRCA or APC genes leading to breast and colon cancers, respectively (Table 1). Occasionally, resolution of L1 integration intermediates causes deletion of genomic sequences [74-76] (Figure 1B) causing mutations that may not be easily attributed to L1 activity in the genome-wide sequencing analysis. Overall, TE-associated insertional mutagenesis can contribute to cancer via germ-line and somatic mutations, either of which can directly lead, or predispose the cells, to the onset of malignant transformation.

Despite the relative decline in mobilization activity over recent evolutionary times [7], human retroelement insertions are still responsible for approximately 0.3% of new human germ-line diseases, which translates into about one germ line retrotransposition event of Alu, L1, and SVA in 20, 100, and 900 births, respectively [77-79]. Currently there is no reliable estimation of the extent of TE-associated insertional mutagenesis in somatic cells or any specific cancer. Data obtained in tissue culture demonstrate that a number of cell lines originated from different types of cancer support retrotransposition of exogenous L1 and Alu elements with varying efficiency [70,80,81]. In general, it has been found that cells that repress apoptosis [80], or cells that have defects in certain DNA repair pathways [69,82], both of which are common changes in human cancers, support higher retrotransposition. Thus, although the endogenous activity of mobile elements in cancers has not been determined, combined with L1 and Alu overexpression detected in many tumor cells, there are many reasons to believe that the insertion rate of these elements could be much higher in tumors than in corresponding normal cells.

Recombination

Even those Alu and L1 elements that appear to have inserted 'harmlessly' have continuing opportunity to wreak havoc on the stability of the human genome. They do so predominately through their contributions to recombination, which is frequently detected in cancer [2]. The most obvious impact on genome stability is through their tendency to serve as sources of homology for non-allelic homologous recombination (NAHR) that preferentially involve Alu elements (Table 2). NAHR events have been well recognized as a major source of DNA damage (reviewed in [83]) that leads to either duplication or deletion of the sequences between the two participating Alu elements. Bioinformatics and reporter gene studies provide experimental evidence that the presence of repetitive sequences, such as Alu elements, within intronic regions of genes promotes 40 to 300 times more recombination-induced deletions in the mutant p53 background relative to the wild type genomic environment [84]. Moreover, it has been found that Alu elements in close proximity with inverted orientations are particularly prone to recombination [84,85]. In a reporter gene assay these inverted Alus are 10,000x more unstable in the absence of functional p53 than in the wild type genetic background [84] providing strong support for significant contribution of repetitive elements to genetic instability during tumorigenesis (Figure 2). In yeast, introduction of a single DSB promotes a boost in genomic instability via recombination events between inverted repetitive elements located as far as 21 kb from one another [86] and other studies show that inverted repeats are unstable even without DSBs [87]. Recombination between inverted Alu repeats frequently does not lead to a clean, homologous recombination event. Instead, it produces recombination events with break points occurring in the vicinity of the two Alu elements, apparently through resolution in a non-homologous manner. Thus, for any individual recombination outcome, it is impossible to determine whether the Alus located in the vicinity of the break point contributed to that particular genomic rearrangement event or not. A growing body of evidence suggests that both

direct and inverted repeats are considerable contributors to recombination-associated mutagenic events, such as loss-of-heterozygosity and other rearrangements identified during cancer progression, particularly in the presence of p53 mutations and probably many other mutations that contribute to genetic instability in cancer.

Numerous examples of Alu/Alu NAHR contributing to cancer have been reported. Table 2 lists a few specific cases of cancers where Alu/Alu NAHR recombination events have led to the same disease numerous times and illustrate a number of interesting points regarding Alu-promoted recombination in cancer. First, several genes that have a large number of Alu elements within their sequence seem to have a high proportion of genetic rearrangements involving Alu/Alu NAHR [88-90]. This phenomenon is specific to Alu repeats because the same formula does not apply to L1 elements. The discrepancy is presumably because of the depletion of L1 sequences in human genes relative to Alu elements that are enriched in human introns and because of the longer distance between adjacent L1 inserts within genes compared to the average proximity of neighboring Alus [7]. For example, 23 out of 29 reported recombination events in the BRCA1 gene, which has an unusually high density of Alu elements (41.5% of the gene) [89] involve Alu elements (reviewed in [88]). Additionally, although a number of instances of hereditary non-polyposis colorectal cancer (HNPCC) caused by Alu/Alu-induced rearrangements in the mutL homolog 1 (mlh1) gene that is relatively Alu-poor have been reported [90,91](Table 2), the majority of rearrangements in this gene are caused by non-homologous recombination that does not involve Alu elements. On the other hand, the majority of rearrangements of the more Alu-rich Mut-S-homolog-2 (msh2) gene, causing HNPCC, are due to Alu/Alu recombination events (Table 2). Two other cancer-promoting genes also have the majority of their rearrangements caused by Alu/Alu NAHR. These are the von Hippel-Lindau tumor suppressor (VHL) gene in the syndrome of the same name [92,93], and the human caspase-activated DNase gene, the key enzyme for nucleosome fragmentation during apoptosis, frequently harboring deletions in hepatoma [94]. Just for the 7 genes shown in Table 2 there have been over 120 different rearrangements reported that involve Alu/Alu recombination. This number far surpasses the number of total reported diseases caused by Alu insertions (reviewed in [8] and Figure 2), making it clear that in the human genome recombination events involving Alu have a much more profound impact on genomic instability than insertion of these elements.

A second important point is that Alu/Alu cancer-related recombination events occur not only in the germ line but also somatically, with some somatic rearrangements occurring on a regular basis. Among the above examples, the HNPCC and VHL mutations represent germ line defects in tumor suppressor genes that greatly increase the risk of those cancers in carriers. On the other hand, the hepatoma data [94] represent somatic recombination cases. The contribution of somatic Alu/Alu recombination events is also demonstrated by a recurring duplication of the MYB (an acronym derived from “myeloblastosis”) locus that can contribute to T-cell acute lymphoblastic leukemia, T-ALL [95], and partial tandem duplications of exons in the myeloid/lymphoid or mixed-lineage leukemia (MLL), a histone methyltransferase gene that contributes to acute myeloid leukemia AML [96]. In the case of MLL, the majority of AML cases that do not show any chromosomal aberrations have this duplication caused by Alu/Alu NAHR [96]. In all of these examples, there are generally multiple Alu elements contributing to these mutagenic events. Even though genes containing large numbers of Alus are more prone to genomic rearrangements via recombination, it is also emerging that some of the Alu elements have a higher propensity than others to participate in recombination events, possibly due to higher levels of sequence identity with nearby Alus.

Finally, one of the more remarkable findings regarding both the MYB and MLL duplication events is that very similar duplication events appear to be occurring at low levels in the blood of the majority, if not all, unaffected humans. This suggests that somatic cells are frequently

undergoing recombination events leading to copy number variations. It seems likely that the lack of disease in these apparently healthy individuals with the MYB or MLL1 duplications normally associated with cancer is either because the mutations are occurring at a late enough stage in the differentiation of the blood cells that prevent them from sufficient proliferation to manifest the disease, or possibly because there are more additional mutations that must occur in these cells before they can become transformed. This observation of somatic copy number variation associated with TE-induced recombination supports the hypothesis that transposable elements can be one of the factors contributing to the step-wise mutagenic process of malignant transformation.

DNA double-strand breaks (DSBs)

The finding that excess DNA double strand breaks (DSBs) are generated by expression of L1 ORF2 [68], opens the possibility that L1 contributes to genomic instability in human cancers in another major mechanism. This supposition is based on the observation that the estimated number of the L1-induced DSBs generated in HeLa cells is at least 10-100-fold higher than the number of the L1 retrotransposition events [68]. At this point, it is not known whether the large surplus of the observed DSBs is the result of abortive insertion events, or simply excess endonuclease expressed from the elements that gets free to attack the genome (Figure 1B).

Very little is known about the fate of the L1 endonuclease-induced DSBs. However, there is strong evidence of a correlation between L1 ORF2 expression in prostate cancer cells and formation of chromosomal translocations that lead to transcripts coding for fusion proteins [55]. Thus, it seems very likely that these elements represent a significant source of DSBs leading to genetic rearrangements in cancer. Because retroelements duplicate the ORF2-endonuclease cleavage site in their flanking regions [68,97], one hypothesized result of these L1-generated DSBs would be the prediction of increased Alu/Alu recombination. Additionally, the loss of check-point mechanisms preventing genome replication in the presence of unrepaired DNA damage that exist in normal cells allows cancer cells to be more successful in moving through the cell cycle even in the presence of DNA lesions some of which could be generated by L1 [98]. Thus, it is possible that the load of mutations associated with the L1-induced DSBs is significantly increased in cancer cells relative to their normal counterparts (Figure 2).

D. DNA repair pathways affecting TE activity

A critical step in the majority of malignant transformations involves defects in various DNA repair pathways that increase genetic instability, leading to more rapid tumor evolution. Thus, it is important to understand how these various genetic components interact with mobile elements expressed in tumors. An indication that cellular environment can fundamentally change L1's ability to retrotranspose originated from the report of efficient retrotransposition of L1 lacking endonuclease activity in chinese hamster ovary cells deficient in non-homologous end joining (NHEJ) repair [82]. NHEJ involvement in L1 retrotransposition is further supported by the recent report of diminished L1 retrotransposition in chicken cells defective in NHEJ repair [73]. Furthermore, cells deficient in ATM did not support efficient L1 integration suggesting that the cellular 'sensing' of the L1 insertion intermediates is important in the L1 life cycle [68].

While some NHEJ signaling appears to be required for successful L1 retrotransposition, another cellular DNA repair protein usually involved in nucleotide excision repair (NER) exhibits the opposite effect on L1 mobilization [69]. Cells deficient in functional ERCC1 support significantly higher L1 retrotransposition than cells that express wild type ERCC1 [69] strongly suggesting that this protein may provide protection against L1 retrotransposition-induced damage to host DNA. Based on the known ERCC1 function [99], together with XPF

it most likely acts on L1 intermediates by cleaving the single strand DNA branch structure or “flap” generated during L1 cDNA synthesis, thus, suppressing L1 retrotransposition (Figure 1B). Interestingly, Alu retrotransposition does not increase in the absence of ERCC1 (Roy-Engel AM., unpublished), even though Alu elements rely on L1 ORF2 protein for their mobilization [23]. This observation parallels previous reports of differential effect of APOBEC proteins on L1 and Alu retrotransposition [70-72] and the discrepancy in the timing required for the completion of Alu and L1 retrotransposition [100]. Because the involvement of DNA repair machinery in the retrotransposition process is only beginning to emerge, many specific details about the role they play in aiding or down-regulating L1, Alu, and SVA mobilization remain unidentified. Selective loss of specific DNA repair pathways in various human cancers may provide an additional level of variability in the TE contribution to genetic instability in a cancer type-specific manner. For example, newly discovered ERCC1 involvement in suppression of L1 retrotransposition becomes particularly relevant for the possibility of increased L1 contribution to genetic instability in human lung cancers that often lack functional ERCC1 [101]. Involvement of DNA repair in TE retrotransposition also suggests that polymorphisms within DNA repair genes may potentially contribute to variation in cancer evolution associated with TE activity.

E. TE sequences and gene expression: changes in the cancer transcriptome

The presence of mobile elements near genes or within the introns of human genes may cause a number of more subtle changes in gene expression, some of which appear to be specific to the transformed state (some major mechanisms are summarized in Figure 3). TE interference with gene expression is orientation dependent and varies relative to the size of the insert [102-104]. As expected, full-length L1 inserts are subject to negative selection because they appear to interfere with normal gene expression the most, particularly in the forward orientation [7,102,103]. They also introduce sense and anti-sense promoter encoded by the L1 5' untranslated region (UTR; Figure 3A) that can drive expression of adjacent genes both in normal and tumor cells [105,106]. Cancer-specific L1-driven hybrid transcripts were detected in two breast cancer and three colon cancer cell lines [107]. Much like L1, the internal pol-III promoter present in Alu elements can also transcribe adjacent sequences (Figure 3B) and is proposed to drive the expression of miRNAs [108]. Expression of some of the normally silenced by methylation miRNAs occurs in cancer cells with hypomethylated genomes [109, 110]. Given that cellular transformation has been shown to relax suppression of transcription of SINE RNA polymerase III promoters [33,52], it is plausible that Alu-driven miRNAs could be up-regulated in transformed cells. Although, the biological relevance of the composite L1- and Alu-driven transcripts in cancer causation or progression is unclear, these RNAs could represent transcriptional interference with cellular genes important for cancer progression.

It is well-established that TEs have played a significant part in reshaping the mammalian genome in the course of evolution (reviewed in [6]). A genome-wide survey of human genes determined that about one fourth of the analyzed human promoter regions contains TE-derived sequences within the 500 bp region upstream of its annotated initiation site [111]. The drift in Alu promoter sequence due to methylation and deamination of CpGs is also reported to generate p53-binding sites [112] suggesting a potential alteration in the transcriptional regulation of the genes harboring these events (Figure 3C). Perhaps this is the reason that Alu sequences are reported to be underrepresented in the first 1,000 bp of human promoters relative to introns, on the other hand, upstream regions beyond 1 kb appear to be enriched in Alus [113] and over 1000 of the human genes may be regulated by Alu-associated CpGs [114].

TE sequences, particularly L1 elements, contain internal polyadenylation (pA) signals, and splice donor or acceptor sites that can be utilized during transcription and as a result contribute to changes in gene expression and produce hybrid L1 transcripts [48-50,106,115,116]. Alu

elements are known to acquire these cis-regulatory signal post insertionally through accumulation of random mutations that introduce functional polyadenylation sites [117-119] or lead to Alu-exonization events [120-122] (Figure 3D). A comprehensive survey of disease-associated cryptic exons demonstrated that about two thirds of them contained TE sequences [122]. Evaluation of the human genome by an automated approach (SERpredict) that detects specific exonized retroelements predicted expression of several RNAs from genes with TE-derived tumor specific isoforms [123]. Recently SVA elements have been reported to provide functional splice sites to generated hybrid transcripts with human genes [124,125]. Although little is known about tumor specific TE-generated RNA or protein isoforms and even less about their potential role in tumorigenesis, there is a potential that some of them may affect cellular response to specific anti-cancer therapeutic agents. For example, a 98 bp Alu-like sequence in the Bcl-rambo beta gene acts as an exon creating a new protein variant that promotes etoposide- and taxol- induced death, which upon removal of the Alu-like sequence loses this function [126].

Because TEs are dispersed throughout many unprocessed transcripts, adjacent repetitive sequences present in the inverted orientation can form double strand RNA (dsRNA) in the nucleus. Double stranded pre-mRNA can be postranscriptionally modified by RNA editing enzymes. The diversity of RNA editing mechanisms includes nucleoside modifications such as cytosine to uridine (C to U) introduced by the APOBEC family of proteins and adenine to inosine (A to I) deaminations by the adenosine deaminase enzymes or ADARs. Inverted Alu sequences within transcripts are subjected to extensive editing by ADARs with a potential impact on the expression of the gene [127,128]. Loss of RNA editing appears to be common in tumors with reported global cancer-related hypo-editing of Alus in tissues from brain, prostate, lung, kidney and testis and with correlation between defects in ADAR activity and cancer progression [129]. Although there are links between RNA editing and cancer, the mechanism and biological consequences of the relationship between these processes remain unclear (reviewed in [130]).

F. Cancer treatment and transposable elements

TE-associated toxicity

Early activation of L1 and Alu elements during tumorigenesis and the reported ability of L1 expression to induce cell cycle arrest, apoptosis, and a senescence-like phenotype in cancer cells [68,80,131,132] could raise the possibility of a “*double-edged sword*” hypothesis associated with TE expression during cancer evolution (Figure 4). Increased DNA damage from up-regulated L1 expression in normal cells or during early stages of transformation may initiate the cascade of events leading to apoptosis or senescence, therefore, potentially preventing cellular progression to malignancy. On the other hand, if the cells have lost the ability to arrest cell cycle progression or apoptosis, L1-induced DNA damage is likely to be one of the factors contributing to genetic instability and potentially cancer evolution toward a more aggressive phenotype. This prospective dual role of L1-associated damage during transformation is supported by the findings that ectopic L1 expression in normal human cells leads to a senescence-like phenotype [29] and that L1-induced toxicity in breast cancer cells can be overcome by suppression of p53 function, Bcl2 expression or treatment with caspase inhibitor, which suppress apoptotic response in these cells [80,131,133]. These studies offer insights into the types of extreme cellular responses to L1 over-expression and provide the means to investigate L1 interaction with cellular environment. The caveat of these findings is that the reported outcomes occurred as a response to acute, transient expression of L1, leaving understanding of the cellular reaction(s) to chronic exposure to L1-induced damage largely unexplored.

TE regulation is not limited to genomic context and programmed responses. Their expression and activity can also be stimulated by cellular changes induced by exposures to external stimuli (reviewed in [134]. For example, Alu expression increases after heat shock, treatments with puromycin, cycloheximide [54], etoposide and cisplatin [135], exposure to UV radiation [135], and in cells infected with adenovirus type 2 [136]. However, an increase in Alu retrotransposition rates has only been suggested for the treatment with etoposide [137]. Similarly, L1 activity has been reported to increase after treatment with either benzo(a)pyrene (BaP) [138] or exposure to ionizing radiation [132]. In addition to these reports, exposures to a number of heavy metals such as nickel, cadmium and mercury increase L1 activity without changing steady-state mRNA levels, acting most likely through the inhibition of cellular factors that limit L1 insertion [139,140]. The TE response to external stimuli brings a question of the potential effects currently used cancer therapeutics may have on TE activity and TE-driven instability. As an example, external stimuli causing genetic damage (such as double strand breaks generated by chemotherapeutic agents) induce a p53 response [98]. Recent data indicate that p53 can activate L1 transcription through p53 binding sites that emerged within L1 promoter of young L1 subfamilies [141]. This p53-driven increase in L1 expression is likely to introduce more DSBs and amplify the p53-mediated DNA damage response (Figure 4). Our current knowledge predicts that many of these treatments may increase TE activity suggesting the possibility of the TE-induced instability contributing to the evolution of the therapy-resistant cancer phenotypes.

It is well-established that transcription of transposable elements is heavily suppressed by methylation and likely other epigenetic modifications [39,142]. One of the most dramatic examples of the loss of epigenetic control of retroelements was reported in the Dnmt3L knockout mouse that presented increased TE transcription due to the loss of *de novo* methylation of these elements [143]. Many cancer therapies include chromatin-modifying and methylation reducing agents such as 5-aza-2'-deoxycytidine (5-Aza-CdR) that inhibit DNA methylation and histone deacetylases (HDAC) [144-146]. The use of "epigenetic therapies" in cancer treatment is reported to have an impact on mobile element regulation [56,147] with potential synergistic and antagonistic results. For example, demethylation of Alu elements in response to 5-Aza-CdR and 4-phenylbutyric acid (PBA) treatment of gastric and bladder cancer cells has been associated with the induction of several miRNAs (Figure 3B) [109,110], one of which, *miR-512-5*, when expressed, suppresses the anti-apoptotic protein *Mcl-1* resulting in the death of the treated gastric cancer cells [110]. Other conventional agents routinely used in cancer therapies such as tamoxifen can also lead to progressive hypomethylation of L1 elements and their increased expression [148]. As epigenetic treatments are common in cancer management, current data on their effect on the regulation of TE expression strongly advocate that a more detailed insight into the potential reactivation of silent retrotransposons is needed in order to understand their role in cancer therapy outcomes.

E. Domesticated Transposable Elements and Cancer

Telomerase reverse transcriptase (TERT) has been proposed to be the product of a domesticated transposable element [149-151]. The reverse transcriptase domain of telomerase enzyme is a relative of the reverse transcriptase of the non-LTR elements, like L1 [152]. In addition, telomere formation uses an RNA encoded at one locus (TER) that combines with a reverse transcriptase (telomerase) made at a different genomic location. This is similar to other non-autonomous retroelements, like Alu, that encode their RNA at one location and steal the reverse transcriptase produced from another location. Much like, L1 and Alu, whose activity is upregulated in cancer cells, the majority of human cancers reactivate expression of telomerase in order to sustain uncontrolled proliferation. Additionally, in species like *Drosophila*, retrotransposons provide an evolutionarily robust non-telomerase mechanism to maintain telomeres (reviewed in [153]).

Humans have also domesticated transposable elements by design and have used them as tools in gene therapy and studies of carcinogenesis [154,155]. Of particular relevance have been a broad range of studies with a DNA transposon named sleeping beauty, that has been engineered to create insertional mutagenesis in mouse model systems to study cancer [156,157]. This type of tool is being used to create animal models of disease, including cancer, and also to utilize the transposon as a genetic tag to isolate the mutated genetic loci that contributed to the specific cancers [158-160].

The Promise of Next Generation Sequencing

The full level of the impact of mobile elements on human genome instability has been extremely difficult to measure, particularly in terms of somatic instability. One of the major difficulties is the presence of massive numbers of L1 and Alu elements in the genome that obscure most approaches to detect small differences in copy number or arrangement between genomes. Next generation sequencing holds tremendous promise for obtaining truly quantitative measures of the amount of insertional mutagenesis and NAHR caused by mobile elements in cancer. Even then, it may be difficult to assess rearrangements caused by recombination events triggered by inverted Alu elements or caused by L1 endonuclease-induced DSBs, as neither of these types of events can be clearly identified on an individual basis as having been induced by the mobile elements. Furthermore, even with next generation sequencing, many of the approaches, like paired-end sequencing, may be biased in detection of certain classes of mobile element-related damage. In addition, until the appropriate bioinformatic pipelines are developed for in depth analysis of sequencing data involving mobile elements, there are likely to be continued difficulties in examinations involving the mobile elements.

Summary

The biological changes associated with malignant transformation are likely to amplify the impact of transposable elements on the stability of the human genome and cancer progression. The rate and scope of TE contribution to the origin and progression of human cancers is poorly quantified to date, although we know a great deal about the types of insertion and recombination events that contribute to this process. However, the field of transposable elements is evolving with accelerated rate toward uncovering a multitude of novel effects of TEs on the stability of the host genome and interactions with various cellular pathways. It is also likely that a considerably better understanding of TE-associated contribution to mutagenesis in cancer will emerge in the near future with accumulation of data generated by the human cancer genome sequencing project. Though the difficulties associated with the analysis of repetitive sequences significantly hinder the progress in identifying specific instances of TE involvement in human cancer, a fast-growing body of studies, heavily focused on the cancer cases with unknown etiology, and genome-wide association studies strongly suggest that transposable elements could be a contributory factor in a number of human cancer with unidentified causation.

Acknowledgments

This publication was made possible by Grants Number P20RR020152 (PLD, VPB and AMR-E), R01GM45668 and EPSCOR/BORSF grant (PLD) and R01GM079709A (AMR-E) from the National Institutes of Health (NIH). VPB is supported by NIH/NIA 5K01AG030074 and The Ellison Medical Foundation New Scholar in Aging award, AG-NS-0447-08. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRN or NIH. Competitive Advantage Funds (2006) from the Louisiana Cancer Research Consortium (LCRC) were also awarded to AMR-E.

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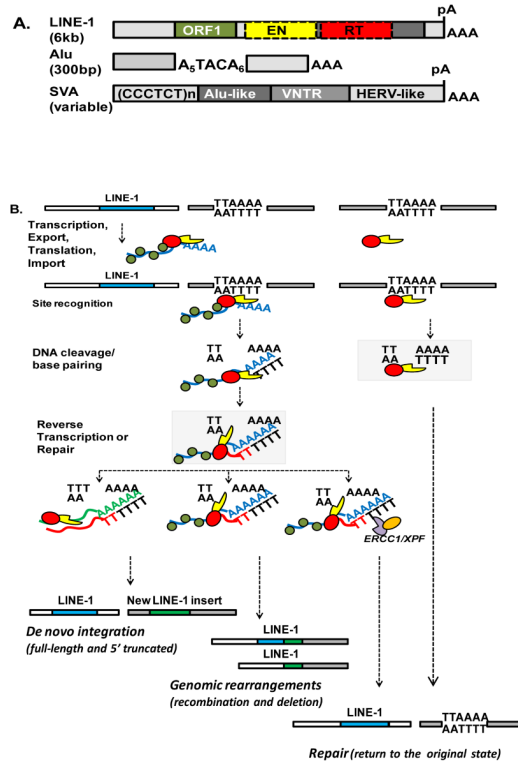


Figure 1.

A. Schematic of the genome organization of active human retroelements. LINE-1 consists of a 5' UTR, an inter ORF region, and 3'UTR (light grey boxes) that ends in a polyadenylation signal (pA) and a run of adenosines (AAA). L1 encodes two open reading frames, ORF1 (green box) and ORF2. ORF2 encodes a protein with endonuclease (EN; yellow box) and reverse transcriptase (RT; red box) activities.

The ~300 bp human SINE, Alu, is composed of two non-identical 7SL-derived monomers (light grey boxes) that are connected by an adenosine (A)-rich linker. The Alu element ends in a variable length stretch of adenosines (AAA). SVA elements consist of four distinct regions: CCCTCT repeats, an inverted Alu-like sequence, VNTR variable number of tandem repeats, and human endogenous retrovirus (HERV) -like sequence. The approximate length of each element is shown in parentheses.

B. L1 integration steps and outcomes. TTA AAAA is the consensus L1 endonuclease recognition site. A blue box represents a transcriptionally active and retrotranspositionally competent L1 locus in the human genome. The L1 RNP (ribonucleoprotein particle) is composed of the L1 mRNA (blue), L1 ORF1 protein (green) and L1 ORF2 protein that contains endonuclease (EN, yellow) and reverse transcriptase (RT, red) domains. The L1 RNP or L1 ORF2 recognizes an L1 target site in the human genome and generates a first DNA nick that at some point during retrotransposition becomes a DNA double strand break (DSB). The polyA tail of the L1 mRNA base pairs with the DNA at the nick that serves as a primer for TPRT (target primed reverse transcription) by L1 RT that generates a cDNA 'flap' (red). This intermediate can be resolved to generate *de novo* L1 integration event (green box), integration-associated genomic rearrangements, or it can be recognized and aborted by ERCC1/XPF complex, a component of the nucleotide excision repair machinery, leading to the restoration of the integration site.

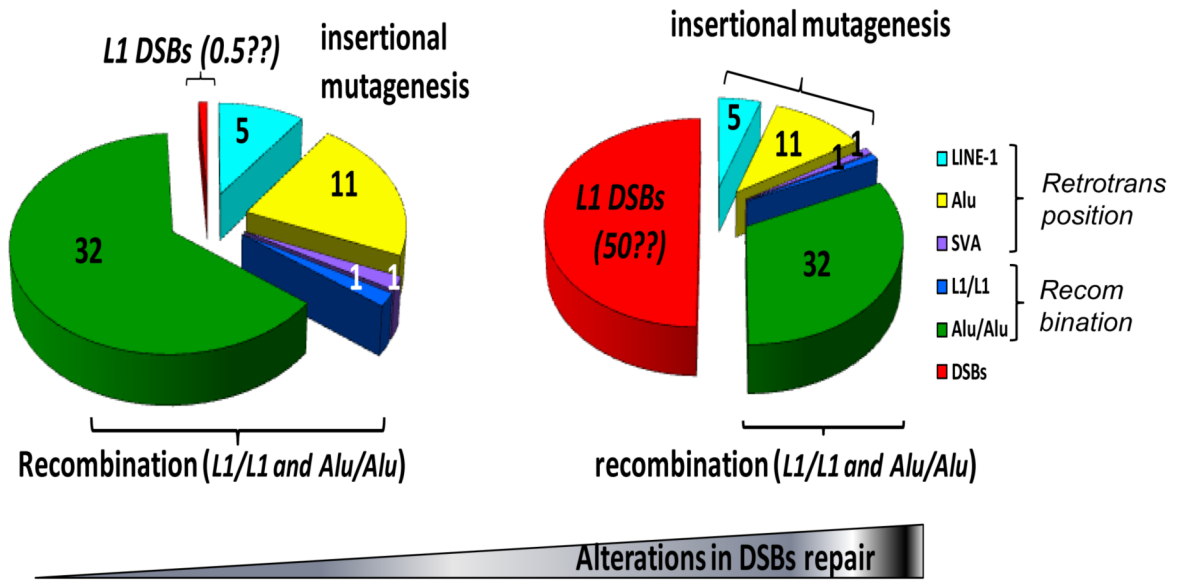


Figure 2. Estimated contribution of various types of retroelement-induced mutagenesis to genomic instability

The three main types of L1-, Alu-, and SVA-related mutagenesis such as insertional, recombination, and error-prone DSB repair are represented. The relative contribution of L1, Alu, and SVA (with SVA set at 1) to insertional mutagenesis (shown in light blue, yellow, and purple, respectively) is estimated based on the reported disease incidence (reviewed in Belancio et.al., 2008). The relative contribution of L1 and Alu to recombination (shown in dark blue and green, respectively) is estimated based on disease and recombination-associated deletions (Deininger and Batzer, 1999, Han K. et.al., 2007, Han K. et.al., 2008). The potential relative mutagenic contribution of the L1-induced DSBs (shown in red) is essentially unknown and example estimates of 0.5 and 50 relative to insertion events are presented for comparison.

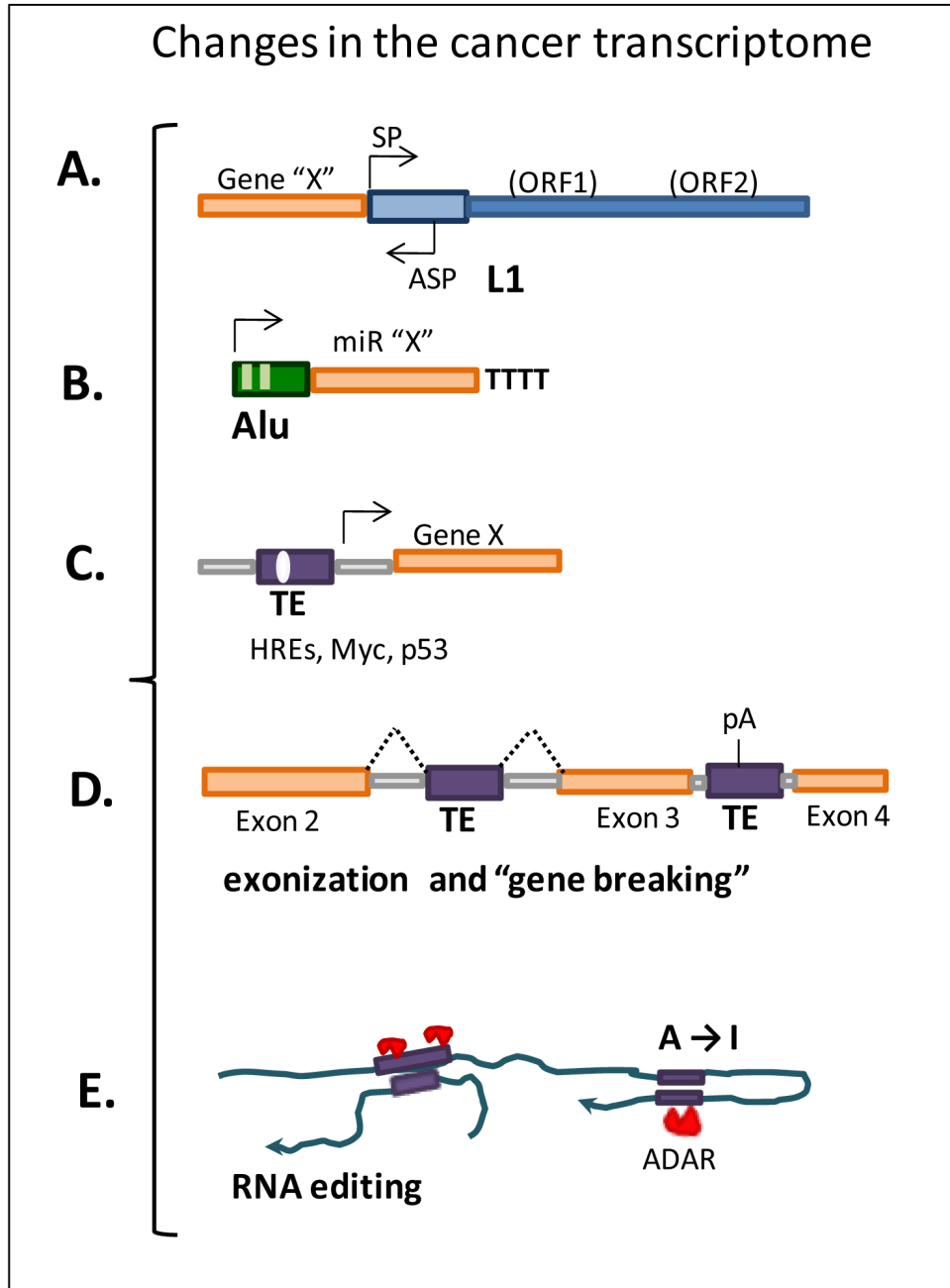


Figure 3. TE-derived changes in the cancer transcriptome

Gene expression can be affected by the presence of mobile element sequences near, or within, genes. The unmethylated status observed in cancer cells usually leads to the loss of transcriptional regulation of TE promoters affecting nearby genes as shown in **A**) where the antisense promoter (ASP) activity of a full-length L1 element (blue) can drive expression of genes immediately adjacent to these sequences (Nigumann et.al. 2002; Speek 2001) and **B**) by the expression of miRs from upstream pol III-containing Alu elements (Borchert et.al. 2006). **C**) TE-derived sequences upstream of genes can supply regulatory sequences such as p53 sites affecting gene expression (Zemojtel et al., 2009); or **D**) introduce splicing and polyadenylation signals changing transcriptional products leading to events such as exonization s that can alter

protein function (Yi et al., 2003). E) The presence of repeated sequences in opposite orientation within transcripts can form double stranded RNA (dsRNA) which is modified by RNA editing enzymes with a potential impact on expression of the gene.

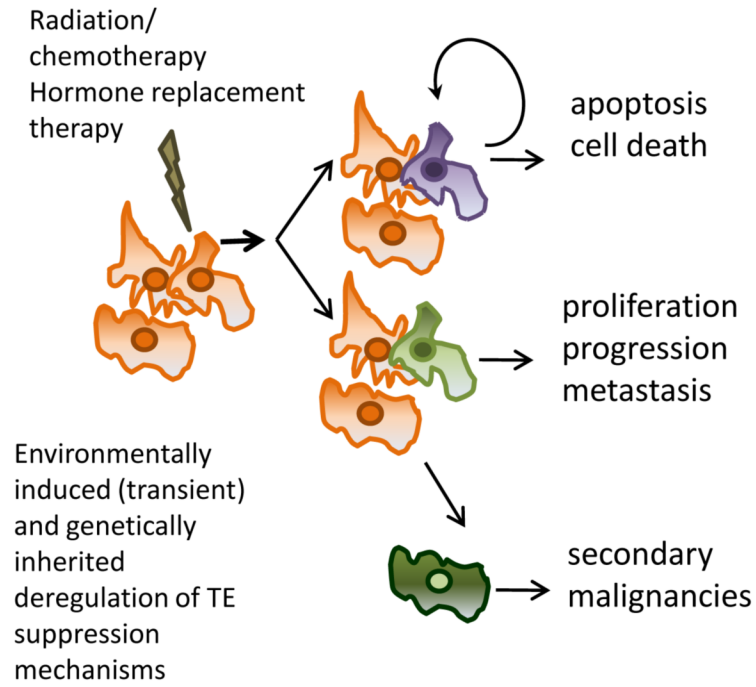


Figure 4. the “double-edge sword” hypothesis

Environmentally induced changes either due to transient exposures (e.g. demethylating agents) alone or in combination with genetic defects, such as DNA repair deficiencies in cancerous cells, can lead to a diminished regulation of mobile elements. The increase in activity and/or deregulated transcription of TE promoters can have an agonistic effect such as promoting apoptosis or alternatively have an antagonistic effect by inducing changes leading to chemotherapeutic resistance, proliferation or even the generation of secondary malignancies.

Table 1

CANCER INSERTIONS

RETROELEMENT	GENE	DISEASE	REFERENCE
Alu	CRB1	retinoblastoma	Den Hollander et al, 1999
	<u>3x</u> BRCA2	breast cancer	Miki et al, 1996; Teugels et al, 2005; Machado et al, 2007
	BRCA1	breast cancer	Teugels et al, 2005
	NF1	Neurofibromatosis	Wallace et al, 1991
L1	APC	Colon cancer	Miki et al, 1992
	MYC	Breast cancer	Morse et al, 1988

Table 2

Alu/Alu RECOMBINATION

GENE	DISEASE	MUTATION ORIGIN	REFERENCE
<u>10x</u> MLH1	HNPCC	Germline	Reviewed in Viel et al, 2002; Aissi-Ben Moussa et al, 2009; Li et al, 2006; van der Klift et al, 2005; Wang et al, 2003
<u>32x</u> MSH2	HNPCC	Germline	Li et al, 2006; Akrami et al, 2005; van der Klift et al, 2005; Apessos et al, 2005; Wang et al, 2003; Charbonnier et al, 2005
<u>30x</u> VHL	VHL	Germline	Franke et al, 2009; Casarin et al 2006
<u>15x</u> hCAD	hepatoma	Somatic	Hsieh et al, 2005
<u>5x</u> MYB	T-All	Somatic and normal	O'Neil et al, 2007
<u>7x</u> MLL1	AML	Somatic and normal	Strout et al, 1998
<u>23x</u> BRCA1	Breast cancer	Germline	Reviewed in Mazoyer S., 2005

TABLE 3

Alterations in gene expression by TE sequences.

Insertion	Examples of the type of impact
5' UTR, promoter region	Sources of new methylation sites (CpGs)
	Alternate promoter sequences (Speek, 2001; Matlik et al., 2006)
	Introduction of potential regulatory sequences (Thomson et al., 2009) and potential response elements (Norris et al., 1995)
	Altered expression of nearby genes (Nigumann et al., 2002; Laperriere et al., 2007)
Introns	Alternative splicing/ exonization (Sorek et al., 2002; Lev-Maor et al., 2008; Lin et al., 2009)
	Introduction of polyadenylation signals and splice sites (Perepelitsa-Belancio and Deininger, 2003; Han et al., 2004; Wheelan et al., 2005; Belancio et al., 2006; Belancio et al., 2008b)
	RNA editing (Kim et al., 2004)
	Introduction of promoters: expression of miRs (Bochert et al., 2006; Saito et al., 2009)
3' UTR	Regulation of nuclear export of mRNAs (Chen et al., 2008; Chen and Carmichael, 2009)
Exons	Disruption of genes (see Table 1 and reviewed in Belancio et al., 2008a)