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## **Mini review: Transposable Elements in Cancer and other Human Diseases**

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**Abstract:** Transposable elements (TEs) are mobile DNA sequences representing a substantial fraction of most genomes. Through the creation of new genes and functions, TEs are important elements of genome plasticity and evolution. However TE insertion in human genomes may be the cause of genetic dysfunction and alteration of gene expression contributing to cancer and other human diseases. Besides the chromosome rearrangements induced by TE repeats, this mini-review shows how gene expression may be altered following TE insertion, for example by the creation of new polyadenylation sites, by the creation of new exons (exonization), by exon skipping and by other modification of alternative splicing, and also by the alteration of regulatory sequences. Through the correlation between TE mobility and the methylation status of DNA, the importance of chromatin regulation is evident in several diseases. Finally this overview ends with a brief presentation of the use of TEs as biotechnology tools for insertional mutagenesis screening and gene therapy with DNA transposons.

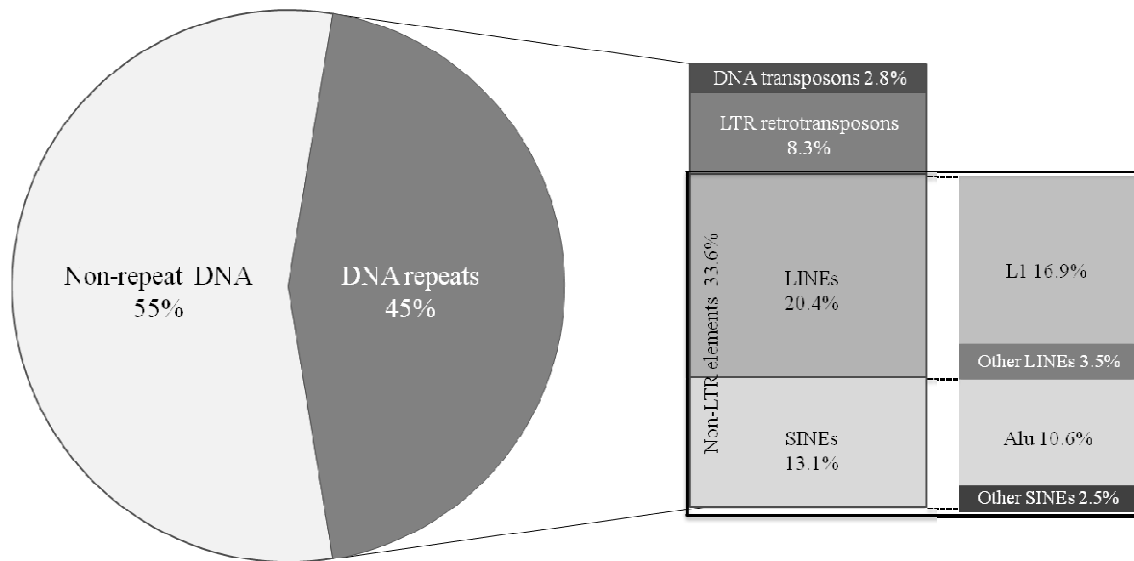
**Keywords:** alternative splicing; gene expression; gene therapy; human disease; promoter; recombination; retrotransposon; transposon

## INTRODUCTION

Transposable elements (TEs) are mobile DNA sequences representing a substantial fraction of most genomes (*e.g.* up to 85% of the maize genome) and at least 45% of the human genome according to the most common data [1-3] and even up to 66% according to the analysis by de Koning et al. [4]. TEs are diverse, and two main classes are identified on the base of their mechanism of displacement. Class-I elements, also called retroelements or retrotransposons, use a reverse transcriptase enzyme to copy an RNA transcript into the host DNA, whereas class-II elements, or DNA transposons, mainly move through a “cut and paste” mechanism involving the excision and reinsertion of the DNA sequence of the element [1-3]. Besides, some other mechanisms of transposition exist that, to the best of our knowledge, are not involved in the mobility of human TEs [1-3, 5]. Both the classes are further subdivided into super-families and then into families on the basis of the transposition mechanism, sequence similarities and/or structural relationships [5]. In addition, a large number of non-autonomous TEs are unable to remove and insert themselves elsewhere in the genome and are dependent of the enzyme(s) encoded by a helper element, which is an autonomous TE. Nevertheless, such non-autonomous TEs have greatly invaded genomes, especially short interspersed nuclear elements (SINEs) that are highly present in mammalian genomes [4].

Class-I elements present in the human genome are divided into Long Terminal Repeats (LTR) and non-LTR elements, the latter being largely predominant (**Fig. 1**) and account for 76% of human TEs and at least 33.6% of the whole human genome [6]. The long interspersed element (LINE)-1 accounts alone for 38% of the human TEs and 16.9% of the entire genome (**Fig. 1**; [6]), followed by the non-autonomous SINE elements, particularly *Alu* repeats, which account for 24% of TEs and 10.6% of the entire genome [4, 6]. Other SINEs found in humans are MIR and MIR3 elements [4, 6]. The main LTR-elements in the human genome are endogenous retroviruses (HERVs, including HERV-I, HERV-K, HERV-L [6]) that resemble retroviruses in both their structure and mobility mechanism, but lack a functional envelope gene that relegates them to an intracellular existence [3, 7].

Class-II elements, or DNA transposons, are present in small amounts in the human genome, accounting for only 6% of TEs and 2.8% of the entire genome (**Fig. 1**). They mainly belong to the TC-1/*mariner* superfamily (*i.e. mariner*, MER2-Tigger, Tc2), *hAT* superfamily (*i.e. MER-1-Charlie*, *Zaphod*), and some *PiggyBac*-like elements [6].



**Fig. (1).**Proportions of transposable elements in the human genome. The percentage of each class or sub-class of transposable element is given with respect to the whole genome. Data according to [6].

Long been considered as junk DNA or parasite DNA, TEs are now accepted as an evolutionary force that contributes to the genetic diversity of organisms [1-3, 8-10]. Indeed, TE activity is a source of genetic innovation through TE exaptation or domestication, *i.e.* the recruitment of TE on behalf of the genome to become a new gene [3, 11-12]. The transposition activity may also contribute to the genetic diversity of organisms by: i) acting as gene regulatory elements by providing their own promoters, ii) introducing alternative splicing, or iii) by altering the state of chromatin (reviewed in [3, 11-13]). TEs can also trigger chromosome rearrangements through ectopic recombination between repeated copies of a TE and cause mutations, resulting in several diseases, *e.g.* cancer, neurological diseases, blood diseases, *etc* [7, 13-19]. TEs can also regulate gene expression in several different ways, and numerous examples of such gene expression changes by TEs are found in all living organisms [1, 9, 12, 20]. Following are some examples of cancer and other human diseases for which TE-related mechanisms of ontology have been described.

## DELETION, DUPLICATION AND CHROMOSOME REARRANGEMENT

TE-mediated genome rearrangements are known in all living organisms and are at the origin of TE discovery by Barbara McClintock [21]. The large fraction of LINE-1 and *Alu* repeats

(i.e. about 17% and 11% of the human genome, respectively) favours ectopic recombination, which causes gene deletions, duplications and chromosome translocations or inversions [1]. Indeed, LINE-1 and *Alu* elements are often observed in the vicinity or even within the breakage points of chromosomal rearrangement [19] and their importance in inversion events and other rearrangement was highlighted by the comparison of human and chimpanzee genomes [22-24]. Furthermore, in a whole genome study of 17 human genomes, Kidd *et al.* showed that LINE-1 retrotransposons account for 19% of the 2081 breakpoints junction analysed [25].

- ***Alu recombination and cancer***

Numerous deletion events have been reported at the *BRCA1* locus and, in a lesser extent, for the *BRCA2* gene related to the unusually high density of *Alu* repeats [26-28] (**Table 1**). Homologous recombination between *Alu* elements results sometimes in gene duplication as reported for the *BRCA1* gene [27, 29], the MLL-1 gene involved in acute myeloid leukemia [30], and the *MYB* proto-oncogene the duplication of which leads to T-cell acute lymphoblastic leukemia [31]. The recombination between *Alu* repeats is also involved in the generation of the Philadelphia chromosome (i.e. translocation between chromosomes 9q34 and 22q11) leading to *BCR/ABL* gene fusions in chronic myeloid leukemia [32]. Another example is the translocation between chromosomes 5q23-31 and 18q12, which is caused by recombination between *Alu* elements in Ewing sarcoma [33].

- ***Alu-mediated deletion causing human diseases***

Other than cancer, other diseases may be due to *Alu*-mediated rearrangements (**Table 1**). Fabry disease, an X-linked recessive disorder of glycosphingolipid catabolism, results from the deficient activity of the lysosomal hydrolase  $\alpha$ -galactosidase A, encoded by an *Alu*-rich gene (12 *Alu* elements for a 12 kb gene) [34]. Among the gene rearrangements at the origin of the disease, only one deletion was initially described as due to *Alu-Alu* recombination, and 10 other *Alu-Alu* rearrangements were identified [34]. More recently, among 50 new mutations at the origin of Fabry disease, one important 3.1 kb deletion including the whole exon 2 was due to *Alu-Alu* recombination [35]. In the case of Sandhoff disease, a recessive lysosomal storage disease due to a deficient  $\beta$ -hexosaminidase activity, Neote *et al.* demonstrated deletion alleles of the *HEXB* gene in 27% of the examined dataset [36]. *HEXB* contains two *Alu* repeats that are involved in the deletion event, which removes about 16kb including the *HEXB* promoter, exons 1 to 5, and part of intron 5. The deletion also resulted in a reconstituted *Alu* sequence, the left half of which comes from the 5' *Alu* sequence and the right half of which from the 3' *Alu* sequence [36]. Deletions involving *Alu* repeats account for

numerous mutations in several other genes, including the apolipoprotein B (*APOB*) gene [37], the LDL receptor (*LDLR*) gene (reviewed in [38]), the caspase-activated DNase (*CAD*) gene [39], the cadherin-1 (*CDH1*) gene [40], the *VHL* gene of von Hippel-Lindau disease [41-42], and the  $\beta$ -globin gene cluster, including  $\gamma$ -,  $\delta$ - and  $\beta$ -globin genes [43-44] (**Table 1**).

- ***LINE-1 and DNA transposons are also source of chromosome rearrangements***

The LINE-1 retroelement is also a source of rearrangement, and a systematic analysis of LINE-1 insertion events in the human genome identified a total of 48 mutations related to L1 retrotransposition causing human genetic disease [45].

Even if they are rare in the human genome, class-II elements may also be responsible of chromosomal rearrangements. Rodic et al. recently reported on ten types of hematolymphoid neoplasms [46]. The conclusion of this work is that most translocations showed no propensity to involve genomic repeats, but 47% of the translocations at the TCF3 locus on chromosome 19 involve a MER20 DNA transposon [46].

One can be sure that increasing knowledge about TEs will lead to the discovery of growing examples of diseases caused by recombination at TE-rich loci.

**Table 1.** Examples human diseases linked to TE-mediated deletions or chromosomal rearrangements.

Type of TE	Gene	Disease or disorder	Type of recombination	References
<i>Alu</i>	<i>BRCA1</i>	Breast/ovarian cancer	deletion and duplication	[26-27, 29]
<i>Alu</i>	<i>BRCA2</i>	Breast/ovarian cancer	duplication	[27-28]
<i>Alu</i>	<i>APOB</i>	Hypo beta lipoproteinemia	deletion	[37]
<i>Alu</i>	<i>CAD</i>	hepatoma	deletion	[39]
<i>Alu</i>	<i>CDH1</i>	Hereditary diffuse gastric cancer	deletion	[40]
<i>Alu</i>	<i>a-GALA</i>	Fabry disease	deletion	[34-35]
<i>Alu</i>	<i>HBB</i>	Thalassemia	deletion	[43-44]
<i>Alu</i>	<i>HEXB</i>	Sandhoff disease	deletion	[36]
<i>Alu</i>	<i>LDLR</i>	familial hyper-cholesterolemia	deletion	[38]
<i>Alu</i>	<i>VHL</i>	Von Hippel Lindau disease	deletion	[41-42]

<i>Alu</i>	<i>MLL1</i>	Acute myeloid leukemia	duplication	[30]
<i>Alu</i>	<i>MYB</i>	T-cell lymphoblastic leukemia	duplication	[31]
<i>Alu</i>	<i>BCR-ABL</i>	Chronic myeloid leukemia	translocation	[32]
<i>Alu</i>	<i>EWSR1-ETV</i>	Ewing sarcoma	translocation	[33]
<i>MER20</i>	<i>TCF3</i>	hematolymphoid neoplasm	translocation	[46]

## CREATING NEW POLYADENYLATION SITES

Human retroelements, such as LINE-1 and *Alu*, may introduce intragenic polyadenylation signals creating new mRNA isoforms that lead to an enrichment of the transcriptome repertoire [47-48]. LINE-1 elements include a polyadenylation signal within their own sequence, and AATAAA sequences are commonly created in the A-rich tails of both SINES and LINES. Interestingly the analysis of the conservation and divergence of different types of alternative polyadenylation sites across human, mouse, rat and chicken sequences have shown that non-conserved poly(A) sites are associated with TEs to a much greater extent than conserved ones, albeit less frequently used [49]. Globally, 8% of total polyA sites are derived from TEs [49]. Approximately 1% of the reported *Alu* insertions in the 3' UTR of functional genes act as a polyadenylation sites, 99% of which are found in forward-oriented *Alu* elements, even though *Alu* inserts in 3' UTRs in either direction with the same probability [50]. Polyadenylation has also been observed in the LTR sequence of HERV-K and HERV-H LTRs in the breast cancer cell line T47D [51], in the HHLA2 and HHLA3 genes [52], respectively. Besides, the 5'-LTR sequence of HERV-F, which is notably expressed in placental and fetal tissue, may provide an alternative polyadenylation site to a Krüppel-related zinc finger gene, ZNF195 [53].

Several diseases have been linked to the introduction of a new polyadenylation site through LINE-1 or *Alu* element insertion in human genes (**Table 2**), such as colon cancer[54], haemophilia A and B [55-56], X-linked dilated cardiomyopathy [57], autoimmune lymphoproliferative syndrome [58], hypo-calciuric hypercalcemia and neonatal severe hyperparathyroidism [59]. Thus, TEs have played a crucial role during evolution in establishing or altering poly-A sites and sometimes generating human diseases.

**Table 2.** Examples of TE-mediated insertions presumed to introduce a new polyadenylation site related to human disease.

Type of TE	Gene name	Disease or disorder	References
<i>Alu</i>	<i>CASR</i>	Hypocalciuric hypercalcemia and neonatal severe hyper-parathyroidism	[59]
<i>Alu</i>	<i>FAS</i>	Autoimmune lympho-proliferative syndrome	[58]
<i>Alu</i>	<i>F9</i>	Hemophilia B	[56]
LINE-1	<i>APC</i>	Colon cancer	[54]
LINE-1	<i>DMD</i>	X-linked dilated cardiomyopathy	[57]
LINE-1	<i>F9</i>	Hemophilia B	[55]

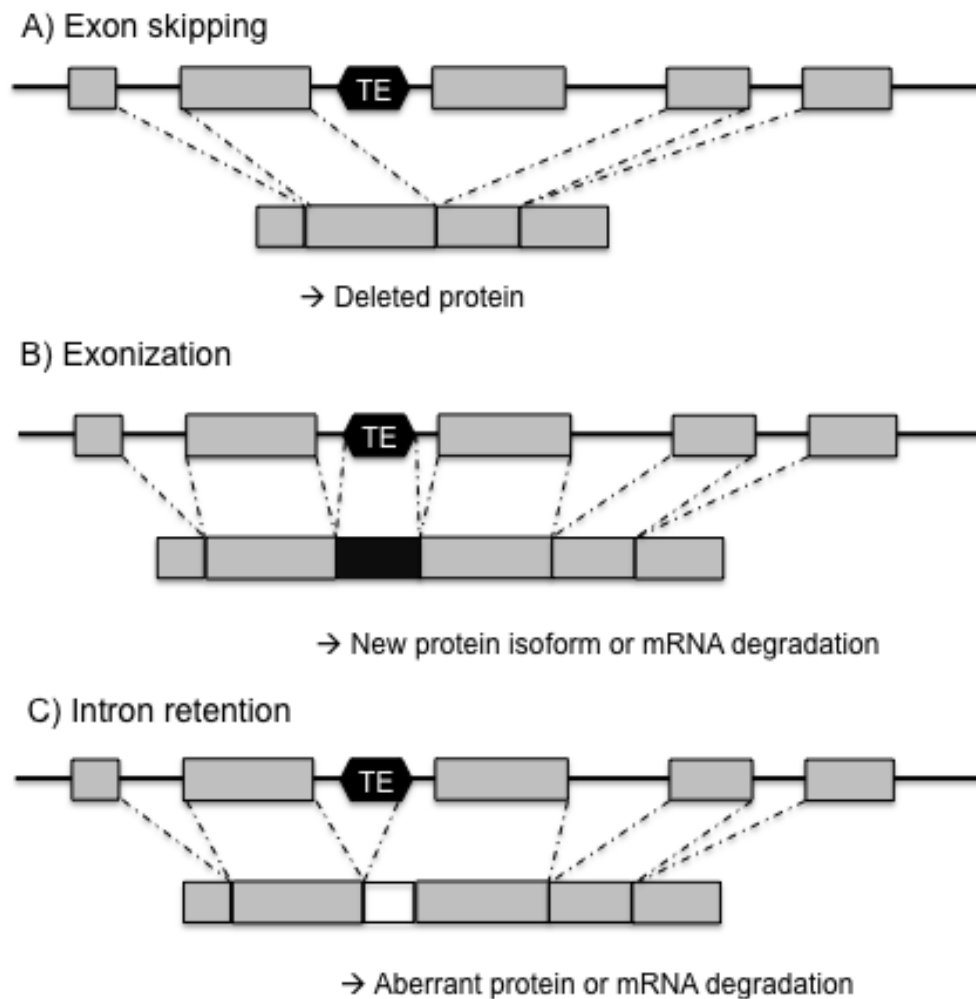
## EXONIZATION AND ALTERNATIVE SPLICING MODIFICATION

Most of recent human exons resemble and have originated from highly repeated sequences, i.e. TEs [60]. The molecular mechanisms and evolutionary consequences of such “exonization” (creation of new exons), as well as the creation of new introns (“intronization”), have been described previously [61-62]. In about 79% of cases, creation of a new exon causes a frameshift or creates premature stop codons, resulting in non-functional sequences, which are often alternatively spliced [61-62]. However, exonization events following LINE-1 or *Alu* insertion may lead to human diseases (**Table 3, Fig. 2**).

**Table 3.** Examples of TE-mediated exonization that cause inactivation of human disease-related loci.

Type of TE	Gene name	Disease or disorder	References
<i>Alu</i>	<i>COL4A3</i>	Alport syndrome	[64]
<i>Alu</i>	<i>GUSB</i>	Sly syndrome	[65]
<i>Alu</i>	<i>OAT</i>	ornithine delta-aminotransferase deficiency	[63]
<i>Alu</i>	<i>U2AF65</i>	Loss of hnRNP C binding leading to aberrant exonization	[66]
LINE-1	<i>CYBB</i>	Chronic granulomatous disease	[71]



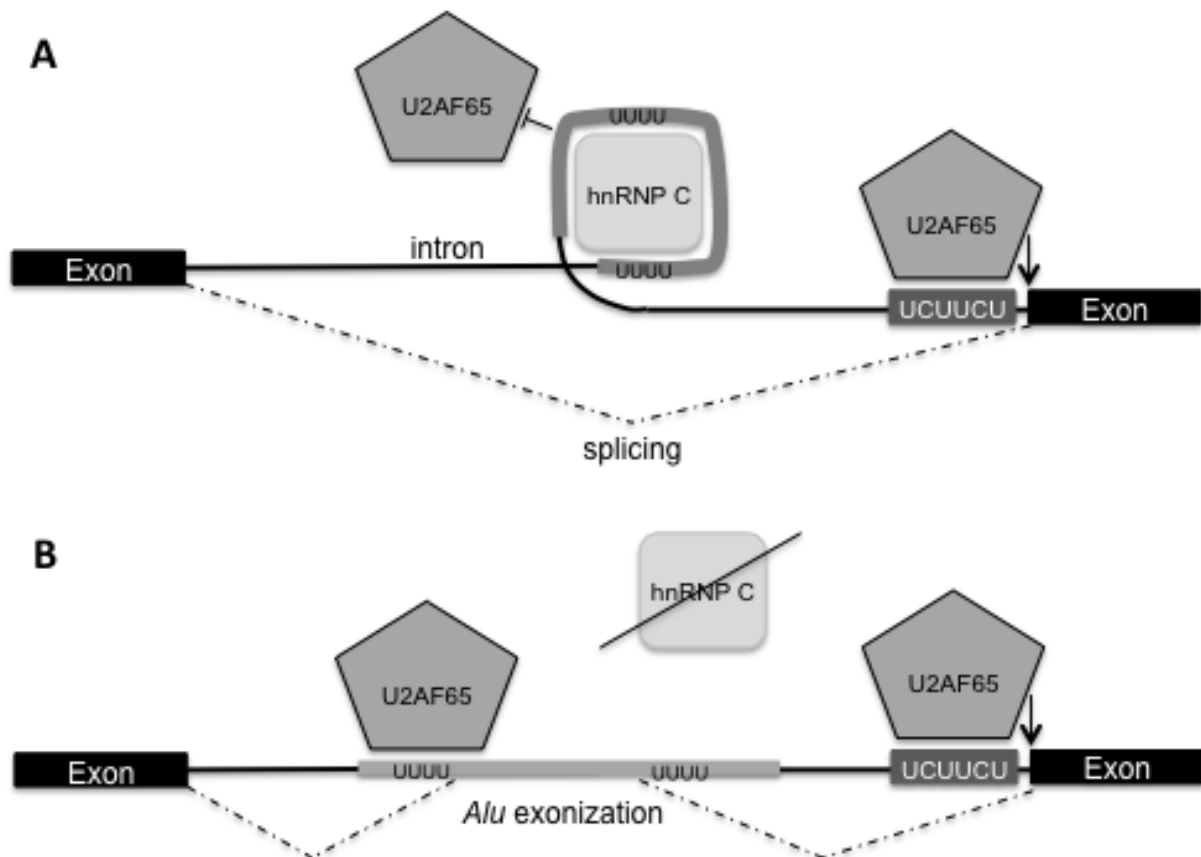


**Fig. (2) Simplified view of the main consequences of *Alu* insertion on mRNA splicing:** A) Exon skipping; B) exonization; C) intron retention. Grey boxes represent the exons, black line the introns and the black boxes are *Alu* elements. Dashed lines indicate the splicing events. See text for further explanation.

- ***Alu*-mediated exonization**

For example, a G-to-C mutation in an *Alu* element present in the intron 3 of the ornithine delta-aminotransferase deficiency (OAT) gene results in the creation of a new donor splice site and potentially to a new exon (**Fig. 2B**) [63]. This *Alu* insertion also causes premature termination, which results in OAT deficiency [63]. Two other examples of *Alu*-mediated exonization have been reported, where mutations in the COL4A3 and GUSB cause Alport [64] and Sly [65] syndromes, respectively. By contrast, a direct competition between the RNA-binding protein hnRNP C and the splicing factor U2AF65 at many genuine and cryptic splice sites has been reported to protect the transcriptome from the exonization of *Alu* elements [66]. Indeed, hnRNP C, which is abundant in the nucleus and associates with all

nascent transcripts [67-68], forms hnRNP particles implicated in the regulation of alternative splicing [69-70]. König et al. showed that hnRNP C represses alternative exons by binding next to the splice sites [68]. Besides, the core splicing factor U2AF65 regulates the inclusion of alternative exons on a genomic scale. Zarnack et al. showed that hnRNP C blocks U2AF65 from cryptic 3' splice sites, thereby preventing the aberrant expression of cryptic exons, including Alu elements (**Fig.3**) [66].



**Fig. (3). Protective role of hnRNP C against Alu exonization.** A) hnRNP C blocks the binding of the splicing factor U2AF65 on 3' cryptic splice site preventing Alu exonization. B) the absence of hnRNP C or mutation(s) in the polyU tracts prevent hnRNP C binding to Alu element and allow Alu exonization. Figure adapted from [66].

- **LINE-1 and ERVs are also source of exons**

The LINE-1 element is also a potential source of exonization as observed for the CYBB gene leading to chronic granulomatous disease [71]. Finally, an obvious example of exonization is provided by the splice acceptor signal and the coding sequence of the C-terminal exon of the human leptin receptor (219.1 isoform), which are derived from the HERV-K homology in a non-autonomous SVA element [72].

- ***Alu- and LINE-1 mediated alternative splicing***

Alternative splicing is often affected by TE insertion (**Table 4**). For example, insertion of the non-autonomous SINE *Alu* element into an intron of the human Factor VIII gene results in exon skipping (**Fig. 2A**) and consequent onset of hemophilia A [73]. In addition, the insertion of a LINE-1 element in the ribosomal S6 kinase 2 (*RSK2*) gene was reported in a patient with Coffin-Lowry syndrome. This LINE-1 insertion at position -8 of intron 3 leads to a skipping of exon 4 and then to a reading-frame shift and the appearance of a premature stop codon [74]. Another example is the insertion of an *Alu* sequence into the intron of the *NF-1* (neurofibromatosis type I) gene that leads to a deletion and reading frame shift in the downstream exon during splicing, possibly associated with neurofibromatosis [75]. The insertion of an *Alu* element within the exon 9, or just upstream of the start of exon 9, of the fibroblast growth factor receptor-2 (*FGFR2*) gene was reported as a cause of Apert syndrome [76]. The latter mutation affects the utilization of the 3' splice site leading to the generation of different, and non-functional, splice forms of *FGFR2* transcripts [76].

The dystrophin (*DMD*) gene is a giant gene (about 2.5 Mb of the X chromosome with more than 60 exons) where numerous TEs have inserted to create deletions or exon skipping [57, 77-81]. For example the insertion of LINE-1 induces skipping of exon 44, causing Duchenne muscular dystrophy [79, 81]. Insertion of an *Alu*-like sequence downstream of intron 11 was observed in individuals with X-linked dilated cardiomyopathy [77]. The insertion induces a rearrangement that activates a cryptic splice site in intron 11 and produces an alternative transcript containing the *Alu*-like sequence and part of the adjacent intron 11, spliced between exons 11 and 12, but not translated due to the presence of numerous stop codons [77]. The LINE-1 element is responsible for a large deletion in the X-linked pyruvate dehydrogenase E1 (*PDHX*) gene, but the LINE-1 insertion may also lead, at last in one patient, to an aberrantly spliced isoform resulting from the use of two cryptic splice sites [82]. Some other examples are cited in **Table 4**, but this list is not exhaustive.

- ***Intron retention: another TE-mediated splicing alteration***

Finally, intron retention (**Fig. 2D**) may happen when an error occurs in normal splicing leading to retention of a transcript-confirmed intron within a transcript-confirmed exon and thus in the mature mRNA [83]. Such events may be affected by TEs, as demonstrated in the case of LINE-1 insertion in the *CYBB* gene that causes chronic granulomatous disease [71]. Thus, TE insertions obviously have an impact on gene expression through alteration of splicing, leading to the absence of expression or production of aberrant proteins.

**Table 4.** Examples of TE-mediated splicing alterations involved in human diseases.

Type of TE	Gene	Disease or disorder	References
<i>Alu</i>	<i>BRCA2</i>	Breast cancer	[84]
<i>Alu</i>	<i>DMD</i>	X-linked dilated cardiomyopathy	[77]
<i>Alu</i>	<i>F8</i>	Hemophilia A	[73]
<i>Alu</i>	<i>FAS</i>	Autoimmune lymphoproliferative syndrome	[58]
<i>Alu</i>	<i>FGFR2</i>	Apert syndrome	[76]
<i>Alu</i>	<i>IDS</i>	Hunter syndrome	[85]
<i>Alu</i>	<i>NF1</i>	Neurofibromatosis type 1	[75]
<i>Alu</i>	<i>OPAI</i>	Autosomal dominant optic atrophy	[86]
LINE-1	<i>CYBB</i>	Chronic granulomatous disease	[71]
LINE-1	<i>DMD</i>	Duchenne Muscular Dystrophy	[79, 81]
LINE-1	<i>FKTN</i>	Fukuyama-type congenital muscular dystrophy	[87]
LINE-1	<i>RP2</i>	X-linked retinitis pigmentosa	[88]
LINE-1	<i>RPS6KA3</i>	Coffin-Lowry syndrome	[74]
LINE-1	<i>PDHX</i>	Pyruvate dehydrogenase complex deficiency	[82]

## TE-DERIVED REGULATORY ELEMENTS

Another way for TE to modify gene expression is to provide *cis*-regulatory elements at promoter and enhancer regions [89-92]. Numerous *cis*-regulatory sequences derive from TE domestication or exaptation [3, 11, 13]. Almost 25% of human promoter regions contain TE-derived sequences [93], and numerous transcription factor DNA binding motifs are enriched in various classes of repeats, such as LINE-2, *Alu* and other SINEs [94-98]. In addition, many of the HERVs present in the human genome have retained functional promoter, enhancer and polyadenylation signals, and these regulatory sequences have the potential to influence the expression of nearby genes.

- ***ERVs, placenta formation and human embryogenesis***

A crucial role of ERVs in placenta formation has been demonstrated in several mammals [99-102]. In humans, the Syncytin-1 and -2 genes are derived from a specific *env* gene contained within an HERV-W insertion. This Syncytin gene is capable of promoting trophoblast cell fusion *in vitro*, and likely plays a functional role in placental development [103]. Furthermore, experimental knockout of mouse syncytin results in a lethal placental defect, demonstrating

the crucial role of ERVs in placental development [104]. The contribution of ERVs to the mammalian genome is also done by providing alternative promoters in their LTRs. LTR exaptation has resulted in a pattern of novel gene expression significantly different from the pattern before LTR insertion or from that of gene orthologs lacking the LTR [90].

In addition, Landry et al. identified a fusion transcript containing the LTR of an HERV-E element linked to the *MIDI* gene and showed that the retroviral promoter (*i.e.* LTR) contributes significantly to the level of *MIDI* transcripts in placenta and embryonic kidney [105]. Furthermore, this study suggests a role for the LTR as a tissue-specific promoter and/or as an enhancer depending on the tissue [105]. Mutations in the *MIDI* gene, which is known to be transcribed from multiple promoters and to use several 5' untranslated exons [106], affect the development of midline structures and are responsible for the X-linked form of Opitz syndrome [107]. More recently, Teng et al. described enhancers from human embryonic stem cells that are significantly enriched in TE derived sequences, probably due to the generally relaxed repressive epigenetic state and the increased activity of TEs in these cells [91].

- ***TE-mediated evolution of the galectin gene cluster***

A recent example of the implication of TEs in gene regulation is given by the study of the evolution of the galectin gene cluster on human chromosome 19, where enrichment in a specific LINE-1 element was found in the placental sub-cluster of galectin genes [108]. This is a complex example of promoter evolution since the LINE-1 insertion may be accompanied by *Alu* insertion, both leading to modified promoters and then to differential expression of the galectin genes [108]. However a direct link with altered galectin expression and preeclampsia remains mainly hypothetical.

- ***Foxa1 and the  $\alpha$ -fetoprotein gene regulation***

Another example of the TE insertion effect in a promoter region was reported by Taube et al., who observed that critical regulatory sites for mouse  $\alpha$ -fetoprotein overlapping the binding sites of three transcription factors (e.g. Foxa1, p53 and SMAD) are located within a 300 bp region lacking DNA methylation due to the insertion of two TEs in which CpG sequences are underrepresented [109]. The transcription factor Foxa1 exploits the lack of methylation of TE sequences to bind and poise chromatin, giving access to secondary transcription factors such as p53 and SMAD, finally leading to  $\alpha$ -fetoprotein gene expression [109]. However, the methylation status of TEs is variable, depending on the abundance of CpG islands in the element and on the structure and age of the insertion [110-111].

## CHROMATIN REGULATION AND TRANSPOSITION

The structure of constitutive heterochromatin, *i.e.* centromere and telomere, which are essential structures for chromosome function and genome integrity, was contributed in part by TEs. In most eukaryotic species, the centromere consists of long tandem arrays of simple sequence repeats (so-called satellite repeats) surrounded by a peripheral region that is rich in TEs. The satellite regions of human centromeres are generally free of TEs, but pericentromeric regions are composed of long blocks of LINEs and SINEs [112]. In addition, subtelomeric heterochromatin that lies adjacent to the telomeric repeats resembles pericentromeric heterochromatin, and is composed of full-length and fragmented TEs [112]. Moreover, epigenetic imprinting is mostly associated with allele-specific DNA methylation of the CpG islands, also called germline differentially methylated regions (gDMRs). In several imprinted loci, the gDMRs may have emerged from the insertion of a TE (retrotransposon or ERV) [113]. Examples of such imprinted genes linked to TE insertion include placental transcripts in mammals [114-115] and the FWA gene in *Arabidopsis* plant model [112, 116-117].

In addition, there is a correlation between the activity of TEs and chromatin regulation, mainly in terms of DNA methylation. Thus TE are repressed in methylated DNA regions and, conversely, TE activation is caused by DNA demethylation [118-120]. This assumption is supported by several observations that LINE-1 elements are active in a hypomethylated DNA context. Early embryogenesis is one of these contexts of DNA under-methylation, and LINE-1 transposition activity has been demonstrated in murine primordial cells between the E11.5 and E13.5 early embryo stages [121]. Somatic mosaicism was demonstrated in mice due to the transfer of LINE-1 mRNA from the parental tissues (*i.e.* oocytes or sperm cells) to progeny where reverse transcription ensures further insertions of LINE-1 element's copies into the genome of the developing mouse during the pre-implantation stage [122]. Thus the early embryonic stages of mammal development appear as a favourable field of TE activation and mobility, as at least demonstrated for LINE-1 elements [123]. Therefore TE mobility and especially retroelement insertions, including those leading to cancer and other diseases, may be considered as the cost of phenotypic diversity formation [124].

The relation between LINE-1 activation and the methylation status of DNA has been particularly investigated in the case of cancers. Indeed the frequent hypomethylation of chromatin in tumor cells is regarded as a facilitator of LINE-1 mobility [110, 118-119, 125].

Recent whole genome studies revealed preferential LINE-1 insertions in hypomethylated DNA regions of cancer cells of different origins [126-129], while several other studies highlighted the hypomethylation of the LINE-1 element itself in several types of cancers [130-134] (**Table 5**). LINE-1 hypomethylation is accompanied by the shortened relapse-free survival of melanoma patients, and global DNA under-methylation influences the metastatic formation of primary melanomas [135]. The analysis of the transcriptome of chronic lymphocytic leukemia showed that TEs are globally derepressed [128]. However the study by Lee et al., which identified almost two hundred somatic insertions of LINE-1 (94.5%), *Alu* (5.0%) and ERV (0.5%) in 43 high-coverage whole genome sequencing datasets from five cancer types (*i.e.* colorectal, glioblastoma, multiple myeloma, ovarian and prostate cancer), shows that all LINE-1 and *Alu* insertions occurred in epithelial cancer but not in blood and brain cancer [127]. The study by Tubio et al. explored 290 cancer samples from 244 patients among twelve tumor types using a bioinformatic algorithm and identified 2756 somatic LINE-1 retrotranspositions [129]. About half of the patients (53%) had somatic LINE-1 retrotransposition, of which 24% were 3' transductions, *i.e.* concomitant transposition of a unique sequence downstream of the LINE-1 element due to continued transcription beyond the LINE-1 repeat [129]. These authors also highlighted that individual LINE-1 activity fluctuated during tumor evolution and correlated with LINE-1 promoter hypomethylation [129]. In addition somatic LINE-1 insertions tend to disrupt the expression of tumor suppressor genes, which are usually mutated in cancer, suggesting that some TE insertions may provide a selective advantage during tumorigenesis [127]. The role of chromatin structure is further shown through the decreased rate of transcription of the  $\beta$ -globin<sub>L1</sub> allele, which originated from the insertion of a full length LINE-1 element in intron 2 of the  $\beta$ -globin gene [136]. Indeed the promoter and enhancer sequences of the  $\beta$ -globin<sub>L1</sub> allele displayed a hypermethylated profile resulting in a transcriptional down-regulation and thus the  $\beta^+$ -thalassemia phenotype [136].

**Table 5.** A few examples of LINE-1 hypomethylation related to human diseases.

LINE-1 hypomethylation related disease	References
Chronic lymphocytic leukemia	[128]
Hepatocellular carcinoma	[131]
Prostate cancer	[130, 133-134]
Colorectal cancer	[132]

Metastatic melanoma	[135]
$\beta$ -thalassemia	[136]

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Finally, the repression of TEs through RNA interference has been documented and involved essentially endogenous small interfering RNA (endo-siRNA) in somatic cells and PIWI-interacting RNA (piRNA) in the germline (reviewed in [112, 137]). Both endo-siRNA and piRNA are transcribed from TEs themselves and, despite their sequence diversity, they do not disturb the expression of protein-coding genes [137]. Tumor cells are known to contain significantly lower quantities of micro-RNAs [138], and the question of the endo-siRNA and piRNA expression pattern in cancer cells has to be addressed since it may affect TE activity.

### **TEs AS SCREENING TOOLS FOR CANCER/PATHOLOGIC GENE CANDIDATES**

Animal models and engineered TEs (mainly *Sleeping Beauty* and *PiggyBac*, both Class-II DNA transposons) were used to support a causative role of TEs in cancer onset, demonstrating that TE insertion can lead to cancer (reviewed in [14]). Recent input includes the demonstration that *Sleeping Beauty* transposon system harboring HRAS, c-Myc and shp53 induces sarcomatoid carcinomas in mouse skin [139]. The identification and validation of CUX1 as a tumor suppressor was obtained recently by using mouse transposon-mediated insertional mutagenesis and *Drosophila* cancer models [140]. Mutagenesis screening allowed the identification of candidate cancer genes (reviewed in [141]), including many established tumor suppressors, oncogenes and genes involved in cell signalling pathways but also new candidates (*e.g.* diacylglycerol kinase, N(alpha)-acetyltransferase-15, ZNF292, *etc.*) in various solid tumors [142], non-melanoma skin cancer [143], colorectal cancer [144], glioma [145] and pancreatic adenocarcinoma [146] (**Table 6**). However, this insertional mutagenesis screening approach is not restricted to cancer and has been recently extended to infectious diseases (*e.g.* [147-149]). Finally, transposon-based gene trapping is an efficient approach for identifying genes involved in diseases or development ontology as recently used in a zebrafish model to characterize both embryonic and adult recessive mutants with cardiac expression [150].



**Table 6.** Examples of TE-based screening for pathologic genes

<b>Methodology</b>	<b>Based-on TE</b>	<b>Model organism</b>	<b>Target genes</b>	<b>Disease</b>	<b>References</b>
Target gene delivery	<i>Sleeping Beauty</i>	mouse	<i>HRAS, c-myc, shp53</i>	Sarcomatoid carcinoma	[139]
Insertional mutagenesis	<i>Sleeping Beauty</i>	mouse	<i>CUX1</i>	Cancer	[140]
Insertional mutagenesis	<i>PiggyBac</i>	mouse	diverse	Cancer	[142]
Insertional mutagenesis	<i>Sleeping Beauty</i>	mouse	<i>Notch1, Nsd1, etc</i>	Non-melanoma skin cancer	[143]
Insertional mutagenesis	<i>Sleeping Beauty</i>	mouse	<i>ZNF292</i>	Colorectal cancer	[144]
Insertional mutagenesis	<i>Sleeping Beauty</i>	mouse	<i>Met, Nf1, etc</i>	glioma	[145]
Insertional mutagenesis	<i>Sleeping Beauty</i>	mouse	<i>Kras, Mll3, Ptk2, etc</i>	Pancreatic adenocarcinoma	[146]
Insertional mutagenesis	<i>Himar</i>	Haemophilus influenzae	<i>iscR, pgdX, pur, etc</i>	Respiratory infections	[147]
Insertional mutagenesis	<i>Himar</i>	Borrelia	diverse	Lyme disease	[148]
Insertional mutagenesis	<i>Himar</i>	Salmonella	diverse	Food-borne gastroenteritis	[149]
Gene trapping	<i>RP2-Gene-break-transposon</i>	zebrafish	diverse	Cardiac organogeny and pathology	[150]

## GENE THERAPY OF CANCER AND GENETIC DISORDERS

Beyond the insertional mutagenesis approach, gene therapy assays have been conducted in animals and human cells in culture using engineered TEs (again mainly *Sleeping Beauty* and *PiggyBac*) that avoid some of the drawbacks of classical retroviral vectors (*i.e.* high mutagenic effect, immune response risk, etc) [151-152]. For example, the anti-angiogenic gene therapy of mice bearing intracranial human glioblastoma was successfully achieved by the transformation of human glioblastoma cells with *Sleeping Beauty* carrying either the soluble vascular endothelial growth factor receptor gene (*sFlt-1*) or the angiostatin-endostatin fusion gene (*statin-AE*), resulting in tumor size reduction and an increased proportion of surviving animals [153]. Otherwise, a gene directed enzyme pro-drug therapy was tested using the *PiggyBac* vector to treat mouse ovarian cancer and human ovarian adenocarcinoma cells [154-155]. Besides cancers, transposon-based gene therapy of several genetic disorders has been assayed in animal models, including haemophilia A and B, inherited tyrosinemia type I, junctional epidermolysis bullosa, pulmonary hypertension, pulmonary fibrosis, Crigler-Najjar syndrome type I, lung allograft fibrosis, and mucopolysaccharidosis type I and VII (**Table 7** and reviewed in [151]). In addition, pathological human cells were tested for *ex vivo* gene therapy, *i.e.* the therapeutic gene construct is introduced into a selected cell population removed from the patient and then the treated cells are infused back into the same patient. Using *Sleeping Beauty* or *PiggyBac* DNA-transposon based systems, efficient transfection and stable transgene expression were achieved in several types of human stem cells, including cord blood-derived CD34<sup>+</sup> hematopoietic progenitors [156-160], primary T cells [161-167], and human embryonic stem cells [168-169].

The first clinical application of the *Sleeping Beauty* system was tested by the group of L.J. Cooper (University of Texas, USA) using autologous T cells genetically modified to redirect specificity to B-lineage malignancies [170]. Several recent improvements have been achieved by the same group. for example eliminating the need to generate patient-specific T cells and thus rendering the vector suitable for administration in multiple recipients [171-172]. However, much remains to be done before a generalization of transposon-based gene therapy in humans.

**Table 7.** Examples of successful DNA transposon-based gene delivery in disease models

DNA-transposon used	Disease	Delivered gene(s)	Model organism or cell type	References
<i>Sleeping Beauty</i>	Hemophilia A	Human clotting factor VIII	Mouse	[173-175]

<i>Sleeping Beauty</i>	Hemophilia B	Human clotting factor IX	Mouse	[176]
<i>Sleeping Beauty</i>	Inherited type I tyrosinemia	Human fumarylacetoacetate hydroxylase	Mouse	[177]
<i>Sleeping Beauty</i>	Glioblastoma	soluble endothelium growth factor receptor and angiotensin-endothelin fusion protein	Mouse	[153]
<i>Sleeping Beauty</i>	Junctional epidermolysis bullosa	Human laminin- $\beta$ 3	Human patient derived cells	[178]
<i>Sleeping Beauty</i>	Pulmonary hypertension	Endothelial nitricoxidesynthase	rat	[179]
<i>Sleeping Beauty</i>	Lung allograft fibrosis	human indoleamine-2-3-dioxygenase	rat	[180]
<i>Sleeping Beauty</i>	Mucopolysaccharidosis, type I and VII	human $\alpha$ -L-iduronase and/or human $\beta$ -glucuronidase	Mouse	[181-182]
<i>Sleeping Beauty</i>	Crigler-Najjar syndrome, type I	Uridine-diphosphoglucuronate glucuronosyl transferase-1A1	rat	[183]
<i>PiggyBac</i>	Ovarian cancer	suicide gene, i.e. <i>Herpes simplex</i> thymidine kinase	Mouse ovarian tumor	[154]
<i>PiggyBac</i>	Ovarian cancer	suicide gene, i.e. <i>Herpes simplex</i> thymidine kinase	Human ovarian adenocarcinoma cell line	[155]
<i>Sleeping Beauty</i>	B-lymphoid malignancies	chimeric antigen receptor	Mouse primary T cells	[184]
<i>Sleeping Beauty</i>	B-lymphoid malignancies	chimeric antigen receptor	Primary human T cells / mouse tumor	[185]
<i>PiggyBac</i>	Solid tumor (murine xenograft)	human epidermal growth factor-2 – chimeric antigen receptor fusion	Human peripheral blood mononuclear cells	[166]
<i>Sleeping Beauty</i>	Fanconi anemia, type C	Fanconi anemiagene C	Human lymphoblastoid cells	[186]
<i>Sleeping Beauty</i>	Skin	Human $\beta$ 1 and $\alpha$ 2	Pig	[187]

<i>Sleeping Beauty</i>	inflammation Osteosarcoma lung metastases	integrin chimeric antigen receptor and interleukin-11 receptor $\alpha$	T cells / mouse tissue	[161]
<i>Sleeping Beauty</i>	Pulmonary fibrosis	microRNA-29	Mouse lung tissue	[188]

## CONCLUSION

The role of TEs in genome plasticity and evolution is now accepted [1-3, 8-10] but, by the same mechanisms, TEs are also responsible for the molecular etiology of several diseases as presented above [7, 14, 16]. In addition, TEs may become activated by the process of cell malignant transformation that promotes increased rates of mutation and recombination in the genome of transformed cells [14]. It seems important to disseminate knowledge of TEs to a wide audience of scientists and physicians, keeping in mind that genomes are continuously evolving due to TEs and that new genetic defects will continue to appear, even at a slow rate in the case of Humans. Some examples have been cited above, but the list presented here of human diseases related to TE insertion events is far from exhaustive. Increasing our knowledge of TEs through extensive genomic approaches may lead to new potential diagnostic markers of diseases and prenatal markers of genetic disorders. Another interesting aspect of TEs is their potential use as biotechnology tools for gene therapy, although further progress is required, such as the control of insertion point. Site-specific insertion has been observed with yeast retrotransposon Ty1 [189-190] and with the bacterial transposon Tn7. Tn7 specifically inserts at the chromosomal attachment site *attTn7* or in non-*attTn7* sites in conjugal plasmids depending on the transposition proteins expressed [191-192]. Indeed, four Tn7 proteins act as target selector; when TnsD is the target selector, Tn7 inserts at high frequency into the *E. coli* *attTn7* site, directly downstream of the essential *glmS* gene, whose expression is preserved [191, 193]. This exceptional property of Tn7 transposon leads to the engineering of mini-Tn7 transposons used as a tool for a single-copy tagging of various bacteria in a site-specific manner at a unique and neutral site without any deleterious effects (reviewed in [194]). Interestingly the site-specific insertion of Tn7 was also found in the human genome but not in yeast [195].

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