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Review

A mobile threat to genome stability: The impact of non-LTR retrotransposons upon the human genome

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ABSTRACT

It is now commonly agreed that the human genome is not the stable entity originally presumed. Deletions, duplications, inversions, and insertions are common, and contribute significantly to genomic structural variations (SVs). Their collective impact generates much of the inter-individual genomic diversity observed among humans. Not only do these variations change the structure of the genome; they may also have functional implications, e.g. altered gene expression. Some SVs have been identified as the cause of genetic disorders, including cancer predisposition. Cancer cells are notorious for their genomic instability, and often show genomic rearrangements at the microscopic and submicroscopic level to which transposable elements (TEs) contribute. Here, we review the role of TEs in genome instability, with particular focus on non-LTR retrotransposons. Currently, three non-LTR retrotransposon families – long interspersed element 1 (L1), SVA (short interspersed element (SINE-R), variable number of tandem repeats (VNTR), and *Alu*), and *Alu* (a SINE) elements – mobilize in the human genome, and cause genomic instability through both insertion- and post-insertion-based mutagenesis. Due to the abundance and high sequence identity of TEs, they frequently mislead the homologous recombination repair pathway into non-allelic homologous recombination, causing deletions, duplications, and inversions. While less comprehensively studied, non-LTR retrotransposon insertions and TE-mediated rearrangements are probably more common in cancer cells than in healthy tissue. This may be at least partially attributed to the commonly seen global hypomethylation as well as general epigenetic dysfunction of cancer cells. Where possible, we provide examples that impact cancer predisposition and/or development.

1. Introduction

Less than a decade ago, with the availability of the first human draft genome sequence, the human genome was considered to be a very stable entity [1]. However, with the identification of structural variations (SVs) as a major cause of inter-individual variation, it is now evident that the human genome is distinguished by a high inter-individual variability [2–6]. SVs are usually at the submicroscopic level and include insertions, deletions, duplications, translocations, and inversions. It is now commonly believed that SVs comprise more nucleotides than single nucleotide polymorphisms (SNPs) in the human genome [3]. Cancer cells are notorious for their genome instability. It is generally accepted that structural rearrangements at the microscopic level are common features of the genome of most human cancers. Recently the full impact of SVs, including those at the submicroscopic level in cancer cells, has become more apparent. Inherited rearrangements have also been associated with cancer predisposition and will be discussed in more detail in this review.

Transposable elements (TEs) are often involved in the genesis of SVs due to their inherent ability to mobilize, their abundance, and their high sequence identity. About half of the human genome is comprised of repetitive sequences, with TEs being the largest contributors [1,7]. The repeat content of the human genome is likely even higher, given that the decay of TEs over time makes the identification and characterization of ancestral TEs difficult if not impossible, and sequencing and assembly is less than perfect in repeat (and transposon)-rich regions [8,9]. Altogether, the impact of TEs onto the human genome has been underappreciated for some time; only recently are we beginning to comprehend the impact of TEs upon genome architecture and, consequently, onto the evolution of the human genome [10].

Abbreviations: TE, Transposable element; SV, structural variation; ERV, endogenous retrovirus; ORF, open reading frame; UTR, untranslated region; LINE 1, L1, long interspersed element 1; SINE, short interspersed element; VNTR, variable number of tandem repeats; SVA, SINE-R/VNTR/*Alu*; TSD, target site duplication; NHEJ, non-homologous end joining; SSA, single strand annealing; DSB, double strand break; HR, homologous recombination; NAHR, non-allelic homologous recombination; SNP, single nucleotide polymorphism; HDGC, hereditary diffuse gastric cancer; AML, acute myeloid leukemia; T-ALL, T-cell acute lymphoblastic leukemia; Dnmt3L, DNA methltransferase 3-like.

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A recent comparative genomics study of two human genomes found that TEs are associated with ~10% of all SVs larger than 100 bp [11]. TEs impact genome integrity in several ways, including TE insertions and rearrangements. TEs are now commonly recognized in genetic diseases (reviewed in [7,12–16]). They have also been associated with cancer genesis; this is not unexpected, as a typical characteristic of cancer cells is their genomic instability. Here, we discuss the impact of TEs – in particular, non-LTR retrotransposons – upon the architecture of the genome; and review how and to what extent TEs have been associated with the genesis of cancer.

2. TE background

2.1. TE classification and activity

To understand the role of TEs within genome instability, it is important to review several key aspects of TE biology. For more detailed information we refer to other reviews (e.g. [7,12,15,17,18]). TEs may be categorized by their mobilization mechanism as either DNA transposons or retrotransposons. DNA transposons propagate via a cut-and-paste mechanism. While active in very early primate evolution, these elements essentially ceased activity in the primate lineage approximately 37 million years ago [19]. In contrast, retrotransposons use an RNA intermediate; are reverse transcribed; and move within the genome through a copy-and-paste mechanism [15,20]. Retrotransposons are further subdivided into two groups on the basis of presence or absence of long terminal repeats (LTRs). The most prominent members of LTR-retrotransposons are endogenous retroviruses (ERVs), which comprise about 8% of the human genome [1]. There is very little (if any) evidence of ongoing ERV retrotransposition in humans [1,7,21]. Their potential role in tumorgenesis is subject to an ongoing debate and covered elsewhere (e.g., [22]). The other group encompasses non-LTR retrotransposons, and is discussed in more detail in this review.

2.2. Non-LTR retrotransposon biology

Three different families of non-LTR retrotransposons are actively mobilized in the human genome. These are long interspersed elements 1 (LINE1s, L1s); *Alu* elements (a short interspersed element, or SINE); and SVAs (named after their composite parts: SINE-R, VNTR (variable number of tandem repeats), and an *Alu*-like sequence) [1,7,12,15,23]. Their success is evident through the fact that non-LTR retrotransposons occupy about one-third of the human genome, making them the most populous TE group in the human genome [1].

L1s are the only currently known autonomous (providing its own enzymatic machinery for retrotransposition) retrotransposons that are currently mobilizing within the human genome. They comprise about 17% (~500,000 copies) of the human genome with evidence of ongoing activity dating back roughly 160 million years [1]. A full-length L1 is about 6 kb in length and contains an internal Polymerase II promoter, two open reading frames (ORFs), and ends in a polyadenylation signal followed by a homopolymeric tract of Adenosines (also known as a polyA-tail; see Fig. 1) [24,25]. ORF1 protein is an RNA-binding protein [26] while ORF2 encodes a protein with both endonuclease and reverse transcriptase activity [27–29]. The majority of L1 insertions are retrotranspositionally incompetent due to variable truncation upon insertion and debilitating mutations [1]. Consequently, only about 80–100 retrotransposition competent L1s have been identified in the human genome [30]. Of those, a few (6–8) "hot" L1s appear to be responsible for the bulk of new insertions [30].

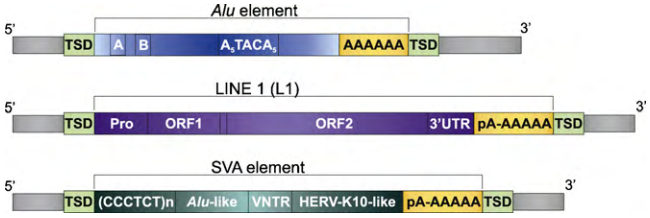

Fig. 1. Structure of non-LTR retrotransposons.
Shown is structure of actively mobilizing retrotransposons in the human genome: an *Alu* element (blue), a full-length L1 (purple), and a full-length SVA (dark green). The non-LTR retrotransposons are not drawn to scale. All full-length non-LTR retrotransposons end in a homopolymeric tract of Adenosines (polyA-tail, yellow). SVA and L1 contain a polyadenylation signal (pA) immediately before the polyA-tail. Insertions are flanked by target site duplications (TSDs, green). *Alu* (blue): The A and B stand for the A and B boxes of the internal promoter. The left and right monomers are linked by a spacer sequence A_5TACA_5. L1 (purple): Pro stands for the internal Polymerase II promoter within the 5′ untranslated region (UTR). A full-length L1 element contains two open reading frames (ORF1, ORF2). SVA (dark green): A full-length composite element contains from 5′ to 3′ a hexamer (CCCTCT), an *Alu*-homologous region of two antisense *Alu* fragments including other sequence of unknown origin, a variable number of tandem repeat (VNTR) region, and ends in a SINE region from parts of HERV-K10, an human endogenous retrovirus.

The human genome contains two actively mobilizing non-autonomous non-LTR retrotransposons: *Alu* elements (member of the SINE family) and SVAs. Non-autonomous elements are believed to rely on the enzymatic machinery of L1s for retrotransposition; e.g., as shown for *Alu* elements [31,32]. With more than 1,000,000 insertions, *Alu* elements are the most successful TE in the human genome by number [1]. This accomplishment is even more remarkable given that *Alu* elements are primate-specific and originated only about 65 million years ago [15]. *Alu* elements are heterodimers made of two non-identical monomers connected by an Adenosine-rich linker [15,33,34]. As shown in Fig. 1, an approximately 300 bp long *Alu* element contains an internal Polymerase III promoter at its 5′ end, and ends in a polyA-tail.

SVA elements, which are altogether less well characterized than other non-LTR retrotransposons, represent the second group of currently mobilizing non-autonomous elements in the human genome. Similar to L1s, SVA insertions are often truncated and terminate in a polyadenylation signal followed by a polyA-tail (Fig. 1) [35,36]. It is now generally believed that SVA elements are transcribed by Polymerase II. However, an internal promoter has not been detected, and SVA transcription might – at least occasionally – take place through promoter activity in the vicinity of the SVA [35–37]. Due to their relatively recent origin (originating less than 25 million years ago), with ~3000 copies, SVA elements show the lowest retrotransposon density in the human genome [7,36].

Non-LTR retrotransposons are thought to typically insert into the human genome through a mechanism referred to as Target Primed Reverse Transcription (TPRT) [7,12,38,39]. During TPRT, the L1-derived endonuclease cuts the minus strand of the host DNA at a loosely recognized target site (5′-TTTT/AA-3′) [28,40]. The polyA-tail of the non-LTR retrotransposon mRNA is proposed to bind to the free 3′ end of the host DNA, and the mRNA is reverse transcribed by the reverse transcriptase encoded by L1 [41]. The next steps of second strand cleavage, second strand synthesis, and ligation are the subjects of ongoing research. However, host repair systems have been implicated in the later stages of L1 retrotransposition [42–46]. A recent tissue culture-based study further supports this, as proteins of the non-homologous end joining (NHEJ) pathway were shown to be involved in L1 retrotransposition [47]. Due to a staggered break of the host DNA at the insertion site, the non-LTR retrotransposon insertion is flanked by short stretches (usually between 6 and 20 bp) of identical host DNA, referred to as target site duplications (TSD) [27,48].

3. Insertional mutagenesis

3.1. Potential impact upon the human genome

Inherited *de novo* TE insertions occur in the germline and/or during early embryogenesis [49–52]. *De novo* retrotransposon insertions account for about 0.3% of all human mutations [7]. The retrotransposition rate compatible with live birth varies greatly between the three retrotransposon families. *Alu* elements have the highest estimated retrotransposition rate, with ~1 in 20 live births, followed by L1 with about 1 in 200, and SVA with ~1 in 900 [7,11]. Consequently, non-LTR retrotransposons have a remarkable impact on genome plasticity and stability.

With few exceptions, retrotransposon insertions are neutral or in some cases even deleterious to the host [53]. Due to the unique properties of non-LTR retrotransposon insertions, an independent *de novo* insertion can be easily discriminated from a shared TE insertion that has been passed down over generations. These include random insertion site (apart from the loose recognition motif of the endonuclease cleavage site), unique TSDs, and identity of subfamily affiliation [15,54]. In the case of L1, the length of the insertion is yet another discriminating factor to discern shared from separate insertions [55]. Because the precise excision of non-LTR retrotransposons is exceedingly rare, the ancestral state is known to be the absence of the element; thus, TEs are generally homoplasy free ([56], reviewed in [57]). Consequently, an insertion shared between two humans at exactly the same genomic location with identical TSDs is testimony to an inherited insertion and a common ancestor.

Deleterious insertions include the disruption of coding or regulatory sequences (reviewed in [12–14,16]). The coding sequence of genes can be disrupted when a non-LTR retrotransposon inserts into an exon, but also can be affected if the insertion occurs within an intron. While the impact of the latter event is often more difficult to demonstrate and may be overlooked, these insertions can potentially disrupt splice sites and cause exon skipping. Intronic TE insertions have been implicated to potentially alter the expression of a gene through introduction of alternative splice sites or polyadenylation signals [58–61]. Intronic insertions have also been associated with destabilization of the mRNA resulting in reduced expression [62]. In addition, insertions into the 5′ and 3′ prime region of genes can possibly alter their expression [63–65], reviewed in [66]. Alterations in gene expression increase the potential for altering equilibrium of regulatory networks, and thus augment susceptibility to certain diseases – including cancer.

3.2. Insertional mutagenesis and disease

All three currently actively mobilizing non-LTR retrotransposon families – L1, SVA, and *Alu* – have been identified as the causative agent of several genetic disorders. These include hemophilia, Alpert syndrome, familial hypercholesterolemia, and colon and breast cancer (reviewed in [7,12,13,16]). Several cancer predisposing mutations caused by retrotransposon insertions are shown in Table 1. Of all TE-causing genetic disorders identified to date, X-linked diseases are disproportionally over-represented compared to the autosomal diseases [7,12,62]. The X-chromosome is particularly enriched in inherited diseases caused by L1. The underlying reasons are the subject of ongoing debate (e.g. [7,12,62,67]). An ascertainment bias likely contributes (at least in part) to this finding, as the insertion on autosomes is commonly masked by the wild-type allele when standard PCR procedures are used. In particular, this is the case for longer TE insertions (L1 and SVA) where the wild-type allele has a much shorter PCR amplicon. However, an insertional bias of L1 insertions toward the X-chromosome has also been reported [67].

The coding sequence of some genes has been disrupted more than once by independent non-LTR retrotransposon insertions [14]. An example is the BRCA2 gene, which is associated with breast/ovarian cancer susceptibility [68,69]. Moreover, some genes have been targeted twice at exactly the same location. The APC gene (associated with colon cancer predisposition) is an intriguing example, as one insertion was caused by an L1 and the other by an *Alu* element (Table 1) [14]. The fact that different genes have been identified with recurrent disease-causing retrotransposon insertions indicates that insertions do not solely occur by chance. Instead, this implies varying susceptibility to non-LTR retrotransposon insertions. The exact reasons for this finding are elusive. However, certain characteristics of these genes likely contribute to their predisposition for multiple retrotransposon insertions. For example, the presence of TEs and the nucleotide composition of introns prone to recurrent *de novo* TE insertions might play a pivotal role. The TE content of genes might be a contributing factor, as genes enriched in TE sequences harbor, on average, more sequences that resemble endonuclease cleavage sites, possibly increasing the proliferation of TEs.

For example, *Alu* elements have been shown to insert upstream of another element or within the polyA-tail of an existing element containing a less than perfect endonuclease cleavage site [70]. The TSD itself, created by classical TPRT, is a source for an additional endonuclease cleavage site. The spacer region (see Fig. 1) of *Alu* elements also closely resembles an endonuclease recognition site. Thus, one byproduct of *Alu* insertions is the creation of additional L1 endonuclease target sites suitable for the insertion of non-LTR retrotransposons. Also, recently inserted full-length L1s in particular contain several nucleotide sequences closely resembling endonuclease cleavage sites. However, TE density alone is likely not a sufficient explanation, as some genes with high TE density do not encounter recurrent *de novo* insertions. The methylation status and expression level of a gene may be other contributing factors as the DNA of genes that are actively transcribed may exist in more open chromatin structures that are more accessible to retrotransposition machinery and, therefore, may be more prone to *de novo* non-LTR insertions.

The origin of a TE insertion can be reconstructed based on the geographical distribution of a TE insertion and its frequency within a population. In the case of cancer, several founder mutations involving *Alu* elements have been identified. For example, an *Alu* insertion into the BRCA2 gene has been identified in the Portuguese population [69]. The *Alu* insertion disrupts exon 3 which results in exon skipping. This skipping of exon 3 has also been found in individuals without the *Alu* insertion, and thus an association of the *Alu* insertion and cancer susceptibility has been questioned. However, a recent study convincingly linked the *Alu* founder mutation in the Portuguese population with cancer susceptibility [71].

3.3. Insertional mutagenesis of TEs in somatic cells

The investigation of TE retrotransposition activity in somatic cells at a comprehensive level was until recently out of reach. Advancements in detection technologies, in particular high-throughput sequencing approaches, are on the verge of changing this. While knowledge about somatic retrotransposition is still sparse, there is increasing evidence of ongoing L1 mobilization in healthy somatic tissues. For example, L1 protein has been identified in adult cells [72], and ongoing retrotransposition causing somatic mosaicism has been demonstrated in embryogenesis and within developing neuronal precursor cells [49–51]. *In vitro* assays using an L1 cassette with an adenovirus vector further indicate that L1 retrotransposition can occur in differentiated human primary cells in G1/S-arrested cells but not in G0-arrested cells [73]. Another

Table 1
Selection of TE insertions associated with cancer predisposition.

Cancer-associated insertional mutagenesis of transposable elements (TEs)					
Locus	Meaning	Cancer association	TE	Distribution	References
Classical TPRT insertions					
APC	Adenomotous polyposis coli gene	Colon cancer	*Alu*	Germline	[135]
APC			L1Ta	Somatic	[132]
BRCA1	Breast cancer 1 gene	Breast cancer/ovarian cancer	*Alu*	Germline	[69]
BRCA2	Breast cancer 2 gene	Breast cancer/ovarian cancer	*Alu*	Germline	[68]
BRCA2			*Alu*	Germline	[69]
MLVI2	Moloney leukemia virus integration 2 homolog	Leukemia	*Alu*	Germline	[136]
NF1	Neurofibromatosis 1 gene	Neurofibromatosis type 1[a]	*Alu*	Germline	[137]
Insertion-mediated deletion					
APC	Adenomotous polyposis coli gene	Cancer of the colon	*Alu*	Germline	[14,84]

[a] ~10% of patients diagnosed with NF1 develop cancer.

study found that L1 retrotransposition using its endogenous L1 promoter requires cell divisions [74].

Furthermore, this research indicates that L1 retrotransposition occurs at (very) low levels in primary human fibroblast cell lines [74]. On the basis of these initial results, it appears likely that the retrotransposition frequency varies individually and also between different tissues. The TE mobilization rate in cancer cells is likely more pronounced in comparison to "normal" tissue due to the likely activation of L1s through demethylation of their promoters (see Section 7). In addition, deleterious retrotransposon insertions might not underlie the same selection criteria in cancer cells compared to healthy tissue. For example, retrotransposon insertions that might typically result in apoptosis in normal cells may not cause cell death in cancer, given that the apoptosis pathway is often impaired in cancerous cells [75]. However, in vitro studies of human carcinoma cells suggest that apoptosis is positively correlated with the presence of retrotransposition-competent L1 [76]. Conceivably, the cell reaction to L1 reactivation is dosage-dependent.

3.4. TE insertions associated with deletion of host DNA

The deletion of host DNA associated with the *de novo* insertion of an L1 or *Alu* element was first demonstrated for L1 in tissue culture and confirmed by comparative genomics studies for both L1 and *Alu* insertions [77–82]. SVA elements have not been sufficiently studied in this context, but probably are equally involved in this mechanism. Two vastly different mechanisms, each with characteristic properties, have been identified causing deletions upon the insertion of non-LTR retrotransposons. These are TPRT-dependent insertion-mediated deletions and endonuclease-independent insertions [41,77,79–82]. The primary difference between the two mechanisms is the dependence on the L1 endonuclease.

3.4.1. Insertion-mediated deletions

Insertion-mediated deletions are endonuclease-dependent and are thought to make use of TPRT [41,77,79,80]. In these instances, an endonuclease cleavage site can be commonly identified at the insertion site. In addition, the 3′ end of non-LTR retrotransposon insertions involved in insertion-mediated deletions (the insertion ends in a polyA-tail) is generally intact [77,79,80]. However, due to the deletion of the host sequence, TSDs are absent in these insertion-mediated events (see Fig. 2). While the precise insertion mechanism(s) of endonuclease-dependent TE insertion-mediated deletions remains elusive, two different mechanisms have been proposed depending on the size of the deleted host DNA [77]. Small deletions of only a few nucleotides could be caused through a nick of the top strand of the DNA to the right of the initial cleavage site resulting in a 5′ overhang [77]. Larger deletions have been proposed to occur when a TE inserts downstream of a double strand break (DSB) [77].

A new line of evidence using *in vitro* assays indicates that disruption of the NHEJ pathway commonly results in deletion of host DNA upon endonuclease-dependent L1insertion [47]. This part of the study was performed with zebrafish L1s in DT40 cells deficient in Ku70. Intriguingly, Ku70 is associated with the protection of DNA from exonucleolytic degradation [83]. This indicates that the host DNA of insertion-mediated deletions is not sufficiently protected from degradation at the endonuclease cleavage site. It is the subject of future studies to determine if this finding represents a typical *in vivo* mechanism of human L1 retrotransposition. However, it strongly supports the hypothesis of competition between the retrotransposon insertion event itself and an attempt by the host to repair the nascent insertion site [10,41].

A number of genetic disorders caused by TE insertion-mediated deletions have been identified (reviewed in [13,14]). *Alu* insertion-mediated deletion has been suggested as a mechanism for deletion in the APC gene, which is associated with colon cancer predisposition [14,84]. Somatic mutations involving this mechanism have not yet been identified. Altogether, the combination of host sequence deletion and a *de novo* retrotransposon insertion represents a large threat to the integrity of the human genome and has a higher potential to be deleterious to the host than insertional mutagenesis alone.

3.4.2. Endonuclease-independent TE insertions

While the bulk of retrotransposon insertions are endonuclease-dependent and thus show the typical hallmarks of TPRT, a small fraction of endonuclease-independent L1 insertions has been observed in tissue culture cells deficient in NHEJ [78]. Although these insertions are also often associated with deletions of host

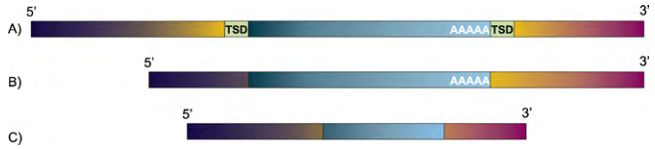

Fig. 2. Illustration of non-LTR retrotransposon insertion mechanisms.
TSDs are shown in green; AAAAA stands for polyA-tail; A) Illustrates a typical non-LTR retrotransposon insertion. These insertions are thought to occur via TPRT. The insertion is 3′ intact (contains a polyA-tail) and is flanked by TSDs. No host sequence is deleted. B) Shown are the typical hallmarks of an insertion-mediated deletion. The non-LTR retrotransposon is 3′ intact, indicated by the polyA-tail. TSDs are absent; upstream (left) of the element, host DNA is deleted. C) Illustrated is an endonuclease-independent insertion with deletion of host DNA 3′ and 5′ of the insertion. However, deletions can be limited to 3′ or 5′ host DNA sequence. The non-LTR retrotransposon usually does not contain a polyA-tail and is not flanked by TSDs.

DNA, these insertion events are not thought to be the products of the retrotransposition pathway; rather they occur by other mechanisms such as DSBs. Recently, comparative genomic studies have shown that endonuclease-independent L1 insertions are not tissue culture artifacts and also occur *in vivo* [81,82]. Up to 0.5% and 0.7% of L1 and *Alu* insertions respectively could be attributed to endonuclease-independent insertion mechanisms [7,81,82]. Apart from the deletion of host sequence and absence of a typical endonuclease cleavage site, these non-LTR insertions are commonly 3′ and 5′ truncated and do not contain TSDs (Fig. 2) [78,81,82]. The average size of endonuclease-independent insertions is also in general smaller (e.g. 572 bp for L1 [81]) than that of recent classical non-LTR retrotransposon insertion events (e.g. 900 bp for L1 [1]) that are thought to have inserted using TPRT.

The deletion of host DNA in conjunction with the structure and mechanism of the TE insertion and the commonly found microhomology between the L1 insertion and the host DNA indicates that endonuclease-independent TE insertions are involved in DSB repair [78,81,82]. Hence, a very small fraction of TEs potentially contribute to cell integrity. A few endonuclease-independent L1 insertions have been identified in genetic disorders (reviewed in [62]). It remains an unanswered question if endonuclease-independent insertions occur only in the germline or if they are also common in somatic cells. While these insertions are probably involved in DNA repair and thus stabilizing, the deletion of host sequence can still be deleterious to the host and (for example) be associated with cancer predisposition. In cancer cells their contribution could be similar to other DSB repair mechanisms that can cause new oncogenes through fusion of two genes or disrupt tumor suppressor genes.

4. Inverted *Alu* elements cause genomic instability

On average, the human genome contains approximately one *Alu* insertion per every 3 kb [1]. However, *Alu* elements are not evenly distributed throughout the human genome [1]. Areas of higher than average *Alu* density have been particularly associated with genomic instability. The abundance of *Alu* insertions and high sequence homology between *Alu* elements (average 71%) [85] makes them targets for genome rearrangements (see section 5). Moreover, inverted *Alu* elements in close proximity to each other are less frequently identified in the human genome than *Alu* insertions in the same orientation [86,87]. This can only partially be attributed to insertional bias of *Alu* insertions in the same orientation [65,83]. Instead, inverted repeats likely represent hotspots of genomic instability, as seen in studies with yeast [86].

Inverted *Alu* elements that are closely spaced appear to build hairpin structures, which can cause DSBs of the DNA and excision of inverted *Alu* elements from the human genome [86,88]. Moreover, hairpin structures involving *Alu* elements appear to cause replication stalling and collapse of the replication fork, which can lead to DSBs and/or intra- or intermolecular template switch [88]. Apart from the distance of two inverted *Alu* insertions, the size of sequence identity between two *Alu* insertions seems to be an important contributing factor [87]. Despite their underrepresentation in the human genome, inverted repeats continue to cause

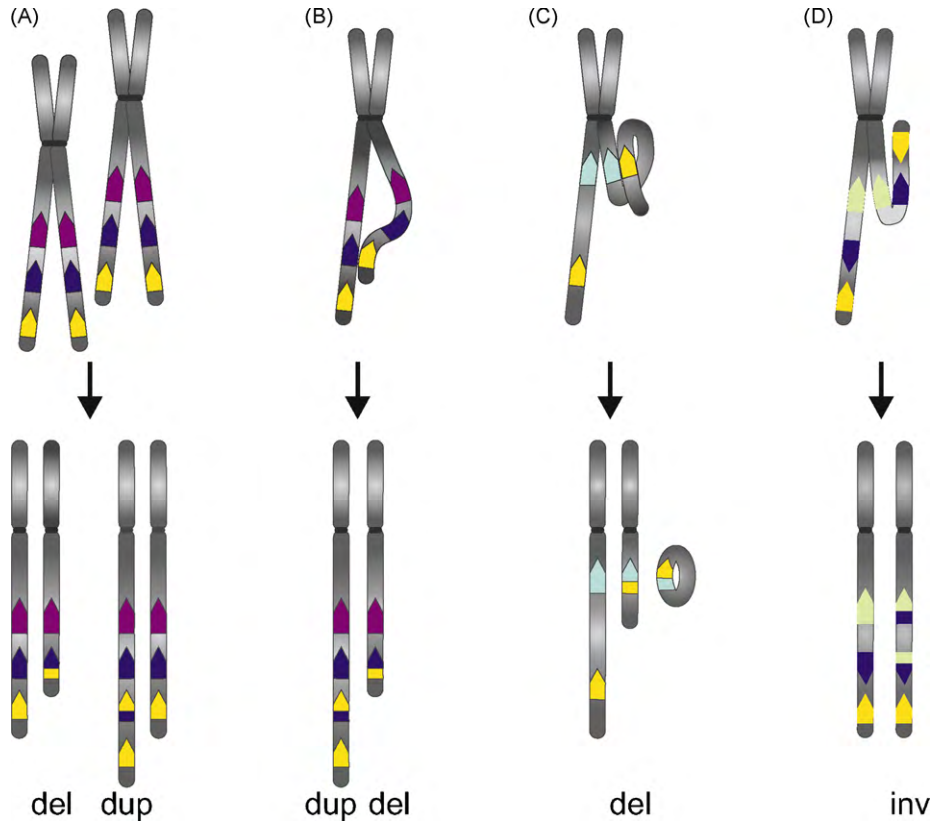

Fig. 3. Typical TE-mediated NAHR models.
The colored arrows represent non-LTR retrotransposons of a given family; e.g. *Alu* elements. The tip of the arrow indicates the 3′ end of the TE. TE-mediated NAHRs create a chimeric TE element (indicated by two different colors within element). The breakage point can be anywhere within the TE. While TE-mediated NAHRs are here shown for two adjacent elements, these events can occur between far removed TE elements in the geography of the chromosome. For A) to C) TEs are in the same orientation; for D) TEs involved in NAHR are inverted. Del stands for deletion, dup for duplication, and inv for inversion. A) Interchromosal TE-mediated NAHR results in reciprocal deletion and duplication. (If two non-homologous chromosomes are involved a translocation can occur.) B) Intrachromosomal TE-mediated NAHR between two sister chromatids creates reciprocal deletion and duplication. C) Intrachromosomal, intrachromatid TE-mediated NAHR produces only a deletion. D) NAHR between two inverted TEs results in inversion of DNA between involved TEs.

genomic instability. Moreover, *de novo Alu* insertions can generate new inverted loci, which pose a potential threat for rearrangements in future human generations. Conceivably, these rearrangements can also occur in somatic cells and potentially contribute to cancer development.

5. TE-mediated recombination and genomic instability

5.1. Role of TE-mediated recombination in the human genome

Chromosomal structural variation is caused by two different general mechanisms: homologous recombination (HR) and NHEJ [89]. HR is highly conserved in a wide array of species, including prokaryotes and eukaryotes, suggesting that HR is a fundamental biological mechanism. Deficiencies involving HR have been associated with cancer development [90,91]. While programmed HR occurs only once during chromosomal crossover in meiosis, HR is commonly involved in the repair of DSBs, preventing an individual from DNA damage (reviewed in [92]). For further information regarding DSB repair mechanisms, we refer to other recent reviews (e.g. [10,92]). If performed accurately, HR will repair the DNA without a trace. However, the process is often undermined by abundant and/or highly homologous sequences including TEs [10,93]. In these instances, two homologous sequences from different genomic locations recombine in a process called non-allelic homologous recombination (NAHR) and consequently cause deletions, duplications, or inversions, as illustrated in Fig. 3 [93–95].

In addition, TE-mediated NAHR can also result in translocations. It would seem reasonable to assume that given the abundance of TEs in the human genome, TE-mediated NAHR translocations would commonly occur by chance. TEs, in particular *Alu* elements, are often found in the vicinity or even within the breakage points of translocations [96]. However, few disease-associated translocations have been identified that clearly suggest the involvement of TE-mediated NAHR (e.g. [97]). This may be due to the requirements of the DSB repair pathways that cause translocations: NHEJ and single strand annealing (SSA) [98]. SSA – which can create *Alu*-mediated NAHR translocations – is likely rarely utilized due to higher sequence homology requirements than usually found between two TEs [98,99]. Instead, NHEJ appears to be the main mechanism in the creation of translocations.

While insertional mutagenesis caused by TEs is occasionally deleterious to the host resulting in genetic disorders including cancer, post-insertional rearrangements of TEs pose altogether a far greater threat to the integrity of the genome [10]. While a TE insertion may disrupt the function of one gene, recombination between two TEs might result in the deletion of a functional region – including several genes, especially if two distant (not necessarily consecutive) TEs are involved. So far, most of our knowledge regarding TE-mediated recombination events is based on germline mutations, some of which cause genetic diseases (also referred to as genomic disorders). Rearrangements involving the germline can be *de novo* or inherited and passed on to future generations [100]. However, somatic structural variation also does occur [101]. TE-mediated NAHRs – both somatic and inherited – have been associated with cancer predisposition and development (e.g. [102–105]). Still, TE-mediated NAHR events are most likely underrepresented in studies involving cancer susceptibility, development, and progression as a consequence of detection challenges.

Comprehensive comparative genomics studies of the human and chimpanzee genomes have shown that L1 and *Alu* elements are commonly involved in TE-mediated recombination events causing deletions [93,94,106]. *Alu* elements are also often involved in NAHR-mediated duplications, as they have been found at chromosomal breakpoints of segmental duplications with a higher frequency (~27%) compared to the average *Alu* density (~10%) in the human genome [107]. Beyond genomic instability caused by *Alu*-mediated NAHRs that cause duplications, segmental duplications themselves represent hotspots for structural variation and genomic instability as they share a very high homology. As a result, they can lead to genetic disease and altered gene expression of genes located within these regions [58,92].

Analysis of the human genome using the chimpanzee genome as a reference indicates that deletions caused by *Alu*-mediated NAHR occur about nine times more often (492 versus 55) than L1-mediated NAHRs in the human genome [93,94]. The analysis of L1-mediated recombination deletions also revealed 18 chimeric L1s showing hallmarks of NHEJ, raising the number of recombination events to 73. In terms of frequency, L1 recombination plays a relatively minor role in the human genome. However, L1-mediated recombination events are significantly larger in size than *Alu*-mediated NAHRs (6132 bp versus 806 bp) [93,94]. L1 recombination-mediated deletions have deleted more DNA sequence from the human genome than the sum of all other TE deletion related events [94]. This includes DNA loss of *Alu* insertion-mediated deletions, L1 insertion-mediated deletions, and *Alu*-mediated NAHR deletions from the human genome over the last 5–6 million years [94]. Thus, L1 recombination-mediated deletions – even though less frequent – are a major contributor to genome instability. In addition, larger recombination events are more likely to involve greater disruption of functional genomic regions, making them more deleterious to the host. Consequently, these events are likely under significant negative selection, which commonly results in the loss of these events from the human population. These deletion events may commonly be so deleterious that affected individuals do not survive to birth. Indeed, to date only a few L1-mediated recombinations have been detected (reviewed in [14]).

5.2. TE-mediated NAHRs in human genetic disorders

TE-mediated NAHR events, in particular those involving *Alu* elements, have been identified in a variety of genetic disorders and play an important role in their genesis (reviewed in [13,16]). Also, several *Alu*-mediated NAHRs with implications for cancer susceptibility have been identified; a subset is shown in Table 2. With few exceptions, NAHR-mediated deletions are more commonly detected than duplications. While in part this might be caused by a detection bias, as deletions are more easily identified compared to duplications, there is also evidence that overall deletions occur more often than duplications (reviewed in [92]). This may in part be explained by the intrachromatid NAHR mechanism which causes only deletions (see Fig. 3).

Through advancements in detection methods for SVs, genomic rearrangements are now recognized in a number of genetic disorders. However, SVs are likely still severely underrepresented due to the required use of more complicated, lengthier, and resource consumptive methods to detect genomic rearrangements. The analysis of the CDH1 gene – associated with hereditary diffuse gastric cancer (HDGC) – is an intriguing example. Up until 2009, only germline single nucleotide or small frameshift mutations were associated with HDGC [108,109]. Yet in individuals with HDGC, mutations were identified in only 30–50% of the cases. Oliveira *et al.* [108] have convincingly shown that some individuals with HDGC harbor structural variations including two *Alu*-mediated NAHRs that disrupt the CDH1 gene. The identification of all mutations including SV in genes associated with cancer is of great importance for the identification of carriers, counseling, appropriate individualized screening and preventive measures. Counseling of breast cancer patients is another intriguing example, as a recent retrospective study found that close to 50% of breast cancer patients with

Table 2
Overview of cancer-associated *Alu*-mediated non-allelic homologous recombination (NAHR) events.

Alu-Alu non-allelic homologous recombination (NAHR) and tumors					
Locus	Meaning	Tumor association	Occurrence	# of events	References
Deletions					
VHL	von Hippel Lindau gene	von Hippel Lindau disease	Germline	Multiple[a]	[102,138]
BRCA1	Breast cancer 1 gene	Breast/ovarian cancer	Germline	Multiple[a]	[105,113], reviewed in [112]
BRCA2	Breast cancer 2 gene	Breast/ovarian cancer	Germline	Few[b]	[105,139]
CHEK	Checkpoint kinase 2 gene	Breast cancer	Germlne	1	[105]
CHEK	Checkpoint kinase 2 gene	Prostate cancer	Germline	1	[140]
MLH 1	MutL E. coli homolog 1 gene	Hereditary non-polyposis colorectal cancer	Germline	Few[c]	[111,141–144]
MSH2	MutS E. coli homolog 2 gene	Hereditary non-polyposis colorectal cancer	Germline	Multiple[a]	[111,142,145–147]
MEN1	Multiple endocrine neoplasia type 1 gene	Multiple endocrine neoplasia type 1	Germline	1	[148]
CDH1	Cadherin 1 gene	Hereditary diffuse gastric cancer	Germline	Few[c]	[108]
RB	Retinobalstoma gene	Association with glioma	Germline	1	[149]
CAD	Caspase activated dnase gene	Hepatoma	Somatic	Recurrent[a]?	[150]
NF1	Neurofibromatosis 1 gene	Neurofibromatosis type 1	Germline	1	[151]
Duplications					
MLL1	Myeloid/lymphoid mixed lineage leukemia gene	Acute myeloid leukemia (AML)	Somatic	Recurrent[a,d]	[103,104,119]
MYB	Transcription factor	T-acute lymphoblasctic leukemia	Somatic	Recurrent[a]?	[118]
BRCA1	Breast cancer 1 gene	Breast cancer/ovarian cancer	Germline	Few[c]	[105,152], reviewed in [112]
BRCA2	Breast cancer 2 gene		Germline	1	[105]
Translocations					
EWSR1-ETV	Ewing sarcoma breakpoint region 1 – ETS variant gene	Ewing sarcoma	Somatic	Recurrent[a]?	[97]

#Number.
[a] More than 10.
[b] More than 1 and less 10.
[c] Commonly detected.
[d] Also detected in healthy blood donors.

BRCA1 mutations eventually develop contra-lateral breast cancer [110].

5.3. Predisposition to TE-mediated NAHRs

It appears that some genes are more prone to TE-mediated NAHRs than others, as multiple recurrent independent recombination events have been identified in these genes [16]. For example, the BRCA1 (and to a lesser extent BRCA2), VHL, MLL1, MLH1, and MSH2 genes encountered recurrent *Alu*-mediated NAHRs (e.g. [102,111,112]). In some genes, 25% or more of cancer predisposing mutations in certain demographic populations are caused by *Alu*-mediated NAHRs (e.g [102,113]). The overall density of *Alu* elements seems to be a contributing factor to recurrent *Alu*-mediated NAHRs as genes with higher than average *Alu* density in general show evidence of a higher *Alu*-mediated NAHR rate. Another factor could be the absence of epigenetic constraints such as CpG methylation and/or histone acetylation.

MLH1 and MSH2, both involved in the mismatch repair system, are two intriguing examples. Point mutations, small insertions and deletions, and genomic rearrangements within these genes are associated with hereditary non-polyposis colorectal cancer, the most common form of inherited colon cancer (e.g. [111,114]). Both genes have a higher than average *Alu* density within their intronic sequence (20% MLH1 and 40% MSH2 compared to 10% genome average) [111]. In the MSH2 gene ~3/4 of rearrangements are caused by *Alu*-mediated NAHRs [111]. In contrast, only about a fourth of the rearrangements of the MLH1 gene contained hallmarks of unequal *Alu* recombination [111]. The majority of the other events show characteristics of NHEJ often with involvement of TEs (in particular *Alu* elements). Another contributing factor to multiple independent *Alu*-mediated NAHRs is the age of the involved *Alu* insertions, as members of the youngest *Alu* subfamily, *Alu*Y, have been disproportionately detected in NAHRs relative to their density in the genome [93]. Occasionally, the same (often young) *Alu* element is involved in several independent NAHRs [102]. This is likely due to a higher homology of the *Alu* element, as younger insertions have on average accumulated fewer point mutations. This is further supported by a recent study that showed that *Alu*Y elements that are fixed in the human genome are associated with an increased local recombination rate [115].

The majority of TE-mediated rearrangements are sporadic and specific to the individual in whom the event was detected, and possibly also in immediate family members. However, some *Alu*-mediated NAHRs founder mutations have been identified in certain populations. For example, out of more than 60 reported BRCA1 rearrangements, the majority of which were caused by unequal recombination of *Alu* elements, six founder mutations have been identified [113,116]. One *Alu*-mediated NAHR event – identified in the Dutch population – accounts for about a quarter of all variations identified in the BRCA1 gene in this demographic population [113]. Due to this founder mutation, the Dutch population has to date the largest fraction of mutations caused by structural variation in the BRCA1 gene. Analysis of the breakage point at the DNA sequence level can discriminate between identical and independent rearrangements. Inherited genomic rearrangements share identical DNA breakage points. The occurrence of two separate identical NAHRs is extraordinarily unlikely. In addition, SNP and/or

microsatellite information in the vicinity of the rearrangement can be used as further support for a shared or separate event.

Rearrangements in the MLL gene are in several ways intriguing. SVs involving this gene are commonly identified in acute myeloid leukemia (AML) [103,104,117]. Beside the recurrence of translocations in certain AML cancers, partial *Alu*-mediated NAHR duplications commonly occur within the coding sequence of this gene [103,117]. The most common in-frame duplication events create in-frame fusions of exons 11 or 12 upstream of exon 5 [103,117]. At the same time, partial deletions involving this region of the genome have not been identified [104]. Thus, it seems likely that another recombination mechanism is involved in the genesis of these partial duplications. Synthesis-dependent strand annealing (see [10]), a repair mechanism of DSBs in mammalian cells, could generate a partial duplication in the MLL1 gene. This mechanism was suggested for the creation of somatic tandem duplications in MYB, a transcription factor that can be associated with T-cell acute lymphoblastic leukemia (T-ALL) [118]. In addition, the MLL gene represents one of a few genes in which several independent somatic *Alu*-mediated NAHRs have been detected. While partial duplications show a high prevalence in patients with AML [103,117], these events have also been identified in hematopoietic cells of healthy blood donors [119]. More recently, it has been shown that suppression of the wild-type allele with expression of the MLL partial duplication allele contributes to the leukemic phenotype [120].

6. L1 induces double strand breaks

Another mechanism by which L1s potentially contribute to genome instability in their human host, is the observation that the L1 endonuclease creates far more DSBs than required for retrotransposition in mammalian cells [43]. To what extent these tissue culture observations will translate to human genomic instability remains elusive, as L1 expression under these experimental conditions was also far greater than under normal physiological conditions. It may not be possible to confirm or refute this mechanism *in vivo* because DSBs caused by L1 are indistinguishable from DSBs caused by other mechanisms [10,43]. Regardless, DSBs compromise DNA integrity, are highly recombinogenic, and exacerbate genomic instability.

7. Methylation status and retrotransposition

In various tumors studied, global demethylation with site-specific hypermethylation has been associated with cancer development and progression (reviewed in [121]). Hypomethylation varies considerably between different cancers, and in some tumor cells hypomethylation is associated with cancer development, while in others with progression [121]. The current model correlates global demethylation with an elevated mutation rate and chromosomal instability [122–124]. TEs including the promoters of L1 elements are often demethylated in cancer cells ([125,126], reviewed in [121]). The methylation of retrotransposons is believed to be a host defense mechanism in somatic cells against ongoing retrotransposition [127]. Indeed, for several cancer cell lines an increased L1 transcription rate has been detected in hypomethylated cancer cells [125,128]. One possible exception may be hepatocellular carcinoma cells, as these cells did not show evidence of this correlation [129].

Several points of evidence further support a correlation between hypomethylation and TE activation and increased recombination rates. The lack of regular *de novo* methylation of LTR and non-LTR retrotransposons through Dnmt3L (DNA methltransferase 3-like) in non-dividing precursors of spermatogonial mouse stem cells resulted in high transcription levels of these TEs and meiotic failure in spermatocytes [130]. Hypomethylated thymic lymphomas in transgenic mice carrying a hypomorphic DNA methyltransferase Dnmt1 allele showed evidence of chromosomal instability [123]. Moreover, somatic retrotransposition of an Intracisternal A Particle, an endogenous retrovirus, into the Notch1 gene was detected in several lymphomas in mice, indicating an activation of endogenous retroviruses through hypomethylation [131]. Finally, a somatic *de novo* L1 insertion into the APC gene has been identified in a human colon tumor [132].

The demethylation of TE promoters (e.g. L1 and ERVs) has possible implications beyond the activation of TE retrotransposition. Through activation of potent TE promoters, transcription factor levels might be globally modified and/or the expression of genes might be altered in the vicinity of demethylated promoters [121]. Moreover, demethylation might result in the activation of the L1 antisense promoter that is also located within the 5′ UTR of a full-length element and which in turn can create chimeric transcripts [133]. Cancer-specific chimeric transcripts derived from L1 antisense promoters have been recently detected [134]. Intriguingly, these transcripts were derived from fixed, older L1s that are likely no longer capable of retrotransposition [134].

8. Conclusions

We have discussed the known manifold roles of TEs with respect to genome instability. Among other things, we have shown that TEs are major contributors to genomic rearrangements. We are just beginning to understand the full impact of TEs upon the genome architecture. It is now recognized that TEs play a role in cancer predisposition, development, and progression. The comprehensive use of recently available technologies such as second-generation sequencing and advanced computational algorithms will allow us to understand the role of TEs in the human genome more deeply. Moreover, it seems highly likely we will soon enter an era of personal genomics. Future projects will greatly increase our understanding of human inter-variability, hotspots of alteration in the human genome, and disease associations. Soon, medical diagnostics and treatment decisions will greatly rely on the analysis of the individual's genome. We are already witnessing the emergence of personalized cancer treatments. In addition, detailed analyses of single cells and/or different tissues are about to become possible for the first time. Using these advancements, we will likely discover a great deal of variation within individual humans. Cancer in particular seems a prime candidate for detailed structural variation studies, as genomic instability and dynamics in genome structure are typical characteristics of cancer cells. A fascinating era employing use of these rapidly evolving technologies lies ahead of us, along with the opportunity to deepen our understanding of TE biology and genome evolution.

Conflict of interest

The authors declare that there are no conflicts of interest.

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