REVIEW

MOBILE GENETIC ELEMENTS AND CANCER. FROM MUTATIONS TO GENE THERAPY

Dedicated to Kyiv and Kyiv region clinical oncologists

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In the present review, an association between cancer and the activity of the non-LTR retroelements L1, *Alu*, and SVA, as well as endogenous retroviruses, in the human genome, is analyzed. Data suggesting that transposons have been involved in embryogenesis and malignization processes, are presented. Events that lead to the activation of mobile elements in mammalian somatic cells, as well as the use of mobile elements in genetic screening and cancer gene therapy, are reviewed.
Key Words: retroelements, transposons, chromosome aberrations, gene therapy.

MOBILE ELEMENTS

Mobile elements (ME), also called junk DNA or transposable elements, are present in the genome of all known eukaryotes. In mammals, MEs make up at least 45% of the genome [1, 2], while their content in other organisms varies from some 2.7% in the fish *Fugu rubripes* [3] to above 90% in some plants [4]. Many authors define MEs as nucleotide sequences capable of changing their position in the host genome [5–7]. Meanwhile, some authors compliment this definition by pointing out the MEs' ability to change also their copy numbers, i.e. to replicate independently from the host genome [8, 9]. Besides, MEs are sometimes referred to as parasitic nucleotide sequences which replicate independently from the host genome and can not be purged of by sexual reproduction [10]. All these definitions, complementing each other, are by no means thorough though, as a versatile definition of MEs would require an exhaustive survey. To summarize, it's also worth accenting that MEs are inherent to the genomes of all organisms, including humans, just like mitochondrial and plastid genomes [11]. Appreciating the role of MEs in their host genomes, they have been portrayed during last years as "genome architects" [12], "genome's treasure" [13], "drivers of evolution" [6, 8], etc. On the one hand, this reflects the understanding of the important role played by MEs. On the other, however, we are yet far from thorough apprehension of their function.

MEs are believed to influence the host genome in several ways. Their active transposition is one of the causative factors of mutation processes [14–16]. MEs' nucleotide sequences can also serve as promoters, enhancers, silencers, as well as sites of epigenetic modifications and alternative splicing, in the host genome [6, 17, 18]. Following molecular domestica-

tion, MEs may lose their autonomy and become part of other host genome's components [19–24]. Large ME numbers in a genome stimulate the formation of deletions, duplications, inversions, or translocations as a result of ectopic recombination [25–27]. Therefore, the effect of MEs on the human genome is diverse: MEs often take part in important genomic functions and provide material for natural selection, and failures and errors in their function lead to genome damage and disease, including cancer.

The general classification based on transposition mechanisms is universal to all eukaryotes [28, 29] and divides MEs into two groups: *transposons* that relocate via the "cut and paste" mechanism, and *retroelements* that make use of RNA intermediates and reverse transcription. The grouping within these classes is also universal to diverse organisms, with retroelements classified into two groups (LTR-, for long terminal repeats, and non-LTR retroelements) and transposons represented by three groups ("rolling circle", "cut and paste", and "self-synthesizing" transposons). However, more detailed classification, on the level of ME families, appears to be host genus-specific [1, 2, 30], though the exceptions of multigenus ME families or families not universal to all the genus members do exist. A number of factors have been pointed out to explain these exceptions, such as horizontal transmission between genera [31], or loss of some ME families during speciation within a genus [32].

Most of non-LTR retroelement families in the human genome are currently inactive, except for three families. LINE-1 (long interspersed nuclear elements), or L1, elements make up near 17% of human genomic DNA, with a total of about 500,000 copies [1]. Full-size L1 elements, stretching for some 6 kb, have two open reading frames encoding proteins required for their transposition and relocation of non-autonomous elements of the SINE (short interspersed nuclear elements) family. L1 have been active in the human genome for near 160 million years [1].

The SINE elements are short (100–400 bp). They contain a promoter for polymerase III and do not encode proteins. The vast majority of known SINE

elements are tRNA derivatives. One exception is the *Alu* element. Over a million copies of this element comprise some 11% of the human genome [1]. This element is specific to primates and has been colonizing primate genomes since 65 million years ago [16].

Another group of non-autonomous retroelements that are active in humans and use the L1 elements' machinery (just like the SINEs) for transposition and are specific to hominids is SVA (SINE-VNTR-*Alu*; VNTR for variable number of tandem repeats) elements [33], which have been colonizing the human genome since relatively recently (less then 25 million years) and currently total near 3,000 copies [34].

NON-LTR ELEMENTS IN TUMOR DEVELOPMENT

A link between the transposition of mobile elements in the human genome and some pathologies, including cancer, was noted a many years ago [28, 35]. For instance, in the 1980s L1 retroelement insertion into the human protooncogene *c-myc* was found in human breast cancinoma cells [35]. Another example of somatic insertions of this mobile element is its integration into the tumor-suppressing gene *apc* (adenomatous polyposis coli), which has been found in colon cancer patients [36]. Insertions of the *Alu* element into the intron of the NF-1 (neurofibromatosis type I) gene lead to a deletion and a reading frame shift in the downstream exon during splicing, which might be associated with neurofibromatosis [37].

ME insertions are not evenly distributed in the genome. There are certain characteristic insertion sites where ME integration is most likely. Thus, the above-mentioned *apc* gene can be target for L1 and *Alu* element insertions [14]. In general, combined L1, *Alu*, and SVA insertions only account for about 0.27% (118 out of 44,000) of all known human mutations [33], so their contribution to mutation processes appears to be rather marginal.

Meanwhile, there are other types of cancer which are linked to MEs indirectly. For example, mobile elements (*Alu*) may play a role in chronic myeloid leukemia, which develops as a result of a translocation between the human chromosomes 9 and 22, as the chromosome breakpoints producing this chromosome aberration contain nucleotide sequences of this element. Therefore, essentially this chromosome aberration results from ectopic recombination between identical sequences of different *Alu* elements [38]. Similarly, an internal tandem duplication of part of the *mll* (myeloid/lymphoid or mixed-lineage leukemia) gene, which results from ectopic recombination between those very *Alu* elements, may trigger a cascade of events which is frequently associated with acute myeloid leukemia [39]. Recombination between *Alu* elements which causes a translocation involving the TRE (USP6, ubiquitin-specific protease 6 (Tre-2 oncogene)) oncogene has been shown to play an important role in Ewing sarcoma development [40]. Being far from complete, this list is still sufficient to illustrate

that ME-linked rearrangements in the human genome may be associated with cancers of various etiology.

THE ROLE OF MEs IN GENOME FUNCTIONING

Although the above-mentioned facts suggest the on involvement of non-LTR mobile elements in tumorigenesis, the question of specific processes that are responsible for triggering ME-linked genome disturbances in the human genome remains open. As it becomes evident from recent studies, there are several ways of ME activation, both in germ and somatic cells. For example, L1 elements are known to actively transpose during early embryogenesis, which is believed to be triggered by total genome demethylation, or the so-called epigenetic reprogramming, which has been shown in muzine primordial cells between the E11.5 and E13.5 early embryo stages [41]. As DNA methylation is known to repress various nucleotide sequences, including L1 elements, demethylation may cause ME activation with the ensuing insertion events. Kano et al. [42] have demonstrated that mRNA from L1 elements transcribed in the parental organism can be passed on through oocytes or sperm cells to progeny where reverse transcription ensures further insertions of the element's copies into the genome of the developing organism during the pre-implantation stage, which leads to somatic mosaicism. It seems, therefore, that at least two ways of L1 activation exist during early stages of mammal development [43]. It can be envisioned that during this early developmental period, as the embryonal cells divide, the retroelement activity aftereffects are tested for compatibility with life. In this way, insertions that survive in somatic tissues create phenotypic diversity without changes in the genome of generative cells. Human neural progenitor cells, in which L1 element activity in embryo brain produces somatic mosaicism [44, 45], are a bright example of this type of somatic retrotranspositions. Such a mosaicism could potentially affect neuron formation and, thus, create individual characteristics and phenotypic diversity of the brain [41]. Therefore, ME activation is rather common during embryogenesis, and retroelement insertions, including those associated with cancer development, may be considered as the cost of phenotypic diversity formation.

There is evidence suggesting that MEs have been important in mammal evolution. In particular, the origin of mammals as a class, specifically the emergence of the genes controlling placenta development, was catered by the domestication of mobile elements [46]. At least 50 of the human genes are known to originate from MEs, predominantly from DNA transposons [1]. Currently active transposons are not known from the human genome, however, as yet mentioned, the human genome contains genes that were formed as a result of transposon domestication [19]. For instance, the genes responsible for somatic diversity formation in the immune system and playing a crucial

role in V(D)J recombination during lymphocyte development (recombination-activating genes RAG1 and RAG2), originate from the nucleotide sequences of the ancient *Transib* superfamily of transposons [47, 48]. The RAG genes still even retain their ability to relocate their nucleotide sequences during V(D)J recombination in the genome of lymphocytes [49]. V(D)J recombination events are biochemically similar to the transposition of the *Hermes* family of transposons, such as *hobo, Activator,* and *Tam3*, which relocate via the "cut-and-paste" mechanism [50]. In fact, a nucleotide sequence fragment cut out during V(D)J recombination resembles transposon DNA, being though, unlike the latter, circularized. Fragments cut out by the RAG proteins usually degrade. However, sometimes the proteins can reinsert these fragments into other sites in the genome [51–53]. Such insertions have been demonstrated, for example, in the *hprt* (hypoxanthine-guanine phosphoribosyl transferase) locus in human T cells *in vivo* [54]. In human cell culture, the frequency of such insertions, according to different estimates, may be 1 per 13,000–50,000 recombination events. If this rate also holds for human lymphocytes, this means 10,000 insertions in a human organism each day [55]. Of course, this rate may well turn out to be an overestimation which cannot be directly extrapolated from cell culture to an organism. However a link between these events and B- and T-cell malignization in the human organism can be tentatively presumed.

Although specific health consequences of RAG-mediated insertions in blood lymphocytes have not been reported so far, a link between V(D)J recombination and the onset of cancer associated with chromosome rearrangements induced by the recombination has been demonstrated [56, 57]. RAG proteins may induce double-strand breaks (DSB) in sites similar in their structure to signal sequences for V(D)J recombination. Such DSBs in DNA are potential players in recombination of the genes or receptors of mature T and B cells. This, in turn, entails deviations in the expression of such protooncogenes as LMO2 (LIM domain only 2 (rhombotin-like 1)) and BCL2 (B-cell lymphoma 2).

However, the list of cancer-linked chromosome rearrangements extends beyond those caused by defects in the functioning of the V(D)J recombination genes. Oncogenic chromosome rearrangements can be formed at fragile chromosome sites due to imperfect functioning of the NHEJ (non-homologous end joining) and homologous recombination reparation systems. The breakpoints during oncogenic translocations, deletions, and other chromosome rearrangements often localize in/near the nucleotide sequences of *Alu* elements [26]. Such events are referred to as cancer-linked *Alu*-mediated events of non-allelic homologous recombination (NAHR). Among such rearrangements, deletions are the most common, duplications occur less frequently, and translocations are the rarest [9]. The existence of ectopic recombination between *Alu*

sequences leading to DNA deletions in germ cells is beyond doubt today, and still such events are rare in somatic cells [58] (see also the examples of acute myeloid leukemia and Ewing sarcoma above). The presence of an *Alu* sequence itself has been found to have little effect. It is the type of this sequence, provided that a recombination-initiating DSB forms within it, that determines what scenario will NHEJ or SSA (single strand annealing) reparation follow [59, 60]. And this, eventually, may determine the final type and complexity of the rearrangement.

ME ACTIVITY AND ENVIRONMENTAL FACTORS

The activity of L1 and L1-dependent MEs may be affected by environmental factors, which can activate the elements. Several chemicals containing mercury (HgS), cadmium (CdS), and nickel (NiO) have been found to elevate the activity of L1 three times in human cell culture [61]. Meanwhile nickel chloride, which increases L1 activity 2.5 times, has no direct effect on the sequence of the element or its proteins, but instead inhibits DNA reparation systems, which eventually leads to L1 transpositions [62]. In general, active ME transposition in various living organisms is known to be induced by a number of environmental factors, like heat shock, viral infection, poisons, detergents, other chemicals, energy metabolism abnormalities, etc [63]. ME transcription and transposition rates have also been found to increase under γ irradiation [64, 65]. Indirectly, through ME activation, therefore, all these agents, as well as those yet not studied for ME activity effects, could potentially contribute to human carcinogenesis. This effect of external factors on ME-mediated carcinogenesis is further supported by the geographic patterns found in these events. For example, a number of studies link the rates of BRCA2 gene expression specific to Portugal population to *Alu* activity [66, 67].

ENDOGENOUS RETROVIRUSES AND CARCINOGENESIS

So far we described ME effects on carcinogenesis caused by transposons and non-LTR retroelements in humans. Another group of mobile elements known to be linked to cancer is human endogenous retroviruses (HERVs). These belong to LTR retroelements and make up near 8.3% of the human genome, with a total of 0.3×10^6 copies. This group of elements is the most diverse one in the human genome and comprises as much as 6 superfamilies, three of which being currently inactive [68]. The structure of these elements incorporates modified main retroviral structural components in the order 5'-gag-pro-pol-env-3'. The *gag* gene encodes the matrix and capsid proteins, *pro* — a protease, *pol* — a reverse transcriptase, the RNAse H and an integrase, and *env* — the envelop proteins. Alongside with these genes, endogenous retroviruses may have other, non-structural genes [69]. Endogenous retroviruses originate from ancient infections, however now they have lost their ability to form self-

contained infectious entities. Still, there is evidence suggesting that at times the infectious property may form spontaneously during cell division [70].

The HTLV-1 (human T cell leukemia virus) retrovirus is known to cause monoclonal leukemia in 1–2% of infected persons, with the latent period sometimes reaching up to 50 years. Proteins of this virus can speed up cell proliferation by interacting with some genes [71]. The retrovirus HTLV-2 is also known to have some carcinogenic potential [72]. High titers of the retrovirus XMRV (xenotrophic murine leukemia virus) have also been detected in patients with prostate carcinoma [73–75].

Data from literature indicate that HERVs are responsible for at least 2 types of human pathologies — autoimmunity and cancer. Animal oncogenic viruses are believed to be able to transform normal cells via three different mechanisms: a) multiplication of an endogenous virus which requires a co-infection with a wild-type virus to provide the necessary machinery, b) insertional mutagenesis interrupting proper functioning of tumor-suppressing genes, c) regulation of the expression of genes controlling cell proliferation and some other processes. All these three mechanisms can only be used by viruses capable of being transferred horizontally, like MLV (mouse leukemia virus), MMTV (mouse mammary tumor virus), FeLV (feline leukemia virus), PERVs (porcine endogenous retroviruses), KoRV (koala retrovirus) [76, 77]. HERV can also influence tumor development indirectly via the immunosuppressive function of the Env proteins. This property has been reported for these proteins in HERV-K, Moloney MLV, and MPMV (Mason-Pfizer monkey virus) [78, 79].

Therefore, based on these data, the HERV activity can be assumed to serve as a co-factor in a complex involved in the multi-step process of tumor development in humans.

ME BEHAVIOR IN THE TUMOR CELL GENOME

The genomic behavior of MEs in transformed tissues deserves separate examination, as it differs from that in normal cells. For instance, the activity of L1 and HERV are known to be higher in tumor cells compared to normal cells, which might potentially lead to higher mutation rates in tumor cells. Rates of recombination are also notably higher in tumor cells, which might partially explain the high rate of chromosome rearrangements in these cells [80, 81]. The activity of MEs in somatic and germinal cells are controlled by a number of repression systems, like post-transcriptional silencing via RNA interference and chromatin modifications [82]. To become activated MEs need to elude this control. As chromatin (both DNA and proteins) is often hypomethylated in tumor cells, which changes its conformation, L1 and HERV promoters may be released with the ensuing he activation of the elements [83]. Also, tumor cells are known to contain significantly lower quantities of micro RNAs [84]. Micro RNAs are involved in RNA interference, so this repression

mechanism is quenched in cancerous cells. Interestingly, high titers of HERV-K RNA and high activity of the reverse transcriptase have been reported in patients with certain forms of lymphomas and breast cancer [85]. Transcripts of the gene *Np9* of the endogenous retrovirus K are found in 50% of cell cultures established from germ cell cancers as well as breast cancer and leukemia tissues [86]. HERV-K-like viruses have been found in human melanomas [87]; iRNA and proteins of these endogenous viruses have been isolated from primary melanomas, melanoma metastases, and cultured melanoma cells [88]. However, the question of the causative nature of this system remains open, i.e., whether it's that increased retrovirus titer that causes tissue transformation or *vice versa*.

Therefore, while MEs may be linked to cancer development, they themselves can get activated by the cell malignization processes, the latter promoting increased mutation and recombination rates in the genome of the transformed cells.

TRANSPOSONS AS A MEANS OF GENETIC SCREENING

Insertional mutagenesis is a tool for identification of genes involved in different functional cellular processes [89]. However, this approach is practically impossible on humans, except, perhaps, for cell cultures. So the most common mammalian models are mice and rats, in which insertional mutagenesis is a means of genetic screening of cell components involved in malignization. In this way, retroviruses are used for identification of mouse cancer-associated genes [90]. Oncogenic retroviruses are represented by two classes: transforming retroviruses invoking the development of acute polyclonal tumor during 2–3 weeks after infection [91] and transforming retroviruses causing non-acute mono- and oligoclonal tumor with the latent period up to 12 month. The latter integrate into the host cell's genome via insertions, and it's these retroviruses that are used in genetic screenings for malignization-linked genes in mammals [92]. However, the applicability of this approach is limited by the insertional predilection of these retroviruses to integrate into the genomes of blood and mammary cells [93].

DNA transposons, which are active in the genomes of many invertebrates, are inactive in vertebrates. These mobile elements have become the basis for genetically engineered transposons capable of transposing in mammalian tissues [94, 95], which has opened a unique perspective for applying such synthetic mobile elements in insertional mutagenesis to reveal as many mammalian (and human) cancer-related genes as possible. The *Sleeping Beauty (SB)* transposon of the *TC1/mariner* family, for instance, was constructed based on an inactive element from fish optimized to transpose in multi-cellular systems, including mouse stem cells [96]. Another transposon, *PiggyBac (PB)*, originating from the cabbage looper *Trichoplusia ni*, has recently been constructed with

the ability to efficiently transpose in mammal cells [97]. Other synthetic transposons have also been constructed (like *Tol2, Mos1, Frog Prince* etc), but *SB* and *PB* have been found to be the most adequate for cancer research [98]. These two transposons differ in that *PB* can carry longer DNA fragments, it has a weaker tendency to transpose locally, and does not leave undesired "footprints" at the sites it cuts off from. *SB* and *PB* also prefer a little different integration sites [99].

These approaches have resulted in eliciting over 20 types of tumors and the identification of new candidate cell malignization-associated genes. Therefore, the main tumorigenesis-controlling mechanisms can be assumed to involve a certain combination of promoters and their genes [100].

TRANSPOSONS AND CANCER GENE THERAPY

Gene therapy is being increasingly applied in cancer treatment. Classic ways to achieve stable expression of alien genes in vertebrates are founded on various methods of gene construct delivery in cell culture, like transfection [101] by electroporation [102], sonoporation [103], needleless injection [104], etc. The main problems with these approaches center around the low integration efficiency and unstable expression of the constructs, which can be explained by the injected DNA concatemerization preceding its integration into the genome [105]. Another problem is that the transgenic cell groups are mosaic. γ retroviral and lentiviral vectors have also been used to integrate foreign DNA into the tumor cell chromosomes [106]. The drawbacks of using such vectors stem from their profound mutagenic effects [107] and the risk of an immune response in patients subjected to this type of gene therapy.

Meanwhile, transposons-based techniques avoid all these problems and ensure safe and non-toxic expression of inserted sequences. For example, the SB transposons-based vectors have successfully been used to deliver the genes *sFlt-1* (soluble vascular endothelial growth factor receptor) and *statin-AE* (angiostatin-endostatin fusion gene) into the human glioblastoma. Such transformation decreased the tumor size and increased the proportion of animals that survived [108]. Antigen-specific T-cells containing receptors to the genes *p53* and MART-1, which had been constructed using an SB-based vector, demonstrated stable expression (50% of the cells) and were functionally efficient against tumor cells [109]. Today, a new generation of "hyperactive" SB-based vectors is used, like SB100X [110–112]. A bright example of the efficiency of such vectors comes from another study in which Kang et al. [113] applied gene-directed enzyme-prodrug therapy (GDEPT) using a PB-based vector to treat ovarian adenocarcinoma. Based on their results, the authors argue that PB is the most efficient transposon for stable genomic integration among the known mammal systems. Whether or not, there exists

a kind of "improvement race" among different vector systems [114] whereby the systems become more and more efficient, and so there is a hope that this race will end up in some reliable cancer treatment techniques.

CONCLUSION

Our understanding of the role of MEs in tumorigenesis has evolved from factors involved in tumor development to methods of genetic screening of cell components involved in malignization and eventually to gene therapy of various forms of cancer. Now, it has become evident that the role of MEs in the initiation of some tumor types in vertebrates should be considered as an inevitable consequence of their vast genomic involvement in the generation of somatic cell diversity. So, like every benefit in nature, the evolutionary contributions of MEs to the host genome come at a price.

ACKNOWLEDGMENT

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