Hepatic Oncology

Retrotransposons and genetic instability in hepatocellular carcinoma

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“L1 elements can play a major role in the genomic instability of cells, as L1s can act as ‘insertional mutagens’ owing to their ability to mobilize themselves and integrate randomly in the genome.”

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Genomic stability is a crucial aspect of cell renewal for faithful perpetuation of cellular DNA and is thus maintained by various repair mechanisms. However, there are several factors leading to increased genomic instability which, if cells survive, can lead to cellular transformation. Cancer is a genetic disease and genomic instability is one of the hallmark causative factors in human cancers [1]. Genomic instability can be caused by extrinsic factors, such as exposure to carcinogens causing genotoxic stress, or intrinsic factors, such as defects in repair pathways or cell cycle checkpoints. Depending upon the causative agent, genomic instability can result in mutations due to defective nucleotide or base excision repair or mismatch repair; chromosome instability due to defects in chromosome segregation apparatus during cell division; chromosomal rearrangements, copy number variations and loss of heterozygosity due to hyper-recombination, single-stranded DNA gaps or double-strand breaks generated as a consequence of replication stress or repeat elements [2,3].

About half of our genome is composed of repeat elements which can be broadly classed as DNA transposon or retrotransposon (copy via RNA intermediate). Among retrotransposons, long interspersed elements-1 (LINE-1 or L1s) account for approximately 17% and short interspersed elements (SINEs), such as Alu and SVA (SINE-VNTR-Alu) elements account for about 11% of the human genome. Due to the great number of identical copies, L1s and SINEs can act as sites of homologous recombination between heterologous sites and lead to genomic rearrangements. L1 elements can play a major role in the genomic instability of cells, as L1s can act as ‘insertional mutagens’ owing to their ability to mobilize themselves and integrate randomly in the genome [4]. There are about 100,000 copies of L1 elements in a human genome but the vast majority of them are inactive due to 5' truncation, internal rearrangements or several internal mutations in their open reading frames. Still, on an average, the human genome harbors about 80–100 full-length

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potentially active L1 elements. Most often than not, the L1 elements' promoter regions in somatic cells are silenced by various epigenetic mechanisms and post-transcriptional regulation, which serves to inhibit the mobilization of these elements and genomic stability is largely maintained [5]. However during embryogenesis, there is a phase of epigenetic reprogramming when epigenetic marks are removed for a time to reset back to a base level. At this time of vulnerability, L1 elements get an opportunity to express themselves and mobilize, leading to somatic mosaicism. Retrotransposons have played a major role in human genome evolution, and despite the genome's defense mechanisms, are still responsible for approximately 0.3% of new human germline diseases, in other words, about one germline retrotransposition event of Alu, L1 and SVA in 20, 200 and 900 births, respectively [6].

Under various pathological conditions, especially those associated with inflammation and malignant transformation, cells lose their defense mechanisms. This allows previously silenced L1 elements to become transcriptionally active, leading to *de novo* insertion of L1 elements and the mobilization of other sequences (host mRNAs and repeat sequences). This leads to retro-pseudogene formation and amplification of other repeat sequences, such as Alu and SVA elements [4]. Upon activation, an L1 element is transcribed as a bicistronic transcript which codes for two proteins: L1-ORF1p (RNA-binding protein) and L1-ORF2p (containing endonuclease and reverse transcriptase activities). Both the proteins are required for active retrotransposition in cis for an L1 element and can also be hijacked by other transcripts and used to facilitate their reverse transcription and reintegration. Occasionally, L1 insertions can lead to large genomic deletions or transduction of flanking DNA sequences, further contributing toward genomic rearrangements [7,8]. Besides insertional mutagenesis, an active L1 element can influence expression of nearby genes due to the presence of an antisense promoter in its 5'-untranslated region. Recently, a 216-bp long translation-competent ORF0 has been identified originating from the antisense promoter [9]. Hence, depending upon the locus of an active L1 element, even the activation of a single L1 promoter can have drastic consequences for genomic stability. Thus, not only full length retrotransposition-competent L1 elements pose a danger, but

individual L1 promoters can also influence cell fate in a plethora of ways. Moreover, some truncated (promoter-less) L1s can get transcribed by nearby gene promoters and L1-ORF2p is by itself sufficient to facilitate retrotransposition of Alu elements [10]. L1-ORF2p also has endonuclease activity and its overexpression leads to single strand breaks in genomic DNA which has been observed by γ H2AX staining in cells *in vitro* [11]. This emphasizes the contribution of active L1 elements in inducing DNA damage and genomic instability.

Transcriptional activation of L1 elements and active retrotransposition is evident in various human tumors of epithelial origin such as colorectal, breast, prostate, ovarian, lung and liver. Hepatocellular carcinoma (HCC) generally develops on a background of chronic liver disease and accounts for about 80% of primary liver cancer cases. Global hypomethylation is a key feature of various cancer types including HCC. L1 promoter hypomethylation has been observed in HCC tumor tissue compared with the surrounding nontumor tissue [12]. L1-ORF1p protein is expressed in several tumor types and about 20% of HCC cases were found to be positive for L1-ORF1p, in a study where immunohistochemical analysis was carried out on archived tissue microarray blocks [13]. However, no correlations were drawn with any clinical data and this ratio is rendered to vary depending upon the HCC background in other words, associated etiology. Furthermore, upregulation of L1 transcripts and active retrotransposition has been well-documented in HCC cases related with Hepatitis B and C Viruses [14]. Upon retrotransposition, L1 elements integrate into *de novo* sites and depending upon the locus, the influence on cellular fate is decided. For example, *de novo* L1 insertion has been observed in an intron of *ST18* where it happens to interrupt a cis-regulatory element thus disrupting a negative feedback loop leading to overexpression of *ST18*. Eventually, *ST18* was found to act as an oncogene in HCC [15], thus *de novo* L1 insertion at this locus might have directly contributed to cellular transformation or tumor progression. In the same study, L1 element insertions were observed in *MCC* as germline insertions in 3 out of 19 patients. *MCC* is a negative regulator of β -catenin and in these tumors, β -catenin was found to be upregulated, thus the presence of an L1 element in an intron of *MCC* might have predisposed these individuals toward

tumor development. Another example of germline insertion influencing HCC prognosis is activation of an L1 element in the second intron of *cMet* [16]. The active L1 promoter leads to the generation of an L1 chimeric transcript, L1-*cMet*. Another example of an L1 chimeric transcript has been described in Hepatitis B Virus-related HCC, where a 647bp HBx-L1 transcript has been detected and shown to promote cell mobility in HCC cells through epithelial–mesenchymal transition and it promoted chemical-induced hepatocarcinogenesis in a mouse model via Wnt/ β -catenin pathway activation [17]. Hence, retrotransposition-associated insertional mutagenesis can contribute to cancer via somatic mutations or germline polymorphisms, either of which can influence intracellular oncogenic pathways leading to the onset of malignant transformation.

Besides generating genomic instability directly, L1 elements influence signaling pathways involved in carcinogenesis through interaction with host proteins. For example, L1-ORF1p has been shown to interact with Smad4 and promote proliferation in HepG2 cells. Also, downregulation of L1 elements in HepG2 led to suppression of xenograft growth in nude mice, thus underscoring the role of L1 elements in liver tumorigenesis [18]. Moreover, knockdown of L1 elements can lead to decrease in expression of *cMyc* and *Klf-4*, key transcription factors regulating telomerase expression. Hence, L1 elements' expression negatively regulates telomerase

activity and their knockdown can reduce tumor survival via shortening of telomere length [19]. Likewise, the treatment of various cancer cell lines and mouse models with compounds having antireverse transcriptase activity have provided enough evidence to consider L1-encoded reverse transcriptase as a novel target in cancer biology [20].

Reactivation of retrotransposons contributes toward genetic instability through chromosomal rearrangements and chromosome instability leading to HCC progression and adverse prognosis, thus controlling transposon activity may be an effective adjunct in cancer management. Hence, further work is needed to understand the mechanisms of retrotransposon activation so as to gain insight into more potential therapies.

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