LINE-1 activity and regulation in cancer

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1. ABSTRACT

LINE-1 retrotransposons are ubiquitous genetic elements interspersed within the primary nuclear genome of modern day humans. Although shorter LINE-1-derived sequences occupy nearly one-in-five nucleotides of our genome, we are just beginning to appreciate the link between these important genetic elements and cancer, perhaps the most well-studied major degenerative disorder affecting humanity today. Herein, I summarize empirical observations linking LINE-1 to tumorigenesis. The work is organized into three major parts. First, I provide an overview of LINE-1 activity in cancer; highlighting major features of LINE-1 life-cycle such as: promoter methylation, transcription, translation, and retrotransposition. Second, I discuss three genetic pathways - epigenetic regulation, interferon signaling, and genome integrity – as they relate to LINE-1 regulation in cancer. Finally, I review most recent body of work linking LINE-1 as not only a diagnostic cancer biomarker, but also a potential therapeutic target.

2. INTRODUCTION

Causal relationships between cancer-related phenotypes and underlying genotype have traditionally been studied using both genetic engineering and epidemiological studies. Because there are likely a couple of hundred retrotransposition-competent active LINE-1 retrotransposons in our genome (1; 2) systematic inactivation of LINE-1 by gene knockout experiments have not been attempted to date. Given this limitation, epidemiologic studies do show a possible putative role of LINE-1 in cancer; a recent review on the topic finds that there is some evidence linking LINE-1 expression to tumorigenesis in several major human cancer types (3; 4). Of note, for an authoritative recent review on mobile DNA in various diseases, not just cancer, the readership is referred to (5).

To date, 1,057 articles focusing on LINE-1 retrotransposons and cancer are available on Pubmed archive. Of those, I identified and manually curated 35 articles focusing on experimental evidence examining some aspect of LINE-1 biology in cancer. Because of widespread availability of bisulfite-treated DNA-based assays in clinical research, vast majority of published reports are studies focusing on LINE-1 promoter hypomethylation as a proxy biomarker for genome-wide DNA hypomethylation, which is in itself a major hallmark of cancer. Of note, LINE-1 promoter-based studies have recently been reviewed in detail here (6). To arrive at a short list of 35 articles, I asked the following three questions: “Is the body of work contributing to mechanistic insight rather than simply reporting LINE-1 as a cancer biomarker?”, “What are the genes that likely modulate LINE-1 activation in cancer?”, and “Is there any evidence that LINE-1 targeting provides putative therapeutic benefit?”. Small portion, or 9 out of 35 articles, link LINE-1 expression in cancer to a potpourri collection of unique empirical observations, such as: micronuclei formation (7), LINE-1
as pre-diagnostic biomarker within circulating tumor cells/tumor DNA (8; 9; 10), gene expression profiles linked to LINE-1 expression (11), putative mechanism of LINE-1-mediated cellular transformation (12; 13), as well as induction of apoptosis and proliferation following LINE-1 expression in cancer (14; 15). To my mind, these nine studies represent preliminary discovery-based work that may prove to be of interest in the future.

3. ON LINE-1 TRANSCRIPTIONAL ACTIVATION IN CANCER

Recent seminal work by Scott et al., 2016, discovered and documented the actual mechanism of how LINE-1 retrotransposon transcriptional activation can drive, at least in part, colorectal carcinoma (16). Studying only the second reported case of APC inactivation by LINE-1 retrotransposition, the authors revealed that LINE-1 insertion in one APC allele complements traditional single nucleotide variant-mediated inactivation of the other allele of APC. Specifically, one allele was inactivated by introduction of unequivocally deleterious premature stop codon, resulting in premature termination at p.R1450*; whereas the other allele was inactivated by LINE-1 mediated somatic retrotransposition of one APC allele by LINE-1 insertion event some 160 bp. By comparing the unique singleton mutation patterns of 295 sequence variants in full-length LINE-1 retrotransposons to that of the LINE-1 insert, the authors identified candidate LINE-1 retrotransposon source, which is located at genome position, chr 17:18776467, of the Hg19 Human Genome assembly. Another important body of work is by Phillippe et al., 2016, identified that only very selected LINE-1 retrotransposons, unique to each individual, contribute to transcriptional activation and subsequently to the bulk of LINE-1 protein expression (17). As high-throughput technology, specifically PacBio-based DNA sequencers, are more widely used in cancer research, we will begin to understand more clearly just when and how LINE-1 transcriptional activation occurs in cancer.

4. ON LINE-1 ENCODED PROTEINS IN CANCER

Work on LINE-1 encoded proteins expression in cancer has largely been based on ORF1p profiling in many human cancer types. Nearly half of all cancers in human support LINE-1 ORF1p expression, which presents pre-diagnostic and/or diagnostic utility in clinical practice today (18). More interestingly, there is evidence that ORF2p encoded reverse transcriptase activity plays a necessary role in TMPRSS2 and ETV1 gene fusion (19), and growth of prostate cancer (19). Finally, there is also evidence that ORF1p expression induces hTERT in tumor cells (20).

5. ON LINE-1 RETROTRANSPOSITION IN CANCER

Despite panoply of intronic and intergenic LINE-1 retrotranspositions in several visceral cancers - including pancreatic ductal adenocarcinoma, colorectal carcinoma, esophageal carcinoma, and prostatic carcinoma – there is limited evidence for LINE-1 retrotransposition causing abberant gene regulation (21; 22; 23; 24). The single example of unequivocal deleterious LINE-1 retrotransposition in cancer has been aforementioned report by Scott et al., 2015 (16), whereby the authors identified that one APC allele was inactivated by traditional single nucleotide mutation and the other allele was mutated by LINE-1 insertion; providing evidence for the classic two-hit hypothesis for mutating APC gene.

6. ON LINE-1-RELATED GENE REGULATORY NETWORKS

The subgroup of 20 identified research articles collectively identify 18 unique genes whose expression is empirically linked to LINE-1 expression in cancer. The gene symbols representing the genes in question are as follows: AID, APOBEC3D, APOBEC3G, DNMT1, DNMT3B, ERCC4, ETS1, IFNA1R, IFNB1, IL-6, PIWILINE-1, RAD21, RB, RNASEL, SIRT6, TERT, TP53, and UHRF1. To better organize the discussion, I performed a biological pathway analysis using a free online database termed Reactome. The interactive server identified that 9 out of 18 genes belong into two groups of distinct biological pathways. The pathways identified pathways are as follows: (i) epigenetic regulation pathway (DNMT1, DNMT3b, UHRF1, and ETS1); and (ii) interferon signaling pathway (IFNA1R, IFNB1, RB, TERT, and IL-6) (Table 1). Remaining 8 genes were determined to belong to the genome integrity pathway.

6.1. ON LINE-1-related epigenetic regulation pathway

There is compelling empirical evidence that genome-wide LINE-1 promoter methylation is dependent on molecular pathway that entails MUC1, DNMT1, and DNMT3B genes (25). Specifically, Li et al., 2015, performed both loss-of-function experiments (by MUC1 shRNA) and gain-of-function experiments (by vector expressing MUC1) to observe that MUC1 is transcriptional activator of both DNMT1 and DNMT3B promoters. In turn, MUC1 expression also causes genome-wide LINE-1 promoter hypomethylation in cultured cancer cells. Nakamura et al., 2016, reported that UHRF1 overexpression led to genome-wide LINE-1 promoter hypomethylation, as well as that, conversely, siRNA UHRF1 knockdown increased the global LINE-1 promoter DNA methylation (26).
Table 1. Biological processes related to L1 retrotransposons

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<th>Epigenetic Regulation</th>
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<td>• DNMT1</td>
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<td>• DNMT3B</td>
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<th>Interferon Signaling</th>
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<td>• IFNA1R</td>
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<td>• IFNB1</td>
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<th>Genome Integrity</th>
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<td>• APOBEC3D</td>
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<td>• APOBEC3DE</td>
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<td>• ERCC4</td>
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<td>• PIWIL1</td>
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<td>• RAD21</td>
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<tr>
<td>• RNASEL</td>
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<td>• SIRT6</td>
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<td>• TP53</td>
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Li et al., 2014, found out that ectopic LINE-1 ORF1p overexpression caused increased RNA expression of ETS1 related genes as well as promoted cell proliferation and anchor-independent growth of LoVo cancer cell line (27). The same group showed that LINE-1 siRNA caused decreased RNA expression of ETS1 related genes as well as reduced the proliferation and anchor-independent growth ability of LoVo cancer cell line. Taken together, there is empirical evidence that genes involved in epigenetic regulation pathway also regulate LINE-1 retrotransposons.

6.2. ON LINE-1-relazzted interferon signaling pathway

Aschacher et al., 2016, observed that LINE-1 knockdown decreases TERT function as measured by both telomere dysfunction foci and telomerase activity (20). Conversely, LINE-1 overexpression increased telomerase activity. Yu et al., 2015, discovered that IFNB1 treatment of cancer cell lines inhibits LINE-1 retrotransposition (28). In addition, knockdown of IFNA1R led to increase in LINE-1 retrotransposition. Separate work by Ishak et al., 2016, shows that genetically engineered mice carrying F832A mutation in RB1 causes both genomewide upregulation of LINE-1 RNA in somatic tissues as well as increased susceptibility to leukemia (29). Taken together, the composite work by Aschacher et al., Yu et al., and Ishak et al., is, to my mind, a collection of the most compelling empirical evidence to date that LINE-1 retrotransposon modify an important aspect of human tumorigenesis. Of note, Gasche et al., 2011, identified that IL-6 treatment of cancer cell line induced genomewide LINE-1 promoter hypomethylation (30).

6.3. ON LINE-1-related genome integrity pathway

The third group of genes associated with LINE-1 biology belong to genome integrity pathway, whose functions include DNA repair and innate cell immunity against viruses. A series of independent reports document the notion that some members of APOBEC (“apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like”) cytidine deaminases regulate LINE-1 retrotransposition in somatic tissue. Specifically, Servant et al., 2017, showed that ERCC4 limits LINE-1 retrotransposition, suggesting that other core components of the nucleotide excision repair (NER) pathway may also play a similar role (31). Liang et al., 2016, also reported that ectopic expression on both APOBEC3 and APOBEC3DE limit LINE-1 retrotransposition (32). Koyama et al., 2013, showed that APOBEC3G inhibit LINE-1 retrotransposition (33). Similarly, Metzner et al., 2012, also showed that AID
inhibits LINE-1 retrotransposition (34). To conclude, multiple studies show that several cytidine deaminase enzyme isoforms regulate LINE-1 retrotransposition in cancer.

Several other genes involved in repair of DNA double-stranded breaks affect LINE-1 retrotransposition. Two studies support the idea that TP53 protein also limits LINE-1 retrotransposition. First, Haoudi et al., 2004, proposed a dichotomous idea whereby LINE-1 retrotransposition in wild-type TP53 cancer cell line causes apoptosis, but not in cancer cell line carrying mutated TP53 allele (35). More recently, Wyulie et al., 2016, presented multiple lines of empirical evidence including gene complementation studies and genetic fish studies to support an assertion that TP53 restricts LINE-1 retrotransposition (36). Xu et al., 2014, reported that Rad21 expression induces increase in LINE-1 retrotransposition, as well as that Rad21 knockdown by siRNA causes reduction in LINE-1 retrotransposition (37). Van Meter et al., 2014, discovered that expression of SIRT6 recruits KAP1 to LINE-1 promoters, thus inducing LINE-1 transcriptional silencing (38). In addition, the authors also observed that senescent cells harbor lower SIRT6 levels and comparably higher LINE-1 RNA levels, relative to young cells. Zhang et al., 2014, examined the role of RNASEL on LINE-1 retrotransposition (39). Expression of wild type RNASEL, but not catalytically inactive protein, reduced many aspects of LINE-1 biology including LINE-1 RNA expression, ORF1p and ORF2p expression, and LINE-1 retrotransposition. Wang et al., 2015, made an interesting observation that PIWILINE-1 protein level is decreased in melanoma cell line with metastatic potential (40). Importantly, overexpression of PIWILINE-1 caused LINE-1 promoter hypermethylation (41).

Taken together, biological pathways affecting epigenetic regulation, interferon signaling, and genome integrity play a role in regulating LINE-1 retrotransposon suppression in somatic tissues and aberrations of some of these genes may, at least in part, be the underlying cause of LINE-1 activity in cancer.

7. ON LINE-1 AS PUTATIVE THERAPEUTIC TARGET IN CANCER

The greatest clinical utility/benefit of any biomarker, is not whether or not a given analyte solely provides diagnostic and/or prognostic information, but is knowing if it can be used as a therapeutic target. To broach that question, a hallmark preclinical studies by Sciamanna et al., 2005 uncovered that pharmacologic LINE-1 inhibition by two reverse transcriptase inhibitors slows down the growth of malignant melanoma and prostatic carcinoma cells (19). Follow-up study by Carlini et al., 2010 similarly demonstrated efficacy of reverse transcription inhibition of prostate cancer growth (42). Most importantly, a clinical trial showed that efavirenz provides therapeutic benefit by increasing progression free survival in a cohort of 53 patients with high-stage castration-resistant prostate cancer (43). The most recent review on the topic suggests that LINE-1 inhibition with reverse transcriptase inhibitors may not only slow down the progression of prostate cancer, but may also play a role in preventing the initiation of tumor growth (44).

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