Long Interspersed Element-1 Methylation Level as a Prognostic Biomarker in Gastrointestinal Cancers

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Abstract

Epigenetic changes play a crucial role in human cancer development. DNA methylation is a central epigenetic process that regulates levels of gene expression. Changes in DNA methylation that occur in human tumors include global DNA hypomethylation and site-specific CpG island promoter hypermethylation. Long interspersed element-1 (LINE-1) is a repetitive DNA retrotransposon that duplicates via a copy-and-paste genetic mechanism. As LINE-1 constitutes approximately 17% of the human genome, the extent of LINE-1 methylation is regarded as a surrogate marker of global DNA methylation. In a variety of gastrointestinal (GI) cancers, LINE-1 hypomethylation is strongly associated with a poor prognosis, supporting its potential role as a prognostic biomarker. In this article, we summarize current knowledge regarding LINE-1 methylation and its prognostic impact in GI cancers.

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Introduction

Epigenetics is a promising and expanding research field in human cancer, and can be defined as the study of heritable changes in gene expression that do not involve changes to the underlying DNA sequence. One of the most widely studied epigenetic changes is DNA methylation, which is the postreplicative addition of a methyl group to the 5th carbon of the cytosine ring in CpG dinucleotides. CpG dinucleotides are widely and nonuniformly distributed throughout the human genome, and typically occur at a frequency of approximately one per 80 dinucleotides. However, isolated CpG-rich regions, referred to as “CpG islands,” comprise 1–2% of the human genome. An estimated 45,000 CpG islands are located close to the promoter regions of various genes. DNA hypermethylation at promoter CpG

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sites suppresses the expression of tumor suppressor genes, thus contributing to cancer development and progression [1].

A second crucial DNA methylation change is genome-wide DNA hypomethylation. Global DNA hypomethylation plays an important role in multistep tumorigenesis in a variety of ways. Accumulating evidence indicates that DNA hypomethylation of repetitive sequences (i.e., short interspersed transposable elements [SINEs or Alu elements] or long interspersed transposable elements [LINEs]) predisposes cells to chromosomal defects and rearrangements that lead to genetic instability [2]. Such increases in chromosomal instability contribute to cancer development and progression. Importantly, given that LINE-1 constitutes a substantial proportion (approximately 17%) of the human genome [3], the degree of LINE-1 methylation is regarded as a surrogate marker of global DNA methylation.

In this review, we summarize the accumulated evidence for LINE-1 methylation level as a prognostic biomarker of gastrointestinal (GI) cancers.

What Is LINE-1?

Repetitive sequences constitute approximately half of the human genome and are subdivided into 2 principal types. One is the tandem repeat, or satellite, in which each repeat unit is immediately adjacent to another [4]. The other consists of interspersed repeats, which are repeated sequences that are spread throughout the genome [5]. Interspersed repeats are derived from transposable elements or mobile DNAs. The transposable elements comprise DNA transposons and retrotransposons, which are subdivided into sequences that contain long terminal repeats (LTR) and those that do not (non-LTR). The majority of mobile element activity in humans likely involves non-LTR retrotransposons, typified by LINE-1. LINE-1 is amplified in the human genome to more than 500,000 copies [6] and is present in a truncated form (mean length = 0.9 kb) on both homologous chromosomes in the human genome. A limited proportion of LINE-1 exists in a potentially active form as full-length elements (~6 kb). Thus, LINE-1 is regarded as a noninformative miscellany from the genetic past, and is often referred to as “junk” or “parasitic” DNA. However, accumulating evidence suggests an important role for LINE-1 in various cellular processes. LINE-1 can continuously rearrange the genome, thereby influencing the expression status of genes in different ways. LINE-1 may induce genetic vari-

ation and polymorphism through the recombination and rearrangement as well as through endogenous mutagenesis [7]. In addition, LINE-1 expression can contribute to transcription disruption, insertion mutations, or DNA breaks, leading to genomic instability in cancer cells. The antisense promoter of LINE-1 can change gene expression status by modulating the transcriptional activity of surrounding genes [8].

Methylation Status of LINE-1

Full-length LINE-1 possesses an internal promoter in its 5'UTR, which ranges from +1 to 909 bp. The initial 460 bp region of the 5'UTR includes 29 CpG sites, the methylation status of which has been extensively examined and shown to be high. Importantly, LINE-1 methylation levels in normal tissues strongly depend on tissue type: the range of LINE-1 methylation levels in certain tissues (e.g., esophagus, thyroid) is broad, while in other tissues (e.g., liver, kidney, breast, stomach, lung) it is limited [9]. In addition, LINE-1 methylation status has been proposed to differ at individual genomic locations: a study in human cancer cell lines has shown that methylation levels vary at 9 LINE-1 loci [10]. Another study evaluated the methylation patterns of 17 LINE-1 loci in several cell types and found that the methylation levels at these loci can be influenced differentially, depending on the location of the particular sequences in the genome [11]. Collectively, the variable methylation statuses of different sets of LINE-1 loci may give rise to different cellular phenotypes.

In 1993, Thayer et al. [12] demonstrated the methylation status of LINE-1 in cancer cells. Since then, tumoral LINE-1 hypomethylation has been reported in various human tumors, including those of the prostate, ovary, head and neck, lung, and testicle. Other research utilizing a broad range of cancer cell lines and clinical samples has demonstrated the wide variability of LINE-1 hypomethylation levels among normal and cancer tissue types [13].

LINE-1 Methylation Level and Prognosis in GI Cancers

Numerous studies have focused on the relationship between LINE-1 methylation level and clinical outcome in patients with human GI cancers (Table 1). In 2008, Ogino et al. [14] were the first to demonstrate that the extent of LINE-1 hypomethylation was linearly associat-

Table 1. LINE-1 hypomethylation and patient prognosis in GI cancers

Cancer type	Number of cases	Selection criterion	Prognostic impact of hypomethylation	Reference
Colon cancer	643	Stage III only	Unfavorable	[14]
Colon cancer	161		Unfavorable	[15]
Colorectal cancer	343		Unfavorable	[16]
Colorectal cancer	207	MSI cases only	Unfavorable	[17]
Colon cancer	129	Patients receiving adjuvant FOLFOX	Unfavorable	[19]
Colorectal cancer	336	Patients receiving adjuvant FOLFOX	Unfavorable	[20]
Colorectal cancer	40	Patients receiving adjuvant FOLFOX	Unfavorable	[21]
Colon cancer	164	Stage II only	Unfavorable	[18]
Gastric cancer	203	Advanced cases only	Unfavorable	[22]
Gastric cancer	198		Unfavorable	[24]
Gastric cancer	434		Unfavorable	[23]
Esophageal cancer	217	Squamous cell carcinoma only	Unfavorable	[25]
Esophageal cancer	105	Squamous cell carcinoma only	Unfavorable	[26]
HCC	208		Unfavorable	[27]
HCC	75		Unfavorable	[28]
Pancreatic cancer	126		No impact	[29]

HCC, hepatocellular carcinoma; MSI, microsatellite instability.

ed with aggressive tumor behavior, in an analysis of 2 independent prospective cohorts with 643 colon cancers. They observed an approximately fivefold difference in cancer-specific mortality, as LINE-1 methylation in the tumor genes ranged from high to low [14]. Subsequent studies have confirmed the relationship between LINE-1 hypomethylation and poor prognosis in resected colorectal cancers [15–18]. In addition, LINE-1 hypomethylation has been reported to correlate with unfavorable outcomes in colon cancer patients receiving oxaliplatin with fluorouracil and folinic acid adjuvant chemotherapy [19–21].

Three studies have described the prognostic value of LINE-1 hypomethylation in patients with gastric cancer. Our group has reported that LINE-1 hypomethylation was associated with shorter survival, utilizing a database of more than 200 cases of gastric cancer, thus suggesting its potential for use as a prognostic biomarker [22]. This was in agreement with the findings of research groups in Korea [23, 24]. Similarly, regarding esophageal squamous cell carcinoma (ESCC), we showed that LINE-1 hypomethylation was significantly associated with lower disease-free survival and cancer-specific survival [25]. This finding was confirmed by Kawano et al. [26].

With respect to hepato-biliary-pancreatic cancers, Harada et al. [27] showed a significantly higher cancer recurrence rate in a LINE-1 hypomethylation group than in a

hypermethylation group in a database of 208 hepatocellular carcinomas. Similar results in hepatocellular carcinoma have since been reported [28].

Only one study to date has focused on LINE-1 hypomethylation in pancreatic cancer. Interestingly, unlike in the other GI cancers, LINE-1 hypomethylation was not significantly associated with overall survival, cancer-specific survival, or disease-free survival in 126 patients with pancreatic cancer [29]. This discrepancy may reflect differences in tumor histology. The effect of LINE-1 methylation level on the survival of pancreatic cancer patients therefore needs to be confirmed in a larger cohort study.

LINE-1 Hypomethylation and Aggressive Tumor Behavior

The mechanism by which LINE-1 hypomethylation affects aggressive tumor behavior remains unclear. The link between LINE-1 hypomethylation and genomic instability represents one possible mechanism. Gaudet et al. [2] showed that transgenic mice carrying a hypomorphic DNA methyltransferase 1 displayed substantial genome-wide hypomethylation in all tissues. Interestingly, the mice developed aggressive T-cell lymphomas with a high frequency of chromosome 15 trisomy, thus supporting a causal role for DNA hypomethylation in

tumor formation via the promotion of chromosomal instability [2]. In our previous study, we investigated LINE-1-hypomethylated and LINE-1-hypermethylated ESCC tumors using array-based comparative genomic hybridization. LINE-1 hypomethylated tumors showed a high frequency of genomic gains at various loci containing candidate oncogenes such as *CDK6*. Taken together, these data suggest that global DNA hypomethylation in ESCC contributes to the acquisition of aggressive tumor behavior through the amplification of oncogenes such as *CDK6* [30]. Another possible mechanism is transcriptional dysregulation, which might activate proto-oncogenes, endogenous retroviruses, or transposable elements that influence tumor aggressiveness. Given the epigenetic regulation of microRNA in human cancers, global DNA hypomethylation might contribute to the acquisition of aggressive tumor behavior via the aberrant expression of microRNA. Furthermore, in addition to acting as a surrogate marker for global DNA methylation, LINE-1 methylation status itself may exert biological effects. As noted above, LINE-1 elements are retrotransposons, which represent alternative promoters and contribute to non-coding RNA expression, thereby

leading to the regulation of the function of multiple genes. Additional studies are needed to identify other potential mechanism(s) by which genome-wide DNA hypomethylation affects tumor behavior.

Conclusions

LINE-1 hypomethylation is associated with poorer prognosis in almost all types of GI cancer, supporting a role for LINE-1 methylation level as a prognostic biomarker. Importantly, epigenetic changes, unlike irreversible genetic changes, may represent reversible molecular targets for both cancer therapy and chemoprevention. Further investigations in this field should provide deeper insights into GI cancer development and contribute to the development of novel therapeutic strategies against these cancers.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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