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5-methylcytosine hydroxylation-mediated LINE-1 hypomethylation: a novel mechanism of proto-oncogenes activation in colorectal cancer?

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Commentary

The human genome comprises the most predominant long interspersed nuclear element-1 (LINE-1) sequences, which are non-long-terminal repeat retrotransposons. The majority of LINE-1 repeats are inactivated through truncation, inversion, and mutation [1]. Importantly, DNA methylation attenuates the transcriptional activation, amplification, and recombination of LINE-1 sequences, thereby maintaining genome stability [2]. Indeed, global DNA hypomethylation has been associated with increased genomic instability and tumorigenesis, suggesting a more important role for hypomethylation in human cancers than the hypermethylation-induced downregulation of some tumor suppressor genes [3, 4, 5]. LINE-1 hypomethylation has been shown to associate with the clinicogenetic features of multiple myeloma [6] and chronic myeloid leukemia [7], poor prognosis in non-small cell lung cancer [8], and with more aggressive progression of colorectal cancer (CRC)[9].

The full-length LINE-1 sequence (6 Kb) includes a sense promoter regulating transcription of two open reading frames and an antisense promoter (ASP) in the 5'-UTR region, which contains a CpG island– usually hypermethylated in normal cells– regulating transcription of adjacent genes in the opposite direction [10, 11, 12]. The ASP has been reported to function as an alternative transcription start site for several proto-oncogenes, whose activation can lead to tumorigenesis in several types of cancer [11, 13]. Additionally, Wolff and colleagues [14] reported that hypomethylation of the promoter of a specific LINE-1 (L1-MET)

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sequence, located within the *c-MET* oncogene, induces transcriptional activation of *c-MET* through an alternative transcription start site in human bladder cancer. Also, hypomethylation of L1-MET promoter was directly associated with increased mRNA and protein levels of c-MET in chronic myeloid leukemia patients [7]. In the present study by Hur and colleagues [15], the authors reported the first evidence of progressively increasing hypomethylation of specific LINE-1 retrotransposable elements, located within introns of protooncogenes, in human primary CRC and liver metastasis. Specifically, they demonstrated that hypomethylation of LINE-1 ASP induces transcriptional activation of *c-MET*, *RAB3IP*, and *CHRM3* oncogenes, which occurs more frequently in liver metastases than primary CRC. The increased c-MET protein expression levels in liver metastases were directly correlated with higher ASP-regulated L1-MET mRNA expression. Of note, c-MET protein expression was correlated with neither c-MET host promoter methylation nor the host c-MET transcript levels; rather it was regulated by methylation status of the LINE-1 ASP. Although *c-MET* gene amplification has been reported previously as the activation mechanism of c-MET expression in CRC [16, 17], Hur et al. [15] ruled out this mechanism as no significant differences in *c-MET* gene copy numbers were observed between matched primary CRC and liver metastases. The findings strongly suggest that while global *c-MET* gene amplification is implicated in the development of primary colorectal tumorigenesis, CRC metastasis may be associated with the activation of specific L1-MET mRNA expression through the hypomethylation of LINE-1 ASP. Taken together, Hur and colleagues [15] provided additional insights into the molecular mechanism underlying the activation of the *c-MET* proto-oncogene and underscored the importance of LINE-1 ASP hypomethylation in the induction of this key proto-oncogene in CRC and liver metastasis.

Although the role of DNA hypomethylation in activating proto-oncogenes such as *c-MET* has been established in several types of cancer [7, 14], little was known about the molecular mechanism that regulates DNA hypomethylation. 5-hydroxymethylcytosine (5-hmc), which is an oxidized product of 5-methylcytosine (5-mc), is generated by the Ten-Eleven-Translocation family of oxygenases that catalyze the transfer of a hydroxyl group [18]. Several reports suggested that the 5-hmc mediates the catalytic conversion of 5-mc to 5-c (5-cytosine), thereby promoting DNA demethylation by inhibiting the formation of chromatin remodeling associated complexes [19, 20, 21]. In this context, Hur and colleagues [15] were the first to interrogate the correlation between the presence of 5-hmc and hypomethylation of LINE-1 sequences in cancer, in an attempt to determine the role of 5-mc hydroxylation in regulating DNA hypomethylation. The findings demonstrated that LINE-1 sequence-specific 5-hmc is directly related to LINE-1 hypomethylation in CRC tissues. Collectively, the authors proposed a model indicating the role of LINE-1 hypomethylation in CRC progression and metastasis. Accordingly, DNA hypomethylation of CpG sites is induced by the conversion of 5-mc to 5c through an intermediate hydroxylation step to generate 5-hmc, which prevents binding of DNA methylation enzymes such as DNMT1 that mediates DNA hypermethylation. This subsequently leads to decreased DNA methylation and accumulation of 5-c. The conversion of 5-mc to 5-c at the constitutively hypomethylated ASP of LINE-1 sequences leads to activation of several downstream proto-oncogenes such as *c-MET*, and possibly the development of CRC metastasis.

The study by Hur and colleagues underscored the role of 5-methylcytosine hydroxylation as a potential mechanism of DNA hypomethylation and activation of protooncogenes in CRC progression and metastasis [15]. Future studies will be important to further investigate the functional and molecular aspects of the proposed mechanism in CRC and other types of tumors. Notably, it is plausible that other epigenetic mechanisms that regulate chromatin remodeling and methylation of LINE-1 retrotransposons could activate downstream protooncogenes in cancer. Nonetheless, this study highlights the previously unrecognized potential of LINE-1 hypomethylation as a diagnostic and prognostic biomarker for cancer.

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