

HYPOTHESIS

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A promising hypothesis of c-KIT methylation/ expression paradox in c-KIT (+) squamous cell carcinoma of uterine cervix ——— CTCF transcriptional repressor regulates c-KIT proto-oncogene expression

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Abstract

We recently reported one interesting case showing mutation-free *c-KIT* proto-oncogene overexpression and paradoxical hypermethylation in 54 cases of primary squamous cell carcinoma of uterine cervix (SCC). However, its molecular mechanisms still remain unknown. We propose the hypothesis that increased methylation at the CpG islands on the promoter near the first exon region might interfere with the binding of CTCF repressor with *c-KIT* promoter that regulates *c-KIT* proto-oncogene expression in such case. Further studies focusing on the status of epigenetic modifications of mutation-free c-KIT (+) tumors are encouraged.

Keywords: Squamous cell carcinoma of uterine cervix, c-KIT proto-oncogene, CTCF repressor

Background

Epigenetic modifications may occur that result in changes in gene expression. Epigenomes represent an attractive therapeutic target. Current use of agents targeting epigenetic changes has become an interesting topic in cancer research. Additional evidence suggests that DNA methyltransferase inhibitors may serve as efficient chemo- and radiosensitizers in solid tumors [1]. Furthermore, tight association of DNA methylation and silencing of gene expression have been already established. Hypomethylation is the mechanism for ectopic oncogene activation whereas hypermethylation is the mechanism for tumor suppressor gene inactivation [2, 3]. In 54 cases of primary squamous cell carcinoma of uterine cervix (SCC) of

uterine cervix, we recently demonstrated one case showing mutation-free, over-expression and paradoxical hypermethylation of the *c-KIT* proto-oncogene [4–6]. Within this afore-mentioned unusual case (no. 26), the DNA methylation ratio of the promoter near the first exon region in c-KIT (+) tumor area (26 T) was higher than that in adjacent c-KIT (-) non-tumor cervical epithelium (26 N). On the other hand, when comparing two cases with different c-KIT expression (no. 12 and no. 26), the DNA methylation ratio in the c-KIT (+) tumor area (26 T) was higher than that in another randomly selected c-KIT (-) tumor area (12 T) and adjacent c-KIT (-) non-tumor cervical epithelium (12 N) [4]. However, the molecular mechanisms for this difference still remain unknown.

The analysis of results from MethHC, a database of DNA methylation and mRNA expression profiles in human cancer showed that hyper-methylation in the promoter region of *c-KIT* proto-oncogene induced the down-regulation of gene expression in most cancer tissues such as colon adenocarcinoma. Conversely, the increased DNA

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methylation ratio in upstream 500 bps of promoter region enhanced the expression of *c-KIT* proto-oncogene in uterine corpus endometrial carcinoma [7, 8]. This phenomenon is similar to our previous findings in the unusual uterine cervix carcinoma case [4]. Intriguingly, this is counter to the current understanding that aberrant DNA methylation tends to silencing of genes.

Transcriptional repressor CTCF also known as 11-zinc finger protein or CCCTC-binding factor is a transcription factor in humans that is encoded by the CTCF gene [9]. Recent references demonstrated that absent or un-bound CTCF was associated with increased DNA methylation at a gene promoter in either normal or cancer cells [10, 11]. In addition, Lai et al. demonstrated that DNA methylation can activate the expression of CTCF-silenced oncogene BCL6 via blocking the binding of CTCF repressor to the first intron region [12]. Conversely, inhibition of DNA methyltransferase decreases BCL6 transcription due to the binding of CTCF to DNA in the methylation-sensitive region. Our previous studies and cumulative references suggested that increased methylation at the CpG islands, which are found on the promoter near the first exon region, might interfere with the binding of CTCF repressor with *c-KIT* promoter that regulates *c-KIT* proto-oncogene expression in such a case.

Presentation of the hypothesis

In this report, we propose a hypothesis for the “paradox” phenomenon between aberrant methylation and expression of *c-KIT* proto-oncogene in SCC uterine cervix. Increased methylation at the CpG islands of *c-KIT* promoter might interfere with the binding of certain repressor proteins such as CTCF transcriptional repressor (TR)

that regulate *c-KIT* proto-oncogene expression. In general, CTCF binds to the CTCF-binding site on the promoter near the first exon region, as shown in Fig. 1. Subsequently, the hypo-methylation of the promoter region results in the silencing of the *c-KIT* proto-oncogene expression through interference with other transcription factors (TFs) to bind the promoter region. When CTCF-binding sites are partly methylated during tumorigenesis in uterine cervix, CTCF is unable to bind the methylated promoter region and loses the repressive function. Therefore, the TFs can smoothly bind to the promoter region and drive *c-KIT* proto-oncogene expression. In addition, when the CpG islands of the *c-KIT* promoter region are hyper-methylated, CTCF and TFs both are unable to bind to their binding sites and then the *c-KIT* proto-oncogene is silenced again (Fig. 1).

Testing the hypothesis

- (1) To test this hypothesis, we must first sequence the methylation status of full length CpG islands of *c-KIT* proto-oncogene on promoter regions in several candidate cervical cancer cells by using bisulfide sequencing or pyrosequencing, and then correlate DNA methylation with the expression levels of *c-KIT* proto-oncogene in these cells. For our testing cell model, we will select the cells that are both hypermethylated at the CpG islands on the promoter near the first exon region and also express higher levels of *c-KIT* mRNA measured by quantitative RT-PCR.
- (2) Second, we will treat the cells with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine

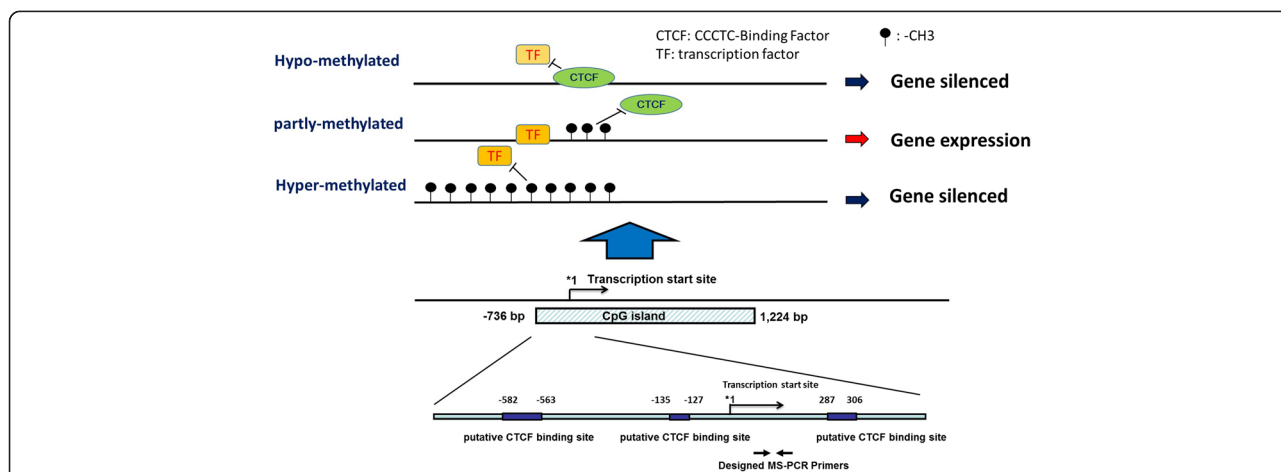


Fig. 1 The analysis of the *c-KIT* proto-oncogene promoter region and the prediction of CTCF binding sites. The *c-KIT* proto-oncogene contains a long CpG island including promoter, first exon and first region ranging from -736 bp to 1224 bp. The CpG island region contains at least three putative transcriptional repressor CTCF binding sites predicted by the CTCFBSDB 2.0 database [13–15]. The methylation-specific (MS) PCR primer sets were designed to be located in the region between the first Exon and first intron by using MethPrimer software [16, 17]. The blue box indicates the putative CTCF binding sites. *1 indicates the transcription start site of *c-KIT* proto-oncogene

(5-Aza-C), and test the change in expression level of the *c-KIT* proto-oncogene by quantitative RT-PCR. We hope to find that the expression of *c-KIT* proto-oncogene is related to the methylation of CpG islands, which are found on the promoter near the first exon region containing three putative CTCF-binding sites.

- (3) Third, we will analyze the amount of CTCF binding to the promoter region with three putative CTCF-binding sites by using a chromatin immunoprecipitation assay with specific primers to test cells treated with various concentration of 5-Aza-C. We will observe that the amount of CTCF binding to the promoter region increases with greater doses of 5-Aza-C. This will mean that de-methylation on the promoter region enhances the binding activity of CTCF on the promoter region with three putative CTCF-binding sites.
- (4) Fourth, we will intensify or diminish the expression of CTCF and then measure the expression of *c-KIT* proto-oncogene to prove whether CTCF could directly bind to the promoter region to regulate the expression of *c-KIT* proto-oncogene.
- (5) Finally, we will assay the DNA methylation status and gene expression of *c-KIT* proto-oncogene in the large-scale clinical samples, and correlate the relationship between DNA methylation status and gene expression in SCC of the uterine cervix.

Implications of the hypothesis

We propose the hypothesis that increased methylation at the CpG islands might interfere with the binding of the CTCF repressor with the *c-KIT* promoter which regulates *c-KIT* proto-oncogene expression in *c-KIT* (+) squamous cell carcinoma of uterine cervix. The paradoxical hypermethylation of the *c-KIT* promoter here suggests that therapeutic strategies using demethylating drugs could be effective against *c-KIT* (+) SCC of uterine cervix. Even though the low frequency (1/54 = 1.86 %) of mutation-free *c-KIT* proto-oncogene over-expression and aberrant methylation suggests that our result may not be representative of a larger population, further studies that focus on the status of epigenetic modifications of mutation-free *c-KIT* (+) tumors are still merited to clarify this point of view.

Abbreviation

SCC: squamous cell carcinoma of uterine cervix.

Competing interests

The authors declare that there are no competing interests.

Authors' contributions

CPH and AR wrote the manuscript; WRC and SWC and PHW supported the work; JCL proposed the idea. All authors read and approved the final manuscript.

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