BRIEF COMMUNICATIONS

Methylation Profile of Von Hippel Lindau Gene Promoter in Acute Lymphoblastic Leukemia Patients

Received: January 2018
Accepted: May 2018

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ABSTRACT

Background: DNA methylation is an epigenetic mechanism that often modifies the function of genes and affects gene expression, which may sometimes lead to cancer development. Tumor suppressor genes such as VHL (Von Hippel-Lindau) may be silenced when affected by epigenetic alteration. This study investigated the DNA methylation of VHL gene in ALL (Acute Lymphoblastic Leukemia) patients with methylation specific PCR (MSP).

Methods: The DNA of peripheral blood and bone marrow cells of 26 ALL patients and 26 healthy control subjects were extracted, treated with bisulfite, and VHL gene methylation was subsequently analyzed using the MSP technique.

Results: None of the patients showed signs of methylation in the VHL gene.

Conclusion: Due to the lack of methylation in the VHL gene promoter in patients and in the healthy control group, it is unlikely that the methylation of this gene can be used in the diagnosis and prognosis of ALL patients.

Keywords: Acute Lymphoblastic Leukemia, von Hippel-Lindau Gene, DNA Methylation, Epigenetics

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BCCR
2018; 10(2):49-53
www.bccrjournal.com

INTRODUCTION:

Leukemia is the most common childhood cancer, and acute lymphoblastic leukemia (ALL) is the most common subtype that accounts for 75–80% of all cases[1]. Genetic changes are central to the development of leukemia, but they are not the only mechanism to distort gene expression. Epigenetic alterations such as DNA methylation could also contribute to this calamity. Previous studies have shown that aberrant promoter methylation of some genes is associated with ALL[2]. Aggire X. et al., 2003 demonstrated the correlation between methylation of the TP53 gene and ALL[3]. Epigenetics essentially control the genome using factors other than DNA sequence. This is mostly achieved through down-regulation of genes[4]. DNA methylation, the most common epigenetic process, is a chemical procedure in which a methyl group is added to a specific part of the DNA. This always occurs on a site known as CpG, where a cytosine is situated next to a guanine base, with a phosphate between them. In mammals, 70-80% of CpG sites are methylated[5]. There are some regions near the promoter called CpG islands that have a higher concentration of CpG and are not methylated in normal cells and yet are highly methylated in neoplastic cells. This abnormality suggested DNA methylation as a cancer diagnostic and therapeutic target[6]. VHL gene is a tumor suppressor gene that codes the VHL protein. In copious amounts of oxygen, this protein, which is an E3 ubiquitin ligase, marks HIF (Hypoxia Inducible Factor) for termination. Studies show that in VHL deficiency, HIF contributes to cancer development, and therefore VHL has been studied in many malignancies such as renal cell carcinoma, pheochromocytoma, and hemangioblastoma[7-9]. However, to the best of our knowledge, no study has been undertaken to elucidate the correlation between VHL methylation and ALL.
Considering the association between this tumor suppressor gene and numerous malignancies, in this study we assessed its methylation in ALL patients to see if it can be used as a therapeutic target in these patients.

METHODS:

The Subjects

This was a case-control study conducted in Shariati Hospital in Tehran. Twenty-six ALL patients and 26 healthy subjects were enrolled in the study. The patients were diagnosed with ALL based on a complete blood count test, a BM biopsy, and subsequent immunology and cytogenetic tests. Any patient who had already begun therapy was excluded. There were 2 B-ALL, 7 T-ALL, and 17 Pre B-ALL patients. The control group comprised 9 children and 17 adults whose BM biopsy and flow cytometry showed no signs of malignancy.

DNA Extraction

The DNA of the peripheral blood and bone marrow cells of patients and healthy donors was extracted following protocol (Qiagen, USA) and its concentration was ultimately measured using a Nanodrop device. Extracted DNA was treated with sodium bisulfite (Qiagen, USA).

MSP and gel electrophoresis

Two sets of primers were crafted for the VHL gene using Methprimer software (**Table1**). For each sample, one set of primers was specifically designed for methylated DNA sequences and the other pair for unmethylated DNA sequence. PCR (Eppendorf, Germany) was then carried out, and MSP-amplified DNA was immediately separated using 2.5% agarose gel electrophoresis. Products were then visualized under ultraviolet light.

RESULTS:

Results from electrophoresis show that in all ALL patients, there is a 200bp product with non-methylat-

Table 1:Forward and reverse methylated and non-methylated primer sequences were crafted using Methprimer software

M.Forward	TGGAGGATTTTTTTGCGTACGC
M.Reverse	GAACCGAACGCCGCGAA
U.Forward	GTTGGAGGATTTTTTTGTGTATGT
U.Reverse	CCCAAACCAAACACCACAAA

ed-specific primers, but no band was seen containing methylated primers. Results from negative adult and children control groups confirmed that subjects in the control group and patients had non-methylated sequences for the VHL gene promoter (**Figure 1**). Also, methylated and un-methylated controls vindicates the occurrence of errors in the reaction, and the negative control disproves contamination.

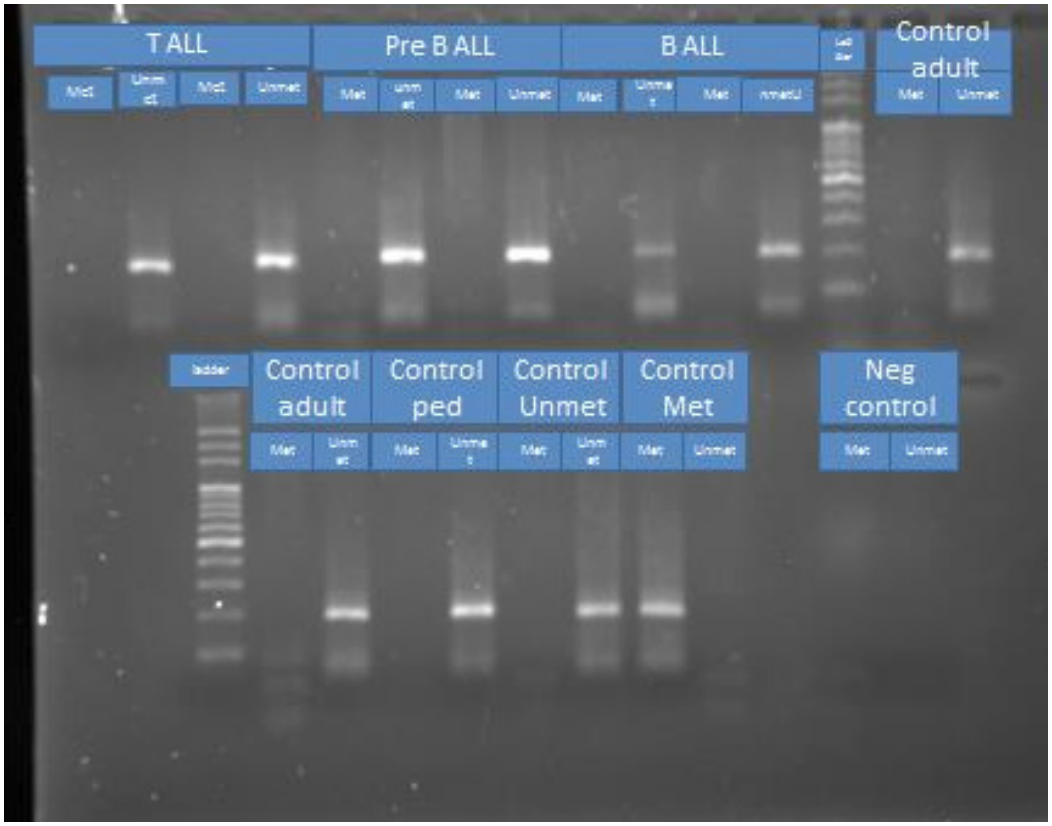

Figure 1. MSP results in patients and control groups. Observing a band in un-methylated sections for ALL and Control adult and Control ped means that VHL promoter of ALL patients and healthy adults and children was not methylated. Control Unmet and Control Met illustrate that PCR reaction was done correctly and Neg control excludes any contamination.

DISCUSSION:

Statistically speaking, the rate of cancer in Iran is on the rise. According to the Iran Ministry of Health, leukemia is the sixth most common cancer in Iran with a 5.76% incidence rate[10]. In the initial profiling study of adult ALL, ten genes such as p15 and p16 etc. were analyzed in a cohort study on 80 patients with ALL. Approximately, up to 85% of patients had methylation of at least one of these genes and 40% had methylation of 3 or more[11]. In a study by Corcoran M. et al. a strong correlation between promoter methylation and transcriptional silencing was shown for certain individual gene promoters such as ZAP70 and HOXA4 in CLL patients[12]. VHL methylation has also been researched in multiple myeloma showing aberrant methylation in plasma cell malignancies[13]. In a study by Azad M. et al. 2013, MSP results for P15 gene before and after the differentiation showed partial methylation[14]. Therefore, it is safe to say that DNA methylation plays a role in many malignancies. Studies using only MSP to analyze methylation will inevitably fail to detect methylation restricted to specific regions of CpG islands and as such imply that methylation cannot be excluded without bisulfite sequence analysis of the entire CpG Island[12]. Utilizing MSP, this study was performed to determine methylation of VHL as a tumor suppressor gene in ALL patients. Our results demonstrate that there was no methylation in any of the samples.

CONCLUSION:

Given the fact that no subjects showed signs of methylation of the VHL gene, it is possible that VHL methylation may not be connected to ALL pathogenesis. It is recommended that further studies be conducted with more sensitive techniques such as LINE-1/pyrosequencing or LC-MS/MS and with a larger sample size.

CONFLICT OF INTEREST:

There are no conflicts of interest to declare.

ETHICAL APPROVAL:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

ACKNOWLEDGEMENT:

We would like to thank Tehran University of Medical Sciences for supporting this project.

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