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Aberrant DNA methylation of key genes and Acute Lymphoblastic Leukemia

Mina Rahmani[a,b], Mehdi Talebi[c], Majid Farshdousti Hagh[c,d], Abbas A.Hosseinpour Feizi[c], Saeed Solali[a,e,f,*]

[a] Department of Immunology, Division of Hematology and Transfusion Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Stem cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

DNA methylation is a dynamic process influencing gene expression by altering either coding or non-coding loci. Despite advances in treatment of Acute Lymphoblastic Leukemia (ALL); relapse occurs in approximately 20% of patients. Nowadays, epigenetic factors are considered as one of the most effective mechanisms in pathogenesis of malignancies. These factors are reversible elements which can be potentially regarded as therapy targets and disease prognosis. DNA methylation, which primarily serves as transcriptional suppressor, mostly occurs in CpG islands of the gene promoter regions. This was shown as a key epigenetic factor in inactivating various tumor suppressor genes during cancer initiation and progression.

We aimed to review methylation status of key genes involved in hematopoietic malignancies such as *IKZF1, CDKN2B, TET2, CYP1B1, SALL4, DLC1, DLX* family, *TP73*, *PTPN6*, and *CDKN1C*; and their significance in pathogenesis of ALL. The DNA methylation alterations in promoter regions of the genes have been shown to play crucial roles in tumorigenesis. Methylation -based inactivation of these genes has also been reported as associated with prognosis in acute leukemia. In this review, we also addressed the association of gene expression and methylation pattern in ALL patients.

1. Introduction

DNA methylation is one of the best-studied epigenetic mechanisms affecting cell fate by regulating genes expression. The variations in DNA methylation status are dynamic processes which occur in both coding and non-coding genomic loci inducing leukemogenesis. Mapping the DNA methylation pattern in particular loci can be a predicting factor for disease aggressiveness or therapeutic outcome [1].

Acute Lymphoblastic Leukemia)ALL(is a malignant disorder of lymphoid progenitor cells characterized by multiple molecular and cytogenetic abnormalities, resulted from genetics and environmental factors [2]. The disease is the most common malignancy and an important cause of mortality in children [3].

ALL occurs both in children and adults; however, it is more prevalent in children aged 2–5 years and in adults of over 50 years [2]. It has been shown that males are more susceptible to ALL than females [4].

B-ALL (B lymphocyte progenitor ALL) constitutes 85% of children and 75% of adult ALLs [5]. Despite advances in therapies, in almost 20% children with BCP-ALL (B Cell Progenitor-ALL), relapse phases occur which complicate further treatment [3].

Defects in genes involved in cellular signaling processes, cell cycle, proliferation, differentiation, apoptosis, and transcription; have been proven in ALL pathogenesis [6]. Evaluation of these defects in regulatory mechanisms of gene expression in main cellular pathways could be a valuable instrument to determine prognosis and development of novel therapeutic strategies [5].

It was evidenced that in almost 80% cases of Ph-positive ALLs, the *IKZF1* locus is deleted [7]. *JAK* mutations have been identified in nearly 10% of *BCR-ABL1* positive ALL patients [8]. *CRLF2* expression was also increased in 6–7% of B-ALL and has been observed in 50–60% of Down syndrome-ALL patients [9–11].

In addition to large-scale chromosomal modifications, other molecular mechanisms cooperate in leukemic transformation of lymphoid progenitors [12].

Extensive studies demonstrated the role of epigenetic factors in gene

* Corresponding author at: Department of Immunology, Division of Hematology and Transfusion Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, East Azerbaijan Province, Tabriz, University Main St, Iran.
E-mail address: ssolali@gmail.com (S. Solali).

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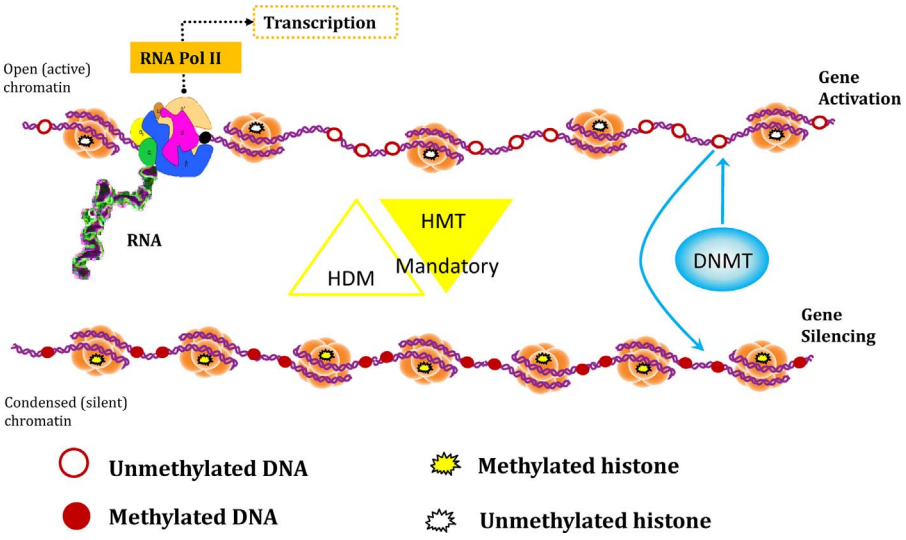

Fig. 1. Epigenetic regulation in human cells. DNA methylation and histone modifications are two epigenetic mechanisms, regulating gene expression. DNA methylation typically occurs at cytosines in CpG-rich regions which converts cytosine to 5′-methyl-cytosine by the functions of DNMTs that located in or near gene promoters, resulting in gene silencing. Post-translational modifications such as histone methylation may regulate chromatin structure and transcriptional status. This reaction is catalyzed by HMT and HDM. Abbreviation: HMT: Histone methyletransferase; HDM: Histone demethylase; DNMT: DNA methyletransferase; RNA POLII: RNA polymerase II.

expression regulation and controlling genes involved in tumorigenesis, without changes in DNA nucleotide sequences [13–15]. Therefore, scientists focused on deciphering the role of epigenetic factors (such as methylation, acetylation, phosphorylation etc.) in tumor development [15,16].

In contrast to structural alternations, changes in gene expression by epigenetic factors are reversible. Various studies focused on epigenetic changes to up-regulate tumor suppressor genes or silence tumor promoting genes [17].

The role of DNA methylation as an epigenetic factor has been proven in regulating genes involved in tumorigenesis; such as breast, prostate and colorectal cancer, as well as in hematologic malignancies including MDS (Myelodysplastic Syndromes), AML (Acute Myelogenous Leukemia), CML (Chronic Myelogenous Leukemia), and ALL [18]. Nevertheless, effective mechanisms related to DNA methylation changes are yet to be discovered [19]. Also, through multiple histone modifications, histone methylation has been considered as an active element regulating transcriptional activity of genes (Fig. 1) [20].

Based on genetic and epigenetic analysis of childhood ALL, it was confirmed that DNA methylation changes hold the key role in leukemogenesis [21]. Methylation of gene promoter CpG sites is associated with their expression in leukemia cell lines or primary ALL cells [12]. CpG sites hypermethylation occurs according in a tissue-specific, non-random manner [22,23].

DNA methylation of cytosine C5 position is one of the key epigenetic alterations in CpG dinucleotides and plays the main role in normal development as well as tumorigenesis [24]. Methyl groups add to DNA through certain enzymes known as DNA methyltransferases (Fig. 1). These enzymes are responsible for the stability of methylation patterns during development (DNMT3A, DNMT3B and their co-factor DNMT3-like); and maintenance of methylation during replication (DNMT1) [25]. The majority of CpG sites were shown to be methylated in mammalian cells. However, there is evidence showing that CpG islands have been mostly deviated from the genomic regular pattern, creating CpG rich regions which are often unmethylated (Fig. 2) [19].

Different methodologies utilized for quantitative and qualitative detection of gene methylation include HPLC (High Performance Liquid Chromatography), MSP (methylation-specific PCR), COBRA (Combined Bisulfite Restriction Analysis), MeDIP (Methylated DNA Immunoprecipitation), RLGS (Restriction Landmark Genomic Scanning), MS-HRM (Methylation- Sensitive High Resolution Melting), and Bisulfite-Pyrosequencing [26,27]. The first known method, MSP, is one of the largely used and well-studied method which is specific and sensitive to detect epigenetic changes distinguishing methylated and unmethylated CpG dinucleotides in promoter regions of genes (critical sites for gene silencing) [28].

Understanding methylation variations in transcription factors involved in hematopoietic normal or malignant processes is an important approach regarding treatment strategy selection and leukemia/lymphoma prognosis evaluation, and is included in the current review.

In this study, we focused on aberrant methylation of tumor suppressor genes involved in solid tumors and hematopoietic malignancies, specifically childhood ALL (Table 1). These key genes include *IKZF1, CDKN2B, TET2, CYP1B1*, *SALL4, DLC1, DLX-2, DLX-3, DLX4, TP72, PTPN6*, and *CDKN1C* (Fig. 3). Genetic and epigenetic changes in these genes can be associated with pathogenesis and prognosis of childhood ALL.

2. DNA methylation of genes

2.1. *IKZF1*

IKZF1(*IKAROS Family Zinc Finger1*) is one of the main regulators of lymphocyte proliferation [29]. Human *IKZF1* locus, with 120 kb length, is located on chromosome 7p12.2 containing 8 exons that forms at least 8 isoforms by alternative splicing [30–32].

*IKZF1*encodes the zinc finger transcription factor known as IKAROS (a member of zinc finger DNA binding protein family) that is essential for the development of all lymphoid lineages [33]. Mutations in IKZF1 as one of the most relevant tumor inhibitors was reported in 28.6% of ALL patients [33,34]. There are many studies determining mutations and polymorphism status of IKZF1, but few are in the context of its epigenetic changes.

Javierre and colleagues did not observe DNA methylation of promoter in the Jurkat cell line (a T cell lineage line), or in ALL and AML patients. Its promoter was hypermethylated in colorectal cancer cell lines, biopsies, and clinical specimens. However, the promoter of this gene was shown to be unmethylated in normal T and B cells [35].

According to previous studies, methylation of the *IKZF1* promoter region in lung cancer was associated with aberrant gene expression;

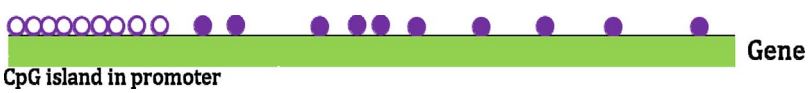

Fig. 2. Typical DNA methylation pattern in human. CpG-rich promoter regions of actively transcribed genes are generally unmethylated. Almost methylations in promoter regions associate negatively with gene expression.