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DNA Methylation in Chronic Myeloid Leukemia

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Abstract

Despite the high efficiency of tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia, 20-25% of patients develop drug resistance resulting in therapy failure. Besides mutations of the BCR-ABL1 kinase domain, the abnormal epigenetic regulation of the expression of critical genes for cell proliferation and survival has a central role in the disease pathogenesis and progression towards the drug resistant phenotype. Such epigenetic changes have the potential to be modulated by specific drugs including demethylating agents and histone deacetylase inhibitors. Here the current knowledge on the BCR-ABL1-associated methylation status is reviewed.

Keywords: Epigenetics; Chromatin Remodeling; Chronic Myeloid Leukemia; BCR-ABL1

DNA Methylation in Chronic Myeloid Leukemia

Epigenomics refers to heritable changes in gene expression not contingent upon changes in DNA sequence. Epigenetic determinants encompass DNA methylation, histone modifications and histone variant deposition in gene bodies. Through the recruitment of transcription-related enzymes to specific promoter regions, they impose an unique chromatin architecture hence controlling transcription factor accessibility and transcriptional permissiveness [1]. DNA methylation is the most stable epigenetic modification. It consists in the attachment of a methyl group (CH_3) at the 5' carbon position of the cytosine ring and predominantly occurs at high density CpG regions named CpG islands. Notably, CpG islands cover the transcriptional initiation sites of approximately 70% of annotated gene promoters, including most housekeeping genes as well as tissue-specific and developmental regulatory genes [2]. DNA methylation patterns are controlled by a family of enzymes, the DNA methyltransferases (DNMTs, encompassing the de novo methyltransferases DNMT3a and DNMT3b and the maintenance DNMT1). By catalyzing the CH_3 group transfer, they establish a permissive landscape for methyl-binding (MBD) proteins, such as MeCP2, MBD1, MBD2 and MBD4, involved in transcriptional repression [3,4]. DNA demethylation mechanisms are still elusive. More recently, the Ten-Eleven Translocation (TET) proteins have been advanced as putative regulators of DNA methylation patterns and transcription through events encompassing 5 methylcytosine conversion (5mC) into 5-hydroxymethylcytosine (5hmC) [5].

Besides its role in cell fate specification during development, DNA methylation has been involved in cancer pathogenesis and progression. Genome wide hypomethylation in transformed cells is largely confined to large "hypomethylation blocks" encompassing repetitive elements, retrotransposons, CpG poor promoters and gene deserts. It contributes to cancerogenesis through aberrant activation of growth-promoting genes and non-coding regions, cryptic transcriptional initiation sites outside gene promoters, mobile element reactivation and loss of imprinting. Moreover, it enhances genomic instability hence promoting chromosomal rearrangements, heterochromatin destabilization at centromeres and telomere length deregulation. Conversely, cancer-associated DNA hypermethylation is mostly restricted to "CpG shores" flanking the CpG islands at gene promoter regions. It drives the transcriptional silence of integral genes for cancer development and progression, including those controlling DNA repair, cell cycle, cell adhesion, apoptosis and angiogenesis [6]. In general terms, it can be assumed that epigenetic patterns, including DNA methylation, histone modifications and chromatin remodeling may serve as the second hit in the Knudson's two-hit model of cellular transformation [7]. From a clinical perspective, such integrated view into cancer genomes should improve the approach to treatment [8].

Chronic myeloid leukemia (CML) provides a paradigm of epigenetics role in transformation. It is, in fact, caused by a single genetic lesion, the BCR-ABL1 rearranged gene originated from t(9;22)(q34;q11) reciprocal translocation, whose p210 kDa chimeric protein has the ABL TK converted into a constitutively activated isoform by the BCR amino acids 1 to 63 [9,10]. Accordingly, the majority of CML patients undergo complete hematologic remission in response to TK inhibitor imatinib (IM) [11]. However, BCR-ABL1 does not play a central role in CML progression towards blast crisis, which involves the accumulation of secondary genomic alterations leading to selection of one or more clones with a fully transformed phenotype [12]. One component of CML clonal evolution is the epigenetic deregulation of expression of genes coding for transcription factors, tumor suppressors and tumor-associated antigens. Such mechanism might play a crucial role in the most primitive cell compartment of leukemic stem cells (LSC), whose self-renewal potential is not contingent upon BCR-ABL1 TK [13].

Aberrant DNA methylation is a common event in CML, with gene hypermethylation prevailing over hypomethylation. In first instance, it concerns the two genes involved in t(9;22) reciprocal translocation which generates the BCR-ABL1 gene. The allele-specific de novo methylation of ABL1 promoter (Pa) nested within the chimeric oncogene is an early marker of hematopoietic progenitor leukemic transformation progressively increasing with the disease progression [14,15]. It is not correlated with BCR-ABL1 transcription rate and most likely drives functional inactivation of tumor suppressor [16]. Conversely, BCR promoter methylation correlates with a better

response to IM through events likely encompassing BCR-ABL1 transcription [17]. Moreover, BCR-ABL1-associated DNA hypermethylation affects genes coding for transcription factors (JunB, IRF-4, CEBPA, HOXA4, PU.1, TFAP2A and EBF2) [18-23], tumor suppressors (SOCS1, PLCD1, DAPK1, PTPRG and DDIT3) [24-28], inhibitors of cell cycle progression (p16INK4a, p15INK4b and p14ARF) [29,30], proapoptotic genes (APAF-1 and BIM) [31-33], protein tyrosine phosphatase (PTPROt) [34], autophagy related gene (ATG16L2) [20], cell adhesion mediator (cadherin-13) [35] and tumor-associated antigen (PRAME) [36]. Notably, the aberrant DNA methylation of multiple genes was associated with the disease progression towards advanced stages eventually resistant to IM in a large cohort of patients [37]. In particular, DNA hypermethylation of several putative tumor suppressor genes is present at the blast crisis outcome in the putative BCR-ABL1+ LSC compartment identified by a CD34+ phenotype hence supports the critical role of epigenetics in clonal evolution of BCR-ABL1+ hematopoiesis towards the fully transformed phenotype [38].

Our recent work was focused on the role of DNA hypermethylation at the promoters of two genes, the BCL2 - interacting mediator (BCL2L11 otherwise referred to as BIM) and Chibby1 (CBY1), in proliferation and survival of BCR-ABL1+ hematopoiesis. BCL2L11is a member of the BH3-only death activator family critical for the regulation of hematopoietic stem cell (HSC) survival [39]. BCL2L11 decreased expression associated with BCR-ABL1 TK is a component of resistance to apoptotic death of CML cells and, accordingly, its upregulation in response to IM accounts for the drug selective cytotoxicity [40,41]. BCL2L11 downmodulation in CML is mostly driven by posttranscriptional events encompassing its proteasome-dependent degradation by ERK1/2 phosphorylation [42,43]. However, it is also contingent upon reduced transcription and epigenetically controlled by the promoter hypermethylation in a percentage of CML patients with unfavourable prognosis [32,41]. Our recent studies identified two critical mechanisms involved in DNA hypermethylation at the BCL2L11 promoter: the loss-of-function of TET2 and the aberrant DNMT1 recruitment. Both events hinder the permissive epigenetic "landscape" for gene transcription and are revoked by the inhibition of BCR-ABL1 TK activity [33,44]. Notably, the expression of BCL2L11 is restored by 5-aza-2'-deoxycytidine (5-Aza-CdR), a drug promoting selective DNMT1 degradation, supporting the central role of BCR-ABL1-associated DNA hypermethylation in prolonged survival of leukemic cells [Leo et al, unpublished results, 32,45].

CBY1 is an antagonist of β catenin which directly interacts with the C-terminal activation domain of β catenin and competes with TCF/LEF1 factors for β catenin binding hence repressing its transcriptional activity [46]. Moreover, it forms a stable tripartite complex with 14-3-3 and β catenin hence promoting the β catenin nuclear exclusion [47]. In a recently published work, we provided evidence of CBY1 reduced expression associated with BCR-ABL1. Notably, CBY1 expression was remarkably reduced in the putative BCR-ABL1+ LSC compartment identified by a $CD34^+$ phenotype compared with more differentiated leukemic progenitors. In this cell context, CBY1 downmodulation was evoked by transcriptional events driven by DNA hypermethylation at the promoter-associated CpG islands [48]. Accordingly, 5-Aza-CdR raises CBY1 expression and concurrently promotes β catenin nuclear export and transcriptional inactivation [49]. Indeed, β catenin and BCL2L11 are central components of resistance to IM and second generation TK inhibitors either of BCR-ABL1+ myeloid progenitors or LSC [13,50,51]. The identification of DNMT1 as a component of such epigenetic trait supports the introduction of DNMT1-targeting agents in CML therapy. The results of clinical trials proved the significant anti-leukemic activity of 5-Aza-CdR (referred to as decitabine), particularly in CML advanced phases or resistant to IM [52-54].

Of course, the matter of epigenetics in the pathogenesis and progression of CML is not restricted to DNA methylation status. DNA accessibility to the transcriptional apparatus is, in fact, regulated by additional events encompassing covalent modifications of core histone terminal tails and microRNAs [55-57]. More extensive information about epigenetic regulation of tumor suppressor expression, in particular, in the putative LSC compartment would let properly integrate the TK inhibitor-based therapy of CML and improve the prognosis of patients who exhibit or will develop drug resistance.

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