Aberrant Methylation in Hematological Malignancies

Venu K. Thirukonda[1,3], Radha Raghupathy[2] and Samir Parekh[1,3]*

[1]Department of Oncology, Montefiore Medical Center, Bronx, NY, USA
[2]Department of Oncology, Chinese University of Hong Kong, Shatin, NT, Hong Kong, China
[3]Albert Einstein Cancer Center, Albert Einstein College of Medicine, Bronx, NY, USA

Abstract

　　Epigenetics encompasses heritable changes in gene expression without alterations in genetic sequence. Among the multiple epigenetic regulators of gene expression, methylation of cytosine in genomic DNA is amongst the most stable and best studied. Expanding knowledge of aberrant methylation in hematological malignancies has increased its applicability in diagnosis, classification, prognostication and prediction of treatment response. Genome-wide methylation profiling can differentiate between clinically relevant subtypes of non-hodgkin's lymphoma (NHL) as well as distinguish monoclonal gammopathy of unknown significance (MGUS) from multiple myeloma (MM). New subcategories of normal karyotype acute myeloid leukemia (AML) with distinct canonical pathways and different disease behavior have also been identified by methylation studies. Methylation scores can predict outcomes in chronic lymphocytic leukemia (CLL). Genes with tumor suppressor function are frequently silenced by methylation and may be amenable to therapeutic intervention. DNA hypomethylating agents azacitidine and decitabine have been approved by the FDA for treatment of myelodysplastic syndrome (MDS). Clinical trials of newer agents including histone deacetylase inhibitors (HDAC-i) are showing great promise in different hematological malignancies. High-resolution methylation analysis using massively parallel sequencing (MPS) is likely to further improve our understanding of disease pathogenesis and identify novel therapeutic approaches in hematological malignancies. In this review article we will describe recent advances in methylation studies and the current and potential future impact of this knowledge on management of hematological malignancies.

Keywords: Biomarker; Epigenetics; Methylation

Introduction

　　Conrad Waddington introduced the term epigenetics in the early 1940s, defining it as 'the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being' [1]. This definition has evolved with epigenetics now referring to stable heritable alterations in chromatin organization and gene expression without underlying modification in the genetic sequence [2]. Epigenetic changes that modify gene expression include DNA methylation, post-translational histone modifications including lysine acetylation, arginine and lysine methylation and serine phosphorylation as well as micro-RNA mediated regulation [2,3]. The best-studied epigenetic modification, DNA methylation, is one of the most stable epigenetic modifications and can be detected in preserved tissue samples [4].

　　DNA methylation most frequently occurs as a covalent modification of the cytosine ring at the 5' position of a CpG dinucleotide with S-adenosyl methionine as the methyl donor. There are CpG dinucleotide rich regions in the human genome called CpG islands that are usually situated in the 5' end of the regulatory region of various genes [5]. Differential methylation of these CpG islands in the DNA plays an important role in physiologic processes such as cell differentiation, embryogenesis and imprinting [6]. Hypermethylation of CpG islands have been correlated with gene repression and hypomethylation has been associated with gene expression [7]. Methylation may inhibit gene expression by directly inhibiting protein or transcription factor binding to the promoter site as well as by methyl-CpG binding proteins attached to these sites that indirectly inhibit transcription factor binding [2]. Large portions of the genome comprising non coding regions are frequently hypermethylated facilitating maintenance of these genes in an inactive state [8]. However the impact of aberrant methylation on gene regulation has emerged to be more complex, with several recent studies demonstrating a locus and context specific association with gene expression. CpG shores have been identified which are important loci for differential methylation and variable gene expression. CpG shores are regions of lower CpG density than CpG islands that are typically located close to the CpG islands. About 76% of the tissue differential methylation regions (T-DMRs) are located in these CpG shores. These T-DMRs are evolutionarily conserved and contain both hyper and hypomethylated segments that determine tissue differentiation and phenotypic expression [9,10]. Methylation also occurs within the gene body or coding regions, which may facilitate transcription. High levels of gene body methylation have been correlated with highly expressed genes [11].

　　In addition to controlling normal growth, differentiation and development, there is a large body of data supporting the role of abnormal differential methylation in carcinogenesis [12,13]. DNA from malignant cells frequently has global hypomethylation [14] with foci of hypermethylated tumor suppressor genes [15]. Global hypomethylation may lead to chromosomal instability with deletions and gene rearrangements, reactivation of transposable elements and loss of imprinting [16-18]. Repression of tumor suppressor genes by methylation also promotes malignant transformation [19,20]. Recently cancer specific differentially methylated regions (C-DMRs) have also been identified involving the CpG island shores and sometimes extending to involve the CpG islands themselves. These C-DMRs overlap with reprogramming DMRs (R-DMRs) in 16% of cases but methylation patterns are opposite in C-DMRs and R-DMRs, suggesting that defective reprogramming may contribute to carcinogenesis [9]. Such aberrant DNA methylation occurs in a non-random, tumor-

type specific manner. The methylation patterns of a set of 12 genes having important roles in tumor suppression, cell cycle regulation, apoptosis, DNA repair and metastatic potential was studied on DNA from over 600 primary tumors of 15 different tumor types including various solid tumors, leukemias and lymphomas [21]. Each cancer was characterized by a unique profile of methylation changes. Some genes were tumor specific whereas others like the cell cycle regulator p16[INK4A] were commonly methylated across different malignancies. Hematologic malignancies had markedly different methylation profiles with high frequency of p73 and p15[INK4b] hypermethylation compared to epithelial solid tumors that did not have such alterations. This profile of CpG hypermethylation or the 'Methylotype' is an epigenetic signature unique for each type of malignancy.

The identification of aberrant methylation in carcinogenesis has led to its study in pathogenesis, diagnosis, development of targeted therapies, predicting response to therapy and prognosis. This review article will focus on aberrant methylation in various hematological malignancies.

Enzymatic Regulation of DNA Methylation

Two processes predominantly control normal methylation patterns, namely de novo DNA methylation and maintenance methylation. De novo DNA methylation occurs in a programmed fashion during embryogenesis facilitating tissue development, differentiation and genomic imprinting. Maintenance methylation occurs during DNA replication transmitting methylation patterns during mitosis. DNA methyl transferases (DNMT) DNMT3A and DNMT3B regulate de novo methylation patterns [22]. DNMT1 facilitates maintenance methylation by acting at the replication fork during the S-phase of the cell cycle copying the methylation patterns onto the daughter strand [7, 23].

Considerable crosstalk exists between histones and DNA methylation. DNMTs recruit histone deacetylases (HDAC), which are transcriptional repressors, to promoters of genes [24, 25]. Conversely trimethylated H3 Lysine 4 (H3K4) histone, an activator of transcription, binds to CpG islands and inhibits methylation by inhibiting recruitment of DNMT3a and DNMT3b to these islands [26,27]. EZH2 (Enhancer of Zeste Homolog 2) is a polycomb repressive protein that acts as a histone methyltransferase contributing to a repressive chromatin state. EZH2 has been shown to recruit DNMTs to EZH2 repressed genes, again demonstrating a mechanistic linkage between the two systems [28].

Other enzymes involved in DNA and histone chromatin demethylation include Isocitrate Dehydrogenase (IDH 1 and 2), Ten Eleven Translocation 2 (TET2) and Activation induced Cytidine Deaminase (AID/APOBEC). IDH catalyzes the conversion of isocitrate to 2-oxyglutarate (2OG), which demethylates histones resulting in gene expression. Mutations of IDH result in gain of function and abnormal conversion of 2OG to the R-enantiomer of 2-hydroxyglutarate (2HG). R-2HG inhibits 2OG dependent histone demethylase causing abnormal hypermethylation phenotypes [29,30]. TET2 catalyzes conversion of 5-methyl cytosine to 5-hydroxymethyl cytosine. Mutations of TET2 result in loss of function and hypermethylation. TET2 is dependent on 2OG for activation and mutations of IDH 1 and 2 also reduce activity of the TET2 enzyme resulting in hypermethylation [31]. The AID/APOBEC family of enzymes also contribute to DNA demethylation as demonstrated in zebrafish and mouse models [32,33].

Knowledge of enzymatic regulation of methylation has been an important tool in development of targeted therapies for hematological malignancies. DNMT inhibitors (DNMTi) such as azacitidine and decitabine have revolutionized therapy of MDS and are also emerging as important agents in managing certain subtypes of lymphomas. HDAC-i include several subclasses of drugs that are derivatives of hydroxamic acid, aliphatic acids, cyclic tetra peptides and benzamides. Suberoylanilide hydroxamic acid (SAHA) and other HDAC-i have shown promise in combination with DNMTi for therapy of MDS and AML [34]. Targeting TET2, IDH 1 and 2 mutations are directions for future exploration in therapy of hematological malignancies.

Assays for DNA Methylation

Studies of DNA methylation on a genomic scale primarily rely on two processes. These include enrichment of differences in methylation followed by interrogation of these differences using a suitable platform [35-37] (Table 1). Enrichment of the differentially methylated DNA fragments can be achieved by different techniques including bisulfite conversion, digestion by methylation-sensitive restriction enzymes and affinity purification of methylated DNA [38]. Bisulfite conversion is the gold standard and is based on bisulfite treatment selectively deaminating all unmethylated cytosine residues to uracil leaving the methylated cytosines unchanged [39]. PCR amplification of converted DNA replaces the uracil with thymine, which can then be analyzed for extent of cytosine methylation. Methylation sensitive restriction enzymes cleave methylated or unmethylated DNA at their restriction sites. For example in the HELP assay (HpaII tiny fragment Enrichment by Ligation-mediated PCR), the HpaII enzyme is methylation sensitive whereas Msp1 enzyme is methylation insensitive. The DNA is digested independently by both enzymes, amplified by PCR and the digested products compared to study the methylation sites [40]. By using an affinity matrix column containing methyl binding domain proteins (MBD) that bind to a single symmetrically methylated CpG pair, it is possible to separate DNA fragments on the basis of degree of methylation [41]. Another technique is chromatin immunoprecipitation, where a monoclonal antibody that specifically recognizes methylated cytosine can be used to immunoprecipitate methylated DNA [42].

These isolated differentially methylated fragments can be further analyzed on a global genomic scale using microarray and high throughput sequencing [43]. DNA microarrays are based on enrichment of differentially methylated segments, amplification of these segments followed by hybridization to oligonucleotide or SNP probes. High throughput MPS involves actual sequencing of methylated DNA. This process eliminates variability due to amplification and hybridization strength as well as quality of the genome sequence on the array. MPS based methods such as HELP-tagged sequencing, Methyl-Sensitive Cut Counting (MSCC) and Reduced representation bisulfite sequencing (RRBS) can interrogate >1.5 million CpGs across the genome, allowing study of intra and intergenic areas beyond the gene promoter [44].

Aberrant Methylation in Specific Hematological Malignancies

Locus-specific and genome-wide methylation studies in various hematological malignancies have revealed specific methylated genes and distinct epigenetic profiles as summarized in Table 2.

Non-Hodgkin's lymphoma

B cell NHL comprises a spectrum of lymphomas with varying malignant potential. Epigenetic studies have facilitated understanding of the genetic and phenotypic variability of this group of malignancies.

Low-grade lymphomas such as follicular (FL) and marginal zone lymphomas have distinct epigenetic signatures compared to normal B

Method	Mechanism	Additional information	Limitations	Assays
Enrichment of differences in Methylation				
Bisulfite conversion	Converts unmethylated cytosines to uracil (and eventually thymine following PCR amplification), while leaving methylated cytosines unconverted.	PCR product may be analyzed by direct sequencing or mass spectrometry to quantify cytosine methylation	-Conversion needs to be complete for accurate results. -Labor intensive & high cost prohibits genome-wide application. Partially overcome by combining with MS-PCR, microarrays & high-throughput sequencing.	Pyrosequencing MassARRAY
Methylation-Sensitive Restriction Digestion Assays	Uses restriction enzyme that identifies methylated DNA.	Often used in initial genome-wide screening	Limited to the analysis of CpG sites located within the enzyme recognition site	HELP, McrBC
Immunoprecipitation-Based Enrichment Assays	Uses antibodies that recognize methylated CpG to enrich methylated DNA	In combination with DNA microarrays (ChIP-chip) & massively parallel sequencing (ChIP-Seq), has made genome-wide analysis easier	Lack of specificity in low CpG-dense regions due to noise	MeDip
Analysis of Methylation				
High Throughput Interrogation				
Bead arrays	Uses two primers each recognizing methylated Vs unmethylated cytosine		Provides less coverage than other array-based methods	Illumina
Short oligonucleotide arrays	Millions of 25-mer probes per chip in a tiling design	Good specificity	Decreased sensitivity, noise	Affymetrix
Long oligonucleotide arrays	60-mer probes, can analyze test and control DNA on the same array	Better balance between sensitivity, specificity and noise		Nimblegen, Agilent
Massively Parallel sequencing	Involves direct sequencing of enriched DNA	-Best-possible resolution and quantitative methylation information (using bisulfite converted DNA) -Data can be re-analyzed if needed		Fluorescent nucleotide-based system (Illumina), MethylC-seq, SMRT (Pacific Biosciences)

PCR – Polymerase chain reaction
MS-PCR – Methylation specific PCR
HELP - HpaII tiny fragment enrichment by ligation-mediated PCR
McrBC - an endonuclease which cleaves DNA containing methylcytosine on one or both strands
MeDIP - Methylated DNA immunoprecipitation
SMRT - Single molecule real time sequencing

Table 1: Common Assays for Methylation Analysis.

cells and high-grade lymphomas. Genome wide methylation studies using bisulfite treated and sequenced DNA fragments from FL cell lines, FL patient samples and normal B cells showed distinct methylation patterns in the FL cells which would explain the survival advantage of malignant lymphocytes in this low grade lymphoma. IRF4, a tumor suppressor gene that inhibits BCL6 transcription, was repressed by methylation in FL resulting in over expression of the BCL6 oncogene [45]. FL also shows methylation and down-regulation of the SHP1 gene that inhibits proliferation of B cells and the androgen receptor that facilitates maturation of lymphocytes. Death-associated protein kinase (DAPK) mediates IFN-γ induced apoptosis and is inactivated by methylation in mucosa-associated lymphoid tissue lymphomas (MALTomas) and FL [46,47].

Intermediate to high-grade lymphomas like Diffuse Large B cell Lymphoma (DLBCL) show aberrant methylation of genes that control cell division, proliferation and cell cycle progression. The p16[INK4A] tumor suppressor gene codes for Cyclin dependent Kinase inhibitor 2A (CDKN2A) that arrests the cell cycle at G1 phase. Methylation of p16[INK4A] is correlated with high grade histology, more aggressive phenotype and greater risk of high grade transformation in NHL [48]. In a study of p16[INK4A] protein expression in 153 NHL samples, loss of expression was detected in 37% of cases, all of which corresponded to high-grade tumors [49]. Progression from MALTomas (31 of 37) or FL (4 of 4) into DLBCL also correlated with loss of expression of p16. The p16[INK4A] promoter CpG island was hypermethlyated in 6 of 23 cases with associated decrease in protein expression. In another study of 25 NHL tumor samples, p16[INK4A] inactivation by methylation was seen in 83% of high grade NHL samples in contrast to only 9% of low grade NHL cases. Loss of CDKN2A expression has also been correlated with poor outcomes after RCHOP therapy for DLBCL [50]. Hypermethylation of CDKN2A can also be used as a marker for relapse risk following therapy [51]. The Activated B cell (ABC) subtype of DLBCL has poorer prognosis compared to the Germinal Center B cell subtype (GCB) and has a distinct epigenetic signature. The TNFα network is dramatically suppressed in ABC and decreased TNFα signaling in ABC may be associated with decreased cytokine response to tumor infiltration as well imbalances in downstream nuclear factor κB (NFκB) signaling and apoptosis regulation [52-54].

Mantle cell lymphoma (MCL) arises from naïve B cells and is associated with a median survival of 4-5 years [55]. Comparison of array-based methylation profiles of MCL patient samples to normal naïve B cells showed 4110 differentially methylated genes involving key cellular proteins such as HDAC1 and NFκB1 transcription factor. The B-cell surface antigen CD37 was hypomethylated and over expressed in MCL and a small-modular immunopharmaceutical to CD-37 showed potent cytotoxicity in MCL, suggesting a novel therapeutic strategy for this disease [56]. Protein arginine methyltransferase (PRMT5) is over expressed in primary MCL and in concert with HDAC2, MBD2 and DNMT3a silences genes with anti-cancer and immune modulatory activities. The growth arrest and apoptosis in MCL cell lines from siRNA-mediated knockdown of PRMT5 suggests another therapeutic target [57].

Methylated Epigenetic biomarker	Clinical Impact	Details	Supporting literature
Follicular lymphoma			
IRF-4	Pathogenesis	Overexpression of BCL-6	Choi et al[45]
SHP-1	Pathogenesis	Proliferation of B-cells	Hayslip et al[47]
Androgen receptor gene	Pathogenesis	Release of immature B-cells	Hayslip et al[47]
DAPK	Pathogenesis	Protection from apoptosis	Hayslip et al[47]
p16^{INK4A}	Grade	Progression to high grade lymphoma	Villuendas et al[49]
Genome-wide methylation profiling	Prognosis and Biology	263 genes differentially methylated between ABC and GCB-DLBCL	Shaknovich et al[52]
Diffuse Large BCell Lymphoma			
p16^{INK4A}	Grade	Correlated with high grade histology, more aggressive phenotype	Herman et al[48]
p16^{INK4A}	Prognosis	Poor outcomes after RCHOP chemotherapy	Jardin et al[50]
p16^{INK4A}	Prognosis	Marker for relapse risk following therapy	Martinez-elgado et al[51]
MGMT	Prognosis	Hypermethylation associated with improved OS and PFS, Improved responses to alkylator therapy	Esteller et al[60]
Mantle Cell Lymphoma			
Genome-wide methylation profiling	Biology	Differential methylation of several genes including CDKN2B, MLF-1, PCDH8 and HOXD8	Leshchenko et al[56]
CD-37	Therapy	Hypomethylated and overexpressed in 13/14 patients. Targeting cytotoxic in cell lines.	Leshchenko et al[56]
Myelodysplastic Syndromes			
DAPK	Pathogenesis	Protection from apoptosis	Voso et al[70]
p15^{INK4B}	Prognosis	Associated with high risk MDS and progression to AML	Tien et al[73]
p15^{INK4B}, CDH1, HIC1, and ER	Prognosis	Hypermethylation more frequent in advanced stages and associated with shorter survival	Aggerholm et al[74]
Multi-gene score	Prognosis	Combined methylation score predicts OS and PFS	Shen at al[76]
SOCS1	Prognosis	Methylated in 54/114 (47.4%) MDS. Associated with higher grade MDS. Correlated with remission/relapse.	Wu et al[75]
Acute Myeloid Leukemia			
Genome-wide methylation profiling	Prognosis/Classification/Biology	Can differentiate prognostic groups. May shed more light on biology of AML.	Figueroa et al[79]
Chronic Lymphocytic Leukemia			
Genome-wide methylation profiling	Prognosis	Several tumor suppressor genes including ABI3, VHL and IGSF4 were hypermethylated in poor prognostic unmutated IgH$_V$ CLL	Kanduri et al[92]
DAPK1	Biology	Hypermethylated and poorly expressed in sporadic and familial CLL	Raval et al[95]
ZAP-70	Prognosis	Hypomethylated and expressed in IgH$_V$ CLL	Corcoran et al[94]
CD38, BTG4, HOXA4	Prognosis	Combined methylation score predicts prognosis independent of other factors.	Irving et al[98]
Multiple Myeloma			
Genome-wide methylation profiling	Prognosis/Classification	Differential methylation between normal plasma cells/MGUS –Myeloma – Plasma cell leukemia	Walker at al[102]
p16^{INK4A}	Prognosis	Methylation associated with poorer OS	Guillerm et al[103]

MRD – Minimal residual disease
OS – Overall survival
PFS – Progression free survival
DAPK – Death associated protein kinase
MGMT – O6-methylguanine DNA methyltransferase

Table 2: Aberrant methylation and impact in various hematological malignancies.

Traditional therapy for lymphomas is alkylator based and methylation analyses may help predict response to therapy. MGMT functions as a DNA repair enzyme, removing alkyl groups from guanine (G) base of the DNA and it's silencing by methylation has been correlated with improved responses to alkylating agents [58,59]. The methylation status of MGMT promoter was retrospectively evaluated in 84 tissue samples from patients with non-HIV associated DLBCL treated with non-rituximab containing alkylator and anthracycline based regimens [60]. MGMT hypermethylation was found in 30 (36%) of the 84 samples and was associated with significantly increased OS and PFS. In a more recent retrospective study of patients receiving RCHOP for DLBCL, MGMT promoter methylation did not retain its prognostic significance, suggesting that rituximab may overcome the reduced efficacy of alkylators in the setting of MGMT over expression.

Epigenetic changes in lymphomas have been targeted using HDACi and DNMTi either alone or in combination with traditional chemotherapy. The HDACi SAHA has shown synergism with bortezomib in treatment of MCL [61]. SAHA has also shown efficacy as a single agent in advanced hematological malignancies including transformed DLBCL and Hodgkins lymphoma [62]. Hypomethylating agents have strong synergism with HDACi in DLBCL mouse models,

with the combination producing a unique gene expression profile compared to the samples treated with single drugs [63]. This is an area for exploration in clinical trials. Azacitidine is being studied in combination with conventional chemotherapy for refractory DLBCL and other low-grade lymphomas.

Myelodysplastic syndromes

MDS includes heterogeneous clonal hematopoietic disorders characterized by bone marrow failure, dysplasia of one or more of the hematopoietic cell lineages, and frequent transformation to AML [64]. The important role of epigenetic changes and methylation in MDS pathogenesis is evident from the survival benefit provided by hypomethylating agent azacitidine in therapy of this disease [65-67]. Multiple genes including tumor suppressors, genes regulating cell adhesion, cell division and motility are methylated and suppressed in MDS [68].

Genome wide studies of marrow samples of patients with MDS and secondary AML revealed significantly increased aberrant methylation patterns in these diseases compared to patients with de novo AML and normal controls. Genes of the WNT signaling pathway and MAP kinase pathway as well as specific genes like CDKN2A were frequently hypermethylated. These are genes controlling normal hematopoietic stem cell division and maturation, under expression of which would explain the aberrant and dysplastic marrow proliferation in this disease [69].

Single locus studies have provided further insight into pathogenesis. In a study of 34 marrow samples of patients with MDS, methylation of the proapoptotic DAPK promoter was seen in 47% (16 of 34) specimens [70]. DAPK silencing may confer survival advantage to dysplastic precursors. Suppression of cell cycle inhibitors is associated with progression of MDS to high risk disease. p15^{INK4B} (CDKN2B) arrests the cell cycle at G1 phase [71,72]. In a study of 50 patients, 13 with FAB low-risk MDS, 32 with high risk MDS and five with AML, p15^{INK4B} promoter methylation was found in 0% of low-risk MDS, 23% of high-risk MDS and 60% of AML evolved from MDS. With progression of time, methylation remained at 0% in low risk MDS but increased to 30% in high risk MDS and 75% in AML evolved from MDS [73]. Among five patients who acquired methylation during the course, all had disease progression. The methylation status of different cell cycle and growth regulators, p15^{INK4B}, CDH1, HIC1, and the ER promoters was studied in 37 MDS patients using the bisulfite reduction method [74]. For all four genes, hypermethylation was more frequent in advanced stages than in early stages of MDS. Concurrent hypermethylation of three or more genes was also more frequent in advanced than in early stages of MDS. Patients with hypermethylation of one or more genes had a significantly shorter survival than patients without methylation of any of the four genes (median 17 vs. 67 months; p 0.002).

The gene for SOCS1, a member of suppressor of cytokine signaling family of proteins, is inactivated by hypermethylation in various hematological neoplasms. SOCS1 negatively regulates the proliferation signals from different cytokine receptors including JAK, IFNγ and different interleukins. The methylation status of SOCS1 was studied in marrow cells from 114 patients with primary MDS [75]. Fifty-four patients (47.4%) showed SOCS1 hypermethylation. Patients with SOCS1 methylation had a higher percentage of marrow blasts and higher risk MDS by FAB or IPSS than those without methylation. On follow-up, two patients who acquired new SOCS1 methylation had disease progression and two patients with disease remission after hematopoietic stem cell transplantation lost SOCS1 methylation. Patients with SOCS1 methylation also had a cumulative higher risk of leukemic transformation.

DNA methylation profiling of 10 genes selected from previous reports (E-cadherin/CDH1, N-cadherin/CDH13, estrogen receptor/ ER, NOR1, nucleoplasmin 2 (NPM2), oligodendrocyte lineage transcription factor 2/OLIG2, p15INK4B, progesterone receptors A & B and PDZ and LIM domain 4) in 317 patients with MDS identified concordant methylation at multiple gene promoters [76]. The author used averaged methylation scores from all genes and showed that overall methylation score was associated with significantly shorter OS and PFS in MDS. They validated these findings in two independent cohorts and were successful in predicting PFS in both cohorts and OS in one of the cohorts. The other cohort had more patients with low-risk MDS and relatively shorter follow-up that may have contributed to the variability in results. Methylation at baseline did not correlate to decitabine response, although decrease in methylation during therapy was associated with clinical responses.

Acute myeloid leukemia

AML is a heterogeneous disease with several subtypes and varied outcomes. Currently used prognostic indicators include age, morphology, cytogenetic findings, the white-cell count, and the presence or absence of an antecedent hematologic disorder with cytogenetics being the most important [77]. 35-50% of patients with AML have normal cytogenetics (CN-AML) with no uniformly accepted prognostication markers [78]. Methylation status has been studied as a prognosticator in AML. However data remains conflicted regarding the correlation of clinical phenotype, cytogenetics and methylation profile in this disease. Genome-wide DNA methylation profiling of leukemic blasts from 344 patients with AML with array-based HELP technique identified 16 clusters with unique methylation signatures different from normal marrow CD34+ cells [79]. Three of these patient clusters corresponded to good prognostic AML subtypes as defined by the World Health Organization classification. Of interest was that 9 patients who did not have t (8:21) also fell into this methylation cluster and had identical survival curves to those who carried the translocation. Eight clusters were enriched for cases harboring specific genetic or epigenetic lesions including NPM or CEBPA mutations. The remaining five clusters could not be explained by any known morphologic, cytogenetic, or molecular features and included patients of different FAB classifications, normal and abnormal karyotypes. Using integrative pathway analysis of the combined aberrantly methylated and aberrantly expressed genes, each cluster was found to have deregulation of different canonical pathways. These clusters also had significant differences in survival. Another study was unable to recapitulate this correlation between cytogenetics and methylation profile. In this study, genome-wide methylation profiling using the Illumina Human Methylation27 Bead Chip in 20 cases of AML analyzing 27,578 promoter CpG sites corresponding to 14,495 individual genes showed an independent positive correlation between the degree of genome wide promoter hypermethylation and OS [80]. Further an increased CR rate was observed in patients with global DNA hypomethylation. However mutational status or karyotype was not significantly associated with promoter-wide or global methylation.

Gene specific methylation patterns may also be helpful in prognostication. Methylation and repression of the cell cycle inhibitor, Deleted in bladder cancer 1 (DBC1) is associated with worse OS in CN-AML [81]. However correlation of methylation of cell cycle inhibitor p15 with AML prognosis remains controversial. While some studies have shown that p15 methylation is associated with poor prognosis, others have contradicted this finding [84,86]. More data appears to favor the poor prognostic impact of p15 methylation, and repression of this gene has been correlated with decreased OS and increased

risk of tumor recurrence in AML [82]. Combining ERα methylation status with p15 methylation status may be more powerful in predicting disease recurrence [83]. Decitabine treatment of AML cell lines results in partial re-expression of p15 suggesting another possible mechanism of action of hypomethylating agents in acute leukemias [71]. Hypermethylation of the transcription factor CEBPA gene has also been shown to be a useful prognostic biomarker. In the absence of CEBPA or NPM mutations, CEBPA hypermethylation predicts improved DFS and OS in AML [84].

Mutations in genes involved in methylation regulation have also been correlated with AML prognosis. Hematopoietic stem cells (HSCs) depend on constitutive DNA methylation for their self-renewal. DNMT3A mutations studied by whole-genome sequencing technology have been reported in 17.8% to 20% of patients with AML younger than 60 years of age, with the incidence in cytogenetically normal AML being slightly higher at 27.2% [85,86]. DNMT3A mutations typically occur in the methyltransferase domain and are associated with unfavorable outcomes [86]. Conversely, interplay between oncogenic fusion proteins in AML such as PML-RARa, MLL and RUNX1/MTG8 and the epigenetic machinery also affects gene expression contributing to leukemogenesis. PML-RARα leads to aberrant gene silencing in RARβ2, a Retinoic Acid Receptor (RAR) target gene by recruiting DNMTs [87].

In elderly patients with AML with multilineage dysplasia and blasts 20-30%, azacitidine improves OS and quality of life compared to conventional therapy (low-dose cytarabine, induction chemotherapy for AML or best supportive care) [65,88]. In addition azacitidine therapy results in higher rates of complete remission, partial remission, hematological improvement and transfusion independence. The use of azacitidine in combination with the HDACi SAHA also yielded promising initial results in a phase I/II study in patients with MDS or AML [89].

Chronic lymphocytic leukemia

CLL results from clonal proliferation and accumulation of neoplastic B-cells. Although no pathogenic mutation has yet been identified in CLL, several molecular alterations including immunoglobulin heavy chain (IgVH) mutation status, CD-38 expression, ZAP-70 status and certain cytogenetic abnormalities with prognostic significance have been described [90].

The first genome wide methylation assessment in CLL was performed using the Restriction Landmark Genomic Scanning with two restriction enzymes [91]. Over 3000 CpG islands were studied and 2-8% were aberrantly methylated in a non-random distribution compared to normal CD19+ cells. Genome-wide methylation in CLL was evaluated using Illumina Infinium HumanMethylation27 Bead Chip array (Illumina) that interrogated upto 27,578 CpGs, covering 14,495 genes [92]. 64 genes were identified as significantly differentially methylated between IgVH mutated and unmutated CLL. Several tumor suppressor genes including ABI3, VHL and IGSF4 were methylated in poor prognostic unmutated CLL. Other genes with important functions in apoptosis, tumor proliferation, and prognosis were also aberrantly methylated. Treatment with DNMTi and HDACi could induce expression of methylated tumor suppressor genes in unmutated CLL primary samples. In contrast another study using methylated CpG island amplification (MCA) coupled with a promoter microarray technique, found no differences in methylation profiles regardless of IgVH mutational status or ZAP-70 expression [93]. Using combined bisulphite restriction analysis (COBRA) technique to study the methylation status of ZAP-70 in CLL, the majority of patients expressing ZAP-70 protein showed lack of methylation in the intron 1-exon 2 boundary region of the ZAP-70 gene and had unmutated Ig VHgenes [94]. Patients with MCL or splenic marginal zone lymphoma with unmutated IgVH genes had neither unmethylated ZAP-70 genes, nor expressed ZAP-70 protein, suggesting ZAP-70 expression is independent of IgVH status.

DAPK-1 gene is a positive mediator of apoptosis, which has been shown to be hypermethylated and poorly expressed in sporadic and in familial CLL, possibly allowing leukemic cells to escape cell death mediated by apoptotic pathways [95]. The HOX family protein HOXB7, which can be aberrantly methylated in CLL can interact with DAPK-1 gene repressing transcription [93].

Methylation of p16^{INK4A} [96] and p15^{INK4B} [97] genes have also been described in small proportion of CLL patients, however the clinical significance from these small studies are yet undefined.

On evaluation of the utility of using the methylation status at three loci namely CD38, BTG4 [which are associated with improved time to first treatment (TFT)] and HOXA4 (associated with reduced TFT) to prognosticate CLL, the combined overall methylation score was a strong predictor of outcome and was independent of other known prognostic factors in multivariate analysis [98].

Given the success with DNA hypomethylating therapy in MDS and AML, decitabine was used in a phase-I trial of 20 patients with relapsed/refractory CLL (n=16) and NHL (n=4) [99]. Global DNA methylation (GDM) was assessed before and after therapy. At doses of 10mg/m 2 Days 1-10 and 15mg/m2 Days 1-5, there was no complete/ partial responses and no change in GDM before and after therapy (days 3, 5, 15, and 22), however the higher doses were associated with dose limiting hematological toxicity in 2/6 patients with CLL. The mechanisms as to why the methylated tumor suppressor genes could not be derepressed needs further exploration.

Multiple myeloma

Multiple myeloma is a neoplastic plasma-cell disorder that is characterized by clonal proliferation of plasma cells in the bone marrow, a monoclonal protein in the blood or urine, and associated organ dysfunction [100]. It may be preceded by a premalignant state termed the MGUS which progresses to myeloma at a rate of 1% per year. About 20,520 cases of myeloma are estimated to occur in 2011, with 10,610 deaths [101].

Genome-wide methylation profiling of plasma cells (PCs) from myeloma cell lines and from patients with MGUS, myeloma and plasma cell leukemia (PCL) were compared to that of normal plasma cells and B-cells using Infinium humanmethylation27 BeadArray (Illumina) [102]. The malignant cells showed more heterogeneous methylation profiles and the overall methylation patterns in these cell types could accurately distinguish between premalignant and malignant cells. There were few methylation changes between normal PCs and the MGUS phenotype, however 1428 genes underwent hypomethylation and 82 probes underwent hypermethylation from MGUS to myeloma with 80% of hypomethylation occurring outside of CpG islands and 58.5% of hypermethylation within CpG islands. Hypermethylation involved three main groups of genes involving developmental processes, cell cycle processes, and transcriptional regulation. In the transition from myeloma to PCL, there was remethylation of previously demethylated genes (between MGUS and myeloma) including CALCA, ONECUT2, GATA4, and CDKN2B. Genes with CpG island hypermethylation were involved in cell-cell signaling, cell development or differentiation and cell adhesion, possibly contributing to release of malignant plasma cells

from the marrow. Patients with t (4,14) over expressed two potential oncogenes, MMSET and FGFR3 and had a similar methylation profile to PCL suggesting the methylation profile could contribute to the aggressiveness of this subgroup. The authors also identified two specific subgroups of hyperdiploid myeloma on the basis of their methylation profile with significant differences in overall survival (OS).

Some of the genes commonly affected by epigenetic silencing in myeloma include p16^{INK4A}, E-Cadherin, Estrogen receptor (ER), SHP and SOCS, DAPK and DNA damage repair genes including MGMT and hMLH-1[27]. In an analysis of 61 myeloma patients followed for a median 8.6yrs, using methylation specific PCR (MS-PCR), methylation of p16^{INK4A} was associated with a significantly poorer OS (p=0.035). The authors found no prognostic impact of p15^{INK4B} methylation in their study [103]. It has also been shown using a MS-PCR assay that patients with a methylated p16 gene had three times higher number of plasma cells in S-phase than those with unmethylated p16 gene, suggesting its important role in cell-cycle regulation in myeloma [104]. However another study using MS-PCR found no apparent effects on either gene-expression level or the cell cycle with p16 methylation in different stages of myeloma questioning its role in pathogenesis [105].

Conclusions and Future Directions

Genome-wide and locus-specific analyses of methylation changes have yielded insights into the pathogenesis and prognosis of different hematological malignancies. However, results of correlation of gene-specific methylation studies with clinical outcomes have been somewhat discordant and further research is needed in this area. Current technologies exist for the confirmation of methylation status of individual genes setting the stage for prospective correlative studies incorporating methylation in future clinical trials. Unlike changes in DNA sequence, aberrant DNA methylation is amenable to therapeutic targeting. Advances in the understanding of the mechanisms contributing to the generation and maintenance of aberrant methylation patterns have identified families of enzymatic targets for therapy. Although the currently available drugs are still non-specific, newer drugs with well-defined impact on the epigenetic machinery are being developed.

Acknowledgements

References

1. Dupont C, Armant DR, Brenner CA (2009) Epigenetics: definition, mechanisms and clinical perspective. Semin Reprod Med 27: 351-357.

2. Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16: 6-21.

3. Khandige S, Shanbhogue VV, Chakrabarty S, Kapettu S (2011) Methylation markers: a potential force driving cancer diagnostics forward. Oncol Res 19: 105-110.

4. Deng D, Liu Z, Du Y (2010) Epigenetic alterations as cancer diagnostic, prognostic, and predictive biomarkers. Adv Genet 71: 125-176.

5. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, et al. (2001) Initial sequencing and analysis of the human genome. Nature 409: 860-921.

6. Jones PA (1999) The DNA methylation paradox. Trends Genet 15: 34-37.

7. Klose RJ, Bird AP (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 31: 89-97.

8. Bird AP, Wolffe AP (1999) Methylation-induced repression--belts, braces, and chromatin. Cell 99: 451-454.

9. Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, et al. (2009) The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nat Genet 41: 178-186.

10. Pollard SM, Stricker SH, Beck S (2009) Preview. A shore sign of reprogramming. Cell Stem Cell 5: 571-572.

11. Ball MP, Li JB, Gao Y, Lee JH, LeProust EM, et al. (2009) Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. Nat Biotechnol 27: 361-368.

12. Baylin SB, Herman JG (2000) DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet 16: 168-174.

13. Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genet 3: 415-428.

14. Feinberg AP, Vogelstein B (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301: 89-92.

15. Esteller M (2008) Epigenetics in cancer. N Engl J Med 358: 1148-1159.

16. Eden A, Gaudet F, Waghmare A, Jaenisch R (2003) Chromosomal instability and tumors promoted by DNA hypomethylation. Science 300: 455.

17. Lee MP, DeBaun MR, Mitsuya K, Galonek HL, Brandenburg S, et al. (1999) Loss of imprinting of a paternally expressed transcript, with antisense orientation to KVLQT1, occurs frequently in Beckwith-Wiedemann syndrome and is independent of insulin-like growth factor II imprinting. Proc Natl Acad Sci USA 96: 5203-5208.

18. Holm TM, Jackson-Grusby L, Brambrink T, Yamada Y, Rideout WM 3rd, et al. (2005) Global loss of imprinting leads to widespread tumorigenesis in adult mice. Cancer cell 8: 275-285.

19. Herman JG, Baylin SB (2003) Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 349: 2042-2054.

20. Esteller M, Fraga MF, Guo M, Garcia-Foncillas J, Hedenfalk I, et al. (2001) DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. Hum Mol Genet 10: 3001-3007.

21. Esteller M, Corn PG, Baylin SB, Herman JG (2001) A gene hypermethylation profile of human cancer. Cancer Res 61: 3225-3229.

22. Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99: 247-257.

23. Holliday R, Pugh JE (1975) DNA modification mechanisms and gene activity during development. Science 187: 226-232.

24. Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T (2000) DNA methyltransferase Dnmt1 associates with histone deacetylase activity. Nat Genet 24: 88-91.

25. Fuks F, Burgers WA, Godin N, Kasai M, Kouzarides T (2001) Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription. The EMBO journal 20: 2536-2544.

26. Ooi SK, Qiu C, Bernstein E, Li K, Jia D, et al. (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature 448: 714-717.

27. Sharma A, Heuck CJ, Fazzari MJ, Mehta J, Singhal S, et al. (2010) DNA methylation alterations in multiple myeloma as a model for epigenetic changes in cancer. Wiley Interdiscip Rev Syst Biol Med 2: 654-669.

28. Viré E, Brenner C, Deplus R, Blanchon L, Fraga M, et al. (2006) The Polycomb group protein EZH2 directly controls DNA methylation. Nature 439: 871-874.

29. Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA , et al. (2011) The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Rep 12: 463-469.

30. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, et al. (2011) Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell 20: 11-24.

31. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, et al. (2009) Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood 114: 144-147.

32. Bhutani N, Burns DM, Blau HM (2011) DNA demethylation dynamics. Cell 146: 866-872.

33. Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, et al. (2008) DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. Cell 135: 1201-1212.

34. Gore SD (2005) Combination therapy with DNA methyltransferase inhibitors in hematologic malignancies. Nat Clin Pract Oncol 1: S30-35.

35. Schones DE, Zhao K (2008) Genome-wide approaches to studying chromatin modifications. Nat Rev Genet 9: 179-191.

36. Adorján P, Distler J, Lipscher E, Model F, Müller J, et al. (2002) Tumour class prediction and discovery by microarray-based DNA methylation analysis. Nucleic Acids Res 30: e21.

37. Gitan RS, Shi H, Chen CM, Yan PS, Huang TH (2002) Methylation-specific oligonucleotide microarray: a new potential for high-throughput methylation analysis. Genome Res 12: 158-164.

38. Zilberman D, Henikoff S (2007) Genome-wide analysis of DNA methylation patterns. Development 134: 3959-3965.

39. Frommer M, McDonald LE, Millar DS, Collis CM, Watt F, et al. (1992) A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proc Natl Acad Sci USA 89: 1827-1831.

40. Bird AP, Southern EM (1978) Use of restriction enzymes to study eukaryotic DNA methylation: I. The methylation pattern in ribosomal DNA from Xenopus laevis. J Mol Biol 118: 27-47.

41. Cross SH, Charlton JA, Nan X, Bird AP (1994) Purification of CpG islands using a methylated DNA binding column. Nat Genet 6: 236-244.

42. Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, et al. (2005) Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 37: 853-862.

43. Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, et al. (2006) Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis. Cell 126: 1189-1201.

44. Figueroa ME, Melnick A, Greally JM (2009) Genome-wide determination of DNA methylation by Hpa II tiny fragment enrichment by ligation-mediated PCR (HELP) for the study of acute leukemias. Methods Mol Biol 538: 395-407.

45. Choi JH, Li Y, Guo J, Pei L, Rauch TA, et al. (2010) Genome-wide DNA methylation maps in follicular lymphoma cells determined by methylation-enriched bisulfite sequencing. PloS one 5: e13020.

46. Rossi D, Capello D, Gloghini A, Franceschetti S, Paulli M, et al. (2004) Aberrant promoter methylation of multiple genes throughout the clinico-pathologic spectrum of B-cell neoplasia. Haematologica 89: 154-164.

47. Hayslip J, Montero A (2006) Tumor suppressor gene methylation in follicular lymphoma: a comprehensive review. Molecular cancer 5: 44.

48. Herman JG, Civin CI, Issa JP, Collector MI, Sharkis SJ, et al. (1997) Distinct patterns of inactivation of p15INK4B and p16INK4A characterize the major types of hematological malignancies Cancer Res 57: 837-841.

49. Villuendas R, Sánchez-Beato M, Martínez JC, Saez AI, Martinez-Delgado B, et al. (1998) Loss of p16/INK4A protein expression in non-Hodgkin's lymphomas is a frequent finding associated with tumor progression. Am J Pathol 153: 887-897.

50. Jardin F, Jais JP, Molina TJ, Parmentier F, Picquenot JM, et al. (2010) Diffuse large B-cell lymphomas with CDKN2A deletion have a distinct gene expression signature and a poor prognosis under R-CHOP treatment: a GELA study. Blood 116: 1092-1104.

51. Martinez-Delgado B, Richart A, García MJ, Robledo M, Osorio A, et al. (2000) Hypermethylation of P16ink4a and P15ink4b genes as a marker of disease in the follow-up of non-Hodgkin's lymphomas. Br J Haematol 109: 97-103.

52. Shaknovich R, Geng H, Johnson NA, Tsikitas L, Cerchietti L, et al. (2010) DNA methylation signatures define molecular subtypes of diffuse large B-cell lymphoma. Blood 116: e81-89.

53. Shaknovich R, Figueroa ME, Melnick A (2010) HELP (HpaII tiny fragment enrichment by ligation-mediated PCR) assay for DNA methylation profiling of primary normal and malignant B lymphocytes. Methods Mol Biol 632: 191-201.

54. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, et al. (2002) The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346: 1937-1947.

55. Ogura M (2010) Current treatment strategy and new agents in mantle cell lymphoma. Int J Hematol 92: 25-32.

56. Leshchenko VV, Kuo PY, Shaknovich R, Yang DT, Gellen T, et al. (2010) Genomewide DNA methylation analysis reveals novel targets for drug development in mantle cell lymphoma. Blood 116: 1025-1034.

57. Parekh S, Weniger MA, Wiestner A (2011) New molecular targets in mantle cell lymphoma. Semin Cancer Biol 21: 335-346.

58. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, et al. (2000) Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 343: 1350-1354.

59. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, et al. (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352: 997-1003.

60. Esteller M, Gaidano G, Goodman SN, Zagonel V, Capello D, et al. (2002) Hypermethylation of the DNA repair gene O(6)-methylguanine DNA methyltransferase and survival of patients with diffuse large B-cell lymphoma. J Natl Cancer Inst 94: 26-32.

61. Heider U, von Metzler I, Kaiser M, Rosche M, Sterz J, et al. (2008) Synergistic interaction of the histone deacetylase inhibitor SAHA with the proteasome inhibitor bortezomib in mantle cell lymphoma. Eur J Haematol 80: 133-142.

62. O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, et al. (2006) Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. J Clin Oncol 24: 166-173.

63. Kalac M, Scotto L, Marchi E, Amengual J, Seshan VE, et al. (2011) HDAC inhibitors and decitabine are highly synergistic and associated with unique gene expression and epigenetic profiles in models of DLBCL. Blood 118: 5506-5516.

64. Komrokji RS, Bennett JM (2007) Evolving classifications of the myelodysplastic syndromes. Curr Opin Hematol 14: 98-105.

65. Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, et al. (2002) Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol 20: 2429-2440.

66. Silverman LR, McKenzie DR, Peterson BL, Holland JF, Backstrom JT, et al. (2006) Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. J Clin Oncol 24: 3895-3903.

67. Kantarjian H, Issa JP, Rosenfeld CS, Bennett JM, Albitar M, et al. (2006) Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer 106: 1794-1803.

68. Issa JP (2010) Epigenetic changes in the myelodysplastic syndrome. Hematol Oncol Clin North Am 24: 317-330.

69. Figueroa ME, Skrabanek L, Li Y, Jiemjit A, Fandy TE, et al. (2009) MDS and secondary AML display unique patterns and abundance of aberrant DNA methylation. Blood 114: 3448-3458.

70. Voso MT, Scardocci A, Guidi F, Zini G, Di Mario A, et al. (2004) Aberrant methylation of DAP-kinase in therapy-related acute myeloid leukemia and myelodysplastic syndromes. Blood 103: 698-700.

71. Herman JG, Jen J, Merlo A, Baylin SB (1996) Hypermethylation-associated inactivation indicates a tumor suppressor role for p15INK4B. Cancer Res 56: 722-7257.

72. Serrano M, Hannon GJ, Beach D (1993) A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366: 704-707.

73. Tien HF, Tang JH, Tsay W, Liu MC, Lee FY, et al. (2001) Methylation of the p15(INK4B) gene in myelodysplastic syndrome: it can be detected early at diagnosis or during disease progression and is highly associated with leukaemic transformation. Br J Haematol 112: 148-154.

74. Aggerholm A, Holm MS, Guldberg P, Olesen LH, Hokland P (2006) Promoter hypermethylation of p15INK4B, HIC1, CDH1, and ER is frequent in myelodysplastic syndrome and predicts poor prognosis in early-stage patients. Eur J Haematol 76: 23-32.

75. Wu SJ, Yao M, Chou WC, Tang JL, Chen CY, et al. (2006) Clinical implications of SOCS1 methylation in myelodysplastic syndrome. Br J Haematol 135: 317-323.

76. Shen L, Kantarjian H, Guo Y, Lin E, Shan J, et al. (2010) DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. J Clin Oncol 28: 605-613.

77. Löwenberg B (2001) Prognostic factors in acute myeloid leukaemia. Best Pract Res Clin Haematol 14: 65-75.

78. Mrózek K, Heinonen K, Bloomfield CD (2001) Clinical importance of cytogenetics in acute myeloid leukaemia. Best Pract Res Clin Haematol 14: 19-47.

79. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, et al. (2010) DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer cell 17: 13-27.

80. Deneberg S, Grövdal M, Karimi M, Jansson M, Nahi H, et al. (2010) Gene-specific and global methylation patterns predict outcome in patients with acute myeloid leukemia. Leukemia 24: 932-941.

81. Alvarez S, Suela J, Valencia A, Fernández A, Wunderlich M, et al. (2010) DNA methylation profiles and their relationship with cytogenetic status in adult acute myeloid leukemia. PloS one 5: e12197.

82. Wong IH, Ng MH, Huang DP, Lee JC (2000) Aberrant p15 promoter methylation in adult and childhood acute leukemias of nearly all morphologic subtypes: potential prognostic implications. Blood 95: 1942-1949.

83. Agrawal S, Unterberg M, Koschmieder S, zur Stadt U, Brunnberg U, et al. (2007) DNA methylation of tumor suppressor genes in clinical remission predicts the relapse risk in acute myeloid leukemia. Cancer Res 67: 1370-1377.

84. Lin TC, Hou HA, Chou WC, Ou DL, Yu SL, et al. (2011) CEBPA methylation as a prognostic biomarker in patients with de novo acute myeloid leukemia. Leukemia 25: 32-40.

85. Shah MY, Licht JD (2011) DNMT3A mutations in acute myeloid leukemia. Nature genetics 43: 289-290.

86. Thol F, Damm F, Lüdeking A, Winschel C, Wagner K , et al. (2011) Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. J Clin Oncol 29: 2889-2896.

87. Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, et al. (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 295: 1079-1082.

88. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, et al. (2009) Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol 10: 223-232.

89. Silverman LR, Verma A, Odchimar-Reissig R, Cozza A, Najfeld V, et al. (2008) A phase I/II study of vorinostat, an oral histone deacetylase inhibitor, in combination with azacitidine in patients with the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Initial results of the phase I trial: A New York Cancer Consortium. ASCO Meeting Abstracts 26: 7000.

90. Mougalian SS, O'Brien S (2011) Adverse prognostic features in chronic lymphocytic leukemia. Oncology (Williston Park) 25: 692-696, 699.

91. Rush LJ, Raval A, Funchain P, Johnson AJ, Smith L (2004) Epigenetic Profiling in Chronic Lymphocytic Leukemia Reveals Novel Methylation Targets. Cancer Res 64: 2424-2433.

92. Kanduri M, Cahill N, Göransson H, Enström C, Ryan F, et al. (2010) Differential genome-wide array-based methylation profiles in prognostic subsets of chronic lymphocytic leukemia. Blood 115: 296-305.

93. Tong WG, Wierda WG, Lin E, Kuang SQ, Bekele BN, et al. (2010) Genome-wide DNA methylation profiling of chronic lymphocytic leukemia allows identification of epigenetically repressed molecular pathways with clinical impact. Epigenetics 5: 499-508.

94. Corcoran M, Parker A, Orchard J, Davis Z, Wirtz M, et al. (2005) ZAP-70 methylation status is associated with ZAP-70 expression status in chronic lymphocytic leukemia. Haematologica 90: 1078-1088.

95. Raval A, Tanner SM, Byrd JC, Angerman EB, Perko JD, et al. (2007) Downregulation of death-associated protein kinase 1 (DAPK1) in chronic lymphocytic leukemia. Cell 129: 879-890.

96. Tsirigotis P, Pappa V, Labropoulos S, Papageorgiou S, Kontsioti F, et al. (2006) Mutational and methylation analysis of the cyclin-dependent kinase 4 inhibitor (p16INK4A) gene in chronic lymphocytic leukemia. Eur J Haematol 76: 230-236.

97. Papageorgiou SG, Lambropoulos S, Pappa V, Economopoulou C, Kontsioti F, et al. (2007) Hypermethylation of the p15INK4B gene promoter in B-chronic lymphocytic leukemia. Am J Hematol 82: 824-825.

98. Irving L, Mainou-Fowler T, Parker A, Ibbotson RE, Oscier DG, et al. (2011) Methylation markers identify high risk patients inIGHVmutated chronic lymphocytic leukemia. Epigenetics 6: 300-306.

99. Blum KA, Liu Z, Lucas DM, Chen P, Xie Z, et al. (2010) Phase I trial of low dose decitabine targeting DNA hypermethylation in patients with chronic lymphocytic leukaemia and non-Hodgkin lymphoma: dose-limiting myelosuppression without evidence of DNA hypomethylation. Br J Haematol 150: 189-195.

100. Palumbo A, Anderson K (2011) Multiple myeloma. N Engl J Med 364: 1046-1060.

101. (2011) Cancer Facts & Figures, American Cancer Society.

102. Walker BA, Wardell CP, Chiecchio L, Smith EM, Boyd KD, et al. (2011) Aberrant global methylation patterns affect the molecular pathogenesis and prognosis of multiple myeloma. Blood 117: 553-562.

103. Guillerm G, Depil S, Wolowiec D, Quesnel B (2003) Different prognostic values of p15(INK4b) and p16(INK4a) gene methylations in multiple myeloma. Haematologica 88: 476-478.

104. Mateos MV, García-Sanz R, López-Pérez R, Moro MJ, Ocio E, et al. (2002) Methylation is an inactivating mechanism of the p16 gene in multiple myeloma associated with high plasma cell proliferation and short survival. Br J Haematol 118: 1034-1040.

105. Gonzalez-Paz N, Chng WJ, McClure RF, Blood E, Oken MM, et al. (2007) Tumor suppressor p16 methylation in multiple myeloma: biological and clinical implications. Blood 109: 1228-1232.