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Epigenetic pathways in hematological malignancies

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It has been recognized for many years that tumors develop as a result of accumulated molecular-genetic or genomic alterations. However, cancer development is not restricted to the genetic changes, but also to epigenetic changes. The inheritance of information based on gene expression levels in known as epigenetics, as opposed to genetics, which refers to information transmitted on the basis of gene sequence. To date, DNA methylation and the post-tranductional modifications of histone proteins are the best studied epigenetic modifications.

DNA methylation

The main epigenetic modification in mammals, and in particular in humans, is the methylation of cytosine nucleotide residue. Cytosine methylation occurs after DNA synthesis, by enzymatic transfer of a methyl group from the methyl donor S-adenosylmethionine to the carbon-5 position of cytosine within the CpG dinucleotide. This enzymatic reaction is performed by DNA methyl transferases (DNMTs). The distribution of CpGs in vertebrates genomes is no uniform. Most of the genome is actually quite depleted of CpGs, a phenomenon termed CpG suppression.[1] By contrast, about 1% of the genome is composed of CpG rich regions termed CpG islands.[1] These CpG islands are usually unmethylated in all normal tissues and frequently span the 5'end (promoter, untraslated region and exon 1) of a number of genes. This lack of methylation in promoter-associated CpG islands permits the expression of the gene, if the appropriate transcription factors are present, and the chromatin structure allows access to them. Methylation of promoter CpG islands is associated with a closed chromatin structure and transcriptional silencing of the associated genes. We can find certain CpG islands normally methylated in at least four cases: imprinted genes, X-chromosome genes in women, germline-specific genes, and tissue-specific genes.[2]

However, this scenario changes substantially when cells became cancerous. Three major phenomena occur in cancer affecting methylation patterns: first, there is an increase in the activity of the methylating enzymes in the malignant cells; second, there is a global hypomethylation of the genome if we compare a tumoral versus a normal cells (this is due mainly to a generalized demethylation in the CpGs scattered in the body of the genes); and third and finally, there are a local and discrete regions that suffer an intense hypermethylation.

CpG islands associated with tumor suppressor genes are unmethylated in normal tissues, but often become hypermethylated during tumor formation. *De novo* methylation of CpG islands induces the silencing of associated tumor suppressor genes and may, in fact, be a critical step during tumor formation. The particular genes that are hypermethylated in tumor cells are strongly specific to the tissue of origin of the tumor.[3] We have described a profile of hypermethylation among various primary human tumors.[4] The genes that undergo abnormal methylation in their 5'-CpG island in human cancer cover the whole spectrum of pathways involved in tumorogenesis from cell cycle and apoptosis to DNA repair and invasiveness ability. Thus, in addition of genetics changes, DNA hypermethylation-associated gene silencing may be a critical step involved in early steps of tumor progression.

In particular, DNA hypermethylation-mediated silencing of tumor suppressor genes occurs in hematological malignancies and these events may constitute early steps in the pathogenesis of these neoplasms. Although the

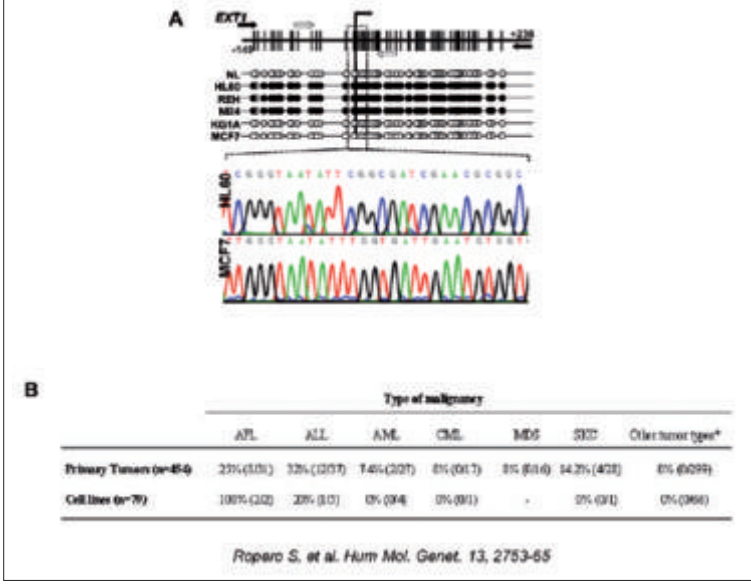

Figure 1. A. Schematic depiction of the EXT1 CpG islands around the corresponding transcription start sites (long black arrows). CpG dinucleotides are represented as short vertical lines. Location of bisulfite genomic sequencing PCR primers are indicated as black arrows and methylation-specific PCR primers as white arrows. Representative examples of the chromatograms obtained by bisulfite genomic sequencing of human cancer cell lines are shown. B. Profile of EXT1 CpG island hypermethylation in primary human malignancies.

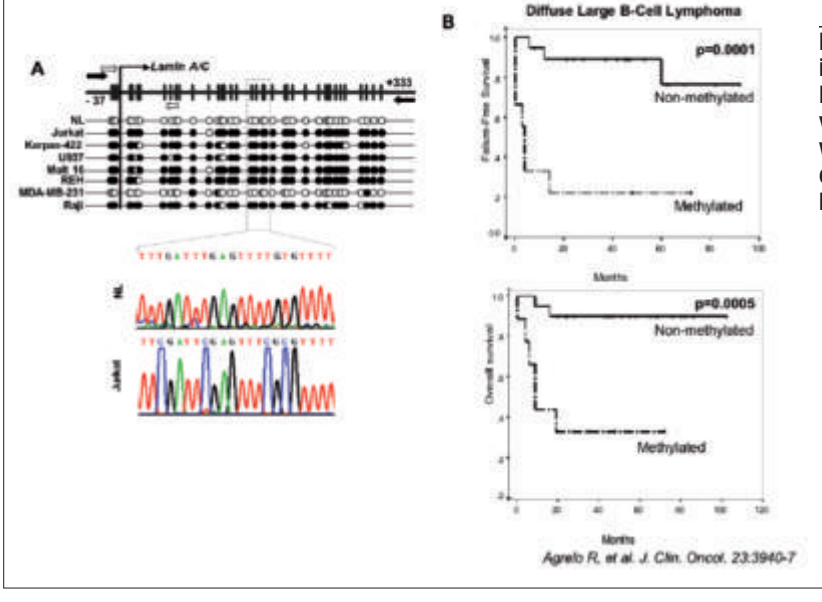

Figure 2. A. Analysis of lamin A/C CpG island promoter methylation status in human cancer cell lines. B. Failure-free survival and overall survival among patients with nodal diffuse large B-cell lymphomas according to the methyaltion status of the lamin A/C promoter.

gene hypermethylation profile of hematological malignances differs from solid tumors, the full spectrum of cancer-related cellular pathways may be deranged.

An example is the case of EXT1, a glycosyltranferase required for the biosynthesis of heparan sulfate glycosaminoglycans (HSGAGs). In our lab we found EXT1 promoter hypermethylation in 25% of acute promyelocitic leukemia (APL), 30% acute lymphocytic leukemia (ALL) and only in 7.4% of acute myelogenous leukemia (AML).[5] An ever-growing number of biological processes are regulated by the interaction of proteins with heparan sulphate (HS). These interactions play important roles in normal physiological processes, such as organogenesis, angiogenesis, blood coagulation, growth factor signalling, lipid metabolism, etc. In the bone marrow, HSGAGs bind growth factors involved in the control of hematopoiesis and thereby regulate leukemic cell differentiation.[6] Consistent with this finding, a HS-associated fraction of the bone marrow matrix induces maturation of leukemia cells *in vitro*.[7] Moreover, the cells from some patients with acute lymphoblastic leukemia, acute promyelocytic leukemia and acute myeloblastic leukemia are defective in their ability to interact with stromal cells and consequently cannot survive in stromal cell-mediated long-term marrow cultures.[6] Thus, the transendothelial migration of undifferentiated leukemic cells from the bone marrow could be explained at least in part by the absence of HS biosynthesis induced by EXT1 promoter hypermethylation. Our results suggest that the epigenetic silencing of EXT1 is a crucial event in the disruption of HS synthesis in trans-

Table 1. A selected list of genes silenced by CpG island hypermethylation in haematological malignances

Gene	*Function*	*Tumor profile*
$p16^{INK4a}$	Cyclin-dependent kinase inhibitor	Multiple types
$p15^{INK4b}$	Cyclin-dependent kinase inhibitor	Leukemia
MGMT	DNA repair of O6-alkyl-guanine	Multiple types
p73	p53 homologue	Lymphoma
RASSF1A	Ras effector homologue	Multiple types
CDH1	E-cadherin, cell adhesion	Breast, stomach, leukaemia
HIC-1	Transcription factor	Multiple types
SOCS-1	Inhibitor of JAK/STAT pathway	Liver, myeloma
DAPK	Pro-apoptotic	Lymphoma, lung, colon
EXT1	Heparan sulphate synthesis	Leukaemia, skin
Lamin A/C	Structural protein	Leukaemia, lymphoma

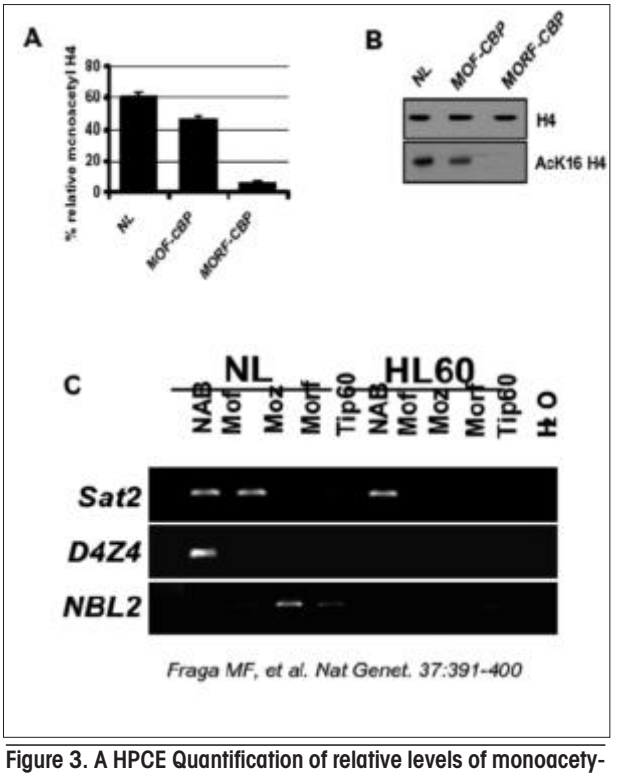

Figure 3. A HPCE Quantification of relative levels of monoacetylated histone H4 in normal lymphocytes (NL) and cells harboring the leukemic fusion proteins MOZ-CBP and MORF-CBP. B. Western blot comparing acetylation levels of lysine 16 of histone H4 in the same samples. C. Chromatin inmunoprecipitation (ChIP) analysis of the Lys16-specific histone acetyltransferases MOF, MOZ, MORF and TIP60 at the repetitive DNA sequences in normal lymphocytes (NL) and HL60 cells.

formed cells and an important step in the development of certain types of leukemia that may contribute to the physiopathologic and clinical features of this group of malignancies.

DNA methylation changes also constitute one of the most promising prognostic and predictive markers. As example of DNA methylation markers of poor prognosis we can mention the cell cycle regulator p15 that has been linked with a poorer outcome in AML.[8]

The expression of the A-type lamins is reduced or absent in cells with low degree of differentiation and/or cells that are highly proliferating, including human malignances, especially leukemias and lymphomas. In our laboratory we have found that epigenetic silencing of the lamina A/C gene by CpG island hypermethylation is responsible for the loss of expression of A-type lamins in leukemias and lymphomas. Moreover, lamina A/C CpG island promoter hypermethylation is a significant predictor of poor outcome in nodal diffuse large B-cell lymphomas.[9]

Post-traductional histone modifications

Another epigenetic modification linked to cancer development is the aberrant pattern of posttralational modifications of histones. In particular, acetylation of lysine residues of histone 3 and histone 4 is one of the best-studied histone modifications. Acetylation levels of key histone amino acid residues result from the balance of the activities of histone acetyltransferase (HAT) and histone deacetylase (HDAC). The acetylated form of lysine residues of histones tails is associated with less condensed chromatin and a transcriptionally active gene status, whereas the deacetylated state is associated with heterochromatin and transcriptional gene silencing. A number of evidence indicates that abnormal HDAC activity results in transcriptional repression of tumor suppressor genes that has been shown to have a crucial role in tumor progression. There is a great number of evidence suggesting that global histone deacetylation may participate in cancer cell

invasion and metastasis. Alterations of expression or structure of HDACs and/or HATs are associated with development of many cancers. Methylation of selected histone amino acids sites is another histone modification controlled by various histones methyltransferases. This modification has different effects on chromatin function, since it can be related for both active and inactive chromatin regions.

With respect to histone acetylation, we have found a loss of acetylation at Lys16 of histone H4 in cancer. This specific histone modification is tightly regulated, and several HATs are implicated, including MOF, MORF, MOZ and TIP60. Since the genes encoding MOZ and MORF are common fusion partners in chromosomal translocations associated with hematological malignances, a direct link with tumorogenesis has been already done. In fact our data show that there is a loss of recruitment of MOZ, MOF and MORF to DNA-repetitive sequences in cancer cells[10] and an association of the fusion proteins.

A similar scenario could be proposed for the trimethylation of lysine 20 of H4. This reaction is catalyzed by two histone methyltransferases (HMTs) Suv4-20h1 and Suv4-20h2, in addition to PR/SET7-SET8. These HMTs could also constitutes targets for disruption in cancer cells, as occurs with another HMT, MLL1, which is translocated to multiple partners in hematological malignancies.[11] The results may have implications for the identification of histone-modifying enzymes as putative targets for cellular transformation.

Conclusions

DNA methylation and histone modifications interact in an epigenetic network that is crucial for the regulation of chromatin structure and gene transcription. A large number of genes involving fundamental cellular pathways may be affected in virtually all types of human cancer by aberrant CpG island methylation in association with transcriptional silencing. Altered methylation patterns can be used as biomarkers for cancer detection, assessment of prognosis, and prediction of response to antitumor treatment. Since DNA methylation and histone deacetylation (HDACs) are potentially reversible by pharmacological inhibition, these epigenetics changes have been recognized as promising novel therapeutic targets in hematopoietic malignances. Furthermore, clinical trials using epigenetically targeted therapies have yielded promising results for leukemias as well as for myelodysplastic syndromes.

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