Focus on Myelodysplastic Syndromes

Highlights from the 45th Annual Meeting of the American Society of Hematology
San Diego, California, December 6–9, 2003

Novel conditioning regimens take the focus in hematopoietic stem cell transplantation in MDS

SAN DIEGO—**Ghulam Mufti, MB, DM, FRCP, FRCPath**, from GKT School of Medicine, Kings College Hospital, London, provided an overview on the current status of hematopoietic stem cell transplantation (HSCT) in myelodysplastic syndromes (MDS) during an education session at the 45th American Society of Hematology annual meeting.

"Allogeneic HSCT is the only therapeutic modality at present that may be delivered with curative intent in MDS," said Dr Mufti. The use of HSCT, however, is limited by the availability of matched donors and by the toxicity of the conditioning regimens. Most MDS patients are elderly and often have concurrent comorbid conditions that prevent the use of standard conditioning regimens for allogeneic HSCT. Therefore, various strategies have been adopted in order to attempt to reduce the toxicities associated with the transplant procedure and to solve the problem of limited donor availability.

Autologous HSCT in MDS is theoretically feasible in patients who achieve a complete remission following induction chemotherapy and in whom sufficient autologous cells can be harvested. Patients over 40 years may

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Presidential symposium sheds light on epigenetics

SAN DIEGO—The presidential symposium at the 45th annual meeting of the American Society of Hematology focused on epigenetics and its impact on health. The human genome project has provided a blueprint of all genes that are expressed and altered in diseases. However, mutations and translocations within genes are not the only causes of cancer. There are facets of gene replication that are now being discovered. Epigenetics adds another layer of complexity. Epigenetics, coined as 'on top of genetics,' are heritable changes in gene expression that occur without a change in DNA sequence. During the symposium, speakers defined the intricacies of epigenetic regulation of transcription.

Arthur L. Beaudet, MD, from the Baylor College of Medicine, Houston, Texas, talked about genomic imprinting and diseases, focusing on the non-cancer aspects of epigenetics. The relevant aspects, according to Dr Beaudet, are the differences between a genetic and an epigenetic disease. Dr Beaudet organized his talk around such questions as whether neural tube defects can be considered an epigenetic disease, whether epigenetics provides a key to understanding complex disease traits, how epigenetics impacts hematology, and whether folic acid intake alters gene expression.

Epigenetics refers to reversible gene regulation over the primary genetic

Methylation during cell division

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Hypermethylation of cell cycle control genes may occur late during differentiation of MDS stem cells

SAN DIEGO—**Wolf-Karsten Hofmann, MD**, from the University Hospital, Frankfurt/Main, Germany, presented a poster on hypermethylation of cell cycle genes in myelodysplastic syndromes (MDS) during the poster session on Myelodysplastic Syndromes: Clinical Investigations at ASH 2003. He compared the methylation pattern of four cell cycle control genes between unselected low-density nonadherent bone marrow cells and CD34+ bone marrow cells from 60 patients with low-risk and high-risk MDS. The genes that were analyzed using a methylation-specific polymerase chain reaction were the cyclin-dependent kinase inhibitors, $p15^{INK4B}$ and $p16^{INK4A}$, $p14^{ARF}$, which block MDM-2 (an inhibitor of p53), and the retinoblastoma protein, critical for hematopoietic proliferation and differentiation.

In unselected low-density nonadherent bone marrow cells, at least one of four genes was methylated in 67% (40/60) of patients. The most frequently hypermethylated gene was $p15^{INK4B}$, which was found in 60% of all patients; in 55% of patients with FAB subtype refractory anemia, and 65% of refractory anemia with excess blasts and refractory anemia with excess blasts in transformation. Much lower incidence of hypermethylation was observed with $p14^{ARF}$ (7%) and $p16^{INK4A}$ (3%). The retinoblastoma gene was hypermethylated in 12% of patients with FAB subtype refractory anemia. Interestingly, no methylation of cell cycle control genes was found in purified CD34+ cells. The authors concluded that hypermethylation of cell cycle control genes in MDS may occur late during the differentiation of myelodysplastic stem cells. ■

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Presidential symposium, *continued*

sequence and is the study of changes in gene function without changes in the DNA sequence. Epigenetics is not under dynamic transcriptional control, but may be regulated posttranscriptionally. The most common epigenetic change observed is a methylation or demethylation at a CpG dinucleotide. During cell division, DNA methylation patterns in the parent DNA strand are maintained in the daughter strand and catalyzed by the enzyme DNA methyltransferase 1 (DNMT1) *(see figure on page 1)*. Similarly, there is more information encoded in the histones (protein structure) on the DNA. Covalent modifications of histones H3 and H4, particularly by the acetylation of lysines, allow the gene to be transcriptionally active. There are similar mechanisms to inherit these modifications from one generation to the next. A number of proteins participate in chromatin modeling, including histone methyltransferases, histone deacetylases, and other DNA binding proteins.

Dr Beaudet's interest in epigenetics stemmed from a teenage patient of short stature who was purported to have cystic fibrosis. The teenager had a microdeletion in chromosome 7; instead of having the usual distribution of maternal and paternal chromosomes, it was interpreted as a monosomic chromosome 7, which should have resulted in a spontaneous abortion. However, the germ cell appeared to have acquired a second copy of chromosome 7. The offspring had a genetic disease with two copies of the cystic fibrosis gene inherited from the mother and an epigenetic disease named maternal uniparental disomy, characterized as the inheritance of two copies of chromosome 7 from the mother and none from the father; this does not normally occur. Dr. Beaudet used this case as an example for genomic imprinting.

In this patient, the epigenetic phenomenon is stature, in which, depending on the sex of the parent from whom the gene is inherited, the activity of a gene is reversibly modified, leading to an unequal expression from the maternal and paternal alleles of a diploid locus where one is on and one is off. This is also manifest in the examples of the differences between a mule and a hinny. Imprinting appears to impact on growth, body size, and behavior. Epigenetics can be described as any change in protein modification; all

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Commentary on epigenetics

***John M. McCarty, MD**, VCU Health System/MCV Hospitals, Richmond, Virginia*

Arguably, hematology has benefitted from the revolution in molecular physiology more than most other disciplines of medicine. Advances in genomics have identified molecular determinants of poor- and standard-risk lymphoma, myeloma, and ALL. Proteonomics has revealed critical intracellular signaling pathways in leukemia and lymphoma towards which directed therapy may be targeted. Now, multiple presentations at ASH show that epigenetics represents an understanding of a critical molecular mechanism relevant to hematology by which gene expression may be altered without changes in DNA sequence. Through reversible, but inheritable methylation of critical cytosine promoter elements and subsequent deacetylation of histone residues, chromatin structure is remodeled to condensed transcriptionally inactive heterochromatin. When methylated, CpG dinucleotides clustered within gene promoters, known as CpG islands, silence gene transcription, resulting in specific functional cell phenotypes. A further layer of translational gene expression control occurs through the reciprocal methylation or acetylation of histones, which affect the accessibility of chromatin to transcription factors. The Presidential Symposium speakers discussed how inherited disorders of gene imprinting resulted from epigenetic abnormalities and how changes in promoter methylation may be an early step in carcinogenesis. Hematologists unfamiliar with these principles have unwittingly witnessed epigenetic-based phenomena for quite some time. In addition to mechanisms for globin gene switching, epigenetic gene expression and repression is responsible for T lymphocyte TH1 and TH2 phenotypic selection. Most recognizably, epigenetics affects the silencing of cell cycle-dependent kinases, such as p15, through hypermethylation. In addition to normal homeostatic conditions, CpG methylation and histone acetylation are found active in the altered genomic and proteonomic phenotypes of leukemia and myelodysplasia. This has led to the development of agents that reverse cytosine methylation, such as azacitidine and decitabine, inhibitors of histone deacetylase, such as phenylbutyrate, and the cyclin-dependent kinase inhibitor flavopiridol. Further understanding of this ubiquitous mechanism will lead to novel combination therapies that will act by resetting pathophysiologic gene expression to its normal homeostatic state. ■

John M. McCarty, MD

A primer on epigenetics

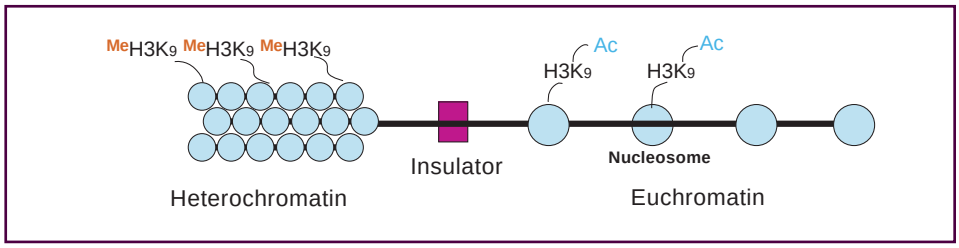
Epigenetics is the change in gene expression without alterations in the genetic sequence. The changes are usually methylation of the cytosine residues and the histone proteins associated with DNA. DNA methyltransferases methylate the 5 position of the phenyl ring in the cytosine residues, but only in cytosines that are next to a guanosine in the DNA sequence. Usually these CpG dinucleotides appear clustered in the promoter regions of genes; these are referred to as CpG islands. Methylation appears to be the dominant epigenetic modification. In cancer, the CpG islands associated with the promoters of tumor suppressors and cell cycle regulatory genes are methylated, silencing the transcription of these genes *(see figure top right)*.

The status of lysine residues in histones, proteins associated with DNA, also have a critical function. If the lysine 9 on histone H3 (H3K9) is methylated, the gene is silenced, and if it is acetylated the gene can be transcribed. In general, acetylation of lysine residues on histone tails is associated with a less condensed, transcriptionally active chromatin (euchromatin), whereas deacetylated histones are associated with a condensed, transcriptionally inactive chromatin (heterochromatin; *see middle figure*). Gary Felsenfeld, MD, has detailed these aspects of histone modification in the presidential symposium on epigenetics *(see story on presidential symposium)*.

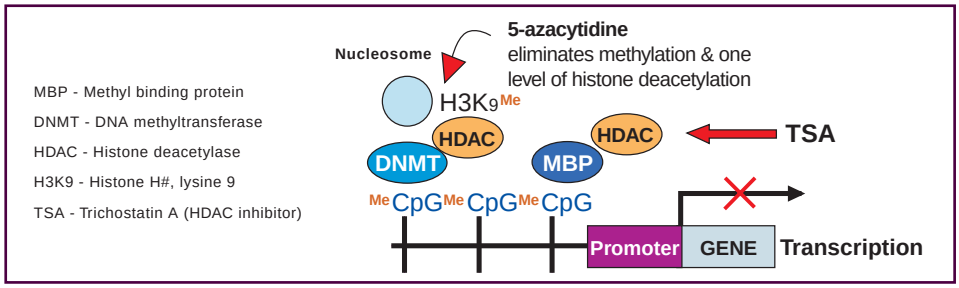
There are two agents available as inhibitors of DNA methylation, 5-azacytidine and 5-aza-2'-deoxycytidine (decitabine). These agents can reverse cytosine methylation and indirectly affect one level of histone deacetylase function. There are many classes of histone deacetylase inhibitors. These can inhibit only histone deacetylases and have no effect on DNA methyltransferases. Blocking the function of histone deacetylases will prevent the methylation enzymes, histone methyltransferases, from methylating free lysine residues *(see bottom figure)*.

It is clear from these schematics, which have been adapted from the work of Scott Baylin, Gary Felsenfeld, and Peter Jones, that a combination treatment may reverse many of these silencing mechanisms and may benefit patients with myelodysplasia and other cancers. ■

DNA methylation



Histone acetylation in chromatin



Action of DNA methyl transferase and histone deacetylase inhibition

Gene array technology reveals differences of gene expression in MDS and AML subtypes

SAN DIEGO—The molecular pathogenesis of myelodysplastic syndromes (MDS) was also featured at the annual meeting of ASH 2003. Using gene array technology, patterns of genes were identified that are up- or down-regulated in subsets of MDS patients. This may further explain the molecular pathogenesis of this disorder and result in identification of new therapeutic targets and additional criteria for accurate disease classification.

During the oral session on Myelodysplastic Syndromes: Biologic Investigations, **Andrea Pellagatti, PhD**, from the LRF Molecular Haematology Unit, NDCLS John Radcliffe Hospital, Oxford, United Kingdom, presented the results of a study using cDNA microarray technology to determine gene expression patterns in neutrophils of 22 MDS patients, 7 of whom had 5q- syndrome, compared to neutrophils of 7 healthy controls. The 5q- syndrome is the most distinct clonal disorder of MDS and is often the sole karyotypic abnormality. Features of the 5q- syndrome include refractory anemia, abnormalities in the megakaryocytic lineage, and a low rate of leukemic transformation.

Neutrophil RNA was prepared from all patients and controls. Serial samples from some MDS patients were obtained during disease progression. A high level of heterogeneity in gene expression patterns was observed, probably reflecting the disease heterogeneity in MDS. Nevertheless, several genes were commonly up- or down-regulated. The most frequently upregulated genes included Rab20, a member of the RAS oncogene family with a function in protein trafficking and cellular signaling, Znf183, ARG1 and ACPL. The most frequently down-regulated genes included Cox-2, an anti-apoptotic gene, CD18, KIAA0001, c-Fos proto-oncogene, and the interferon-induced protein 56. Many genes were differentially expressed in MDS patients in transformation to acute leukemia and/or in the high-risk MDS group as compared to low-risk group. A subset of genes was identified that was able to discriminate patients with 5q- syndrome from the other patients with MDS.

In another presentation involving gene

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