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INVITED REVIEW

A role for epigenetics in the formation of chromosome translocations in acute leukemia

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In general, the field of cancer genetics seems to have shifted its focus from cancer-associated genes to cancer-associated epigenetic activity. An abundance of evidence suggests that epigenetic malfunction, such as aberrant histone modification, and altered DNA methylation, is at the root of much, if not most aberrant gene expression associated with cancer. However, a role for epigenetics in physical DNA changes, such as chromosome rearrangements, is less obvious, and certainly less well understood. A growing body of evidence suggests that epigenetics may play a role in many of the steps of aberrant chromosome recombination, especially chromosome translocations, associated with cancers such as acute leukemias.

Keywords Etoposide, Acute Leukemia, Chromosome translocations, Epigenetics, Nucleosome

Unequivocally, cancer is a disease stemming from gene mutation. In fact, many subtypes of cancer now have an identified collection of common, consistent DNA sequence abnormalities associated with them. These DNA sequence changes range from single nucleotide substitutions to extensive, complex chromosome rearrangements. More than 20 years of molecular genetic analysis have elucidated hundreds of "cancer-associated" genes whose normal function contributes to cell cycle regulation, DNA repair, cell death, angiogenesis, cell adhesion, and genomic stability. The newest frontier of cancer genetics is *epigenetics*, which includes the various molecular modifications of chromosome elements that affect DNA packaging and gene expression but that do not alter DNA sequence. It is now clear that cancer results from both DNA mutation and epigenetic alterations.

Understandably, the bulk of epigenetic-based cancer research has focused on the dysregulation of gene expression, primarily in oncogenes and tumor suppressor genes (1). Less emphasis has been placed on understanding epigenetic factors associated with, but which precede or follow, oncogenic DNA changes, such as chromosome rearrangements. Likewise, our understanding of epigenetic changes associated with cancer treatment is in its infancy.

This review aims to summarize some recently discovered or newly interpreted epigenetic phenomena that may play a role in cancer-associated chromosome rearrangements, primarily in acute myeloid leukemia (AML). A major focus is chromosome translocations involving the lysine-specific methyltransferase 2A gene (*KMT2A*) also known as the myeloid lymphoid leukemia (*MLL*) gene. Some discussion focuses on the epigenetic consequences of cancer treatment. In some cases, such as *KMT2A* gene rearrangements in secondary AML, the two are interconnected.

Chromosome translocations and cancer

Consistent, recurring chromosome rearrangements, most often reciprocal translocations, are a hallmark of hematopoietic malignancies, and will likely prove to be a common genetic abnormality in most solid tumors as well. (2). These nonhomologous recombination events place two genes in proximity of one another, either disrupting the expression of one or both genes, or creating fusion genes. When abnormal expression of proto-oncogenes or formation of oncogenic fusion genes result from chromosome translocations, the cell involved acquires a proliferative or survival advantage.

Chromosome translocation requires DNA double-strand breaks (DSBs) and aberrant joining of DNA ends. Surprisingly little is understood about the molecular mechanisms underlying chromosome translocations, although most studies support illegitimate joining via the nonhomologous end-joining (NHEJ) repair mechanism (3,4). Many studies have focused on determining the order of events in the formation of chromosome translocations. Some experiments support DNA

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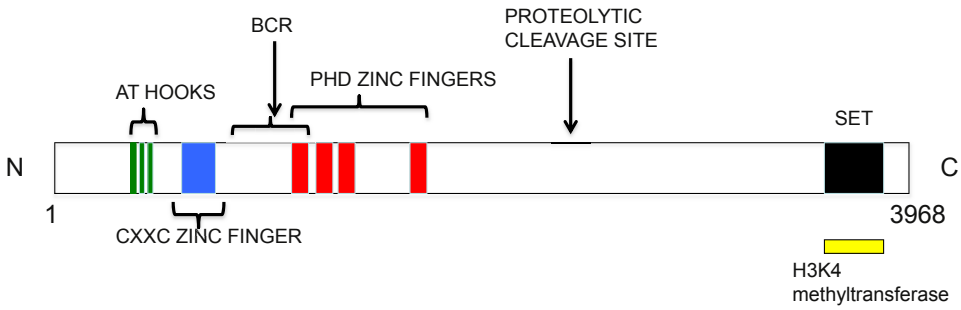

Figure 1 A simple schematic showing some domains of the KMT2A protein. General transcriptional or epigenetic activity is shown beneath the sketch. Numbers refer to amino acids. *Abbreviation*: PHD, plant homeodomain/zinc fingers.

"breakage first," whereas others support the idea that chromosomes involved in translocations must be in proximity prior to breaking—the so-called "contact-first" or "position-first" hypothesis. Regardless of the order of events, only translocations that confer a selective advantage to the cell are readily apparent and important in the etiology of cancer (5,6).

Epigenetics in DNA double-strand breaks

KMT2A chromosome rearrangements: teasing out mechanisms using topoisomerase II inhibitors: newly discovered epigenetic activities

The *KMT2A*, gene represents one of the most interesting cancer-associated genes with respect to cancer epigenetics. The *KMT2A* gene encodes a large, multidomain protein that has a C-terminal SET domain with histone H3, Lysing 4 (H3K4) methyltransferase activity. It therefore is itself an epigenetic modifier with transcription activation activity. The active KMT2A protein is a complex of proteolytic fragments of the full length KMT2A protein, and a major target of gene activation is the HOX gene family (7). The KMT2A protein has two different types of zinc finger domains. Its CXXC-type zinc finger domain distinguishes between methylated and nonmethylated CpG DNA and preferentially binds nonmethylated CpGs in the *HOXA9* promoter (8). Interestingly, the KMT2A protein shows features of an epigenetic transcriptional repressor as well, in its ability to recruit histone deacetylases (HDACs) and Polycomb group proteins (PcGs) (9) (Figure 1).

Prior to the identification of the KMT2A protein's activity as an epigenetic modifier or transcription regulator, the *KMT2A* locus at chromosome 11 band q23 gained fame for involvement in aggressive leukemia associated with its frequent rearrangement involving an extraordinary variety of chromosome translocation partner genes. A recent study identified 121 unique *KMT2A* translocation partners. Of these loci, 79 have already been studied at the molecular level, although the vast majority of *KMT2A* translocations involve only six partner genes, which are located on chromosomes 4, 6, 9, 10, and 19. *KMT2A* also forms an intragene rearrangement creating a partial tandem duplication (PTD) of the 5′ end of the gene (10). *KMT2A* rearrangements are associated with approximately 70% of acute myeloid, acute lymphoid, and mixed-lineage leukemia in infants and young children. *KMT2A* rearrangements are poor prognostic markers in AML and acute lymphocytic leukemia (ALL) in adults as well (11). Likewise, 5–15% of chemotherapy-related secondary AML shows a *KMT2A* gene rearrangement, with a strong correlation with prior exposure to DNA topoisomerase II (topo II) inhibitors (12). All translocations stem from breakage within an 8.3-Kb region of this approximately 90-Kb gene, which is referred to as the *KMT2A* breakpoint cluster region (*KMT2A* BCR) (13,14). Within the *KMT2A* BCR, breakpoints tend to cluster into two regions correlating with de novo or secondary AML and with the age of the patient at diagnosis (15) (Figure 2).

Numerous studies of the *KMT2A* BCR have attempted to identify features contributing to its propensity to break or initiate nonhomologous recombination. Sequence analysis reveals only weak homology to any known common recombinogenic signal sequences. Moreover, thorough studies to map specific DNA-binding proteins in the *KMT2A* BCR have not been done. However, the correlation of exposure to topo II inhibitors, such as etoposide, with breaks in the *KMT2A* BCR suggested topo II likely binds in the BCR (16). Etoposide is a topo II poison that forms a cleavable DNA complex with topo II and inhibits its ability to reseal DSBs. Several cell-free and cell line studies provide evidence for topo II binding sites within the *KMT2A* BCR. It is assumed that in *KMT2A*-rearranged secondary AML, etoposide allows topo II–mediated DSBs to accumulate in hematopoietic precursors and that aberrant NHEJ repair leads to leukemogenic *KMT2A* chromosome translocations in these cells (17,18).

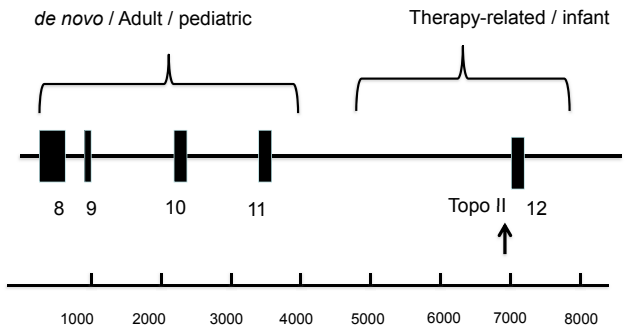

Figure 2 An illustration of an *KMT2A* gene breakpoint cluster region that consists of 8.3 kb of DNA, including exons 8–12. Numbers represent base pairs of DNA. Exons are represented by filled black boxes. Breaks in AML distribute to two distinct regions, depending on age and whether the AML is de novo or therapy-related. A large arrow indicates the proposed position of topo II binding/etoposide cleavage site.