Chromosomal Rearrangements Leading to MLL Gene Fusions: Clinical and Biological Aspects

David P. Harper and Peter D. Aplan

Abstract

Rearrangements of the MLL gene located at 11q23 are common chromosomal abnormalities associated with acute leukemia, especially infant and therapy-related leukemias. A variety of chimeric oncoproteins resulting from these rearrangements has been described; all of these include the NH2-terminal region of MLL implicated in protein-protein interactions and transcriptional repression. Although the molecular basis for the oncogenic activity of MLL chimeric proteins is incompletely understood, it seems to be derived, at least in part, through activation of clustered homeobox (HOX) genes. Here, we survey MLL gene rearrangements that are associated with acute leukemia and discuss molecular pathways leading to these rearrangements. [Cancer Res 2008;68(24):10024–7]

Introduction

Chromosomal rearrangements involving the MLL (MLL1, ALL1, TRX, and HTRX) gene, including balanced and unbalanced translocations, inversions, insertions, and a partial tandem duplication, have been associated with a heterogeneous group of lymphoid, myeloid, and mixed lineage leukemias (1). The MLL gene, a homologue of the Drosophila trithorax, is located at chromosome 11q23, consists of 36 exons, and encodes a protein of 3,969 amino acid residues, with an estimated molecular mass of 430 kDa (1). Most MLL gene rearrangements map to an 8.3-kb breakpoint cluster region (bcr; ref. 2), and result in production of a chimeric oncoprotein, which fuses the amino terminal portion of MLL with the carboxy terminal portion of a partner gene (Fig. 1). MLL can be regarded as a highly “promiscuous” oncogene because >70 different 11q23 chromosomal partners have been identified, and at least 50 of these have been cloned and characterized on a molecular level.3

Clinical Findings

Although MLL rearrangements are associated with a wide spectrum of leukemias, and numerous translocation partners, there are several unique subsets of MLL leukemias that are defined by associated clinical and laboratory findings. These subgroups include infant leukemia, therapy-related leukemia, MLL-amplified leukemia, and T-cell acute lymphoblastic leukemia (ALL). Infant leukemia. Leukemia in infancy (ages <12 months), whether classified as ALL, acute myelogenous leukemia (AML), or mixed lineage, is often associated with MLL gene rearrangements (3). It seems likely that infant leukemia with and without MLL are different diseases with different clinical characteristics at presentation, different responses to therapy, and very different genetic profiles (4). When molecular diagnostic techniques are used, MLL gene fusions can be identified in ~80% of all infant leukemia patients (4). Although >90% of infant ALL patients achieve a complete remission, despite the use of aggressive intensification regimens and allogeneic stem cell transplantation, event-free survival (EFS) for MLL-rearranged infant ALL patients remain in the 30% to 40% range (4). Several lines of evidence have shown that the MLL rearrangement in most infants with leukemia occurs in utero. First, MLL rearrangements have been identified in congenital infant leukemia, and even in aborted fetuses. Second, the concordance rate for infant leukemia between identical twins is very high, suggesting that the MLL rearrangement occurred initially in one twin in utero, and subsequently “metastasized” to the second twin (5). This hypothesis has been confirmed for numerous twin pairs that had identical immunoglobulin gene rearrangements (5). Finally, MLL gene fusions have been identified in archived neonatal blood spots (“Guthrie” cards) of infants who subsequently developed leukemia (6).

Therapy-related acute myeloid leukemia. In the 1990s, the use of the epipodophyllotoxins became linked to the development of therapy-related acute myeloid leukemia (t-AML; ref. 7). Remarkably, a very high proportion of patients who developed t-AML after therapy with topoisomerase II (topo II) poisons had translocations including the MLL gene (7). This observation led to speculation that topo II poisons were directly involved in the generation of MLL rearrangements. The MLL partner genes involved in t-AML, including AF9, AF4, and ENL, are similar to those involved in de novo AML, except for the CBP gene, which is almost always associated with t-AML (8). Although less common than t-AML, therapy-related ALL and lymphoblastic lymphoma with MLL gene rearrangement have also been reported.

MLL amplification. Amplification of the MLL gene, either at 11q23, distant chromosomal regions (called segmental jumping translocations), or double minute chromosomes were identified using fluorescence in situ hybridization probes (9). In these cases, no rearrangement of the MLL gene has been reported, rather, MLL has been amplified as part of a large ampiclon encompassing up to 10 Mb of genomic sequence. Of note, a wild-type MLL transcript is reported to be overexpressed in these cases, as is expression of several documented target genes for leukemogenic MLI fusion proteins, such as HOXA9 and MEIS1.

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T-cell ALL. Although T-cell ALL with **MLL** gene rearrangements are uncommon, this is an interesting subgroup as this group of translocations involve relatively few partner genes, almost exclusively t(11;19) that generate a **MLL-ENL** fusion. In addition, these patients tend to have a good prognosis, with close to 90% long-term EFS in one published series (10).

**Effects of MLL Fusion Genes**

The MLL protein is widely expressed during development, and continues to be expressed in most adult tissues, including myeloid and lymphoid cells (1). MLL is required for normal development and body pattern formation, as deletion of **MLL** in mice leads to homeotic transformation (1). MLL is processed in the cytoplasm by the threonine-aspartase (TASPASE1) enzyme into a 320-kDa amino-terminal fragment (**MLL-N**) and an 180-kDa carboxy-terminal fragment (**MLL-C**), which remain noncovalently bound (Fig. 1; ref. 1). The MLL-N fragment is thought to bind DNA as part of a multisubunit complex that includes components of the basal transcription machinery (1). Recent experiments have suggested that MLL-N binds to regulatory regions of clustered **homeobox (HOX)** genes, including **HOXA9** and **HOXC8**. Upon binding DNA, MLL-N can mediate transcriptional repression of the target gene, likely dependent on recruitment of additional cofactors such as **BMI1**. However, in the presence of MLL-C, the MLL-N complex can lead to transcriptional activation (1).

Although no single theme connects all of the **MLL** translocation partners, several are known or putative transcription factors (**AF9,** **ENL,** **CBP,** and **P300**). Other partners, such as **AF1P** and **GAS7,** have no known transcription factor motifs, and have instead hydrophobic coiled-coil domains with the potential to form oligomers. Useful clues as to how MLL fusion proteins might be leukemogenic have been identified by gene expression profiling. Leukemic cells that express **MLL** gene fusions have a gene expression profile that distinguishes them from ALL and AML without **MLL** gene rearrangements; among the genes most differentially expressed are **HOXA5** and **HOXA9** (1). Expression of MLL fusion proteins such as **MLL-ENL** or **MLL-CBP** in mouse bone marrow leads to overexpression of **Hoxa7,** **Hoxa9,** and **Meis1,** and it is thought that overexpression of these genes is important for leukemic transformation (11). **Hoxa9** is normally expressed in primitive hematopoietic cells, becomes down-regulated as cells differentiate, and promotes hematopoietic stem cell self-renewal (12). Enforced expression of **Hoxa9** in mouse bone marrow cells is leukemogenic, and leukemic transformation is accelerated by coexpression of **Meis1** (12). However, expression of **Hoxa9** is not required for leukemic transformation by MLL fusion proteins, as expression of an **MLL-**

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**Figure 1.** Structure of the **MLL** gene and protein. A, schematic representation of **MLL** gene showing location of the partial tandem duplication (**PTD**; see text) and breakpoint cluster region (**BCR**), which encompasses almost all known **MLL** translocation breakpoints. Exon size and intronic distances are not to scale. B, location of **MLL** protein domains in relation to **BCR**, **PTD**, and fusion partners. **AT-hook** [DNA binding motif that binds adenosine-thymidine (**AT**)-rich DNA], speckled nuclear localization sites (**SNL**), DNA methyltransferase domain (**DNMT**), plant homeodomains (**PHD**), transactivation domain (**TAD**), and **Suppressor of variegation/Enhancer of zeste/Trithorax (**SET**) domain]. Cleavage by Taspase (Threonine-aspartase) 1 divides **MLL** in **NH2-** and **COOH-terminal** fragments.
GA7 fusion in Hoxa9-deficient bone marrow remains leukemogenic (13). Given the redundancy of the clustered Hox genes, it is possible that up-regulation of other Hox genes (such as Hoxa7 or Hoxa10) mediates leukemic transformation in this setting.

Several approaches have been used to show that MLL fusion proteins are leukemogenic in mice. Using retroviral-mediated gene transfer to murine hematopoietic cells, followed by transplantation into irradiated mice, a number of MLL fusions, including MLL-AF9, MLL-GAS7, MLL-ENL, and MLL-CBP have been shown to be leukemogenic (13). Using ES cell gene targeting strategies, an MLL-AF9 fusion, under the regulatory control of endogenous MLL sequences, was leukemogenic (14). Interestingly, although the targeted ES cells expressed the MLL-AF9 fusion ubiquitously, the only malignancies noted were hematopoietic, suggesting that MLL fusions might only be oncogenic in hematopoietic cells (14).

Mechanisms of MLL Gene Rearrangement

Because MLL gene fusions lead to leukemia, it is important to understand the causes of MLL gene fusions. It should be noted that for any chromosomal rearrangement to be recognized clinically, two criteria must be fulfilled. First, the region involved must undergo a DNA double strand break (DSB) and religation. Second, the break and religation must in some way confer a clonal growth advantage to the cell. If both criteria are not fulfilled, then the translocation will not produce a clonal population, and not be recognized clinically. Proposed mechanisms to account for MLL translocations include recombination between Alu elements, recombination mediated by topo II poisons, and an error prone nonhomologous end joining (NHEJ) of DNA DSB.

Although chromosomal translocations caused by interchromosomal recombination between Alu elements have been implicated in a case of an MLL-AF9 translocation, these seem to be relatively rare. However, the MLL partial tandem duplication (also known as MLL self-fusion), which leads to a duplication of MLL exons 2 to 6, is commonly mediated via inter or intrachromosomal recombination between Alu elements within the MLL locus, via a mechanism that is consistent with the single-strand annealing repair pathway (15).

The association of t-AML with MLL translocations and topo II poisons has led to the hypothesis that these translocations are directly caused by topo II poisons (16). Topo II normally functions as a homodimeric enzyme that introduces a 4-bp staggered nick in double-stranded DNA resulting in a short-lived intermediate in which the topo II monomer is covalently bonded to the DNA phosphodiester backbone. Topo II poisons stabilize this short-lived intermediate, which becomes recognized by the cell as a DNA DSB, which triggers an apoptotic cell death (16). Because topo II normally functions as a homo-dimer, it has been proposed that perfect (i.e., no net gain or loss of genetic material) or near-perfect (gain or loss of four or fewer nucleotides) reciprocal translocations could occur via an exchange of topo II subunits and covalently linked chromosomal DNA (17). Chromosomal translocations showing this type of perfect or near-perfect interchromosomal exchange have been identified in patients with t-AML after chemotherapy regimens that included topo II poisons. However, it should be noted that these cases may be the exception rather than the rule, as more extensive surveys of MLL translocation breakpoints, from de novo or t-AML patients, do not contain large numbers of samples with these near-perfect reciprocal interchromosomal exchanges (18).

Site-specific cleavage of the MLL bcr has been identified in leukemic patient samples and cell lines after treatment with topo II poisons (19). Although this cleavage site maps close to a consensus topo II cleavage site, it seems likely that this site encompasses a region that is generally susceptible to DNA DSB, as cleavage can be induced by DNase I, or more generally by the high molecular weight DNA fragmentation that occurs in cells undergoing apoptosis (19). Taken together, these observations have led to speculation that an “aborted” apoptosis program could lead to MLL gene rearrangement after abnormal repair at this cleavage site (19). Interestingly, PCR products consistent with MLL gene rearrangements produced by improper repair of DNA double strand cleavage at this site can be identified after treatment with genotoxic (etoposide) or nongenotoxic (Fas ligand) triggers of apoptosis (20).

Breakpoint nucleotide sequence data suggests that a majority of the leukemogenic translocations involving MLL are mediated by inappropriate repair of DNA DSBs via an “error-prone” NHEJ pathway (18). However, the proximate cause(s) of the DNA DSBs within the MLL bcr remain unclear. Moreover, it remains unknown whether the MLL bcr is extraordinarily susceptible to DNA DSBs (i.e., one of the most susceptible regions throughout the human genome), whether the MLL bcr is uniquely susceptible to aberrant, error-prone NHEJ repair of DNA DSBs, or whether neither of these are true, and the frequent presence of MLL translocations in leukemic samples is due to a remarkable growth/survival advantage conferred by oncogenic MLL fusion proteins.

Summary

Chromosomal translocations leading to MLL gene fusions are a common event in patients with acute leukemia, and are particularly common in infants with AML or ALL, and patients with t-AML. MLL is a “promiscuous” oncogene, with numerous partner genes involved in MLL fusions. The molecular mechanisms that lead to MLL gene rearrangements remain obscure but seem to involve aberrant repair of DNA DSB breaks via NHEJ. The normal MLL protein undergoes proteolytic cleavage, and can function as a transcriptional repressor or activator; the activation function is associated with its ability to covalently modify histones. Expression of MLL gene fusions is oncogenic in mice, and seems to be due, at least in part, to up-regulation of clustered homeobox genes such as Hoxa9. The presence of MLL fusions are currently used in risk-stratification schemes for childhood leukemia, and the observation that MLL fusion proteins modify histones suggests that this activity might be a target for modulation by small molecules.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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