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Chromosomal translocations involving the *MLL* gene: molecular mechanisms

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Abstract

A wide array of recurrent, non-random chromosomal translocations are associated with hematologic malignancies; experimental models have clearly demonstrated that many of these translocations are causal events during malignant transformation. Translocations involving the *MLL* gene are among the most common of these non-random translocations. Leukemias with *MLL* translocations have been the topic of intense interest because of the unusual, biphenotypic immunophenotype of these leukemias, because of the unique clinical presentation of some *MLL* translocations (infant leukemia and therapy-related leukemia), and because of the large number of different chromosomal loci that partner with *MLL* in these translocations. This review is focused on the potential mechanisms that lead to *MLL* translocations, and will discuss aberrant VDJ recombination, Alu-mediated recombination, non-homologous end joining, as well as the effect of DNA topoisomerase II poisons and chromatin structure.

Keywords

MLL; chromosomal translocation; infant leukemia; DNA topoisomerase II; non-homologous end-joining

1. Introduction

The study of recurrent, non-random chromosomal translocations has proven to be a rich source of insights into the biology of hematologic malignancies [1,2]. A number of themes concerning these chromosomal translocations has become apparent over the past decades [3]. For instance, particular translocations are associated with particular subtypes of leukemia; the t(15;17) is exclusively associated with acute promyelocytic leukemia (APL) [4], and the t(1;19) is associated only with pre-B cell acute lymphoblastic leukemia (ALL) [5]. Additionally, each gene involved in chromosomal translocations has a preferred partner, with perhaps 2–4 additional, infrequent partners. For instance, *E2A* is typically fused to *PBX1* but less frequently to *HLF* [6], and *RARA* is most commonly involved in translocations with *PML*, but can also be fused to *PLZF* or *NPM* [4]. Chromosomal translocations involving the *MLL* (for Mixed Lineage Leukemia, also called *ALL-1*, *HRX*, and *Htrx*) locus on chromosome 11 band q23 provide exceptions to these two aforementioned generalizations. *MLL* translocations are associated with a wide array of hematologic malignancy, including acute myelogenous leukemia (AML), T-cell ALL, B lineage ALL, myelodysplastic syndrome (MDS), lymphoblastic lymphoma, and Burkitt's lymphoma [7–9]. In addition, *MLL* has been labeled a “promiscuous” oncogene since over 60 partner genes or regions have been identified [9].

The *MLL* gene was initially cloned by virtue of its involvement in t(4;11), t(11;19), and t(9;11) translocations [7,9]. The *MLL* genomic structure consists of 36 exons distributed over 100 kb, and produces a 12 kb mRNA that encodes a 3968 aa protein with an estimated molecular weight of 430 kD [7]. The *MLL* protein is widely expressed in the developing embryo, and is expressed at lower levels in most adult tissues. The predicted amino acid sequence of *MLL* indicates that it is homologous to the trithorax gene of *D. melanogaster*. Recent experiments have indicated that *MLL* is normally processed via a cytoplasmic cleavage event into a 320 kD amino terminus (*MLL-N*), and a 180 kD carboxy terminus fragment [7]. A number of protein motifs/domains have been identified in the primary structure of *MLL*, including AT hooks, a DNA methyltransferase domain, PHD domains, a transactivation domain, and a SET domain. Several lines of evidence indicate that one important function of *MLL* is in the maintenance of HOX gene expression during embryonic development. Loss of *MLL* function in flies leads to homeotic transformation, and deletion of *MLL* in mice leads to embryonic lethality and homeotic transformation [7]. Biochemical analysis of *MLL* suggests that it normally functions as a transcription regulator, and expression of *MLL* fusion proteins has been shown to be leukemogenic in mice. A detailed discussion of these aspects is beyond the scope of this review, which will focus on mechanisms that lead to *MLL* translocations; the reader is referred to several excellent reviews for an overview of these aspects [7,9,10].

2. Clinical observations

2.1 Clinical overview

MLL translocations are among the most common translocations in hematologic malignancy. Approximately 3–10% of patients with AML have *MLL* translocations, and 8–10 % of patients with B-lineage ALL have *MLL* translocations [7,9]. Patients with AML and *MLL* translocation have an intermediate prognosis compared to all cases of AML, whereas patients with B-lineage ALL and *MLL* translocations tend to have a poor prognosis. In contrast, although *MLL* translocations are relatively rarely associated with T-ALL, these patients tend to have a very good prognosis [11]. Two distinct forms of acute leukemia are associated with *MLL* translocations and will be discussed in more detail below. *MLL* translocations are exceedingly common in infants with AML and ALL, and have been identified in up to 80% of all infant acute leukemia cases [12]. In addition, *MLL* translocations are seen in approximately 25% of patients with therapy-related leukemias, particularly those associated with the use of chemotherapeutic agents that target DNA topoisomerase II (topo II) [13].

2.2 Infant leukemia

Current models of malignant transformation suggest that multiple pathways, including those governing cell growth, differentiation, death, and responsiveness to external signals need to be disrupted in order to generate any malignancy [14]. It has been suggested that myeloid leukemias require at least two collaborating mutations, one that impairs blood cell differentiation, and a complementary one that leads to increased proliferation and/or decreased apoptosis [15]. Support for this conceptual framework comes both from clinical observations (patients with APL often have *PML-RARA* fusions, which impair differentiation, and *FLT3* mutations, which lead to increased proliferation), as well as murine bone marrow transduction/transplantation experiments, in which bone marrow cells transduced with both *BCR-ABL* (which leads to increased proliferation) and *NUP98-HOXA9* (which impairs differentiation) were leukemic, whereas cells transduced with only *BCR-ABL* were not [15]. Further support for this framework comes from the observation that many leukemic fusions can be detected in hematopoietic cells from healthy individuals. For instance, *AML1-ETO* fusions can be detected in long term survivors who are presumably cured of their disease, and *TEL-AML1* fusions can be detected in cord blood from healthy newborns [16]. Moreover, “knock-in” mice that express an *AML1-ETO* fusion gene do not develop leukemia until they are exposed to mutagens, which

presumably cause mutations that complement *AML1-ETO* [17]. Taken together, these findings support the hypothesis that a single genetic event is not sufficient to cause acute leukemia.

In this context, *MLL* fusions are unusual in that they display a very brief latent period. Leukemias with *MLL* fusions have been detected in newborns [18,19], and even in aborted fetuses [20], indicating that the *MLL* translocations can occur *in utero*. Moreover, the concordance rate of leukemia in monozygotic twins who have *MLL* fusions is almost 100% suggesting that the disease is highly penetrant [21]. These observations suggest that either complementary mutations occur very rapidly in cells that have undergone *MLL* fusions, or that an *MLL* gene fusion is sufficient to cause malignancy, and does not require additional, complementary events. The observations that infants with *MLL* fusions often have *FLT3* mutations as well as the *MLL* fusion, and that *MLL-AF9* “knock-in” mice develop leukemia only after a protracted latency period argue it is unlikely that cells with *MLL* fusions do not require additional mutations. Therefore, it seems more likely that complementary mutations occur very rapidly in cells that have undergone *MLL* fusions. At least two mechanisms have been suggested to explain this hypothesis [10]. It is possible that *MLL* fusions could lead to a “mutator phenotype” Although there is no direct experimental evidence to support this hypothesis, *MLL* fusions have been shown to impair DNA double strand break recognition [22] and cell cycle checkpoints [23,24], both of which could lead to increased accumulation of mutations. Alternatively, the insult (for instance, exposure to topo II poisons; see below) that caused the *MLL* translocation could cause additional mutations, especially if the individual had an inherited deficiency in DNA repair and/or toxin metabolizing enzymes. This possibility is supported by studies which show that certain isoforms of *NQO1* [25] or *CYP3A4* [26] confer an increased risk of infant or therapy-related leukemia respectively.

2.3 therapy-related leukemia

One of the tragic side-effects of successful cytotoxic chemotherapy treatment of any malignancy is the development of a therapy-related neoplasm. Therapy-related acute myeloid leukemia (t-AML) generally presents either early (within 3 years of cessation of all chemotherapy), or late (seven or more years after completion of therapy). The “early” t-AML often display balanced translocations, especially involving the *MLL* gene, whereas the “late” t-AML often shown unbalanced rearrangements, and often have an antecedent myelodysplastic syndrome [27]. Although the analysis of t-AML was confounded because most patients had received multi-agent chemotherapy, multivariate analysis strongly implicated topo II poisons, particularly the epipodophyllotoxins etoposide and teniposide, in the generation of balanced *MLL* translocations in the patients with “early” t-AML [13,28]. The role of topo II poisons in the generation of *MLL* translocations was reinforced by case reports of t-AML with *MLL* translocations in patients who received single agent therapy with etoposide [29], and by studies showing that etoposide could generate chromosomal rearrangements in cultured lymphocytes [30]. Data from the initial series of cases with t-AML related to topoisomerase II poisons suggested that the epipodophyllotoxins were more highly associated with t-AML than other topo II poisons, such as the anthracyclines, and that t-AML was both dose and schedule dependent [31].

3. Not all gross chromosomal rearrangements involving *MLL* are translocations

Although most studies of gross chromosomal rearrangements (GCR) involving *MLL* have focused on chromosomal translocations; chromosomal insertions, duplications, and “jumping” translocations involving *MLL* have also been described in patients with AML. Insertions of a megabase region of chromosome 11 material, including the 5' portion of *MLL*, into chromosome 5q31 leads to production of an *MLL-AF5Q31* fusion gene; this insertion has thus

far only been reported in infants with AML [32]. A partial tandem duplication (PTD), in which a genomic fragment containing several *MLL* exons becomes duplicated, is commonly seen in patients with AML, particularly those with trisomy 11 or normal karyotypes [33,34] (fig. 1). As a consequence of this rearrangement, *MLL* exon 6 sequences become fused to *MLL* exon 2, generating an *MLL* “self-fusion” protein. In contrast to the *MLL* PTD and *MLL* insertions, jumping translocations involving *MLL* do not produce an *MLL* fusion gene. Instead, these jumping translocations result from integration of an amplicon of chromosome 11 on one or more chromosomes, resulting in cells that have multiple additional copies of the *MLL* gene [35].

4. *MLL* translocation breakpoints

The first step in identifying possible mechanisms that lead to any chromosomal translocation is simply to localize and characterize the translocation breakpoints. Most (but not all) *MLL* translocations associated with hematologic malignancy can be mapped to an 8.3 kb breakpoint cluster region (bcr). This region is bounded by BamHI restriction sites, and encompasses *MLL* exons originally designated exons 5–11 [36]. Subsequent studies revealed that *MLL* exon 4 actually consisted of 3 discrete exons (now designated exons 4A, 4B, and 4C). Therefore, although *MLL* exon 5 is actually the 7th exon (as per Ensemblv37, http://www.ensembl.org/Homo_sapiens/exonview?db=core;transcript=ENST00000359313), this review will use the older nomenclature to allow direct comparison to the original studies and exon designation.

When studying any chromosomal translocation, it is important to remember that for any translocation to be recognized clinically as a recurrent chromosomal translocation, two criteria must be fulfilled. First, the region involved must undergo a DNA breakage and religation event. Second, the breakage and religation must confer a growth or survival advantage to the cell. If both criteria are not fulfilled, then the translocation will not be recognized clinically. Translocations between any two non-oncogenic loci, such as those encoding actin and spectrin, may be quite common, but not recognized clinically since they are not leukemogenic, and never become a clonal population of cells. Therefore, the fact that *MLL* breakpoints in leukemic cells typically localize to this 8.3 kb bcr does not mean *a priori* that this region is highly susceptible to DNA double strand breakage and chromosomal translocation.

A large number of *MLL* translocation breakpoints have been cloned and the breakpoints sequenced [37]. The nucleotide sequence at the breakpoints has provided clues as to the potential mechanism(s) leading to the translocation. In general, although the translocations often appear reciprocal, or balanced, at a cytogenetic level, analysis of the nucleotide sequence demonstrates that there are often sub-microscopic deletions, inversions, and amplifications [37].

5. Proposed molecular mechanisms

Several molecular mechanisms have been proposed to account for the recurrent translocations that occur within the *MLL* bcr. Some of these mechanisms are speculations based on characterization of sequence motifs found at or near translocation breakpoints, whereas other proposed mechanisms have been tested in experimental systems. It should be noted that the proposed mechanisms that are described below are not mutually exclusive, and it seems likely that no single mechanism can explain all *MLL* translocations.

5.1 Aberrant VDJ recombination

Gu and colleagues noted nucleotide sequences that resembled heptamer and nonamer sequences near the t(4;11) breakpoints in two cell lines derived from patients with B-cell

precursor ALL and *MLL* translocations [38]. They proposed that the VDJ recombination machinery may have been directly involved in the genesis of these translocations. However, although the cryptic heptamer sequences were near the breakpoints (within 20–30 nucleotides) they were not at the breakpoints, and there was no evidence of non-templated nucleotide addition; these findings are in contrast to other examples of illegitimate VDJ recombination [39,40]. Moreover, cloning of additional breakpoints from patients with has not identified cryptic heptamers near the breakpoints.

5.2 Recombination between *Alu* elements

Alu elements are repetitive sequences that comprise over 10% of the human genome. Intrachromosomal rearrangements between *Alu* elements have been well documented in meiotic cells (for review, see [41]). In addition, interchromosomal translocations can be induced in cultured mammalian cells by introducing DNA double strand breaks (DSBs) adjacent to *Alu* elements [42]. In this system, the majority of the translocations identified were mediated via the single-strand annealing (SSA) pathway. However, although chromosomal translocations caused by interchromosomal recombination between *Alu* elements in somatic cells has been implicated in complex *BCR-ABL* translocations [43] and in a single case of AML with an *MLL-AF9* translocation [44], these seem to be relatively rare [41]. In contrast the partial tandem duplication of *MLL* (*MLL* PTD) is commonly mediated via inter or intra chromosomal recombination between *Alu* elements within the *MLL* locus. In seven of nine *MLL* PTD cases analyzed, the recombination took place between *Alu* elements within the *MLL* locus, consistent with repair via the single-strand annealing pathway; the remaining two cases showed one break within an *Alu* sequence and the second break within a non-*Alu* sequence [45].

5.3 Recombination mediated via DNA topoisomerase II

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. This is an attractive hypothesis, since topo II normally functions as a homodimeric enzyme that catalyzes the relaxation of supercoiled DNA. It does so in a three-step reaction, consisting of DNA double-strand cleavage, strand passage, and strand religation. In the first step, each monomer of topo II introduces a 4-bp staggered nick in double stranded DNA. This reaction results in a normally short-lived intermediate known as the cleavable complex. Topo II poisons stabilize the cleavable complex, which is subsequently recognized by the cell as damaged DNA. Upon recognition of this damaged DNA, the cell is eliminated through an apoptotic pathway [46]. Given that chromosomal translocations are the result of DNA double strand cleavage and religation events, it seems reasonable to think that topo II, which is known to catalyze DNA cleavage and religation reactions, would be a likely candidate for directing chromosomal translocations.

Although preferred sites of topo II cleavage *in vivo* do not consistently correlate with preferred cleavage sites *in vitro* [47], an *MLL* translocation breakpoint cloned from a patient with AML and a t(9;11) was noted to be a preferred site for epipodophylotoxin-induced topo II cleavage *in vitro* [48], leading to the suggestion that cleavage at this site triggered the translocation. Since topo II normally functions as a homodimer, and the transient cleavable complex contains topo II monomers covalently bound to the phosphodiester backbone, it has been proposed that perfect (ie, no net gain or loss of genetic material) or near-perfect (gain or loss of 4 or fewer nucleotides) reciprocal translocations could occur via an exchange of the topo II subunits and covalently linked chromosomal DNA (figure 2) [49]. This model is supported by observations that DNA recombination events consistent with this hypothesis have been detected *in vitro*, using purified topo II and lambda phage DNA substrates [50]. In addition, a near-perfect reciprocal chromosomal translocation was detected in Chinese hamster ovary cells treated with the topo II poison amsacrine [49]. More recently, chromosomal translocations showing this

type of perfect or near-perfect interchromosomal exchange have been identified at the *NUP98* [51] and *MLL* [52] loci in patients with t-AML following multiagent chemotherapy regimens that included one or more topo II poisons. However, it should be noted that these cases are probably the exception rather than the rule, as more extensive surveys of *MLL* translocation breakpoints, from either de novo leukemia patients [37] or t-AML patients [53], do not contain large numbers of samples with these near-perfect reciprocal interchromosomal exchanges. Interestingly, preliminary studies have suggested that individuals with common polymorphisms of enzymes involved in drug metabolism (*CYP3A* and *GSTP1*) may be more likely to develop t-AML, perhaps due to ineffective elimination of topo II poisons and/or active metabolites [26,54].

5.4 Translocation mediated via non-homologous end joining

Marschalek and colleagues have cloned a large number of *MLL* translocation breakpoints, primarily from patients with a t(4;11) translocation, and shown that the breakpoints often display features consistent with DNA repair via non-homologous end joining (NHEJ) [37, 55]. These features include short deletions, inversions, insertions of “filler” DNA or direct repeats, and microhomology at the breakpoints. These features were found in both infant and adult patients with t(4;11) translocations involving *MLL*. They conclude that the translocations were likely initiated by DNA double or single-strand breaks, followed by an error prone repair process (such as NHEJ).

5.5 Site-specific *MLL* cleavage within the breakpoint cluster region

As discussed above, almost all *MLL* translocations are localized to a 8.3 kb breakpoint cluster region (bcr). This bcr can be further subdivided into a centromeric and telomeric portion. The *MLL* breakpoints in cases of infant leukemia and t-AML tend to be clustered near the telomeric portion of the bcr, whereas the breakpoints in patients with de novo AML tend to be near the centromeric portion of the bcr [37,56]. A number of years ago, site-specific cleavage of the *MLL* bcr was noted to occur in patients, cell lines, and naked DNA following treatment with topo II poisons [57,58]. Intriguingly, this cleavage site mapped close to the telomeric portion of the *MLL* bcr, near a sequence that was an 18/18 bp match for a consensus topo II cleavage site, leading to the hypothesis that this cleavage may be an initial step in chromosomal translocation involving the *MLL* gene [57].

However, it seems likely that this site is a marker for a region that is generally susceptible to DNA DSB. Subsequent studies showed that cleavage at this site could be induced by DNase I, or more generally initiated during the high molecular weight (HMW) DNA fragmentation that occurs in cells undergoing apoptotic cell death [58,59]. As a cell undergoes apoptosis, prior to the oligonucleosomal degradation of genomic DNA, high molecular weight (HMW) DNA is broken into fragments of ~50 kb [60]. It has been proposed that these fragments are produced by excision of chromosomal “loops” via cleavage at scaffold/matrix attachment regions (S/MARs) [61–63]. In this context, it is important to note that this *MLL* cleavage site maps to a S/MAR, leading to the proposal that this *MLL* cleavage site is located at the base of a S/MAR, and is in a more “open” configuration, and thus more susceptible to cleavage induced by a variety of agents, including apoptotic nucleases [58]. This cleavage is clearly directed by sequences within the *MLL* bcr, and not more long-range effects, since episomes containing the *MLL* bcr are cleaved at the same site when cells containing the episomes undergo apoptosis [64].

Taken together, these observations have led several investigators to speculate that an “aborted” apoptosis program could lead to GCRs following abnormal repair at this cleavage site [58, 65,66]. Although apoptosis has traditionally been thought of as irreversible once initiated, studies in *C. elegans* suggest that apoptosis might be reversible [67–69]. Intriguingly, two

recent studies have used inverse PCR to identify PCR products consistent with GCRs produced by improper repair of DNA double strand cleavage at this site caused by treatment with fas ligand or etoposide [70,71].

6. Summary

Although rare cases may be mediated via SSA involving *Alu* elements, or topo II subunit exchange, nucleotide sequence data supports the contention that most of the leukemogenic translocations involving *MLL* are mediated by inappropriate repair of DNA DSBs via an “error-prone” NHEJ pathway. However, the proximate cause of the DNA DSBs remains unclear. Epidemiologic data suggests that the *MLL* bcr may be more susceptible to DNA DSBs, and a site within the *MLL* bcr that is generally susceptible to DNA DSBs has been identified. It remains unclear whether the *MLL* bcr is remarkably susceptible to DNA DSBs (ie, one of the very most susceptible regions throughout the entire genome), whether the *MLL* bcr is more susceptible to aberrant NHEJ repair of DNA DSBs, or whether neither of these are true, and the frequent presence of *MLL* translocations in leukemic samples is simply due to a remarkable growth/survival advantage conferred by *MLL* fusion proteins.

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Figure 1. *MLL* partial tandem duplication (PTD)

The germline (GL) *MLL* locus is shown; exons 1–16 are indicated (the remaining 3' exons are omitted for clarity). Regions of *Alu* repeats are indicated, as is the *MLL* breakpoint cluster region (bcr). Centromere to telomere orientation is indicated. The *MLL* partial tandem duplication (PTD) is depicted. A recombination event occurs between *Alu* sequences in intron 1 and intron 6, leading to a duplication of exons 2–6 between exons 6 and 7. Exon sizes and intronic distances are not to scale.

Figure 2. Chromosomal translocation consistent with a DNA topoisomerase II subunit exchange
 The germline *NUP98*, *TOP1*, and both derivative chromosomes from a patient with t-AML were cloned and sequenced [51]. In this model, topo II monomers initially bound to *NUP98* are indicated with solid ovals; monomers initially bound to *TOP1* are indicated with cross-hatched ovals. A 4-bp staggered DNA DSB occurs as indicated, and an inter-chromosomal strand exchange occurs, with the *NUP98* and *TOP1* sequences aligning as indicated. Religation occurs as indicated, leaving 2 nucleotide mismatches (T-G on the der 11; C-A on the der 20). Repair of the mismatches leads to the final sequences shown as the der 11 and der 20. Note that the 4 nucleotides in bold italics (CACC) are present at the breakpoint on both the der 11 and der 20 chromosomes.