REVIEW

Extending the repertoire of the mixed-lineage leukemia gene *MLL* in leukemogenesis

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Chromosomal¹ translocations are key elements in tumor etiology, as these somatically created abnormal chromosomes have activated oncogenes where the chromosome breakpoints occur (for review, see Rabbitts 1994). Most often, gene fusions are caused by chromosomal translocations, which frequently break within the exons of the two involved chromosomes, allowing the transcription product to encompass the linked exons, for post-transcriptional processing to splice these exons to create a tumor-specific fusion mRNA and in turn fusion protein. This type of event is common in both leukemias and in sarcomas (Rabbitts 1994; Look 1997). The leukemias divide into chronic and acute forms of cancer. The translocation genes involved can be roughly categorized into distinct types, with those involved in acute leukemia often being transcription regulators (Cleary 1991) whose role as master genes in cell fate determination is a key element in their role in leukemias (Rabbitts 1991). Thus, activated oncogenes or gene fusions influence differentiation in cell-specific ways and the tropism of specific chromosomal translocations for specific cell types is a manifestation of this role.

The ubiquitous *MLL* gene and its multitude of chromosomal abnormalities

Among the most common chromosomal breakpoint regions in human leukemias is the long arm (q) of chromosome 11 at band q23 (Rowley 1993, 1998; Look 1997; Ayton and Cleary 2001). A gene called the mixed-lineage leukemia gene, *MLL* (or *HTRX*, *HRX*, *TRX1*, *ALL-1*; Ziemer-van der Poel et al. 1991; Djabali et al. 1992; Gu et al. 1992; Tkachuk et al. 1992; Dörmers et al. 1993), is found at the breakpoints of the many and disparate chromosomal translocations of 11q23 (as well as cases of internal duplication of 11q23, often associated with trisomy 11; Schichman et al. 1994, 1995; Caligiuri et al. 1996; Schnittger et al. 2000). Chromosomal breakpoints

are found in a consistent region of *MLL* and cause the synthesis of an *MLL* fusion protein in the damaged cells, which leads to leukemia of those cells. Chromosomal translocations (or other abnormalities) alter the *MLL* gene structure, often by creating a fusion between *MLL* and a gene from a different chromosomal region, and, uniquely among the translocation genes, more than 30 different *MLL* fusions have been described (for review, see Ayton and Cleary 2001; Collins and Rabbitts 2002). The most frequently found are *MLL-AF4* and *MLL-AF9* (Fig. 1A). A critical piece of *MLL* fusion protein biology is the association of specific versions with either acute myeloid leukemias (AML) or acute lymphoid leukemias (ALL). *MLL* gene abnormalities are found in childhood leukemias (~10% of all pediatric leukemias) and in adults (~5% of acute leukemias; Look 1997; Huret et al. 2001).

Overall, therapeutic regimens have become very successful with complete remission rates ~45%–75% for AML (Stone 2002) and cure of childhood ALL up to 80% (Downing and Shannon 2002; Pui et al. 2002). However, in addition to spontaneously arising leukemias, there are also *MLL*-associated leukemias related to the therapies applied to ablate leukemias, which often therefore arise as new leukemias unrelated to the original disease for which the patient was given high-dose chemotherapy (Bloomfield et al. 2002). Around 5%–10% of *MLL*-associated leukemias are therapy related (t-leukemia). These therapy-related cancers can be acute myeloid leukemia (t-AML) and, more rarely, therapy-related acute lymphoblastic leukemia (t-ALL), myelodysplasia (t-MDS), or chronic myelo-monocytic leukemia (t-CMML). Of these t-leukemias, approximately half are made from various chromosomal translocations and the other half, the most frequent *MLL* fusion partners, are *ELL* (eleven nineteen lysine-rich leukaemia gene), *AF4* (ALL1 fused gene from chromosome 4), *AF9* (ALL1 fused gene from chromosome 9), and *AF10* (ALL1 fused gene from chromosome 10; Fig. 1B; Huret et al. 2001). These *MLL*-associated t-leukemias occur after treatment with topoisomerase II targeting drugs (Felix 2001) but also occur with a variety of other treatments including radiotherapy and regardless of the primary cancer type, that is, leukemias, sarcomas, and solid tumors (Bhatia and Sklar 2002).

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Article and publication are at <http://www.genesdev.org/cgi/doi/10.1101/gad.1195504>.

Figure 1. The frequency and diversity of *MLL*-associated chromosomal abnormalities involving *MLL* in acute leukemias. *MLL*-associated chromosomal abnormalities (translocations, inversions and interstitial duplications, or deletions) occur in ~5%–10% of human leukemias (Look 1997; Huret et al. 2001). *MLL* is associated with AML and ALL leukemias, and more than 30 different chromosomal translocations have been described (Huret et al. 2001) resulting in multifarious *MLL* fusion genes. These occur at differing frequencies in spontaneous (A) and therapy-related (B) leukemias (Huret et al. 2001; see also Atlas of Genetics and Cytogenetics in Oncology and Haematology at <http://www.infobiogen.fr/services/chromcancer/Anomalies/11q23ID1030.html> and <http://www.infobiogen.fr/services/chromcancer/Anomalies/11q23secondLeukID1131.html>). Approximately 5%–10% of all *MLL* translocations are therapy-related leukemias. The major fusion partners (i.e., *AF4*, *AF9*, *AF10*, and *ELL*) are associated with *MLL* translocations in either mainly ALL (*MLL*–*AF4*) or AML (*MLL*–*AF9*, *MLL*–*AF10*, and *MLL*–*ELL*).

The *MLL* protein is large, multidomain, and versatile

MLL is a fairly large gene of ~100 kb, and chromosomal abnormalities involved in this gene (translocations, inversions, and interstitial duplications) are clustered in a major break region (MBR) just after the repression domain (Fig. 2A). The most remarkable feature of *MLL* in leukemias is the diversity of fusion partners (Ayton and Cleary 2001; Collins and Rabbitts 2002). *MLL* can fuse to at least 36 partner genes with more than 60 recurrent chromosomal translocations (Huret et al. 2001; see also Atlas of Genetics and Cytogenetics in Oncology and Haematology, <http://www.infobiogen.fr/services/chromcancer/Anomalies/11q23ID1030.html>). However, chromosomal translocations are not the only mechanism of conversion of *MLL* into an oncogene, as other recurrent events have been found, most especially a partial tandem duplication close to the plant homeodomain (PHD) domain (Schichman et al. 1994; Caligiuri et al. 1996; Schnittger et al. 2000; Fig. 2B). Translocations, inversions, and interstitial duplications are present in patients with myeloid or lymphoid leukemias.

MLL encodes a protein with many domains and forms part of a multiprotein supercomplex involving many

components of the TFIID transcription complex (Nakamura et al. 2002). Three AT hooks are found near the N terminus of *MLL* that mediate binding to the minor groove of AT-rich DNA (Zelevnik-Le et al. 1994), and the transcriptional repression domain (Fig. 2A, RD1, RD2) contains a DNMT1 DNA methyltransferase homology domain including the CXXC zinc-finger domain (Ma et al. 1993; Prasad et al. 1995; Xia et al. 2003). This domain remains in all *MLL* fusions following translocations and is required for transformation capacity of the fusion proteins (Slany et al. 1998). *MLL* is a member of the trithorax family and recruits the polycomb repressor proteins HPC2 and BMI-1, as well as the corepressor protein CTBP, providing repression activity independent from RD2 (Xia et al. 2003). RD2 mediates transcriptional repression through recruitment of histone deacetylases HDAC1 and HDAC2, which can also interact with part of RD1 directly (Xia et al. 2003). Thus, the *MLL* supercomplex remodels, acetylates and deacetylates, and methylates nucleosome-attached histone (Ernst et al. 2001; Milne et al. 2002; Nakamura et al. 2002; Xia et al. 2003). However, following chromosomal translocations, the histone H3-K4 methylation, which occurs through the C-terminal SET [Su(var)3-9, Enhancer-of-zeste, Trithorax] domain of *MLL* (Milne et al. 2002; Nakamura et al. 2002), is lost, to be replaced by a fusion partner. Following chromosomal translocations, the main region of gene fusion lies just beyond the exons encoding the C-terminal side of the repression domain, defining the MBR (Fig. 2A), which is adjacent to PHD zinc-finger regions and the BROMO domain (Rowley 1998). This has consequences for target gene activity as the *MLL*-mediated H3 and H4 methylation of the *HOXA9* and *Hoxc8* promoter cannot occur after chromosomal translocations (Milne et al. 2002; Nakamura et al. 2002), although it could theoretically do so in the cases of trisomy 11 with the internal duplication of *MLL* (Fig. 2B).

A newly defined threonine protease cleaves *MLL* downstream of the MBR

In earlier work in which *MLL* fusions were created by knock-in technology (see below), the issue was raised that a principal feature of *MLL* fusions was their intrinsic stability compared with native *MLL* or with truncated *MLL* proteins (Dobson et al. 2000). Biochemical studies of *MLL* protein synthesis showed that indeed the native protein is cleaved post-translationally into two parts and thus does not accumulate in cells in its ~500-kD native form (Nakamura et al. 2002; Yokoyama et al. 2002; Hsieh et al. 2003b). Elegant biochemical isolation work has recently resulted in isolation and gene cloning of a novel threonine protease, designated taspase 1 (an endopeptidase with an asparaginase 2 homology domain), responsible for proteolysis of *MLL* (Hsieh et al. 2003a). The mature *MLL* protein is cleaved by taspase 1 at amino acid residues 2666/2667 (CS1) and 2718/2719 (CS2; Fig. 2A; Hsieh et al. 2003a) yielding N300/320- and C180-terminal polypeptides. These fragments reassociate through the FYRN domain (amino acids 1975–

Figure 2. Structure of the MLL protein and derivatives after chromosome 11 rearrangements. (A) Domains and interactions of the MLL protein. The *MLL* gene is located on chromosome 11, long-arm band q23.3, and the 36 exons span a region of ~89 kb. The mRNA of ~11.9 kb encodes a massive protein of 3969 amino acids. The MLL protein is a multidomain molecule with regions of homology to diverse proteins. Three AT hooks are found near the N terminus of MLL, which mediate binding to the minor groove of AT-rich DNA (Zeleznik-Le et al. 1994) to stabilize protein–DNA complexes or mediate protein–protein interactions through binding to the DNA. Two nuclear localization signals occur downstream of the AT hooks (SNL1 and SNL2) followed by a large transcriptional repression domain that is retained in all MLL fusions and is required for transformation by the fusion proteins (Slany et al. 1998). The repression domain has three structurally different subdomains forming two mechanistically different repression domains. RD1 contains the DNMT1 DNA methyltransferase homology domain including the CXXC zinc finger domain, which recruits the polycomb repressor proteins HPC2 and BMI-1, and the corepressor CTBP (Xia et al. 2003). RD2 mediates repression through recruitment of histone deacetylases HDAC1 and HDAC2, which can also interact with part of RD1 (Xia et al. 2003). The mature MLL protein is cleaved by a threonine protease (taspase; Hsieh et al. 2003a), which cuts the protein at amino acid residues 2666/2667 (CS1) and 2718/2719 (CS2; Hsieh et al. 2003b). The resultant N300/320- and C180-terminal polypeptides are associated through the FYRN and FYRC domains plus part of the SET domain (amino acids 3656–3876; Nakamura et al. 2002; Hsieh et al. 2003b). The nuclear cyclophilin CYP33 (which negatively affects *HOXC8* and *HOXC9* transcription; Fair et al. 2001) interacts with the third PHD zinc-finger domain of MLL (Fair et al. 2001). CYP33 also interacts with HDAC1 in the RD2 domain (Xia et al. 2003). On C180, there is a conserved transcriptional activation domain (TAD) that binds directly to the coactivator CBP (CREB-binding protein; Bannister and Kouzarides 1996). CBP is an acetyltransferase, possibly acetylating H3 and H4 in connection with MLL binding to promoters of *HOX* genes, as demonstrated for *HOXA9* and *Hoxc8* (Milne et al. 2002; Nakamura et al. 2002). Interaction of the TAD residues 2829–2883 with CBP residues 581–687 facilitates CBP binding to phosphorylated CREB (Ernst et al. 2001). There is a SET domain near the C terminus of MLL, which is a histone methylase of histone 3 at Lys 4 (H3-K4; Milne et al. 2002; Nakamura et al. 2002). The H3-K4 methylation status correlates with an active state of transcription, so it is likely the *HOX* gene promoters (including *HOXA9* and *HOXC8*) are H3-K4 methylated through the MLL SET domain, playing a direct role in the transcriptional activation of *HOX* genes. Protein interactions possible after chromosomal translocation are shaded in green and those that are lost in the MLL fusion proteins are shaded in purple. (B) Consequences of recurrent chromosomal aberrations on the MLL protein. Following chromosomal translocations, the main region of gene fusion lies just beyond the exons encoding the C-terminal side of the repression domain, defining the MBR, and is adjacent to PHD zinc-finger regions and the BROMO domain (BD; Rowley 1998). (Top) The normal chromosome 11 with the *MLL* gene located at 11q23 and the diagrammatic structure of the encoded MLL protein. (Middle) An example of an *MLL*-associated chromosomal translocation with the derivative chromosome 11(der) after translocation with chromosome 9p22 (the *AF9* gene). The *MLL* fusion shown is a typical fusion product emanating from a break within the break region of *MLL*. The MLL portion includes the N-terminal AT hooks, SNL1, SNL2 and repression domains, followed by one of the many different fusion partners. (Bottom) The *MLL* gene internal duplication, which is often associated with trisomy 11 and which results in an internal tandem duplication of *MLL* coding sequences (Schichman et al. 1994; Caligiuri et al. 1996; Schnittger et al. 2000) as indicated.

2158) and FYRC domain plus part of the SET domain (amino acids 3656–3876; Hsieh et al. 2003b). The break region is located upstream of the taspase 1 sites; thus, MLL fusion proteins are not a substrate for this protease. In addition, there is an apparent discrepancy between loss of the taspase sites after chromosomal translocations and their retention in cases of internal duplication of MLL (Fig. 2B). Part of the explanation may be that stabilization of MLL fusions occurs in the former by loss of taspase proteolysis sites and in the latter by effectively displacing the taspase proteolysis sites to the C-terminal end of a huge, potentially stable protein that would still be ~430 kD after taspase cleavage of the C-terminal sites. It is possible that the important event of chromosomal abnormality is disturbance of homeostasis of the MLL supercomplex, which would be a facile explanation for the huge variability of MLL alterations (including the deletion of the simple first PHD finger; Lochner et al. 1996) leading to the deregulation of a very specific set of target genes.

HOX gene regulation by MLL fusion proteins

Insights into MLL fusion protein function have been obtained by gene targeting experiments in mice. Inactivation of the *Mll* gene in mice by homologous recombination (knock-out) methods showed that Mll is a regulator of hematopoiesis but is also a master regulator in embryonic development (Yu et al. 1995; Hess et al. 1997; Yagi et al. 1998), accompanied by *homeobox* (*Hox*) gene expression abnormalities, which were most significant for *Hoxa7*, *Hoxa9*, *Hoxa10*, *Hoxc4*, *Hoxc8*, and *Hoxc9* in whole embryos or fetal liver (Yu et al. 1995; Yagi et al. 1998). Analysis of the MLL supercomplex demonstrated direct binding of MLL to *HOXA9* and *Hoxc8* promoters (Milne et al. 2002; Nakamura et al. 2002). Gene targeting of the mouse homologs of genes encoding fusion partners of MLL gave further evidence of regulators of cell differentiation. The null mutation of the mouse homolog of AF9 showed its importance for embryonic patterning without influencing hematopoiesis (Collins et al. 2002), whereas the mouse *Af4* gene influences lymphoid development (Isnard et al. 2000). These observations suggested that the MLL fusion proteins, created by the leukemia-associated chromosomal abnormalities, are themselves transcription regulators that may be determinants of *HOX* gene expression in abnormal arrangements (Ayton and Cleary 2001; Collins and Rabbitts 2002). Target gene assessment lends very strong support to this model of MLL fusion protein function (Ayton and Cleary 2001; Collins and Rabbitts 2002). An especially pertinent function of MLL, and of its fusion protein derivatives, is as a major regulator of class I *HOX* gene expression (for review, see Ernst et al. 2002). *HOX* genes are a major group of transcription factors and participate in the control of embryonic development and hematopoietic cell differentiation with a specific expression pattern in different hematopoietic lineages at various differentiation stages (Sauvageau et al. 1994; Lawrence et al. 1996; Pineault et al. 2002). In leukemias with MLL fusion proteins, distur-

bance of *HOX* expression patterns has been observed, subverting a major role of *HOX* gene expression in hematopoietic lineage regulation (Yu et al. 1995; Hess et al. 1997; Yagi et al. 1998). All three major types of MLL gene aberrations (Fig. 2) are implicated in *HOX* gene deregulation as the mediator of leukemia.

An important link between *Hox* gene expression and MLL–ENL (eleven nineteen leukemia) fusion protein-mediated leukemogenesis was made using retroviral transduction of bone marrow progenitors (Ayton and Cleary 2003). In these studies, embryonic stem (ES) cells that were null for *Hoxa7* or *Hoxa9* had reduced in vitro myeloid immortalization but bone marrow transplantation (BMT) of *Hoxa9*^{−/−} bone marrow (BM) cells transduced with retroviral expressing MLL–ENL failed to develop leukemias, whereas wild-type BM cells with MLL–ENL developed AML. Finally, the ability of *Hoxa9*^{−/−} BM cells to become leukemic in vivo was rescued by expression of both *Hoxa9* and MLL–ENL. This showed unequivocally that leukemia only occurs if the *Hoxa9* gene is present and suggests an absolute requirement of *Hoxa9*, and possibly of *Hoxa7*, for the development of AML mediated by MLL–ENL (Ayton and Cleary 2003). However, the situation with Mll–AF9 may be different because Mll–AF9 knock-in mice develop leukemia with similar rates in the presence or the absence of the *Hoxa9* gene showing that *Hoxa9* is not required for leukemia in the KI (knock-in using homologous recombination) model (Kumar et al. 2004). It is intriguing that the Mll–AF9 leukemias in the absence of *Hoxa9* have a more immature phenotype (Kumar et al. 2004). This difference between the outcomes of the two fusions may reflect the different models used (i.e., BMT for MLL–ENL and KI for Mll–AF9) or may reflect biological differences mediated through the two fusion proteins.

Hoxa7 and *Hoxa9* expression was also observed in BMT in vitro models with fusion proteins MLL–GAS7, MLL–AF1P, MLL–AF9, and dimerized MLL. In addition, *Hoxa7* and *a9* gene expression was reversibly deregulated in a dimerization model in which MLL dimerization was required for a blockage of BM myeloid cell differentiation (see below; Martin et al. 2003). Addition of the inducer of dimerization caused MLL fusion to bind with the *Hoxa9* promoter, linking MLL-induced leukemogenesis with *HOX* gene regulation. An intriguing feature of *Hox* gene expression profiles in these Mll fusion gene models is that the *Hox* gene expression is very similar in all models regardless of which fusion partner was expressed in the chimeric protein. This suggests that the MLL fusion gene characteristics are the dominant feature of this type of leukemia, as opposed to other transformation mechanisms. Data from gene expression profiling studies with human leukemias strengthen this conclusion. All MLL-associated leukemia subtypes had *HOXA9* and *MEIS1* expression (Armstrong et al. 2002; Yeoh et al. 2002; Debernardi et al. 2003; Ferrando and Look 2003). Different MLL tumor lineages share very similar deregulated *HOX* gene expression patterns (Armstrong et al. 2002; Yeoh et al. 2002; Debernardi et al. 2003; Ferrando and Look 2003), and the pattern is inde-

pendent from the specific fusion partner, as found for the mouse models. ALLs were divided into subgroups according to their chromosomal translocation associated with a highly specific cluster of overexpressed genes (Armstrong et al. 2002; Yeoh et al. 2002; Ferrando and Look 2003). Thus, the profiling data suggest that *HOX* genes are targeted by the MLL fusions, but the nature of the fusion partner does not contribute to the specificity of the *HOX* gene subset target.

MLL dimerization as a mechanism of leukemogenesis

Formation of a stable MLL protein coupled to dimerization may be a crucial constituent of MLL-mediated tumorigenesis. Several observations add verisimilitude to this inference, including the partial duplication of *MLL* in some patients suffering from AML and ALL (Schichman et al. 1994; Caligiuri et al. 1996; Schnittger et al. 2000), which are effectively duplications of the N-terminal region to just ahead of the PHD fingers (Fig. 2B). In addition, a subgroup of MLL fusion partners that are joined by chromosomal translocations have oligomerization domains such as leucine zippers and α -helical coiled-coil domains (Prasad et al. 1994; Chaplin et al. 1995; Saha et al. 1995; Linder et al. 2000; Pegram et al. 2000; Sano 2001; DiMartino et al. 2002; Ono et al. 2002; Slater et al. 2002). The first experimental evidence for molecular dimerization as a mode of MLL tumorigenicity came from the finding that mice with the *lacZ* gene (encoding β -galactosidase) knocked into *Mll* at the place corresponding to the break region of human *MLL* developed AML (Dobson et al. 2000) and that these mice express Mll- β -galactosidase protein with β -galactosidase enzyme (which is a tetramer) activity. Furthermore, an N-terminal segment of Mll has no transforming capacity, as an artificially created truncation of Mll at exon 8 in the break region has no influence on hematopoietic differentiation or on tumor propensity (Corral et al. 1996). This suggested that addition of material to the N-terminal portion of MLL may be a mechanism to elicit a tumorigenic effect and that stabilization of the N-terminal MLL protein and dimerization could be the functional consequence of such fusions (Prasad et al. 1994; Chaplin et al. 1995; Dobson et al. 2000).

In recent publications, dimerization of MLL has been directly tested as a biochemical function involved in tumorigenicity. Two fusion partners of MLL are GAS7 and AF1P (So et al. 2003b), which are normally cytoplasmic proteins with different functions but both possessing coiled-coil domains capable of oligomer formation, potentially creating nuclear, oligomerized MLL fusions. This was directly tested using retroviral transduction of BM hematopoietic stem cells followed by transplantation into recipient mice (So et al. 2003b). Both fusion products resulted in leukemia but, significantly, the presence of the GAS7 or AF1P coiled-coil domains fused to the MLL portion was sufficient for transformation in mice. Further, an inducible dimerization of N-terminal MLL affected hematopoietic differentiation, which was reversible by withdrawing a pharmacological dimeriza-

tion agent (Martin et al. 2003; using retroviral transduction of mouse BM cells with the MLL fusion partner FKBP12, which is a FK506 binding protein that oligomerizes in the presence of AP20187). These studies support the conclusions about the earlier *Mll-lacZ* KI mice that MLL dimerization is a key element of its leukemogenic function (Dobson et al. 2000). As first observed with the *Mll-lacZ* KI model (Dobson et al. 2000), all of these models of MLL oligomerization have reduced oncogenicity, compared with "normal" Mll fusions, which suggests further specific biological contributions of the fusion partners in dynamics and penetrance of tumorigenesis.

Models of MLL fusion-induced leukemia in mice

Biological models of MLL-mediated cancer should mimic underlying human disease as closely as possible. The laboratory mouse provides a suitable system as genetically homogeneous strains can be used and the mouse genome is amenable to alteration either by transgenesis or by homologous recombination techniques. Manipulation of the mouse genome has been used to assess the cell of origin of MLL fusion-induced leukemia, the effects that the MLL fusion has on these hematopoietic cells, and whether specific fusions have lineage-specific developmental properties. There are several hypotheses to explain the role of MLL fusions in defining tumor phenotype (summarized in Fig. 3). Hematopoiesis occurs by a developmental program from the multipotent, self-renewing SCs (Fig. 3A, HSC), which give rise to progenitors of the lymphoid lineage (the common lymphocyte precursor, CLP) or of the erythro-myeloid lineage (the common myeloid precursor, CMP). In turn, each of these committed progenitors differentiates into the mature cells. B cells and T cells develop from CLPs through a series of stages (for review, see Weissman et al. 2001; Fig. 3A, designated pro-B and pro-T, respectively). Myeloid cells (granulocytes and monocyte lineage) emanate from the granulocyte-monocyte precursor (GMP) and erythrocytes and megakaryocytes from their precursor (MEP). MLL fusions created somatically in this tissue can be instructive or noninstructive in determining tumor phenotype. In the instructive model, the acquisition of a fusion is followed by a developmental signal through the MLL fusion protein (Fig. 3B). Thus, for instance, MLL-AF4, which typically occurs in B-lymphoid tumors, would provide proliferative signals for B-cell differentiation. This would imply that *MLL-AF4* translocations occur in the HSC or progeny. Alternatively, MLL-AF9 has a cell fate effect giving rise to myeloproliferation and eventual myeloid leukemias (Fig. 3B). A variant of this model is the postulate of a biphenotypic cell that comes from the HSC and is the target of MLL fusions (Cumano et al. 1992; Montecino-Rodriguez et al. 2001; Fig. 3C), leading to either ALL or AML depending on which fusion is found. Finally, the noninstructive model attributes no cell fate decisions to the fusion but rather indicates that translocations will be oncogenic when in a permissive cell (Fig. 3D). Thus, MLL-AF9 would be oncogenic in the myeloid precursors and MLL-AF4 in lymphoid precursors.

Figure 3. Instructive vs. noninstructive models of MLL fusion proteins in leukemia. Chromosome abnormalities involving MLL are somatic events that lead to leukemia. These occur during normal hematopoiesis summarized in A. (A) Definitive hematopoiesis. Hematopoiesis occurs from pluripotent, self-renewing stem cells (HSC) found mainly in BM (Weissman et al. 2001). These cells give rise to progenitors of myelo-erythroid cells (the common myeloid progenitor, CMP) and of lymphoid cells (the common lymphoid progenitor, CLP); these progenitors are committed to differentiation decisions producing the two classes of lymphocyte, namely the T and B cells (as well as NK cells) via the pro-T and pro-B intermediates from the CLP. Differentiation for the CMP is more complex as a larger set of differentiated cells must be produced. CMP can produce the lineage committed granulocytic/monocytic-restricted progenitors (GMP) and megakaryocytic/erythroid-restricted progenitors (MEP), which in turn differentiate into the granulocyte/monocyte cells or erythrocytes and megakaryocytes, respectively. (B,C) Instructive model of MLL-mediated leukemogenesis. This hypothesis posits that the target cell for translocation and MLL fusion gene is a common, uncommitted progenitor and the specific MLL fusions have different effects in hematopoietic differentiation; for example, MLL-AF9 gives myeloproliferation resulting in myeloid leukemia whereas MLL-AF4 gives B-lymphoproliferation resulting in B-cell leukemia. In one version of this model, the chromosomal abnormality occurs in an HSC (B); a variation is the proposition of an uncommitted, multipotent progenitor (which is not the HSC) in which the effects of MLL fusions are manifest (Armstrong et al. 2003). (D) Noninstructive model of MLL-mediated leukemogenesis. This model has the MLL fusion only effective in leukemogenesis in a permissive cell, such that the target cell in which a fusion gene can be effective is different in the case of different MLL fusions; for example, if MLL-AF9 translocations occur in myeloid progenitors (i.e., permissive for MLL-AF9), this is tumorigenic, whereas if MLL-AF9 translocations occur in lymphoid progenitors (i.e., nonpermissive for MLL-AF9) this is not tumorigenic. Alternatively, the target cell in which translocation can occur is different in the case of different MLL fusions (e.g., MLL-AF9 translocations occur in myeloid progenitors and MLL-AF4 translocation occurs in B-lymphoid progenitors).

The mouse model of MLL-associated acute myeloid leukemia achieved by KI technology supported the instructive model. The KI mouse design uses fusion partner gene sequences, corresponding to the human fusion, integrated into one *Mll* allele via homologous recombination in ES cells, leaving the other allele as wild type. The KI of the 3' end of human *AF9* into mouse *Mll* was achieved by gene targeting in ES cells, and this led to acute myeloid leukemia in chimeric and heterozygous mice (Corral et al. 1996). An important feature was that the *Mll-AF9* fusion elicited an early myelo-proliferation,

which preceded the onset of AML in both chimeric and heterozygous KI mice, and could be detected as early as 6 d after birth (Corral et al. 1996; Dobson et al. 1999). Myelo-proliferative impairment could be detected prenatally but was not sufficient at any time point (prenatally or postnatally) to induce leukemia in transplantation experiments. The late onset of overt leukemia suggests that secondary mutational events (not directly on *Mll*) were necessary for the malignant transformation of the myelo-proliferative cell pool. Similarly, the use of retroviral transduction of BM progenitors and transplantation

of such cells into syngeneic mouse recipients models lineage specific leukemia (Ayton and Cleary 2001). In this way, transduction of hematopoietic stem cells with retrovirus expressing the *MLL-ENL* fusion caused leukemia and myelo-proliferation preceding the leukemia (Lavau et al. 1997; Slany et al. 1998).

In different circumstances, retroviral transduction of BM has also been used to show that both *MLL-ENL* and *MLL-GAS7* can induce a biphenotypic leukemia using controlled culture conditions (So et al. 2003a; Zeisig et al. 2003). Transplantation gave biphenotypic tumors expressing lymphoid and myeloid cell surface markers and displaying biphenotypic gene expression profiles (So et al. 2003a; Zeisig et al. 2003). Similarly, when murine hematopoietic stem cells enriched for early progenitors were transduced with the *MLL-GAS7* fusion gene and directly transplanted into syngeneic mice, oligoclonal AML (80%) and ALL (10%) and multiclonal acute biphenotypic leukemia (ABL; 10%) resulted. Multiclonality of the latter suggests that the transduction procedure affected several different progenitors resulting in multilineage leukemia and supports a noninstructive model (Fig. 3D). This approach has been taken further with refined experiments transducing *MLL-ENL*-expressing retrovirus in purified, committed myeloid progenitors (CMP and GMP; Cozzio et al. 2003) as well as multipotent HSC. In these experiments, myeloid leukemias resulted in each case, implying that the “permissive” environment for oncogenicity of *MLL-ENL* fusion proteins is provided by both noncommitted progenitors or committed progeny (Cozzio et al. 2003).

The limitation of the KI gene fusion and BMT is circumvented by the translocator mouse model in which chromosomal translocations are produced *de novo* (Smith et al. 1995; van Deursen et al. 1995) during mouse development (Buchholz et al. 2000; Collins et al. 2000). *Mll*-involved translocations were evaluated in a translocator model of *Mll-Enl* gene fusions (Forster et al. 2003). Somatic occurring reciprocal translocations involving rearrangement between *Mll* and *Enl* loci were achieved using Cre-mediated interchromosomal recombination between *loxP* sites introduced in intronic regions of mouse *Mll* and *Enl*, corresponding to human breakpoints (Forster et al. 2003). This experimental strategy results in *de novo Mll-Enl* chromosomal translocations and fusion genes in their normal genomic context and under control of their normal transcriptional controlling elements. This led to myeloid leukemias with a rapid onset in all mice (mean survival 2–4 mo; Forster et al. 2003). Reciprocal translocations were present in all tumor cells and could be detected as early as 12 d after birth suggesting that the translocation events occur early and frequently to allow time for disease manifestation (Forster et al. 2003). These results were obtained using a Cre-expressing mouse line in which Cre was expressed in stem cells and early progenitors from the Lmo2 promoter (Warren et al. 1994), suggesting that the translocations (which appeared very early) can occur in uncommitted cells and thus supporting an instructive role for *Mll-Enl* fusion in this setting. Continued study of the

cells of origin of the translocations in this model will shed light on this issue. Furthermore, additional mouse models are needed to investigate the possible specifying effects of the fusion partners, ideally translocators with different Cre-expressing mice; thus, lineage-specific Cre expression would be informative about the accessibility of hematopoietic cells at different stages of differentiation to the influence of the *Mll* fusion proteins. A good test for the instructive vs. noninstructive models should be an *Mll-Af4* translocator mouse, as *MLL-AF4* in humans typically manifests as an ALL phenotype.

The diverse *MLL* fusions, and related mutations, found in human leukemias shows the disparate means by which chromosomal abnormalities can affect gene structure to result in malignant transformation. Because *MLL* fusions have been found in leukemias with various hematopoietic phenotypes, the function of this mixed lineage gene in leukemogenesis remains a conundrum. Several different mouse models and many molecular studies have yet to clarify definitively whether *MLL* fusions work by instructing cell fate decisions or whether these fusion proteins are only oncogenic in specific cellular environments. Furthermore, a mixed situation may be invoked, whereby a particular fusion is instructive if the chromosomal abnormality occurs in a multipotential precursor and noninstructive (i.e., simply oncogenic) if the chromosomal abnormality occurs in a committed cell of the permissive phenotype. Either way, noninstructive models would not allow for oncogenicity of a particular fusion in a committed cell of the nonpermissive phenotype.

Future perspectives

Ultimately, the molecular and animal models of *MLL*-derived leukemia will exemplify how *MLL* fusions contribute to leukemia and are further aimed at developing new therapies for these prevalent cancers. Mouse models for human cancer are of importance to understand the determinants of the phenotypic characteristics of the tumors but also form the basis for developing rational therapeutic strategies by providing preclinical setting to test new therapeutic molecules or approaches that cannot be assessed in cancer patients. Furthermore, complex mouse leukemia models may help to define early obligatory genetic events that could be used for early detection. In conjunction with molecular biology aimed at classifying leukemias more precisely using molecular markers and expression signatures, it should be possible to stratify therapies according to effectiveness of therapy regimes. Finally, development of new therapeutics based on molecular studies, which will target molecules or particularly complexes, could provide a new type of therapeutic reagent that could be used alone or in conjunction with existing therapies as anti-cancer agents.

Acknowledgments

The authors are supported by the Medical Research Council. We thank Annette Lenton for preparation of the artwork.

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Extending the repertoire of the mixed-lineage leukemia gene *MLL* in leukemogenesis

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Genes Dev. 2004, **18**:

Access the most recent version at doi:[10.1101/gad.1195504](https://doi.org/10.1101/gad.1195504)

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