REVIEW ARTICLE

The molecular biology of mixed lineage leukemia

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ABSTRACT

Mixed lineage leukemia is a very aggressive blood cancer that predominantly occurs in pediatric patients. In contrast to other types of childhood acute leukemias, mixed lineage leukemia presents with a dismal prognosis and despite the availability of advanced treatment methods cure rates have stagnated over the last years. Mixed lineage leukemia is characterized by the presence of MLL fusion proteins that are the result of chromosomal translocations affecting the *MLL* gene at 11q23. These events juxtapose the amino-terminus of the histone methyltransferase MLL with a variety of different fusion partners that destroy normal histone methyltransferase function of MLL and replace it by heterologous functions contributed by the fusion partner. The resulting chimeras are transcriptional regulators that take control of targets normally controlled by MLL with the clustered *HOX* homeobox genes as prominent examples. Recent studies suggested that MLL fusion partners activate transcription by two different mechanisms. Some of these proteins are themselves chromatin modifiers that introduce histone acetylation whereas other fusion partners can recruit histone methyltransferases. In particular, histone H3 specific methylation at lysine 79 catalyzed by DOT1L has been recognized as a hallmark of chromatin activated by MLL fusion proteins. Interestingly, several frequent MLL fusion partners seem to coordinate DOT1L activity with a protein complex that stimulates the elongation phase of transcription by phosphorylating the carboxy-terminal repeat domain of RNA polymerase II. The discovery of these novel enzymatic activities that are essentially involved in MLL fusion protein function presents potential new targets for a rational drug development.

Key words: MLL, proteins, leukemia.

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Mixed lineage leukemia - a clinical primer

Apart from the fascinating molecular biology underlying the pathogenesis, mixed lineage leukemia mainly presents a clinical challenge. More than 30 years ago, physicians realized that certain subsets of patients initially diagnosed with acute lymphoblastic (ALL) or acute myeloid leukemia (AML) fared far worse than others. In the pediatric field, one of these *high-risk* leukemias stood out particularly amongst all remaining cases of childhood leukemia. A cohort of ALLs diagnosed in newborns and infants (younger than one year) fell into a group with similar clinical aspects and an extremely dismal prognosis. With the advent of fluorescent activated cell sorting (FACS) it was revealed that the leukemic blasts of these aggressive leukemias frequently expressed surface markers of both the lymphoid and the myeloid lineage. Sometimes even a complete lineage switch was observed during treatment and a leukemia initially diagnosed as ALL could relapse as AML.[1] Accordingly the term mixed lineage leukemia was coined.[2-4] Even before this, cytogeneticists had noted that translocations affecting the locus 11q23, and in particular the translocation t(4;11), characterize a special subset of ALL that was associated with poor survival.[5-7] Soon thereafter it became clear that these translocations of the locus 11q23 are also typical for mixed lineage leukemia. Whereas treatment of non-mixed lineage leukemia in children has become the textbook success story of modern medicine with 5-year survival rates approaching 90%,[8] mixed lineage leukemia treatment seems to have hit a roadblock with hardly 40% of all infants surviving five years after diagnosis (Figure 1).[8-11]

Mixed lineage leukemia reaches a second peak of incidence later in life, particularly in patients who have been treated previously for an unrelated neoplastic disease with topoisomerase inhibitors like etoposide (so-called therapy related

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leukemia; t-AML or t-ALL).[12] In total, 11q23 abnormalities occur in up to 70% of infant ALL, and in approximately 10% of all other ALL cases.[13] Therapy related leukemia almost always manifests as AML with about 10% of therapy induced AML and 3% of *de novo* AML carrying an 11q23 translocation.[14] Intriguingly, the *MLL* gene is also the target of a second type of aberration that creates a short repeat within the MLL coding sequence resulting in an internal partial tandem duplication (PTD). As a consequence, an extra amino-terminus is added in frame to full length MLL. MLL-PTD occurs predominantly in AML. Judging from gene expression patterns and clinical parameters, MLL-PTD seems to cause a different disease from that induced by *classical* MLL fusions. MLL-PTD has been covered by a recent publication[15] and therefore this aberration will not be subject of this review.

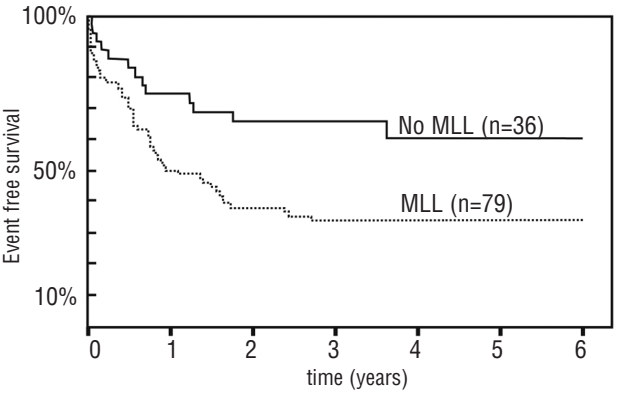

Figure 1. Event free survival of infants with ALL separated by *MLL* status. Redrawn after Hilden *et al*.[9] Please note that the data correspond to event free survival (a more stringent criterion) and do not include children older than one year.

Normal MLL - a histone methyltransferase necessary for efficient transcription

Guided by the chromosomal aberrations, four groups independently succeeded in cloning the gene spanning the translocation breakpoint at 11q23.[16-19] From sequence comparison it became immediately clear that this gene encoded a homolog of a known fly gene named trithorax (*Trx*). Because of this relationship and the involvement in leukemia, the human gene was initially labeled either *HRX* (human trithorax), *ALL-1* (acute lymphocytic leukemia-1) or *MLL* (mixed lineage leukemia). Later it was agreed to use *MLL* as the standard name. Drosophila *Trx* mutants displayed a very suggestive phenotype with homeotic changes in all three breast segments reminiscent of *Hox* gene mutations and indeed *Hox* gene expression was perturbed in *Trx* negative flies.[20-22] This function was conserved in mammals as *MLL* knockout embryos also showed skeletal transformations and misexpression of *Hox* genes before they died *in utero* around day 10.5-16.5 p.c., depending on the particular knockout allele.[23-25] Fly *Trx* was also isolated in a genetic screen that was set up to identify genes that counteracted genetic silencing.[26] Since Hox gene expression was correctly initiated in $Trx^{-/-}$ flies as well as in $MLL^{-/-}$ mice but later deteriorated during embryogenesis, it was thought that Trx/MLL is a specific maintenance factor for *Hox* genes. However, nowadays it is known that MLL serves a much more general function. The breakthrough came with the identification of the highly conserved SET domain (an acronym for Suppressor of variegation, Enhancer of zeste, Trithorax) at the C-terminus of MLL as the site of a histone methyltransferase activity that specifically methylates histone H3 at lysine 4.[27] MLL was found to be incorporated into a large macromolecular complex that was purified from mammalian nuclei.[28,29] The complex showed conservation across phyla all the way down to *Saccharomyces cerevisiae* where SET1, the yeast counterpart of MLL, was also present in a similar complex called COMPASS (complex of proteins associated with Set1).[30,31] Interestingly, MLL is post-translationally processed by proteolytic cleavage.

The large MLL protein is cut by an aspartic protease called taspase into an N-terminal 320kDa fragment and a C-terminal 180kDa moiety that are both core components of the MLL complex (Figure 2).[32-35] Within this molecular machinery a *division of labor* exists. The MLL^{C} subunit associates with at least four proteins that help in preparing chromatin for efficient transcription. One of these proteins is the histone H4 lysine 16 specific acetyltransferase MOF that loosens up chromatin by histone charge neutralization.[36] The WDR5 protein in turn recognizes the histone H3 lysine 4 methyl-mark introduced by MLL and it has therefore been suggested that WDR5 ensures the processitivity of histone modification.[37-39] And finally the proteins RBBP5 and ASH2L appear to be necessary for efficient methyltransferase activity by stabilizing an active conformation of MLL allowing allosteric control.[40] The histone acetyltransferase CBP and the INI1 subunit of the SWI/SNF nucleosome remodeling complex have also been identified as interaction partners of MLL^{C} in interaction screens,[41,42] although these proteins did not copurify with MLL^{C} in biochemical experiments.

MLL^{N} on the other side contains features essential for correct targeting of the MLL complex. At the outmost amino-terminal end of MLL a binding site for menin, the product of the tumor suppressor gene *multiple endocrine neoplasia* is present.[43-45] Menin and MLL form an interaction surface for LEDGF (lens epithelium derived growth factor) and LEDGF makes contact to chromatin via a PWWP domain.[46] Interestingly, LEDGF is also involved in HIV pathogenesis where it assists integration of HIV proviruses into chromatin.[47,48] In addition, MLL^{N} codes for several AT-hooks, a minor groove DNA binding motif that preferentially recognizes DNA with distortions like bends or kinks.[49] Further downstream a CxxC domain can be found. CxxC domains occur in proteins that discriminate the methylation status of DNA, and indeed, also the MLL CxxC moiety binds specifically to unmethylated CpG dinucleotides.[50] Swap experiments between MLL and the highly homologous MLL2 indicated that the CxxC

domain seems to be a major determinant of subnuclear localization and target gene selection.[51] In addition, the CxxC region also has been shown to recruit repressive factors like histone deacetylases and polycomb group proteins.[52] This interaction appeared to be regulated by conformational changes elicited by the prolyl-isomerase cyclophilin 33 (Cyp33) that interacts further carboxy-terminal with the *plant homeodomain* (PHD) of MLL^N.

In summary therefore, the MLL complex coordinates three major mechanisms of chromatin modification: methylation, acetylation and nucleosome remodeling. Most likely transcription factors recruit the MLL complex to initiate RNA synthesis. Examples are p53 and β-catenin that have been found to associate with *MLL* during transcriptional activation.[36,53] H3K4 methylation is universally introduced around the transcription start site of all transcribed genes, and next to *MLL* several other confirmed or putative H3K4 methyltransferases (*MLL2, MLL3, MLL5, SET1A, SET1B*, and *ASH1L*) have been identified in mammalian cells. If all of these proteins have a comparable number of cellular targets, and with an estimated 10,000 genes transcribed at any present moment under standard conditions, each H3K4 methyltransferase should be responsible for more than 1,000 loci. Although this is a greatly oversimplified prediction, nonetheless it seems to be confirmed by emerging experimental data that identified several hundred genomic loci bound by MLL and ASH1L in ChIP experiments.[54,55] Obviously certain genes, like the *Hox* genes, depend more on MLL mediated chromatin modification than others, and therefore they stand out in the MLL loss of function phenotype.

The origin of 11q23 translocations

Much has been speculated about the origins of the chromosomal aberrations that convert an innocuous chromatin modifier into a pernicious oncogene. Several lines of evidence point to a mishap in non-homologous end joining of double strand breaks as the most likely reason for 11q23 translocations.

For one, the characteristic peak of mixed lineage leukemia in patients treated with etoposide is highly suggestive for an involvement of DNA double strand lesions in the etiology of MLL fusions. Etoposide inhibits topoisomerase II and therefore causes breaks in both DNA strands. Indeed, it could be shown that the locus 11q23 is particularly susceptible to this kind of assault in cells treated with topo II inhibitors.[56,57] Alternatively, a break might be introduced at early stages of apoptotic DNA fragmentation that was later aborted and repaired. Published data also provide some support for this scenario, as breaks preferentially occur at 11q23 in early apoptotic cells.[58]

Whatever the reason for the initiating event, an aberrant non-homologous end joining (NHEJ) process most likely causes the cross-wise sealing of the DNA ends. A close examination of the breakpoint junctions revealed that they frequently code for non-templated nucleotides,[59] a hallmark of NHEJ repair as known from generation of antibody and T-cell receptor diversity. Despite the attractions of this hypothesis as an explanation for the origin of 11q23 translocations it does not take into consideration that many double strand breaks induced by background radiation are continuously repaired in each cell without dire consequences. In this respect, a publication might be important showing that double strand breaks lead to chromosomal aberrations only in cells with impaired ATM-dependent DNA-damage signaling, whereas normal cells are able to join free ends correctly.[60] The potential involvement of DNA repair pathway defects in mixed lineage leukemia is almost completely unexplored and would be a rewarding topic for future research.

MLL fusion proteins; transcriptional elongation and chromatin modification versus dimerization

The first and most striking property of MLL fusion proteins is their incredible diversity.

MLL has been found in 73 different translocations and 54 partner genes have been cloned (*http://atlasgeneticsoncology.org/Genes/MLL.html; last update 5/08*). Despite this variety most cases of mixed lineage leukemia present as a clinical entity and gene expression signatures in leukemic blasts do not separate MLL fusions according to the fusion partner.[61-64] Therefore, it was a long stand-

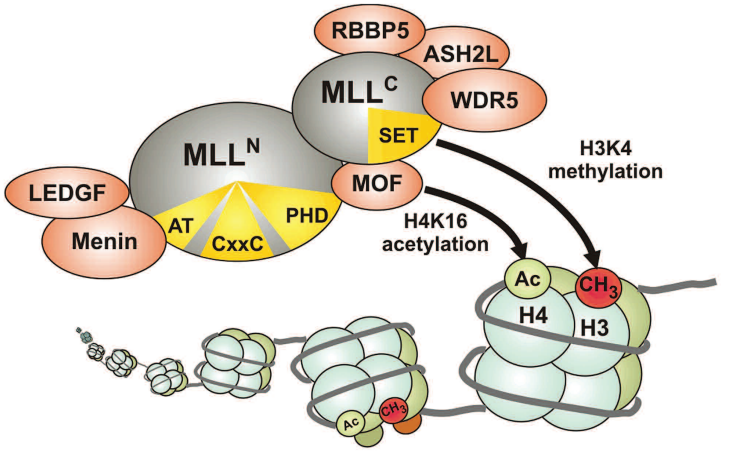

Figure 2. The MLL complex. After post-transcriptional proteolytic processing amino-terminal and carboxy-terminal portions of MLL are incorporated in a macromolecular complex with histone methyltransferase and histone acetyltransferase function. Functional domains in MLL are indicated in yellow. AT = AT-hooks, a DNA binding domain, CxxC = motif recognizing unmethylated CpG dinucleotides, PHD = plant homeodomain, SET = histone methyltransferase active site. Proteins associated with MLL are explained in the text.

ing question how a multitude of different proteins could cause the same disease. Two facts gave early clues to this problem. Firstly, all MLL fusion proteins share a common structure with the respective partners invariably fused in frame to MLL^{N} right after the CxxC domain but excluding the PHD fingers. Secondly, proteins joined to MLL clearly fall into two classes. Only 6 frequent partner proteins (*AF4, AF9, ENL, AF10, ELL, AF6*) constitute the bulk (> 85%) of all clinical cases of mixed lineage leukemia[65,66] (Table 1) whereas the remaining fusions were cloned each from a few isolated, mostly adult patients. This distinction is mirrored by the biology of the respective proteins. With the exception of AF6, all frequent MLL partners are nuclear while cytoplasmatic localization predominates amongst the rarely occurring MLL fusions. Therefore it was expected that at least two different mechanisms should be responsible for MLL fusion function. Further clarification of these pathways was promoted by the development of an *in vitro* assay that was able to measure the biological readout of MLL fusion activity.[67]

This serial replating assay records an inhibition of hematopoietic differentiation as surrogate parameter for transformation activity. A block in differentiation can be visualized as enhanced clonogenic capacity of hematopoietic precursor cells after repeated replating in semisolid medium. With respect to the MLL portion included in the fusions, deletion studies demonstrated that the LEDGF-menin binding motif and the CxxC domain were absolutely necessary for the overall function of MLL fusions.[68,69] In addition, it was mandatory that the breakpoint in MLL was upstream of the PHD fingers because artificial MLL fusions including this domain lost their transforming capacity.[70,71] This explains the strict conservation of the fusion breakpoints found in leukemic blasts. Further studies suggested that the leukemogenic potential of truncated MLL could be activated in at least four different ways (Figure 3).

The "common" nuclear fusion partners (ENL, AF9, AF4, ELL, and AF10) - transcriptional elongation meets histone methylation

Early reports showed that MLL fusions function as a novel type of general transcription factor that is able to indiscriminately activate many different promoters.[72] The search for a common *MLL machinery* revealed that the close homologs ENL and AF9 were both able to interact with other MLL fusion partners like AF4, the AF4-homolog AF5 and probably also with AF10. In addition, it was realized that ENL could bind to histone H3, indicating a potential shared link of these proteins with chromatin modification.[73] A breakthrough concerning the normal function of these proteins came from the purification of the *ENL associated protein* complex (EAP).[74,75] In this complex, ENL was not only linked with all members of the AF4 protein family that occur as MLL fusion partners (AF4, AF5q31, LAF4) but also with positive transcription elongation factor b (pTEFb) and the histone methyltransferase DOT1L. pTEFb is a dimer of cyclin dependent kinase 9 (CDK9) and a cyclin T that phosphorylates the carboxy-terminal repeat domain of RNA polymerase II. This activity is essential for efficient transcriptional elongation.[76] DOT1L methylates histone H3 at lysine 79, a modification that is introduced also during transcriptional elongation.[77] This immediately invoked parallels to AF10 as it had been demonstrated that AF10 binds DOT1L and that DOT1L recruitment was essential for the transforming activity of MLL-AF10.[78] A dramatically increased H3K79 methylation has been demonstrated for the *HOXA9* gene activated by MLL-ENL[79] and this result was corroborated on a global scale in two recent studies by genome-wide ChIP where the majority of MLL target genes defined by MLL fusion protein binding also showed increased H3K79 modification.[80,81] Another connection to transcriptional elongation is defined by the fusion partner ELL, as this protein had been identified before as elongation factor.[82] This idea was later dismissed because domains in ELL necessary for elongation activity were dispensable for transformation by MLL-ELL.[83] However, ELL also interacts with proteins that are homologous to AF4, and ELL might therefore make indirect contact to the EAP elongation machine.[84,85] In summary, the common MLL fusion partners with clinical importance all seem to participate in the same biological process if not in the same macromolecular complex responsible for control of transcriptional elongation. Although not yet formally proven, it is tempting to speculate that MLL fusions might recruit EAP to genomic loci to achieve ectopic target gene expression. Indeed, there are hints that the association of ENL and AF9 with AF4 family members is essential for survival of mixed lineage leukemia cells as small peptides disrupting this interaction proved to be toxic for MLL transformed cells but

Table 1. A selection of MLL fusion partners with known functions or domains.

Name	Gene alias	Features	Localization
ENL[b]	*MLLT1*	Binds histone H3, assembles EAP[a] elongation/chromatin modification complex	Nuclear
AF9	*MLLT3*	ENL homolog, also found in EAP	Nuclear
AF4	*AFF1, MLLT2*	Founder of AF4 family, member of EAP	Nuclear
AF5q31	*AFF4, MCEF*	AF4 homolog, found in EAP	Nuclear
LAF4	*AFF3*	AF4 homolog, found in EAP	Nuclear
ELL		Elongation factor, interacts with a protein related to AF4	Nuclear
AF10	*MLLT10*	Interacts with DOT1L histone methyltransferase	Nuclear
CBP	*CREBBP*	Histone acetyl-transferase, in therapy related MLL fusions	Nuclear
P300	*EP300*	CBP homolog, in therapy related MLL fusions	Nuclear
AF1p	*EPS15*	Dimerization domain	Cytoplasma
GAS7		Dimerization domain	Cytoplasma
AF6	*MLLT4*	Dimerization domain	Cytoplasma
ABI1		Interacts with ENL when imported to nucleus	Cytoplasma
EEN		Interacts with histone arginine methyltransferase PRMT1 when imported to nucleus	Cytoplasma

[a]EAP: "ENL associated proteins"; [b]the six most frequent fusion partners are printed in bold.

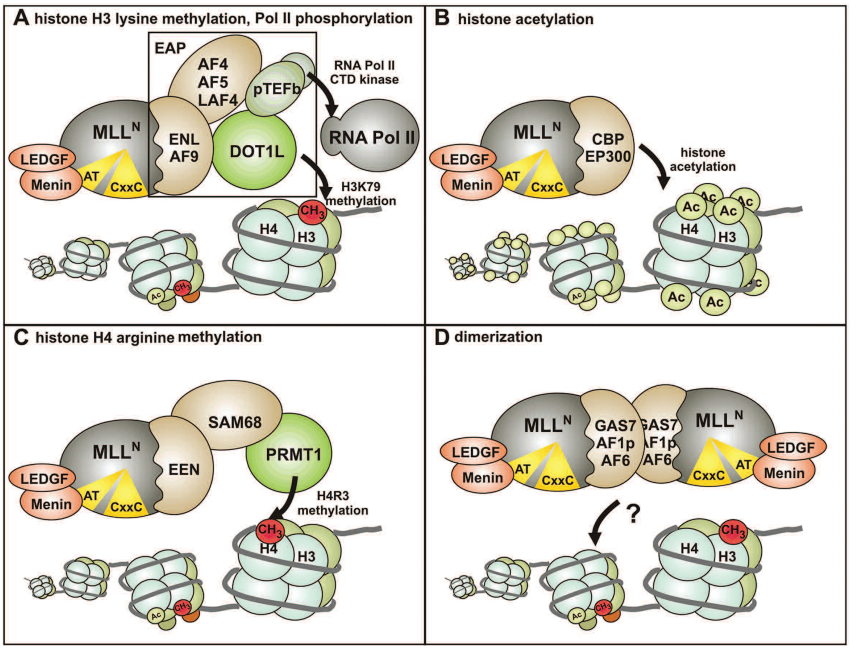

Figure 3. Molecular pathways leading to oncogenic activity of MLL fusion proteins. MLL fusions are aberrant transcription factors that activate gene expression. Four different mechanisms have been suggested as to how fusion partners might induce transcriptional activation. (A) The most frequent fusion partners of the ENL and AF4 family are members of the EAP complex that combines histone H3K79 methyltransferase activity catalyzed by DOT1L with transcriptional elongation stimulation by pTEFb (positive transcription elongation factor b, a dimer of CDK9 and a cyclinT) that phosphorylates the C-terminal repeat domain of RNA polymerase II. It is speculated that MLL fusion proteins aberrantly recruit this complex to target chromatin. (B) Active histone acetyltransferases are fused to MLL in the MLL-CBP and MLL-p300 proteins. (C) MLL-EEN indirectly recruits the histone H4R3 arginine methyltransferase through binding of the adaptor SAM68. (D) Dimerization of MLL via coiled-coiled or other dimerization domains supplied by the fusion partner activates target genes by unknown mechanisms.

not for blasts of a different etiology.[86,87] The potential usefulness of these peptides as therapeutic intervention is under active investigation.[88]

CBP and p300 - MLL fusions and histone acetylation

Interestingly, fusions of MLL with the histone acetyltransferases CREB binding protein (CBP) and the related p300 have been observed. Although these MLL derivatives have only been found in a few cases of therapy induced secondary leukemia, they allowed immediate insight into a possible activation mode for MLL.[89,90] Structure function analyses clearly singled out the bromo- and histoneacetyltransferase domains of CBP as necessary and sufficient for the oncogenic function of the respective fusion proteins.[91] Unfortunately, the consequences of MLL-CBP/p300 expression for target chromatin have not yet been investigated. However, it seems reasonable to assume that the permanent recruitment of HAT activity will result in a hyperacetylation of chromatin and an increased transcriptional output.

The special case of MLL-EEN arginine specific histone methylation

Although the EEN fusion partner was cloned only from a single case of mixed lineage leukemia and has never been found again, the resulting MLL fusion was studied in great detail. Surprisingly, a biochemical interaction study revealed that EEN bound the arginine methyltransferase *PRMT1* through the adaptor protein SAM68.[92] *PRMT1* is an arginine specific methyltransferase that shuttles between nucleus and cytoplasma. Next to several cytoplasmic substrates, *PRMT1* also methylates histone H4 at arginine 3. This modification has in turn been shown to be correlated with an increased histone acetylation.[93] Therefore MLL-EEN might feed into the same pathway as suggested for MLL-CBP or MLL-p300.

Cytoplasmatic fusion partners and dimerization

MLL joined to proteins of cytoplasmatic origin seem to be more weakly transforming as fusions with nuclear proteins. *Cytoplasmatic* fusions are found preferentially in older patients where more time has elapsed from the initiating pre-leukemic event to the outbreak of acute leukemia allowing additional secondary mutations to occur. Consequently several of these MLL fusions do not read out in the standard *in vitro* assays that measure MLL fusion activity.[94,95] Nevertheless MLL fusions with the cytoplasmatic proteins GAS7, AF1p and AF6 could be assayed in this system, and therefore the minimally necessary contributions of the respective fusion partner could be localized to a coiled-coiled dimerization domain.[96] In addition, also fusion of MLL to an artificial inducible dimerization domain caused activation of the transforming potential.[97] Unfortunately, up to now it is not known how dimerized MLL fusion proteins activate target genes. With respect to cytoplasmatic fusion partners, it must be taken into consideration that all MLL fusions will be imported to the nucleus because of the strong nuclear import signals in MLL^N.[98] This might cause aberrant protein-protein interaction of the fusion partner, a situation that has been demonstrated for the ABI1 protein. Normally localized in the cytoplasma, ABI1 interacts with ENL after import to the nucleus.[99] In this way, also cytoplasmatic fusion partners might feed into pathways used by the nuclear partner proteins.

MLL fusion downstream targets and the problem of pediatric leukemia

Normal MLL performs an important task necessary for the transcription of many genes. Because all domains within MLL^N thought to be involved in target selection are retained in the fusion proteins, it seems likely that

MLL fusions will share many target loci with wild type MLL. This assumption has been confirmed for the clustered *HOX* homeoboxgenes that are under control of MLL as well as of MLL fusion proteins. In addition to the *HOX* cluster, MLL-AF4 has been found on a genome-wide scale on more than 1,000 promoters that also showed a corresponding H3K79 methylation pattern as an indication for a functional interaction of MLL-AF4 with chromatin.[80,81] This number matches approximately the amount of loci occupied by MLL; [55] however, the potential overlap has never been determined. Surprisingly, experiments searching for MLL fusion controlled transcripts on the RNA level uncovered only a relatively small number (<100) of genes with a significant response to MLL fusion presence.[64,100,101] Obviously, many genes are largely resistant to MLL fusion induced modifications and only some of them, like the *HOX* genes, are susceptible to e.g. elongation stimulation. This fits well with the fact that a subset of genes controlling embryonic development and cellular differentiation preferentially are occupied by RNA polymerase II also in the non-transcribed state. These genes are *poised* for transcription, and release of the stalled polymerase allows a fast response without the need to recruit transcription factors and histone modifiers to free the chromatin and allow assembly of the transcriptional initiation complex.[102] Whatever the exact number and identity of all fusion targets will be, undoubtedly, *HOX* deregulation is the most important factor for MLL fusion induced leukemogenesis.[79,100,101,103-109] HOX proteins, especially HOXA9, and its dimerization partner MEIS1, are major hematopoietic oncoproteins that are overexpressed in a wide variety of different leukemias and that act, at least partially, through activation of the proto-oncogene *c-Myb*.[110] In general, HOX transcription factors are not only master controls of embryonic development but they also direct normal hematopoietic differentiation. *HOX* expression is high in stem cells and early precursors and needs to be down-regulated for maturation. Therefore, a continuous ectopic *HOX* expression will block differentiation and create a rapidly proliferating pre-leukemic precursor pool (Figure 4). Secondary mutations will have to occur to convert this *smoldering* state into an acute leukemia. Such mutations have been found in murine experimental models[111] and also in patient cells that frequently carry an activating mutation in the receptor tyrosine kinase Flt-3.[112,113] In a very surprising development, it has also recently been suggested that increased glycogen-synthase-kinase 3 activity is involved in the etiology of mixed lineage leukemia, an unexpected finding because GSK3 normally is a tumor suppressor gene.[114] It is very interesting that mixed lineage leukemia tends to be a pediatric disease in contrast to many other tumors that arise later in life, because several years are needed to accrue the mutations necessary to convert a normal cell into a cancerous state. An attractive hypothesis to answer this question has been brought forward by Greaves and colleagues[115]. They speculated that a persistent genetic assault during gestation first produces MLL translocations. Once these are transcribed, the presence of the fusion proteins might sensitize cells to further mutations induced by the same mutagens that created the fusion before. In this way, secondary events would accumulate very rapidly and congenital leukemia would ensue. Indeed, there are experimental hints that MLL fusions increase susceptibility to mutagenic influence,[115] and a few reports suggest that this might be due to inhibition of the tumor suppressor protein p53.[116-118]

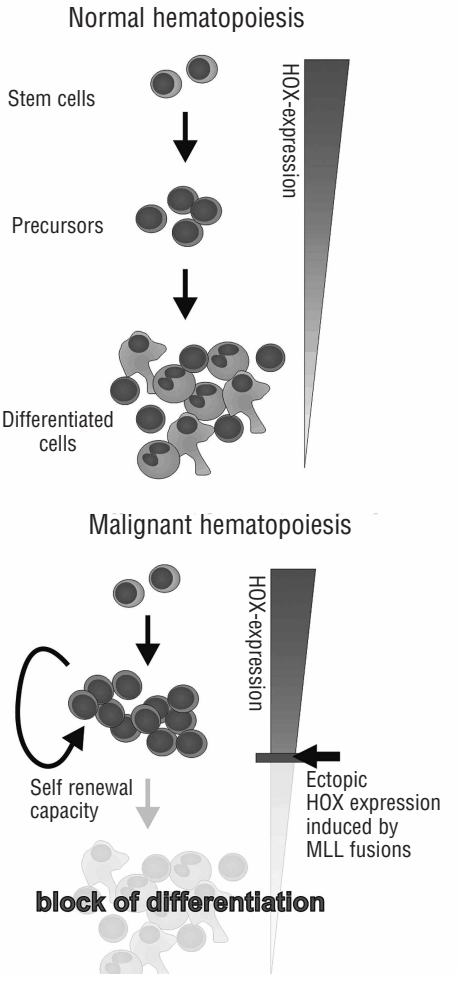

Figure 4. The role of HOX proteins in control of hematopoiesis. HOX transcription factors control hematopoietic differentiation. *HOX* expression must be terminated for maturation to occur, and therefore ectopic presence will block maturation and cause a population of self-renewing precursor cells to expand.

Unsolved questions and future directions

While the molecular pathways triggered by MLL fusion proteins slowly emerge, other major questions still wait for answers.

1. Certainly, the most pressing problem with respect to the dismal prognosis of this disease is if new rational treatments can be devised by targeting the enzymatic activities necessary for MLL fusion activity.

One possible approach would be to interfere with the protein interactions necessary for proper MLL fusion protein function. Here, either the interaction with

menin or LEDGF could be disrupted or, at least for the fusions employing the EAP complex, the protein-protein interactions stabilizing EAP might be targeted. Proof of principle experiments have been performed with peptides as binding site mimetics[86,87] but if this is a feasible approach in patients remains to be seen. Theoretically, also the interaction surface between the MLL CxxC domain and DNA might be a point of attack, since this interaction has been analyzed in detail by X-ray crystallography.[119] The largest concern with these strategies, however, is that apart from technical difficulties delivering peptides, likely also the vital natural function of MLL will be abrogated leading to toxicity. A small molecule approach that can be finely tuned to turn down the local hyperactivity of the enzymatic functions of MLL recruited EAP seems more promising. In this regard, specific methyltransferase inhibitors blocking DOT1L might be valuable tools. Also kinases like CDK9 or maybe GSK3 are potential targets for a rational therapeutic approach. Finally, also activities downstream of MLL fusions or cooperative oncogenic pathways might be druggable. It has been shown, for example, that inhibitors of the FLT-3 receptor tyrosine kinase are remarkably efficacious in mixed lineage leukemia animal models,[120,121] although the role of FLT-3 in the etiology of MLL fusion induced leukemia is not exactly clear.[122]

2. It is not known how histone modifications introduced by MLL and MLL fusions actually support transcription. In particular, H3K79 methylation still remains enigmatic.

3. HOX proteins are transcription factors at the top of a regulatory cascade. We need to know what is downstream of HOX and which other proteins cooperate with these regulators.

4. It would be interesting to know if MLL fusions are indeed only hyperactive MLL molecules and how these molecules find their appropriate binding sites.

With advanced molecular biology at hand it is to hope that 18 years of MLL research will finally translate into better survival chances for patients that all but too often face a bleak prospect after their diagnosis.

Authorship and Disclosures

The author reported no potential conflicts of interest.

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