Review

**MLL-SEPTIN gene fusions in hematological malignancies**

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**Abstract**

The mixed lineage leukemia (MLL) locus is involved in more than 60 different rearrangements with a remarkably diverse group of fusion partners in approximately 10% of human leukemias. MLL rearrangements include chromosomal translocations, gene internal duplications, chromosome 11q deletions or inversions and MLL gene insertions into other chromosomes, or vice versa. MLL fusion partners can be classified into four distinct categories: nuclear proteins, cytoplasmatic proteins, histone acetyltransferases and septins. Five different septin genes (SEPT2, SEPT5, SEPT6, SEPT9, and SEPT11) have been identified as MLL fusion partners, giving rise to chimeric fusion proteins in which the N terminus of MLL is fused, in frame, to almost the entire open reading frame of the septin partner gene. The rearranged alleles result from heterogeneous breaks in distinct introns of both MLL and its septin fusion partner, originating distinct gene fusion variants. MLL-SEPTIN rearrangements have been repeatedly identified in de novo and therapy related myeloid neoplasia in both children and adults, and some clinicopathogenetic associations are being uncovered. The fundamental roles of septins in cytokinesis, membrane remodeling and compartmentalization can provide some clues on how abnormalities in the septin cytoskeleton and MLL deregulation could be involved in the pathogenesis of hematological malignancies.

**Keywords:** fusion genes; hematological malignancies; MLL; septins.

**The MLL gene**

The mixed lineage leukemia protein-1 gene (MLL) is the mammalian homolog of *Drosophila melanogaster* trithorax (trx), the founding member of the trithorax group proteins. In humans, there are several proteins homologous to MLL, such as MLL2, MLL3, MLL4, MLL5, Set1A and Set1B, and all are members of the evolutionarily conserved SET1 family of histone H3 lysine 4 (H3K4) methyltransferases (Milne et al., 2005; Terranova et al., 2006; Malik and Bhaumik, 2010). The MLL gene maps to chromosome 11q23, is approximately 89 kb long and consists of 37 exons (Ayton and Cleary, 2001; Kriptsov and Armstrong, 2007). It encodes a 3969 amino acid nuclear protein with a complex domain structure that is involved in chromatin remodeling and positively regulating multiple homeobox transcription factors, including *HOXA9* and *MEIS1* (Imamura et al., 2002; Wang et al., 2006). The maintenance of appropriate homeobox *HOX* gene expression patterns during hematopoiesis and development is crucial for the survival of hematopoietic stem and progenitor cell populations (Yokoyama and Cleary, 2008).

**MLL protein structure**

The wild-type MLL protein structure has been intensively studied in recent years (Popovic and Zeleznik-Le, 2005; Meyer et al., 2006; Yokoyama and Cleary, 2008). The 500 kDa full length MLL precursor undergoes evolutionarily conserved site-specific proteolysis to generate a mature MLL heterodimer (Meyer et al., 2009). The mature MLL protein (3968 amino acids) consists of two non-covalently associated subunits, MLLN (300 kDa) and MLLC (180 kDa), produced by the cleavage of nascent MLL by the endopeptidase Taspase 1 (Hsieh et al., 2003a; Liu et al., 2008).

The fragments associate through the FYRN and FYRC domains, and translocate into the nucleus where they remain non-covalently associated into a high molecular mass complex (Yano et al., 1997; Nakamura et al., 2002; Hsieh et al., 2003b). The N-terminus contains three short AT-hook motifs fingers, which are thought to mediate binding to the minor groove of AT-rich genomic DNA sequences (Yokoyama et al., 2002) and a cysteine-rich CxxC zinc-finger motif with homology to DNA methyltransferases that binds selectively to non-methylated CpG rich DNA sequences (Fair et al., 2001). Both domains can contribute to the localization of MLL protein complexes to chromatin and this interaction appears to be mediated by Menin, a protein coded by the tumor suppressor gene MEN1, and the lens epithelium-derived growth factor (LEDGF), a chromatin associated protein (Chandrasekharappa et al., 1997; Ayton et al., 2004). The plant homology domain (PHD) zinc-finger motifs might mediate binding of the cyclophilin, CYP33 and potentially other proteins (Hughes et al., 2004).

The transcriptional activation (TAD) domain recruits the transcriptional co-activator CBP (CREB-binding protein) that contains histone and transcription factor acetylation
activities (Arai et al., 2010). The TAD domain precedes a C-terminal evolutionarily conserved SET [Su(var), enhancer-of-zeste, trithorax] domain that possesses histone H3 lysine 4 (H3K4) methyltransferase activity and is structurally homologous to Drosophila trithorax (trx) (Briggs et al., 2001; Ernst et al., 2001).

**MLL protein function**

Under normal circumstances, *MLL* encodes a histone methyltransferase that, like other methyltransferases, has been reported to assemble a supercomplex of proteins of varied function involved in transcriptional regulation (Milne et al., 2002; Yokoyama et al., 2004). Although not completely elucidated, current evidence suggests that *MLL* binds DNA in a non-sequence specific manner through the AT-hook domains and the domain homologous to DMT, and is a major regulator of class I homeobox (HOX) gene expression, directly interacting with HOX promoter regions (Briggs et al., 2001). HOX genes are transcription factors involved in the specification of cell fate during development, playing a key role in the regulation of hematopoietic development (Dou et al., 2005; Jude et al., 2007), and it seems plausible that deregulation of *MLL* protein activity might result in abnormal patterns of HOX gene expression in hematopoietic stem cells or progenitors. Normally, HOX genes are expressed in lineage and stage specific combinations during hematopoiesis; however, cell commitment to myeloid or erythroid lineages is accompanied by global down-regulation of HOX gene expression (Pineault et al., 2002; McMahon et al., 2001). A failure to down-regulate HOX expression can inhibit hematopoietic maturation and can lead to leukemia (Pineault et al., 2002). As HOX genes are the best characterized *MLL* targets, the significance of their deregulation in *MLL* leukemia has been extensively studied. However, HOX deregulation does not appear to be required in all cases of *MLL* fusions. For instance, HOX7, HOXA9 and MEIS1 up-regulation is a prerequisite for *MLL-MLLT1* initiated leukemia (Grier et al., 2005) but is dispensable for leukemia induced by *MLL-MLLT3* and *MLL-GAS7* (Kumar et al., 2004; Zeisig et al., 2004). Therefore, it is likely that deregulation of critical pathways other than HOX genes plays an instrumental role in *MLL* leukemia. Indeed, gene expression profiling suggests the existence of a number of other potentially important target genes in *MLL*-rearranged leukemia (So et al., 2004; Stam et al., 2010).

**MLL rearrangements: incidence and clinical relevance in leukemia**

The *MLL* locus is involved in more than 60 different chromosomal rearrangements with a remarkably diverse group of fusion partners (Meyer et al., 2006, 2009) and is associated mostly with the acute myeloid leukemia FAB (French-American-British) subtypes M4 or M5. According to the new WHO (World Health Organization) classification of tumors of hematopoietic and lymphoid tissues, patients with *MLL* rearrangements are classified as a distinct entity (Arber et al., 2008). *MLL* rearrangements include chromosomal translocations (the most common mechanism), gene internal duplications, chromosome 11q deletions or inversions and *MLL* gene insertions into other chromosomes, or vice versa (Meyer et al., 2006, 2009). Overall, *MLL* rearrangements are found in approximately 10% of human leukemia (Huret et al., 2004). *MLL* rearrangements are found in >70% of infant leukemia, whether the immunophenotype is more consistent with acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML), but are less frequent in leukemia from older children (Biondi et al., 2000; Daser and Rabbits, 2005). *MLL* translocations are also found in approximately 10% of adult AML and can also be found in a proportion of patients with therapy related leukemia after treatment for other malignancies with topoisomerase II inhibitors, such as anthracyclines (e.g., doxorubicin and epirubicin) and epipodophyllotoxins (e.g., etoposide and tenoposide) (Biondi et al., 2000; Daser and Rabbits, 2005). Independently of their association with other high-risk features at presentation, 11q23 rearrangements are in most cases predictive of poor clinical outcome (Biondi et al., 2000; Harrison et al., 2010; Tamai and Inokuchi, 2010). Children with *MLL*-rearranged AML have a particularly poor outcome compared with children with other forms of ALL, and *MLL*-rearranged leukemia that occurs after treatment with topoisomerase II inhibitors has a similarly poor prognosis (Chen et al., 1993). Interestingly, *MLL-MLLT3* rearranged AML has an intermediate survival and one that is superior to AML with other 11q23 translocations (Felix et al., 1995; Rubnitz et al., 2002), showing that, at least in some cases, the fusion partner is relevant to the phenotype of *MLL*-rearranged AML.

**MLL fusion partners in leukemia**

One of the most notable features of *MLL* is the extraordinary diversity of its fusion partners. To date, of the 71 genetic loci shown by conventional and molecular cytogenetic analysis to be involved in rearrangements with 11q23, where the *MLL* gene is located, 66 could be molecularly characterized and the respective fusion partner cloned (Meyer et al., 2009; Park et al., 2009; Cerveira et al., 2010). In ALL the most frequent, accounting for approximately 94% of all *MLL*-rearranged cases, are the fusion genes *MLL-AFF1*, *MLL-MLLT1*, *MLL-MLLT3*, and *MLL-MLLT10*. In AML the most common fusions, accounting for almost 77% of all reported cases, are *MLL-MLLT3*, *MLL-MLLT10*, *MLL-ELL*, *MLL-MLLT1*, *MLL-MLLT6*, and *MLL-SEPT6* (Meyer et al., 2009).

*MLL* fusion partners can be classified into four distinct categories. The first category, which accounts for most of *MLL*-rearranged leukemia, includes fusions with nuclear proteins (e.g., AFF1, MLLT3, MLLT10, and MLLT1) (Krivtsov and Armstrong, 2007; Meyer et al., 2009). A second group comprises fusions with cytoplasmatic proteins, such as GAS7, SH3GL1, EPS15, MLLT4 and FOXO4, which may possess coiled-coil oligomerization domains that are impor-
tant for their transformation potential (So et al., 2003; Krivtsov and Armstrong, 2007; Meyer et al., 2009). A third group includes histone acetyltransferases, EP300 (Ida et al., 1997) and CREBBP (Taki et al., 1997), which when fused to MLL retain histone acetyltransferase activity. Finally, a fourth group includes fusions with five septin family members (SEPT2, SEPT5, SEPT6, SEPT9 and SEPT11) involved in rearrangements with the MLL gene, making the septins the protein family with most members involved in MLL-related leukaemia (Megonigal et al., 1998; Osaka et al., 1999; Taki et al., 1999; Ono et al., 2002; Kojima et al., 2004; Cerveira et al., 2006).

Structure and function of MLL fusion proteins

MLL translocations, as many other translocations found in leukemia, are probably the result of a failure of appropriate DNA double strand break repair in developing hematopoietic cells (Richardson and Jasin, 2000). Breaks in MLL cluster between introns 8 and 13, in the 8.3 Kb breakpoint cluster region (BCR), resulting in loss of the PHD and distal domains and fusion to one of many different translocation partners. Presumably, the breaks are limited to this region because the BCR contains topoisomerase II cleavage sites along with nuclear matrix attachment regions that are likely to contribute to the mechanism by which translocations occur (Strissel et al., 1998), but this could also be because more proximal or distal breaks are not compatible with transformation (Hess, 2004). Indeed, the presence of the PHD fingers in the MLL fusion protein MLL-MLLT3 can block immortalization of hematopoietic progenitors, leading to reduced association with the HOXA9 locus and suppressing HOXA9 up-regulation in hematopoietic progenitors, providing an explanation for why MLL translocation breakpoints exclude the PHD fingers and suggesting a possible role for these domains in the regulation of the function of the wild-type MLL protein (Muntean et al., 2008). In addition, all identified MLL fusions contain the first 8–13 exons of MLL and a variable number of exons from a fusion partner gene. Accordingly, the fusion genes encode chimeric proteins harboring the NH2-terminal amino acids of MLL and the COOH-terminal amino acids of the partner protein, with the rearrangements always occurring in a way that an in-frame chimeric protein is produced.

The major contribution of the fusion partners investigated so far appears to be to convert the rearranged MLL protein to a potent transcriptional activator and it is usually accepted that deregulation of the MLL protein function is the key event in MLL-mediated leukemogenesis (Marschalek, 2011). Despite similarities between some of the more common MLL translocations, many MLL fusion partners are not transcription factors (Meyer et al., 2009; Marschalek, 2010, 2011). The finding of self-association motifs among these MLL fusion proteins suggests that, in these cases, the dimerization of truncated MLL converts it into an extremely potent transcriptional transactivator that has increased binding affinity for HOX gene promoters (So et al., 2003). In this way, it may be that all that is necessary for MLL fusions to be oncogenic is the ability of the fusion partner to direct oligomerization of the fusion protein. However, there is increasing evidence supporting the hypothesis that MLL fusion partners are not randomly chosen but rather functionally selected (So et al., 2003; Marschalek, 2010, 2011). For instance, the most frequent MLL fusion partners AFF1, MLLT3, MLLT1 and MLLT10 have been shown to belong to the same nuclear protein network (Mohan et al., 2010). Furthermore, the carboxyl-terminal domain of ELL and MLLT10 was shown to be required for the leukemic transformation associated with the MLL-ELL and MLL-MLLT10 fusion proteins, respectively (DiMartino et al., 2000, 2002). This suggests that AFF1, MLLT3, MLLT10, MLLT1, ELL and presumably other fusion partners possess activity beyond simple oligomerization.

Septins as MLL fusion partners

Septins are an evolutionarily conserved family of GTP-binding proteins that associate with cellular membranes and the actin and microtubule cytoskeletons (Hall and Russell, 2004). In humans, 13 septin genes have been characterized to date (SEPT1 to SEPT12 and SEPT14) (Leipe et al., 2002; Russell and Hall, 2005; Versele and Thorner, 2005; Peterson et al., 2007). They map throughout the human genome, are strikingly similar and they all possess a loop-based GTP-binding (G) domain flanked by a polybasic domain and, with a few exceptions, a C-terminal coiled-coil region (SEPT1, 2, 4–8, 10, and 11) and/or a long N-terminal extension rich in proline residues (SEPT4, 8, and 9) (Leipe et al., 2002; Versele and Thorner, 2005; Peterson et al., 2007). The polybasic domain has been implicated in phosphoinositol phosphates binding and membrane dynamics and the coiled-coil domain in protein-protein interactions (Hall and Russell, 2004; Russell and Hall, 2005). The function of the GTP-binding domain has not been fully elucidated but there is some evidence that it might have signaling properties or structural properties associated with oligomerization between septins or between septins and other proteins (Leipe et al., 2002; Spiliotis and Nelson, 2006). The characterization of the crystal structure of the human SEPT2-SEPT6-SEPT7 small core complex showed that it assembles into a hexamer composed of two copies of each septin protein, with SEPT6 sandwiched between SEPT2 and SEPT7, and that complex and filament formation is dependent on the GTPase domains rather than, as had been previously proposed, on the coiled-coil domains (Gladfelter and Montagna, 2007; Sirajuddin et al., 2007; Weirich et al., 2008). All septin transcripts contain multiple translation initiation sites and are alternatively spliced giving origin to multiple septin isoforms, some of which are tissue specific (Hall and Russell, 2004). With the exception of SEPT14, which has its expression restricted to the normal testis, septins are expressed in all tissue types but some show
high expression in lymphoid (SEPT1, 6, 9, and 12) or brain tissues (SEPT2, 3, 4, 5, 7, 8, and 11) (Hall et al., 2005; Peterson et al., 2007).

The first septin identified as a MLL fusion partner was SEPT9; subsequently four other septin genes (SEPT2, SEPT3, SEPT6, and SEPT11) were also identified as MLL fusion partners giving rise to chimeric fusion proteins in which the N terminus of MLL is fused, in frame, to almost the entire open reading frame of the septin partner gene (Table 1) (Megonigal et al., 1998; Osaka et al., 1999; Taki et al., 1999; Ono et al., 2002; Kojima et al., 2004; Cerveira et al., 2006). The rearranged genes result from heterogeneous breaks in distinct introns of both MLL and its septin fusion partner and as a result, distinct gene fusion variants can be observed (Table 1). The identification and detailed characterization of these molecular variants, although essential for accurate molecular sub-typing at diagnosis and subsequent patient follow-up, is not believed to have a significant impact at the biological and clinical level.

The MLL-SEPT2 gene fusion

Our group was the first to describe the MLL-SEPT2 gene fusion as the molecular abnormality subjacent to the translocation, t(2;11)(q37;q23), in a case of therapy-related acute myeloid leukemia (t-AML) of the M4 FAB subtype (Table 1, Figure 1, Cerveira et al., 2006). The molecular characterization of the fusion transcript by RT-PCR and sequencing analyses showed a fusion between MLL exon 10 and SEPT2 exon 3 (type I fusion transcript). Subsequently, a second MLL-SEPT2 fusion variant involving the fusion between MLL exon 9 and SEPT2 exon 3 (type II fusion transcript) was identified in a patient with t-AML of the M2 FAB subtype (van Binsbergen et al., 2007), and a third MLL-SEPT2 alternative fusion variant (MLL exon 11 with SEPT2 exon 3 – type III fusion transcript) was uncovered by our group in a case of therapy-related myelodysplastic syndrome (t-MDS) (Cerveira et al., 2008b; Snijder et al., 2008). Recently, a fourth case of the MLL-SEPT2 fusion (with the type III variant) was described in a patient that developed a t-AML of the M5 FAB subtype 26 months after treatment for osteosarcoma (Bielorai et al., 2010). Interestingly, although the breakpoints in the MLL BCR can be quite diverse involving distinct exons, in SEPT2 they always occur in intron 2, leading to the formation of a fusion protein where the N-terminus of MLL is always fused to almost the entire open reading frame of SEPT2, except for the first three amino acids (Figure 1). Interestingly, when RNA expression analysis was performed in MLL-SEPT2 patients, a significant down-regulation of both SEPT2 and MLL was found (Cerveira et al., 2009).

The MLL-SEPT5 gene fusion

The MLL-SEPT5 fusion gene was, to our knowledge, described in only three patients with de novo AML (Megonigal et al., 1998; Tatsumi et al., 2001) (Table 1, Figure 1). It was first described as a MLL fusion partner in two infant twins with a t(11;22)(q23;q11) and AML of the M1 and M2 subtypes. Molecular characterization by RT-PCR and sequencing analysis revealed in both patients an in-frame fusion of MLL exon 10 with SEPT5 exon 3 (type I fusion transcript) (Megonigal et al., 1998). Subsequently, a third MLL-SEPT5 fusion was detected in an adult with AML-M2 and a t(11;22)(q23;q11) (Tatsumi et al., 2001). In this case, molecular analysis showed a fusion between MLL exon 9 and SEPT5 exon 4 (type II fusion transcript).

The MLL-SEPT6 gene fusion

Rearrangements recombining 11q23 and chromosomal band Xq24 that result in MLL-SEPT6 fusions are usually complex and sometimes cryptic as a result of the opposite orientation of MLL and SEPT6 on the respective chromosome arms. As a consequence, several different types of chromosomal rearrangements that can generate MLL-SEPT6 in-frame fusions were described including translocations and insertions (Table 2) (Borkhardt et al., 2001; Ono et al., 2002; Slater et al., 2002; Fu et al., 2003; Kim et al., 2003; Kadkol et al., 2006; Strehl et al., 2006; Cerveira et al., 2008a; De Braekeleer et al., 2010). So far, four different types of in-frame MLL-SEPT6 fusion variants were identified in 14 AML patients (Table 1). These fusion transcripts, named type I to IV, correspond to fusions between MLL exons 9, 10, 11, and 12 and SEPT6 exon 2 (Figure 1). Interestingly, this is the only group of patients with MLL-SEPTIN fusions where the presence of out-of-frame alternative splicing variants was detected, although its biologic relevance remains unclear (Kadkol et al., 2006; Cerveira et al., 2008a). However, as in all other MLL-SEPTIN fusions reported, the MLL-SEPT6 fusion is expected to give rise to chimeric fusion proteins, where almost the entire open reading frame of SEPT6 (with the exception of the first ten amino acids), containing all the three septin function-defining domains, is fused with the N-terminal moiety of MLL. Interestingly, as in MLL-SEPT2 patients, when RNA expression of MLL and SEPT6 was evaluated in MLL-SEPT6 patients, a statistically significant down-regulation of both MLL and SEPT6 was observed (Santos et al., 2010a).

The MLL-SEPT9 gene fusion

The MLL-SEPT9 fusion, resulting from a translocation between 11q23 and 17q25, has been described to date in 12 cases (Table 1, Figure 1) (Osaka et al., 1999; Taki et al., 1999; Yamamoto et al., 2002; Shih et al., 2006; Kreuziger et al., 2007; Kurosu et al., 2008; Saito et al., 2010; Santos et al., 2010b). As opposed to other cases with MLL-SEPTIN fusions, MLL-SEPT9 patients are very heterogeneous regarding both age (4 months–72 years) and clinical presentation: one MDS case, two t-AML cases and nine cases of de novo AML. Four distinct fusion variants were described (types
## Table 1
Clinical, karyotype, FISH and RT-PCR data on all known hematological malignancy patients with MLL-SEPTIN fusions.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Karyotype</th>
<th>FISH</th>
<th>MLL-SEPT</th>
<th>Fused exons (MLL/SEPT)</th>
<th>Transcript type</th>
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<tbody>
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<td>1</td>
<td>57 years</td>
<td>F</td>
<td>t-AML-M4</td>
<td>46,XX,t(2;11)(q37;q23)</td>
<td>MLL+</td>
<td>MLL-SEPT2</td>
<td>exon 10/exon 3</td>
<td>I</td>
<td>Cerveira et al., 2006</td>
</tr>
<tr>
<td>2</td>
<td>68 years</td>
<td>M</td>
<td>t-AML-M2</td>
<td>46,XY,t(2;11)(q37;q23)51,ident,+8,+17,+21,+22,+mar</td>
<td>MLL+</td>
<td>MLL-SEPT2</td>
<td>exon 9/exon 3</td>
<td>II</td>
<td>van Binsbergen et al., 2007</td>
</tr>
<tr>
<td>3</td>
<td>56 years</td>
<td>M</td>
<td>t-MDS</td>
<td>46,XX,X,del(X)(q24q28)t(2;11)(q37;q23),del(7)(q22q36)</td>
<td>MLL+</td>
<td>MLL-SEPT2</td>
<td>exon 11/exon 3</td>
<td>III</td>
<td>Cerveira et al., 2008b; Sjijder et al., 2008</td>
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<td>4</td>
<td>11 years</td>
<td>M</td>
<td>t-AML-M5</td>
<td>46,XY,t(2;11)(q37;q23)</td>
<td>MLL+</td>
<td>MLL-SEPT2</td>
<td>exon 11/exon 3</td>
<td>III</td>
<td>Bielorai et al., 2010</td>
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<tr>
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<td>11 months</td>
<td>F</td>
<td>AML-M2</td>
<td>46,XX,t(11;22)(q23;q11)</td>
<td>MLL+</td>
<td>MLL-SEPT5</td>
<td>exon 10/exon 3</td>
<td>I</td>
<td>Megonigal et al., 1998</td>
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<tr>
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<td>11 months</td>
<td>F</td>
<td>AML-M2</td>
<td>46,XX,t(11;22)(q23;q11)</td>
<td>MLL+</td>
<td>MLL-SEPT5</td>
<td>exon 10/exon 3</td>
<td>I</td>
<td>Megonigal et al., 1998</td>
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<tr>
<td>7</td>
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<td>46,XX,t(11;22)(q23;q11)</td>
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<td>MLL-SEPT5</td>
<td>exon 10/exon 3</td>
<td>I</td>
<td>Tatsumi et al., 2001</td>
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<td>exon 11/exon 2</td>
<td>II</td>
<td>Slater et al., 2002</td>
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<td>MLL-SEPT5</td>
<td>exon 8/exon 2</td>
<td>OF</td>
<td>Cerveira et al., 2008a</td>
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<tr>
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<td>M</td>
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<td>46,XX,t(11;22)(q23;q11)</td>
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<td>exon 8/exon 2</td>
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<tr>
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<td>11 months</td>
<td>F</td>
<td>AML-M2</td>
<td>46,XX,t(11;22)(q23;q11)</td>
<td>MLL+</td>
<td>MLL-SEPT5</td>
<td>exon 8/exon 2</td>
<td>OF</td>
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<tr>
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<td>F</td>
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<td>MLL-SEPT5</td>
<td>exon 8/exon 2</td>
<td>OF</td>
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<td>10 months</td>
<td>M</td>
<td>AML-M2</td>
<td>46,XX,t(11;22)(q23;q11)</td>
<td>MLL+</td>
<td>MLL-SEPT5</td>
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<td>3 months</td>
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<td>46,XX,t(11;22)(q23;q11)</td>
<td>MLL+</td>
<td>MLL-SEPT5</td>
<td>exon 8/exon 2</td>
<td>OF</td>
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<tr>
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<td>7 months</td>
<td>M</td>
<td>AML-M2</td>
<td>46,XX,t(11;22)(q23;q11)</td>
<td>MLL+</td>
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<tr>
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<td>6 months</td>
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*Patients 5 and 6 were twins.

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*Patients 5 and 6 were twins.

The MLL-SEPT11 gene fusion

The t(4;11)(q21;q23) is a recurring chromosomal translocation observed in acute leukemia that usually fuses the MLL gene on 11q23 with the AFF1 gene on chromosome 4q21 (Meyer et al., 2006, 2009). AFF1 is the most frequent translocation partner gene of MLL in acute leukemia (approximately 42% of all MLL-rearranged cases) and is characteristic of ALL, where it can be found in nearly 34% and 90% of pediatric and adult cases with MLL rearrangements, respectively (Meyer et al., 2009). Interestingly SEPT11, which shares the chromosomal band localization with AFF1, was identified as a fusion partner of MLL in the leukemia cell line, CNLBC1 derived from a 63 year old female with chronic neutrophilic leukemia (CNL) in transformation and a t(4;11)(q21;q23) (Table 1) (Kojima et al., 2004). In this particular case, MLL exon 9 was fused with SEPT11 exon 2 (Type I fusion transcript), leading to the retention of all three septin defining domains in the MLL-SEPT11 protein, as it appears to occur with all cases of MLL-SEPTIN fusions (Figure 1). Recently, a second case of the MLL-SEPT11 fusion, a type II variant involving the fusion of MLL exon 11 to SEPT11 exon 2 was described in a patient that developed a t-ALL after treatment for a previous nephritic syndrome (Figure 1) (Stevens et al., 2010). As in the previous case, the MLL-SEPT11 fusion is cytogenetically indistinguishable from the recurrent t(4;11)(q21;q23), an observation, which strongly supports that patients with hematological malignancies should be characterized by both cytogenetic and molecular approaches even in cases where a known recurrent translocation is presumed.

Genesis of MLL-SEPTIN fusions

Leukemia presenting MLL rearrangements can be subdivided into reciprocal chromosomal translocations, 11q deletions, 11q inversions and insertions of 11q23 sequences into another chromosome (Marschalek, 2011). These genetic abnormalities require two DNA double-strand breaks (DSB) and are probably the result of inappropriate DSB DNA repair in developing hematopoietic cells by the non-homologous end-joining (NHEJ) repair pathway (Slany, 2009). In agreement with this hypothesis is the identification by detailed molecular analysis of identical microhomologies in MLL-SEPT6 patients (Cerveira et al., 2008a). Indeed, the presence of identical microhomologies or non-templated nucleotides at breakpoint junctions is a hallmark of NHEJ (Gillert et al., 1999). DSBs can also result from exposure to drugs targeting topoisomerase II and there is strong evidence supporting the
Figure 1  Schematic representation of the localization of the breakpoints (arrowheads) in MLL and SEPTIN partner genes in all reported patients with MLL-SEPTIN associated hematologic malignancies. Although the breakpoints in the MLL breakpoint cluster region (BCR, introns 8–13) can be quite diverse, the breaks in the SEPT partner gene always occurs in the 5' region, leading to fusion proteins where the N-terminus of MLL is fused to almost the entire open reading frame of the septin partner.

hypothesis that topoisomerase II inhibitor related leukemia is closely associated with the presence of MLL gene rearrangements (Pui and Relling, 2000). Interestingly, all four patients with the MLL-SEPT2 fusion developed t-MDS/t-AML after chemotherapy for a previous neoplasia with topoisomerase II inhibitors (Cerveira et al., 2006; van Binsbergen et al., 2007; Snijder et al., 2008; Bielorai et al., 2010). The mapping of two sequences with 94.4% homology with the topoisomerase II consensus cleavage site to MLL intron 7 and to SEPT2 intron 2 in one of the MLL-SEPT2 patients provides support to a link between topoisomerase II inhibitor therapy and the origin of the MLL-SEPT2 fusion gene (Cerveira et al., 2006). The same topoisomerase II signature was also detected in SEPT6 intron 1 in patients with the MLL-SEPT6 rearrangement (Cerveira et al., 2008a), giving additional support to the hypothesis that exposure to drugs targeting topoisomerase II can result in double-strand DNA breaks that trigger the error-prone NHEJ pathway. However, with the exception of one case (a 43-year-old male with AML), all MLL-SEPT6 patients are very young children (0–29 months) and none showed evidence of therapy related disease (Table 1) (Borkhardt et al., 2001; Ono et al., 2002; Slater et al., 2002; Fu et al., 2003; Kim et al., 2003; Kadkol et al., 2006; Strehl et al., 2006; Cerveira et al., 2008a; De Braekeleer et al., 2010). Nevertheless, there is supporting evidence of a causal relationship between infant leukemia induced in utero and maternal exposure to dietary compounds that can act as topoisomerase II poisons (Cimino et al., 1997; Strick et al., 2000; Alexander et al., 2001), which lead us to speculate that children with MLL-SEPT6, as appears to be the case of MLL-SEPT2 patients, were also the result of exposure to topoisomerase II inhibitors (Cerveira et al., 2008a). Further studies in larger series of MLL-SEPT2 and MLL-SEPT6 patients are required to confirm or refute this relationship.

Clinical characteristics of MLL-SEPTIN leukemia

MLL-associated AML patients are usually classified as FAB M4 or M5 subtypes showing features of monocytic differentiation (DiMartino and Cleary, 1999). When considered as
a whole, the incidence of monocytic features in MLL-SEPTIN cases is 54% (14 out of 26 FAB-typed cases). The incidence is higher in the MLL-SEPT9 group (75%, 6 out of 8 cases) but lower in the MLL-SEPT6 group (50%, 6 out of 12 cases) (Table 1) (Osaka et al., 1999; Taki et al., 1999; Borkhardt et al., 2001; Ono et al., 2002; Slater et al., 2002; Yamamoto et al., 2002; Fu et al., 2003; Kim et al., 2003; Kadkol et al., 2006; Shih et al., 2006; Strehl et al., 2006; Kreuziger et al., 2007; Cerveira et al., 2008a; Kurosu et al., 2008; De Brackelee et al., 2010; Saito et al., 2010; Santos et al., 2010b). An interesting finding is the association of a particular MLL-SEPTIN fusion with specific patient characteristics. For instance, 13 of the 14 reported patients with a MLL-SEPT6 rearrangement are children, with an age range of 0–29 months (Table 1) (Borkhardt et al., 2001; Ono et al., 2002; Slater et al., 2002; Fu et al., 2003; Kim et al., 2003; Kadkol et al., 2006; Strehl et al., 2006; Cerveira et al., 2008a; De Brackelee et al., 2010). Interestingly, the majority (65%) of pediatric patients with MLL rearrangements have ALL (Meyer et al., 2009), which led us to hypothesize that the SEPT6 domains of the MLL-SEPT6 chimeric protein might be involved in myeloblastic leukemogenesis in children. Another interesting finding is the association of MLL-SEPT2 fusion and therapy related leukemia, with all the four published MLL-SEPT2 cases being t-AML or t-MDS patients (Table 1) (Cerveira et al., 2006; van Binsbergen et al., 2007; Snijder et al., 2008; Bielorai et al., 2010). The prognostic impact of MLL-SEPTIN fusions remains unknown due to the small number of cases reported in each subgroup and the lack of availability of follow up data in most cases (Table 1). However, and at least for AML, it appears that the prognostic effect of an MLL rearrangement could depend on the particular translocation partner involved. Indeed, several studies in children and adults with MLL-MLLT3 AML have shown superior event free and overall survival as compared to patients with other MLL rearrangements (Mrózek et al., 1997; Swansbury et al., 1998; Chen et al., 2010, Burnett et al., 2011). In contrast, AML patients with an MLL-MLLT10 have been shown to have a poor outcome in some studies (Dreyling et al., 1998) but superior long-term survival in others (Lillington et al., 1998; Chen et al., 2010; Burnett et al., 2011).

Models of MLL-SEPTIN associated leukemogenesis

In vitro and in vivo models of the MLL-SEPT6 fusion have provided some clues to MLL-SEPTIN induced leukemogenesis (Ono et al., 2005a,b). Fusion partner-mediated homooligomerization of MLL-SEPT6 was shown to be essential to immortalize hematopoietic progenitors in vitro (Ono et al., 2005b). However, MLL-SEPT6 could induce myeloproliferative disease with long latency in mice but not acute leukemia, implying that, at least in this model, secondary events are required to develop overt leukemia (Ono et al., 2005b). Indeed, transformation of hematopoietic progenitors and acute leukemia with short latency in vivo only occurred in the simultaneous presence of MLL-SEPT6 and activated FLT3 (Ono et al., 2005b). Furthermore, loss of SEPT6 did not alter the phenotype of myeloproliferative disease induced by MLL-SEPT6, suggesting that SEPT6 does not function as a tumor suppressor gene (Ono et al., 2005a). The development of additional in vivo and in vitro models of MLL-SEPTIN leukemia could help to resolve some of these questions.

Concluding remarks

Some septins have been repeatedly identified as in-frame fusion partners of the MLL gene in de novo and therapy related myeloid neoplasia (mostly AML), both in children and adults. The involvement of the septin protein family in MLL-related leukemia does not appear to be a chance event, a hypothesis that is supported by several observations. First, 5 out of the 13 known septins are involved in rearrangements with the MLL gene. Second, although chromosomal translocations disrupting the MLL gene are usually associated with both AML and ALL, corresponding to 27% and 73% of all MLL rearranged cases (Meyer et al., 2009), respectively, patients with MLL-SEPTIN fusions show a clear bias towards AML (30 out of 35 cases – 86%, with the remaining patients being one MDS, two t-MDS, one t-ALL and one CNL in blastic phase) (Table 1). Third, although the breakpoints in MLL usually cluster in the BCR, the breaks in the fusion partner can occur in its 5′ or 3′ region (in some genes, at both its 3′ and 5′ extremities) (Meyer et al., 2009), which is in contrast with septin genes where the breaks are always found at the very 5′ end of known septin open reading frames (Table 1, Figure 1). The fundamental roles in cytokinesis, membrane remodeling and compartmentalization can provide some clues on how abnormalities in the septin cytoskeleton and MLL deregulation could be involved in the pathogenesis of hematological malignancies.

Acknowledgments

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