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The role of *CALM-AF10* gene fusion in acute leukemia

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Abstract

Chromosomal translocations are important genetic perturbations frequently associated with hematologic malignancies; characterization of these events has been a rich source of insights into the mechanisms that lead to malignant transformation. The t(10;11)(p13;q14–21) results in a recently identified rare but recurring chromosomal translocation seen in patients with ALL as well as AML, and results in the production of a *CALM-AF10* fusion gene. Although the details by which the CALM-AF10 fusion protein exerts its leukemogenic effect remain unclear, emerging data suggests that the CALM-AF10 fusion impairs differentiation of hematopoietic cells, at least in part via an upregulation of *HOXA* cluster genes. This review discusses the normal structure and function of *CALM* and *AF10*; describes the spectrum of clinical findings seen in patients with *CALM-AF10* fusions; summarizes recently published *CALM-AF10* mouse models; and highlights the role of *HOXA* cluster gene activation in *CALM-AF10* leukemia.

Keywords

CALM-AF10; acute leukemia; chromosomal translocations; t(10;11)

Introduction

Acute leukemia is associated with a wide spectrum of gross chromosomal rearrangements. These acquired mutations include balanced and unbalanced chromosomal translocations, as well as chromosomal inversions, deletions, and amplifications.[1–4] The analysis of chromosomal translocations has proven to be especially useful in understanding the biology of hematologic malignancies, leading to improved diagnosis and classification, as well as identification of novel targets for therapy.[5] Balanced chromosomal translocations typically result in either dysregulated gene expression or production of a chimeric gene formed from two unrelated genes[2], and analysis of numerous recurrent translocations has revealed that many of the genes locates at the translocation breakpoint are transcription factors involved in normal blood cell differentiation.[1] In terms of clinical relevance, cloning of the *BCR-ABL* fusion gene highlighted the role of tyrosine kinases in leukemic transformation and led to the subsequent development of imatinib, a tyrosine kinase inhibitor.[6] Furthermore, chromosomal abnormalities are now routinely used to help classify leukemia patients for risk-directed therapy.[7]

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The t(10; 11) translocation was first observed in a patient with diffuse histiocytic lymphoma[8]; the U937 cell line was established from this patient and has been used extensively as an *in vitro* model of monocyte differentiation.[9] The presence of a reciprocal translocation between chromosomes 10 and 11, a t(10; 11)(p13–14; q14–21) in the U937 cell line was confirmed and subsequently refined by cytogenetic analysis.[10, 11] Using positional cloning techniques to narrow the chromosome 10 breakpoint to a 3 cM region, *AF10* was identified as a candidate gene on chromosome 10 potentially involved in this translocation. Subsequent studies showed that the *AF10* gene was indeed disrupted by the t(10;11) translocation, and identified *CALM* as the *AF10* fusion partner in this recurrent translocation.[12]

Structure and function of *CALM*

*CALM (*for *C*lathrin *A*ssembly *L*ymphoid *M*yeloid; also known as *PICALM)* is located on chromosome 11q23, is ubiquitously expressed, and encodes a 652 aa protein with multiple domains involved in endocytosis (Fig. 1).[12] These domains include the epsin N-terminal homology (ENTH) domain (Asp-Pro-Phe), a DPF (ASP-Pro-Phe) motif, an NPF (Asn-Pro-Phe) motif, and type I and type II clathrin-binding sequences (CBS I and II).[13–15] The predicted CALM protein is similar to the neuronal specific monomeric clathrin assembly protein AP180, which was first identified in coated vesicles of bovine brain.[16, 17] CALM homologues have been identified in rat, mouse and cow.

Endocytosis is the active cellular process of removing proteins from the plasma membrane.[18] The active formation of transport vesicles that shuttle cytosolic cargo, such as transmembrane and luminal proteins from the plasma membrane and the trans-Golgi network (TGN) to endosomes requires participation of clathrin coated pits.[19] Clathrin is composed of three major proteins; a 192 kDa heavy chain bound to two ~30 kDa light chains that forms a structure known as a triskelion.[19] In order for coat proteins to function properly in vesicle transport, they link to adaptor proteins (APs).[20] The role of APs is complex: First, studies suggest that AP-3 likely functions as a clathrin adaptor protein and aids in signal mediated protein sorting events.[21] Secondly, AP1 and AP2 function to attach clathrin to the plasma membrane and help form complexes in the TGN.[20] AP180 homologues have been observed in yeast[22] and *Drosophila*[23] where they were identified as participants in clathrin binding and endocytosis respectively. Likewise, *AP180/CALM* homologues identified in *C. elegans* presumably regulate endocyte vesicle size.[23] Finally, point mutations in the *CALM* gene led to ineffective hematopoiesis, functional iron deficiency, and altered growth in mice, suggesting that CALM may play a role in endocytosis-mediated iron transport.[15]

In addition to an established role in endocytosis, a recent study suggested that CALM may interact with nuclear proteins.[24] In this study, the novel protein CATS (CALM interacting protein, expressed in thymus and spleen) was shown to interact with a region of the CALM protein that is retained in the CALM-AF10 fusion protein and increased the nuclear localization of both CALM and a CALM-AF10 fusion protein. This study further suggested that CATS may be important for malignant transformation mediated by CALM-AF10, through either mis-localization of the CALM-AF10 protein, or via transcriptional regulatory properties of the CATS protein.[24]

A single case report described an *MLL-CALM* fusion in a 12-week-old Caucasian female who presented with rapidly progressive AML.[25] In this study, the authors used panhandle PCR to determine that *MLL* was fused to *CALM* at 11q14–q21. By assaying RNA from the patient's leukemic cells, fusion transcripts were identified. The breakpoints for this particular translocation were within *MLL* intron 7 (in the known *MLL* breakpoint cluster region) and *CALM* intron 7. This finding is intriguing since, in this case, *CALM* sequences are present at the 3′ portion of the fusion gene, in contrast to *CALM-AF10* fusions, where *CALM* sequences

form the 5′ portion of the fusion gene. In addition the breakpoint lay within *CALM* intron 7, whereas the breakpoints for *CALM-AF10* fusions typically occur within *CALM* introns 17–19.

Structure and function of *AF10*

The *AF10* (also known as *MLLT10*) gene is located on chromosome 10p12 and encodes a 109-kDa protein containing 1,027 aa residues, and was initially cloned as an *MLL* partner gene in the recurrent t(10;11)(p13;q23) translocation.[26] It is important to note that the reference sequence for human *AF10* (NM_004641 or U13948) is missing 124 nucleotides between exons 22 and 23; these 124 nucleotides are present in most human expressed sequence tags (ESTs) and a homologous 124 nucleotide sequence is present in the mouse reference sequence (NM_010804). Insertion of these 124 nucleotides leads to a frameshift of the COOH terminal portion of the AF10 protein. Studies in both humans and mice have demonstrated that *AF10* expression is highest in the testis, and is also expressed in the thymus, ovary, colon, peripheral blood, brain, and kidney.[27, 28] AF10 is a member of a small highly conserved protein family that includes the AF17, BR140, and CEZF proteins.[27, 29] Although the precise function of AF10 is not known, both structural and functional data suggest that it functions as a transcription factor (Figure 1). The AF10 protein contains PHD (Plant Homeo Domains) fingers, which are structurally conserved domains present in a number of known transcription factors that are involved in chromatin-mediated gene regulation, including the CBP, MLL, TRX, and Drosophila Polycomb group (PCL) proteins.[30] In addition to the N-terminal PHD domain, AF10 contains an extended PHD domain also known as a leukemia-associated protein domain (LAP) that functions in homo-oligomerization and is conserved in several other proteins including MLL, AF17, and BR140.[29] AF10 also contains an AT-hook motif, a motif that was initially described in the high-mobility group of non-histone chromosomal proteins and other DNA-binding proteins, and is thought to mediate protein binding to cruciform DNA.[31] In addition to these domains, AF10 contains a bipartite nuclear localization signal (NLS) and a C-terminus glutamine-rich region. However, due to alternative splicing the latter is not present in all isoforms.[29, 32]

A role for *AF10* in leukemic transformation was proposed as the gene was initially cloned as an *MLL* chromosomal translocation partner in 3 patients with AML-M5 and an *MLL-AF10* fusion.[27] An additional report presented two cases of pediatric AML-M5 in which there was paracentric inversion of the 11q region with chromosome 10p12.[33] Although pediatric acute megakaryoblastic leukemia (AML-M7) is considered rare, two independent case reports demonstrated fusions between *MLL* and *AF10* in pediatric patients.[33, 34] Deletion analysis of the *MLL-AF10* fusion protein has demonstrated that the leucine zipper (LZ) motif in the carboxy terminus of AF10 is essential for leukemogenesis [35], and the H3K79 methyltransferase hDOT1L has been shown to be involved in *MLL-AF10* mediated leukemogenesis through interaction of hDOT1L and AF10.[36]

Clinical Features of leukemia with *CALM-AF10* fusions

Although the t(10;11)(p13:q14–21) translocation was initially observed in a patient with histiocytic lymphoma, Groupe Français de Cytogénétique Hématologique (GFCH) subsequently identified the t(10;11) translocation as a recurrent reciprocal translocation in patients with T-cell acute lymphoblastic leukemia.[37] *CALM-AF10* translocations appear to be most frequently associated with T-cell ALL, specifically T-cell ALL of either γ/δ or immature phenotypes. In the largest series of its kind, 20 cases of *CALM-AF10* fusion leukemias were identified in a study of 144 patients with T-ALL.[38] Patients with a *CALM-AF10* fusion ranged from 3–43 years of age (mean 20.7), and were more frequently male (M:F=2.8). WBC at presentation ranged from 3.3–556 × 109/L, and 8 of 20 patients had a mediastinal mass. Although the numbers were small, patients with a *CALM-AF10* fusion and an immature

phenotype had a poor prognosis, as 10/12 patients either did not respond to therapy or relapsed. In contrast, 8/8 patients with a *CALM-AF10* fusion and a TCR γ/δ phenotype were in complete remission at the time of the report. In this series, *CALM-AF10* fusions were present in 12/29 cases with an immature TCR γ/δ phenotype and 8/32 cases with a TCR γ/δ phenotype, but in 0/78 α/β or IM β T-ALLs, demonstrating a marked bias toward T-cell ALL of the γ/δ lineage. This study also suggested that the *AF10* content of the *CALM-AF10* fusion might correlate with the stage of maturation arrest. All but the final four amino acid residues of the protein encoded by the *CALM* gene are retained in most *CALM-AF10* fusion transcripts (Figure 1)[12, 38], although due to alternate splicing, 20, 35, or 55 amino acid residues are excluded in some *CALM-AF10* fusions. At least four common *AF10* fusion sites have been described.[38] The resultant *CALM-AF10* fusion transcripts can be classified as either 5′ or 3′, depending on how much *AF10* sequence is included in the fusion transcript. 5′ fusion transcripts, which retain almost all of the *AF10* coding sequence, are associated with TCRγδ T-cells, whereas 3′ fusion transcripts, which contain less *AF10* sequence, are associated with more immature T-cells that do not express a TCR on the cell surface.[38]

CALM-AF10 fusions have also been observed in a variety of myeloid leukemias including AML-M0, M1, M2, M4, M5, and M7 (non-Down's M7 patients), as well as eosinophilic leukemia and granulocytic sarcoma (Table 1).[39–45] Interestingly, most of the AML M0, M1, and M2 cases that have been analyzed show clonal *IGH*, *TCRG*, and/or *TCRD* gene rearrangements, suggesting that the target for malignant transformation may have been a multipotential hematopoietic precursor cell.[38, 46] *CALM-AF10* translocations have been identified in both children and adults with AML; specific lesions include splenomegaly, hepatomegaly, mediastinal masses, and CNS leukemia.[47] Cytochemical and immunophenotypic data varies with the diagnosis but includes markers for lymphoid as well as myeloid antigens.[40, 44, 45, 47]

Mouse Models of *CALM-AF10* Leukemia

Animal models have provided tractable systems with which to study a wide range of human diseases, including the recurrent chromosomal translocations involved in hematologic malignancies.[48] Given the shared physiology between mice and humans, the mouse has become a standard animal model used to study pathophysiology and explore novel therapeutic approaches. Chromosomal translocations have been studied in mouse models using a variety of techniques including retroviral transduction and bone marrow transplantation as well as gene-targeting and transgenic approaches.[48–50] Recently, three groups have published reports that used mice to study *CALM-AF10* leukemia; findings from each of these reports are summarized in Table 2.

The first study used a vector based RNA interference system to "knock down" *CALM-AF10* expression in the U937 cell line, which expresses a CALM-AF10 fusion protein. The "knockdown" clones proliferated less rapidly *in vitro*, and showed a modest survival benefit following xeno-transplantation into NOD/SCID mice (median survival 27 vs. 19.5 days post transplant).[51]

A second study used retroviral transduction and bone marrow transplantation to generate acute leukemia in mice.[46] Recipient mice transplanted with bone marrow cells that expressed a *CALM-AF10* fusion gene developed disease a median of 110 days following transplantation. Mice with leukemia were anemic and had circulating blasts. Parenchymal infiltration by leukemic myeloid cells was noted in a variety of organs and these cells were positive for the myeloid markers myeloperoxidase and chloracetate esterase. Further analysis of leukemic cells from these mice demonstrated that myeloid cells positive for Mac1 and Gr1 were also positive for the lymphoid marker B220 and had clonal immunoglobulin DH-JH rearrangements,

consistent with the observation of clonal *IGH* gene rearrangements in patients with *CALM-AF10* fusions, and suggesting that the target cell for malignant transformation was multi-potential. Interestingly, these authors went on to show that AML could be propagated from a leukemic cell with lymphoid features, as Mac1+ myeloid leukemia could be established by serial transplantation of Mac1+/B220+ or Mac1−/B220+ leukemia cells.[46]

A third recent study reported a *CALM-AF10* transgenic mouse model that developed acute leukemia after a long latency period with incomplete penetrance.[52] In this study a *CALM-AF10* cDNA was expressed under *Vav* regulatory elements in the hematopoietic compartment, including thymus, spleen and bone marrow. Depending on the transgenic line, at least 40 or 50% of the F1 generation mice developed acute leukemia, at a median age of 12 months. Prior to the onset of acute leukemia, clinically healthy *CALM-AF10* mice displayed a variable, at times severe, inhibition of myeloid and T-cell maturation. *CALM-AF10* mice had reduced numbers of CD4+/CD8+ ("double positive") and increased numbers of immature CD4−/CD8− ("double negative") cells; interestingly, the proportion of immature CD4−/CD8− thymocytes seemed to increase with age. Leukemic mice typically had enlarged spleens; invasion of parenchymal organs including liver, lung, kidney, and brain with cells that stained positive for myeloid markers such as myeloperoxidase, Mac1, and Gr1. Similar to the findings with the retroviral transduction model described above, many, but not all, of these leukemias expressed both Mac1 and B220, and about half of the leukemic samples had clonal *Igh* gene rearrangements. Two distinct populations, a B220 "bright" and B220 "dim" population could be detected in the leukemic spleens. The B220 "bright" population was positive for CD19 and IgM, suggesting that these represent contaminating normal B lymphocytes. However, the B220 "dim" population was negative for CD19 and IgM, but positive for CD117 and CD24, suggesting that they represent a progenitor cell not irreversibly committed to the B-cell lineage. This finding is consistent with reports which suggest that B220 is expressed on non-B cell populations progenitor cell populations.[45, 53] Additionally, some leukemias arising in *CALM-AF10* transgenic mice showed features consistent with T-cell differentiation such as CD3 staining or clonal *Tcrb* or *Tcrd* gene rearrangements. Taken together, these data suggest that the B220 expression observed in leukemic cells from *CALM-AF10* transgenic mice represent a cell type other than committed B-lineage cells.

The long latency period prior to the onset of leukemia, and the incomplete penetrance of the leukemic phenotype suggest that additional genetic events are needed to complement the *CALM-AF10* transgene and complete the process of leukemic transformation. It is tempting to speculate that these proposed additional events may explain the phenotypic differences seen between individual *CALM-AF10* mice, in terms of degree of B220 expression, CD3 expression, and clonal rearrangements of antigen receptor genes. In this context, it is interesting to note that some patients with leukemia and *CALM-AF10* fusion have clonal *IGH* and *TCRB, TCRG,* and *TCRD* gene rearrangements.[38, 46]

HOXA cluster genes as *CALM-AF10* targets

Mammalian *HOX* genes are members of the evolutionarily conserved homeodomain family of genes that are structurally organized into four discreet clusters (A, B, C, and D), located on four different chromosomes in mammals, that encode for transcription factors.[54, 55] In addition to their well-studied role as executive regulators of anterior-posterior body pattern organization during early embryonic development, *HOX* genes play a fundamental regulatory role in hematopoietic system organization and development.[56] Of the distinct clusters, A, B, and C, but not D are thought to be important for normal hematopoiesis.[57] Gene ablation studies have shown specifically that *Hoxa9, Hoxc8,* and *Hoxb6* are necessary for hematopoietic cells to progress though development.[57] In addition to their role in normal development, *HOX* genes also play roles in cell cycle control, cell adhesion, and cell death. Given the central role of

HOX genes in normal development, it is not surprising that *HOX* genes have been implicated in a number of neoplastic and non-neoplastic disease processes.[58]

Several lines of evidence, based on clinical observations as well as data from experimental animal models, suggest that *HOX* genes are involved in leukemic transformation. Chromosomal translocations lead to fusions of *NUP98* with *HOXA9*, *HOXA11*, *HOXA13*, *HOXC11*, *HOXC13*, *HOXD11*, and *HOXD13* in patients with myelodysplastic syndrome (MDS) or AML.[59] Chromosomal translocations also lead to ectopic expression of *HOXA* cluster genes by juxtaposition of *TCRA* regulatory elements with *HOXA* coding sequences in some patients with T-cell ALL.[60, 61] In addition to these translocations which directly lead to overexpression of *HOX* genes, a number of recent studies have demonstrated that chromosome translocations which result in *MLL* fusions lead to unscheduled expression of *HOX* genes in both AML and T-cell ALL patients.[62] In addition to these studies showing *HOX* gene dysregulation resulting from chromosomal translocations, global gene expression profiling has identified *HOX* genes as being consistently overexpressed in AML.[63, 64] Finally, a number of *Hox* genes and *Hox* co-factors, including *Hoxa7*, *Hoxa9*, *Meis1* and *Pbx1* are among the most common upregulated genes in murine leukemias that result from retroviral insertional mutagenesis.[65]

Direct experimental evidence supporting a role for *Hox* gene dysregulation in myeloid leukemia comes from several mouse models. Transplantation of bone marrow cells that overexpressed *Hoxa5*, *Hoxa9*, or *Hoxa10* consistently led to myeloid expansion, and, in collaboration with the *Hox* cofactor *Meis1*, AML.[57, 58] Transplantation of bone marrow cells that overexpressed *Nup98-Hox* fusions led to abnormal myeloid differentiation and AML, again in collaboration with *Meis1*.[66–68] Lastly, transgenic mice that expressed a *NUP98-HOXD13* fusion showed upregulation of *Hoxa7*, *Hoxa9*, and *Hoxa10*, and developed a myelodysplastic syndrome (MDS) that progressed to acute leukemia in about half of the mice.[69]

It seems likely that the *CALM-AF10* fusion gene, similar to *MLL* fusions, exerts its leukemic effect, at least in part, through upregulation of *HOX* genes. *HOXA* cluster genes, including *HOXA5*, *HOXA9*, and *HOXA10* were recently shown to be upregulated in the leukemic cells of patients with *CALM-AF10* fusions.[70] In addition, U937 cells (which express a *CALM-AF10* fusion) transduced with a siRNA directed to the *CALM-AF10* fusion showed a modest decrease in *HOXA5*, *HOXA7*, *HOXA9*, and *HOXA10* expression.[51] Consistent with those findings, transgenic mice that expressed a *CALM-AF10* transgene showed 8-fold or greater upregulation of *Hoxa5*, *Hoxa7*, *Hoxa10*, *Hoxa11*, and *Meis1* in bone marrow from clinically healthy *CALM-AF10* mice, and further upregulation (as much as 500-fold) of these genes in myeloid leukemia cells from *CALM-AF10* mice.[52]

Summary

CALM-AF10 fusions result from a rare but recurring t(10;11) chromosomal translocation seen in both adult and pediatric patients with AML or ALL, and are often associated with a poor prognosis. Recently, it has been shown that *CALM-AF10* fusions induce an acute leukemia in mice. Although the exact mechanism by which the *CALM-AF10* fusion exerts its leukemogenic effect on hematopoietic cells remains unknown, evolving evidence suggests that the *CALM-AF10* fusion leads to upregulation of specific *HOXA* cluster genes, and interferes with normal hematopoietic differentiation. In the context of a current working model for AML, in which complementary mutations that lead to either impaired differentiation or increased proliferation collaborate to produce a fully leukemic clone,[71] it seems likely that the *CALM-AF10* fusion functions to impair differentiation. The corollary of this hypothesis is that *CALM-AF10* leukemias should acquire spontaneous mutations leading to increased proliferation. The

challenge before us is to use this knowledge to develop more effective and less toxic therapies for patients with *CALM-AF10* fusions.

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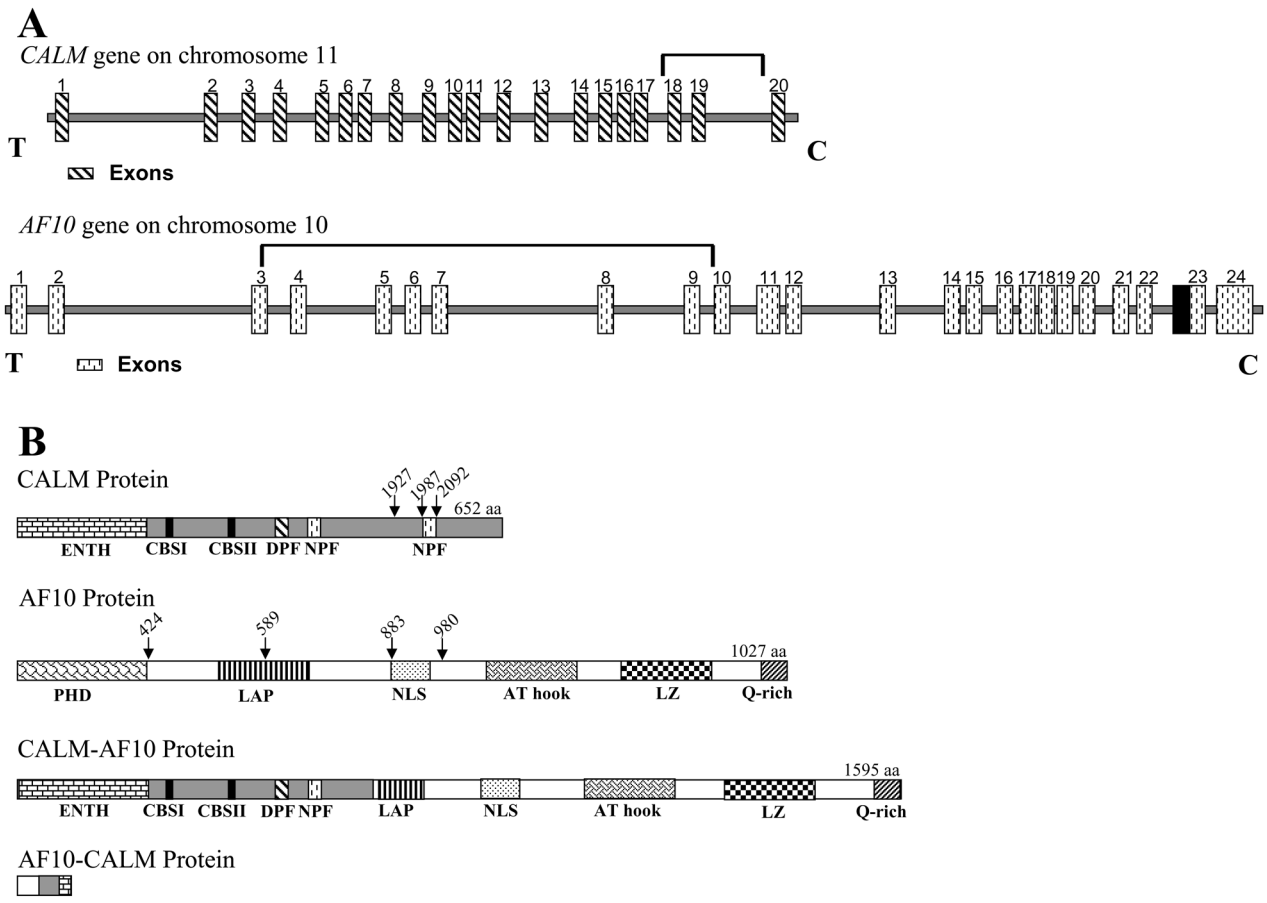

Figure 1. Structure of *CALM, AF10* and *CALM-AF10* fusions

(A) Schematic representation of the human *CALM* and *AF10* genes based on the reference sequences NM_007166 and U13948 respectively. *CALM* exons 1–20 are indicated by striped rectangles. The bracket spanning introns 18–19 indicate the genomic region where translocations are known to occur within *CALM*. *AF10* exons 1–24 are indicated by stippled rectangles. The bracket spanning introns 3–9 indicate the genomic region where translocations are known to occur within *AF10*. The black box immediately adjacent to exon 23 represents 124 bp of exon sequence this is present in most ESTs but missing from the reference sequence (U13948). Centromere (C) and telomere (T) orientation is as indicated. (B) Schematic representation of the predicted protein structure for CALM, AF10 and the two chimeric proteins CALM-AF10 and AF10-CALM. Protein domains are indicated in each structure: ENTH (espin N-terminal homology; Asp-Pro-Phe), DPF (ASP-Pro-Phe) motif, NPF (Asn-Pro-Phe) motif, CBS I & II (clathrin binding sequence I & II), PHD (plant homology domain), LAP (leukemia-associated protein), NLS (nuclear localization signal), LZ (leucine zipper), and Q (glutamine)-rich region. Vertical arrows indicate known nucleotide breakpoint regions.

Table 1

Characteristics of patients with the t(10:11) *CALM-AF10* translocation

Patient	Diagnosis	CALM-AF10 Breakpoint(s)	References
U937	Histiocytic Lymphoma	CALM 2091; AF10 423	Dreyling, 1996
M/19	AML-M1	CALM 1926; AF10 883, CALM 2091; AF10 883	Bohlander, 2000
M/47	AML-M0	CALM 1926; AF10 883	Bohlander, 2000
F/21	AML-M1	CALM 1926; AF10 589, CALM 2091; AF10 589	Bohlander, 2000
M/22	T-ALL	CALM 2091; AF10 589	Bohlander, 2000
F/16	pre T-ALL	CALM 1926; AF10 883	Bohlander, 2000
M/5	pre T-ALL	CALM 2091; AF10 979	Bohlander, 2000
1	ALL	CALM 1926; AF10 980	Kumon, 1999
2	ALL	CALM 2091; AF10 883	Kumon, 1999
3	ALL	CALM 1926; AF10 589	Kumon, 1999
4	AML-M0	CALM 2091; AF10 589	Kumon, 1999
M/12	AmoL	NR	Kobayashi, 1997
F/10	ALL	NR	Kobayashi, 1997
M/25	LBL	NR	Kobayashi, 1997
F/30	Granulocytic sarcoma	NR	Kobayashi, 1997
M/6	AML-M7	CALM 1926; AF10 424	Jones, 2001
F/10	Acute eosinophilic leukemia	NR	Salmon-Nguyen, 2000
F/17	T-ALL	NR	Salmon-Nguyen, 2000
M/16	AML-M4	CALM 1926; AF10 424	Nakamura, 2003
M/20	AML-M4	NR	Abdou, 2002
F/44	AML-M0	CALM 2091; AF10 424	Carlson, 2000
F/28	AML-M1	CALM 2091; AF10 883	Carlson, 2000
M/41	AML-M5	CALM 2091; AF10 979	Carlson, 2000
M/26	T-ALL	CALM 2091; AF10 979	Carlson, 2000
M/12	T-ALL	CALM 1926; AF10 589	Carlson, 2000
F/23	NHL Tcell LBL AML	NR	Carlson, 2000
F/4	AML-M7	CALM 2091; AF10 423	Abdelhaleem, 2007
M/2	AML-M7	CALM 2091; AF10 796	Abdelhaleem, 2007
M/33	T-ALL TCRγδ	AF10 424	Macintyre, 2003
M/29	T-ALL TCRγδ	AF10 589	Macintyre, 2003
M/20	T-ALL TCRγδ	AF10 883/979	Macintyre, 2003
M/15	T-ALL TCRγδ	AF10 589	Macintyre, 2003
F/11	T-ALL TCRγδ	AF10 589	Macintyre, 2003
M/7	T-ALL TCRγδ	NR	Macintyre, 2003
M/6	T-ALL TCRγδ	AF10 589	Macintyre, 2003
M/3	T-ALL TCRγδ	AF10 424	Macintyre, 2003
M/43	T-ALL IMγ	AF10 589	Macintyre, 2003
M/37	T-ALL IMγ	AF10 883/979	Macintyre, 2003
M/28	T-ALL IMγ	AF10 883/979	Macintyre, 2003
M/26	T-ALL IMγ	AF10 424	Macintyre, 2003
M/25	T-ALL IMγ	AF10 883/979	Macintyre, 2003
F/25	T-ALL IMδ	AF10 883/979	Macintyre, 2003
M/24	T-ALL IMγ	AF10 883/979	Macintyre, 2003
M/23	T-ALL IMγ	AF10 424	Macintyre, 2003
F/20	T-ALL IMγ	AF10 883/979	Macintyre, 2003
M/14	T-ALL IMδ	AF10 883/979	Macintyre, 2003
F/12	T-ALL IMδ	AF10 883/979	Macintyre, 2003
F/12	T-ALL IMγ	AF10 883/979	Macintyre, 2003
F/78	B-ALL	AF10 883/979	Macintyre, 2003
M/18	AUL	AF10 883/979	Macintyre, 2003
M/18	AUL	AF10 883/979	Macintyre, 2003
F/29	AUL	AF10 589	Macintyre, 2003
M/25	AML-M1	AF10 883/979	Macintyre, 2003
F/16	AML-M1	AF10 424	Macintyre, 2003
M/22	AML-M0	CALM 2091; AF10 883	Deshpande, 2007
F/33	AML-M2	CALM 1926; AF10 883	Deshpande, 2007
F/36	AML-M1	CALM 2091; AF10 978	Deshpande, 2007
M/39	AUL	CALM 2091; AF10 424	Deshpande, 2007
M/19	AML-M1	CALM 2091; AF10 424	Deshpande, 2007
M/47	AML-M1	CALM 2091; AF10 424	Deshpande, 2007
F/12	AML-M1	CALM 2091; AF10 424	Deshpande, 2007
F/19	AML	CALM 2091; AF10 1048	Deshpande, 2007
F/4	AML-M5a	NR	Deshpande, 2007
F/12	AML-M1	CALM 2091; AF10 424	Starza, 2006
M/13	T-ALL	CALM 2091; AF10 424	Starza, 2006
M/36	AML-M2	NR	Starza, 2006
M/27	AML-M0	CALM 2091; AF10 424	Starza, 2006
F/38	T-ALL	CALM 2091; AF10 589	Starza, 2006
M/47	AML-M1	CALM 2091; AF10 424	Starza, 2006
M/19	AML-M1	NR	Starza, 2006

Patient	Diagnosis	CALM-AF10 Breakpoint(s)	References

NR; not reported

Table 2

Summary of *CALM-AF10* Mouse Models

Model	Mouse Strain	Disease	Survival Median (range)	Immunophenotype Markers	Tissue/ Organ Involvement	Antigen Receptor Gene Rearrangement	Serial Transplantation	Reference
Xenograft of siRNA clones	NOD/SCID	AML	19.5 d (16–23d) control 27d (22–33d) siRNA clones	CD45	Spleen, Kidney, Pancreas	not applicable	nd	Okada, 2006
Retroviral infection and bone marrow transplantation	C57Bl/ 6xC3H	AML	110 d (46–366d)	Mac1++, Gr1++, B220+/−, MPO++	Bone Marrow, Spleen, Liver, Lung, Kidney, Brain, Intestine	*Igh*	yes	Deshpande, 2006
Transgenic	FVB	AML	12 mo (6–18 mo)	Mac1++, Gr1++, B220 +/−, MPO++, CD24+/−, CD117+/ −, CD3 rare +, F4/80 rare +	Bone Marrow, Spleen, Liver, Lung, Kidney, Brain, Lymph node	*Igh; Tcrb; Tcrd*	nd	Caudell, 2007

nd is not done