CARMA1 is a novel regulator of T-ALL disease and leukemic cell migration to the CNS

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Pediatric acute lymphoblastic leukemia (ALL) is the most common type of malignancy in children with 15% bearing markers for the T-lineage (T-ALL) and 20% of T-ALL patients still suffering disease relapse. Many genes are implicated in T-ALL disease, including Notch, and Notch mutations have been identified in a majority of T-ALL patients. Patients with T-ALL relapse with disease usually in the central nervous system (CNS). Despite the use of intrathecal therapy and cranial radiation, successful management of CNS disease continues to be problematic in T-ALL. Few molecules are known to be associated with T-ALL CNS disease including CCR7, a chemokine receptor normally expressed on naive T cells. CXCR4 has also recently been shown to have a role in T-ALL initiation and progression. The involvement of chemokine receptors as well as other molecules regulating cell motility suggests that regulation of T-ALL cell migration is likely to have an important role in T-ALL.

Cell signaling molecules that drive normal T-cell proliferation, such as PKCθ, can regulate T-ALL. PKCθ activates CARMA1 (also known as CARD11) bringing together Bcl10 and MALT1 leading to B-cell activation. Although higher expression of CARMA1 has been seen in a small subset of T-ALL patients, little is known about how CARMA1 might regulate T-ALL. To study the potential role of CARMA1 in T-ALL disease, we used shRNA to knock down CARMA1 expression in the human CEM T-ALL cell line expressing luciferase and GFP (called CARMA1KD), with the level of knockdown ~ 50% (Supplementary Figure 1A). We transferred the CARMA1KD CEM cells into the immunodeficient NOD.Cg-Prkdcsid Il2rgtm1Wjl/Siz, IL2RG (NSG) mice. Animals receiving CARMA1KD T-ALL cells survived significantly longer than animals receiving parental CEM cells (Figure 1a). We also used a Notch1 ΔE-induced mouse T-ALL model in which hematopoietic precursors from WT C57Bl/6 (called WT/WT; Notch1 ΔE; labeled WT) or CARMA1−/− (WT/CARMA1−/−; Notch1 ΔE; labeled CARMA1KO) animals were transduced with Notch1 ΔE-GFP and develop T-ALL. Like the xenograft model, we found that WT/CARMA1−/− (Notch1 ΔE) CARMA1KO animals survived significantly longer than WT/WT (Notch1 ΔE; WT; Figure 1b).

We then asked whether CARMA1 is also involved in human T-ALL disease. Using microarray analyses from patient T-ALL cells, we compared CARMA1 expression in bone marrow (BM) cells from T-ALL patients with BM cells from a non-leukemia control patient. CARMA1 expression was significantly higher in the T-ALL patients compared with non-leukemic cells (Figure 1c) and other tumor types including lung, liver and breast (Figure 1d).

These results, along with the animal models, demonstrate that CARMA1 is an important regulator of T-ALL disease.

We assessed proliferation of CARMA1KD cells compared with parental CEM T-ALL cells. Surprisingly, we found that CARMA1KD showed no difference in basal in vitro proliferation measured by cell growth in culture (Supplementary Figure 1B) or baseline T-ALL cell apoptosis measured by Annexin V staining (Supplementary Figure 1C) compared with parental CEM T-ALL cells. We also assessed tumor growth using in vivo bioluminescent luciferase imaging of CEM cells. Animals that received parental CEM cells showed a higher T-ALL tumor load than animals receiving CARMA1KD CEM cells at weeks 1, 2 and 3 (Supplementary Figures 2A and B). We examined T-ALL cell accumulation in specific tissues using anti-human CD45 and GFP expression to identify CEM cells by flow cytometry. CARMA1KD CEM cells were significantly decreased in the liver, but we saw no difference in the BM or in spleens between parental and CARMA1KD CEMs (Figure 1e). Using the NOTCH-induced T-ALL model, we found that CARMA1−/− T-ALL cells accumulated less in lymph node, but not in BM, spleen or liver (Supplementary Figure 2C).

Our previous results suggested CARMA1 may regulate differential migration patterns to specific organs. We used the transwell migration assay to compare the migration of parental and CARMA1KD CEM cells in vitro. We assessed T-ALL cell migration toward CCL21, the ligand for CCR7 and found that CARMA1KD CEM cells showed a significant decrease in migration to CCL21 and LFA-1 ligand ICAM1 compared with parental CEM cells (Figure 2a). CCR7 has been shown to regulate T-ALL migration specifically to the CNS. Using the CEM xenograft model, we could detect the presence of parental CEM cells in both the calvarial BM (indicated by *) as well as the meninges of the CNS (arrows, Figure 2b). In contrast, animals receiving CARMA1KD CEM cells showed little to no accumulation in the meninges, but still showed accumulation in the BM. We measured the total area of meningeal T-ALL infiltration in four coronal sections per mouse and parental CEM cells showed higher levels of cell infiltration compared with CARMA1KD CEM cells (Supplementary Table 1A). We also analyzed CNS infiltration of T-ALL cells in the NOTCH model of T-ALL and found similar results to the xenograft model (Supplementary Figure 3, Supplementary Table 1B). These data show that CARMA1 expression in T-ALL cells leads to an increase in T-ALL cell infiltration into the CNS.

We then evaluated CARMA1 expression in a cohort of T-ALL patients who initially presented with T-ALL cells in the CNS versus...
patients who showed no CNS disease at time of diagnosis. T-ALL patients with CNS involvement showed significantly higher expression of both CARMA1 probes in the microarray compared with patients with no CNS involvement (Figure 2c). These results suggest that CARMA1 may specifically regulate T-ALL cell migration to the CNS.

In this study, we show that CARMA1 is a novel regulator of T-ALL disease in animal models as well as data from T-ALL patients. CARMA1 expression is associated with increased T-ALL disease severity as well as leukemia cell accumulation in the CNS. Many studies, including whole-genome studies, have identified novel regulators of T-ALL disease. A previous study showed that CARMA1 is upregulated in a small group of T-ALL patients but did not find a specific role for CARMA1 in CNS disease. Only a few molecules including chemokine receptors CCR7 and CXCR4 have been specifically shown to be involved in CNS disease in T-ALL. We find that animals with CARMA1−/− T-ALL show similar survival advantage to CCR7−/− T-ALL (Supplementary Figure 4). We also find that CARMA1 colocalizes with CCR7 (Supplementary Figure 5) and CARMA1KD cells show decreased migration to the CCR7 ligand CCL21 in vitro. Although some of our results suggest that CARMA1 affects migration of T-ALL cells directly downstream of CCR7, it is also possible that CARMA1 and CCR7 independently regulate T-ALL cell migration. In T cells, CXCR4 has also been
colocalized with ZAP70, suggesting that cross talk between chemokine receptors and intracellular signaling pathways such as CCR7 and CARMA1 may act both in normal and leukemic cells to regulate cell migration.13

In normal T cells, TCR activation activates PKCθ phosphorylation of CARMA1, leading to NF-κB activation.9 We previously showed that PKCθ and CARMA1 regulate naive T-cell migration14,15 and PKCθ has been shown to have a role in T-ALL.8 We find that animals receiving PKCθKD CEM cells show similar survival to those receiving parental CEM cells (Supplementary Figures 6A and B). This suggests that unlike naive T cells, in which PKCθ activates CARMA1, T-ALL cells may activate CARMA1 independently of PKCθ. CARMA1 may be aberrantly upregulated in T-ALL, leading to disease progression and migration of leukemic cells to the CNS. In the future, inhibitors of CARMA1 or pathways that lead to CARMA1 activation may prove therapeutically useful in controlling CNS disease in T-ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Expansion of Th1-like Vγ9Vδ2 T cells by new-generation IMiDs, lenalidomide and pomalidomide, in combination with zoledronic acid

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Multiple myeloma (MM) still remains incurable even with the implementation of novel therapeutic modalities, leading to the idea to develop various forms of immunotherapies. One such approach is the application of γδ T cells. Similar to NK cells, γδ T cells are important effectors in the first-line defense against infections and tumors, and have a critical role in host defense and tumor surveillance.1–3 In human, γδ T cells are divided into two predominant subpopulations by T-cell receptor V gene usage, namely Vβ7, Vβ7 cells and Vγ9Vδ2 γδ T cells. Vδ2 T cells

Figure 1. γδ2 T-cell expansion and activation by Zol in combination with new-generation IMiDs. (a, b) γδ2 T-cell expansion by LEN and Zol in combination. PBMCs isolated from a normal donor (# 1) were cultured for 1 week alone or in the presence of Zol at 1 μM with or without LEN at 1 μM or IL-2 at 100 IU/ml as indicated (a). Then, the cells were analyzed by flow cytometry to determine a distribution of γδ2 T cells. Vδ2-positive and CD3-positive cells accumulated in the right upper columns represent γδ2 T cells. PBMCs isolated from additional six normal donors (# 2–7) (left) or 6 MM patients (right) were cultured for 1 week alone or with Zol at 1 μM in combination with either LEN at 1 μM or IL-2 at 100 IU/ml, followed by flow cytometric analysis to determine the expansion of γδ2 T cells (b). Results are expressed as a % distribution of γδ2 T cells within CD3-positive cells. (c) Induction of γδ2-like γδ T cells by LEN and Zol in combination. γδ T cells expanded by Zol in combination with LEN were stained by PE-labeled anti-Vδ2 monoclonal antibody together with FITC-labeled monoclonal antibodies for the indicated molecules. Intracellular levels of IFN-γ and Foxp3 in γδ T cells are indicated in red lines (upper). Surface levels of NKG2D and DNM-1 and intracellular levels of perforin and granzyme B in γδ T cells are shown in red lines (lower). Baseline expression of these molecules in γδ T cells without treatment with LEN plus Zol was indicated by blue lines. Gray areas indicate background staining with IgG isotype controls. (d) The cytotoxic activity against MM cells of γδ2 T cells expanded with LEN and Zol in combination. PBMCs from a normal donor were cultured for 1 week with LEN and Zol in combination. After expansion, whole PBMCs were used as effectors and added at the indicated effector to target (E:T) ratios and co-cultured in triplicate with PKH26-lebeled MM cell lines (BM1, RPMI8226; □, KMS-11; ▣, U266) (upper), and PKH26-lebeled primary MM cells (lower). The cells were then stained with 7-aminoactinomycin D (7-AAD) to detect dead cells within the PKH26-lebeled MM cells by flow cytometry. Results are expressed as a % distribution of dead cells within MM cells. (e) γδ2 T-cell expansion by Zol in combination with LEN or POM. PBMCs isolated from normal donors (# 8–17) were cultured for 1 week alone or upon treatment as indicated, followed by flow cytometric analysis to determine the expansion of Vδ2 γδ T cells. Zol, LEN, POM and IL-2 were added at 1, 1, 0.1 μM and 100 IU/ml, respectively. Results are expressed as a % distribution of γδ T cells in CD3-positive cells.

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