Acute lymphoblastic leukemia of the central nervous system: on the role of PBX1

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Central nervous system (CNS) infiltration is rarely detected at initial diagnosis of pediatric acute lymphoblastic leukemia (ALL). This is mainly due to a lack of sensitivity of cerebrospinal fluid (CSF) diagnostics by cytology. Currently, all patients receive extensive CNS-directed chemotherapy regardless of the presence of leukemic cells in the CSF, which is associated with a number of neurological toxicities. Some high-risk patients also receive cranial irradiation, which carries an increased risk for secondary malignancies. On the other hand, omitting this type of CNS-directed therapy will lead to CNS relapses in a vast majority of the patients[1] suggesting that CNS tropism is an omnipresent feature in ALL blasts. Even though the prognosis of pediatric ALL has been continuously improving on contemporary treatment protocols, the CNS compartment is affected in roughly one-third of ALL relapses.[2] Importantly, CNS involvement at relapse occurs mainly in patients who were CNS negative at initial diagnosis.[3] This suggests that ALL cells that are refractory to therapy or particularly receptive to microenvironment-derived protective signals are able to survive in the CNS niche for prolonged periods of time as extramedullary minimal residual disease. In order to solve the mechanistic problem of CNS disease in ALL, factors influencing the homing and the survival of leukemic cells in this protective sanctuary are increasingly being investigated.

In this issue of *Haematologica*, Gaynes *et al.* report a set of CNS-related genes identified by a screening approach in NALM-6 B-cell precursor (BCP)-ALL cells recovered from the bone marrow and CNS compartments of NOD/SCID-gamma (NSG) mice.[4] They further investigated the role of the homeobox gene PBX1, one of the genes up-regulated in the CNS by using BCP-ALL cell lines in a co-culture model of the blood brain barrier (BBB) and in xenografts. They found PBX1 protein levels to be up-regulated in different BCP-ALL cell lines in co-culture with murine choroid plexus cells and in the CNS of xenografts. In co-culture, PBX1 conferred chemotherapy resistance to cytarabine and methotrexate, both of which are important drugs for CNS-directed therapy in ALL. Targeting PBX1 by RNA interference decreased the protective effects induced in this co-culture model pointing at a role of PBX1 as a niche-specific survival factor. As expected, ectopic expression of PBX1 enhanced the colony-forming ability of ALL cell lines *in vitro* and, importantly, CNS infiltration *in vivo*.

PBX1 is known to support long-term self-renewal abilities in hematopoietic stem cells and, as a translocation partner for E2A in t(1;19) positive BCP-ALL, it leads to an arrest of B cells at the pro-/pre-B-stage and is also able to enhance self-renewal capacity in pre-leukemic B-cell progenitors.[5] It has been previously shown that enforced expression of PBX1 has no transforming potential in NIH3T3 cells.[6] In contrast, the oncogenic potential of the E2A-PBX1 fusion has been demonstrated in a variety of experimental models.[5,7] Interestingly, E2A-PBX1 rearranged BCP-ALL has a particular propensity to enter the CNS.[8] The E2A-PBX1 fusion includes almost the entire coding sequence of PBX1, is predominantly localized in the nucleus, and acts as a constitutive transcriptional activator.[9] E2A-PBX1 binds a subset of targets also bound by PBX1, raising the possibility that both regulate similar genes, which may also be relevant for CNS disease in ALL. The TAM-receptor MER is over-expressed in E2A-PBX1 rearranged BCP-ALL and has been found to mediate a quiescent and chemo-resistant phenotype in leukemic cells in the CNS of pediatric t(1;19) positive patients.[10] There is, however, no evidence for a direct regulation of MER by either E2A-PBX1 or PBX1. In contrast, E2A-PBX1 was found to induce pre-B-cell receptor (pre-BCR) signaling by activating the transcription of the pre-BCR kinases SYK, LCK and ZAP70 in a subset of murine leukemias in a transgenic E2A-PBX1 BCP-ALL mouse model[5,11] and in human BCP-ALL.[12] The E2A-PBX1 transgene caused the acquisition of a number of secondary activating mutations, most notably in the JAK/STAT and RAS pathways, suggesting that E2A-PBX1 induces genomic instability.[5] Interestingly, activated JAK/STAT signaling and constitutive activation of the RAF/RAS/MEK/ERK pathway may be distinguishing features of certain leukemias invading the CNS: interleukin-15, shown to be predictive of CNS involvement and relapse in patients,[13] induces both ERK1/2 and STAT5, thereby up-regulating leukocyte trafficking molecules.[14] Activating mutations in the RAS pathway have been found to be highly prevalent in relapsing childhood ALL and led to CNS infiltration in xenografts which could be abrogated by MEK1/2 inhibition.[15] Recently, ZAP70 kinase has been associated with CNS disease in ALL, in part *via* ERK1/2-mediated activation of the chemokine receptors CCR7 and CXCR4.[16] A summary of the signaling pathways induced in E2A-PBX1 positive BCP-ALL and their potential role in CNS leukemia is presented in Figure 1. Whether CNS leukemogenesis by PBX1 without the E2A-PBX1 rearrangement is based on similar molecular mechanisms remains to be investigated.

It has recently been suggested that CNS infiltration is a universal feature of leukemic blasts based on pre-clinical experimental data.[17] While it is highly possible that the majority of ALL blasts are able to cross the BBB and home to the CNS, pre-clinical and clinical data suggest that ALL sub-clones expressing niche-specific survival factors may be more relevant from a mechanistic point of view as these cells may be quiescent and chemotherapy-resistant (Figure 2, model 1). On the other hand, it is also possible that only ALL cells expressing specific homing markers can enter the CNS compartment and that they activate pro-survival signaling in that niche in a second step (Figure 2, model 2). Whether PBX1-mediated CNS leukemia reflects the first or the latter mechanism remains to be elucidated using further *in vivo* modeling, primary patient samples, and clinical cohorts.

The work of Gaynes *et al.*, together with our own work and that of others, illustrates a number of challenges in the field of CNS leukemia research.

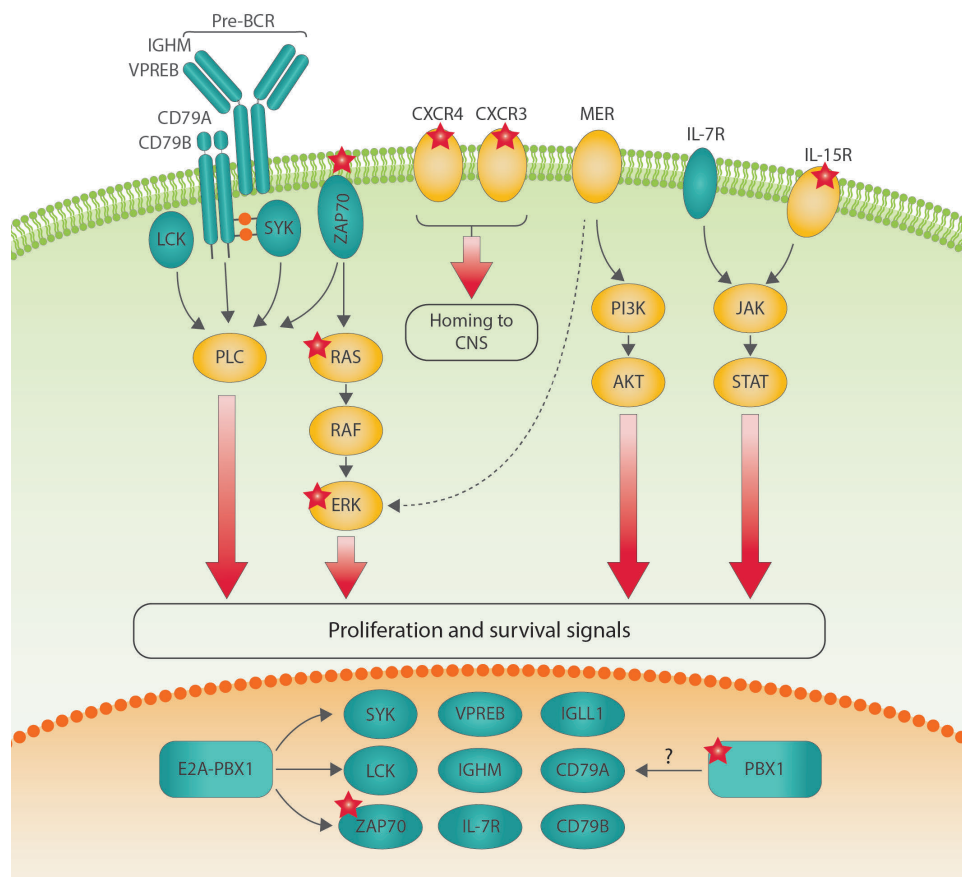First, there have been attempts to identify target genes in

Figure 1. Surface receptors and signaling pathways involved in central nervous system (CNS) leukemia. E2A-PBX1 transcriptional targets are depicted in green. Many of the E2A-PBX1 target genes are important for pre-BCR signaling, which has also been connected to CNS leukemia. Molecules for which there is direct evidence for a role in CNS leukemia are illustrated with red stars. The overlap between genes regulated by E2A-PBX1 and by PBX1 alone remains unclear.
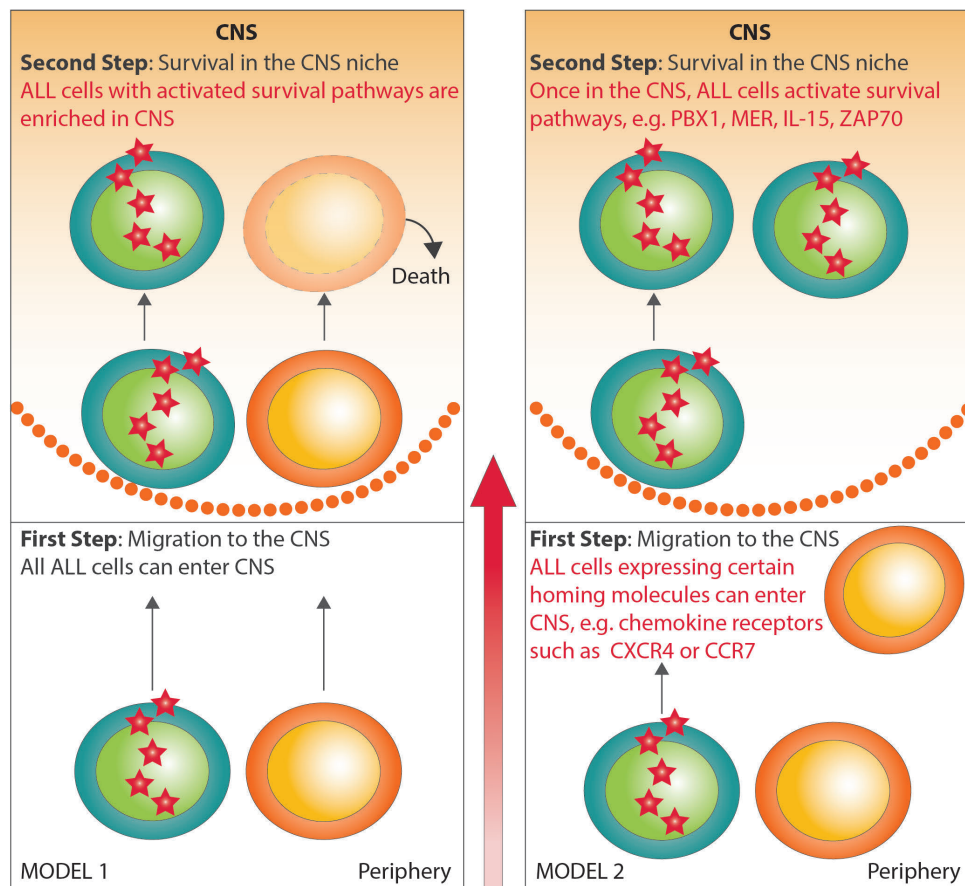

Figure 2. Models of central nervous system (CNS) leukemia. In model 1, every acute lymphoblastic leukemia (ALL) cell has the ability to migrate to the CNS, but only cells with up-regulated survival pathways cause CNS leukemia. In model 2, ALL cells with up-regulated homing markers (eg. chemokine receptors) can migrate to the CNS. In a second step, they activate the survival pathways necessary to maintain CNS disease. It is essential to clarify which model is true, as this has important implications for CNS-directed prophylaxis and therapy in ALL.

blasts directly isolated from patient CSF which could confirm, among others, JAK/STAT pathway[18,19] as well as ERK and BCR activation.[19] Due to limited availability of material and restricted cell integrity in primary patient CSF, model systems will increasingly be needed. Co-culture models of the BBB naturally do not reflect the complexity of its composition, and the leukemic BBB may be entirely different from a healthy one. Clearly, immunodeficiency of xenograft mice can affect the modeling of CNS leukemia[20] and high degrees of immunodeficiency may favor heavy ALL infiltration in all target organs, including the CNS. Also, modeling of the leukemic CNS compartment in xenografts may have to take place during chemotherapy as it remains completely unclear at which time point before, during or after therapy initiation CNS infiltration occurs in patients.

Second, the functions of novel biomarkers indicating CNS leukemia are sometimes not easy to distinguish. It has been shown that chemokine receptors are responsible for the homing of mainly T-ALL cells into the CNS,[16,21] and this is also possible, although a lot less clear, in BCP-ALL. In fact, similarly to the situation for PBX1 in the current study, it is often obscure as to whether a parameter influences the homing and/or the survival of ALL cells in the CNS niche or both.

Third, parameters identified in a pre-clinical model need to be confirmed in clinical patient series. Due to the rare detection of CNS infiltration at diagnosis, patient numbers are often too small to be able to come to any valid conclusions on a specific marker. Even patient cohorts of large study groups are biased due to selection of CNS positive patients. It is important that a CNS biomarker reliably diagnoses or excludes CNS infiltration. It must also be very clear if a novel biomarker indicates CNS infiltration at initial diagnosis or if it is predictive for CNS relapse. Both scenarios may be fundamentally different regarding their mechanism.

A fourth and final point is that diagnostic strategies for the detection of CNS infiltration need to be refined independently of novel biomarkers. Microscopic examinations of CSF samples are the conventional gold standard; however, novel techniques such as flow cytometry in the CSF and genetic methods for the detection of minimal residual disease in the CNS compartment need to be thoroughly evaluated.

Taken together this work provides important insights into the biology of CNS infiltration in ALL which is important in the context of the previous findings on CNS leukemia of the past few years. PBX1 is established as a novel mechanism of survival and chemotherapy resistance for ALL in the CNS compartment independent of the E2A-PBX1 translocation but with important implications for this entity. Work like this is fundamental in order to unravel the molecular mechanisms of CNS leukemia to ultimately identify two patient subgroups: 1) patients not needing such an intensive CNS-directed therapy, thereby avoiding significant toxicity; and 2) patients with ALL cells prone to survive in the CNS. These are patients who may benefit from intensive CNS-directed therapy and novel, more specific, approaches in the future.

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