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SPECIAL REPORT

Insights into the pathophysiology and therapy of myeloproliferative neoplasms from mouse models

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CML (chronic myeloid leukemia) is genetically simple cancer, having a very few point mutations or copy number variations in its early chronic phase genome. Therapies using ABL1 tyrosine kinase inhibitors (TKIs) have dramatically altered the prognosis in CML. The major goal of current molecular research on CML is to understand TKI resistance and disease progression. CML mouse models can provide information and insights into the pathophysiology of this disease that are difficult or impossible to obtain when one is limited to working with human cells. There are two widely used mouse models of CML: binary conditional BCR-ABL1 transgenic mice[1,2] and the retroviral bone marrow transplant (BMT) model of CML.[3–5] In these models, BCR-ABL1-induced CML-like myeloproliferative neoplasm originates from stem/progenitor cells with multi-lineage repopulating activity.[5] This neoplasm is transplantable and can progress to blast crisis;[5,6] it is responsive to kinase inhibitors and immunological therapy.[7,8] In this summary, I will focus on three examples where mouse model systems have revealed crucial details about the pathophysiology of CML.

Role of STAT5 in CML

STAT5 is a transcription factor encoded by two closely linked genes in mammals, *STAT5a* and *STAT5b*. STAT5 is phosphorylated by JAK family kinases during cytokine and growth factor signaling, and is activated in malignant cells from patients with a wide variety of lymphoid and myeloid malignancies including CML.[9] Conditional *Stat5* null mutant mice are perinatal lethal due to severe anemia and lung abnormalities,[10] and are also characterized by a lack of CD8+ and γ/δ T cells, a profound block in early B-lymphoid development,[11] and a normal phenotypic hematopoietic stem cell (HSC) frequency but severe lymphoid and moderate myeloid repopulation defects.[12,13] Upon BM-specific deletion of *Stat5a/b* using Mx-Cre;*Stat5a/b*$^{fl/-}$ donor animals, we observed abolishment of detectable BCR-ABL1 leukemia. Notably, however, BCR-ABL1 signaling was maintained in *Stat5* null HSC, indicating that these cells were unable to drive the massive expansion of myeloid cells that would have resulted in the development of CML in the absence of STAT5 signaling.[14] In serial transplant experiments with BCR-ABL1$^+$*Stat5*$^{-/-}$ BM cells, recipient animals engrafted and subsequently developed B-cell lymphoblastic leukemia that arose from the transplanted BCR-ABL1$^+$*Stat5*$^{-/-}$ stem cells. These data suggest that STAT5 is required for the pathogenesis of CML; however, STAT5 deficiency does not eliminate BCR-ABL1$^+$ leukemic stem cells in mice, nor prevent disease progression.

Targeting the niche in CML

It is known that human osteoblasts can support hematopoiesis in *ex vivo* culture systems,[15] and acute deletion of osteoblastic cells results in abnormal hematopoiesis.[16] It was also found that osteopontin, an acidic glycoprotein synthesized by preosteoblasts, osteoblasts and osteocytes, which is an important component in the formation of bone, negatively regulates stem cell pool size.[17] Osteoblasts form one part of the niche and are responsive to parathyroid hormone (PTH) signaling. We have utilized an animal model expressing constitutively active receptor for PTH and PTH-related peptide (PTHRP) under the Collα promoter.[18] Coll-caPPR (PPR) mice have greatly increased trabecular bone turnover and numbers and functionality of HSCs in these animals are increased. When PPR mice were used as the recipients for transplantation with BCR-ABL1-transduced HSCs, we observed prolonged latency of CML-like myeloproliferative disease, and reduced frequency and cycling status of BCR-ABL1$^+$ progenitor cells.[19] In secondary transplantation of CML, in which BM cells from wild-type saline- or PTH-treated primary donor mice were transplanted in limiting dilution, we confirmed that CML-initiating cells were reduced 15-fold in a PTH-treated BM environment. We also found that transforming growth factor beta 1 (TGFβ1) was increased in Col1-caPPR BM, and knockdown of TGFβRI in transplanted BCR-ABL1$^+$ BM 'rescued' CML-like disease in Coll-caPPR recipients. We, therefore, proposed that PTH, through bone remodeling and pharmacological levels of TGFβ1, negatively regulates the leukemic stem cell compartment but does not have an effect on normal stem cells. These results demonstrate for the first time that the niche differs between normal and CML stem cells, and suggests that modulating the niche is a novel approach to eliminate CML stem cells, leading to disease eradication.

Role of Ikaros in pathogenesis and therapy of CML lymphoid blast crisis and Ph$^+$ B-ALL

In B-cell acute lymphoblastic leukemia (B-ALL), mutations in transcription factors regulating B-lymphoid development (PAX5, E2A, EBF1, IKZF1) are frequent.[20] Deletions and dominant-negative mutations in IKZF1 (Ikaros) are extremely frequent (~85%) in Ph$^+$ B-ALL,[21] and are indicators of poor prognosis.[22] 'Ph-like' B-ALL is characterized by a gene expression profile similar to Ph$^+$ B-ALL and is accompanied by frequent mutational activation of Janus kinase 2 (JAK2), Abelson kinase 1 (ABL1), platelet-derived growth factor receptor beta (PDGFRβ) and erythropoietin receptor (EPOR).[23] The Ikaros gene (*IKZF1*) encodes a family of hemopoietic-specific zinc-finger proteins, and is a central

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regulator of lymphocyte differentiation. *IKZF1* is required for pre-B-cell differentiation,[24] and conditional inactivation of its DNA-binding domain in early pre-B cells (IkE5$^{\Delta/\Delta}$ mice) resulted in the expansion of large pre-B cells without disruption of BM myeloid/erythroid functions. IkE5$^{\Delta/\Delta}$ pre-B cells were found to be highly adherent to stroma, but remained proliferative with active B-cell proliferation signaling. Importantly, integrin signaling and integrin-FAK signaling in adherent cells were upregulated, and IkE5$^{\Delta/\Delta}$ pre-B cells progressed to lymphoblastic leukemia. We also found that BCR-ABL1 cooperates with *IKZF1* mutation, and IkE5$^{\Delta/\Delta}$ BCR-ABL1^{+} ALLs have stromal resistance to imatinib but are sensitive to a FAK inhibitor. We conclude that Ikaros mutations result in accumulation of non-malignant large pre-B cells that, with a secondary event (either mutated tyrosine kinase or some dysregulated downstream pathway), transform into a stromal-adherent mutant B cell that has active BCR-ABL1 signaling and is resistant, in this case, to imatinib mesylate. This suggests a new model of high-risk precursor B-lymphoid leukemia characterized by *IKZF1* mutation, stromal adhesion and chemotherapy resistance, and validates FAK as a new target for therapy in this aggressive disease.

CONFLICT OF INTEREST

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