SPECIAL REPORT

Myeloid differentiation and the leukemia-initiating cell

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Acute myeloblastic leukemia (AML) is defined by a block in the differentiation of multipotent proliferating progenitor cells into mature granulocytes. C/EBPα (CCAAT enhancer binding protein alpha) is a basic leucine zipper transcription factor and one of the central factors controlling differentiation. C/EBPα is required for adult hematopoietic stem cell (HSC) quiescence, and low levels of C/EBPα lead to enhanced HSC function. C/EBPα is a crucial regulator of granulopoiesis, and its absence, as shown in C/EBPα knockout (KO) mice, results in a block in the differentiation of common myeloid progenitor cells into granulocyte–macrophage progenitor cells accompanied by an absence of mature granulocytes. C/EBPα induces cell cycle arrest and has the capacity to inhibit proliferation by inducing p21, inhibiting cyclin-dependent kinases, mediating Calpain cleavage of cyclin A, interacting with and inhibiting E2F, and downregulating C-myc. We have shown that *CEBPA* is mutated in 16% of AML-M2 patients. Mutations that occur at the N-terminus result in a frameshift and loss of the full-length 42-kDa C/EBPα protein. A nonfunctional 30-kDa C/EBPα form that is initiated downstream of the frameshift is still produced. The N-terminal mutation acts as a dominant negative inhibitor of full-length *CEBPA*.¹ To generate a mouse model that recapitulates the dominant negative function of C/EBPα, which results in AML, a combination of C-terminal mutation and N-terminal mutation was used (C/EBPα K/L).² The central question in the molecular etiology of AML is: which cell is transformed in AML, HSC or committed progenitor? Is myeloid differentiation required for the formation of the 'leukemic stem cell' in AML? C/EBPα KOs mimic human AML and although the mice have, by definition, human AML (blasts in bone marrow and blood), the mice do not develop a malignant disease.^{3–5} It is known that C/EBPα suppresses targets such as Bmi-1 and N-myc.^{3–5} Our efforts focused on identifying other targets that may contribute to the phenotype. A short hairpin RNA (shRNA) screen done on Lin – kit +Sca1+ (LSK) cells isolated from C/EBPα KO bone marrow identified Sox4 as a C/EBPα target-mediating serial replating ('self-renewal') and inhibiting differentiation. Sox4 is a member of the SOX transcription factor family with a highly conserved high mobility group domain. Sox4 is a frequent target of insertional mutagenesis in retrovirally induced myeloid leukemias. Sox4 is also required for self-renewal and transformation pathways during leukemic evolution of multiple murine AML models (MOZ-TIF2, AML1-ETO and NUP98-HOXA9).^{6–9} It is known that Sox4 expression is reciprocal to that of C/EBPα in wild-type mice, and C/EBPα binds to the Sox4 promoter directly to represses its expression (like Bmi-1 and N-myc). Therefore, we were interested in comparing the phenotypes of C/EBPα and Sox4 KOs to better understand the relationship between these two transcriptional factors in the context of myeloid differentiation. We have

observed that Sox4 was upregulated in C/EBPα KO LSK and that Sox4 KO rescued myeloid progenitor expansion and increased proliferation of C/EBPα KO LSK (and rescued suppression of Bmi1 and N-myc). Importantly, in humans, enhanced SOX4 levels are observed in AML patients with dysfunctional *CEBPA*.¹⁰ Moreover SOX4 knockdown was confirmed to rescue myeloid differentiation in human *CEBPA*-mutant AML, and human C/EBPα mutants derived from patients did not repress Sox4 RNA levels in C/EBPα KO LSK and did not repress Sox4 promoter activity.¹¹ In summary, our studies showed that Sox4 mediates serial replating ('self-renewal') of both C/EBPα KO LSK and C/EBPα-mutant AML (K/L). In addition, Sox4 is upregulated in human primary blasts with C/EBPα mutation (modeled by K/L) or silenced (modeled by C/EBPα KO). shRNA downregulation of Sox4 induces differentiation of human primary blasts with C/EBPα dysfunction.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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