Transcription program differentiates AML cells, halts proliferation

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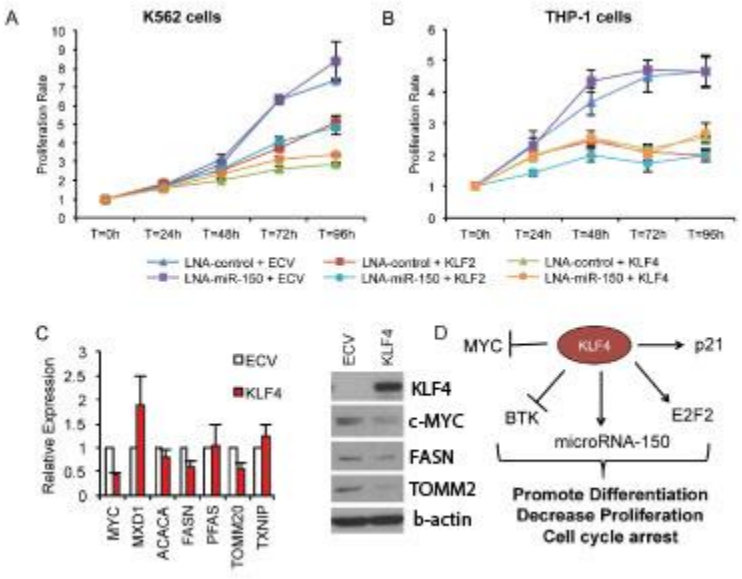
J Herman



KLF4 decreases leukemia cell proliferation by regulating transcription of multiple gene targets. KLF2 and KLF4 expression slows the proliferation rate in both (A) CML and (B) AML cell lines, and is not reversed by miR-150 inhibition. (C) KLF4 expression reduces MYC expression, alters expression of MYC targets associated with metabolism, and stimulates MXD1 transcription, a combination that is known to limit proliferation. (D) Schematic of a gene network regulated by KLF4 to limit proliferation and promote differentiation.

Image provided by Dr. Valerie Morris

In acute myeloid leukemia (AML) myeloid progenitor/stem cells fail to differentiate and instead remain in an immature and hyper-proliferative state. Traditional therapies used to treat AML and other types of cancer have been cytotoxic agents that induce cell death or apoptosis. As our understanding of these malignancies grows new therapeutic approaches become viable. In the case of AML, researchers are also exploring the possibility of differentiation therapy. In this approach leukemic cells are forced to differentiate into a more mature cell type, essentially converting the proliferating leukemia cells into a benign cell with a limited life span. Central to the differentiation process are transcription factors, which have been well studied in AML. Of recent interest has been the family of Krüppel-like factors (KLF). In normal hematopoiesis KLF2 and KLF4 drive myeloid cell differentiation and limit proliferation by increasing expression of the tumor suppressor and cyclin-dependent kinase inhibitor p21. Moreover, it was recently shown in a small number of AML patient samples that KLF4 expression is silenced. In a study from the Oehler Laboratory (Clinical Research Division) published in *Molecular and Cellular Biology*, researchers elucidated a network of microRNA and proteins downstream of KLF4 that regulate proliferation and differentiation in AML cells.

First, the scientists confirmed that KLF4 expression is commonly decreased in AML cells using publicly available microarray data sets of AML patient samples. KLF4 expression was significantly reduced in most AML samples compared to $CD34^+$ hematopoietic progenitors from healthy patients, though patient-to-patient heterogeneity was common. Researchers further verified that KLF4 and to a lesser extent KLF2 expression induced differentiation and reduced proliferation of the AML patient derived cell line THP-1. Surprisingly, gene expression changes in KLF4 transduced cells were similar to changes observed when cells are transduced with the microRNA, miR-150. Decreased expression of miR-150 has also been commonly observed in AML and re-expression of this miRNA in a murine leukemia model inhibited cancer cell growth. These findings led researchers to explore if expression of miR-150 is regulated by KLF4. The first step was to identify the promoter region of miR-150 and then to predict transcription factor binding sites, which among a dozen sites included two GC-rich elements that commonly bind KLF transcription factors. Using the miR-150 promoter to drive luciferase expression it was shown that KLF2 and KLF4 increased luciferase production, and mutation to either of the GC-rich elements prevented this activity. Thus KLF4 and KLF2 positively regulate miR-150 transcription.

The initial hypothesis from these results was that KLF4-mediated differentiation in AML was the result of miR-150 expression. However, genomic knock out of miR-150 with CRISPR/Cas9 did not prevent KLF4-mediated differentiation. KLF4 also activates the tumor suppressor p21 (also called CDKN1A), so researchers performed a double knockout of miR-150 and CDKN1A in KLF4 expressing cells. The double knockout also failed to prevent differentiation, suggesting KLF4's effects on differentiation are the result of other uncharacterized downstream regulators. To identify these unknown KLF4 targets microarray expression profiles were determined for KLF4 positive cells expressing three unique CRISPR sgRNA: non-targeting control, miR-150, and CDKN1A. From these gene expression profiles, researchers identified alterations that were common to all three conditions, as each condition is anti-proliferative and differentiates. Numerous cytokines and transcription regulators were included in the group, such as TNF, NFkB, and E2F2; however, of most interest was the inverse relationship between MYC and MXD1. Elevated KLF4 levels repressed MYC and activated MXD1 expression. These two related transcription factors have opposing effects on cells: MYC activates proliferation and metabolism, while MXD1 promotes cell cycle arrest. KLF4 expression also decreased BTK levels, which is overexpressed in AML and also contributes to MYC activation. This network of KLF4 regulated proteins can now be further dissected to understand how they contribute to differentiation and cell cycle arrest. Moreover, targeting genes in this network could be exploited as a therapeutic strategy. While this research focused on AML, Dr. Valerie Morris (Clinical Research Division) commented that such a therapy may apply to other cancers as well, "KLF4 has been described as a tumor suppressor regulating proliferation, apoptosis and

differentiation in other blood cancers, including both T-cell acute lymphoblastic leukemia and B-cell lymphomas, as well as various solid tumors, including colon and lung cancer." Such a therapy could be even further reaching and benefit any cancer with low KLF4 levels, "KLF4 expression is low or absent in a number of other malignancies including various gastrointestinal malignancies, brain tumors and lung cancer." Said Dr. Vivian Oehler (Clinical Research Division).

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Morris VA, Cummings CL, Korb B, Boaglio S, Oehler VG. (2015). Deregulated KLF4 expression in myeloid leukemias alters cell proliferation and differentiation through microRNA and gene targets. *Mol Cell Biol.* doi: 10.1128/MCB.00712-15. [Epub ahead of print.]