1-26-2017

From EMT to HSC to AML: ZEB2 is a cell fate switch.

Sara E. Meyer
Thomas Jefferson University

From EMT to HSC to AML: ZEB2 is a cell fate switch

Sara E. Meyer
Department of Cancer Biology, Thomas Jefferson University

In this issue of *Blood*, complimentary studies by J. Li et al[1] and H. Li et al[2] identify the transcription factor ZEB2 as a critical regulator of multi-lineage differentiation in both normal and malignant hematopoiesis. In particular, these studies show that ZEB2 is an inhibitor of normal granulocyte production, and in acute myeloid leukemia (AML) antagonizing ZEB2 function releases the granulocytic differentiation block creating an anti-leukemic therapeutic effect.

ZEB2 is most widely known as an inducer of epithelial-to-mesenchymal transition (EMT), the process of converting from a mature epithelial-like state to an immature mesenchymal state with increased migratory and invasive behavior. EMT occurs normally in tissue morphogenesis, including during embryonic organ development and wound healing. Several transcription factors including Snail/Slug, Twist, and Zeb1/2 contribute to EMT, and these pathways remain intact in many cancer cells. ZEB2 expression can directly induce EMT in a variety of solid tumor types and can thereby promote metastasis[3,4]. Consistent with the EMT function of ZEB2, new findings by J. Li et al and H. Li et al uncover a crucial theme to ZEB2 biology in regulating the balance between stemness and differentiation in normal hematopoiesis and leukemia.

Previously, it was shown that Zeb2 expression is essential for embryonic hematopoietic stem and progenitor cell (HSPC) differentiation in the fetal liver[5]. With the use of a mouse model harboring conditional deletion of *Zeb2* in adult hematopoietic cells, J. Li et al[1] report that Zeb2 controls hematopoietic stem cell (HSC) numbers as well as differentiation of myeloid progenitors, B-cell and myeloid precursors, and terminally differentiated cells. Strikingly, *Zeb2* knockout results in loss and/or aberrant morphology of many mature cell types including monocytes, B-cells, erythrocytes, and platelets, but causes a significant increase in the number of granulocytes[1] (Figure 1A). Moreover, conditional *Zeb2* knockout mice develop splenomegaly accompanied by HSPC infiltration with a corresponding increase in extramedullary hematopoiesis. Evidence from J. Li et al[1] suggests that, at least in part, Zeb2 functions to integrate signals from different cytokines to mediate its effects on differentiation.

The fundamental changes to HSPCs giving rise to hematologic malignancies include attenuated hematopoietic differentiation and gain of self-renewal. The resulting abnormal blasts accumulate and move from the bone marrow into the peripheral blood and colonize additional sites such as the spleen and liver, a process reminiscent of EMT in metastatic disease. Using high-throughput shRNA screens comparing AML and non-AML cancer cell lines *in vitro* and validation in a murine model of AML *in vivo*, H. Li et al[2] identified *ZEB2* as an AML-specific dependency. Interestingly, knockdown of *ZEB2* in AML cells promoted the expression of genes associated with granulocytic differentiation, and in addition to displaying some morphological features of differentiation, arrested leukemia cell growth (Figure 1B). Finally, H. Li et al[2] show the miR-200 negative-feedback loop present in solid cancers that potentiates ZEB2-mediated EMT[6,7], is also intact in AML cells.

These studies by J. Li et al and H. Li et al corroborate and complement one another in several ways. First, each report that *ZEB2* is highly expressed in HSPCs and in AML. Second, a granulocyte differentiation threshold or blockade is released when ZEB2 is repressed or deleted in normal and malignant hematopoietic cells. Thus, cancer cells co-opt ZEB2 to inhibit differentiation and promote stemness. In agreement with these findings, another recent study identified invasion, migration, and metastasis genes, including *Zeb2*, associated with aggressive, early-onset, HSC-derived AML, which were not present in slower growing, more differentiated, progenitor-derived AML[8]. Altogether, these studies remind us of the complexity of balancing stemness and differentiation with many other processes such as migration, invasion, and proliferation in hematopoiesis and leukemia. But is ZEB2 a master “on” “off” switch that integrates all these processes? Moving forward, additional work needs to be done to more precisely elucidate the molecular mechanisms at play, and to investigate the potential development and use of ZEB2 pathway inhibitors as an anti-leukemic therapy. Such a therapy will likely have a wide range of applicability not only for AML, but also in early T-cell precursor acute lymphoblastic leukemia (ETP-ALL)[9] and solid cancers such as breast[10], colorectal[11], and liver[12]. However, careful consideration will need to be given to limit the toxicity of this type of differentiation therapy on the remaining normal immune cell populations which also rely on ZEB2. In sum, these findings solidify a role for ZEB2 as an essential regulator of normal and malignant hematopoietic differentiation, and suggest a closer

look at EMT pathways as therapeutic candidates in leukemia and in other malignant contexts is warranted.

Figure 1. ZEB2 is a differentiation switch in normal hematopoiesis and leukemogenesis. (A) Cartoon illustration of *Zeb2* "ON" and "OFF" expression switches (depicted as green and red, respectively) indicating that with Zeb2 expression, all hematopoietic stem, progenitor, and mature lineages are produced at normal numbers (++). However, upon deletion of *Zeb2* expression, multi-lineage differentiation and progenitor production are perturbed. Red arrows highlight increased HSC, MEP, and granulocytes in the absence of *Zeb2*. (B) Left: Illustration of *ZEB2* expressing AML cells undergoing proliferation and self-renewal. Right: *ZEB2* shRNA-mediated knockdown results in differentiation and decreased proliferation of AML cells. HSC, hematopoietic stem cell; GMP, granulocyte-monocyte progenitor; MEP, megakaryocyte-erythrocyte progenitor; Mk, mature megakaryocyte; Mo, monocyte; Gr, granulocyte; Er, erythrocyte; B, B-cell.

References

1. Li J, Riedt T, Goossens S, et al. The EMT transcription factor Zeb2 controls adult murine hematopoietic differentiation by regulating cytokine signaling. Blood 2016.
2. Li H, Mar BG, Zhang H, et al. The EMT regulator ZEB2 is a novel dependency of human and murine acute myeloid leukemia. Blood 2016.
3. Comijn J, Berx G, Vermassen P, et al. The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol Cell 2001;7:1267-78.
4. Vandewalle C, Comijn J, De Craene B, et al. SIP1/ZEB2 induces EMT by repressing genes of different epithelial cell-cell junctions. Nucleic Acids Res 2005;33:6566-78.
5. Goossens S, Janzen V, Bartunkova S, et al. The EMT regulator Zeb2/Sip1 is essential for murine embryonic hematopoietic stem/progenitor cell differentiation and mobilization. Blood 2011;117:5620-30.
6. Korpal M, Lee ES, Hu G, Kang Y. The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem 2008;283:14910-4.
7. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008;10:593-601.
8. Stavropoulou V, Kaspar S, Brault L, et al. MLL-AF9 Expression in Hematopoietic Stem Cells Drives a Highly Invasive AML Expressing EMT-Related Genes Linked to Poor Outcome. Cancer Cell 2016;30:43-58.
9. Goossens S, Radaelli E, Blanchet O, et al. ZEB2 drives immature T-cell lymphoblastic leukaemia development via enhanced tumour-initiating potential and IL-7 receptor signalling. Nat Commun 2015;6:5794.
10. Bindels S, Mestdagt M, Vandewalle C, et al. Regulation of vimentin by SIP1 in human epithelial breast tumor cells. Oncogene 2006;25:4975-85.
11. Kahlert C, Lahes S, Radhakrishnan P, et al. Overexpression of ZEB2 at the invasion front of colorectal cancer is an independent prognostic marker and regulates tumor invasion in vitro. Clin Cancer Res 2011;17:7654-63.
12. Kim T, Veronese A, Pichiorri F, et al. p53 regulates epithelial-mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. J Exp Med 2011;208:875-83.