

Oncogenic Drivers and Development

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In this issue of Cancer Discovery, Lopez and colleagues show that the aggressive acute leukemic phenotype caused by the chimeric transcription factor CBFA2T3-GLIS2 varies depending on the developmental stage of the cell transformed (i.e. fetal versus adult). This is likely a general principle in pediatric cancers and begins to explain why some cancer phenotypes are more common in infants and young children, whereas others are more frequent in older individuals.

Genomic rearrangement can generate novel fusion oncogenes. These fusion genes either dysregulate protein expression or produce chimeric proteins with abnormal functionality. Interestingly, some fusion oncogenes are exclusively acquired in utero and the disease is limited to childhood (1-3). These fusion oncogenes are associated with diverse cancer phenotypes including pediatric acute myeloid leukemia (AML)(4). Approximately 5% of pediatric AML cases are characterised by a cryptic inversion of chromosome 16 (inv(16)(p13.3q24.3)) that confers a poor prognosis (5,6). This chromosome 16 inversion produces a chimeric protein where the nuclear corepressor CBFA2T3 (also known as ETO2) is fused with the zinc finger DNA-binding transcription factor GLIS2. Lopez et al show that the phenotype of CBFA2T3-GLIS2 associated acute leukemia varies with age (7). In infants and young children, it is more likely to present as an acute megakaryoblastic leukemia (AMKL) (mean age of presentation 1.6 years of age), whereas in older children it presents as an acute myeloid leukemia (AML) (mean age 8.5 years). Rarely, it is associated with both AMKL and AML phenotypes in the same patient.

To understand the cellular and molecular basis of this phenotype variation the authors created a novel doxycycline inducible ETO2-GLIS2 (iEG) expressing transgenic mouse model. EG expression produced transplantable megakaryoblastic and myeloid leukemias, thus phenocopying the human disease. The authors then hypothesized that the developmental stage of the hematopoietic cell transformed by iEG helps to determine the phenotype of the resulting leukemia. To test this hypothesis, they transplanted fetal liver (FL) and adult bone marrow (ABM) cells into primary recipients. Importantly, iEG transformed FL cells primarily gave rise to CD41⁺ AMKL, whereas iEG transformed ABM cells primarily gave rise to a Gr1⁺/CD11b⁺ AML phenotype. Furthermore, FL-derived leukemia was markedly more aggressive with a shorter latency than ABM derived disease. Thus, the developmental stage of the transformed cells modifies the phenotype of iEG driven leukemia.

The authors then further dissected if the differentiation stage of the iEG transformed cells altered the lineage phenotype. At the apex of the hemopoietic cellular hierarchy reside hemopoietic stem cells (HSC) that give rise to all blood cells. Downstream of HSCs are a series of lineage-biased multipotential progenitor cells (MPP) known as MPP2-MPP4 (8). In vitro, MPP2 give rise predominantly to erythroid-megakaryocytic cells; MPP3 to granulocyte-monocyte myeloid cells and finally MPP4 to lymphoid cells. FL iEG transduced HSCs and MPP2 gave rise to cells expressing CD41⁺ (a megakaryocyte marker), whereas iEG transduced FL MPP3 cells gave both CD41⁺ and Gr1⁺ (granulocyte marker) cells. In ABM the situation was a little different. Like FL, ABM HSC gave rise to mainly CD41⁺ cells both when tested as a population and as single cells. ABM MPP2, MPP3 and MPP4 all gave rise to mixed CD41⁺ and Gr1⁺ cells with progressively more Gr1⁺ cells in MPP3 and MPP4 compared to MPP2. Collectively, this suggests that differentiation stage of the transformed cells also modifies the phenotype of the cells produced.

To begin to understand the mechanism of phenotype differences, the authors performed single cell RNA sequencing in FL and ABM HSC and MPP4. They also performed ATAC-Seq on fetal cell populations after EG induction in vitro. As expected, prior to EG expression the baseline transcriptional programs differed in development, between FL and ABM; and during differentiation stage, between HSC and MPP. There was increased expression of the megakaryocyte transcription factor *Gata1* and the stem cell transcription factor *Erg* in FL cells and the myeloid transcription factor *Cebpa* in ABM. These data suggest a hypothesis that immortalisation of FL cells, with higher *Gata1* expression, skews the leukemia phenotype to AMKL, whereas immortalisation of ABM cells, with higher *Cebpa* expression skews the leukemia phenotype to AML.

Supportive of this suggestion patient CBFA2T3-GLIS2 AMKL cells have higher GATA1 expression and patient CBFA2T3-GLIS2 AML cells have higher CEBPA levels. Direct experimental support of this hypothesis was provided by the observation that ectopic expression of GATA1 and EG in ABM cells promoted cells with a megakaryocyte phenotype rather than a myeloid phenotype. Finally, analysis of mRNA showed that induction of EG expression resulted in more pronounced expression of *Erg* in HSC compared to MPP consistent with the notion that EG promoted a stem cell program, and this is easier to induce in cells with a pre-existing stem cell transcriptional program, see figure 1.

Taken together, this suggests that both the developmental- and differentiation-stage ground state of transcriptional factor driven transcriptional networks cooperate with EG to determine the leukemic phenotype. The authors also present data supportive of this concept with another fusion oncogene MLL-AF9. Others have also recently published findings supportive of ontogeny dependent leukemic phenotype with *NUP98-HOXA9* (9) and *KMT2A-ENL* (10) fusion oncogenes.

Finally, the authors also showed that sustained expression of the chimeric EG protein was required to maintain the transplantable leukemia. Withdrawal of doxycycline lead differentiation of leukemic blasts and mice survived. This suggests that CBFA2T3-GLIS2, and the pathway downstream of it, could be a therapeutic target.

These experiments raise the question why fetal derived and young adult stem progenitor cells are exquisitely sensitive to EG transformation. Why does EG not transform cells in older adults? Similarly, why are gene mutations that transform cells in older adults not detected in infants and young children? Is it due to cancer cell autonomous differences (e.g. differences in transcriptional ground states between cells in children/young adults or differences in DNA repair mechanisms during aging?). Alternatively, are there differences in the microenvironment or the inflammatory/immune state of the younger versus older host. Given the power of gene editing and the variety of experimental models now available these questions can now be directly studied.

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Figure Legend

Figure 1. A) The fusion oncogene *ETO2-GLIS2* is associated with a megakaryoblastic (AMKL) or myeloid (AML) leukaemia in an age dependent manner. *ETO2-GLIS2*⁺ AMKL cells are characterized by a higher expression of *GATA1* and *ERG* mRNA than *ETO2-GLIS2*⁺ AML cells. B) Analysis of a novel inducible *ETO2-*

GLIS2 transgenic mouse model determined that transformation of fetal liver hematopoietic stem cells leads to an aggressive disease that transcriptionally resembles AMKL, whereas transformation of adult multipotential cells (MPP3-4) leads to a myeloid leukemia (AML).