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ETO2-GLIS2: A Chimeric Transcription Factor Drives Leukemogenesis through a Neomorphic Transcription Network

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Acute megakaryoblastic leukemia (AMKL) is a heterogeneous disease with a relatively poorly understood pathogenesis. In this issue of *Cancer Cell*, Thirant and colleagues systematically examine unique transcriptional and functional effects of ETO2-GLIS2, an oncogenic fusion protein frequently encountered in AMKL, and elucidate a therapeutic vulnerability in this poor-prognosis leukemia.

Acute myeloid leukemias (AMLs) are a heterogeneous group of hematopoietic malignancies with complex phenotypes. While all acute leukemias are defined by a differentiation block and the proliferation of immature blast cells, the exact clinical entity encountered can depend on both the cell of origin and the specific molecular aberrations. Acute megakaryoblastic leukemia (AMKL) is a subtype of AML arising in the megakaryocytic lineage. AMKL is predominantly a pediatric disorder and has two major subgroups: AMKL arising in Down syndrome patients (DS-AMKL) and AMKL arising de novo (non-DS-AMKL). While both subgroups of AMKL have overlapping cytochemical and immunophenotypic characteristics, the prognosis and genetic alterations are substantially different. DS-AMKL is a relatively well-characterized AML that arises early in life in DS patients, uniformly involves mutations in the gene encoding the erythroid/megakaryocyte transcription factor GATA1, and has a good prognosis (Athale et al., 2001). Conversely, non-DS AMKL is a genetically varied disease harboring a spectrum of mutations and chromosomal rearrangements and has a very poor prognosis. How these genetic lesions contribute to such an aggressive AMKL pathogenesis is a central question in the field.

Recent genetic studies of non-DS-AMKL have identified a number of recurrent chromosomal translocations, gene fusions, and mutations involving genes encoding critical transcriptional regulators (Gruber et al., 2012; Thiollier et al., 2012). The most frequently identified genetic event in non-DS-AMKL is a cryptic inversion on chromosome 16 [inv(16)(p13.3q24.3)] that produces a novel gene fusion product between the corepressor ETO2 and GLI-family transcription factor GLIS2. This fusion is found in 20%–30% of non-DS-AMKL cases, which carry a very poor prognosis and have a distinct transcriptional profile from those not harboring the inversion (Gruber et al., 2012; Gruber and Downing, 2015). While ETO2 has a well-documented role in transcription regulation in hematopoietic stem cells and in megakaryopoiesis (Doré and Crispino, 2011), the role of GLIS2, a component of the Hedgehog signaling pathway, in hematopoiesis has yet to be fully elucidated. Moreover, no study to date has identified how fusion of these factors leads to aberrant transcriptional programs in megakaryocytic progenitors.

In a series of elegant experiments, Thirant and colleagues clearly demonstrate a novel transcriptional program and unique functional properties induced by ETO2-GLIS2 (Thirant et al., 2017). First, the authors illustrated that while expression of GLIS2 or ETO2-GLIS2 induced megakaryocytic differentiation in primary hematopoietic progenitor cells, ETO2-GLIS2 resulted in an additional phenotype of significant self-renewal capacity. Furthermore, this phenotype required intact DNA binding by the GLIS2 moiety and transcription factor complex recruitment via an NHR2 domain of ETO2. Next, the authors showed that ETO2-GLIS2 induces a transcriptional program that is distinct from those induced by GLIS2 or ETO2 overexpression alone. This deregulated network included aberrant expression of several key transcription factors. Of particular interest were significant changes in the expression and transcriptional activity of two transcription factors, Gata1 and Erg, which are known to be critically important in megakaryopoiesis. Specifically, expression of ETO2-GLIS2 led to marked downregulation and depressed activity of Gata1, while the levels of Erg and its target genes increased. This Gata1/Erg imbalance was further validated in primary samples from patients with AMKL harboring the *ETO2-GLIS2* fusion, indicating that this may be an important transcriptional event in disease pathogenesis.

To develop a better understanding of how ETO2-GLIS2 initiated these aberrant AMKL transcriptional networks, whole-genome occupancy of the endogenous fusion protein was queried with chromatin immunoprecipitation and sequencing (ChIP-seq). In these experiments, the authors made several important observations: (1) that ETO2-GLIS2 bound in two classes of sites, those bound by ETO2 normally (shared sites) and a larger set of novel binding sites not known to be bound by ETO2 in megakaryocytes; (2) that motifs for factors known to complex with ETO2, including those for RUNX1, ERG, and GATA1, were found in shared sites; (3) that motifs for GLIS2 and members of the ZIC family of zinc finger transcription factors were highly enriched at all ETO2-GLIS2 binding sites; and (4) that ERG bound to approximately half of all ETO2-GLIS2 sites. Importantly, this aberrant binding pattern appeared to have unique transcriptional consequences: while ~20% of genes bound by ETO2-GLIS2 were deregulated, only half of these transcription changes could be explained by overexpression of GLIS2 alone or ETO2 alone, or by co-expression of both factors, indicating that the fusion protein had a unique transcriptional behavior from either of its component moieties. Moreover, genes residing near de novo ETO2-GLIS2 binding sites tended to be upregulated, while genes near shared binding sites between ETO2 and ETO2-GLIS2 showed a trend toward downregulation. Thirant and colleagues went on to show that de novo binding sites tended to occur in super-enhancers and to be co-occupied by ERG, while shared sites were enriched at proximal promoters.

These results suggested that the abnormal transcription network induced by ETO2-GLIS2 possibly resulted from aberrant ERG and ETO2 activity. Consistent with this hypothesis, CRISPR-Cas9-mediated inactivation of *ERG* blocked the ability of ETO2-GLIS2 to induce expression of target genes such as *KIT*, blocked in vitro proliferation of ETO2-GLIS2-expressing AMKL cells, and led to reduced in vivo proliferation of xenografted cells from AMKL patients. Similarly, by ectopically expressing the NHR2 domain-containing peptide NC128, which blocks oligomerization and thus function of ETO factors, Thirant and colleagues found substantial reductions in the proliferative capacity of AMKL cells, increased megakaryocytic differentiation, and a reversal of the aberrant *GATA1/ERG* ratio induced by ETO2-GLIS2. Most strikingly, expression of NC128 completely rescued the survival of mice xenografted with *ETO2-GLIS2*-expressing AMKL cells.

This study by Thirant et al. represents an important milestone in the understanding of AMKL pathogenesis. Through a series of well-controlled assays and whole-genome approaches, they systemically identified a neomorphic transcriptional network induced by ETO2-GLIS2. They further characterized how this fusion mediates a block in terminal megakaryopoietic differentiation and an increase in self-renewal through inhibition of normal ETO2 function, through aberrant activation of super-enhancer-related genes, and by generating an imbalance in critical megakaryocyte transcription factors such as GATA1 and ERG. While the molecular details involved in this aberrant transcriptional network—including the exact composition of these novel transcriptional complexes, how the presence of the fusion protein alters the kinetics and dynamics of component binding, and how these complexes manipulate polymerase activity at target genes—still need to be explored in future studies, this work represents an important step at identifying critical transcriptional pathways involved in non-DS-AMKL pathogenesis. Most importantly, however, this work illustrates that the observed transcriptional effects may indeed be "druggable," by blocking critical interaction domains and association of the oncogenic fusion protein with other ETO family members, and may thus provide the basis for the development of novel approaches to pharmacologically target this AML subtype with particularly poor prognosis.

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