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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Fetal MLL-ENL Initiation Induces Developmental Stage-Specific Programs That Restrict Transformation and Depend on MLL3

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Infant leukemias present as either acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) and are commonly driven by *KMT2A/ MLL* rearrangements (MLLr). MLLr account for ~70% of infant ALL and ~50% of infant AML. Most of the MLLr that drive infant leukemias occur before birth and require very few cooperating mutations. These observations suggest that MLLr transform fetal/neonatal progenitors with great efficiency. However, this model introduces important paradoxes. First, the incidence of MLLr infant leukemia is ~20-fold lower than the incidence of adult MLLr leukemias. Second, fetal leukemias are exceedingly rare despite the fact that MLLr can arise before birth and require a low secondary mutation threshold for transformation. Third, even in mouse models that demonstrate efficient leukemogenesis, the fraction of MLLr progenitors that actually give rise to AML is quite small. Fourth, in our prior studies, we developed a doxycycline (DOX) inducible MLL-ENL mouse model (Tet-ME) to activate MLL-ENL and found that transformation efficiency is higher when MLL-ENL is induced shortly after birth rather than before birth (PMID: 31405949). These observations raise the question of whether fetal/neonatal progenitors carry intrinsic protection against transformation.

To test whether fetal identity conveys protection against MLLr leukemia initiation, we activated MLL-ENL at embryonic day 10.5 (E10.5) or postnatal day 14 (P14), by removing DOX. We then assessed the leukemogenic potential of postnatal progenitors in transplantation assays. Strikingly, progenitors that expressed MLL-ENL from E10.5 onward could engraft efficiently but did not give rise to AML in recipient mice. In contrast, progenitors that expressed MLL-ENL induced from P14 onward gave rise to rapid, fully penetrant AML in recipient mice (Figure 1A). Our data show that MLL-ENL induces heritable epigenetic changes in fetal progenitors, that limit transformation potential after transplantation. To assess the unique response of fetal progenitors to MLL-ENL activation, we used our Tet-ME model and induced MLL-ENL before or after birth. We isolated P28 progenitors, repeated transplantation/survival assays and performed Cellular Indexing of Transcriptomes and Epitopes (CITE-seq), in parallel, to test whether MLL-ENL differentially reprograms pre- and post-natal progenitors (i.e., do P28 progenitors have distinct expression programs depending on when MLL-ENL is induced?). Hematopoietic progenitors had similar differentiation trajectories by pseudotime analysis, but distinct gene expression profiles after fetal and postnatal MLL-ENL induction. Some known MLL-ENL targets, including Hoxa9 and Pim1, were maintained at similar levels following fetal and postnatal induction. Other targets, including Meis1, Pbx1 and Sox4, were only expressed in the HSC/MPP cluster following postnatal induction. Metabolic and differentiation states also differed. As in the initial survival assays, MLL-ENL expressing progenitors only gave rise to AML following postnatal induction.

We next sought to identify epigenetic regulators that enforce non-malignant cell fates following fetal MLL-ENL induction. We focused our screen on epigenetic factors with known tumor suppressor roles and identified the SET methyltransferase MLL3 as a candidate effector of fetal protection. Specifically, heterozygous Kmt2c deletions greatly accelerated AML initiation after fetal MLL-ENL induction, but not postnatal induction (Figure 1B). Notably, the MLL3/4 COMPASS-like complex was recently shown to antagonize the KMT2A-MENIN interaction and promote differentiation in AML cells.

Our data reinforce the notion that there are ontological windows that permit or repress leukemic transformation. Quite surprisingly, fetal progenitors appear to be more resistant to transformation than neonatal progenitors, potentially accounting for the paucity of fetal MLLr leukemias. Infant leukemias that do occur must bypass heritable protective mechanisms, either via mutations or epigenetic reprogramming.

Disclosures No relevant conflicts of interest to declare.

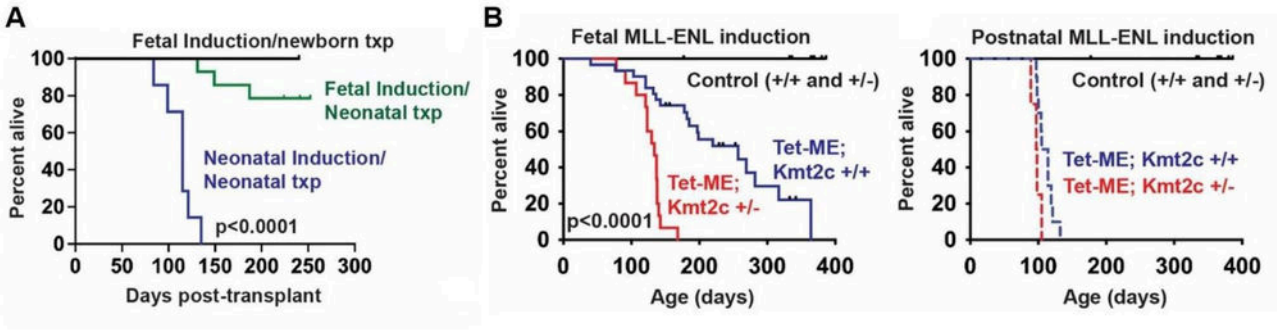

Figure 1: MLL-ENL induction before birth causes heritable resistance to transformation that depends on MLL3. (A) Survival of recipient mice following fetal or postnatal MLL-ENL induction, and transplantation at P0 or P14. (B) Survival curves of Tet-ME mice after fetal (left) or postnatal (right) MLL-ENL induction for the indicated *Kmt2c* genotypes.

Figure 1

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