Review of: "RUNX1 isoform disequilibrium in the development of trisomy 21 associated myeloid leukemia"

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This study by Gialesaki, Brauer-Hartmann, et al. addresses important topics in the molecular biology of the RUNX1 gene and its encoded transcription factor and in the pathogenesis of Down syndrome-associated myeloid leukemias (ML-DS). The topic entails a unique situation in which GATA1 mutations drive leukemogenesis, but only in the trisomy 21 (T21) genetic background. While the chromosome 21-linked RUNX1 gene has been suspected (largely a priori) to be involved in this situation, direct positive supporting data have been slow to emerge. The current preprint describes convincing data supporting a key role for RUNX1, but interestingly not via its generic over-expression but rather through a shift in relative amounts of specific differentially spliced isoforms, i.e., the RUNX1A:RUNX1C ratio.

The evidence presented is of several types, both correlative and functional, and the data figures are quite clear and convincing. The first functional experiment was a CRISPR-Cas9 screen, which revealed RUNX1 as being the top “hit” (among chromosome 21 genes) required for survival of a ML-DS cell line. The authors then examined RUNX1 isoforms and discovered an increased A:C ratio in ML-DS cases, compared to normal hematopoietic stem/progenitor cells. So, they pursued this hypothesis further in experiments using lentiviral expression vectors to modulate the A:C ratio in HSPCs, and then in mouse fetal liver cells that they had engineered to contain Gata1s mutations. Readouts of these manipulations, including assays of cell proliferation and differentiation, plus transcriptomics, all converged on the conclusion that RUNX1 “isoform dysequilibrium” is indeed sufficient to drive ML-DS. Moreover, biochemical analyses using pull down assays and mass spectrometry were also done - which revealed protein interactions - including relevant RUNX1A-specific ones such as an association with MYC:MAX, and differential interactions of specific RUNX1 isoforms with GATA1. Lastly, the authors pursued the idea that MYC:MAX would be a potential therapeutic target specifically in ML-DS, with evidence for a therapeutic window of sensitivity, compared to normal HSPCs. For this reviewer, all of the data figures are convincing, thus supporting the authors' conclusion that - “through detailed functional validation, we discovered that, rather than RUNX1 gene dosage, a disequilibrium of RUNX1 isoforms and RUNX1A bias is key to T21-associated leukemogenesis.”

After this important work, the main as yet unanswered question seems to be - how does T21 lead to the specific shift in RUNX1 isoforms? The effects of T21 on alternative promoter epigenetic states, and alternative splicing of mRNAs, has become an active general research area (e.g., Wang, Y., et al., Front. Cell Dev. Biol., 30 September 2021; Palmer et al. PNAS 2021 Vol. 118 No. 47 e2114326118; Muskens et al., Nature Comm. (2021) 12:821), so answers to this question should soon emerge.