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Super-enhancing AML with Trib1

Yoshino et al have deciphered the mechanism involved in HoxA9-associated acute myeloid leukaemia (AML) linking an Erg specific super-enhancer with the Trib1-C/EBPalpha axis. It has been previously shown for TRIB proteins, including TRIB1 and TRIB2, that they are AML oncoproteins capable of driving disease development. Degradation of the p42 isoform of C/EBPalpha (an important myeloid transcription factor), and increased MAPK/ERK signalling (a proliferation and survival indicator) are key molecular mechanisms for Trib oncogenic activity [1]. Cooperation with Hoxa9 leads to accelerated AML development [2,3], which is important as Hoxa9 overexpression alone is unable to drive AML *in vivo.* The role TRIB proteins may have across the wide heterogeneity of AML phenotypes is still under-appreciated, however given that deregulated Hox signalling is associated with ~70% of all AMLs, it is important to understand the involvement of Trib oncogenic activities in Hoxa9-mediated AML. It remained an open question how TRIB proteins cooperate with Hoxa9 at the mechanistic level. In this issue, Nakamura and colleagues use the genetic Trib1-ROSA26-Cre knockout murine model and retroviral mediated Hoxa9 overexpression together with or without Trib1 re-expression (Trib1 Hi and Trib1 null) to investigate the mechanistic basis for Hoxa9-mediated AML [4].

Previous data has shown Hoxa9 is organized into enhanceosomes containing lineage-restricted transcription factors including C/EBPalpha [5]. Together with what is known about TRIB1 oncoprotein function this led the authors to hypothesize that Trib1-mediated degradation of C/EBPalpha p42 isoform would lead to enhanced remodelling at HoxA9-associated genomic loci, and thus be a key event in HoxA9-Trib1 cooperation in AML. The authors tested this using microarray gene expression and ChIP-Seq analysis, and could show that in the absence of Trib1 (Trib1 null) in HoxA9-expressing cells, C/EBPalpha p42 is expressed and a HoxA9-C/EBP complex and H3K27ac is present at distal enhancers. The HoxA9-C/EBP complex is associated with gene activation and repression, and known to contribute to the aberrant proliferative phenotype in an AML model which required co-expression of MEIS1 [6]. In this Blood paper, the authors now advance our understanding of HoxA9 leukaemogeneis; HoxA9 DNA-binding peaks and H3K27ac peaks were not significantly different between TRIB1 hi and null cells but there was HoxA9 associated super-enhancers specific to TRIB1 hi AML cells that were enriched, most notably at the Erg +85 super-enhancer together with H3K27ac. Importantly, it was the removal of C/EBPalpha p42 isoform mediated by Trib1 that occurred at these specific Hoxa9 associated super-enhancer genomic loci leading to enhanced H3K27ac and elevated Erg expression. There is a requirement for C/EBPalpha expression in HoxA9-associated and TRIB-mediated AML [6,7] which can appear to be counterintuitive. However, this is explained in the literature by the expression and opposing functions of C/EBPalpha p42 and p30 isoforms. The degradation of p42 isoform whilst p30 expression remains intact is a feature of AML [8], and consistent with the literature

here it is shown to be a driving force for enhancer modification at the Hoxa9-binding specific Erg super-enhancer.

The authors have tested the therapeutic targeting of this mechanism with the use of the BRD4 inhibitor JQ1, as BRD4 is found abundantly at super-enhancers. JQ1 suppressed the leukaemic super-enhancer activity of TRIB1 hi AML cells *in vitro* and *in vivo* consistent with a block in Erg target gene expression. How well linked are the expression of Hox proteins and Trib proteins across AML remains an open question. Using mRNA expression has not revealed strong correlations between Hoxa9, Trib1 or 2 and Erg, but as elegantly shown in this paper, it is the oncoprotein activity and protein isoform expression that are the key factors distinguishing AML with highly aggressive features which can be therapeutically targeted. Erg expression is associated with poor prognosis in AML [9], and a more aggressive phenotype [10] therefore its elevated expression as a result of Trib1 function in HoxA9-associated leukaemia has important implications for patient prognostication and potentially AML stratification.

There is no conflict of interest to declare.

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Figure legend

Schematic depiction of Erg +85 super-enhancer in Trib1 null (top) and Trib1 Hi (bottom) Hoxa9 expressing leukaemia cells.