



The 66th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

A Novel Form of AEBP2-Containing PRC2.2 Is a Genetic Dependency in Germinal Centre B-Cell Lymphoma and Its Targeting Can Overcome Acquired Resistance to EZH2 Inhibition

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Introduction

EZH2 is the catalytic subunit of Polycomb Repressive Complex 2 (PRC2) and plays a central role in B-cell lymphopoiesis. EZH2 is an oncogene in B-cell lymphoma with recurrent change-of-function hotspot mutations causing decreased levels of di-methylation of H3K27 (H3K27me2), coupled with increased levels of H3K27me3. Although enzymatic inhibitors of EZH2 are entering the clinic for the treatment of selected patients with B-cell lymphoma, their utility is limited by disease progression due to acquired resistance. Overcoming acquired resistance to EZH2 inhibitors will assist in their application in the clinic and improve patient outcomes.

Materials and Methods

To explore the mechanistic basis of acquired resistance to EZH2 inhibitors, a genome-wide CRISPR screen was performed in B-cell lymphoma cell lines treated either with or without Tazemetostat, an FDA approved EZH2 inhibitor drug. An additional, more focussed CRISPR tiling screen was performed to identify the specific domains within EZH2 and its 10 associated PRC2 proteins that are functionally essential in B-cell lymphoma. Subsequent validation work using small guide RNAs (sgRNAs) and short hairpin RNAs (shRNAs) were employed to validate both novel genetic dependencies, such as AEBP2, and genes that conferred resistance, such as NSD2. Immunoprecipitation coupled with mass spectrometry (IP-MS) of endogenous PRC2 was performed to profile the forms and compositions of PRC2 in lymphoma cells. Chromatin Immunoprecipitation (ChIP-seq) and CUT&RUN-seq of histone post-translational modifications and PRC2 components, as well as RNA-seq, were performed to understand the mechanisms of action of AEBP2-PRC2.2 in lymphoma cells.

Results

The genome-wide CRISPR screen identified AEBP2 as a gene whose loss sensitises lymphoma cells to treatment with EZH2 inhibitor Tazemetostat, while NSD2 was identified as a gene whose loss confers resistance to Tazemetostat. Both NSD2 knockout and EZH2 inhibitor resistant mutant lymphoma cells were sensitive to AEBP2 depletion, indicating that AEBP2 is a candidate target for overcoming acquired resistance. A PRC2-tiling CRISPR screen identified AEBP2's Zinc finger domains, which interact with chromatin, and its PRC2 interaction domain as being vital for its function. IP-MS established that AEBP2 forms part of a novel form of PRC2.2 that lacks JARID2. In contrast with loss of EZH2, loss of AEBP2 leads to increased genome-wide levels of H3K27me3 with distinct effects on gene transcription. We explore these mechanisms, including an apparent role of AEBP2-PRC2.2 in promoting H3K27me2 deposition at intergenic regions.

Conclusion

We identified a novel AEBP2-containing PRC2.2 subcomplex, which lacks JARID2, as a genetic dependency in B-cell lymphoma. The loss of AEBP2-PRC2.2 overcomes acquired resistance to Tazemetostat. We propose a mechanism whereby loss of AEBP2-PRC2.2 results in genome-wide disruption of H3K27 methylation marks with adverse effects on cellular proliferation. These data indicate that pharmacological inhibitors or degraders of AEBP2 could be a promising strategy to treat germinal centre lymphomas and overcome resistance to EZH2 inhibitors.

Disclosures No relevant conflicts of interest to declare.

<https://doi.org/10.1182/blood-2024-205382>