Abstract: S125

Title: CELL-INTRINSIC INFLAMMATION SHAPES CELL-STATE HETEROGENEITY AND DRIVES THERAPEUTIC RESISTANCE IN HUMAN ACUTE MYELOID LEUKEMIA

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Background:

Cell-state heterogeneity is increasingly recognized as a critical driver of therapeutic resistance in cancer. In acute myeloid leukemia (AML), this heterogeneity is pervasive across the myeloid developmental hierarchy and evolves during therapeutic resistance, posing a substantial clinical challenge. Despite its importance, the biological cues that shape cell-state heterogeneity and their implications for therapeutic design remain obscure.

Aims:

We aim to elucidate a previously unrecognized role of cell-intrinsic inflammation in shaping cell-state heterogeneity in human AML and the mechanisms driving this process, with the goal of exploring strategies to manipulate cell-state heterogeneity for therapeutic opportunities.

Methods:

Integrated single-cell and bulk RNA-seq analyses were used to reveal the association between inflammation and cell-state heterogeneity. shRNA and overexpression systems were used to assess the role of RBM47 in inflammation and cell-state heterogeneity. Multiplexed single-cell RNA-seq, multi-color flow cytometry were employed to illustrate shifts in the myeloid developmental hierarchy at transcriptome and surface proteome levels. eCLIP-seq and RNA-seq were used for mechanistic studies. Finally, CDX and PDX models were built to study the impact of manipulating the RBM47/inflammation axis on cell-state heterogeneity and venetoclax sensitivity *in vivo*.

Results:

We discovered a surprising correlation between the strength of inflammatory signaling and the maturation of cell states along the myeloid developmental hierarchy of AML. RNA processing pathway as well as RNA-binding protein RBM47 was identified among the top dysregulated pathways and genes in inflammation-high versus low primary AMLs. Subsequent analysis found RBM47 drove cell-intrinsic inflammation in AML as indicated by NFκB reporter activation, upregulation of inflammatory gene sets, and increased secretion of inflammatory cytokines. Importantly, overexpression or knockdown of RBM47 in a collection of AML cell lines and primary AMLs representing a diverse spectrum of myeloid developmental hierarchy demonstrated a global shift towards more mature or primitive states, respectively. Mechanistically, RBM47 directly bound to mRNA transcripts of TNFα, IL8, IFNGR2, and others, promoting their expression. Blockade of TNFα-mediated inflammatory signaling abrogated RBM47-induced maturation of myeloid cell states, suggesting this process is inflammation-dependent. Finally, we explored the therapeutic potential of this finding. We found that the overexpression of RBM47 decreased the ratio of BCL2/(MCL1+BCL2A1), while the knockdown of RBM47 led to the opposite, and this translated into venetoclax resistance and sensitivity, respectively. In multiple primary AML and PDX models, we showed that inhibition of RBM47 or its

downstream TNFα/NFκB signaling reversed cell-state maturation towards a more primitive stage and restored BCL2 dependency, ultimately leading to venetoclax sensitivity.

Summary/Conclusion:

Together, our results reveal RBM47-driven cell-intrinsic inflammation as a critical signal shaping the cell-state heterogeneity of human AML. Inhibition of the RBM47/TNFα/NFκB/Inflammation axis can shift the cell-state heterogeneity of human AML towards a more primitive, BCL2-dependent state, resulting in venetoclax sensitivity. A combination of venetoclax plus TNFα/NFκB blockade therapy represents a promising strategy to treat therapeutic resistance resulting from the evolving cell-state heterogeneity of human AML.

Keyword(s): Acute myeloid leukemia | Inflammation | Venetoclax | Tumor necrosis factor alpha (TNFa)