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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

KDM6A Modulates Anti-Tumor Immune Response By Integrating Immunogenic Cell Death in Human Acute Myeloid Leukemia

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Identifying tumor cell-epigenetic regulation of immune activation in acute myeloid leukemia (AML) would be instrumental in developing next-generation of targeted therapy. AML cells have low mutational burden; inefficient 'cross-presentation' through conventional dendritic cells (DC) and immune activation are the major challenges for AML immunomodulation. 'Immunogenic cell death' (ICD) represents changes in the composition of the tumor cell surface as well as the release of 'damage associated molecular patterns' (DAMPs) in the tumor microenvironment that stimulates the dysfunctional immune system. DAMPs in turn operate on a series of receptors expressed by DCs and natural killer (NK) cells and contribute to long-lasting, protective anti-tumor immunity. For example, plasma membrane exposure of endoplasmic-reticulum (ER)-translocated calreticulin (ecto-CRT), which functions as an 'eat me' signal, is an important hallmark of ICD. Previous study suggested that calreticulin (CALR) promotes immunity and type I interferon-dependent survival in mice with AML (Fucikova, Blood 2016; Chen, Oncoimmunology 2017). In addition, ecto-CRT on malignant blasts correlated with improved NK cell-mediated cytotoxicity in AML patients with superior overall survival (Truxova, Haematologica 2020). Ecto-CRT has also been identified as an endogenous sterile ligand for NK cells (Sen Santara, Nature 2023). Nevertheless, molecular regulation of ICD in the context of epigenetic derangements and inflammation and its implication in AML immunotherapeutic modulation is poorly understood. KDM6A is a histone 3 lysine 27 demethylase that plays tumor suppressor function in AML (Gozdecka, Nat Genet 2018; Sera, Blood 2021). KDM6A escapes X-chromosome inactivation, and *loss-of-function* deletion or point mutations associate with resistance to '3+7' chemotherapy (Stief, Leukemia 2020). We have identified that in comparison to control AML cells, deficiency of KDM6A (*KDM6A-kd*) associated with daunorubicin/etoposide-induced ICD, which was manifested by significant increase (2-3 fold , $P<0.05$) in ecto-CRT, plasma membrane exposure of pE1F2α, HSP70 and HSP90 with extracellular release of HMGB1 and ATP. Similarly, pharmacological inhibition of JmjC catalytic function using GSK-J4 sensitized human AML lines as well as primary AML patient bone marrow-derived CD34 $^{+}$ hematopoietic stem/progenitor cells to ICD. Induction in ICD was accompanied by an elevated type I interferon production that was functionally active in promoting interferon regulated gene expression. Interestingly, treatment with acute recombinant IFN-β caused an increase in activated STAT1 and inflammatory gene expression in *KDM6A-kd* AML cells compared to control AML. Corroborating these results, gene set enrichment analysis revealed a significant enrichment (NES=1.708, P=0.007) of upregulated type I interferon signatures (n=40) in *Kdm6a* $^{-/-}$ primary leukemia cells. Re-analysis of ChIP-seq results indicated 1647 Kdm6a occupied genes, which were upregulated upon *Kdm6a* loss. Gene ontology (GO) analysis of the 1647 genes further suggested an enrichment ($P<0.001$) of type I interferon-responsive GO terms, indicating Kdm6a role in interferon signaling.

Ecto-CRT level relies on premortem ER stress. In OHSU AML, KDM6A expression correlates with ER stress/unfolded protein response. Mechanistically, quantitative-ChIP and promoter-reporter assays indicated that *KDM6A-kd* de-repressed ICD induced expression of ATF4 and HSPA5 through chromatin and transcriptional remodeling. In agreement with these findings, AML blasts-healthy immune cells *ex vivo* co-culture experiments revealed that increase in ICD in *KDM6A-kd* AML was associated with activation (~2-foldincrease inCD80 $^{+}$CD86 $^{+}$, $P<0.05$) and phagocytic function of DCs. Loss of KDM6A was shown to bestow 'BRCAness' (Boila, Leukemia 2023), and PARP inhibition itself can cause ICD and augment tumor immunity. We observed that there was a significant induction in ICD and expression of NKG2D ligands ULBP1, ULBP5 in *KDM6A-kd* AML cells treated with olaparib. In addition, olaparib and GSK-J4 treatment showed additive effects in ICD and immune activation

using primary AML CD34 $^{+}$ cells. Together, we illustrate that KDM6A regulates ICD through epigenetic mechanisms, while KDM6A deficient AML subtypes could be sensitized with immunomodulatory targeted therapy.

Disclosures No relevant conflicts of interest to declare.

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