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P400 PROTEOGENOMIC CHARACTERIZATION OF 5-AZACYTIDINE EFFECTS ON ACUTE MYELOID LEUKEMIA IMMUNOPEPTIDOME

Topic: 03. Acute myeloid leukemia - Biology & Translational Research

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Background:

Hypomethylating agents like 5-azacytidine (AZA) are licensed for the treatment of acute myeloid leukemia (AML) patients ineligible for allogeneic hematopoietic cell transplantation. While previous reports suggested that AZA promotes the recognition of AML blasts by cytotoxic T cells, the mechanism behind this improved recognition is not fully understood. Specifically, AZA is assumed to promote the expression of transcripts repressed by genomic methylation, namely cancer-testis antigens (CTA) and endogenous retroelements (ERE), resulting in the presentation of immunogenic MHC-I-associated peptides (MAPs, collectively referred to as the immunopeptidome) derived from the translation of these transcripts. However, the presentation of such MAPs after AZA treatment has not been firmly demonstrated thus far.

Aims: Our study aims to characterize how AZA treatment shapes the identity of MAPs presented by AML cells.

Methods: Four different AML cell lines, THP-1, OCI-AML3, SKM-1, and MOLM-13 were treated with a non-cytotoxic dose of AZA for 3 days. Their transcriptome has been characterized by high-coverage RNA sequencing (RNA-seq) and their immunopeptidome by shotgun mass spectrometry (MS). To identify MAPs deriving from unannotated genomic regions, we have designed a cutting-edge proteogenomic pipeline using the RNA-seq data to build personalized MS databases that enabled the identification of non-canonical MAPs such as ERE-derived MAPs.

Results:

Paired transcriptomic comparisons between treated and untreated cells showed that AZA induces a large-scale gene upregulation (87% differentially-expressed transcripts were upregulated). Among them, 38% were EREs and 6% were CTAs, suggesting that AZA-induced MAPs have greater chances of deriving from EREs than from CTAs. However, we could not identify a single ERE-derived upregulated MAP among the immunopeptidome of AZA-treated AML cells while multiple CTA-derived MAPs (0.4 - 0.7% of the upregulated MAPs) were presented at greater levels by AZA. Because a GO-term analysis of the upregulated protein-coding genes evidenced a robust innate immune response in AZA-treated cells, we conclude that AZA-induced enhanced CTL recognition is more dependent on CTA- than on ERE-derived MAP presentation and that ERE enhanced expression could rather trigger the observed innate immune response. An in-depth analysis of the immunopeptidome showed that MAPs having an altered presentation following AZA treatment derived only partially from transcripts whose expression was affected by AZA. The analysis of the other AZA-induced MAPs showed that they resulted preferentially from the activity of the constitutive proteasome (vs the immunoproteasome) and derived preferentially from proteins having less aromatic residues (and therefore more prone to misfolding). Because the degradation of misfolded proteins by the constitutive proteasome can indicate that AZA induces an unfolded protein response, we have examined whether AZA stimulates autophagy, a process frequently triggered in association with such stress. Accordingly, flow cytometry assays on AML cells treated with AZA for 24h evidenced a robust autophagy induction.

Summary/Conclusion: Altogether our results show that AZA promotes the presentation of CTA-derived rather than ERE-derived MAPs and that autophagy induction could enable the survival of AML cells to AZA-induced proteotoxic

stress. Our findings suggest that autophagy inhibitors could synergize with AZA in AML therapy.