COMBINED MULTI-OMICS ANALYSIS DELINEATES THE IMPACT OF EPIGENETIC THERAPIES ON THE IMMUNOPEPTIDOME OF ACUTE MYELOID LEUKEMIA

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Background Epigenetic modifying therapies, including hypomethylating agents (HMA) and histone deacetylase (HDAC) inhibitors, have shown promising results in the treatment of acute myeloid leukemia (AML). Recent data point to an immunological mode of action suggesting that HMA-induced gene expression of endogenous retroviral element (ERV)-encoded promoters and various cancer/testis antigens (CTA) might result in the presentation of novel peptides on human leukocyte antigen (HLA) molecules. These treatment-induced antigens could represent targets for immune surveillance in malignant disease and guide the development of novel combinatorial T-cell based immunotherapies. Thus, we here investigated the impact of the DNA methyltransferase inhibitor decitabine (DAC) and the HDAC inhibitor vorinostat (VOR) on the immunopeptidome of primary AML cells.

Methods Implementing label-free quantitation mass spectrometry, we assessed HLA class I and II peptide presentation of (i) AML blasts of patients treated with DAC (n=3), or (ii) primary AML samples (n=7), (iii) AML cell lines (n=3), and (iv) peripheral blood mononuclear cells (PBMC) of healthy donors (n=4), after in vitro treatment with DAC, VOR, or a combination of both.

Results Flow cytometry-based quantification showed no loss or downregulation of HLA surface expression on AML blasts under DAC or VOR treatment. In total, 638,209 HLA class I binders and 331,765 class II presented peptides were identified with 75,619 and 58,098 unique identifications, respectively. A comparative analysis of DAC and/or VOR treated samples with their untreated controls revealed 16,251 treatment exclusive HLA class I binders and 14,788 treatment exclusive HLA class II peptides. Overlap analysis of the HLA class I binders with our in-house benign tissue (n=404, >140,000 peptides) and untreated malignant tissue (n=552, >240,000 peptides) databases revealed that 5,271 (32%) treatment-exclusive peptides were never identified on benign or malignant tissues before confirming that these HLA ligands were induced upon treatment. Focusing on alternated CTA-expression of patients treated in vivo with DAC, HLA ligands from several CTA (ATAD2, KIAA0100, CNOT9, CNTN2, CTNA2) were found to be exclusively presented after the epigenetic modifying therapy. By integrating mass spectrometry-based immunopeptidomics with genome-wide CpG methylation screening, and deep short- and long-read RNA sequencing (RNA-seq) technologies followed by reference guided transcriptome assembly, treatment-induced HLA-ligands from canonical proteins as well as novel open reading frames (ORF) were identified.

Conclusions Our results demonstrate that epigenetic therapy modifies the immunopeptidome of primary AML cells by inducing treatment-exclusive novel HLA ligands. These treatment-associated antigens will be further evaluated for their eligibility as suitable targets for immunotherapeutic combinatorial approaches in AML.

Ethics Approval The study was performed according to the guidelines of the Declaration of Helsinki and the local ethics committees in Tübingen (713/2018BO2, 406/2019BO2). Participants gave informed consent before taking part.

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