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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Identifying Surface Protein Markers for AML Immunotherapy

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Background: Acute Myeloid Leukemia (AML) is a hematologic malignancy characterized by clonal expansion and differentiation arrest of myeloid precursor cells in the bone marrow. It results in the accumulation of abnormal and immature myeloblasts, which impairs the production of normal blood cells, including erythrocytes, leukocytes, and platelets. Chimeric Antigen Receptor T-cell therapy (CAR-T therapy) and Antibody-Drug Conjugate (ADC) immunotherapies have shown significant success in the treatment of hematological malignancies. However, their efficacy in AML is limited due to the disease's genetic diversity, lack of uniform antigen targets and immunosuppressive microenvironment. Single-cell RNA sequencing (scRNA-seq) is a powerful technique that can be utilized for surface antigen discovery in various cell types, including immune cells and cancer cells.
Methods: To identify potential AML cell-surface markers, we employed a comprehensive approach that involved the utilization of 3' single-cell RNA sequencing (scRNA-seq), bulk RNA sequencing (RNA-seq), and enhanced whole exome sequencing (eWES). A total of 21 samples were analyzed, comprising 18 distinct AML patients and 3 healthy bone marrow aspirates, serving as controls.
Results: Using enhanced whole exome sequencing (eWES) on tumor/normal paired samples, we defined the significant driver mutations within our sample cohort, notably DNMT3A (N=4, 21%), FLT3 (N=3, 16%), TET2 (N=3, 16%), KRAS (N=2, 11%), and GATA2 (N=2, 11%). Subsequently, we conducted scRNAseq on 21 samples, yielding a total of 185,106 high-quality cells, which were effectively clustered into 38 unbiased groups. Each sample demonstrated an average capture of 8862 cells, showcasing robust data acquisition. Our scRNAseq analysis revealed a diverse representation of major lymphoid and myeloid lineages, with prominent populations of AML blasts, T/NK cells, and progenitor cells. Notably, tumor clusters exhibited highest copy number variations and formed separate clusters from other non-tumor immune cells. To uncover key functional pathways, we conducted pathway enrichment analysis comparing tumor cells (N=131,196 cells) to non-tumor cells (N=53,910 cells). Our findings demonstrated a significant overrepresentation of cancer hallmarks, such as Allograft rejection (FDR= 0.000002), IL2/STAT5 signaling (FDR= 0.0000005), IFN-gamma response (FDR= 0.00000001) and apoptosis (FDR= 0.00000001) within the AML blasts, suggesting their potential involvement in AML pathogenesis. To identify AML specific markers, we developed analysis pipeline and performed an unbiased systematic search of the scRNAseq dataset for genes with unique expression patterns in tumor cells compared to the healthy bone marrow controls. Our analysis identified 302 differentially expressed genes, with 8 genes displaying relative cell and tissue specificity. Notably, among these genes, we unveiled key potential targets with cell surface specificity, including novel markers alongside previously known ones. Noteworthy candidates, such as *CD86*, *CSF1R*, *CD33*, *CLEC12A*, and *FLT3*, were prominently and significantly expressed, showcasing their potential significance as targets for engineered immunotherapy. To validate and consolidate our discoveries, we conducted a cross-referencing exercise by comparing our scRNAseq data against bulk RNA data from primary human AML samples and cell lines. Bulk global proteomics and flow cytometry-based studies are planned to confirm our genetic discoveries.
Summary: Our rigorous computational and experimental approach provides valuable insights into tumor-specific cell surface proteins, which hold promise as potential targets for innovative and precision immunotherapeutic interventions in the realm of AML treatment.

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