Deciphering the early events molecular effectors and signaling pathways of the Anti-CD19 CAR-T cell therapy using Phosphoproteomics analysis

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Introduction: A pioneer in the field of cell immunotherapy is the chimeric antigen receptor T-cell (CAR-T) therapy which utilizes genetically modified T-lymphocyte cells expressing receptors against certain targets on tumor cells, including CD-19, to induce cytotoxicity and the death of malignant cells. We generated an Anti-CD19 CAR-Jurkat, a T cell line, using a locally produced second-generation CAR-CD19 construct. This cell line allowed us to analyze early phosphoproteomic changes that are crucial for understanding the signaling pathways and mechanism of action of this CAR.

Materials and Methods: SILAC-heavy tagged RAJI B-cells and anti-CD19 CAR-Jurkat T-cells were co-cultured for ten minutes. The phosphoproteomic profiles were acquired via the DIA methodology on the Orbitrap Astral LC-MS/MS platform. Based on the SILAC isotopically labeled peptides, the data were processed by DIA-NN and arranged to separate the proteins and phosphopeptides from the effector CAR-Jurkat and the target RAJI cells.

Results and Discussion: The phosphoproteome was extensively covered by the analysis of six biological replicates, which resulted in the identification and quantification of about 8,800 proteins and 20,000 phosphorylation sites, respectively. As anticipated, the effector CAR-Jurkat cells showed some early proteomic changes. Still, there were not many statistically significant changes that suggested unique antigen presentation mechanisms by CD74 and other MHC molecules. On the other hand, the target RAJI B-cells exhibited more statistically significant alterations, such as changes in CD28 and adhesion molecules, which are important for T-cell survival and activation. It was also noted that some proteins had unreported direct relationships with T-cell interaction; these could be intriguing molecules that control the CAR response. Regarding the phosphoproteomic changes, the effector CAR-Jurkat and the target RAJI cells showed a large number of regulated phosphosites, and notable ones were validated using Immunoblotting. About 60 kinases were identified with unique consensus sites involved in the regulated phosphosites that mediated telomerase capping regulation, G2/M cell cycle, Fc receptor signaling, T cell receptor signaling, MAPK cascade, immune-response regulating/activating cell surface receptor signaling, transmembrane receptor protein tyrosine kinase signaling, and apoptotic process, etc. Remarkably, a network analysis revealed that the spliceosome and splicing-related proteins had changed significantly in both cells. Splicing and T-cell activation have been linked recently, but it's remarkable how early these processes occur in CAR-T cell

function.

Conclusion: Our research has led us to identify proteins and phosphosites that function as molecular effectors of anti-CD19 CAR-T cell therapy during the initial phases of CAR-T-target cell engagement. These findings are advancing our knowledge of the mechanism and signaling pathways that will support CAR development and enhance the effectiveness of this ground-breaking cancer treatment approach which can enhance the discovery of adjuvant control of CAR-T responses.

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