Proteomics approach for developing new CAR-T therapeutic methods

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A T cell receptor (TCR) mediates antigen-induced signaling through its associated CD3 ε , d, g, and z, but the contributions of different CD3 chains remain elusive. An exploration of tyrosine dynamic phosphorylation patterns of the immunoreceptor tyrosine-based activation motif (ITAM) of all CD3 chains may provide key information for a comprehensive understanding of the functions of different CD3 chains. There are ten ITAM TCR - CD3 receptor complex domain with 20 phosphorylation site, implementation under the time resolution and quantitative analysis of the whole phosphorylation sites is technically challenging. In order to directly compare the phosphorylation patterns under different TCR stimulation and precisely map the dynamic process of all tyrosine phosphorylation in TCR, we developed a novel Absolute Quantitative assay with Targeted ip-multiplex - light-absolute -Quantitative Mass Spectrometry (Timlaq-MS). Using this method, we discovered that a subpopulation of CD3 ε ITAMs was mono-phosphorylated, owing to Lck kinase selectivity, and specifically recruited the inhibitory Csk kinase to attenuate TCR signaling, suggesting that TCR is a self-restrained signaling machinery containing both activating and inhibitory motifs. Incorporation of the CD3 ε cytoplasmic domain into a second-generation chimeric antigen receptor (CAR) improved antitumor activity of CAR-T cells.