Costimulatory domains tune actin remodeling and force generation at early CAR immune synapse

Our immune system constantly patrols the body for abnormal cells, and T cells are among its key players: they recognize these cells and eliminate them. However, tumor cells adapt and evade this surveillance leading to their progression. Among recent therapeutic advances, cellular immunotherapies have revolutionized the treatment such tumors. Patient's own T cells are collected and engineered to carry a synthetic receptor, called a Chimeric Antigen Receptor (CAR), which allows them to recognize and kill tumor cells. Yet these therapies still fail in many cancers. Understanding why is the key to improve their efficacy.

Current efforts have mostly focused on understanding how these CAR-T cells signal upon activation, while the biomechanical dimension of T cell activation remains underexplored. However, T cells sense the mechanical properties of their targets and respond to them by generating forces that enhance their membrane deformation and promote their killing. Whether CAR-T cells can mount a mechanical response as effectively as conventional T cells is unclear and is particularly relevant since tumor cells mechanical properties vary greatly upon cancer progression

Using a custom micropipette rheometer, we quantified force generation by second-generation CD19-targeting CARs incorporating either CD28 or 4-1BB costimulatory domains (19-28ζ and 19-BBζ, respectively) upon activation by a model target coated with CD19. After a latency following contact with a bead coated with CD19, CAR-T cells initiated a directional pushing protrusion as the first detectable mechanical response. We uncovered distinct mechanical signatures dictated by the CAR costimulatory domain: 19-28ζ CAR-T cells exhibited shorter latency and faster pushing than 19-BBζ CAR-T cells. Applying a small oscillatory force (100±50 pN at 1 Hz) through real-time piezoelectric feedback also allowed us to measure cell stiffness during force generation. This response is strongly influenced by the costimulatory domain: 19-28ζ CAR-T became markedly stiffer during protrusion formation than 19-BBζ cells. CAR-T cells expressing a truncated CAR without signaling domain (CAR CD19Δ) does not show any mechanical response. Using confocal microscopy, we correlated these differences in force generation to distinct patterns of actin dynamics between 19-28ζ and 19-BBζ CAR-T cells. We are now investigating the molecular determinants driving this divergence.

Together, these results uncover distinct, costimulation-dependent CAR-T cells mechanical signatures. Understanding and engineering the mechanobiology of CAR-T cells could open new avenues to overcome the mechanical barriers of solid tumors.