A LYMPHOCYTE BASED CELL-TO-CELL THERAPEUTIC DELIVERY SYSTEM

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With their ability to sense and integrate a wide range of signals, and actuate context-dependent responses, engineered cell-based systems are promising next-generation therapeutics. Cytotoxic lymphocytes (CLs) are an ideal chassis for developing such systems for two reasons: (i) CLs possess a unique cell-to-cell molecular transfer system in the granzyme-perforin pathway; and (ii) T-cell receptors (TCRs), or chimeric antigen receptors (CARs), can endow CLs with an exquisite level of specificity in controlling activation of this pathway, and in targeting a cell population defined by its antigen profile.

We are developing a cell-to-cell therapeutic delivery system by engineering the granzyme-perforin pathway, which, unmodified, involves CL secretion of granzyme B (GzB) and perforin, followed by perforin facilitated GzB target cell entry and apoptosis induction. We are engineering CLs to transfer a GzB-payload fusion protein to targeted cells, where the GzB motif of the fusion protein acts as a chaperone to ensure appropriate packaging, trafficking and delivery to the target cell.

We first showed the feasibility of this approach by demonstrating that a GzB-tdTomato fusion protein is transferred from NK-92MI natural killer cells to target K562 cells. We are now developing this system as a novel cancer therapeutic for apoptosis resistant tumor cells, a major challenge in cancer therapy. A common resistance mechanism is overexpression of the inhibitor of apoptosis protein XIAP, which we have shown renders target cells resistant to NK lysis. We have constructed various GzB-toxin fusions, and functionally verified their toxicity in HeLa cells. We are now evaluating the efficacy of GzB-toxin mediated NK cell killing of K562 cells that overexpress XIAP.

Engineering the granzyme-perforin pathway represents a completely novel approach to molecular delivery, which, when combined with CAR or TCR targeting, could pave the way to a new class of cell-based therapeutics, capable of executing complex in vivo therapeutic activity.