A 5th Generation CAR T-Cell: MicroRNA Guided Radiogenetics for T-Cell Engineering

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An emerging “fifth pillar” of cancer therapy is CAR T-cell therapy. This therapy involves the removal and engineering of a patients’ CD8 T-cells to express chimeric antigen receptors (CARs) to target specific proteins or molecules. While CAR T-cell therapy is effective in hematologic cancers, it has not been translated to solid tumors due to off-target reactivity, creating severe side effects. These side effects are caused by a lack of control of CAR T-cell activity, making CAR T-cell therapy unviable for use. We proposed a fifth generation CAR T-cell with additional layers of control, utilizing a synthetic 3’ untranslated region (UTR) with two microRNA (miR) binding sites and a synthetic 5’ UTR with regulatory promoter elements in a pBRAKE vector. Under normal conditions, CAR expression is suppressed by miRs. “Release” of the brakes occurs with radiation induction, allowing for CAR expression. pBRAKE vectors were cloned and transfected in four human CD8 T-cell models (human embryonic kidney 293 (HEK 293) cell line, mouse splenocytes, mouse CD8 T-cells, and Jurkat cell line). HEK 293 cells were reverse transfected with control, HIF-1a, HIF-1a +miR-a, and p53. Splenocytes, CD8 T-cells, and Jurkats were electroporated with wild-type CD3 and CArG. Gene expression was measured. Data showed that HIF-1a +miR-a yielded a nine-fold increase in gene expression with radiation ($p=0.04$). Results demonstrated CAR expression can be controlled using radiation and external factors, making pBRAKE viable to optimize CAR T-cell therapy for solid tumors and reduce side effects.

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