TRANSCRIPTOME-GUIDED DESIGN OF LOGIC-GATED CAR T CELLS FOR TREATMENT OF ACUTE MYELOID LEUKEMIA

Tess Woodring*, Katherine A Freitas, Elena Sotillo, Crystal L Mackall, Rebecca M Richards. University of Wisconsin-Madison, Madison, WI, USA; Stanford University School of Medicine, Stanford, CA, USA

Background In acute myelogenous leukemia (AML), CD93 is an appealing target for chimeric antigen receptor (CAR) T-cell therapy, offering leukemic killing without myelosuppression in preclinical studies.1 However, CD93 CAR T-cell development is hampered by CD93 expression on healthy endothelium. Our group previously demonstrated the efficacy of an inhibitory CAR (iCAR) to counteract off-tumor CD93-directed activating CAR (aCAR) signaling in a model system.1 Here, we identify endothelial-specific iCAR targets in resting and cytokine-activated states to minimize off-tumor toxicity for logic-gated (a NOT b) CD93 CAR T cells.

Methods We analyzed differentially expressed genes (DEGs) from RNA-Seq of 3 AML (Kasumi-1, THP-1, NOMO-1) and 2 endothelial (HUVEC, TIME) cell lines, incubated with and without proinflammatory cytokines (IFNγ, TNFα). We applied a statistical filter (FDR<0.05 & log2FC>10) to identify genes specific to endothelial cells and selected surface-expressed genes using annotations from a surfaceome database (figure 1).2 DEGs present in both resting and cytokine-activated conditions were reviewed as potential iCAR targets and cross-referenced with CD93 in a healthy transcriptome atlas (Tabula Sapiens) and 3,225 pediatric AML samples (TARGET-AML).3,4

Results From the thousands of DEGs in resting and cytokine-activated conditions, statistical and surfaceome filters reduced DEGs by 99%, with only half of remaining genes (N=16) upregulated in both conditions. Among these, we identified 3 potential iCAR targets (CHD5, KDR, TEK) encoding well characterized, single-pass receptors with strong endothelial cell expression (figure 2A). Validating our cell line data, we found iCAR targets expressed along with CD93 in all 21 organ-specific endothelial cell subpopulations in Tabula Sapiens (figure 2B). By contrast, median expression of iCAR targets was extremely low in TARGET-AML samples (<0.2 transcripts per million [TPM] for all targets), nearly 200 times less than that of aCAR target CD93 (figure 3). Notably, CD93 was detected in nearly all AML samples (97.2%, 3134/3225, TPM >1), with median expression twice that of existing AML immunotherapy target CD33 (CD93, 36.7 TPM; CD33, 17.2 TPM).

Conclusions Our findings suggest that VE-cadherin (CHD5), VEGF-R2 (KDR), and Tie2 (TEK) could function as iCAR targets in NOT-gated CD93 CAR T cells for AML, predicted to protect endothelium without sheltering leukemic cells. Limited overlap of endothelial-specific DEGs in resting and cytokine-activated conditions reinforces the importance of screening iCAR targets that remain stably expressed on healthy tissue during inflammation anticipated with immunotherapy. Overall, our work demonstrates an efficient and generalizable transcriptome-guided approach to design logic-gated CAR T cells.

REFERENCES
Abstract 319 Figure 2

Abstract 319 Figure 3

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