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

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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 (iHUVEC, 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 (*CDH5*), VEGFR-2 (*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.

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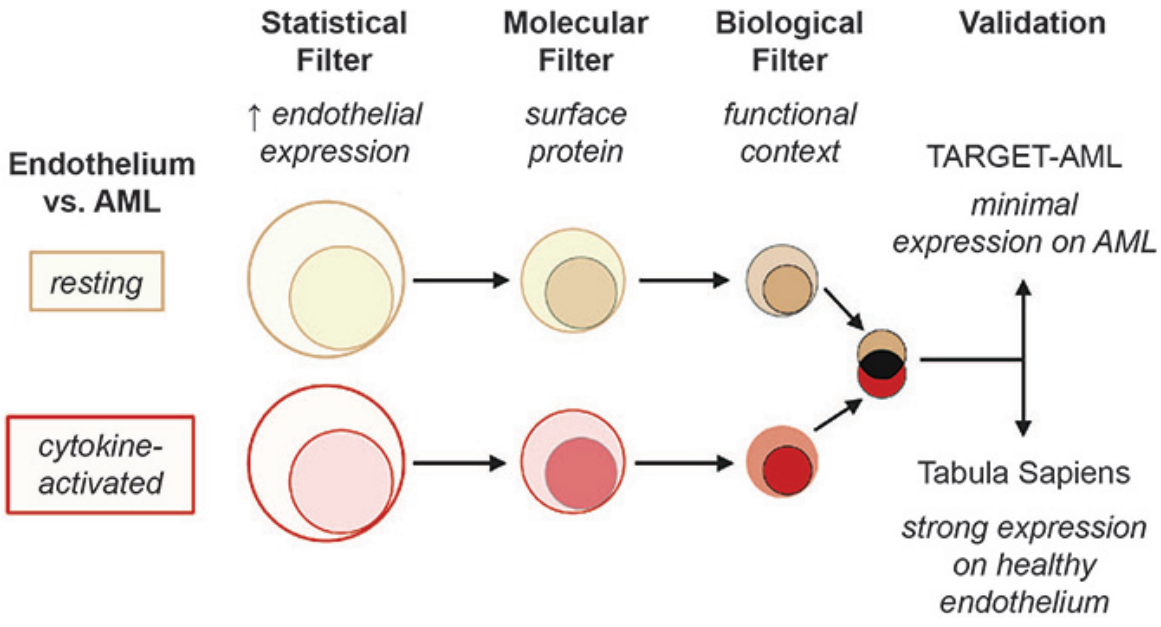

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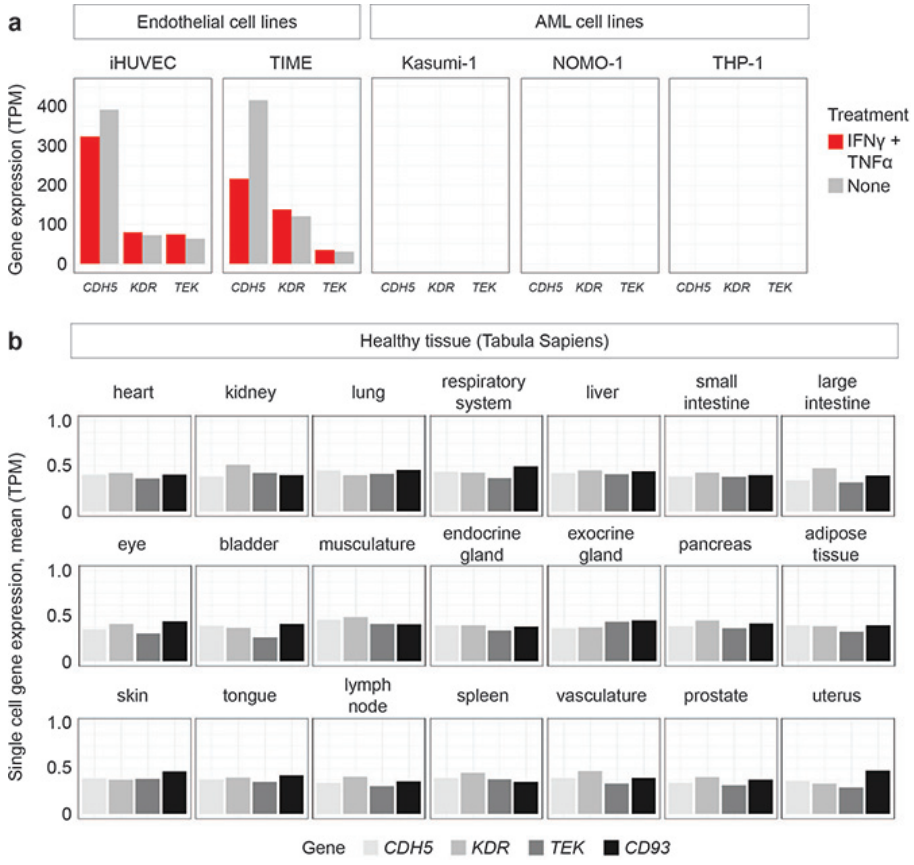

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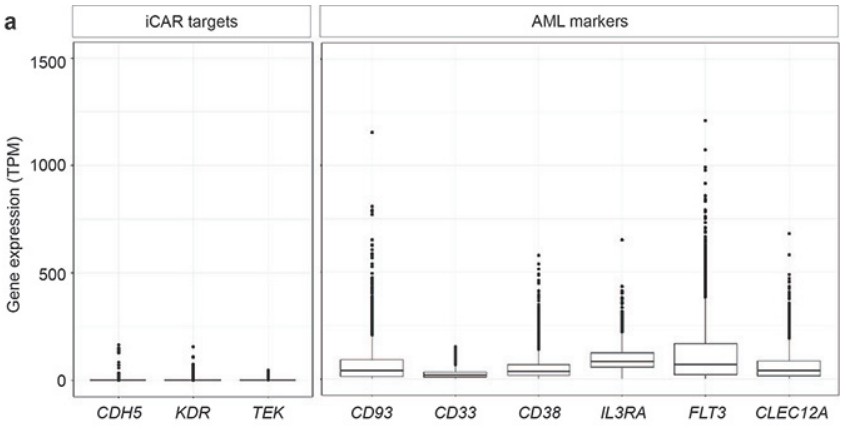

b

Gene	Absolute expression (TPM) *Median (range)*	*25%ile*	*75%ile*	Median expression relative to *CD93*
CDH5	0.17 (0.0 - 1667)	0.07	0.43	0.005
KDR	0.08 (0.0 - 156)	0.02	0.32	0.002
TEK	0.19 (0.0 - 45)	0.07	0.50	0.005
CD93	36.7 (0.0 - 1154)	11.5	89.1	--
CD33	17.2 (0.0 - 152)	8.50	31.2	0.47
CD38	33.9 (0.0 - 579)	17.6	66.4	0.92
IL3RA	81.4 (0.8 - 652)	53.9	121	2.22
FLT3	65.2 (0.0 - 1210)	20.0	164	1.78
CLEC12A	37.8 (0.0 - 680)	13.8	85.3	1.03

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