Chimeric antigen receptor (CAR)-expressing T cells are patient-derived blood lymphocytes genetically engineered to express a fusion protein that consists of an antigen-specific single chain antibody as the extracellular domain linked to intracellular signaling domains that mediate T-cell activation. Owing to this unique construction, CARs combine the effector functions of T lymphocytes with the ability of antibodies to recognize predefined surface antigens with high specificity and avidity, independent of MHC restriction.1,2 Recently, dramatic tumor regressions in patients with blood-borne malignancies have been achieved with CAR-expressing T cells targeting the B-cell antigen CD19.3,4 This has resulted in growing enthusiasm to apply the same approach to treat solid tumors.

However, CAR-expressing T cells, similar to endogenous effector T cells or tumor-infiltrating lymphocytes (TILs) expanded ex vivo, will face a number of challenges within the microenvironment of solid tumors that are likely to limit their efficacy (Fig. 1A). These obstacles include: (1) the immunosuppressive effects of multiple tumor-infiltrating cells, including macrophages, neutrophils, myeloid-derived suppressor cells, and regulatory T cells; (2) the inhibitory effects of tumor-derived soluble factors, such as transforming growth factor β (TGFβ), prostaglandin E2 (PGE2), and adenosine; (3) metabolic challenges, such as restrictive amounts of arginine or tryptophan; and (4) a microenvironment characterized by hypoxia and low pH. Moreover, previous studies with mouse and human TILs suggest that 2 additional inhibitory mechanisms will limit the antineoplastic activity of CAR-expressing T cells. First, as these cells encounter their cognate antigen within the tumor microenvironment, they are likely to upregulate inhibitory receptors causing the inhibition of their tumoricidal activity. A growing number of these receptors (and the corresponding ligands) are being characterized, including CTLA4/B7–1, PD-1/PD-L1, LAG3/MHC class II molecules, 2B4/CD48, and TIM3/Galectin-9. Second, T cells are endowed with a robust intracellular inhibitory system that can be induced upon T-cell activation, hence limiting T-cell receptor (TCR)-dependent signaling pathways and functions (presumably as a means to prevent autoimmune reactions). Components of this system that have been shown to operate in TILs include: (1) phosphatases that oppose immunostimulatory kinases, such as SHP-1, which dephosphorylates some of the TCR-associated kinases; (2) deubiquitinases (i.e., cbl-b); and (3) inhibitory kinases, such as diacylglycerol kinases (DGKs), which physically translocate to subcellular compartments important for TCR signaling.5

Many studies (in both mouse and human systems) have shown that, owing to these mechanisms, TILs become hypofunctional (i.e., unable to kill malignant cells and release immunostimulatory cytokines) upon recruitment to neoplastic lesions. We believe the same phenomenon would affect CAR-expressing T cells. This idea is supported by recent preclinical studies from our laboratory showing that human CAR-expressing T cells injected into immunodeficient mice bearing human tumors developed profound functional defects. Thus, for CAR-based
therapies to succeed in the clinic, it will be important to design strategies that will render T cells more resistant to tumor-induced functional impairment. To date, this goal has been approached by engineering T cells with genes other than the CAR-coding one, including genes that encode cytokines (i.e., IL-12), stimulatory proteins (i.e., constitutively active AKT1), or antagonists of inhibitory proteins (i.e., a dominant-negative TGFβ receptor). It should also be possible to block the expression or activities of inhibitory factors such as PD-1, SHP-1, or CBL-B using short-hairpin RNAs or intracellular antibodies.

We recently evaluated the role of the isoforms of one intrinsic inhibitor of TCR signaling, namely DGKs, on effector T-cell functions. DGKs are key enzymes that inactivate diacylglycerol (DAG), the essential second messenger of signal transduction cascades that are essential for T-cell activation, most notably the RAS/ERK pathway. DAG activity is terminated through its conversion into phosphatidic acid by 1 of 2 isoforms of DGK present within T cells, DGKα or DGKζ. The deletion of DGKs has been shown to counteract anergy in CD4+ cells, and DGKs are known to be upregulated in functionally impaired TILs (Fig. 1B).

In our recent study, we investigated T cells from mice deficient in 1 or both isoforms of DGK. We found that the deletion of DGK-coding genes has profound effects...
on effector T cells, both downstream of the TCR in ovalbumin-specific T cells and downstream of the CAR in T cells expressing a CAR specific for the tumor-associated antigen mesothelin. The ablation of DGKζ augmented ERK activation in retrovirally-transduced CAR-expressing T cells upon antigen ligation, resulting in enhanced cytokine production and target cell killing relative to their wild-type counterparts. The ablation of both DGKα and DGKζ resulted in an even greater enhancement of the effector functions of CAR-transduced cells and prolonged the persistence of adoptively transferred T cells, ultimately resulting in superior antineoplastic effects in mice. In addition, the deletion of DGKs was observed to protect T cells from inactivation by inhibitory stimuli such as PGE₂, adenosine, and TGFβ. Importantly, using an in vitro model of functional impairment of human CAR-expressing T cells, pharmacologic inhibition of DGKs was also shown to prevent the loss of cytotoxic activity and cytokine release.

Our study demonstrates that targeting specific enzymes that inhibit T-cell signaling (DGKs) has the potential to blunt tumor-induced functional defects in T cells. Given the ease by which additional genes can be inserted into CAR-expressing T cells, we believe that knocking-down or inhibiting DGKs could be a useful approach to improve adoptive T cell transfer-based strategies for the treatment of human neoplasms. We are currently attempting to genetically suppress DGK activity in human CAR-expressing T cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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