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Commentary on Kong et al., p. 5949

Model T Muscle CARs Can Treat Brain Tumors

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Despite standard treatment with resection, radiation, and chemotherapy, glioblastoma remains a deadly disease with a dismal prognosis. Redirecting patient T cells to target the glioblastoma-associated antigen, IL13Rα2, offers a promising translational immunotherapy with the potential to make a meaningful impact for patients with this disease. *Clin Cancer Res; 18(21); 5834–6. ©2012 AACR.*

In this issue of *Clinical Cancer Research*, Kong and colleagues (1) show use of a second-generation chimeric antigen receptor (CAR) modified to retarget autologous T cells to the interleukin-13 receptor α2 (IL13Rα2), preferentially expressed on glioma tumors.

Malignant primary brain tumors account for more deaths than cancer of the kidney or melanoma, and now represent the most common cause of cancer-related death in children and young adults. Current therapy for glioblastoma, the most common and malignant of these tumors, consists of surgical resection followed by radiation and chemotherapy, which is limited by toxicity to systemic tissues and surrounding eloquent brain. Despite aggressive therapy, these tumors remain universally fatal; therefore it is important to develop alternate therapies for this deadly disease.

In contrast with current nonspecific therapies, immunotherapy, which has recently been shown to be effective in several phase III clinical trials for patients with metastatic melanoma and prostate cancer, offers a specific targeted approach for the eradication of tumors (2, 3). Natural selection and evolution have provided us with an immune system capable of responding to cancerous cells and destroying them so long as they can be identified as "dangerous." However, an ongoing evolutionary arms race driven by mutations in rapidly dividing cancerous cells has also resulted in tumors with the ability to "hide" from the immune system by suppression of the MHC and various antigen-presentation mechanisms associated with this pathway and to attenuate the immune response against them. In addition, many tumor-associated antigens are overexpressed "self" antigens, shared between tumors and normal tissues. Because of the basic immunologic process of thymic selection, most T cells equipped with receptors that would otherwise recognize and destroy these antigens have already been eliminated to prevent autotoxicity. Thus, the remaining pool of potentially tumor-reactive T cells tend to be fairly infrequent in number and of low avidity and reactivity for the selected antigen, so mount a poor endogenous antitumor immune response.

One particular type of immunotherapy can overcome each of these problems. It involves adoptive transfer of genetically redirected T cells and stands out as a promising treatment able to eliminate brain tumor deposits in patients with metastatic cancer (4, 5). CARs combine *ex vivo* engineered antigen recognition regions with T-cell signaling molecules that induce T-cell activation upon encounter of target antigen (6). Expressing CARs in T cells results in T-cell activation very similar to that observed by triggering the endogenous T-cell receptor (TCR). However, unlike TCR, CAR receptors are not MHC restricted, and therefore, the same CAR receptor can be used for any patient regardless of haplotype and can additionally circumvent tumor-mediated T-cell escape mechanisms, such as the loss of MHC molecules. In addition, CARs can be further engineered, adding costimulatory and antiapoptotic molecules, such as CD28 and 4-1BB, resulting in second- and third-generation constructs, respectively, conferring improved activity and survival to T cells.

Early clinical trials with CARs have generally used first-generation vector designs whereby T cells did not persist (7), or showed high potency against shared self-tissue antigens (HER2/ERBB2) resulting in severe toxicity (8). However, recent clinical trials using improved second- and third-generation CAR vectors targeting CD19 to treat patients with disseminated lymphoma have shown these agents to be safe and produced dramatic clinical responses in patients with advanced disease (9–11). As CARs are remarkably powerful, shown by the elimination of all target antigen-expressing cells, including those in nonmalignant tissues in patients receiving CAR-transduced cells (9), the target antigen must be carefully selected to reduce the risk of life-threatening autoimmunity.

Although tissues outside the central nervous system (CNS) may undergo collateral damage without causing death, in the brain this toxicity would likely be fatal. To avoid CNS toxicity while targeting brain tumors, selected antigenic targets must be present only on tumors, and these

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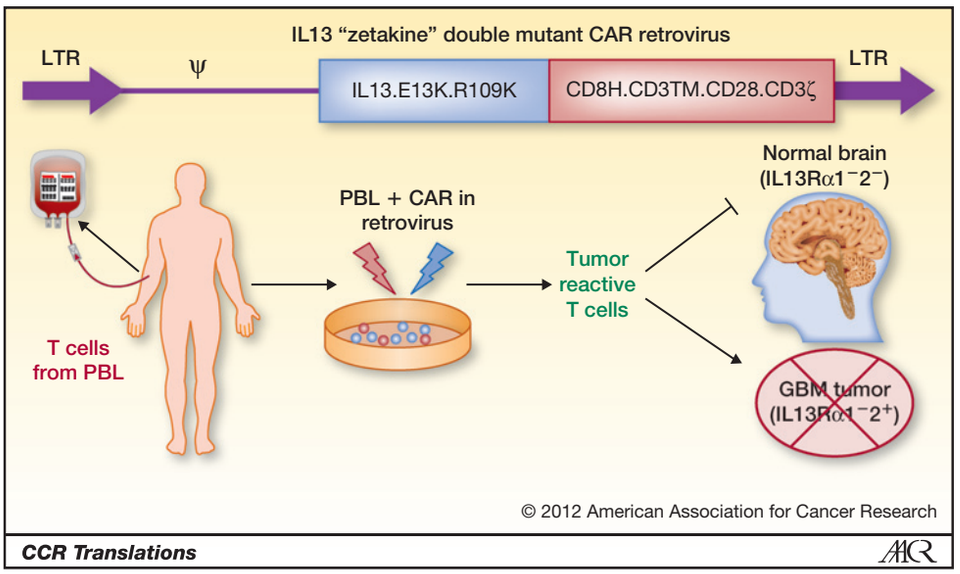

Figure 1. *CAR* gene engineered tumor immunotherapy. Autologous T cells present in patient peripheral blood leukocytes (PBL) are gene engineered to express an antigen-specific receptor, double-mutant IL13 (E13K.R109K), with increased binding to IL13Rα2 on tumors, while minimizing binding to IL13Rα1 on normal tissues. *Ex vivo* PBLs are transduced with mutated IL13 encoding retrovirus, incorporating an additional CD28 costimulatory motif tied to the CD3ζ T-cell signaling chain. Resulting IL13Rα2 reactive T cells are then expanded before intratumoral injection into glioblastoma tumor–bearing hosts, specifically destroying IL13Rα2^{+} tumors, while sparing IL13Rα1^{-}2^{-} normal brain tissues. LTR, long terminal repeat.

must be recognized with exquisite specificity by the immune system. Brain tumors, are known to express tumor-specific antigens, including IL13Rα2; in comparison, normal tissues tend to express IL13Rα1.

In this issue, Kong and colleagues (1) have built upon previous works (12) using "zetakine" CARs to target IL13Rα2. Here, the authors have generated a second-generation IL13 "zetakine" CAR, incorporating CD28 costimulation, with dual IL13 mutations specifically designed to decrease binding to IL13Rα1, while increasing avidity for IL13Rα2 (Fig. 1; ref. 1). They show specificity and function against IL13Rα2-expressing gliomas *in vitro*, resulting in type I immune cytokine production, T-cell proliferation, and target cell lysis. *In vivo*, using direct intratumoral CAR T-cell injection, this translates into increased T-cell infiltration of tumor, and treatment of 6-day established intracranial glioma in a nude rat xenograft model, shown by brain scan imaging, immunohistochemistry, and increased animal survival.

Many potentially active therapeutic agents for brain tumors are ineffective by systemic administration because they are unable to cross the blood–brain barrier (BBB). As intracerebral administration bypasses the BBB, it increases the amount of treatment that can be successfully delivered into the brain, while minimizing the potential for systemic toxicity and improving treatment effect. In their experiments, Kong and colleagues deliver CAR T cells directly to the tumor bed *in vivo*. This is clinically relevant, as intracavitary bolus injection and convection-enhanced delivery are regularly used clinically to deliver therapeutic agents directly intratumorally to patients with brain tumors.

Although such potent therapeutic intervention should be undertaken with caution, this type of gene-engineered tumor immunotherapy has the potential to be translated into meaningful treatment of patients with brain tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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