Review

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Synthetic biology in cell-based cancer immunotherapy

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The adoptive transfer of genetically engineered T cells with cancer-targeting receptors has shown tremendous promise for eradicating tumors in clinical trials. This form of cellular immunotherapy presents a unique opportunity to incorporate advanced systems and synthetic biology approaches to create cancer therapeutics with novel functions. We first review the development of synthetic receptors, switches, and circuits to control the location, duration, and strength of T cell activity against tumors. In addition, we discuss the cellular engineering and genome editing of host cells (or the chassis) to improve the efficacy of cell-based cancer therapeutics, and to reduce the time and cost of manufacturing.

Emergence of cellular immunotherapy

The intricate relationship between tumors and the immune system has been the subject of intense research, providing both insight into cancer progression [1,2] and an arena for therapeutic intervention [3]. The immune system can directly attack tumors, and harnessing this power to eradicate tumors is a major goal in immunotherapy. The involvement of the immune cells in combating tumors was demonstrated when a lower rate of relapse was observed in cancer patients who underwent a hematopoietic stem cell transplant (HSCT) (see Glossary) to replace their bone marrow after chemotherapy [4–6]. This effect has been attributed to fresh T cells from the transplant engaging and killing the tumor in a graft-versus-tumor (GVT) response. However, this response is also correlated to graft-versus-host disease (GVHD), wherein the donor T cells begin to attack the tissue of the host. This potential autoimmune response has limited the use of stem cell transplants for cancer treatment as a universal solution. Instead, aiding the immune system of the patient to fight cancer may provide more viable and widespread therapies. However, cancer cells have also evolved strategies to oppose immune action [2,3,7]. As such, a major goal of cancer immunotherapy is to overcome these immunosuppressive mechanisms, including the use of cytokines to promote T cell proliferation [8–10] or antibody checkpoint blockers to prevent the signaling of inhibitory or apoptotic pathways of a T cell [11–13]. Cytokines and checkpoint blockers have shown great promise in therapy [14,15], and several high-profile drugs have been approved in recent years. These forms of immunotherapy aid the response of the body against cancer, but immune cells can also be directly used as therapeutic agents.

Cells are inherently capable of carrying out complex computations and responses, and the immune system in particular is composed of cells designed to perform cytotoxic tasks through careful assessment of targets. Adoptive T cell therapy, the use and engineering of a patient's T cells as therapeutic agents, has emerged as a promising branch of immunotherapy (Figure 1A). Much of the current success in adoptive T cell therapy is derived from the genetic engineering of tumor-targeting receptors. However, synthetic sensors, switches, and circuits are also being explored to improve efficacy and safety by providing greater control over the location, duration, and magnitude of T cell activity (Figure 1C). Synthetic biology, an emerging discipline aimed at reprogramming living organisms through the combined use of genetics, engineering principles, and systems and computational analysis [16–18], is primed to deliver the genetic tools necessary to enhance the control of these living therapies and explore T cell behavior [19].

Glossary

Chimeric antigen receptor (CAR): engineered receptor that fuses an extracellular single-chain variable fragment (scFv) of an antibody to intracellular T cell signaling domains.
Cytotoxic T lymphocyte-associated protein 4 (CTLA-4): an inhibitory receptor that downregulates T cell response.
Epitope: fragments of proteins expressed in a cell that are presented on the surface by the major histocompatibility complex (MHC) for detection by T cells. Epitopes that represent pathogenic organisms to the T cell trigger T cell activation upon binding.
Fatty acid oxidation: cascade of β-oxidation reactions that converts fatty acids in the mitochondria to produce acetyl-CoA.
Hematopoietic stem cell transplant (HSCT): transplant of blood cells from the bone marrow that give rise to all other blood cells. HSCT is usually performed in patients with blood or bone-marrow cancers.
Immunogenicity: the potential for a molecule to elicit an immune response. Proteins expressed in a cell can be processed into smaller fragments, termed epitopes, to be presented at the surface of the cell as potential antigens by the MHC. T cells assess these MHC-peptide complexes through their T cell receptor (TCR), which are selected to distinguish epitopes derived from self-proteins and those derived from foreign organisms. If the TCR recognizes an epitope as a foreign antigen, it will activate the T cell and drive the death of the antigen-presenting cell. In T cell therapy, engineered T cells can be targeted by other immune cells owing to the expression of foreign proteins as part of the receptors or circuit components of non-human origin.
Programmed cell death 1 (PD-1): a cell surface receptor that negatively modulates the T cell response by promoting apoptosis.
Ribozyme: RNA molecules that can act as catalytic agents.
T cell receptor (TCR): receptors expressed on the surface of T cells to drive recognition of pathogenic organisms through epitope–MHC binding.
Tumor-infiltrating lymphocytes (TILs): T cells that have been able to penetrate the tumor.

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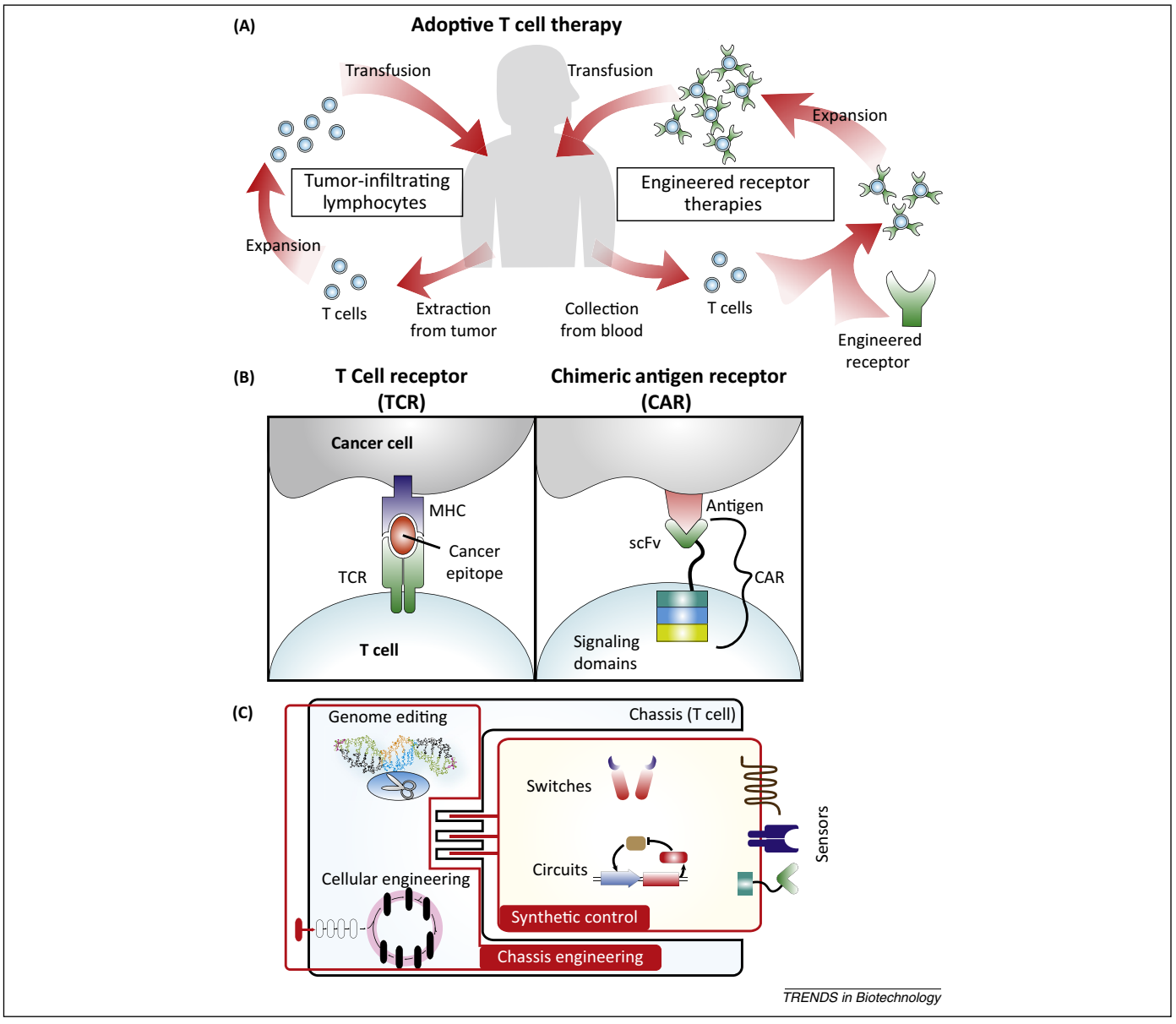

Figure 1. Adoptive T cell therapy for cancer treatment. **(A)** Several approaches for the adoptive transfer of a patient's own T cells for cancer therapy. Tumor-infiltrating lymphocytes (TILs) involve extraction of T cells directly from the tumor, *ex vivo* expansion, and then transfusion back into the patient. For engineered receptor therapies, T cells are collected from the blood, genetically modified to express a cancer-targeting receptor, expanded, and then transfused back into the patient. **(B)** Receptors engineered to target cancer cells. T cell receptors (TCRs) naturally recognize protein epitopes presented by the major histocompatibility complex (MHC) of a target cell. Engineering a TCR to detect cancer epitopes 'teaches' the T cell to detect cancer cells. Chimeric antigen receptors (CARs) are composed of a single-chain variable fragment (scFv) from an antibody fused to intracellular T cell signaling domains that trigger activation and proliferation of the T cell. CARs recognize markers expressed at the surface of a cell, and, by choosing a cancer-specific scFv, can be made to trigger killing of the cancer cell upon binding to the target antigen. **(C)** Engineering T cells for improvement of adoptive T cell therapy. Generating novel receptors and circuits can enable increased control over cell-based therapies, and techniques to engineer the chassis, such as genome editing and cellular engineering, can drive the development of more powerful treatments.

In addition to the introduction of exogenous sensors and circuits, the endogenous machinery of the host cell (chassis) presents numerous opportunities for tinkering and optimization (Figure 1C). Therefore, cellular engineering and genome editing of T cells are also under active investigation [20,21]. Much akin to the role synthetic chemistry plays in transforming the development of small-molecule drugs, synthetic-biology approaches are becoming a major engine in driving the progress of adoptive T cell therapy.

Genetic engineering and cellular immunotherapy: a potent combination against tumors

One of the most promising and earliest forms of adoptive T cell therapy involves the use of a patient's tumor-infiltrating lymphocytes (TILs), which are T cells extracted from the tumor. These isolated TILs were expanded *ex vivo*, and then transfused back into the patient to treat cancer [22]. Owing to their inherent ability to locate and traffic to the tumor site, TILs have had some success against melanoma in clinical trials [23,24]. However, the identification and isolation of TILs in sufficient quantity from a patient is challenging, limiting their potential [25]. The shortcomings of TILs have accelerated efforts to redirect the specificity of T cells towards cancer rather than relying on the isolation of T cells with inherent tumor-targeting capability. T cells from a patient can be modified with genes that encode tumor-targeting receptors that will 'teach' the T cell to