CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Turbocharging the T Cell to Fight Cancer

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Human tumors express antigens, and T cells specific for these antigens have been detected in patients with cancer. However, we know that tumor-reactive T cells found in tumors are generally nonfunctional or ineffective because, despite their presence, tumors often progress and eventually cause death. One therapy being pursued to establish effective antitumor immunity is adoptive T-cell transfer: the administration of millions of in vitro–generated, highly functional, tumor-reactive T cells to patients with cancer. Tumor-reactive T cells can be isolated from patients' own tumors, stimulated and expanded in vitro, and infused back into the patient. Alternatively, autologous T cells can be engineered in vitro to become tumor-reactive by the introduction of genes that encode receptors specific for tumor antigens (either T-cell receptors [TCRs] or chimeric antigen receptors [CARs]).

Although impressive successes with adoptive T-cell transfer have been observed in subgroups of patients with cancer and in various cancer types, most patients, especially those with solid tumors, still do not have long-term responses. Why? When T cells enter tumors, they become exposed to the immune-suppressive tumor microenvironment and to persistent tumor antigen. Microenvironmental signals, persistent antigen encounter, and chronic TCR stimulation drive T cells into a hyporesponsive state, also referred to as T-cell exhaustion or dysfunction.[1] T cells then stop proliferating and lose their ability to produce the effector cytokines (tumor necrosis factor α [TNF-α], interferon-γ, and interleukin-2) and cytotoxic molecules (granzymes and perforin) that are necessary for effective attack and elimination of tumor cells (Fig. 1).

Can T cells be engineered to resist dysfunction? A recent study by Legut and colleagues[2] suggests that they can. Technological advances, including genomewide perturbation screens, are enabling precise dissection of the molecular mechanisms that regulate tumor-induced T-cell dysfunction. However, such screens have largely been limited to loss-of-function screens that target negative regulators[3]; more challenging are gain-of-function screens for the identification of positive regulators of T-cell function. Legut et al. conducted a genome-scale gain-of-function screen in primary human T cells, discovering genes and pathways that boost T-cell proliferation, the production of effector cytokines, and tumor killing in vitro (Fig. 1).

The authors transduced primary human T cells from heathy donors with a lentiviral library containing 12,000 bar-coded genes to determine the effect of each gene on T-cell proliferation. (Barcoding of genes involves the purposeful inclusion of a unique, short DNA sequence that can be thought of as a marker for the gene that accompanies it. It is easy to "read" and thus analogous to the use of bar codes by shops.) By sequencing the bar codes in the most proliferative T cells, the researchers were able to identify and rank candidate genes that drive T-cell proliferation. Some of the top-ranked genes encoded known regulators of T-cell proliferation, but surprisingly, the gene with the strongest effect was *LTBR*, which encodes the protein lymphotoxin-β receptor (LTBR). This receptor is a member of the TNF-receptor family and is typically expressed on the surface of cells of epithelial and myeloid lineages but not on T cells.

Next, they asked whether these genes would also improve other aspects of T-cell function, including cytotoxicity and the secretion of interleukin-2 and interferon-γ. Indeed, they did, and again the strongest effect was observed with

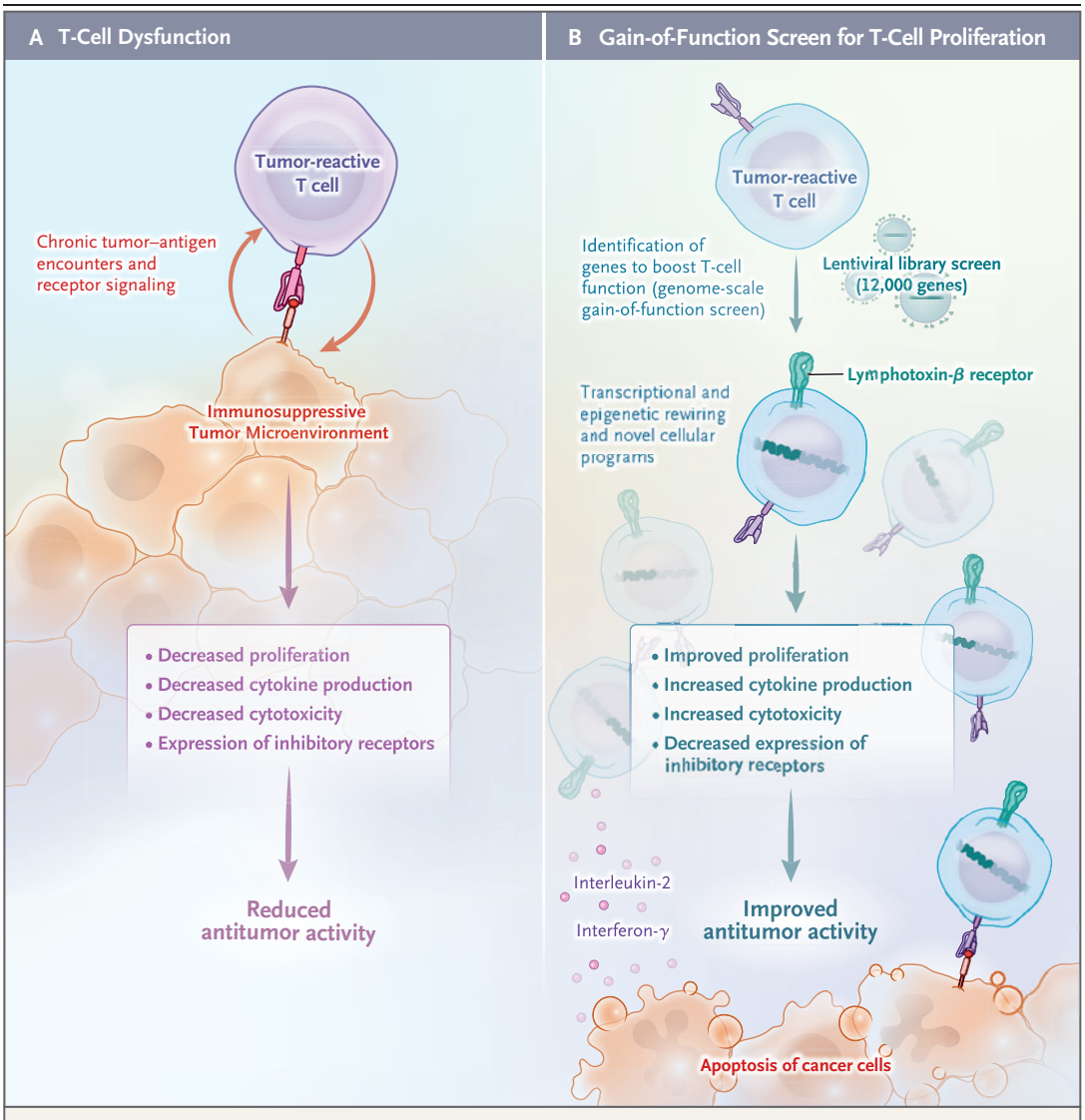

Figure 1. Reprogramming the T Cell for Therapeutic Effectiveness.

Tumor-reactive T cells within progressing tumors become dysfunctional owing to persistent tumor antigen encounter and chronic T-cell receptor (TCR) or chimeric antigen receptor (CAR) signaling and immunosuppressive factors in the tumor microenvironment (Panel A). Dysfunctional T cells fail to proliferate and to produce effector cytokines or cytotoxic molecules, and they express multiple inhibitory receptors (e.g., programmed death 1 [PD-1] and cytotoxic T-lymphocyte–associated antigen 4 [CTLA4]). Positive regulators of T-cell function can be identified by a genome-scale gain-of-function screening platform (Panel B). Legut et al.[2] expressed more than 12,000 genes in primary human T cells (1 gene per cell). They selected T cells with the highest proliferative potential and then determined the identity of the transduced genes within those T cells. Their top hit, *LTBR* (the gene encoding the lymphotoxin-β receptor), boosted T-cell proliferation, drove novel transcriptional gene circuits and epigenetic programs, and activated pathways important for T-cell cytotoxic function in vitro.

LTBR-expressing T cells. In a series of subsequent experiments, the authors assessed how LTBR works in T cells. They developed a sequencing technology called OverCITE-seq, which allows for the identification of the overexpressed gene and the simultaneous capture of granular genetic and molecular data at single-cell resolution. Combined with epigenetic and functional studies, OverCITE-seq revealed that LTBR induces profound genomewide transcriptional and

epigenetic rewiring, activates the nuclear factor κB pathway, and drives the activation of several key transcription factors, including one that is known to be critical to T-cell self-renewal and longevity.

The researchers then tested whether the overexpression of top-ranked genes could increase the antitumor effector function of CAR T cells. They expressed LTBR together with (Food and Drug Administration–approved) CD19-targeting CARs in T cells from healthy donors and in T cells from patients with diffuse large B-cell lymphoma. LTBR-expressing, CD19-targeting CAR T cells from both healthy donors and patients showed higher functionality and improved cytotoxicity against CD19-expressing tumor cells in vitro. These are certainly exciting findings. One question that remains is whether genes such as *LTBR* will also be able to turbocharge T cells in vivo, in preclinical models, and more importantly, in patients with cancer, in whom T cells are exposed to tumor antigen and microenvironmental immunosuppression for weeks or even months. Moreover, given that these genes drive cell proliferation, is there an increased risk of inducing malignant transformation of T cells? Nonetheless, the study by Legut et al., in addition to other recently published studies of gain-of-function screens,[4,5] highlight the power of large-scale screening platforms for the identification of novel genes for improved next-generation cellular therapies, including for the treatment of solid tumors, for which effective therapies involving adoptive cell transfer remain a challenge.

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