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Commentary

COMMENTARY

Improving T cell therapy: *in vivo* CRISPR-Cas9 screens tell us how to do

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Editor's note

A commentary on "*In vivo* CD8$^+$ T cell CRISPR screening reveals control by Fli1 in infection and cancer".

In recent years, various genetic manipulation techniques have been described for gene and cell therapy, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases, and the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR-Cas9) system.[1] Among these genome-editing technologies, CRISPR-Cas9 technology allows for rapid and targeted genome editing at nearly any loci. Moreover, it is simpler and quicker. Importantly, it appears to have a limited off-target effect. Due to those unique characteristics and exceptional potential, CRISPR-Cas9 technology for genetic screens in mammalian cells has become a powerful tool for the unbiased discovery of critical genes.[2,3]

In the last decades, many large-scale genetic screens have been performed *in vitro*.[4] Even though these approaches can generate numerous potential disease targets, these candidates are not developed within the context of the physiologic microenvironment. As such, *in vitro* screens have limited power to discover the bona fide essential genes. *In vivo* CRISPR screens offer a strategy for identifying novel therapeutic targets for various diseases. To facilitate the discovery of new therapeutic targets for cancer, multiple *in vivo* CRISPR screens have been performed to identify genes involved in cancer progression, including tumorigenesis,[5,6] drug resistance[7,8] and synthetic lethality.[9] These *in vivo* studies delivered a large quantitative of transformative new discoveries. However, to date, there have not been many large-scale screens done to enhance immunotherapy.

CD8$^+$ T lymphocytes are of great importance in host protective immunity against pathogens and tumors. During chronic infections and cancer progression, CD8$^+$ T cells are constantly exposed to antigens and inflammation, and eventually develop functional exhaustion.[10] T cell exhaustion is a state of T cell dysfunction defined by deteriorated effector function, sustained inhibitory receptors expression and a distinct transcriptional state from that of functional effector or memory T cells, which prevents optimal control of infection and tumors.[11] Adoptive cell therapy using chimeric antigen receptors (CAR) T cells has achieved prominent success in the treatment of malignancies. Beyond endogenous CD8$^+$ T cell exhaustion, therapeutic failures for CAR T cells are also associated with exhaustion.[12,13] Many different molecular and cellular mechanisms contribute to the failure of exhausted T cells to eradicate the tumor.

Exhausted T cells express high levels of inhibitory receptors, including programmed cell death protein 1

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(PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), and other inhibitory molecules. Blockade of the PD-1 or CTLA-4 pathway restores T-cell function and improves host defense against tumors. The advent of immune checkpoint blockade (ICB) therapy has transformed the cancer treatment landscape. Patients with multiple cancer types benefit from immune checkpoint inhibitors. Blocking either PD-1 or CTLA-4 can acquire unprecedented durable responses, even without continuous treatment.[14–16] However, only a minority of patients achieve the long-term response because resistance will develop eventually. Currently, a key question at the forefront of immunotherapy is to investigate why only a fraction of patients can respond to immune checkpoint inhibitors. Of note, recent publications suggest that not all exhausted T cells are responsive to checkpoint blockade. A subset of cells among the pool of exhausted T cells, defined as $TCF1^+$ exhausted T cells, display self-renewing capacity and are responsible for the boost of immunity following checkpoint blockade.[17] Therefore, revealing the underlying mechanisms that control T-cell fate decision is helpful for clarifying the mechanism of ICB resistance and developing novel therapeutic interventions.

The role of transcription factors in regulating differentiation of effector T cells (T_{EFF}), memory T cells (T_{MEM}) and exhausted T cells (T_{EX}) has been intensively investigated. Even though previous studies have accumulated some knowledge, the mechanisms that safeguard against commitment to T_{EFF} differentiation are still far from clear. CRISPR-Cas9 approaches have also been employed in genetic manipulation of immune cells to potentiate immune responses.[18] Recently, another elegant study published in *Cell* by Chen *et al.*[19] reported a $CD8^+$ T cell CRISPR screening platform used $Cas9^+$ antigen-specific $CD8^+$ T cells combined with an optimized retroviral based-sgRNA expression strategy. They used this platform to identify transcriptional factors that govern T_{EFF} and T_{EX} cell differentiation. They constructed domain-focused guide RNA library against 120 transcriptional factors, and assessed gRNA enrichment after *in vivo* selection with acutely resolving LCMV Arm (Arm) or chronic Cl13. Fli1 stood out as the strongest hits in repressing T_{EFF} differentiation in the large-scale screen. In further studies, they defined a central role for Fli1 in T_{EFF} responses. As expected, loss of Fli1 in $CD8^+$ T cells enhances immunity to pathogens. In preclinical tumor models, Fli1-deficient $CD8^+$ T cells robustly controlled the tumor growth. There was a significant increase of T_{EFF} and T_{EX} in both tumor microenvironment and spleen tissues. Even though they did not investigate whether targeting Fli1 could sensitize the ICB, this is a good way to find novel combinational strategies with ICB.

Cellular metabolic programming is intrinsically linked to T-cell development, differentiation, quiescence and activation.[20–22] Understanding immune cell metabolic engagement would develop new targets for immunotherapy. Even *in vitro* culture systems and *ex vivo* analysis could provide informative insights into T-cell metabolism, as during T-cell activation, there are various differences for central carbon metabolism *in vivo* and *in vitro*.[23] To systemically uncover metabolic factors orchestrating terminal effector cells (TE) and memory precursor cells (MP), Huang *et al.*[24] designed an *in vivo* CRISPR-Cas9 screening platform. They transduced a pooled guide RNA library of 3017 metabolism-associated genes into Cas9 + antigen-specific $CD8^+$ T cells, followed by LCMV infection. They found loss of amino acid transporters *Slc7a1* and *Slc38a2* promoted MP formation. Mechanistically, amino acid transporters could tune T_{MEM} responses by promoting mTORC1 activation. Another metabolism-associated candidate gene they found in the screens for TE differentiation and MP formation was Pofut1. Pofut1 deficiency simultaneously promoted T_{EFF} and T_{MEM} responses. In this article, they did not show targeting Pofut1 could enhance immunotherapy; however, they indicated in their discussion that Pofut1-dependent signature was inversely correlated with survival of certain cancer patients. Therefore, large-scale CRISPR-Cas9 screening for metabolic reprogramming is another way to find critical genes to enhance immunotherapy.

Nonetheless, a high-throughput CRISPR screening approach allows us to systematically investigate the antitumor functions of $CD8^+$ T cells in animal models closely recapitulating the human disease. Undoubtedly, more faithful target genes can be found for immunotherapy. In the future, several important questions should be considered: (1) Beyond CTLA-4 and PD-1 in the T-cell exhaustion, many other immune checkpoint inhibitor molecules should be screened out for future therapy. (2) How to identify more unknown and previously characterized regulators of $CD8^+$ T-cell function. Beyond transcription factors and metabolic regulators, epigenetic factors could be considered, because the epigenetic profile of exhausted T cells differs substantially from those of T_{EFF} and T_{MEM}.[25,26] (3) The density of $CD8^+$ tumor-infiltrating lymphocytes (TILs) is a predictive marker for the efficacy of PD-1 inhibitors.[27,28] Strategies augmenting TILs in the tumor microenvironment represent ways to enhance ICB therapy. Importantly, tumor-cell-intrinsic factors could affect the immune cells' infiltration into the tumor microenvironment.[29–31] It is intriguing to make use of a high-throughput CRISPR screening approach to find out import tumor-cell-intrinsic factors, which could regulate the influx of TILs. Eventually, increased sensitivity to ICB therapy will be achieved by targeting these factors. (4) Because CRISPR-Cas9 enables genome modification, it can be used to generate mutations *in vivo*, which then recreates human disease in animal models. Construction of a CRISPR-Cas9 animal model can also be used to screen new strategies for immunotherapy. (5) It is urgent currently to develop more combinational strategies for ICB-resistant patients. For rapid translation, the

targets for large-scale screening should be easily druggable. Collectively, this high-throughput CRISPR screening approach could help to guide clinical translation and precision medicine strategy.

Conflict of interest

None declared.

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