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RESEARCH HIGHLIGHT

Transcriptional suppression of CD8$^+$ T cell exhaustion for improving T cell immunotherapy

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As the major effector of cellular adaptive immune response, CD8$^+$ T cells play crucial roles in the host's defense against tumor and infection. Two recent studies revealed that basic leucine zipper ATF-like transcriptional factor (BATF) not only blocks the exhaustion of CD8$^+$ engineered chimeric antigen receptor (CAR)-T cells, but also regulates the differentiation of stem-like CD8$^+$ T precursor cells into effector cells during chronic infection [1, 2]. These results emphasized the important roles of transcriptional regulation in promoting T cell effector functions and preventing T cell exhaustion as well, suggesting the potential application of motivating transcriptional regulators such as BATF to improve the efficacy of adaptive T cell immunotherapy of cancer and chronic infection.

In response to antigenic peptide stimulation, naive T cells proliferate and differentiate to effector T cells to eliminate malignant cells and invading pathogens, part of which further transform to memory T cells to provide long-term immune protection [3]. When the stimulation persists in the tumor microenvironment (TME) and chronic infections, CD8$^+$ T cells can gradually enter an exhausted state characterized by high expression of inhibitory receptors as well as the overall epigenetic and metabolic profile changes, leading to the failure of clearing tumor cells and pathogens [4]. Therefore, discovering the key transcriptional regulators to T cell exhaustion will provide the potential target to improve the therapeutic effect of cancer immunotherapy by blocking or reversing the exhaustion of effector T cells.

Transcription factor nuclear factor of activated T cells (NFAT) has been recognized as the crucial regulator of

Abbreviations: BATF, basic leucine zipper ATF-like transcriptional factor; CAR, chimeric antigen receptor; TME, tumor microenvironment; NFAT, nuclear factor of activated T cell; AP-1, activator protein 1; ATAC-seq, assay for transposase-accessible chromatin using sequencing; IRF4, interferon regulatory factor 4; ChIP-seq, chromatin immunoprecipitation-sequencing; AICE, AP-1-IRF composite element; LCMV, lymphocytic choriomeningitis virus; RNA-seq, RNA-sequencing; CUT & Tag-seq, Cleavage under targets and tagmentation sequencing; ICB, immune checkpoint blockade; MAFF, MAF basic leucine zipper transcription factor F; CyTOF, cytometry by time of flight; Bhlhe40, basic helix-loop-helix family member e40; Ccl5, C-C motif chemokine ligand 5; Elk4, ETS-like transcription factor 4; Eomes, Eomesodermin; Foxo1, Forkhead box o1; E2f4, E2f transcription factor 4; Gzmb, granzyme B; Icos, Inducible T cell costimulatory; Ifnar1, Interferon-alpha and beta receptor subunit 1; Klf2/3, Kruppel-like factor 2/3; Lag3, Lymphocyte activation gene 3 protein; Pd-1, Programmed cell death-1; Nr4a2, Nuclear receptor subfamily 4 group A member 2; Runx1, RUNX family transcription factor 1; Runx3, RUNX family transcription factor 3; Tcf-1, T-cell factor 1; Tim3, T cell immunoglobulin and mucin domain 3; TIL, Tumor-infiltrating lymphocyte; Tox, Thymocyte selection-associated high mobility group box factor

effector responses of T cells by interacting with its partner activator protein 1 (AP-1) [5]. Based on a preliminary screening of transcription factors that promote NFAT-AP-1 activity, Seo *et al.* [1] constructed CAR-T cells overexpressing three candidates (BATF, JUN and MAF basic leucine zipper transcription factor F [MAFF]) and then transferred them to mice inoculated with B16F0-hCD19 tumors. As a result, they found that CAR-T cells overexpressed with BATF, a cellular transcription factor that participates in the regulation of various immune cells differentiation, showed the most significant antitumor abilities with the enhanced effector functions and increased infiltration in the TME. Transcriptome results confirmed that BATF-overexpressing CAR-T cells manifest with increased tumor infiltration, intratumor expansion, effector functions and decreased exhaustion tendency. In addition, BATF-transduced CAR-T cells can survive several weeks after tumor clearance and acquire similar phenotype of central memory T cells, rendering mice more resistant to the secondary occurrence of tumor. Further assays for transposase-accessible chromatin using sequencing (ATAC-seq) showed decreased accessibility of exhaustion-related chromatin regions in BATF overexpressing cells, which was consistent with the reduced exhaustion tendency of CAR-T cells by cytometry by time of flight (CyTOF) analysis. Next, Seo and colleagues affirmed that BATF exert the regulatory function by binding with interferon regulatory factor 4 (IRF4), which is essential for the survival and expansion of BATF-transduced CAR-T cells in the TME. Surprisingly, chromatin immunoprecipitation-sequencing (ChIP-seq) analysis results showed that in BATF-overexpressing CAR-T cells, the reduced IRF4 can redistribute and preferentially bind at higher-affinity AP-1-IRF composite element (AICE) sites of chromatin to promote the expression of T cell effector-related genes. Conclusively, overexpression of BATF blocked CAR-T cell exhaustion to enhance CAR-T cells proliferation and cytotoxic capacity, with the underlying mechanisms by forming BATF-IRF4-DNA complexes to alter chromatin accessibility of target genes, indicating the potential application of BATF for improving the effectiveness of CAR-T cells in treating solid tumors.

In another independent study, Chen *et al.* [2] sorted virus-specific splenic $GP_{33\text{-}41}{}^{+}CD8^{+}$ T cells, a pool of heterogeneous exhausted T cells from chronic lymphocytic choriomeningitis virus (LCMV) infection. On the basis of the distribution and activity of transcription factors and the corresponding binding targets, they further divided the above-mentioned cell population into three major $CD8^{+}$ T cell subsets: $Ly108^{+}$ progenitor (T_{PRO}) with the stem-like signature, CX_3CR1^{+} cytolytic (T_{EFF}) and $CX_3CR1^{-}Ly108^{-}$ exhausted (T_{EXH}) $CD8^{+}$ T subsets. Then, they focused on the epigenetic characteristics of three subsets by analyzing the chromatin accessibility as well as the activity of promoter and enhancer regions in each subtype using multiple sequencing tools including single-cell RNA-sequencing (RNA-seq), cleavage under targets and tagmentation sequencing (CUT & Tag-seq), and ATAC-seq. Notably, the authors also discovered BATF is mostly enriched within the CX_3CR1^{+} effector T subset, which shows a potential role of BATF in promoting T_{EFF} cell differentiation and avoiding T_{EXH} cell formation. Further analysis showed that BATF mainly bound to the active enhancer regions during the differentiation of T_{PRO} and T_{EFF} cells, therefore, promoting the expression of key effector genes. While in BATF-deficient virus-specific $CD8^{+}$ T cells, the proportion and cytotoxicity of T_{EFF} subsets both significantly reduced, which was accompanied by the remarkably decreased active enhancer regions of effector genes. Collectively, Chen and colleagues demonstrated that BATF promotes the differentiation from T_{PRO} cells to T_{EFF} cells and suppresses the generation of T_{EXH} cells upon the sustained viral infection.

However, there are still several questions that need to be addressed. Although both studies described that BATF regulated the expression of key effector genes accompanied with altered chromatin accessibility, neither study elucidated whether and how BATF epigenetically affected the structure of exhaustion-related chromatin regions. Therefore, whether there exists a regulatory network among epigenetic changes induced by BATF and other T cell exhaustion characteristics such as metabolic changes, and whether BATF-IRF4 interaction has the crosstalk with other transcription regulatory factors need further exploration [6]. In addition, previous studies have reported that BATF can be induced by $CD4^{+}$ T cell-derived IL-21 signaling pathway. Since $CD4^{+}$ T cells mostly exhibit dysfunctional in the TME and chronic infection, which might promote $CD8^{+}$ T exhaustion due to decreased IL-21 production [7]. Therefore, whether restoration of $CD4^{+}$ T function and supplementation of IL-21 have similar effects as BATF overexpression, or whether there are other BATF regulators in these pathological conditions still require further clarification. Currently, immune checkpoint blockade (ICB) therapy has been widely used to treat patients with different malignancies [8], which are based on monoclonal antibodies targeting inhibitory receptors to change T cell exhaustion state and restore its effector functions. Thus, it is worth investigating whether there is a synergistic effect by combining BATF overexpression with checkpoint inhibitors. Besides, the authors did not show the long-term effect of BATF overexpression in human CAR-T cells as well as the unintended adverse effects such as cytokine release syndrome and neurologic toxicity.

Altogether, both studies demonstrated that BATF can block the $CD8^{+}$ T cell exhaustion pathway and facilitate

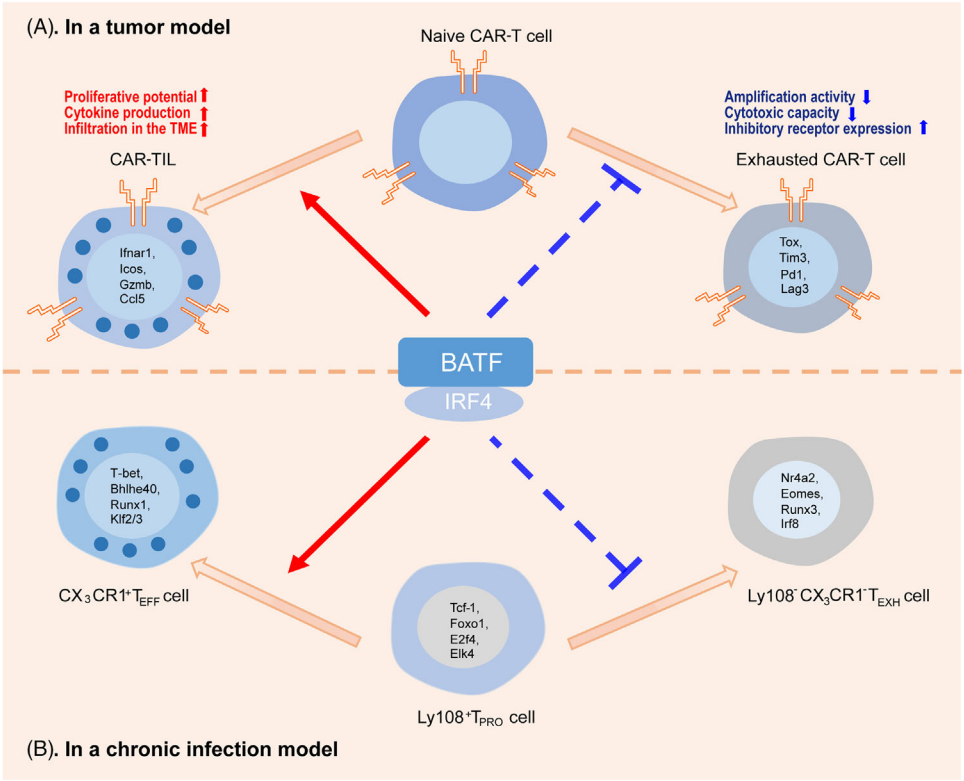

FIGURE 1 The transcription factor BATF epigenetically regulating the exhaustion and differentiation of $CD8^+$ T cells in a solid tumor model case and chronic infection case. (A) In a CAR-T therapy of solid tumor model, BATF binds with IRF4 at AICE site to reshape the chromatin landscape, therefore, inducing chromatin accessibility changes to promote the mRNA expression of effector genes and suppress the mRNA expression of exhaustion genes. Consequently, BATF-transduced CAR-T cells can show enhanced anti-tumor effect with increased TME infiltration, proliferation capacity and cytokine production, as well as the decreased exhaustion tendency. (B) In a chronic viral infection model, transcription factor BATF promotes $Ly108^+$ progenitor T (T_{PRO}) cell subset to differentiate into CX_3CR1^+ cytolytic (T_{EFF}) cells by improving chromatin accessibility in the enhancer regions and blocks the formation of $CX_3CR1^-Ly108^-$ exhausted (T_{EXH}) cells in the meantime. Abbreviations: BATF, basic leucine zipper ATF-like transcriptional factor; AICE, AP-1-IRF composite element; Bhlhe40, basic helix-loop-helix family member e40; CAR, Chimeric antigen receptor; Ccl5, C-C motif chemokine ligand 5; Elk4, ETS-like transcription factor 4; Eomes, Eomesodermin; Foxo1, Forkhead box o1; E2f4, E2f transcription factor 4; Gzmb, Granzyme B; Icos, Inducible T cell costimulatory; Ifnar1, Interferon alpha and beta receptor subunit 1; IRF4, Interferon regulatory factor 4; Klf2/3, Kruppel-like factor 2/3; Lag3, Lymphocyte activation gene 3 protein; Pd-1, Programmed cell death-1; Nr4a2, Nuclear receptor subfamily 4 group A member 2; Runx1, RUNX family transcription factor 1; Runx3, RUNX family transcription factor 3; Tcf-1, T-cell factor 1; Tim3, T cell immunoglobulin and mucin domain 3; TIL, Tumor-infiltrating lymphocyte; Tox, Thymocyte selection-associated high mobility group box factor.

the effector phenotype of $CD8^+$ T cells, suggesting the great potential of BATF in preventing $CD8^+$ T cell exhaustion (Figure 1). In immunotherapy for solid tumors and chronic viral infection with Epstein-Barr virus, hepatitis B virus or human papillomavirus, which is significantly related to the carcinogenesis, $CD8^+$ T cell exhaustion has been recognized as the main obstacle of adaptive immune response [9]. Therefore, inhibiting $CD8^+$ T cell exhaustion to restore immune system function could have important implications for the treatment of virus-related diseases and tumors. According to these two papers, BATF plays an important role in the regulation of $CD8^+$ T cell exhaustion, implicating its potentiality in clinical application. It is impressive that both articles performed comprehensive bioinformatics including single-cell transcriptomics and genome-wide epigenetics to analyze the individual cell characteristics from a microscopic perspective and discovered the shift of regulatory networks among different $CD8^+$ T cell subsets. The discovery of BATF-overexpressing CAR-T cells displaying outstanding anti-tumor effects provides new insights into the potential use of transcriptional suppressors of T cell exhaustion for specifically improving CAR-T cancer immunotherapy effectiveness.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

X.Y. and B.W. drafted the manuscript. X.C. supervised and revised the manuscript. All authors read and approved the final manuscript.

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