Immunity & Inflammation

RESEARCH HIGHLIGHT

Open Access

Ubiquitin E3 ligase KLHL6 brings exhausted T-cells back into action

Zhuoyao Chen[1], Alex N. Bullock[1] and Benedikt M. Kessler[1,2*]

Abstract

Persistent antigen stimulation drives $CD8^+$ T-cell exhaustion in cancer and chronic infection, limiting immunotherapy efficacy. Two recent studies identify the ubiquitin E3 ligase Kelch-like protein KLHL6 as a key suppressor of T-cell exhaustion. KLHL6 is maintained in progenitor and memory-like T cells but lost upon chronic TCR signaling through PI3K–AKT–mediated inhibition of FOXO1. By targeting TOX and mitochondrial regulators, such as PGAM5, KLHL6 preserves T-cell function, and its restoration rescues antitumor immunity. This discovery reveals the relevance of KLHL6 mediated ubiquitylation not only in B-lymphocytes, but also in T-cells, thereby highlighting a promising new avenue for immunotherapeutic intervention.

Keywords Ubiquitin, $CD8^+$ lymphocytes, T-cell exhaustion, Kelch-like protein, E3 ligase

Main text

Persistent antigen stimulation and associated suboptimal T-cell responses are defining features of chronic infection and cancer. Tumor infiltrating lymphocytes (TILs) and antiviral T-cells are exposed to sustained T cell receptor (TCR) signaling. How this is molecularly wired to $CD8^+$ T cell exhaustion has remained incompletely understood. Interfering with checkpoint inhibitor ligands, such as programmed death-ligand 1 (PD-L1), has yielded a successful inroad into immunotherapy, but this is working for a small subset of cancer patients only and is confronting resistance mechanisms [1].

Other ways to reverse T-cell exhaustion and reactivate them, in particular in a tumor context, have been of considerable interest. For instance, alternative checkpoint receptors that may interfere with TCR-CD3 activation and manipulation of TCR downstream signaling have been explored (reviewed in [2].

Two recent reports [3, 4] have investigated the molecular basis of T-cell exhaustion more systematically by reanalysing single-cell RNA-seq data from chronically infected mice and combining it with in vitro and in vivo models of $CD8^+$ T-lymphocyte exhaustion. In particular, the authors have taken advantage of a computational analysis that appeared powerful and very fruitful in this case. They reanalysed two published data sets reporting on two tumor specific T-cell responses and exhaustion studies, as well as antigen specific T-cell responses against Listeria and LCMV late-stage infection. Combined, these analyses revealed the ubiquitin proteasome system (UPS) as one of the main pathways generally involved in T-cell dysfunction.

The exact involvement of ubiquitin processing enzymes, in particular ubiquitin E3 ligases, in longer-term effects following T-cell activation, has not yet been well understood. To explore this, the authors designed a CRISPR screen for ubiquitin E3 ligases that was set up on active versus exhausted T cells that were positive or negative for the surface markers PD-1 and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) in order to compare these populations, also potential differences in mitochondrial dysfunction. In the two reports,

*Correspondence:
Benedikt M. Kessler
benedikt.kessler@ndm.ox.ac.uk
[1] Centre for Medicines Discovery, Nuffield Department of Medicine, Target Discovery Institute, University of Oxford, Roosevelt Drive, Oxford OX3 7FZ, UK
[2] CAMS Oxford Institute, Chinese Academy of Medical Sciences & Peking Union Medical College, Nuffield Department of Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK

they describe novel ubiquitin E3 ligases associated with activated or exhausted T-cell populations, clearly indicating that there must be strong ubiquitin and proteostasis components involved in molecular processes that lead to exhaustion, reflected by a decreased ability to respond to chronic antigen stimulation. In particular, they identified the E3 ubiquitin ligase Kelch-like protein 6 (KLHL6) as a key suppressor of exhaustion via control of T-cell progression to terminal exhaustion and mitochondrial fitness (Fig. 1).

This finding extends previous observations showing that several ubiquitin E3 ligases were capable of conjugating ubiquitin onto protein substrates critical to downstream TCR signaling pathways after acute activation [5]. For instance, TNF receptor-associated factor 6 (TRAF6/RNF85), identified as another hit from the author's CRISPR screen, acts as a positive regulator by being recruited to the immunological synapse to facilitate K63-linked ubiquitylation of the adapter protein-linker for activation of T cells (LAT), enhancing TCR signaling and nuclear factor of activated T cells (NFAT) activation. Casitas B-lineage lymphoma-b (Cbl-b), is the primary gatekeeper of T-cell activation. It dampens signaling by ubiquitylating the p85 regulatory subunit of phosphoinositol-3 kinase (PI3K) and phospholipase gamma 1 (PLCγ1), and by promoting TCR internalization. Loss of Cbl-b allows $CD8^+$ T cells to activate without CD28 co-stimulation, significantly enhancing anti-tumor immunity. Ubiquitin E3 ligase Itchy homolog (ITCH), a HECT-type ligase that negatively regulates TCR signaling, induces K33-linked polyubiquitylation of TCR-ζ, which hinders its interaction with zeta-chain-associated protein kinase 70 (ZAP70) without causing degradation. Gene related to anergy in lymphocytes (GRAIL/RNF128) is a transmembrane ligase localized in endosomes that facilitates T-cell anergy. It ubiquitylates and degrades the CD3ζ chain and activates RhoGDI through non-degradative ubiquitin chains, which impairs cytoskeleton rearrangement and IL-2 secretion. The E3 ligase NRDP1 impairs proximal TCR signaling by adding K33-polyubiquitin chains to ZAP70. This modification recruits phosphatases (STS1/STS2) that inactivate ZAP70 (Fig. 1). All these ubiquitin E3 ligase-dependent processes are proximal to the TCR-CD3 complex. Therefore, the discovery of KLHL6's effects on chronic T-cell stimulation acting further downstream, offers a novel molecular snapshot into how T-cell exhaustion might be controlled.

KLHL6 has been previously described to play a role in B-cell receptor (BCR) signaling and B-lymphocyte activation including the development of germinal centres [6]. The present reports now extend its function to progenitor-exhausted and memory-like T lymphocytes, where it is selectively lost in terminally exhausted cells following repeated stimulation. Mechanistically, they identified forkhead box protein O1 (FOXO1) as a direct transcriptional activator of *Klhl6*. Chronic TCR signaling

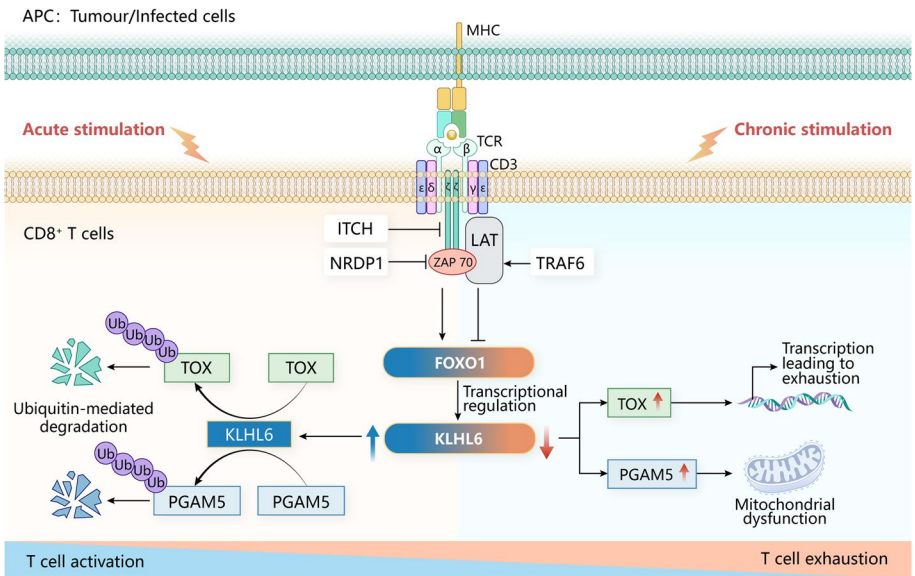

Fig. 1 KLHL6 ubiquitin E3 ligase as key switch for T-cell exhaustion. Schematic diagram illustrating acute versus chronic TCR engagement that leads to $CD8^+$ T functional activation (in blue) versus terminal cell exhaustion (in red). APC: antigen presenting cell; FOXO1: Forkhead box O1 transcription factor; ITCH: Itchy homolog; KLHL6: Kelch-like protein 6 ubiquitin E3 ligase; LAT: linker for activation of T cells; MHC: major histocompatibility complex; NRDP1: neuregulin receptor degradation protein-1; PGAM5: Phosphoglycerate mutase family member 5; TCR: T cell receptor; TOX: Transcription factor Thymocyte selection-associated high mobility group box protein; TRAF6: TNF receptor-associated factor 6; Ub: Ubiquitin; ZAP70: zeta-chain (TCR) associated protein kinase 70

activates the PI3K-AKT pathway, leading to FOXO1 phosphorylation, nuclear exclusion, and loss of promoter occupancy, thereby transcriptionally silencing *Klhl6*. To understand mechanism of action, the authors used a ubiquitin E3 proximity substrate labelling approach (E-STUB) to discover KLHL6 ubiquitin E3 ligase targets such as the transcription factor thymocyte selection-associated high mobility group box protein (TOX). TOX ubiquitylation and degradation attenuates transition from progenitor to terminally exhausted T-cells. In addition, ubiquitylation of the phosphoglycerate mutase family member 5 (PGAM5) leads to changes in mitochondrial morphology and the preservation of performance [3]. Deletion of *Klhl6* in mice increased TOX and PGAM5 levels and consequentially reduced T-cell responses to tumors. This could be rescued by triggering KLHL6 expression to reactivate exhausted T-cells, which improved anti-tumoral immunity. Together, these findings establish KLHL6 as a central driver of T-cell fate and a critical player in anti-tumor immune responses.

As the authors have discussed, their discoveries offer a novel clinical route to reversing T-cell exhaustion in cancer immunotherapy by reactivating KLHL6 expression. Treating T cells with an AKT inhibitor AKTi-1/2 proved effective in restoring KLHL6 expression by modulating the PI3K-AKT-FOXO1 axis [4]. Importantly, KLHL6 itself harbours ligandable pockets that could be exploited by small molecule activators. For example, molecular glues have been designed to stabilise the interaction of β-catenin with its cognate E3 ligase Skp1-Cullin-F-box and β-transducin repeat-containing protein ($SCF^{\beta\text{-}TrCP}$) [7], and could be similarly developed to enhance KLHL6 interactions with TOX and PGAM5. Alternatively, the ubiquitin E3 ligase activity of KLHL6 depends on its BTB domain interaction with cullin3 (CUL3) which enables its assembly into a Cullin-RING Ligase (CRL3) complex. Studies on the KLHL6 paralog, Kelch-like ECH-associated protein 1 (KEAP1), have identified a covalent binder of the BTB domain that acts to increase CUL3 binding affinity resulting in enhanced E3 ligase activity [8]. The small molecule compound derivative VVD-130037, referred as an "allosteric" molecule glue, is being investigated in phase 1 clinical trials. The clinical strategy is to explore cancers with high nuclear factor erythroid 2-related factor 2 (NRF2) levels with the aim to degrade NRF2. Conceptually, a similar approach could be developed for KLHL6.

Alternatively, proteolysis-targeting chimeras (PROTACs) that target many KLHL6 substrates for ubiquitylation and subsequent degradation, including TOX and PGAM5, could be developed to reactivate the function of tumor infiltrating lymphocytes. In addition to immunomodulatory effects in T-cells, KLHL6 also affects the degradation of CD79B (subunit of the BCR complex), Roquin2 and NOTCH2, critical for B-cell function and germinal centre formation [9]. Therefore, developing specific small-molecule agents that enhance KLHL6 expression or activity may offer further benefits by acting on both B-cell and T-cell functions, thereby having a potentially greater impact on adaptive immune responses against tumors and pathogen infections.

Furthermore, ways to activate E3 ligase activity may be considered by enforcing KLHL6- deubiquitylase (DUB) interactions to stabilize KLHL6 protein levels. DUBs associated with KLHL6 are currently not known. However, loss of *KLHL6* promotes diffuse large B-cell lymphoma (DLBCL) growth and survival by stabilizing the mRNA decay factor Roquin2 [9]. A similar DLBCL signature involves OTULIN, a DUB involved in trimming linear ubiquitin chains [10], suggesting a potential cross-connection through intersecting ubiquitin pathways. More generally, the consideration of other E3 ligases and DUBs through targeted protein degradation or stabilisation may offer novel therapeutic opportunities in cancer immunotherapy.

In summary, the unexpected discovery of KLHL6 ubiquitin E3 ligase having a specific role in regulating T-cell exhaustion, next to its previously characterised function in B-cell biology, has tangible consequences for antitumor immunity. FOXO1 and KLHL6 deficiencies drive profound exhaustion, impaired cytokine production and loss of central memory T-cells, which subsequently lead to poor tumor control. Remarkably, enforced KLHL6 expression substantially rescues these defects, positioning KLHL6 as a critical downstream effector of FOXO1-mediated T cell fitness.

Acknowledgements
We thank Professor Gu Yan for expert help with the conceptual design and artwork shown in Fig. 1.

Authors' contributions
B.M.K. conceptualised the research highlight and wrote the initial draft of the paper. Z.C. and A.N.B. made comments and edited the manuscript.

Funding
This work was supported by Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Science (2024-I2M-2–001-1).

Data availability
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 10 January 2026 Revised: 12 February 2026 Accepted: 19 February 2026
Published online: 05 March 2026

References

1. Zemek RM, Anagnostou V, da Pires Silva I, et al. Exploiting temporal aspects of cancer immunotherapy. Nat Rev Cancer. 2024;24:480–97.
2. Sharma P, Goswami S, Raychaudhuri D, et al. Immune checkpoint therapy—current perspectives and future directions. Cell. 2023;186:1652–69.
3. Cheng H, Su Y, Pan X, et al. The ubiquitin ligase KLHL6 drives resistance to CD8+ T cell dysfunction. Nature. 2026. https://doi.org/10.1038/s41586-025-09926-8.
4. Pan X, Pan Y, Su Y, et al. Chronic TCR signaling-driven suppression of the FOXO1-KLHL6 axis promotes T cell exhaustion. Immunity and Inflammation. 2026;2(1):8.
5. Cammann C, Israel N, Slevogt H, Seifert U. Recycling and reshaping—E3 ligases and DUBs in the initiation of T cell receptor-mediated signaling and response. Int J Mol Sci. 2022;23:3424.
6. Kroll J, Shi X, Caprioli A, et al. The BTB-kelch protein KLHL6 is involved in B-lymphocyte antigen receptor signaling and germinal center formation. Mol Cell Biol. 2005;25:8531–40.
7. Simonetta KR, Taygerly J, Boyle K, et al. Prospective discovery of small molecule enhancers of an E3 ligase-substrate interaction. Nat Commun. 2019;10:1402.
8. Roy N, Wyseure T, Lo IC, et al. A covalent allosteric molecular glue suppresses NRF2-dependent cancer growth. Cancer Discov. 2025. https://doi.org/10.1158/2159-8290.CD-25-1187.
9. Choi J, Lee K, Ingvarsdottir K, et al. Loss of KLHL6 promotes diffuse large B-cell lymphoma growth and survival by stabilizing the mRNA decay factor roquin2. Nat Cell Biol. 2018;20:586–96.
10. Li Y, Zhao S. A novel ubiquitination-based molecular signature predicts prognosis in diffuse large B-cell lymphoma. Ann Hematol. 2025;104:5201–15.

Publisher's Note