PBK phosphorylates MSL1 to elicit epigenetic modulation of CD276 in nasopharyngeal carcinoma

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Abstract

CD276 (also known as B7-H3, an immune checkpoint molecule) is aberrantly overexpressed in many cancers. However, the upregulation mechanism and in particular, whether oncogenic signaling has a role, is unclear. Here we demonstrate that a pro-oncogenic kinase PBK, the expression of which is associated with immune infiltration in nasopharyngeal carcinoma (NPC), stimulates the expression of CD276 epigenetically. Mechanistically, PBK phosphorylates MSL1 and enhances the interaction between MSL1 and MSL2, MSL3, and KAT8, the components of the MSL complex. As a consequence, PBK promotes the enrichment of MSL complex on CD276 promoter, leading to the increased histone H4 K16 acetylation and the activation of CD276 transcription. In addition, we show that CD276 is highly upregulated and associated with immune infiltrating levels in NPC. Collectively, our findings describe a novel PBK/MSL1/CD276 signaling axis, which may play an important role in immune evasion of NPC and may be targeted for cancer immunotherapy.

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common head and neck cancers in southeast Asia and north Africa1,2. The standard treatment for patients with NPC is concurrent chemoradiation preceded or followed by systemic chemotherapy, according to the National Comprehensive Cancer Network (NCCN) guidelines. Although the local control rate has been significantly improved, approximately 30–40% of patients with locoregionally advanced NPC eventually develop distant metastasis after receiving radical treatment3. Novel strategies are alarmingly needed for NPC patients with a high risk of distant metastasis.

Evading immune destruction is described as the hallmark of cancer4. NPC originated from the epithelium of the nasopharyngeal, which is regarded as a highly immunogenic tumor characterized by heavy tumor-infiltrating lymphocytes (TILs), therefore at one time called "lymphoepithelial neoplasia"5. Furthermore, Epstein–Barr virus (EBV) infection is a major risk factor for the development of NPC in the endemic regions6,7, upregulation of programmed cell death-ligand 1 (PDL1) was found in EBV-driven NPC cells, it is reported that PDL1 (CD274) expression on NPC is associated with a poor outcome8, this suggests that the development of NPC may be closely related to the immune escape of tumor cells. In pursuit of the novel effective treatment, several single-arm trials investigate that PD1 (CD279) inhibitors are effective in only 20–30% of recurrent or metastatic NPC patients9,10, it is urgent to develop more biomarkers to reconstruct the immune surveillance, which would be exceedingly beneficial for the clinical intervention.
CD276, also known as B7–H3, is a type I membrane protein with its sequence similarity to the extracellular domain of other B7 family members, which modulate T-cell function in a co-stimulatory or co-inhibitory manner. The CD276 protein is rarely expressed and is only found at low levels in normal human tissues. By contrast, recent studies found aberrant high CD276 expression on the many common malignancies, including stomach, lung, prostate, kidney, ovary, and endometrium. The high expression of CD276 was also correlated with advanced disease and poor outcome. Moreover, CD276 protein is also frequently overexpressed on tumor vessels of human lung, breast, colon, endometrial, renal, and ovarian cancer, but not in the angiogenic tumor vessels of human lung, breast, colon, endometrial, renal, and ovarian cancer.

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Moreover, CD276 protein is also frequently overexpressed on tumor vessels of human lung, breast, colon, endometrial, renal, and ovarian cancer, but not in the angiogenic vessels of the normal ovary. Notably, an increasing number of studies support a pro-oncogenic role for CD276 in various human cancer types that is independent of its immune function. Overexpression of CD276 makes it an attractive target for the development of therapeutic agents, and in fact, CD276-targeting therapies are currently under clinical investigation in several children and adult tumors. The level of TILs is an independent predictor of sentinel lymph node status and survival in many cancers including NPC.

To explore the role of PBK in immune regulation of NPC, we investigated whether PBK expression was correlated with immune infiltration levels in NPC. ImmuCellAI, a highly accurate method, was used to estimate the infiltration abundance of immune cells from RNA-seq expression profiles of two NPC cohorts (GSE102349 and GSE68799). Interestingly, correlation analysis showed that PBK expression was significantly correlated to the infiltration of neutrophil and monocyte cells (Fig. 1a), which were reported to promote the immune evasion of tumors, but negatively correlated to the infiltration of natural killer (NK) and B cells (Fig. 1b), which were reported to repress the immune evasion of tumors. Moreover, ImmuCellAI was designed to estimate the abundance of 18 T-cell subsets that are major players in the tumor microenvironment. Notably, PBK expression has significant correlations with CD8-naive cell, nTreg cell, and Th2 cell (Fig. 1c), but was negatively correlated to the infiltration of Th1 cell, CD4+ T cell, and Th17 cell (Fig. 1d). Based on these results, it is supposed that high PBK expression is closely associated with compromised immune microenvironment in NPC.

PBK regulates the transcription of CD276

Given the pivotal roles of T-cell subsets in cancer initiation, progression, and immunotherapy, we focus on how PBK modulates the infiltration of T-cell subsets. Accumulating evidence has indicated that cancer cells could upregulate a series of immune inhibitory factors to repress the proliferation, cytokine production, and effector activities of T cells, in turn leading to immune evasion. Thus, we investigated whether previously reported immune inhibitory molecules of T cells, including PDL1 (CD274), PDL2 (CD273), CD276, IL10, and IDO1, could be downstream targets of PBK.

The results of quantitative polymerase chain reaction (qPCR) showed that only CD276 was significantly downregulated in CNE2 cells after PBK knockdown by siRNA in our primary screening (Fig. 2a and Fig. S1). Further, qPCR and immunoblotting assays revealed that the mRNA and protein level of CD276 were downregulated in CNE2 and SUNE1 cells with PBK depletion by two siRNAs (Fig. 2b and Fig. S2a), but were upregulated in HK1 and CNE1 cells with PBK overexpression (Fig. 2c and Fig. S2b), suggesting that CD276 is a bona fide downstream target of PBK.

To investigate whether the PBK kinase activity is indispensable for the regulation of CD276, we treated CNE2 and SUNE1 cells with OTS514, a PBK kinase-specific inhibitor, followed by qPCR and immunoblotting.
assays, and found that compared to DMSO treatment, OTS514 treatment decreased the mRNA and protein level of CD276 in a dose-dependent manner (Fig. 2d and Fig. S2c). According to previous reports, we construct two PBK mutants, including kinase-active mutants (PBK-T198E) and kinase-dead mutants (PBK-T198A). Both qPCR and immunoblotting assays revealed that PBK-WT and PBK-T198E but not PBK-T198A could rescue the repression of CD276 mediated by a PBK siRNA that targets the 3′ UTR region (Fig. 2e and Fig. S2d). These data

Fig. 1 PBK mRNA level is associated with immune cell infiltration. A The scatterplots show that the mRNA level of PBK (lymphokine-activated killer T-cell-originated protein kinase) is significantly correlated with the infiltration of neutrophil cells (left), monocyte cells in NPC (nasopharyngeal carcinoma). B The scatterplots show that the mRNA level of PBK is negatively correlated with the infiltration of NK (natural killer) cells (left) and B cells in NPC. C The scatterplots show that the mRNA level of PBK is significantly correlated with the infiltration of CD8-naive cell (left), nTreg cell (middle), and Th2 cell. D The scatterplots show that the mRNA level of PBK is negatively correlated with the infiltration of Tfh cell (left), CD4+ T cell (middle), and Th17 cell. The method called ImmuCellAI was used to estimate the abundance of 24 immune cell types from two integrated NPC RNA-seq data (GSE102349, GSE68799). A Pearson correlation test was used (two-tailed) (n = 159).
show that PBK regulates CD276 transcription dependent on its kinase activity.

Furthermore, correlation analysis showed that the mRNA expression of PBK and CD276 is positively correlated in our NPC cohort detected by qPCR ($r = 0.45$, $n = 41$, $p = 0.0018$, Fig. 2f) as well as in the NPC RNA-seq dataset ($r = 0.32$, $n = 113$, $p = 0.0006$, Fig. 2g). Notably, pan-cancer correlation analysis between PBK and CD276 was conducted using the mRNA expression data from TCGA. The result reveals that the mRNA level of PBK is
positively correlated with CD276 in several common cancers (Fig. S3), including adrenocortical carcinoma, brain lower-grade glioma, kidney renal clear-cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, and thyroid carcinoma, indicating that this is a general mechanistic upregulation of CD276 by PBK in cancers.

PBK regulates CD276 transcription dependent on MSL1
Considering that PBK is a kinase, not a transcription regulator, there is a mediator involved in CD276 transcription regulation by PBK. In order to identify the mediator, we carried out immunoprecipitation and mass spectrometry analysis and identified MSL1 as an interacting partner of PBK (Fig. 3a). Co-immunoprecipitation assays validated the exogenous interaction between PBK and MSL1 in 293T cells (Fig. 3b). To further confirm the in vivo interaction, total proteins from CNE2 and SUNE1 cells were extracted, and immunoprecipitation was performed with an antibody against PBK followed by immunoblotting with an antibody against MSL1, demonstrating that MSL1 was efficiently co-immunoprecipitated with PBK (Fig. 3c). Since PBK has serine/threonine kinase activity, we investigated whether MSL1 is a phosphorylation target of PBK. Immunoprecipitation assays using an antibody against phosphorylated serine/threonine followed by immunoblotting analysis using MSL1 antibody demonstrated that the phosphorylation level of MSL1 was enhanced in PBK-overexpression cells (Fig. 3d and Fig. S4a), but was decreased in PBK-knockdown cells (Fig. 3e and Fig. S4b) as well as in cells treated with PBK inhibitor (Fig. 3f and Fig. S4c). These data show that PBK indeed interacts with and phosphorylates MSL1.

Furthermore, qPCR and immunoblotting results showed that MSL1 depletion decreased the expression of CD276 (Fig. 3g and Fig. S4d) and MSL1 overexpression increased the expression of CD276 (Fig. 3h and Fig. S4e), suggesting that MSL1 is involved in the transcriptional regulation of CD276. More importantly, the knockdown of MSL1 repressed the upregulation of CD276 mediated by PBK overexpression (Fig. 3i and Fig. S4f). MSL1 contains multiple serine/threonine residues, six of which (Ser66, Ser126, Ser205, Ser393, Thr396, and Ser442) were reported as phosphorylation sites. Given that the target sites of MSL1 phosphorylated by PBK are unknown, we replaced all of those MSL1 serine/threonine residues noted above individually with an alanine residue (S/T > A-MSL1). The result of qPCR showed that MSL1 wild-type but not MSL1-S/T > A mutant overexpression could rescue the downregulation of CD276 after PBK depletion in CNE2 and SUNE1 cells (Fig. 3j and Fig. S4g), indicating that PBK controls the transcription of CD276 dependent on the phosphorylation status of MSL1.

PBK promotes the enrichment of MSL complex on CD276 promoter
MSL1 acts as a scaffold to tether MSL2, MSL3, and KAT8 (MOF) together for the formation of the MSL histone acetyltransferase complex, in turn leading to histone H4 Lys16 acetylation (forming H4K16ac), thereby efficiently increasing the gene expression. Apart from the dosage-compensation role of the MSL complex, accumulating evidence suggests that MSL proteins may be involved in additional functions, such as transcription regulation. To address the role of PBK in the MSL complex, we conducted immunoprecipitation experiments using an antibody against MSL1 followed by immunoblotting with antibodies against the components of the MSL complex in PBK-overexpression or PBK-knockdown cells, respectively. The results demonstrated that PBK overexpression could enhance the physical association between MSL1 and MSL2, MSL3, and KAT8 (Fig. 4a and Fig. S5a), whereas PBK depletion obviously inhibited these interactions (Fig. 4b and Fig. S5b). Moreover, quantitative chromatin immunoprecipitation (qChIP) analysis using specific antibodies against MSL1 or KAT8 showed that the occupancy of MSL1 and KAT8 on the CD276 promoter region is increased in PBK-overexpression cells (Fig. 4c) but decreased in PBK-deficient cells (Fig. 4d). Consistently, the acetylation level of histone H4 Lys16 on the promoter region is increased in PBK-overexpression cells but decreased in PBK-deficient cells, as indicated by qChIP results (Fig. 4e, f).

High expression of CD276 is associated with the immune infiltration in NPC
Since the role of CD276 in NPC remains unknown, we performed a qPCR assay and found that CD276 mRNA expression in NPC tissues was higher than in the noncancerous NP tissues (Fig. 5a). In addition, in agreement with our qPCR result, gene expression profiles derived from the GEO (GDS3341, GSE40290, and GSE53819) showed that compared to noncancerous NP tissues, the CD276 mRNA level was obviously elevated in the NPC tissues (Fig. 5b). Previous reports showed that CD276 has been associated with co-stimulatory as well as co-inhibitory functions in regulating T-cell responses. In NPC, correlation analysis showed that CD276 mRNA level was positively associated with the infiltration level of CD8-naive cell, nTreg cell, and Th2 cell (Fig. 5c), but was negatively associated with the infiltration level of CD4 T cell, Th1 cell, and Th1 cell (Fig. 5d). In addition, CD276 expression has a significant correlation with monocyte and neutrophil cells and a negative correlation with NK and B cells (Fig. 5e). Taken together, our findings demonstrated that CD276 is upregulated in NPC and associated with immune cell infiltration.
PBK reduces T-cell-mediated cytotoxicity dependent on CD276

In order to investigate whether the PBK/CD276 axis plays an important role in tumor immune evasion, we conducted the cytotoxic T-lymphocyte assay. Since the NPC cell lines used above did not express HLA-A2 that is important for tumor cell recognition by HLA-A2+ T cells, we selected another NPC cell line TW03 with HLA-A2 positive for the cytotoxic T-lymphocyte assay. The results showed that PBK knockdown significantly increased the percentage of lysis TW03 cells when the tumor cells were cocultured with CD3/CD28-activated
expression. Simultaneously, the in
NPC based on the results that the expression of
CD276
mRNA expression. These correlation patterns are at least
further to be elucidated.

especially anti-PD1 and anti-PDL1 antibodies, have
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tumor-repressing leukocytes, including NK, B, Tfh, CD4
and Th2 cells, were positively correlated with

PBK, a dual-specificity serine/threonine kinase, plays
an important role in the activation of the Raf/MEK/ERK
pathway to promote cell proliferation, colony formation,
and cancer development. For example, PBK facilitates
transformation by upregulating and activating ERK2
through an increase in AP-1 (c-Jun) transcriptional
activity and increased tumorigenesis of HCT116 color-
rectal cancer33,71. PBK can also directly bind to and
phosphorylate AP-1 (c-Jun) after solar ultraviolet expo-
sure33,73. Besides a potential central player of the MAPK
cascade mechanism, PBK binds with other proteins and
promotes various cancer-related processes, such as PBK
promotes tumor dissemination by direct phosphoryla-
tion of p53-related protein kinase (PRPK)74. PBK reg-
ulates autophagy by phosphorylatingULK1 and
promotes glioma resistance to TMZ (temozolomide)75.
Thus, more research is necessary to identify potential
downstream targets of PBK. Herein, we identify MSL1 as
a previous unknown substrate of PBK. Indeed, PBK
phosphorylates MSL1 and promotes the formation of the
MSL complex. However, the speci-
cification levels of diverse immune cells in NPC. Speci-
cifically, the infiltration levels of tumor-promoting leuko-
cytes, including neutrophil, monocyte, CD8-naïve, nTreg,
and Th2 cells, were positively correlated with PBK mRNA
expression. Simultaneously, the infiltration levels of
tumor-repressing leukocytes, including NK, B, Tfh, CD4+
T cells, and Th17, were negatively correlated with PBK
mRNA expression. These correlation patterns are at least
partly attributed to the transcription regulation of the
CD276 gene by PBK. Although the role of CD276 in
antitumor immunity has been controversial with con-
flicting co-stimulatory and co-inhibitory functions,
CD276 may exert a protumor effect on tumor progression
in NPC based on the results that the expression of CD276
is much higher in tumor samples than normal samples
and is associated with the compromised immune micro-
environment. Since several immunomodulatory agents,
especially anti-PD1 and anti-PDL1 antibodies, have
shown great promise in treating advanced NPC, the role
of the PBK/CD276 axis in immunotherapy response is
further to be elucidated.
Fig. 4 PBK promotes the formation of MSL complex on the promoter of CD276. A Immunoprecipitation assays with MSL1 antibody and immunoblotting analyses with the indicated antibodies were performed in PBK-overexpression stable cell lines. Left, CNE1, right, HK1. B CNE2 and SUNE1 cells were transfected with PBK siRNAs or NC (nonspecific siRNA) for 48 h. Immunoprecipitation assays with MSL1 antibody and immunoblotting analyses with the indicated antibodies were performed. Left, CNE2, right, SUNE1. C ChIP-qPCR (chromatin immunoprecipitation-quantitative PCR) analysis of CD276 promoter using antibodies against MSL1 and KAT8 in PBK-overexpression or control cell lines. Left, CNE1, right, HK1. D ChIP-qPCR analysis of CD276 promoter using antibodies against MSL1 and MOF in PBK-knockdown or control cells. Left, CNE2, right, SUNE1. E, F ChIP-qPCR analysis of CD276 promoter using antibodies against H4K16ac (acetylation of histone H4 on lysine 16) in PBK-overexpression (E) or -knockdown cells (F). In C–F, ChIP-qPCR results were presented as the fold change over the vector/IgG or NC/IgG group. Error bars represent the mean ± SD (standard deviation) for three biological replicates. ***P < 0.001 (student’s t test for two groups, one-way ANOVA for three groups). All the experiments were performed independently three times with similar results and the data are representative of three independent experiments.
CD276 is upregulated and associated with immune infiltration in NPC. A The graphs show that CD276 is significantly upregulated in NPC samples (n = 41) compared to normal samples (n = 20) via qPCR analysis. ***P < 0.001 was determined by Student’s t test. B The graphs show that CD276 is significantly upregulated in NPC samples compared to normal samples, as indicated by four public datasets from GEO (Gene Expression Omnibus database). **P < 0.01, ***P < 0.001 was determined by Student’s t test. C Correlation analysis between the CD276 mRNA level and the tumor-promotion T-cell subset infiltration, including CD8-naive cell (left), nTreg cell (middle), and Th2 cell using publicly available NPC RNA-seq data. A Pearson correlation test was used (two-tailed). D Correlation analysis between the CD276 mRNA level and the tumor-repression T-cell subset infiltration, including CD4 T cell (left), Tfh cell (middle), and Th1 cell using publicly available NPC RNA-seq data. A Pearson correlation test was used (two-tailed). E Correlation analysis between the CD276 mRNA level and the infiltration level of monocyte, neutrophil, NK, and B cells using publicly available NPC RNA-seq data. A Pearson correlation test was used (two-tailed).
Specifically, PBK phosphorylates MSL1 and promotes the enrichment of MSL complex on the promoter region of CD276, in turn leading to an increase in histone H4 Lys16 acetylation, thereby activating the transcription of CD276 and suppressing the cytotoxic T-cell function (Fig. 6C). Further investigations need to be conducted to clarify which transcription factors are essential for CD276 transcription.
In summary, what is noteworthy is that this is the first demonstration of PBK modulation of a co-inhibitory signal CD276 induction to escape from immunosurveillance in NPC. Our results highlight the potential clinical benefits of targeting both PBK and CD276 in NPC patients with a high risk of progression.

Materials and methods

Cell culture
The 293T cells and the human NPC cell lines, including CNE2, SUNE1, HK1, and CNE1, were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS at 37°C and 5% CO2. All the cell lines were tested for negative mycoplasma contamination.

Sample collection
Fresh frozen tissues for qPCR analysis were resected from histopathologically and clinically diagnosed NPC patients. All samples were obtained from the tumor resource bank of Guangzhou Medical University Affiliated Cancer Hospital and Institute. All patients supplied informed consent.

Plasmids and mutagenesis
Full-length human PBK cDNA was amplified and cloned into a pcDNA6B-his-myc vector (ECORI and XholI) or plenti-puro vector (ECORI and XholI). Full-length human MSL1 cDNA was amplified and cloned into pCMV-N-flag (BamHI and ECORI). ClonExpress II One Step Cloning Kit (Vazyme) was used according to the manufacturer’s instructions.

PBK(T198E), and pCMV-N-MSL1(S/T > A) mutants were constructed using a QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer’s instructions. The sequences were as follows:

PBK forward, 5′-GGAGAATGCAGGAGCTGAG-3′
PBK reverse, 5′-ACATGTCAGGCTGAGGCTA-3′

CD276 forward, 5′-GGGAATACGAGCTGAGGAGG-3′
CD276 reverse, 5′-CCCTTCATACACCAGACCG-3′

PDL1 forward, 5′-ACATGTCAGGCTGAGGCTA-3′
PDL1 reverse, 5′-TTGTTGTTGTTGCTTACCTAC-3′
PDL2 forward, 5′-CAGGTGGTGGTTCTTCATACCTAC-3′
PDL2 reverse, 5′-GGTGGTGGTTCTTCATACCTAC-3′
IDO1 forward, 5′-CAGGGTGTTGTTCTACCTGC-3′
IDO1 reverse, 5′-CCCTCTACCTGAGGAGGAGG-3′
IL10 forward, 5′-ATCAAGCGGAGCTGAGGAGGAGG-3′
IL10 reverse, 5′-CATACCTGAGGAGGAGGAGGAGG-3′

siRNA transfection
The specific siRNAs and control nonspecific siRNA (NC) were obtained from Guangzhou RiboBio Co., Ltd. Cancer cells were counted and seeded into 6-well plates with 2 × 105 cells/well. After 24 h, the cells were 30–40% confluent and transfected with siRNAs using RNAiMAX transfection reagents (Invitrogen) according to the manufacturer’s instructions. The cells were harvested for further experiments after 24 or 48 h. The siRNA targeting sequences were as follows:

PBK#1: 5′-GAATATGGCAAGAGGGTTAAA-3′
PBK#2: 5′-GGGAATACGAGCCACCTATTAA-3′
PBK#3: 5′-GAAGTGTTGCTGTAATAAATA-3′
MSL1#1: 5′-CACCAGGATGAGAATAGGAGGAATAG-3′
MSL1#1: 5′-ATGTTATCAGTCTGTAATAATGGA-3′

RNA extraction and qPCR
Total RNA was extracted using the TRIzol Reagent (Invitrogen) according to the manufacturer’s instructions. Reverse transcription was performed using a cDNA Synthesis Kit (Thermo, K1622). The quantitative real-time polymerase chain reaction was conducted using ChamQ SYBR Color qPCR Master Mix (Vazyme, Q411-02). The sequences of the PCR primers used for amplification were as follows:

ACTB forward, 5′-AAGGTCATCCCTAGAGCTGA-3′
ACTB reverse, 5′-TGACAAAGTGTTGAGTTAGG-3′
PDK forward, 5′-GGCGAAGACTCTCGAGAC-3′
PDK reverse, 5′-CTGCATAAAGGGAGGCCCG-3′
CD276 forward, 5′-GGGAATACGAGCTGAGGAGG-3′
CD276 reverse, 5′-GGCAAGGTAGGAGGCTGAGG-3′
PDL1 forward, 5′-ACATGTCAGGCTGAGGCTA-3′
PDL1 reverse, 5′-TTGTTGTTGTTGCTTACCTAC-3′
PDL2 forward, 5′-CAGGTGGTGGTTCTACCTAC-3′
PDL2 reverse, 5′-GGTGGTGGTTCTACCTGC-3′
IDO1 forward, 5′-CAGGGTGTTGTTCTACCTGC-3′
IDO1 reverse, 5′-CCCTCTACCTGAGGAGGAGG-3′
IL10 forward, 5′-ATCAAGCGGAGCTGAGGAGGAGG-3′
IL10 reverse, 5′-CATACCTGAGGAGGAGGAGGAGG-3′

Immunoblotting
Protein lysates were electrophoresed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).
Co-immunoprecipitation and mass spectrometry analysis

For co-immunoprecipitation (co-IP), treated cells were lysed in IP lysis buffer (50 mM Tri-Cl (pH 7.4), 150 mM NaCl, 0.5% NP-40, and 5 mM EDTA) supplemented with PMSF (Sigma, USA) and protease inhibitor cocktail (Roche). The cell lysates were incubated with the indicated antibodies or Flag-beads (Sigma) overnight at 4 °C and washed with lysis buffer 4 times. Proteins were eluted and detected using immunoblotting assays with mouse antibody to Flag (Sigma, M2), an antibody to MSL1, and other appropriate antibodies.

For mass spectrometry analysis, 293T cells were transfected with Flag-PBK or vector control for 48 h. Then treated cells were lysed in IP lysis buffer (50 mM Tri-Cl (pH 7.4), 150 mM NaCl, 0.5% NP-40, and 5 mM EDTA) supplemented with PMSF (Sigma, USA) and protease inhibitor cocktail (Roche) and incubated with Flag-tagged affinity agarose beads (Sigma, M2) overnight at 4 °C. The beads were then washed 4 times with IP lysis buffer. The immunoprecipitates were eluted and separated by SDS-PAGE and then stained with silver staining, and the indicated bands were subjected to mass spectrometry analysis.

qChIP assay

The ChIP assays were carried out with a ChIP assay kit (Upstate Biotechnology, Lake Placid, NY, USA) according to the manufacturer’s instructions. The cultured NPC cells were fixed with 1% formaldehyde for 10 min at room temperature. Then, the ultrasonic breaker was set to 10 s per ultrasonic cycle with 10-s intervals with 15 cycles. Subsequently, the fragments underwent centrifugation (30,237×g) at 4 °C (part of the DNA fragments were used as input). The supernatant was collected and added with IgG control (1 µg/ml) or indicated antibodies, including anti-MSL1 (1 µg/ml, MerckMillipore, ABE469), anti-MOF (1 µg/ml, PTG, 13842-1-AP), and anti-H4K16ac (1 µg/ml, CST, #13534), followed by incubation at 4 °C overnight. Protein Agarose/Sepharose was used to precipitate the endogenous DNA–protein complex. After a short period of centrifugation (1000×g), the supernatant was removed, and the nonspecific complex was washed. Following de-cross-linking at 65 °C overnight, DNA fragments were extracted, purified, and retrieved with phenol/chloroform.
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References

1. Caniò, G. et al. Global trends in nasopharyngeal cancer mortality since 1970 and predictions for 2020. focus on low-risk areas. Int. J. Cancer 140, 2256–2264 (2017).
2. Ferlay, J. et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer 136, E359–E386 (2015).
3. Hui, E. P. et al. Lung metastasis alone in nasopharyngeal carcinoma: a relatively favorable prognostic group. A study by the Hong Kong Nasopharyngeal Carcinoma Study Group. Cancer 101, 300–306 (2004).
4. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. Cell 144, 646–674 (2011).
5. Wang et al. published maps and institutional affiliations.
6. Fang, W. et al. Camrelizumab (SHR-1210) alone or in combination with chemotherapy accompanies this paper at (https://doi.org/10.1038/s41438-020-00293-9).
7. Sun, M. et al. Characterization of mouse and human B7-H3 genes. Front. Immunol. 3, 56–62 (2016).
8. Wu, C. P. et al. Relationship between co-stimulatory molecule B7-H3 expression and gastric carcinoma histology and prognosis. World J. Gastroenterol. 12, 457–459 (2006).
9. Altan, M. et al. B7-H3 expression in NSCLC and its association with B7-H4, PD-L1 and tumor-infiltrating lymphocytes. Clin. Cancer Res. 23, 5202–5209 (2017).
10. Zykova, T. A. et al. T-LAK cell–originated protein kinase (TOPK) phosphorylation of Prx1 at Ser-32 prevents UV-induced apoptosis in RPMI/751 melanoma cells through the regulation of Prx1 peroxidase activity. J. Biol. Chem. 285, 29138–29146 (2010).
11. Herbert, K. J., Ashston, T. M., Prevo, R., Pirovano, G. & Higgins, G. S. T-LAK cell–originated protein kinase (TOPK): an emerging target for cancer-specific therapeutics. Cell Death Dis. 9, 1089 (2018).
12. Wang, L. et al. The potential role of B7-H3 in the regulation of T-cell function. Front. Immunol. 9, 1334 (2019).
13. Hsu, C. et al. Safety and antitumor activity of pembrolizumab in patients with programmed death-ligand 1-positive nasopharyngeal carcinoma: results of the KEYNOTE-028 study. J. Clin. Oncol. 35, 4050–4056 (2017).
14. Fang, W. et al. Carmeloxim (SHR-1210) alone or in combination with gemcitabine plus cisplatin for nasopharyngeal carcinoma: results from two single-arm, phase 1 trials. Lancet Oncol. 19, 1538–1550 (2018).
15. Wang, L., Kang, F. B. & Shan, B. E. B7-H3-mediated tumor immunology: friend or foe? Int. J. Cancer. 134, 2764–2771 (2014).
16. Hofmeyer, K. A., Ray, A. & Zang, X. The contrasting role of B7-H3. Proc. Natl Acad. Sci. USA 105, 10277–10278 (2008).
17. Seaman, S. et al. Erdafitinib of tumors through simultaneous ablation of CD276/B7-H3-positive tumor cells and tumor vasculature. Cancer Cell 31, 501–515 e508 (2017).
18. Wu, C. F. et al. Relationship between co-stimulatory molecule B7-H3 expression and gastric carcinoma histology and prognosis. World J. Gastroenterol. 12, 457–459 (2006).
19. Altan, M. et al. B7-H3 expression in NSCLC and its association with B7-H4, PD-L1 and tumor-infiltrating lymphocytes. Clin. Cancer Res. 23, 5202–5209 (2017).
20. Zykova, T. A. et al. T-LAK cell–originated protein kinase (TOPK) phosphorylation of Prx1 at Ser-32 prevents UV-induced apoptosis in RPMI/751 melanoma cells through the regulation of Prx1 peroxidase activity. J. Biol. Chem. 285, 29138–29146 (2010).
21. Herbert, K. J., Ashston, T. M., Prevo, R., Pirovano, G. & Higgins, G. S. T-LAK cell–originated protein kinase (TOPK): an emerging target for cancer-specific therapeutics. Cell Death Dis. 9, 1089 (2018).
22. Ishikawa, C., Senba, M. & Mori, N. Mitotic kinase PKB/TOPK as a therapeutic target for adult T cell leukemia/lymphoma. Int. J. Oncol. 53, 801–814 (2018).
23. Pirovano, G. et al. TOPK modulates tumour-specific radiosensitivity and correlates with recurrence after prostate radiotherapy. Br. J. Cancer. 117, 503–512 (2017).
24. Wang, M. Y. et al. PDZ2 binding kinase (PKB) is a theranostic target for nasopharyngeal carcinoma: driving tumor growth via ROS signaling and correlating with patient survival. Oncotarget 7, 26604–26616 (2016).
25. Chang, C. F. et al. PBK/TOPK expression predicts prognosis in oral cancer. Int. J. Mol. Sci. 17, 1007 (2016).
26. Kim, D. J. et al. Novel TOPK inhibitor H6-TOPK-032 effectively suppresses colon cancer growth. Cancer Res. 72, 3060–3068 (2012).
27. Matsuoka, Y. et al. TOPK inhibitor induces complete tumor regression in xenograft models of human cancer through inhibition of cytokinesis. Sci. Transl. Med. 6, 259ra145 (2014).
28. Gao, G. et al. ALPHA-07 suppresses solar ultraviolet-induced skin carcinogenesis by directly inhibiting TOPK. Mol. Cancer Ther. 16, 1843–1854 (2017).
29. Azimi, F. et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. J. Clin. Oncol. 30, 2678–2683 (2012).
30. Xiao, X. et al. ImmuCellAI: a unique method for comprehensive T-cell subsets abundance prediction and its application in cancer immunotherapy. Adv. Sci. 7, 1902880 (2020).
31. Szczerba, B. M. et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. Nature 566, 553–557 (2019).
32. Coifet, S. B., Wellenstein, M. D. & de Visser, K. E. Neutrophils in cancer: neutral no more. Nat. Rev. Cancer 16, 431–446 (2016).
33. Olingy, C. E., Dinh, H. Q. & Hedrick, C. C. Monocyte heterogeneity and functions in cancer. J. Leukoc. Biol. 106, 329–332 (2019).
34. Hodgens, J. J., Khan, S. T., Park, M. M., Auer, R. C. & Ardolino, M. Killers: NK cell therapies at the forefront of cancer control. J. Clin. Aver. 129, 3499–3510 (2019).
35. guilell, C., Huntington, N. D. & Smyth, M. J. Targeting natural killer cells in cancer immunotherapy. Nat. Immunol. 17, 1025–1036 (2016).
51. Ding, T., Yan, F., Cao, S. & Ren, X. Regulatory B cell: new member of immunosuppressive cell club. *Hum. Immunol.* **76**, 615–621 (2015).
52. Kochenderfer, J. N. et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J. Clin. Oncol.* **33**, 540–549 (2015).
53. Luckheram, R. V., Zhou, R., Verma, A. D. & Xia, B. CD4(+)-T cells: differentiation and functions. *Clin. Dev. Immunol.* **2012**, 925135 (2012).
54. Kamphorst, A. O. et al. Proliferation of PD-1+CD8 T cells in peripheral blood after PD-1-targeted therapy in lung cancer patients. *Proc. Natl Acad. Sci. USA* **114**, 4993–4998 (2017).
55. Vinay, D. S. et al. Immune evasion in cancer: mechanistic basis and therapeutic strategies. *Semin. Cancer Biol.* **35**, S185–S198 (2015).
56. Wilky, B. A. Immune checkpoint inhibitors: the linchpins of modern immunotherapy. *Clin. Cancer Res.* **20**, 687–692 (2015).
57. Alachkar, H. et al. T-LAK cell-originated protein kinase presents a novel therapeutic target in FLT3-ITD mutated acute myeloid leukemia. *Oncotarget* **6**, 33410–33425 (2015).
58. Ikeda, Y. et al. T-LAK cell-originated protein kinase (TOPK) as a prognostic factor and a potential therapeutic target in ovarian cancer. *Clin. Cancer Res.* **22**, 6110–6117 (2016).
59. Bao, Y. N. et al. Urokinase-type plasminogen activator receptor signaling is critical in nasopharyngeal carcinoma cell growth and metastasis. *Cell Cycle* **13**, 1959–1964 (2014).
60. Zhou, H. et al. Toward a comprehensive characterization of a human cancer cell phosphoproteome. *J. Proteome Res.* **12**, 260–271 (2013).
61. Zykova, T. A. et al. The T-LAK cell-originated protein kinase signal pathway promotes colorectal cancer metastasis. *Elife* **3**, e02104 (2014).
62. Smith, E. R. et al. A human protein complex homologous to the Drosophila MSL complex is responsible for the majority of histone H4 acetylation at lysine 16. *Mol. Cell Biol.* **25**, 9175–9188 (2005).
63. Rohe, E. et al. Suppression of the solar ultraviolet-induced skin carcinogenesis by TOPK inhibitor HI-TOPK-032. *Oncogene* **39**, 4170–4182 (2020).
64. Li, X. et al. The histone acetyltransferase MOF is a key regulator of the embryonic stem cell core transcriptional network. *Cell Stem Cell* **11**, 163–178 (2012).
65. Liu, K. et al. Sunlight UV-induced skin cancer relies upon activation of the p38alpha signaling pathway. *Proc. Natl Acad. Sci. USA* **104**, 17899–17904 (2007).
66. Zhao, J. et al. Bcl-2 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat. Immunol.* **4**, 899–906 (2003).
67. Conrad, T. & Akhtar, A. Dosage compensation in human liver phosphoproteome. *Mol. Cell Biol.* **25**, 9175–9188 (2005).
68. Nygren, M. K. et al. Identifying microRNAs regulating B7-H3 in breast cancer: the clinical impact of microRNA-29c. *Br. J. Cancer* **110**, 2072–2080 (2014).