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HDAC3 modulates cancer immunity via increasing PD-L1 expression in pancreatic cancer

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is the second leading cause of cancer-related deaths worldwide. Despite immune checkpoints based immunotherapy highlights a new therapeutic strategy and achieves a remarkable therapeutic effect in various types of malignant tumors. Pancreatic cancer is one of the non-immunogenic cancers and is resistant to immunotherapy. Programmed death ligand 1 (PD-L1) is expressed on the surface of tumor cells and its level is a key determinant of the checkpoint immunotherapy efficacy. Here, we reported that the specific inhibitor of histone deacetylase 3 (HDAC3) decreased the protein and mRNA level of PD-L1 in pancreatic cancer cells. Furthermore, we showed that HDAC3 was critical for PD-L1 regulation and positively correlated with PD-L1 in PDAC patient specimens. Finally, we demonstrated that HDAC3/signal transducer and activator of transcription 3 (STAT3) pathway transcriptionally regulated PD-L1 expression. Collectively, our data contributes to a better understanding of the function of HDAC3 in cancer immunity and the regulatory mechanism of PD-L1. More importantly, these data suggest that the HDAC3 inhibitors might be used to improve immunotherapy in pancreatic cancer.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the second leading cause of cancer-related deaths worldwide [1,2]. Resistance to conventional therapies, such as traditional chemotherapy, targeted therapy and radiotherapy; are responsible for the poor prognosis of PDAC, with a 5-year survival rate less than 5% [3]. Herein, innovative therapeutic options are urgently needed for prolonging the disease free survival and overall survival rate of PDAC.

Immunotherapy highlights a new therapeutic strategy beyond surgery, chemotherapy, and radiation treatment for cancer [4]. Despite immune checkpoints based immunotherapy, including cytotoxic T lymphocyte-associatedantigen-4 (CTLA-4) and programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1), makes great progress in the treatment of many types of malignancies [5]. However, the monotherapy of immune checkpoints blockade has so far been disappointing in PDAC [6–8].

It has been well documented that the CTLA-4 and PD-1 are located in the T cell surface, but the PD-L1 is expressed in tumor cells. PD-L1 expression is associated with poor prognosis in PDAC [9]. The mechanism underlying pancreatic cancer resistance to anti-PD-1/PD-L1 immunotherapy is still poorly understood, but it has been suggested that the expression level of PD-L1 in tumor cells is a key determinant of checkpoint immunotherapy efficacy [10].

In this study, we sought to explore the novel regulatory mechanism of PD-L1 and search for the small molecular inhibitors that suppress the expression of PD-L1 in pancreatic cancer cells. We found that the specific inhibitor of HDAC3 decreased the protein and mRNA level of PD-L1 in a dose- and time-dependent manner. Moreover, we showed that HDAC3 was critical for PD-L1 regulation and positively correlated with PD-L1 in PDAC patient specimens. Finally, we demonstrated that HDAC3 modulated PD-L1 expression through STAT3 signaling pathway. Taken together, our data contribute to a better understanding of the regulatory mechanism of PD-L1 and suggest that the HDAC3 inhibitors might be used to overcome the resistance of immunotherapy in pancreatic cancer.

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Material and methods

Cell lines, cell transfection and chemicals

The pancreatic cancer cell lines (MIA PaCa-2 and BxPC-3) were purchased from the Chinese Academy of Science Cell Bank and cultured in Dulbecco's Modified Eagle Medium (DMEM) medium (Invitrogen, USA) containing 10% fetal bovine serum (HyClone, USA). All cell lines were routinely maintained in an incubator at 37 °C with 5% CO_2. Transfections were preformed by using the lipid-based method (Lipofectamine 2000, Thermo Fisher Scientific, USA) following the manufacturer's instructions. RGFP966 was purchased from MedChem Express (Shanghai, China).

Western blot

Cells were harvested and lysed with lysis buffer (Beyotime, China) containing 1% protease and phosphatase inhibitors as described previously [11]. The protein concentration was determined with a protein assay kit (Pierce Biotechnology, USA). Equal amounts of protein for each sample were separated using SDS-PAGE gels and transferred onto PVDF membranes (Pierce Biotechnology, USA). The membranes were blocked in 5% non-fat milk for 1 h at room temperature and then incubated with a primary antibody overnight at 4 °C. The membranes were then washed with 1xTBST and incubated with a secondary antibody for 1 h. Finally, the membranes were treated with ECL detection reagents and exposed to X-ray film. The following primary antibodies were used: PD-L1 (Cell Signaling Technology, 13684, USA, dilution 1: 1000), STAT3 (Cell signaling Technology, 9139, USA, dilution 1:1000), p-STAT3 T705 (Cell signaling Technology, 9131, USA, dilution 1:1000), HDAC3 (Cell signaling Technology, 85057, USA, dilution 1:1000), and β-Tubulin (Abcam, ab179513, USA, dilution 1:5000).

Real-time RT-PCR

Total RNA was extracted from cells using Trizol reagent (Thermo Fisher Scientific). First-strand cDNA was synthesized from 2 μg of RNA using a cDNA reverse transcription kit, and the real-time PCR analysis was performed with a PCR kit according to the manufacturer's protocols. The two kits were purchased from Takara Bio Inc. (Shigo, Japan). All signals were normalized against β-Actin, and the 2-△Ct method was used to quantify the fold change [12]. The following primers were used: *PD-L1*, forward 5' - TATGGTGGT GCCGACTACAA -3′ and reverse 5' - TGCTTGTCCAGATGACTTCG -3'; and *β-actin*, forward 5′-CCCTGGCTCCTAGCACCAT -3′ and reverse 5′-AGAGCCACCAATCCACACAGA-3'.

RNA interference

Lentivirus-based control and gene-specific shRNAs were purchased from Sigma-Aldrich. Lipofectamine 2000 was used to transfect 293T cells with shRNA plasmids and viral packaging plasmids (pVSV-G and pEXQV). Then, 24 h post-transfection, the medium was replaced with DMEM containing 10% FBS. The virus culture medium was collected 48 h post-transfection and added to cells. The cells were harvested 48 h after the virus infection and puromycin selection [13]. The following shRNA sequences were used: shHDAC3-˜˜1,CCGGCCTTCCACAAATACGGAAATTCTCGAGAATT TCCGTATTTGTGGAAGGTTTTT; shHDAC3-2,CCGGCAAGAGTCTTAATG CCTTCAACTCGAGTTGAAGGCATTAAGACTCTTGTTTTTTG; shHDAC6-1,CCGGCCTCACTGATCAGGCCATATTCTCGAGAATATGGCCTGATCAGTG AGGTTTTT; shHDAC6-2, CCGGCGGTAATGGAACTCAGCACATCTCGA GATGTGCTGAGTTCCATTACCGTTTTT; shSTAT3-1,CCGGGCAAAGAATC ACATGCCACTTCTCGAGAAGTGGCATGTGATTCTTTGCTTTTT; shSTAT3-2,CCGGGCTGACCAACAATCCCAAGAACTCGAGTTCTTGGGATTGTTGGT CAGCTTTTT.

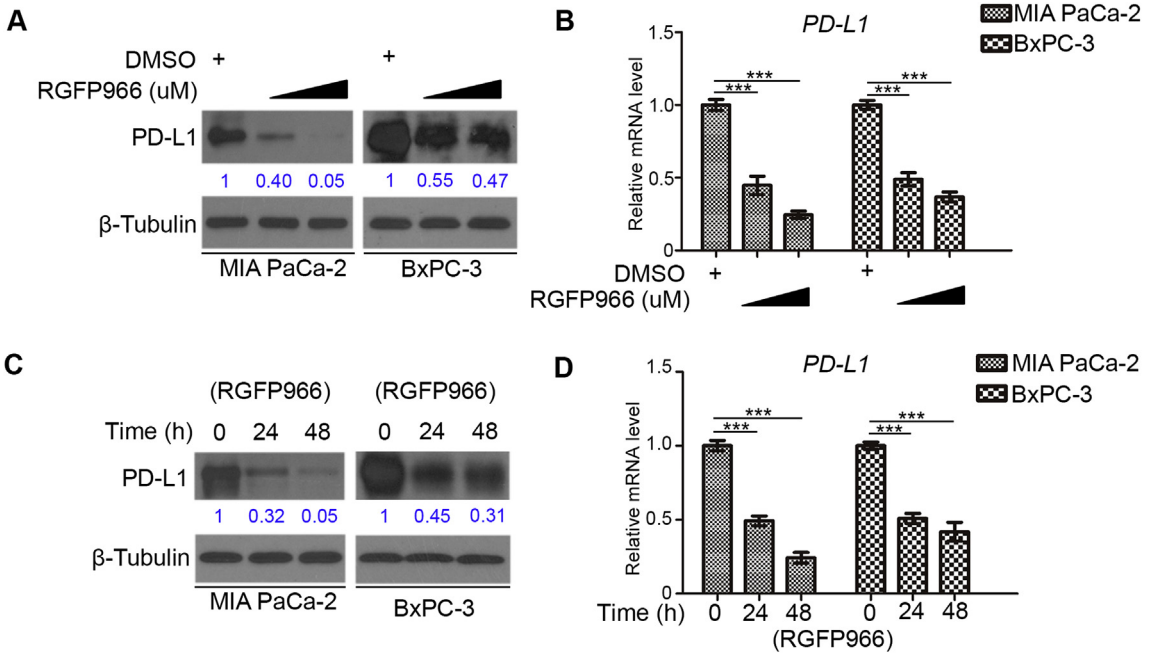

Fig. 1. RGFP966 decreases PD-L1 expression in a time- and dose-dependent manner in pancreatic cancer cells. (A and B) MIA PaCa-2 and BxPC-3 cells were treated with DMSO, 1 μM or 5 μM RGFP966.24 h after treatment, cells were harvested for western blotting analysis (A) and RT-qPCR analysis (B). For panel A, PD-L1 proteins were quantified by ImageJ software and normalized to the quantified value of β-Tubulin. For panel B, data are mean ± S.D. from experiments with three replicates. ***, $p < 0.001$. (C and D) MIA PaCa-2 and BxPC-3 cells were treated with or without RGFP966 (3 μM), and at different time points, cells were harvested for Western blots (C) and RT-qPCR analysis (D). For panel C, PD-L1 proteins were quantified by ImageJ software and normalized to the quantified value of β-Tubulin. For panel B, data are mean ± S.D. from experiments with three replicates. ***, $p < 0.001$.

Chromatin immunoprecipitation (ChIP) and ChIP-qPCR

ChIP was performed following the manufacturer's instructions for the Chromatin Extraction Kit (Abcam, ab117152, USA) and ChIP Kit Magnetic - One Step (Abcam, ab156907, USA) [14]. STAT3 (Cell signaling Technology, 9139, USA, dilution 1:100) was used for the ChIP assay. The purified DNA was analyzed by real-time PCR with a PCR kit (Takara Bio Inc., Japan) according to the manufacturer's protocols [15]. The following primers were used: PD-L1 ChIP primer, forward 5' - CAAGGTGCGTTCAGATGTTG -3′ and reverse 5' - GGCGTTGGACTTTCCTGA -3'.

Tissue microarray and immunohistochemistry (IHC)

The tissue microarray slides were purchased from Outdo Biobank (Shanghai, China) (lot no. XT14-029). The tissue microarray specimens were immunostained with PD-L1 (Cell Signaling Technology, 13684, USA, dilution 1: 1000) and HDAC3 antibodies (Cell signaling Technology, 85057, USA, dilution 1:1000) as described previously. Staining intensity was scored in a blinded fashion: 1 = weak staining at 100 × magnification but little or no staining at 40 × magnification; 2 = medium staining at 40 × magnification; 3 = strong staining at 40 × magnification [16]. The degree of immunostaining was reviewed and scored by two independent pathologists who were blinded to the clinical details. The scores were determined by the percentage of positive cells multiplied by the staining intensity.

Correlation analysis using GEPIA web tool

The online database, Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancerpku.cn/index.html) [17], was used to analyze the RNA sequencing expression data related to our project based on The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects. GEPIA performs survival analyses based on gene expression levels and uses a log-rank test for hypothesis evaluation. GEPIA performs a pairwise gene correlation analysis for any given sets of TCGA and/or GTEx expression data using Pearson correlation statistics.

Statistical analysis

Statistical analyses were performed with the two-sided paired Student's *t*-test for single comparisons and one-way ANOVA or a post hoc test for multiple comparisons. p values < 0.05 were considered statistically significant. All values represent the means ± SD.

Results

RGFP966 decreases PD-L1 expression in a time- and dose-dependent manner in pancreatic cancer cells

Since inhibiting the expression of PD-L1 is important to regulate the anti-tumor immunity in cancer related immunotherapy [18],

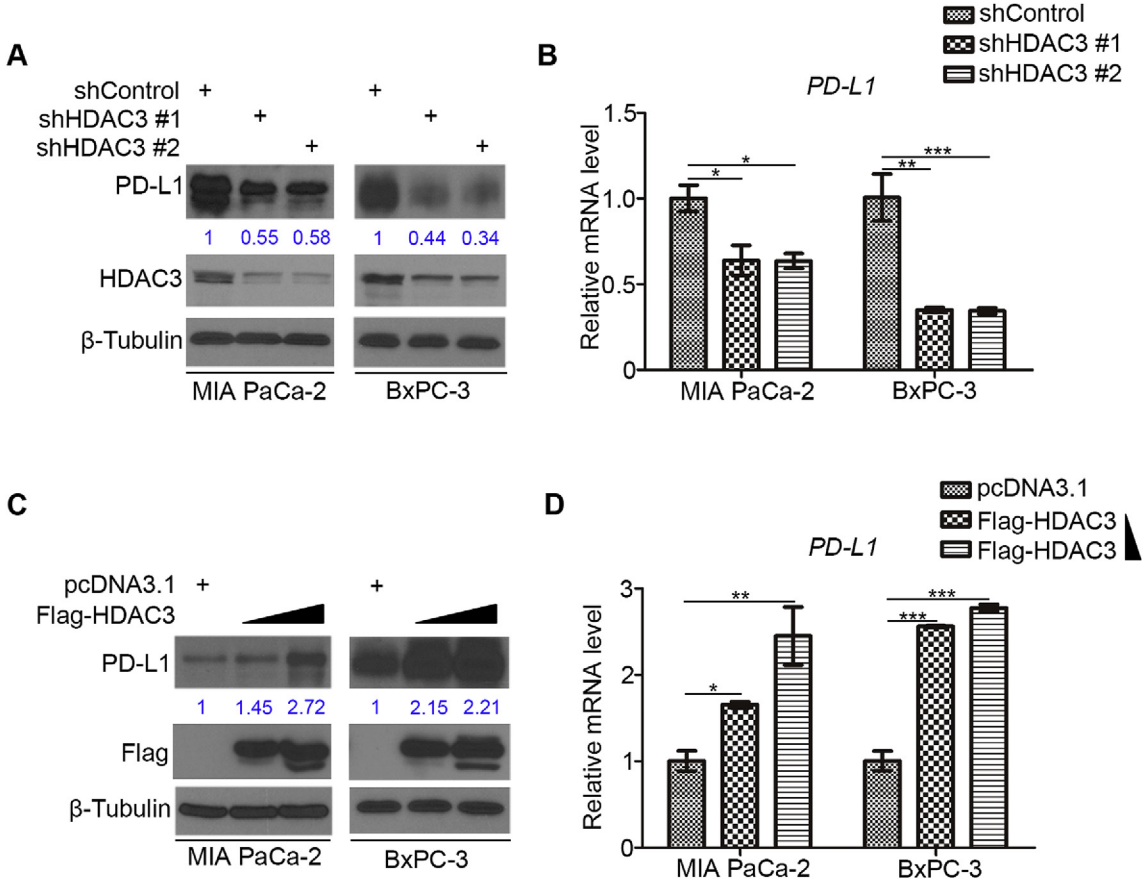

Fig. 2. HDAC3 regulates PD-L1 expression at the transcriptional level in pancreatic cancer cells. (A and B) MIA PaCa-2 and BxPC-3 cells were stably infected with control or two independent HDAC3-specific shRNAs for 3 days and harvested for Western blots (A) and RT-qPCR analysis (B). For panel A, PD-L1 proteins were quantified by ImageJ software and normalized to the quantified value of β-Tubulin. For panel B, data are mean ± S.D. from experiments with three replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. (C and D) MIA PaCa-2 and BxPC-3 were transfected pcDNA3.1, 1 μg or 3 μg Flag-HDAC3 for 48 h and cells were harvested for Western blots (C) and RT-qPCR analysis (D). For panel C, PD-L1 proteins were quantified by ImageJ software and normalized to the quantified value of β-Tubulin. For panel D, data are mean ± S.D. from experiments with three replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

identifying the novel small molecular inhibitors suppressing the PD-L1 expression gives a promising therapeutic strategy in pancreatic cancer. Interestingly, we found that the specific inhibitor of HDAC3, which is RGFP966 [19], significantly decreased the protein level of PD-L1 in MIA PaCa-2 and BxPC-3 pancreatic cancer cells in a dose-dependent manner (Fig. 1A). Then, we further showed that RGFP966 down-regulated the PD-L1 expression at the transcriptional level in both pancreatic cancer cells (Fig. 1B). Furthermore, we also found that RGFP966 undermined the expression of PD-L1 in a time-dependent manner in both MIA PaCa-2 and BxPC-3 cells (Fig. 1C and D). Together, our data indicate that RGFP966 may be a novel small molecular inhibitor represses the expression of PD-L1 in pancreatic cancer cells.

HDAC3 regulates PD-L1 expression at the transcriptional level in pancreatic cancer cells

Given that RGFP966 designing to specifically target and modulate the function of HDAC3, we sought to determine whether HDAC3 has regulated the expression of PD-L1 in pancreatic cancer cells. Firstly, we knocked down of HDAC3 by two independent short hairpin RNA and found that PD-L1 mRNA and protein expression was decreased after knockdown of HDAC3 in both MIA PaCa-2 and BxPC-3 cells (Fig. 2A and B). In contrast, using a gain of function approach, we demonstrated that the overexpression of HDAC3 increased the PD-L1 protein and mRNA levels in both MIA PaCa-2 and BxPC-3 cells (Fig. 2C and D). Therefore, our data suggest that HDAC3 regulates the PD-L1 expression at the transcriptional level in pancreatic cancer cells.

HDAC3 is positively correlated with PD-L1 in pancreatic cancer patients

To further investigate the relationship between HDAC3 and PD-L1, we sough to determine the correlation between HDAC3 and PD-L1 in pancreatic cancer specimens. We examined the expressions of

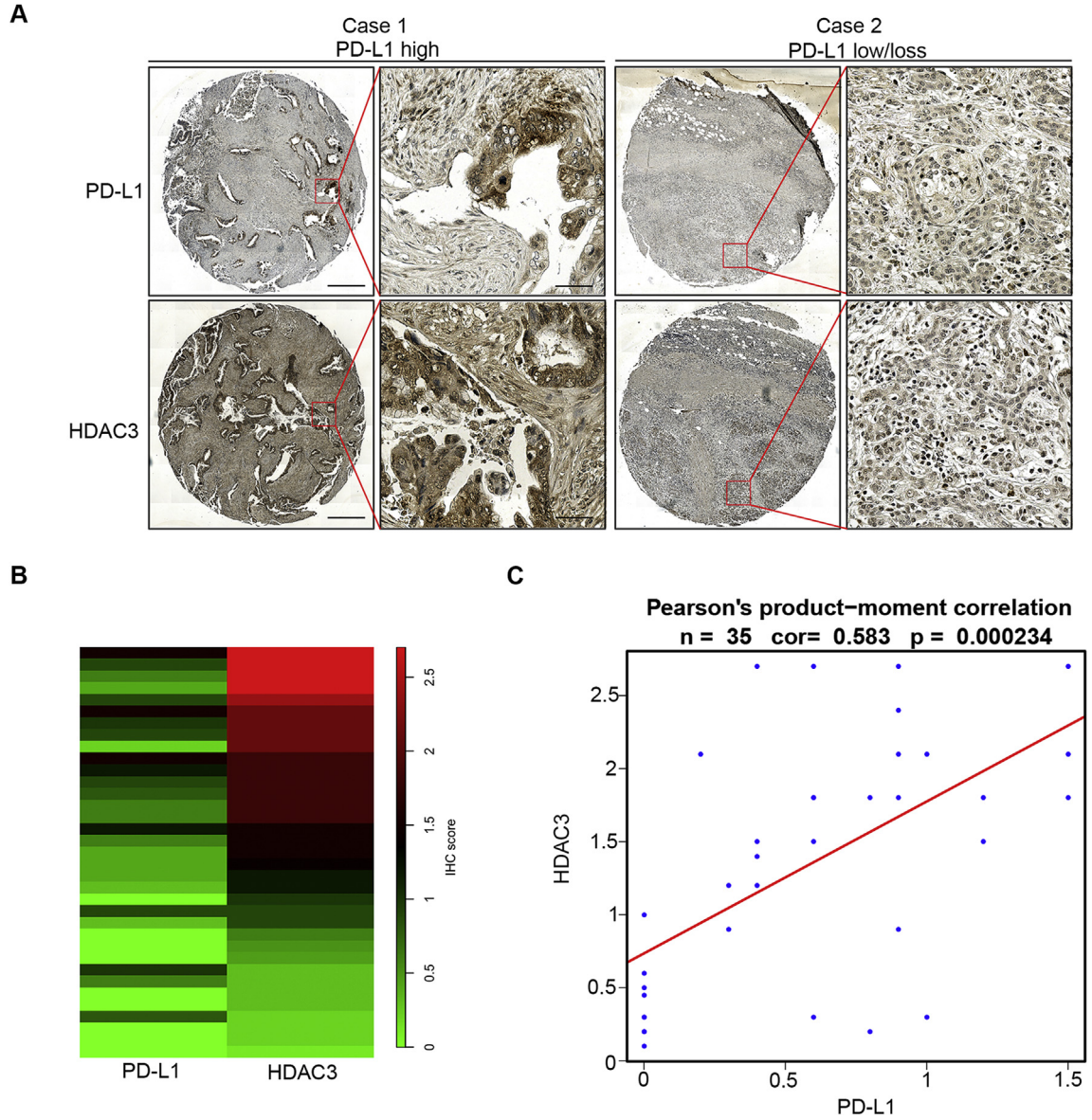

Fig. 3. HDAC3 is positively correlated with PD-L1 in pancreatic cancer patients. (A) Representative images of IHC for HDAC3 and PD-L1 on TMA (n = 35) of pancreatic cancer specimens. Scale bars are indicated. (B) Heatmap showing the staining index (SI) of HDAC3 and PD-L1 proteins in TMA. (C) Correlation analysis of the SI of HDAC3 and PD-L1 proteins in TMA.

these two proteins by performing immunohistochemistry (IHC) on a tissue microarray (TMA) containing a cohort of pancreatic cancer samples (n = 35). IHC staining index (SI) was calculated by multiplying the percentage of positively stained cells and staining intensity [15]. Representative images of high and low/no staining of HDAC3 and PD-L1 are shown in Fig. 3A. The level of PD-L1 was usually absent or low in the majority of pancreatic cancer specimens, only about 10%–20% of patient specimens showed high level of PD-L1 staining (Fig. 3B), which is consistent with the findings reported by the other laboratory [20,21]. Moreover, we demonstrated that the PD-L1 expression was positively correlated with HDAC3 level (Pearson's product-moment correlation coefficient r = 0.583, $p = 0.000234$) (Fig. 3B and C). Thus, these data suggest that although only small proportion of patients showed high level of PD-L1 expression, PD-L1 is positively correlated with HDAC3 in pancreatic cancer patients in our tissue microarray. Then, we further analyzed the correlation between HDAC3 and PD-L1 in pancreatic cancer in GEPIA web tool (Supplementary figure 1A).

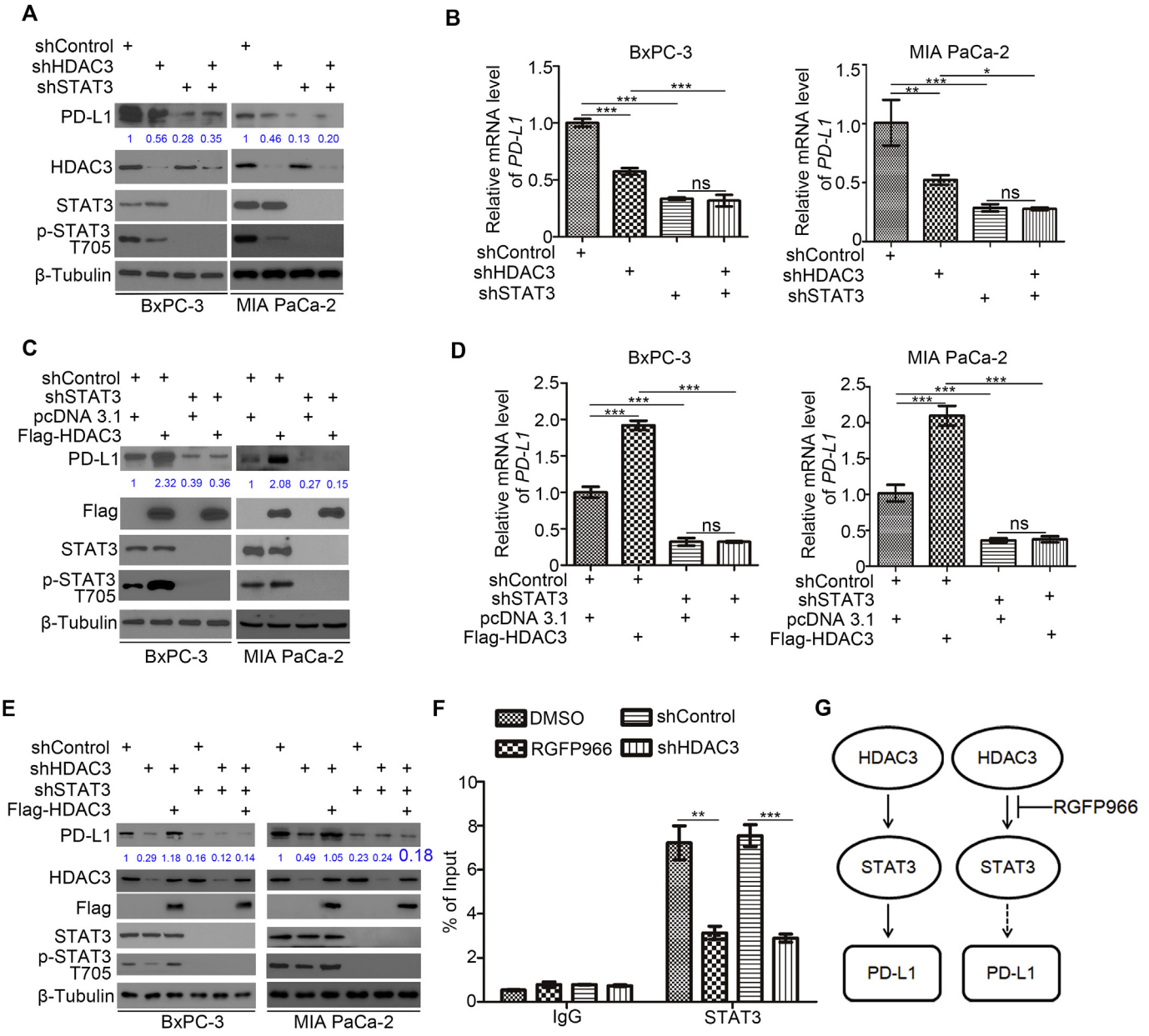

Fig. 4. HDAC3 modulates PD-L1 expression through STAT3 signaling pathway. (A and B) BxPC-3 and MIA PaCa-2 cells were infected with indicated constructs. After 72 h, cells were harvested for Western blotting (A) and RT-qPCR analysis (B). For panel A, the PD-L1 proteins were quantified by ImageJ software and normalized to the quantified value of β-Tubulin. For panel B, data are mean ± S.D. from experiments with three replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, ns, not significant. (C and D) BxPC-3 and MIA PaCa-2 cells were infected with shControl or shSTAT3 lentivirus constructs. After 48 h infection and puromycin selection, cells were transfected with pcDNA 3.1 or Flag-HDAC3 plasmids for 24 h and cells were harvested for Western blotting (C) and RT-qPCR analysis (D). For panel C, the PD-L1 proteins were quantified by ImageJ software and normalized to the quantified value of β-Tubulin. For panel D, data are mean ± S.D. from experiments with three replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, ns, not significant. (E) BxPC-3 and MIA PaCa-2 cells were infected with shControl, shSTAT3 and shHDAC3 respectively or both. After 48 h infection and puromycin selection, cells were transfected with pcDNA 3.1 or Flag-HDAC3 plasmids for 24 h and cells were harvested for Western blotting. The PD-L1 proteins were quantified by ImageJ software and normalized to the quantified value of β-Tubulin. (F) ChIP-qPCR analysis of STAT3 binding at the PD-L1 gene promoter in BxPC-3 after treated with DMSO or RGFP966 (3 μM) for 24 h or infected with indicated constructs for 48 h. Data are mean ± S.D. from experiments with three replicates. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. (G) A hypothetical model depicting that HDAC3 regulates PD-L1 expression through STAT3 signaling pathway. The HDAC3 inhibitors disrupts HDAC3/STAT3/PD-L1 pathway through inactivating HDAC3.

However, there was no direct positive correlation between HDAC3 and PD-L1 in the published data set. Since it has been documented that HDAC6 regulated PD-L1 expression [22]. Notably, there was no direct positive correlation between HDAC6 and PD-L1 in the GEPIA data set (Supplementary Figure 1B). Furthermore, we showed that the knockdown of HDAC6 decreased the protein and mRNA levels of PD-L1 in MIA PACA-2 cells (Supplementary Fig. 1C and 1D), which was consistent with the findings in melanoma cells. The above phenomenon may be attributed to individual differences in patients with pancreatic cancer.

HDAC3 modulates PD-L1 expression through STAT3 signaling pathway

Given that HDAC3 regulating PD-L1 expression at the transcriptional level, but the underlying mechanism is still unclear. Sorts of transcriptional factors, including STAT3 [23], BRD4 [18] and p65 [24], are responsible for regulating the PD-L1 expression. It has been documented that HDAC3 inhibition inactivated the STAT3 cascade in cancer cells [25]. Therefore, we sought to investigate whether STAT3 is the key mediator "bridge" HDAC3 with PD-L1 in pancreatic cancer cells. The knockdown of HDAC3 decreased the protein and mRNA level of PD-L1 expression and this effect was diminished after the knockdown of STAT3 in both BxPC-3 and MIA PaCa-2 cells (Fig. 4A and B). Furthermore, we showed that ectopic expression of HDAC3 significantly increased the PD-L1 expression but this effect was not obvious after the knockdown of STAT3 in both BxPC-3 and MIA PaCa-2 cells (Fig. 4C and D). Then, the gain of function assay further confirmed the effect of HDAC3 on the regulation of PD-L1 level in pancreatic cancer cells (Fig. 4E). Moreover, we demonstrated that the treatment with RGFP966 or the knockdown of HDAC3 in BxPC-3 cells attenuated the STAT3 binding to the promoter of PD-L1 via using ChIP-qPCR assay (Fig. 4F). Collectively, these data indicate that HDAC3/STAT3 pathway regulates PD-L1 expression in pancreatic cancer, and administration of the HDAC3 inhibitors, RGFP966, disrupts HDAC3/STAT3/PD-L1 signaling (Fig. 4G).

Discussion

The non-immunogenic characteristic of pancreatic cancer is responsible for the failure of immunotherapy, especially the immunosuppressive microenvironment, poor T cell infiltration, and a low mutational burden which contributes to the immune privilege in pancreatic cancer [4]. A growing body of evidence suggests that the expression level of PD-L1 in pancreatic cancer is critical for the efficacy of anti-PD-L1 therapy [10]. Therefore, searching the small molecular inhibitors that influence the PD-L1 expression provide new clues to regulate the efficacy of immunotherapy in pancreatic cancer cells. It has been previously reported that the BET inhibitors [26], MEK inhibitors [27] and mTOR inhibitors [28] decreased PD-L1 expression in vitro and in vivo. In our study, we identified a novel small molecular inhibitor, the specific inhibitor of HDAC3, which also suppressed the PD-L1 expression in pancreatic cancer cells.

A previous study reported that class I HDAC inhibitors upregulated the PD-L1 expression in melanoma cells [29] whereas HDAC1 and HDAC2 silence could not decrease the PD-L1 level [30]. It has been documented that HDAC6 increased the PD-L1 expression through regulation the phosphorylation of STAT3 [22]. Similarly, our data demonstrated that HDAC3 regulated the PD-L1 expression via STAT3 in pancreatic cancer cells. These data indicated that STAT3 acted as a key meditator for HDACs modulating the expression of PD-L1. Meanwhile, it has been reported that HDAC inhibition improves the immune response and outcome of pancreatic cancer in mice [31]. In consistent with previous findings, our data demonstrated that the HDAC3 specific inhibitor, RGFP966, repressed the PD-L1 expression in pancreatic cancer cells.

Multiple types of pathways, such as AKT-mTOR pathway [32], KRAS pathway [27] and NF-kB signaling pathway [33] are reported to have participated in regulating the PD-L1 expression in tumor cells. Meanwhile, a kind of well-known transcriptional factors, including BRD4 [18], MYC [34] and STAT3 [23], could directly bind to the promoter of PD-L1 and initiate the transcription of PD-L1. Recently, we have found that RB [35] and FUBP1 [36] could regulate the PD-L1 expression via NF-kB and Myc pathway in pancreatic cancer respectively. Moreover, it has been documented that miR-142-5p and MLL1-H3K4me3 axis regulated the expression of PD-L1 in pancreatic cancer [37,38]. In addition to the regulation of PD-L1 at the transcriptional level, sorts of protein, including SPOP [39], β-Trcp [40],CMTM4/CMTM6 [41] or CSN5 [42], were responsible for the stability of PD-L1. It has been reported that HDAC3 is important for modifying the activity of STAT3 via interaction with STAT3 [25]. HDAC3 silence or deletion decreases the level of p-STAT3(Y705) and impairs the function of STAT3 in cells [25]. Thus, HDAC3 forms a complex with STAT3 and this complex is important for STAT3 signaling pathway. In our study, our data suggest that HDAC3/STAT3 signaling is important for PD-L1 regulation in pancreatic cancer cells.

Collectively, our study demonstrates that the HDAC3 inhibitors decrease the protein and mRNA level of PD-L1 in pancreatic cancer cells. Then, we identify that HDAC3/STAT3 pathway is responsible for transcriptionally modulating the PD-L1 expression and PD-L1 is positively correlated with HDAC3 in pancreatic cancer patient specimens. These findings uncover important aspects of the function of HDAC3 in cancer immunity and elucidated the specific mechanism regulating PD-L1 expression. Thus, the HDAC3 inhibitor may be an ideal small molecular inhibitor to modulate the immunotherapy efficiency of pancreatic cancer.

Author contribution

Guofu Hu and Nan He performed the experiments and wrote the paper; Chuanqi Cai, Fei Cai and Ping Fan collected data; Xin Jin and Zhikun Zheng wrote the paper and analyzed the data.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2019.01.011.

References

[1] Morrison AH, Byrne KT, Vonderheide RH. Immunotherapy and prevention of pancreatic cancer. Trends Cancer 2018;4:418–28.
[2] Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin 2015 2015;65:5–29.
[3] Lin QJ, Yang F, Jin C, Fu DL. Current status and progress of pancreatic cancer in China. World J Gastroenterol 2015;21:7988–8003.
[4] Torphy RJ, Zhu Y, Schulick RD. Immunotherapy for pancreatic cancer: barriers and breakthroughs. Ann Gastroenterol Surg 2018;2:274–81.
[5] Cheng B, Yuan WE, Su J, Liu Y, Chen J. Recent advances in small molecule based cancer immunotherapy. Eur J Med Chem 2018;157:582–98.

[6] Vonderheide RH. The immune revolution: a case for priming, not checkpoint. Cancer Cell 2018;33:563–9.
[7] Banerjee K, Kumar S, Ross KA, Gautam S, Poelaert B, Nasser MW, et al. Emerging trends in the immunotherapy of pancreatic cancer. Cancer Lett 2018;417:35–46.
[8] Feng M, Xiong G, Cao Z, Yang G, Zheng S, Song X, et al. Pd-1/pd-l1 and immunotherapy for pancreatic cancer. Cancer Lett 2017;407:57–65.
[9] Tessier-Cloutier B, Kalloger SE, Al-Kandari M, Milne K, Gao D, Nelson BH, et al. Programmed cell death ligand 1 cut-point is associated with reduced disease specific survival in resected pancreatic ductal adenocarcinoma. BMC Canc 2017;17:618.
[10] Winograd R, Byrne KT, Evans RA, Odorizzi PM, Meyer AR, Bajor DL, et al. Induction of t-cell immunity overcomes complete resistance to pd-1 and ctla-4 blockade and improves survival in pancreatic carcinoma. Cancer Immunol Res 2015;3:399–411.
[11] Jin X, Yang C, Fan P, Xiao J, Zhang W, Zhan S, et al. Cdk5/fbw7-dependent ubiquitination and degradation of ezh2 inhibits pancreatic cancer cell migration and invasion. J Biol Chem 2017;292:6269–80.
[12] Jin X, Tian S, Li P. Histone acetyltransferase 1 promotes cell proliferation and induces cisplatin resistance in hepatocellular carcinoma. Oncol Res 2017;25:939–46.
[13] Jin X, Pan Y, Wang L, Ma T, Zhang L, Tang AH, et al. Fructose-1,6-bisphosphatase inhibits erk activation and bypasses gemcitabine resistance in pancreatic cancer by blocking iqgap1-mapk interaction. Cancer Res 2017;77:4328–41.
[14] Fan P, Wang B, Meng Z, Zhao J, Jin X. Pes1 is transcriptionally regulated by brd4 and promotes cell proliferation and glycolysis in hepatocellular carcinoma. Int J Biochem Cell Biol 2018;104:1–8.
[15] Jin X, Yan Y, Wang D, Ding D, Ma T, Ye Z, et al. Dub3 promotes bet inhibitor resistance and cancer progression by deubiquitinating brd4. Mol Cell 2018;71:592–605 e594.
[16] Jin X, Pan Y, Wang L, Zhang L, Ravichandran R, Potts PR, et al. Mage-trim28 complex promotes the warburg effect and hepatocellular carcinoma progression by targeting fbp1 for degradation. Oncogenesis 2017;6:e312.
[17] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z, Gepia. A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45:W98–102.
[18] Zhu H, Bengsch F, Svoronos N, Rutkowski MR, Bitler BG, Allegrezza MJ, et al. Bet bromodomain inhibition promotes anti-tumor immunity by suppressing pd-l1 expression. Cell Rep 2016;16:2829–37.
[19] Bieszczad KM, Bechay K, Rusche JR, Jacques V, Kudugunti S, Miao W, et al. Histone deacetylase inhibition via rgfp966 releases the brakes on sensory cortical plasticity and the specificity of memory formation. J Neurosci 2015;35:13124–32.
[20] Mace TA, Shakya R, Pitarresi JR, Swanson B, McQuinn CW, Loftus S, et al. Il-6 and pd-l1 antibody blockade combination therapy reduces tumour progression in murine models of pancreatic cancer. Gut 2018;67:320–32.
[21] Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Canc Res 2007;13:2151–7.
[22] M L, P PV, T K, M P, E S, J P, et al. Essential role of hdac6 in the regulation of pd-l1 in melanoma. Mol Oncol 2016;10:735–50.
[23] Sasidharan Nair V, Toor SM, Ali BR, Elkord E. Dual inhibition of stat1 and stat3 activation downregulates expression of pd-l1 in human breast cancer cells. Expert Opin Ther Targets 2018;22:547–57.
[24] Maeda T, Hiraki M, Jin C, Rajabi H, Tagde A, Alam M, et al. Muc1-c induces pd-l1 and immune evasion in triple-negative breast cancer. Cancer Res 2018;78:205–15.
[25] Lu XF, Cao XY, Zhu YJ, Wu ZR, Zhuang X, Shao MY, et al. Histone deacetylase 3 promotes liver regeneration and liver cancer cells proliferation through signal transducer and activator of transcription 3 signaling pathway. Cell Death Dis 2018;9:398.
[26] Hogg SJ, Vervoort SJ, Deswal S, Ott CJ, Li J, Cluse LA, et al. Bet-bromodomain inhibitors engage the host immune system and regulate expression of the immune checkpoint ligand pd-l1. Cell Rep 2017;18:2162–74.
[27] Qiu XY, Hu DX, Chen WQ, Chen RQ, Qian SR, Li CY, et al. Pd-l1 confers glioblastoma multiforme malignancy via ras binding and ras/erk/emt activation. Biochim Biophys Acta 2018;1864:1754–69.
[28] Lastwika KJ, Wilson 3rd W, Li QK, Norris J, Xu H, Ghazarian SR, et al. Control of pd-l1 expression by oncogenic activation of the akt-mtor pathway in non-small cell lung cancer. Cancer Res 2016;76:227–38.
[29] Woods DM, Sodre AL, Villagra A, Sarnaik A, Sotomayor EM, Weber J. Hdac inhibition upregulates pd-1 ligands in melanoma and augments immunotherapy with pd-1 blockade. Cancer Immunol Res 2015;3:1375–85.
[30] Booth L, Roberts JL, Poklepovic A, Kirkwood J, Dent P. Hdac inhibitors enhance the immunotherapy response of melanoma cells. Oncotarget 2017;8:83155–70.
[31] Edderkaoui M, Chheda C, Soufi B, Zayou F, Hu RW, Ramanujan VK, et al. An inhibitor of gsk3b and hdacs kills pancreatic cancer cells and slows pancreatic tumor growth and metastasis in mice. Gastroenterology 2018;155:1985–98. e1985.
[32] Zhang X, Zeng Y, Qu Q, Zhu J, Liu Z, Ning W, et al. Pd-l1 induced by ifn-gamma from tumor-associated macrophages via the jak/stat3 and pi3k/akt signaling pathways promoted progression of lung cancer. Int J Clin Oncol 2017;22:1026–33.
[33] Asgarova A, Asgarov K, Godet Y, Peixoto P, Nadaradjane A, Boyer-Guittaut M, et al. Pd-l1 expression is regulated by both DNA methylation and nf-kb during emt signaling in non-small cell lung carcinoma. OncoImmunology 2018;7. e1423170.
[34] Casey SC, Tong L, Li Y, Do R, Walz S, Fitzgerald KN, et al. Myc regulates the antitumor immune response through cd47 and pd-l1. Science 2016;352:227–31.
[35] Jin X, Ding D, Yan Y, Li H, Wang B, Ma L, et al. Phosphorylated rb promotes cancer immunity by inhibiting nf-kappab activation and pd-l1 expression. Mol Cell 2018;73(1):22–35.
[36] Fan P, Ma J, Jin X. Far upstream element-binding protein 1 is up-regulated in pancreatic cancer and modulates immune response by increasing programmed death ligand 1. Biochem Biophys Res Commun 2018;505:830–6.
[37] Jia L, Xi Q, Wang H, Zhang Z, Liu H, Cheng Y, et al. Mir-142-5p regulates tumor cell pd-l1 expression and enhances anti-tumor immunity. Biochem Biophys Res Commun 2017;488:425–31.
[38] Lu C, Paschall AV, Shi H, Savage N, Waller JL, Sabbatini ME, et al. The mll1-h3k4me3 axis-mediated pd-l1 expression and pancreatic cancer immune evasion. J Natl Cancer Inst 2017;109.
[39] Zhang J, Bu X, Wang H, Zhu Y, Geng Y, Nihira NT, et al. Cyclin d-cdk4 kinase destabilizes pd-l1 via cullin 3-spop to control cancer immune surveillance. Nature 2018;553:91–5.
[40] Li CW, Lim SO, Xia W, Lee HH, Chan LC, Kuo CW, et al. Glycosylation and stabilization of programmed death ligand-1 suppresses t-cell activity. Nat Commun 2016;7:12632.
[41] Mezzadra R, Sun C, Jae LT, Gomez-Eerland R, de Vries E, Wu W, et al. Identification of cmtm6 and cmtm4 as pd-l1 protein regulators. Nature 2017;549:106–10.
[42] Lim SO, Li CW, Xia W, Cha JH, Chan LC, Wu Y, et al. Deubiquitination and stabilization of pd-l1 by csn5. Cancer Cell 2016;30:925–39.