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IFI16 promotes cervical cancer progression by upregulating PD-L1 in immunomicroenvironment through STING-TBK1-NF-kB pathway

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ABSTRACT

Cervical cancer remains one of the leading causes of cancer death worldwide. Immunotherapy is the most promising cancer therapeutics in recent years and has gain positive results in several cancers in the clinic. This study was aimed to investigate the roles and mechanism of IFI16 in cervical cancer immunotherapy. We observed an abnormally high expression of Programmed cell death 1 ligand 1 (PD-L1) and Interferon-inducible 16 (IFI16) in Human papillomavirus (HPV) positive cervical cancer cells compared with HPV negative cervical cancer cells. Moreover, IFI16 promotes cervical cancer development in vitro and in vivo as the oncogenic role of PD-L1. In the subsequent mechanism investigation, we found that IFI16 activated STING-TBK1-mediated immunoregulation, and subsequently activated downstream NF-kB pathway, which interacted with the proximal region of PD-L1 promoter to facilitate PD-L1 expression. In conclusion, we found that IFI16 positively regulate PD-L1 through STING-TBK1-NF-kB pathway, thus promoting cervical cancer progression. The roles of IFI16 in cervical cancer progression deserve further investigation and hold the promise of being developed as a novel immunotherapy target in the future.

1. Introduction

Cervical cancer remains one of the most common malignancy in women all of the world [1]. Human papillomavirus (HPV) infection highly heighten the risk of cervical cancer through encoding E6 and E7 viral oncoproteins and disturbing p53 signaling pathway [2]. HPV vaccines and cervical cancer screening largely reduced the incidence of cervical cancer [3], limited therapeutic approaches result in poor prognosis though [4].

The coordinates interaction of tumor cells and immune system [5] was critical for tumor cells to escape from the host's immune recognition via elaborate mechanisms [6]. Stimulator of IFN genes (STING) is the transmenbrane protein detecting cytosolic double-strand DNA [7] and triggering cascade signaling including TANK-binding kinase-1 (TBK1) and interferon regulatory factor 3 (IRF-3) [8]. STING plays a critical role in innate immune which can be activated by Interferon-inducible 16 (IFI16), a AIM2-like receptor [9]. IFI16 was reported to serve as a viral DNA sensor that can activate the STING-TBK1 signaling pathway for viral defense [9]. Activated STING signaling is important in promoting antitumor immunity in cancer patient [10].

Recently, immune checkpoint inhibitors [11] including Programmed cell death 1 ligand 1 (PD-L1) acquired great efficacy in cancer treatment including cervical cancer [12]. However, lack of immunogenicity and tumor antigen recognition still limited the utilization of checkpoint therapy [13]. PD-L1 is a biomarker on the surface of multiple cells including tumor cell which helps tumor cell to escape the attack of immune cells. Previous studies have reported many ways in PD-L1 regulation [14] and HPV infection was highly correlated to PD-L1 expression in cervical cancer [15]. The underlying mechanism of immunoregulation in HPV positive cervical cancer is supposed to study.

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Table 1
antibodies used in western-blot.

Antigen name	Commercial source	Catalog number (clone number)
AREG	Proteintech	16036-1-AP
GAPDH	Abways	AB0037
PD-L1	GenomeMe	IHC411
IFI16	Cell Signaling	#14970
Ki67	Proteintech	27309-1-AP
PCNA	Proteintech	10205-2-AP
p-STING	Cell Signaling	#19781
STING	Cell Signaling	#13647
p-TBK1	Cell Signaling	#5483
TBK1	Proteintech	67211-1-Ig
p-IRF3(ser396)	Cell Signaling	#4947
IRF3	Cell Signaling	#4302
p-p65(ser536)	Cell Signaling	#3033
p65	Proteintech	10745-1-AP

Table 2
Primers used in qRT-PCR.

Genes	Forward (5′–3′)	Reverse (5′–3′)
GAPDH	CAAGGTCATCCATGACAACTTTG	GTCCACCACCCTGTTGCTGTAG
IFI16	TAGAAGTGCCAGCGTAACTCC	TGATTGTGGTCAGTCGTCCAT
PD-L1	GCTGCACTAATTGTCTATTGGGA	AATTCGCTTGTAGTCGGCACC

2. Method

2.1. Tissue samples collection and cell line

Human cervical cancer tissue (n = 30) was collected from 30 HPV positive cervical cancer patients. Human HPV-positive cervical cancer cell lines (CaSki, SiHa, Hela) and HPV-negative cervical cancer cells (C33A) were purchased form the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All of cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 10 % FBS (Hyclone) and 1 % penicillin/streptomycin (Santa Cruz) at 37°C with 5 % CO_2.

2.2. Transfection

Full-length PD-L1 cDNA was inserted into pCDNA3.1 vector (pCDNA3.1-PD-L1). PD-L1, IFI16, p65 and IRF3 short hairpin RNA and scrambled shControl were synthesized and cloned into pLKO.1 TRC vector by Genechem. Expression plasmid and lentiviruse containing shPD-L1 and shIFI16 were transfected to cell using Lipofectamine 2000 reagent (Invitrogen). transfection of corresponding empty vector and shControl were performed as negative control.

2.3. Western blot

Proteins were quantified using BCA reagent (Biotime Biotechnology, Shanghai, China) before western-blot. Proteins were then separated in 10 % sodium dodecyl sulfate polyacrylamide gel (Willget Biotech, Shanghai, China) and transferred onto nitrocellulose menbrane (Millipore, Shanghai, China). Then the menbrane was blocked by 5 %

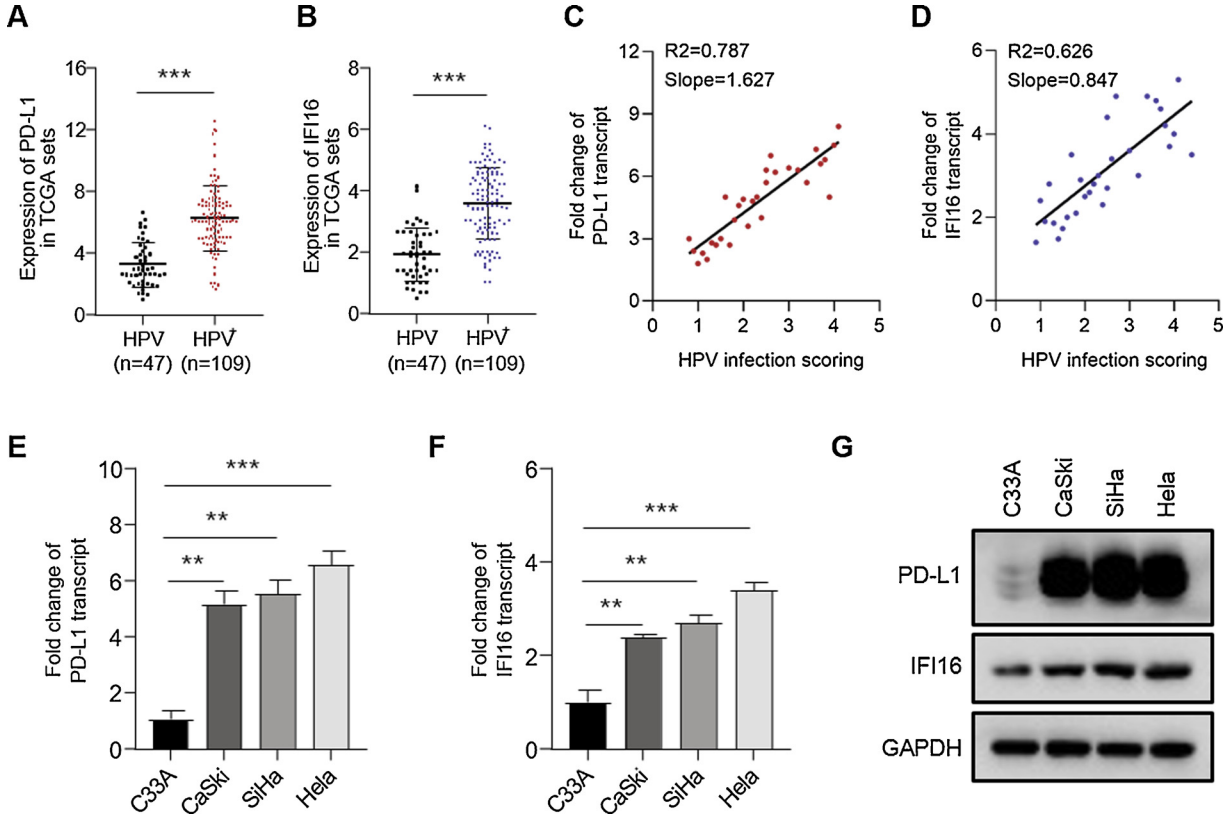

Fig. 1. PD-L1 and IFI16 are upregulated in HPV-positive cervical cancer. (A) Analysis of PD-L1 mRNA expression in TCGA cohort. (B) Analysis of IFI16 mRNA expression in TCGA cohort. (C) A positive correlation between PD-L1 expression and HPV infection in cervical cancer. (D) A positive correlation between IFI16 expression and HPV infection in cervical cancer. (E–F) qRT-PCR analysis of PD-L1 and IFI16 mRNA expression was increased in HPV-positive cervical cancer cell lines (CaSki, SiHa, Hela) compared with HPV-negative cervical cancer cells (C33A). (G) Western blot analyzed PD-L1 and IFI16 expression in HPV-positive cervical cancer cell lines (CaSki, SiHa, Hela) and HPV-negative cervical cancer cells (C33A). *P < 0.05. **P < 0.01 (Student's *t*-test).

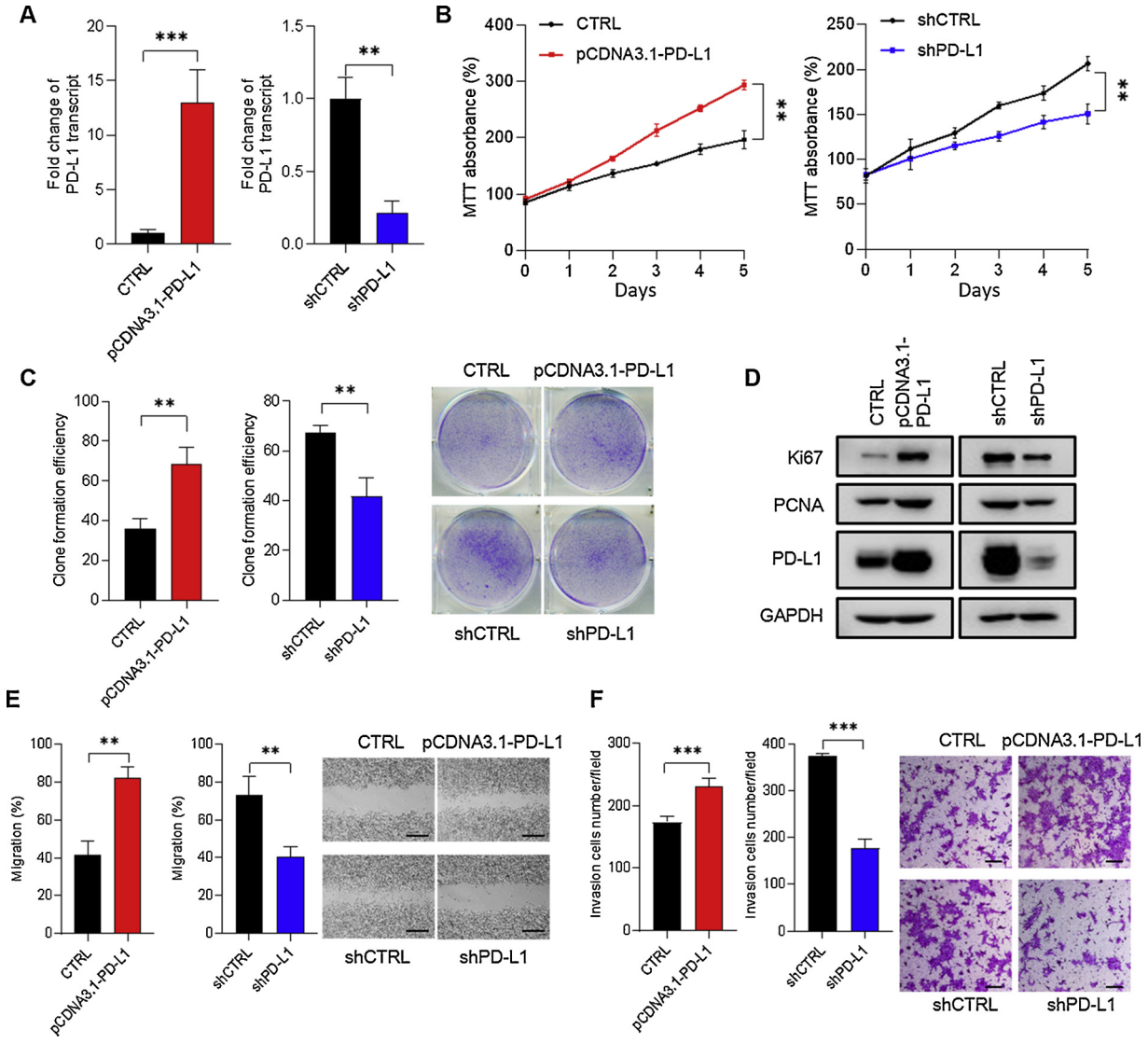

Fig. 2. PD-L1 is required for cervical cancer cells proliferation and invasion. (A) The efficiency of overexpression and inhibition of PD-L1 mRNA expression. (B) MTT assays revealed that downregulation of PD-L1 significantly reduced the growth rate, while overexpression of PD-L1 significantly increased the growth rate. (C) Colony formation assay showed that downregulation of PD-L1 significantly reduced the mean colony number, while overexpression of PD-L1 increased the mean colony number. Representative images of SiHa cells. (D) Western blot analyzed Ki67 and PCNA expression in PD-L1-overexpression or PD-L1-knockdown SiHa. (E) Wound healing assay showed that downregulation of PD-L1 significantly reduced cell migration rate, while overexpression of PD-L1 significantly increased cell migration rate. Representative images of SiHa measured at 48 h or 72 h. Scale bars, 100 μm. (F) Transwell invasion assay showed that downregulation of PD-L1 significantly reduced cell invision number, while overexpression of PD-L1 significantly increased cell invasion number. Representative images of SiHa measured at 48 h or 72 h. Scale bars, 50 μm. Data are shown as the mean ± standard deviation (n = 3) and representative of three independent experiments. *P < 0.05. **P < 0.01 (Student's *t*-test).

defatted milk for 1 h and added with primary antibody at 4°C over night. The next day, menbrane was washed by tris buffered saline tween (TBST) and added with secondary antibody conjugated with horse radish peroxidase at RT for 1 h. Antibodies used in western blot experiment were listed in Table 1.

2.4. Real-time polymerase chain reaction

Total RNA was extracted by TRIzol (Sigma) and cDNA was synthesized by SuperScript III first-strand synthesis system (Invitrogen). qPCR was carried out with SYBR-Green PCR Master Mix (Thermo Fisher Scientific). Primers used were manifested in Table 2. mRNA expression was correlated to HPV infection score including 0–1 (negative), 1–2 (weak), 2–3 (moderate), 3–4 (strong) according to previous study [16].

2.5. MTT

Survival of cells was measured by MTT assay. Cell were patched in 96-well plate and cultured over night. the next day, 0.5 % MTT (Beyotime, China) was added into each well followed with 4 -h incubation. Then the supernatant was discarded and DMSO was added followed with 10-min shaking. Optical density (OD) at 490 nm was measured on a microplate reader.

2.6. Clonal formation assay

Cells were digested with trypsin and resuspended with DMEM containing 10 % FBS and 1 % penicillin/streptomycin. Then cells were transferred into 6-well plate with 5000 cells per well and cultured for 2 weeks. Cells were then fixed with 4 % formaldehyde and stained by 1 % crystal violet. Microscope was used for observation.

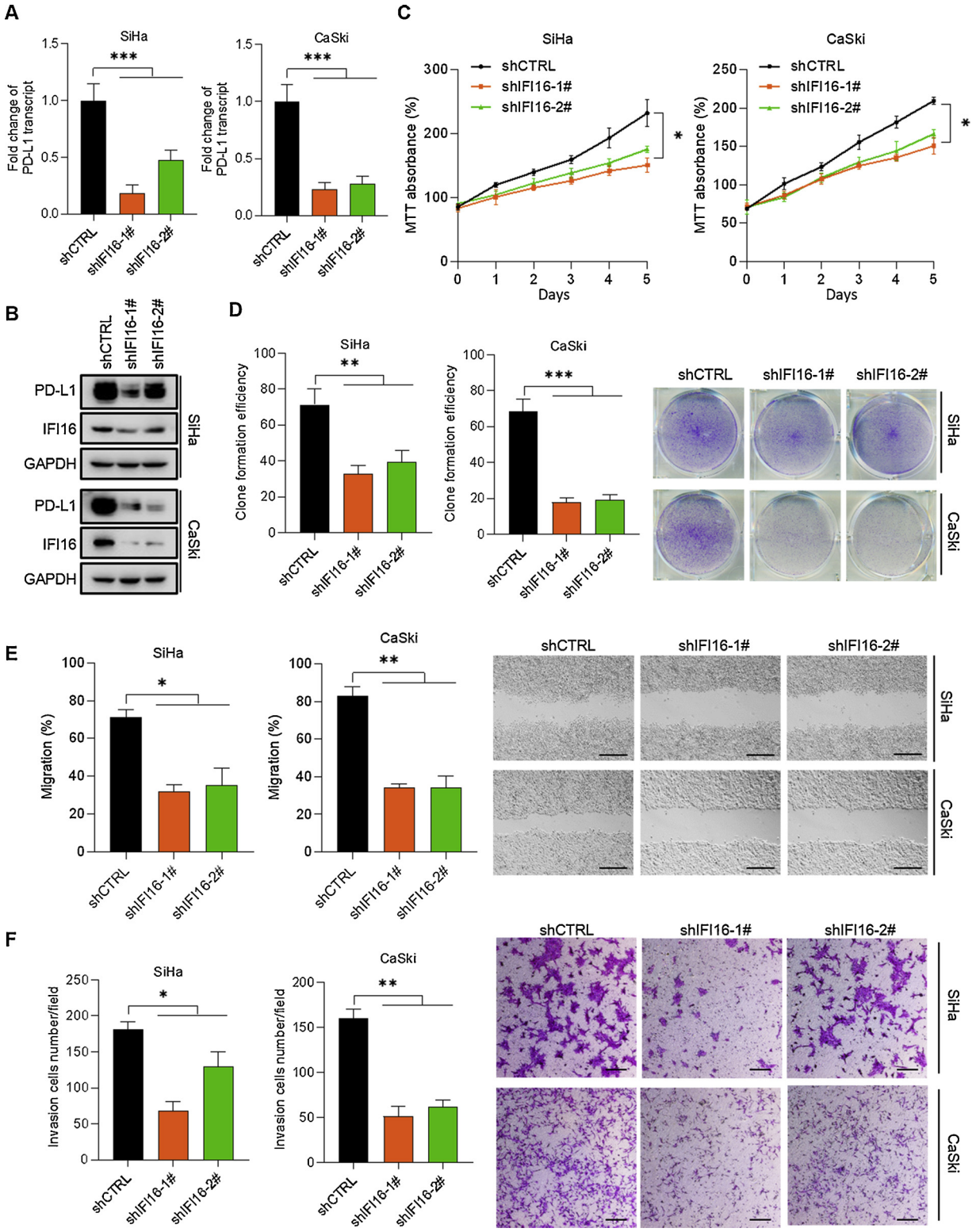

Fig. 3. Knockdown of IFI16 decreases PD-L1 expression and inhibits HPV-positive cervical cancer cells proliferation and invaion. (A–B) qRT-PCR and western blot showed that PD-L1 expression was reduced in IFI16-knockdown cervical cancer cell lines. (C) MTT assays revealed that downregulation of IFI16 reduced the growth rate. (D) Colony formation assay showed that downregulation of IFI16 reduced the mean colony number. Representative images. (E) Wound healing assay showed that downregulation of IFI16 significantly reduced cell migration rate. Representative images measured at 48 h or 72 h. Scale bars, 100 μm. (F) Transwell invasion assay showed that downregulation of IFI16 significantly reduced cell invision number. Representative images measured at 48 h or 72 h. Scale bars, 50 μm. Data are shown as the mean ± standard deviation (n = 3) and representative of three independent experiments. *P < 0.05. **P < 0.01 (Student's *t*-test).

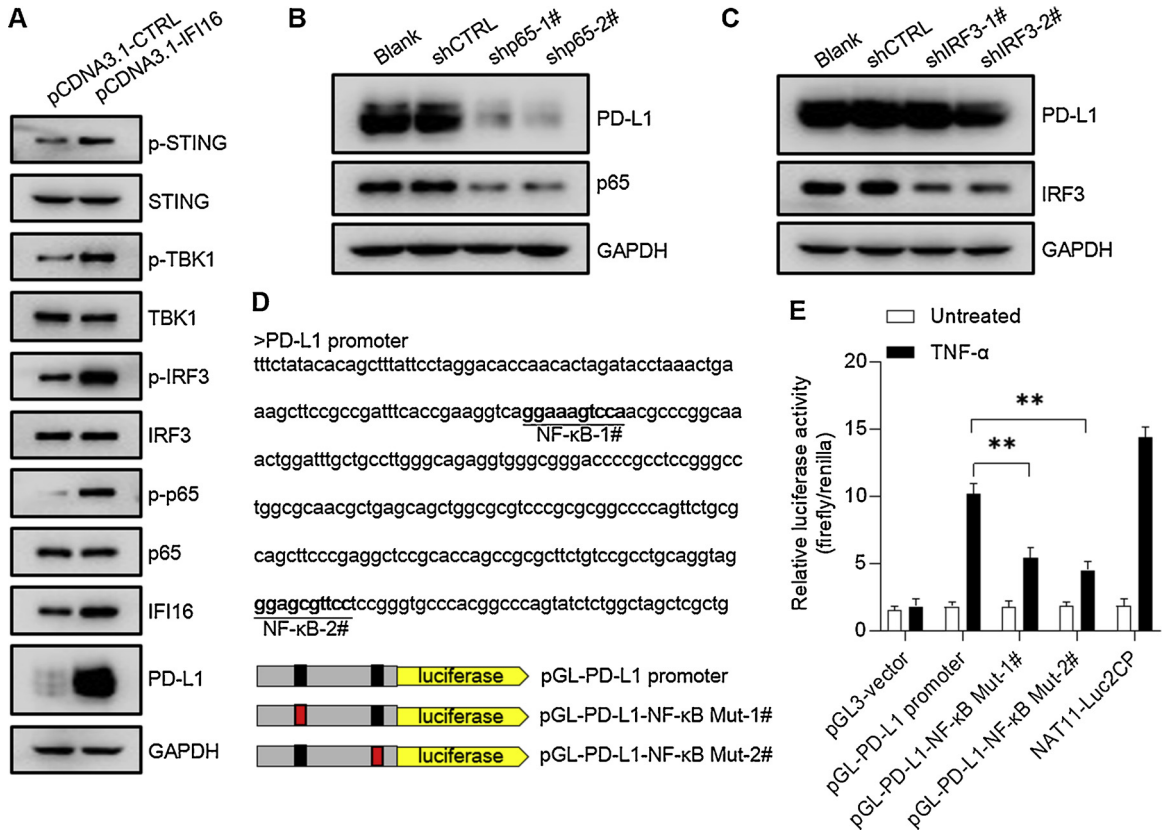

Fig. 4. Overexpression of IFI16 activates downstream signaling STING-TBK1-NF-KB to induce PD-L1 expression. (A) Western blot showed that overexpression of IFI16 activated STING-TBK1-NF-KB/IRF3 signaling pathway in cervical cancer cell lines. (B–C) Western blot showed that knockdown of NF-KB reduced PD-L1 expression in IFI16-overexpressed cervical cells, while knockdown of IRF3 had no influence. (D) Sequence of the PD-L1 promoter showing the position of the most representative putative NF-KB binding sites of the promoter. Schematic of putative NF-KB binding sites in the proximal region of PD-L1 promoter. (E) PD-L1 promoter transient reporter assay including deletions of the putative binding sites. Results are represented as normalized relative luciferase units (RLUs). *P < 0.05. **P < 0.01 (Student's *t*-test).

2.7. Transwell

Cells were transferred into serum-free medium 24 h before Transwell assay. Cells were diluted with medium containing 1 % FBS to a concentration of 1 × 10 [5]/ml. 500ul of the suspension were transferred to the uper chamber and 750ul medium containing 10 % FBS was added to the lower chamber of the Transwell followed with 24 or 48 -h culture at 37℃. Then 4 % formaldehyde was used for fixation and 0.5 % crystal violet was used for 30-min staining. The cells were observed under a microscope and counted.

2.8. Wound healing

Cells were transferred into 6-well e-plate and cultured over night. The next day, a straight linear was made by 200ul pipette tip and subsequently washed by 1 × PBS. Cells were observed under microscope. Then cells were cultured for 24 or 48 h followed with observation under microscope.

2.9. Luciferase assay

Wild type and mutant NF-κB binding site in the proximal region of PD-L1 promoter were designed and subclone into pGL3 Basic vector (Promega). Empty pGL3 vector was used to serve as negative control and Luc2CP vector harbouring NAT11 was used to serve as positive control. Recombinant vector was transfected into cervical cancer cells using Lipofectamine 2000 reagent (Invitrogen). Cells were then stimulated with TNF-α. Dual-Luciferase™ Reporter (DLR™) Assay Systems (Promega) was used to detect the luciferase activity.

2.10. Tumor xenografts

The NOC/SCID mice (male, 6 weeks) were provided by Charles river, peking, China. SiHa cells performed with transfection of sh-PD-L1, sh-IFI16 and sh-NCs were subcutaneously implanted into the lower flank of nude mice. Tumor volume was record every 5 days and the mice would be sacrificed after at day 45.

2.11. Statistical analysis

All data were calculated based on 3 independent experiments and represented as mean ± SD. The variation between 2 groups was compared by Student's *t*-test. Data were considered significant different when P value < 0.05.

3. Results

3.1. PD-L1 and IFI16 highly expressed in HPV positive cervical cancer cells

Abnormally high expression of PD-L1 and IFI16 in HPV positive rather than HPV negative cervical cancer was discovered by TCGA dataset analysis from 156 paitent samples (Fig. 1A, 1B). The correlation between HPV infection and PD-L1 or IFI16 was further confirmed by transcripts analysis in HPV positive cervical cancer patients (n = 30) (Fig. 1C, D). Consistently, the mRNA expression of PD-L1 and IFI16 were significantly higher in HPV positive cells compared with HPV negative cells (Fig. 1E, F). Protein expression also showed the same situation (Fig. 1G). These results showed a potential link between HPV infection and PD-L1 or IFI16.

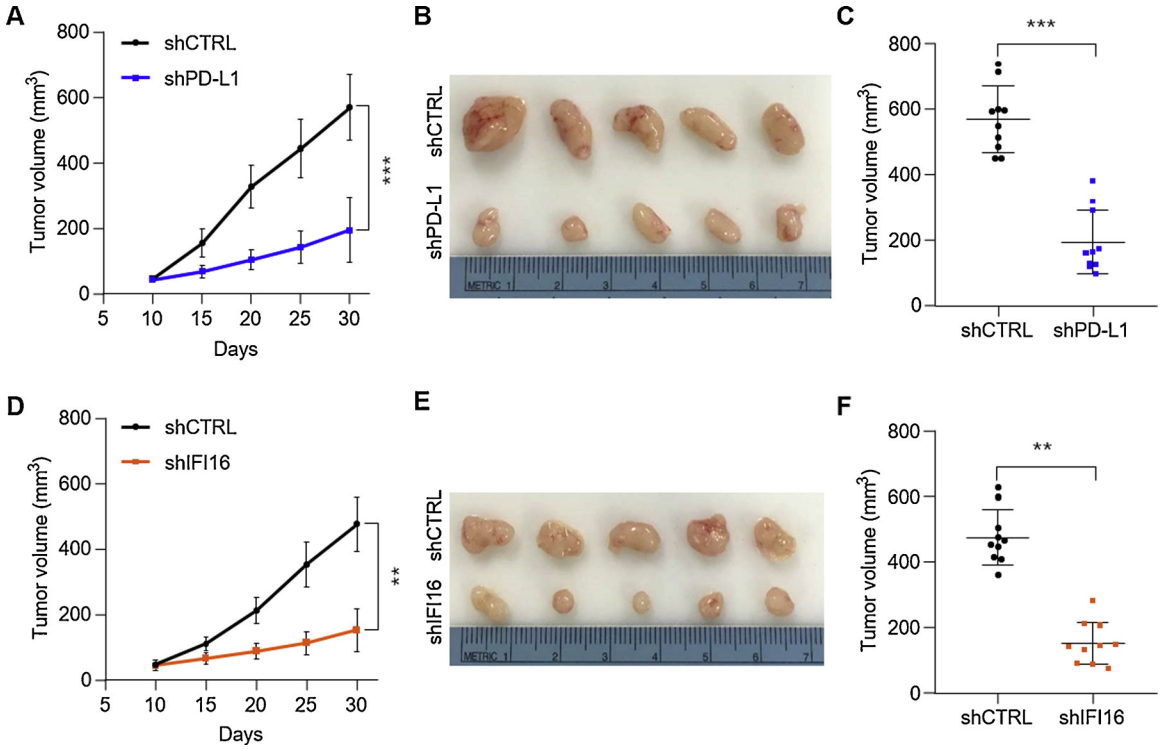

Fig. 5. Knockdown of IFI16 or PD-L1 inhibits the growth of cervical cancer cells in vivo. (A) NOD/SCID mice (6 weeks old; 10 per group) were inoculated subcutaneously with SiHa cells (2 × 106 per animal) pre-transfected with shCTRL or shIFI16. Tumor volumes were measured as indicated. Data points are presented as mean volume ± s.d. values. (B–C) Comparative statistics of tumor end volumes and images of excised tumors from five NOD/SCID mice at 45 days used in A. (D) NOD/SCID mice (6 weeks old; 10 per group) were inoculated subcutaneously with SiHa cells (2 × 106 per animal) pre-transfected with shCTRL or shPD-L1. Tumor volumes were measured as indicated. Data points are presented as mean volume ± s.d. values. (E–F) Comparative statistics of tumor end volumes and images of excised tumors from five NOD/SCID mice at 45 days used in D. *P < 0.05. **P < 0.01 (Student's *t*-test).

3.2. PD-L1 facilitates tumor growth and metastasis in vitro

Since PD-L1 was widely reported to play an oncogenic role in many cancers, we first did overexpression and knockdown of PD-L1 in SiHa cells to figure out the role of PD-L1 in HPV positive cervical cancer cells (Fig. 2A). A positive correlation between PD-L1 expression and cell proliferation (Fig. 2B) or tumorigenesis ability (Fig. 2C) were represented. Moreover, Ki67 and PCNA were significantly increased in cells with higher PD-L1 expression (Fig. 2D). Further, Wound healing and transwell showed an enhanced cell migration and invasion respectively in PD-L1 high expressing cells (Fig. 2E, F). These results suggested that PD-L1 played an oncogenic role in HPV positive cervical cancer.

3.3. IFI16 facilitate PD-L1 expression and promote tumor progression in vitro

IFI16 overexpression and knockdown were performed in SiHa and CaSki cells (Fig. 3A) to investigate the role of IFI16 in cervical cancer. Interestingly, a decreased PD-L1 protein level was observed after IFI16 knockdown (Fig. 3B). Besides, cell proliferation and colony formation were also inhibited after IFI16-knockdown (Fig. 3C, D). More importantly, both cell migration and invasion were inhibited after IFI16 knockdown (Fig. 3E, F) showing a reduced tumor metastasis ability after IFI16 knockdown. These results indicated that IFI16 facilitates PD-L1 expression and promotes HPV positive cervical cancer progression in vitro.

3.4. IFI16 facilitate PD-L1 expression through STING-TBK1-NF-kB pathway

IFI16 was assumed to act as an oncogenic role according to previous results (Fig. 3). To further explore the underlying mechanism of IFI16 in HPV positive cervical cancer, many downstream signaling pathways were detected. Western blot revealed increased PD-L1 and activated STING-TBK1, IRF3 and NF-kB pathway by IFI16 (Fig. 4A). Although both IRF3 and NF-kB were activated by STING-TBK1, subsequent western blot demonstrated that PD-L1 was activated by NF-kB (Fig. 4B) but not IRF3 (Fig. 4C). Moreover, two binding sites of NF-kB in the proximal region of PD-L1 promoter were predicted by bioinformatic methods (Fig. 4D) which was confirmed by subsequent promoter transient reporter assay (Fig. 4E). These results demonstrated a regulation mechanism of IFI16 that IFI16 promoted STING-TBK1 which activated downstream NF-kB pathway leading to PD-L1 expression thus promoting cervical cancer progression.

3.5. PD-L1 and IFI16 promote tumor growth in vivo

To better understand the role of PD-L1 and IFI16 on cervical cancer development in vivo, SiHa cells were subcutaneously injected into NOD/SCID mice for tumor xenografts. The results suggested that tumor growth was slowed down after PD-L1 (Fig. 5A) knockdown or IFI16 knockdown (Fig. 5D). The final tumor volume was significantly dropped after PD-L1 (Fig. 5B, C) or IFI16 knockdown (Fig. 5E, F) as well. Both PD-L1 and IFI16 knockdown significantly restrained the growth of SiHa-derived tumor in vivo. Taken together, we found that IFI16, playing an oncogenic role, promote cervical cancer progression by regulating PD-L1 through STING-TBK1-NF-kB pathway.

4. Discussion

The innate immune system is the first line of host defense that can deploy pattern-recognition receptors (PRRs) against viral infection. STING was the endoplasmic reticulum adaptor that can facilitate innate

immune signaling [17]. After activated by several viruses as well as intracellular DNA, STING recruits and activates the downstream TBK1 to initiate a cascade signaling thus activates interferon regulatory factor (IRF) and NF-κB [18]. Studies have showed that STING signaling could prevent the development of cancers [19]. In some HPV positive cancer, STING response was dampened result from the high-risk HPV 16E7 oncogene thus causing poor prognosis [20]. But this immunoregulation in cervical cancer remains unclear. In this research, we identified a mechanism of STING-TBK1 mediated immunoregulation in HPV positive cervical cancer.

Many DNA sensors have been reported for cytosolic DNA detection after the discovery of STING. One of them was IFI16 who was the first reported PRR. Recent studies had reported that IFI16 to be a tumor suppressor by activating p53 signaling pathway and inflammasome in some cancers such as Hepatocellular Carcinoma [21]. In HPV positive disease, IFI16 expression was rather higher than HPV negative disease [22] which is consistent with our study. In our research, we found that HPV infection was positively correlated with IFI16. Also, we observed that IFI16 promotes cervical cancer development in vitro and in vivo as an oncogenic role like PD-L1, which is unprecedented and deserve further investigation.

Finally, a IFI16-STING-TBK1-NF-κB pathway in this process was identified. Many researches reported the role of NF-κB family, it is made up of many transcription factors that have essential effects on immunity, inflammation and cancer progression et,al. NF-κB was activated in most tumors [23]. Inhibition of NF-κB could reverse radioresistance in cervical cancer [24]. In this research, we firstly found that NF-kB played a critical role on IFI16-STING-TBK1 mediated PD-L1 regulation thereby promoting HPV positive cervical cancer progression. Two binding sites of NF-kB in the proximal region of PD-L1 promoter was also identified which expanded the understanding of NF-kB.

In tumors, cells can express coinhibitory receptors including PD-L1 thus exploiting the immune checkpoints to create the immunosuppressive tumor microenvironment, thereby helping tumor cells escape from antitumor immunity [5]. Recent studies on PD-L1 immunotherapy have acquired efficacy [25]. But lack of immunogenicity and tumor antigen recognition still limited the utilization of checkpoint therapy. Our findings expanded the understanding of IFI16 in cancer immunoregulation and give new insight into immunotherapy. Also, IFI16 hold the promise of being developed as a novel immunotherapy target in the future, benefiting a wider cancer patient population.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

All authors declare that they have no financial and non-financial conflicts of interest.

References

[1] S. McGuire, World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015, Adv. Nutr. (Bethesda, Md) 7 (2) (2016) 418–419.
[2] M. Narisawa-Saito, T. Kiyono, Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and E7 proteins, Cancer Sci. 98 (10) (2007) 1505–1511.
[3] A. Goodman, HPV testing as a screen for cervical cancer, BMJ 350 (2015) h2372.
[4] C. Guitarte, I. Alagkiozidis, B. Mize, E. Stevens, G. Salame, Y.C. Lee, Glassy cell carcinoma of the cervix: a systematic review and meta-analysis, Gynecol. Oncol. 133 (2) (2014) 186–191.
[5] R.D. Schreiber, L.J. Old, M.J. Smyth, Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion, Science (New York, N. Y.) 331 (6024) (2011) 1565–1570.
[6] J.M. Pitt, M. Vetizou, R. Daillere, M.P. Roberti, T. Yamazaki, B. Routy, et al., Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors, Immunity 44 (6) (2016) 1255–1269.
[7] L. Sun, J. Wu, F. Du, X. Chen, Z.J. Chen, Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway, Science (New York, N. Y.) 339 (6121) (2013) 786–791.
[8] D.L. Burdette, K.M. Monroe, K. Sotelo-Troha, J.S. Iwig, B. Eckert, M. Hyodo, et al., STING is a direct innate immune sensor of cyclic di-GMP, Nature 478 (7370) (2011) 515–518.
[9] L. Unterholzner, S.E. Keating, M. Baran, K.A. Horan, S.B. Jensen, S. Sharma, et al., IFI16 is an innate immune sensor for intracellular DNA, Nat. Immunol. 11 (11) (2010) 997–1004.
[10] A.R. Lam, N.L. Bert, S.S. Ho, Y.J. Shen, L.F. Tang, G.M. Xiong, et al., RAE1 ligands for the NKG2D receptor are regulated by STING-dependent DNA sensor pathways in lymphoma, Cancer Res. 74 (8) (2014) 2193–2203.
[11] T.N. Schumacher, R.D. Schreiber, Neoantigens in cancer immunotherapy, Science (New York, N. Y.) 348 (6230) (2015) 69–74.
[12] E. Borcoman, C. Le Tourneau, Pembrolizumab in cervical cancer: latest evidence and clinical usefulness, Ther. Adv. Med. Oncol. 9 (6) (2017) 431–439.
[13] S.J. Rodig, D. Gusenleitner, D.G. Jackson, E. Gjini, A. Giobbie-Hurder, C. Jin, et al., MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma, Sci. Transl. Med. 10 (450) (2018).
[14] V.A. Boussiotis, Molecular and biochemical aspects of the PD-1 checkpoint pathway, N. Engl. J. Med. 375 (18) (2016) 1767–1778.
[15] C. Liu, J. Lu, H. Tian, W. Du, L. Zhao, J. Feng, et al., Increased expression of PDL1 by the human papillomavirus 16 E7 oncoprotein inhibits anticancer immunity, Mol. Med. Rep. 15 (3) (2017) 1063–1070.
[16] F. Chen, Q. Long, D. Fu, D. Zhu, Y. Ji, L. Han, et al., Targeting SPINK1 in the damaged tumour microenvironment alleviates therapeutic resistance, Nat. Commun. 9 (1) (2018) 4315.
[17] H. Ishikawa, G.N. Barber, STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling, Nature 455 (7213) (2008) 674–678.
[18] T. Abe, G.N. Barber, Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF-kappaB activation through TBK1, J. Virol. 88 (10) (2014) 5328–5341.
[19] L. He, X. Xiao, X. Yang, Z. Zhang, L. Wu, Z. Liu, STING signaling in tumorigenesis and cancer therapy: a friend or foe? Cancer Lett. 402 (2017) 203–212.
[20] M.H. Shaikh, V. Bortnik, N.A. McMillan, A. Idris, cGAS-STING responses are dampened in high-risk HPV type 16 positive head and neck squamous cell carcinoma cells, Microb. Pathog. 132 (2019) 162–165.
[21] W. Lin, Z. Zhao, Z. Ni, Y. Zhao, W. Du, S. Chen, IFI16 restoration in hepatocellular carcinoma induces tumour inhibition via activation of p53 signals and inflammasome, Cell Prolif. 50 (6) (2017).
[22] M. Mondini, S. Costa, S. Sponza, F. Gugliesi, M. Gariglio, S. Landolfo, The interferon-inducible HIN-200 gene family in apoptosis and inflammation: implication for autoimmunity, Autoimmunity 43 (3) (2010) 226–231.
[23] M. Karin, Y. Cao, F.R. Greten, Z.W. Li, NF-kappaB in cancer: from innocent bystander to major culprit, Nat. Rev. Cancer 2 (4) (2002) 301–310.
[24] S. Tilborghs, J. Corthouts, Y. Verhoeven, D. Arias, C. Rolfo, X.B. Trinh, et al., The role of Nuclear Factor-kappa B signaling in human cervical cancer, Crit. Rev. Oncol. Hematol. 120 (2017) 141–150.
[25] J. Bellmunt, T. Powles, N.J. Vogelzang, A review on the evolution of PD-1/PD-L1 immunotherapy for bladder cancer: the future is now, Cancer Treat. Rev. 54 (2017) 58–67.