The IFN-γ-IDO1-Kynureine Pathway-Induced Autophagy in Cervical Cancer Cell Promotes Phagocytosis of Macrophage

Shao-Liang Yang
Obstetrics and Gynecology Hospital of Fudan University

Hai-Xia Tan
Obstetrics and Gynecology Hospital of Fudan University

Tian-Tian Niu
Obstetrics and Gynecology Hospital of Fudan University

Yu-Kai Liu
Obstetrics and Gynecology Hospital of Fudan University

Chun-Jie Gu
Obstetrics and Gynecology Hospital of Fudan University

Da-Jin Li
Obstetrics and Gynecology Hospital of Fudan University

Ming-Qing Li (✉ mqli@fudan.edu.cn)
Obstetrics and Gynecology Hospital of Fudan University

haiyan Wang (✉ haiyanwang2002@163.com)
Obstetrics and Gynecology Hospital of Fudan University  https://orcid.org/0000-0002-9685-0522

Research

Keywords: IFN-γ, IDO1, kynurenine, autophagy, cervical cancer, phagocytosis, macrophage

DOI: https://doi.org/10.21203/rs.3.rs-27592/v1

License: ☝️ This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background: Cervical cancer is a common malignant disease in female patients accompanied by active autophagy in tumor cells. However, much remains unknown about the regulatory factors of autophagy and its effect on immune response.

Methods: Autophagy in HeLa and SiHa cells treated with IFN-γ, tryptophan depletion, kynurenine, and epacadostat was detected by western blot and autophagy detection kit. After co-cultured with pre-treated HeLa and SiHa cells, U937 was detected by ow cytometry to analyze the CD80, CD86, CD163, CD206 expression and the phagocytosis.

Results: IFN-γ could significantly increase the autophagy level of HeLa and SiHa cells by promoting the indoleamine-2,3-dioxygenase-1 (IDO1) expression. HeLa and SiHa cells treated with recombinant human IFN-γ could significantly increase the phagocytosis and CD80, CD86 expression of U937, and this effect was associated with the autophagy in tumor cells. IFN-γ treatment and IDO1 overexpression promote tryptophan depletion and kynurenine accumulation in cervical cancer cells, and the latter was proved to be more potent in inducing autophagy of cervical cancer cells and promoting phagocytosis of macrophage. In vivo, IDO1 overexpression could significantly restrict the tumor growth in C57 mice, and the phagocytosis of macrophage was enhanced.

Conclusions: IFN-γ promotes the autophagy of cervical cancer cell possibly through IDO1 expression and kynurenine metabolism, and further promotes macrophage phagocytosis in cervical cancer.

Background

Cervical cancer, a major cause of women morbidity and mortality, is currently the second most popular malignant diseases in worldwide women [1, 2]. With the popularization of regular cervix screening, more and more patients can be diagnosed at the early stage of cervical cancer, and the outcome of cervical cancer has been improved [3]. However, as for advanced cervical cancer patients with recurrence and metastasis, effect of surgery or radiotherapy is quite limited. It was reported that large amount of immune cells infiltrated the tumor tissue, and immune microenvironment in cervical cancer could determine their clinical outcome [4]. Therefore, immunotherapy, which has achieved great progress in many malignant diseases, such as melanoma, non-small cell lung cancer and so on [5, 6], is now regarded as a potential treatment strategy to improve the survival of these patients. In order to shed light in the direction of immunotherapy for cervical cancer, investigating the interaction between tumor cells and immunocytes is indispensable.

Indoleamine-2,3-dioxygenase (IDO1) is an enzyme which can catalyze tryptophan into kynurenine. As it can induce immune suppression of T cells in some diseases, IDO1 is deemed a check point which has been implied in clinical trial in melanoma, metastatic breast cancer, acute myeloid leukemia, and so on [7]. It was found that IDO1 was also highly expressed in cervical tumor cells [8]. To explore the role of IDO1 in the progress of cervical cancer growth could provide evidence for clinical trials of IDO1 inhibitors.
As an important professional antigen-presenting cell, macrophage plays indispensable roles in T cell stimulation and immune regulation. Activated macrophage could enhance the anti-tumor effect of cytotoxic T cells, while tolerated macrophage could promote tumor growth, angiogenesis, and metastasis [9-11]. Phagocytosis of macrophage was the first step in tumor specific antigen processing, and the antigens after process combined with co-stimulatory molecule CD80/CD86 effectively activate CD4+ and CD8+ T cells with the restrict of major histocompatibility complex (MHC). During the tumorigenesis of cervix, human papillomavirus (HPV) could inhibit the phagocytosis of macrophage to induce immune evasion[12]. And the “don't eat me” signal CD47, which can inhibit the phagocytosis of macrophage, is expected to be an efficacious target in the treatment of cervical cancer[13].

Autophagy is a highly conserved process in eukaryotic cells which could dispose unnecessary or dysfunctional components for reuse. As tumor cells are commonly in a condition of rapid proliferation, nutrition deficiency and hypoxia, the autophagy level is usually upregulated. In some studies, autophagy in cancer cells was reported to contribute to the immune suppression and tumor growth[14], while in other studies, autophagy could promote the antigen presentation and immune activation[15]. In our previous work, we found that autophagy suppression in endometrial stromal cells could inhibit the cytotoxicity of NK cells [16]. However, there is no consensus on the role of autophagy in the progression of cervical cancer. Additionally, whether autophagy of cervical cancer contributes to the crosstalk between cervical cancer cells and NK cells remains unknow.

In this article, we investigate the role of IFN-γ-IDO1-kynurenine pathway in autophagy of cervical cancer cell and the function of macrophage, which may provide clues in finding potential treatment strategies in cervical cancer.

**Methods**

**Cell culture**

The HeLa, SiHa (human cervical cancer cell line), TC-1(HPV-16 E6/E7 and c-Ha-Ras co-transformed mouse lung epithelial cell line) and U937 (human monocyte cell line) were bought from American Type Culture Collection. HeLa, SiHa and TC-1 cells were cultured with DMEM/F12 and U937 in RPMI 1640 (HyClone Laboratories, Logan, UT, USA), containing 10% fetal bovine serum (Gibco Cell Culture, Carlsbad, CA, USA) and 1% Antibiotic-Antimycotic (Gibco Cell Culture, Carlsbad, CA, USA). Cells were passaged depending on their densities. The temperature of the incubator was stabilized at 37°C and CO₂ concentration was 5%.

**Co-culture of cervical cancer cells with U937 cells**

To identify whether IFN-γ, rapamycin or kynurenine treated cancer cells impact the phagocytic activity and polarization of human monocyte / macrophage cell line U937, HeLa and SiHa cells were pretreated
with recombinant human IFN-γ protein (rhIFN-γ, 10ng/ml, PeproTech), rapamycin (2μmol/l, Sigma), or kynurenine (500μmol/l, Sigma) for 48 hours, supernatant was discarded and cells were washed with PBS. Then, fresh medium and U937 cells were added to the plate and cervical cancer cells were co-culture directly with U937 cells for 48 hours at the ratio of 1:1. After 48 hours, cells were harvested and analyzed by FCM.

**Western blot**

Cells were washed with PBS for three times, and lysed with lysis buffer (Beyotime Biotechnology, Shanghai, China), containing protease inhibitor cocktail (MedChemExpress, Shanghai, China) and phosphatase inhibitor cocktail (MedChemExpress). Protein concentrations were detected using a BCA protein assay kit (Beyotime Biotechnology). After that, protein was diluted with loading buffer (Beyotime Biotechnology) and heated to 95°C for 10 minutes; denatured protein was stored at -20°C. For western blot, equal amounts of protein calculated according to the concentration were electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gels (Epizyme Scientific, Shanghai, China), transferred to nitrocellulose membranes (BioRad, Hercules, CA, USA), blocked by 5% non-fat milk for 2 h at room temperature, and incubated with corresponding primary LC3B, IDO1 and GAPDH antibody (1:1000, Cell Signaling Technology, Danvers, MA, USA) overnight at 4 °C. The membrane was washed three times with TBS-T for 15min and incubated with HRP-linked Anti-rabbit IgG (Cell Signaling Technology, Danvers, MA, USA) for 1 h at room temperature. After washing for three times with TBS-T, protein bands were wetted with Immobilon Western Chemiluminescent HRP Substrate (Millipore, Darmstadt, Germany) and detected by Luminescent Image Analyzer LAS 4000 (FUJIFILM, Japan).

**Flow cytometry (FCM)**

HeLa, SiHa or U937 cells collected from wells were centrifuged at 1500 rpm for 6 min, and incubated with APC-conjugated anti-human CD45, PE-conjugated anti-human CD86, and PE/CY7-conjugated anti-human CD163, FITC-conjugated anti-human CD80, and BV421-conjugated anti-human CD206 (eBioscience, San Diego, CA, USA). Specifically, HeLa and SiHa cells were fixed, permeabilized, and then stained with APC-conjugated anti-human IDO1 antibody. After that, the cells were washed twice with PBS, and resuspended for FCM analysis. In parallel, the isotopic IgG antibodies were used as controls.

In animal experiment, tumor tissue was mechanical cut, digested with collagenase, and filtrated by sieve to prepare monoplast suspension. Cells were centrifuged at 1500 rpm for 6 min, and incubated with Percp-conjugated anti-mouse CD45, APC-conjugated anti-mouse CDF4/80, BV605-conjugated anti-mouse CD11b, FITC-conjugated anti-mouse CD80, PE-conjugated anti-mouse CD86, Pecy7-conjugated anti-mouse CD206 (Biolegend, San Diego, CA, USA)

Data were collected in FACS Calibur flow cytometer (Beckman Coulter CyAn ADP or Beckman Coulter Cytoflex, North Carolina, USA) and analyzed with FlowJo 7.6. Each experiment was performed for three
times independently. Statistical analysis was performed by using isotype matched controls as references. Typically, less than 1% positive cells were permitted beyond the statistical marker in the appropriate controls.

DAP Green autophagy detection

Cells were seeded in an appropriate dish overnight. Discard the supernatant and wash the cells with culture medium once. Add the diluted DAP Green solution (0.1 μmol/l, Dojindo Laboratories, Japan), incubate at 37°C for 30 minutes. Discard the supernatant and wash the cells with culture medium twice. Then add the medium in different group and treat cells for 4 hours. Discard the supernatant, dye the nucleus with DAPI (Sigma-Aldrich, USA) for 10 minutes and wash the cells with culture medium twice. Observe fluorescence and take pictures under a fluorescence microscope (Leica, Munich, Germany). Multiple fields of view were randomly selected through fluorescence microscope observation, and then the number of autophagosomes was calculated.

Phagocytosis assays

HeLa and SiHa cells were planted in 24-well plates and treated with rhIFN-γ (10ng/ml, PeproTech), epacadostat(50nmol/L, MedChemExpress), kynurenine(500μmol/l, Sigma) or tryptophan free medium() as shown in results for 48 hours. Then cells were harvested and re-suspended in PBS supplemented with 5 μmol/l of 5, 6-carboxyfluorescein diacetate succinimidyl ester (CFSE, eBioscience, San Diego, CA, USA) for 8 min at 37°C with 5% CO₂. After washed with PBS, the CFSE-labelled cells were co-cultured with U937 cells for 2 hours at the ratio of 1:1, then cells were harvested and incubated with APC-conjugated CD45 antibody to label U937. The phagocytic ratio was tested by flow cytometry. CFSE⁺CD45⁺ cells were regarded as U937 cells which had swallowed CFSE⁺ cancer cells.

Lentivirus transfected in HeLa and SiHa cells

HeLa and SiHa cells were transfected with IDO1 overexpression lentivirus or negative control lentivirus respectively. Briefly, HeLa and SiHa cells were seeded at a density of 5 × 10⁵ cells/well in 6-well plates and adhered overnight. At the density of 50%, cells were transfected in triplicate with lentivirus at the MOI of 1, 10, and 100. Choose appropriate MOI based on the fluorescence intensity. Puromycin was continuously used to filter the successfully transfected cells for 2 weeks until the purity was more than 90%. TC-1 cells were transfected with mouse IDO1 lentivirus in the same way.

High-performance liquid chromatography (HPLC)-tandem mass spectrometry (LC-MS/MS) quantification of
**Tryptophan and kynurenine**

Qualitative assessment of tryptophan metabolites by MS (metabolomics) was performed by the Institute of Biomedical Sciences, Fudan University. HeLa and SiHa cells treated with IFN-γ or transfected with IDO1 overexpression lentivirus were washed with PBS, trypsinized and collected in 1.5ml centrifuge tube. Cells were lysed by adding 300µl deionized water, freezed and thawed for three times. Add 900µl methanol to the lysis solution, well mixed and then centrifuged at 20000g for 10 minutes. Collected the supernatant and volatilized to get dry power. Samples and tryptophan/kynurenine standards were detected in TSQ-Vantage triple quadrupole mass spectrometer (Thermo), using a ShimazuLC (LC-20AB pump) system and a C18 column (250mm×2.1mm I.D., 3µm particle size, ULTIMATE). Selected reaction monitoring (SRM) scan mode was applied, and transitions evaluated were Trp, 205.09–188.0, Kyn, 209.063–94.2. The results were analyzed in Analyst Software.

**Animal mode and treatment**

Animals used in this study were approved by the Ethical Committee of the Obstetrics and Gynecology Hospital, Fudan University. Four-week-old female C57BL/6 mice were obtained from Animal Laboratory (Shanghai, China). After one week’s adaptation, 16 mice were randomly divided into two groups, injected subcutaneously on the back with 200µl TC-1 cells (5×10^6 cell per mouse, IDO1 overexpression or negative lentivirus transfected). When palpable, tumors were measured every other day and tumor volume was calculated as 1/2(length*width*width). Mice were euthanized when the tumor width was over 20mm or ulceration of tumor was observed. The tumor tissue was minced, digested and subjected for FCM.

In epacadostat experiment, 28 mice were randomly divided into four groups, injected subcutaneously on the back with 200µl TC-1 cells (5×10^6 cell per mouse, IFN-γ overexpression+PBS, IFN-γ overexpression+epacadostat, negative lentivirus+PBS, negative lentivirus+epacadostat). In IFN-γ and kynurenine group, 26 mice were randomly divided into three groups, injected subcutaneously on the back with 200µl TC-1 cells (5×10^6 cell per mouse). Mice in different groups were intraperitoneal injected with epacadostat (50mg/kg), IFN-γ (100ng/animal), or kynurenine (100mg/kg) every day after the tumor was palpable. Tumor volume was detected every three days.

**Statistical analysis**

All of the data are shown as mean ± SEM. Comparison between controls and treatments was analyzed by Student’s *t*-test. Comparison between groups more than two was analyzed by One-way ANOVA. All analyses were performed using Graphpad Prism software (Graphpad Software, San Diego, CA, USA) for Windows. Differences were considered to be significant at *P*<0.05.

**Results**
IFN-γ induces autophagy of cervical cancer cells and promotes phagocytosis and activation of macrophage

To explore the effect of IFN-γ on the autophagy level of cervical cancer cells and the function of macrophage, HeLa and SiHa cells were treated with IFN-γ at the concentration of 10ng/mL, and the autophagy activity was measured by western blot and autophagosome detection kit. LC3BII was the most common used marker to indicate the activity of autophagy. Here, we found that LC3BII was significantly promoted by IFN-γ both in HeLa and SiHa cells, shown as LC3BII/GAPDH (Fig.1A). Autophagosome (green dot pointed by yellow arrow) labeled with green fluorescence was also increased in IFN-γ treated cells (Fig.1B). To investigate the effect of IFN-γ on macrophage, we co-cultured the treated cancer cells tracked by CFSE with U937 cells for 2 hours to test phagocytosis, or 48 hours to measure the polarization. It was found that cervical cancer cells treated with IFN-γ were more likely to be swallowed by U937 cells, as we can see in the picture, that CD45^+CFSE^+ cells were increased in IFN-γ treated group (Fig.1C-D). What’s more, we confirmed that IFN-γ treated HeLa and SiHa cells could promote the CD86 expression of U937, and treated SiHa cells inhibited the CD163 expression, which was not significantly changed in HeLa cells (Fig.1E-F). And CD80 and CD206 were not significantly changed in HeLa and SiHa cells (data not shown).

Autophagy of cervical cancer cells promotes the phagocytosis and activation of macrophage

To investigate the relationship between autophagy in cervical cancer cells and function of macrophage, HeLa and SiHa cells pretreated with rapamycin was co-cultured with U937 cells. First, we verified the autophagy-inducing efficiency of rapamycin in HeLa and SiHa cells. The results showed that rapamycin could significantly increase the autophagy level in HeLa and SiHa cells (Fig.2A-B). HeLa and SiHa cells treated with or without rapamycin were collected, labeled with CFSE, and then co-cultured with U937 cells for 2 hours to test the phagocytosis. Cells phagocytosed by U937 were increased in rapamycin treated group (Fig.2C-D). After 48 hours co-culture, the polarization of U937 cells was measured by FCM. As shown, CD80 and CD86 expression in U937 cells co-cultured with rapamycin-pretreated cancer cells were increased compared to the control group, meanwhile, CD163 expression was decreased, in both HeLa and SiHa cells co-culture units (Fig.2E-F). Rapamycin-pretreated HeLa cells decreased the expression of CD206 in U937 cells, while there was no difference in U937 cells co-cultured with SiHa cells pretreated with rapamycin or not. These results demonstrate that autophagy of cervical cancer cells promotes the activation of macrophage in vitro.

IFN-γ induces autophagy of cervical cancer cells by up-regulating IDO1 expression
To identify the possible regulatory mechanism of IFN-γ and IDO1 on autophagy in cervical cancer cells, IDO1 expression in HeLa and SiHa cells treated with IFN-γ or not was detected by western blot and FCM (Fig. 3A-B). It was found that HeLa and SiHa cells rarely expressed IDO1 without the stimulation of IFN-γ. While stimulated, the IDO1 expression was significantly up-regulated. As shown, the identification of IDO1 overexpression was confirmed by PCR and western blot (Fig. 3C-D). IDO1 overexpression significantly increased the LC3BII expression in HeLa and SiHa cells (Fig. 3D), suggesting that IDO1 promotes the autophagy of cervical cancer cells. Of note, epacadostat, an inhibitor of IDO1, could significantly suppress the IFN-γ-induced cell autophagy, verified by western blot and autophagy detection probe (Fig. 3E-F). These data indicate that IFN-γ induces autophagy of cervical cancer cells possibly by promoting the IDO1 expression.

**IDO1 in cervical cancer cells promotes phagocytosis and activation of macrophage**

To investigate the role of IDO1 in cervical cancer, the phagocytosis ability of U937 cells to control or IDO1-overexpressed HeLa and SiHa cells was analyzed. As shown, cervical cancer cells transfected with IDO1 overexpression lentivirus were phagocytosed more compared with control group (Fig. 4A). CD45 was used to distinguish cancer cells and U937 cells in co-culture system. It was found that CD80 was significantly increased in U937 cells in IDO1 overexpress group, and CD86 was increased in U937 cells co-cultured with IDO1-overexpressed HeLa cells (Fig. 4B-C). However, CD163 and CD206 expression were not significantly different in the two groups in both HeLa and SiHa (Fig. 4D-E). These finding implies that IDO1 in cervical cancer cells promotes the phagocytosis of macrophage towards them, and the activation of macrophage should be involved in this process.

**The accumulation of kynurenine and consumption of tryptophan catalyzed by IDO1 promote phagocytosis and activation of macrophage**

IDO1 is an enzyme which can catalyze tryptophan to kynurenine. To explore the change of tryptophan and kynurenine in IFN-γ-treated or IDO1-overexpressed cervical cancer cells, mass spectrometric detection was performed to detect the level of tryptophan and kynurenine in HeLa and SiHa cells. As shown, tryptophan was significantly decreased and kynurenine was notably increased in IFN-γ-treated cancer cells, as well as in IDO1-overexpressed groups (Fig. 5A-B). These results were coincident with the fact that IFN-γ and IDO1 overexpression lentivirus could increase IDO1 expression in HeLa and SiHa cells. Autophagy was detected in HeLa and SiHa cells treated with kynurenine (500μmol/L) or tryptophan free medium. The results showed that both tryptophan depletion and kynurenine addition could promote the level of autophagy in cervical cancer cells (Fig. 5C-D). In addition, HeLa and SiHa cells pretreated with kynurenine significantly increased the CD80 and CD86 expression in U937 cells after 48 hours co-culture.
(Fig. 5E). And we found that the effect of tryptophan depletion was not as obvious as kynurenine addition. The ratio of phagocytic cervical cancer cells in kynurenine treatment group was significantly increased (Fig. 5F). The effect of tryptophan depletion was also significant up-regulated in HeLa cells, but weaker than kynurenine addition (Fig. 5F). In conclude, IFN-γ and IDO1 may promote the phagocytosis and activation of macrophage through accumulation of kynurenine, rather than consumption of tryptophan.

**IDO1 inhibits tumor growth and promotes macrophage phagocytosis in vivo**

To investigate the role of IDO1 in cervical cancer cells in vivo, we constructed subcutaneous tumor model in C57BL/6J mice using TC-1 cells transfected with mice IDO1 overexpression or negative control lentivirus. The efficiency of transfection was verified by FCM (Fig. 6A). Autophagy of TC-1 cells transfected with lentivirus was detected by western blot and the results was similar with IDO1 in HeLa and SiHa cells (Fig. 6B). The tumor volume was significantly smaller in IDO1-overexpressed group, so as the tumor weight (Fig. 6C-E). CD45⁺CD11b⁺F4/80⁺ was used to identify macrophages in tumor tissue, and GFP⁺ macrophages were regarded as those which had phagocytosed tumor cells (Fig. 6F-G). It was found that TC-1 cells phagocytosed by macrophages in IDO1-overexpressed group were elevated compared to negative control (Fig. 6G). Moreover, CD206 expression in macrophage in IDO1-overexpressed group was decreased, and CD80 was not significantly changed (Fig. 6H). Consistent with this, intraperitoneal injection of epacadostat could significantly increase tumor volume, tumor weight, and inhibit CD80 expression of macrophage in tumor tissue (Fig. 6I-L). But intraperitoneal injection of IFN-γ or kynurenine could not restrict the growth of subcutaneous tumor in mice (Fig. SF1). This result may be related to the route of administration, and the direct effect of IFN-γ and kynurenine on immune cells.

**IFNG and IDO1 expression were related to a better survival in cervical patients**

To clarify the effect of IFNG and IDO1 on the survival of cervical patients, we analyzed the results in TCGA online database (http://ualcan.path.uab.edu). As shown, the expression of IFNG was significantly higher in primary tumor than normal cervix (Fig. 7A). When cancer stage was considered, IFNG expression was also increased in all stages of cervical cancer (Fig. 7B). Higher IFNG expression was related to a better survival (Fig. 7C). Similarly, IDO1 expression was also notably increased in cervical tumor than normal cervix tissue (Fig. 7D), in all stages of patients (Fig. 7E), and IDO1 was linked to a better survival (Fig. 7F). Immunohistochemistry of tissue array from HumanProteinAtlas (https://www.proteinatlas.org) showed that more than half of cervical cancer patients (7/12) expressed medium or high level of IDO1 in tumor cells(Fig. 7G), and IDO1 protein level was generally more intensive in cervical cancer tissue than normal cervix (Fig. 7H). Correlation analyze showed that IDO1 expression in cervical cancer was associated with IFNG, the correlation coefficient was 0.63 (Fig. 7I). These results indicate that IDO1
expression in cervical cancer cells is related to IFNG, and both IFNG and IDO1 are linked to a better survival of cervical cancer patients.

**Discussion**

In our research, we found HeLa and SiHa cells rarely expressed IDO1 without the stimulation of IFN-γ. As a matter of fact, IDO1 expression could be detected in cervical cancer tissue, and was higher than normal cervical epithelium; additionally, IDO1 expression is related to a better survival, especially in the first 3000 days. Similar result was also reported by Venancio et al [17]. The expression of IDO1 in cervical tumor cells may be induced by the local environment, such as IFN-γ, HPV and so on [8, 18].

IDO1 is known to induce immune suppression in local tumor environment by promoting the differentiation of regulatory T cell and inhibiting the function of effector T cells [19]. And IDO1 is commonly co-expressed with programmed death ligand 1(PD-L1) in cervical squamous carcinomas [20]. But in clinical study, adding IDO1 inhibitor to PD-L1 inhibitor could not significantly improve the outcome, and IDO1 inhibitor showed little effect compared to placebo group, in various tumor types including cervical cancer. [21]. IDO1 is an enzyme that exists in almost all of the cells, and inhibitor of IDO1 not only influenced the metabolism of tryptophan in immune cells, but also tumor cells. As mention above, we found that IDO1 could promote the autophagy in cervical cancer cells, which may further promote the phagocytosis and activation of macrophages in tumor tissue. Inhibitor of IDO1 may impair the autophagy of tumor cells and the phagocytosis of macrophage, even the antigen presenting function. As a matter of fact, Heeren et al found that IDO1+ tumors had higher CD8+Ki67+ T cell rates (P = 0.004), and IDO1 expression is linked to improved disease-free (DFS) (P = 0.017) and disease-specific survival (P = 0.043) [8]. This may partly explain why IDO1 inhibitor could not effectively improve the ratio of cervical patients who achieved response. These results remind us to consider the side effects of immunotherapy which may act on tumor cells.

Autophagy is a conservative procedure existed in eukaryocytes. Cellular material is enclosed in autophagosome, delivered to lysosomes and got degraded, leading to the basal recycle of cell components and providing energy and substrates. It is universally accepted that autophagy actively exists in tumor cells, but there is controversy about the role of autophagy in the progression of tumor [22]. Some researchers believed that autophagy in cancer cells could inhibit the immune reaction, promote tumor growth or induce drug resistance [23-26]; while others confirmed that autophagy played a role in stimulating tumor antigen cross-presentation, increasing immune cell activation, and improving the survival of patients [15, 27, 28]. That is to say, the role of autophagy was not always constant, but differed based on the condition. In this study, we found that in cervical cancer, autophagy promoted the phagocytosis of macrophage, induced the CD80, CD86 expression, and could lead to the antigen presentation and immune activation, thus limit the tumor growth.

IDO1 was reported to induce autophagy in different mechanisms. The first one was tryptophan depletion. Tryptophan is an essential amino acid in human body, deficiency of tryptophan can cause the
accumulation of uncharged Trp-tRNA in cells, which could be sensed by integrated stress response kinase General Control Non-depressible 2 (GCN2) [29]. And GCN2 was a potent driver of autophagy which can initiate autophagy when activated [30]. The fact that tryptophan-deficiency signal cause autophagy through GCN2 was also found in T cells, podocyte and kidney epithelial cells, and IDO1-GCN2-autophagy signals was regarded as a common circuit existed in human inflammatory disease [29, 31, 32]. Apart from this, tryptophan deficiency signaling caused by IDO1 activity can lead to the inhibition of metabolic regulator mTOR and protein kinase C independent of GCN2 [33]. And reduced mammalian target of rapamycin (mTOR) signaling was associated with decreased glycolysis, lower oxidative stress, as well as increased autophagy [34].

Kynurenine accumulation was also an important signal disseminated in cells when IDO1 was overexpressed. Kondrikov et al. found that at physiological levels (10 and 100 μmol/l), kynurenine could inhibit autophagy of bone marrow mesenchymal stem cells via Aryl hydrocarbon receptor (AhR) signaling [35]. This study verified the regulatory effect of kynurenine on autophagy through AhR. Actually, at the presence of IFN-γ or overexpression of IDO1, kynurenine concentration at local environment was notably increased to a level hundreds of times higher than the physiological concentration, as showed in our study. And the effect on autophagy may also differ in physiological and pathological conditions. And kynurenine could be catalyzed to several other metabolites, including kynurenic acid, quinolinic acid and NAD+. It was reported that kynurenine metabolites were related to mitochondrial dysfunction and reactive oxygen production, which may leads to cell autophagy [36]. In our research, kynurenine accumulation was more effective than tryptophan depletion. This result may be related to the sensitivity of cervical cancer cells to the stimulation. The kynurenine-AhR signaling or the metabolites of kynurenine in HeLa and SiHa cells may be more important in regulating the function of macrophage.

Autophagy of tumor cells was involved in the antigen presentation. Li et al. found that inhibition of autophagy in tumor cells abolished the cross-presentation by dendritic cells almost completely, whereas induction of autophagy enhanced the cross-presentation of tumor antigens. Additionally, purified autophagosomes were found to be efficient antigen carriers for antigen presentation [37]. Hahn et al. found that alpha-tocopheryloxyacetic acid could promotes antigen cross-presentation by triggering tumor autophagy, and autophagosome-enriched fractions of tumor cells efficiently cross-primed antigen-specific CD8+T cells [38]. Similar results was also found by Li et al [39]. These results demonstrated that autophagy in tumor cells can promote the antigen processing of antigen presenting cells. As phagocytosis of tumor cells and the expression of costimulatory molecules are of importance in the process of antigen processing and presentation. So it's understandable that autophagy could promote the phagocytosis and activation of macrophages. And the mechanism how autophagy of tumor cells regulate the function of macrophage still need to be studied.

IFN-γ and kynurenine effectively induced the autophagy of cervical cancer cells and promote the activation *in vitro*, while they were proved to be invalid to restrict the tumor growth *in vivo* by intraperitoneal injection. Several reasons may contribute to this result. Intraperitoneal injection of IFN-γ may not be an ideal method of administration. This kind of cytokine may be degraded inside the
abdomen. Knock down of IFN-γ or IFN receptor was more common used in animal experiment. Actually, we established an IFN-γ overexpression TC-1 cell line, while the tumor was not formed successfully, and the negative control TC-1 did. This may partly be attributed to the effect of IFN-γ. As for kynurenine, intraperitoneal injection may influence both tumor cells and immune cells. Kynurenine may suppress the immune response by affecting the immune cells. What’s more, the absorption of kynurenine by tumor cells may restrict its intracellular concentration. And low concentrations of kynurenine may not effectively induce the autophagy of tumor cells.

The IDO1 inhibitor should not be an ideal treatment for cervical cancer, but immunotherapy is still the potential strategy to improve the outcome of advanced cervical cancer patients. Bevacizumab and pembrolizumab, two approved therapies that have been added to the standard of care for these patients, represented the fruits after decades of efforts in researching. And other drugs are still currently under study or in clinical trials. An in-depth understanding of the molecular biology and biomarkers may help us to explore the potential immunotherapy. And effective activation of macrophages or inhibition of autophagy in tumor cells may be the key target of immunotherapy, which may bring light in cervical cancer.

Conclusions
Collectively, as shown in Fig. 8, IFN-γ can promote the IDO1 expression in cervical cancer cells, and promotes the activity of IDO1 that catalyzes tryptophan to kynurenine. The accumulation of kynurenine promotes the autophagy of cervical cancer cells, and further induces the activation and phagocytosis of macrophage towards them. As a result, the tumor growth is restricted. The activation and the phagocytosis of macrophage should be interacted each other. Therefore, the IFN-γ-IDO1 axis-mediated kynurenine accumulation enhances the phagocytosis of macrophage and restricts the progression of cervical cancer possibly by up-regulating the autophagy of cervical cancer cells.

Abbreviations
IFN-γ: interferon gama
IDO1: indoleamine-2,3-dioxygenase-1
LC-MS/MS: High-performance liquid chromatography-tandem mass spectrometry
MHC: major histocompatibility complex
HPV: human papillomavirus
FCM: flow cytometry
CFSE: 5, 6-carboxyfluorescein diacetate succinimidyl ester
PD-L1: programmed death ligand 1

DFS: disease-free survival

GCN2: General Control Non-depressible 2

mTOR: mammalian target of rapamycin

AhR: Aryl hydrocarbon receptor

**Declarations**

**Acknowledgments**

There are no acknowledgements.

**Authors’ contributions**

Writing the manuscript, Shao-Liang Yang; performing animal experiment, Hai-Xia Tan.; preparing the figures, Tian-Tian Niu and Yu-Kai Liu; editing the figures, Chun-Jie Gu; editing the manuscript, Hai-Yan Wang and Da-Jin Li; writing and editing of the manuscript and figures, Ming-Qing Li. All authors read and approved the final manuscript.

**Funding**

This study was supported by the National Natural Science Foundation of China NSFC (No.81373868) to Hai-Yan Wang; the NSFC (No. 31970798 and 31671200), the Innovation-oriented Science and Technology Grant from NPFPC Key Laboratory of Reproduction Regulation (CX2017-2) and the Program for Zhuoxue of Fudan University to Ming-Qing Li.

**Availability of data and materials**

The datasets involved in our study are available on reasonable request.

**Ethics approval and consent to participate**

All the mouse experiments were approved by the Institutional Animal Care and Use Committee of Fudan University and carried out in accordance with the 'Guide for the care and use of laboratory animals' published by the US National Institutes of Health (Publication no.85–23, revised 1996).
Consent for publication

All authors agree to publish.

Competing interests

The authors declare that there is no potential conflict of interest.

Author details

1. Department of Gynecology of Integrated Traditional Chinese and Western Medicine, Hospital of Obstetrics and Gynecology, Fudan University, Shanghai, 200011, People’s Republic of China;
2. Department of Obstetrics and Gynecology, Zhangye People’s Hospital of HeXi College, Zhangye, Gansu, 734000, China
3. Laboratory for Reproductive Immunology, Hospital of Obstetrics and Gynecology, Fudan University, Shanghai, 200011, People’s Republic of China;
4. Key Laboratory of Reproduction Regulation of NPFPC, SIPPR, IRD, Fudan University, Shanghai 200032, People’s Republic of China;
5. Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Shanghai, 200011, People’s Republic of China.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F: Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015, 136:E359-386.
2. Ginsburg O, Bray F, Coleman MP, Vanderpuye V, Eniu A, Kotha SR, Sarker M, Huong TT, Allemani C, Dvaladze A, et al: The global burden of women’s cancers: a grand challenge in global health. Lancet 2017, 389:847-860.
3. AM dC, D H, JHTG F, E W: Overall survival and time trends in breast and cervical cancer incidence and mortality in the Regional Health District (RHD) of Barretos, São Paulo, Brazil. BMC cancer 2018, 18:1079.
4. S D-E, LE C, SS N, AV Y, G Y, A D, M M, P E, A J, LL L, et al: Kinetics of Intratumoral Immune Cell Activation During Chemoradiation for Cervical Cancer. International journal of radiation oncology, biology, physics 2018, 102:593-600.
5. JJ L, KT F, A R, GV L: Targeted agents and immunotherapies: optimizing outcomes in melanoma. Nature reviews Clinical oncology 2017, 14:463-482.
6. V A, KN S, PM F, N N, R B, J W, T Z, V A, J P, N W, et al: Evolution of Neoantigen Landscape during Immune Checkpoint Blockade in Non-Small Cell Lung Cancer. Cancer discovery 2017, 7:264-276.

7. Zhai L, Ladomersky E, Lenzen A, Nguyen B, Patel R, Lauing KL, Wu M, Wainwright DA: IDO1 in cancer: a Gemini of immune checkpoints. Cell Mol Immunol 2018, 15:447-457.

8. Heeren AM, van Dijk I, Berry D, Khelil M, Ferns D, Kole J, Musters RJ, Thijsse NL, Mom CH, Kenter GG, et al: Indoleamine 2,3-Dioxygenase Expression Pattern in the Tumor Microenvironment Predicts Clinical Outcome in Early Stage Cervical Cancer. Front Immunol 2018, 9:1598.

9. Pedraza-Brindis EJ, Sanchez-Reyes K, Hernandez-Flores G, Bravo-Cuellar A, Jave-Suarez LF, Aguilar-Lemarroy A, Gomez-Lomeli P, Lopez-Lopez BA, Ortiz-Lazareno PC: Culture supernatants of cervical cancer cells induce an M2 phenotypic profile in THP-1 macrophages. Cell Immunol 2016, 310:42-52.

10. van der Sluis TC, Sluijter M, van Duikeren S, West BL, Melief CJ, Arens R, van der Burg SH, van Hall T: Therapeutic Peptide Vaccine-Induced CD8 T Cells Strongly Modulate Intratumoral Macrophages Required for Tumor Regression. Cancer Immunol Res 2015, 3:1042-1051.

11. Heeren AM, Kenter GG, Jordanova ES, de Gruijl TD: CD14+ macrophage-like cells as the linchpin of cervical cancer perpetrated immune suppression and early metastatic spread: A new therapeutic lead? Oncoimmunology 2015, 4:e1009296.

12. Liu F, Dai M, Xu Q, Zhu X, Zhou Y, Jiang S, Wang Y, Ai Z, Ma L, Zhang Y, et al: SRSF10-mediated IL1RAP alternative splicing regulates cervical cancer oncogenesis via mIL1RAP-NF-kappaB-CD47 axis. Oncogene 2018, 37:2394-2409.

13. Krishnan V, Schaar B, Tallapragada S, Dorigo O: Tumor associated macrophages in gynecologic cancers. Gynecol Oncol 2018, 149:205-213.

14. PS, JS, GM, AG, L L, DL C, CJ W, K J, SG, P M, et al: Noncanonical autophagy in dermal dendritic cells mediates immunosuppressive effects of UV exposure. The Journal of allergy and clinical immunology 2019.

15. WJ Z, KK C, K W, HL Y, J M, F X, DJ L, MQ L: Rapamycin Synergizes with Cisplatin in Antiendometrial Cancer Activation by Improving IL-27-Stimulated Cytotoxicity of NK Cells. Neoplasia (New York, NY) 2018, 20:69-79.

16. Mei J, Zhou W-J, Zhu X-Y, Lu H, Wu K, Yang H-L, Fu Q, Wei C-Y, Chang K-K, Jin L-P, et al: Suppression of autophagy and HCK signaling promotes PTGS2-high FCGR3 " NK cell differentiation triggered by ectopic endometrial stromal cells. Autophagy 2018, 14:1376-1397.

17. Venancio PA, Consolaro MEL, Derchain SF, Boccardo E, Villa LL, Maria-Engler SS, Campa A, Discacciati MG: Indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase expression in HPV infection, SILs, and cervical cancer. Cancer Cytopathol 2019, 127:586-597.

18. Mittal D, Kassianos AJ, Tran LS, Bergot AS, Gosmann C, Hofmann J, Blumenthal A, Leggatt GR, Frazer IH: Indoleamine 2,3-dioxygenase activity contributes to local immune suppression in the skin expressing human papillomavirus oncoprotein e7. J Invest Dermatol 2013, 133:2686-2694.

19. Yeung AW, Terentis AC, King NJ, Thomas SR: Role of indoleamine 2,3-dioxygenase in health and disease. Clin Sci (Lond) 2015, 129:601-672.
20. Chinn Z, Stoler MH, Mills AM: **PD-L1 and IDO expression in cervical and vulvar invasive and intraepithelial squamous neoplasias: implications for combination immunotherapy.** *Histopathology* 2019, **74**:256-268.

21. Jung KH, LoRusso P, Burris H, Gordon M, Bang YJ, Hellmann MD, Cervantes A, de Olza MO, Marabelle A, Hodi FS, et al: **Phase I Study of the Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitor Navoximod (GDC-0919) Administered with PD-L1 Inhibitor (Atezolizumab) in Advanced Solid Tumors.** *Clin Cancer Res* 2019, **25**:3220-3228.

22. Levy JMM, Towers CG, Thorburn A: **Targeting autophagy in cancer.** *Nature Reviews Cancer* 2017, **17**:528-542.

23. Lebovitz CB, Robertson AG, Goya R, Jones SJ, Morin RD, Marra MA, Gorski SM: **Cross-cancer profiling of molecular alterations within the human autophagy interaction network.** *Autophagy* 2015, **11**:1668-1687.

24. Eritja N, Chen BJ, Rodriguez-Barrueco R, Santacana M, Gatius S, Vidal A, Marti MD, Ponce J, Bergada L, Yeramian A, et al: **Autophagy orchestrates adaptive responses to targeted therapy in endometrial cancer.** *Autophagy* 2017, **13**:608-624.

25. Fukuda T, Oda K, Wada-Hiraike O, Sone K, Inaba K, Ikeda Y, Miyasaka A, Kashiyama T, Tanikawa M, Arimoto T, et al: **The anti-malarial chloroquine suppresses proliferation and overcomes cisplatin resistance of endometrial cancer cells via autophagy inhibition.** *Gynecol Oncol* 2015, **137**:538-545.

26. Ran X, Zhou P, Zhang K: **Autophagy plays an important role in stemness mediation and the novel dual function of EIG121 in both autophagy and stemness regulation of endometrial carcinoma JEC cells.** *Int J Oncol* 2017, **51**:644-656.

27. Y L, T H, K G, ZH C, A T, J T, HM H, ET A: **The vitamin E analogue α-TEA stimulates tumor autophagy and enhances antigen cross-presentation.** *Cancer research* 2012, **72**:3535-3545.

28. S L, D E, L S, F G, V P-C, K C, M S, M C, F P-L, L A, et al: **The presence of LC3B puncta and HMGB1 expression in malignant cells correlate with the immune infiltrate in breast cancer.** *Autophagy* 2016, **12**:864-875.

29. McGaha TL: **IDO-GCN2 and autophagy in inflammation.** *Oncotarget* 2015, **6**:21771-21772.

30. Ravindran R, Khan N, Nakaya HI, Li S, Loebbermann J, Maddur MS, Park Y, Jones DP, Chappert P, Davoust J, et al: **Vaccine activation of the nutrient sensor GCN2 in dendritic cells enhances antigen presentation.** *Science* 2014, **343**:313-317.

31. Fougeray S, Mami I, Bertho G, Beaune P, Thervet E, Pallet N: **Tryptophan depletion and the kinase GCN2 mediate IFN-gamma-induced autophagy.** *J Immunol* 2012, **189**:2954-2964.

32. Labadie BW, Bao R, Luke JJ: **Reimagining IDO Pathway Inhibition in Cancer Immunotherapy via Downstream Focus on the Tryptophan-Kynurenine-Aryl Hydrocarbon Axis.** *Clin Cancer Res* 2019, **25**:1462-1471.

33. Metz R, Rust S, Duhadaway JB, Mautino MR, Munn DH, Vahanian NN, Link CJ, Prendergast GC: **IDO inhibits a tryptophan sufficiency signal that stimulates mTOR: A novel IDO effector pathway targeted by D-1-methyl-tryptophan.** *Oncoimmunology* 2012, **1**:1460-1468.
34. Bottcher M, Hofmann AD, Bruns H, Haibach M, Loschinski R, Saul D, Mackensen A, Le Blanc K, Jitschin R, Mougiakakos D: Mesenchymal Stromal Cells Disrupt mTOR-Signaling and Aerobic Glycolysis During T-Cell Activation. *Stem Cells* 2016, **34:**516-521.

35. Kondrikov D, Elmansi A, Bragg RT, Mobley T, Barrett T, Eisa N, Kondrikova G, Schoeinlein P, Aguilar-Perez A, Shi XM, et al: Kynurenine inhibits autophagy and promotes senescence in aged bone marrow mesenchymal stem cells through the aryl hydrocarbon receptor pathway. *Exp Gerontol* 2020, **130:**110805.

36. Sas K, Szabo E, Vecsei L: Mitochondria, Oxidative Stress and the Kynurenine System, with a Focus on Ageing and Neuroprotection. *Molecules* 2018, **23**.

37. Li Y, Wang LX, Yang G, Hao F, Urba WJ, Hu HM: Efficient cross-presentation depends on autophagy in tumor cells. *Cancer Res* 2008, **68:**6889-6895.

38. Hahn T, Akporiaye ET: alpha-TEA as a stimulator of tumor autophagy and enhancer of antigen cross-presentation. *Autophagy* 2013, **9:**429-431.

39. Li Y, Hahn T, Garrison K, Cui ZH, Thorburn A, Thorburn J, Hu HM, Akporiaye ET: The vitamin E analogue alpha-TEA stimulates tumor autophagy and enhances antigen cross-presentation. *Cancer Res* 2012, **72:**3535-3545.

**Figures**
Figure 1

IFN-γ induces autophagy of cervical cancer cells and promotes phagocytosis and activation of macrophage (A) HeLa and SiHa cells were treated with recombinant human IFN-γ at a concentration of 10ng/mL for 48 hours. LC3B was measured by western blot. (B) The autophagy level of HeLa and SiHa cells after treatment with IFN-γ was detected by DAP autophagy kit and observed with fluorescence microscope. Yellow arrows represented autophagosomes. Multiple fields of view were randomly selected through fluorescence microscope observation, and then the number of autophagosomes was calculated. (C, D) HeLa and SiHa cells were pretreated with IFN-γ or not, and then labelled with CFSE, and further co-cultured with U937 cells for 2 hours. The phagocytosis of U937 cells to HeLa and SiHa cells was tested by FCM. (E, F) HeLa and SiHa cells were pretreated with IFN-γ or not, and then co-cultured with U937 cells for 48 hours, and the expression of CD86 and CD163 on U937 cells were measured by FCM. IFN-γ: recombinant human IFN-γ. The results were expressed as mean ± SEM. NS: not significant difference, ** means P<0.01, and **** means P<0.0001.
Autophagy of cervical cancer cells promotes the phagocytosis and activation of macrophage (A) HeLa and SiHa cells were treated with rapamycin at a concentration of 2 μmol/l for 48 hours. LC3B was measured by western blot. (B) The autophagy level of HeLa and SiHa cells after treatment with rapamycin was detected by DAP autophagy kit and observed with fluorescence microscope. Yellow arrows represented autophagosomes. Multiple fields of view were randomly selected through fluorescence microscope observation, and then the number of autophagosomes was calculated. (C, D) HeLa and SiHa cells were pretreated with rapamycin or not, and then labelled with CFSE, and further co-cultured with U937 cells for 2 hours. The phagocytosis of U937 cells to HeLa and SiHa cells was tested by FCM (E, F) HeLa and SiHa cells were pretreated with rapamycin or not, and then co-cultured with U937 cells for 48 hours, and the expression of CD80, CD86, CD163 and CD206 on U937 cells were measured by
FCM. The results were expressed as mean ± SEM. Rapa represents for rapamycin, NS: not significant difference, * means P<0.05, ** means P<0.01, *** means P<0.001, and **** means P<0.0001.
Figure 3

IFN-γ induces autophagy of cervical cancer cells by up-regulating IDO1 expression (A, B) HeLa and SiHa cells were treated with IFN-γ or not, and the IDO1 expression was detected by western blot and FCM. (C) Transfection efficiency of IDO1 overexpression lentivirus was verified by PCR. (D) LC3B expression in HeLa and SiHa cells transfected with lentivirus was measured by western blot. (E) HeLa and SiHa cells were treated with IFN-γ at the presence of epacadostat or not, and the LC3B expression was measured by western blot. (F) HeLa and SiHa cells were treated with IFN-γ at the presence of epacadostat or not, and the autophagy level were detected by DAP autophagy kit. Autophagosomes were observed with...
fluorescence microscope. Yellow arrows represented autophagosomes. Multiple fields of view were randomly selected through fluorescence microscope observation, and then the number of autophagosomes was calculated. The results were expressed as mean ± SEM. Epa represents for epacadostat, I+Epa represents for IFN-γ+ epacadostat. * means P<0.05, ** means P<0.01, and **** means P<0.0001.
Figure 4

IDO1 in cervical cancer cells promotes phagocytosis and activation of macrophage (A) HeLa and SiHa cells were transfected with IDO1 overexpression or negative control lentivirus, then labeled with CFSE, and further co-cultured with U937 cells for 2 hours, and the phagocytosis of U937 cells was detected by FCM. (B-E) HeLa and SiHa cells transfected with IDO1 overexpression lentivirus or negative control were co-cultured with U937 cells for 48 hours, and the CD80, CD86, CD163 and CD206 expression of U937 were detected by FCM. The results were expressed as mean ± SEM. NS: not significant difference, **** means P<0.0001.
Figure 5

The accumulation of kynurenine and consumption of tryptophan catalyzed by IDO1 promote phagocytosis and activation of macrophage (A) HeLa and SiHa cells were treated with IFN-γ or not; tryptophan and kynurenine level were measured by LC-MS/MS quantification. (B) HeLa and SiHa cells were transfected with IDO1 overexpression or negative control lentivirus; tryptophan and kynurenine level were measured by LC-MS/MS quantification. (C) HeLa and SiHa cells were treated with kynurenine (500μmol/l) or tryptophan free medium, and LC3B expression was measured by western blot. (D) HeLa and SiHa cells were treated with kynurenine or tryptophan free medium, and the autophagy level was detected by DAP autophagy kit. Autophagosome was observed with fluorescence microscope. Yellow arrows represented autophagosomes. Multiple fields of view were randomly selected through fluorescence microscope observation, and then the number of autophagosomes was calculated. (E) HeLa and SiHa cells were treated with kynurenine or tryptophan free medium, then co-cultured with U937 cells for 48 hours, and the CD80, CD86, CD163 and CD206 expression of U937 cells were measured by FCM. (F) HeLa and SiHa cells were treated with kynurenine or tryptophan free medium, then labelled with CFSE, and further co-cultured with U937 cells for 2 hours. The phagocytosis was tested by FCM. The results
were expressed as mean ± SEM. −Trp represents for tryptophan free, Kyn represents for kynurenine. NS; not significant difference, * means P<0.05, ** means P<0.01, and **** means P<0.0001.

**Figure 6**

IDO1 inhibits tumor growth and promotes macrophage phagocytosis in vivo (A) TC-1 cells were transfected with mus IDO1 overexpression or negative control lentivirus. And the IDO1 expression was measured by FCM. (B) LC3B in TC-1 cells transfected with lentivirus was measured by western blot. (C-E) TC-1 cells transfected with IDO1 overexpression or negative control lentivirus were injected subcutaneously in mice. Tumor volume was detected every other day. (F) Strategy of gating macrophage in tumor tissue. (G) Phagocytosis of macrophage towards GFP+ tumor cells were analyzed by FCM. (H) CD80 and CD206 were detected in macrophage (I-K) TC-1 cells transfected with IFNG overexpression or negative control lentivirus were injected subcutaneously in mice. And mice were administrated with epacadostat or control solvent every day. Tumor volume was detected every three days. (L) CD86 expression was detected in macrophage. The results were expressed as mean ± SEM. Epa represents for epacadostat. NS: not significant difference, * means P<0.05, ** means P<0.01, and **** means P<0.0001.
Figure 7

IFNG and IDO1 expression were related to a better survival in cervical patients (A) IFNG expression in normal cervix and primary cervical cancer, data from TCGA database (http://ualcan.path.uab). (B) IFNG expression in normal cervix and primary cervical cancer based on the cancer stage (C) Kaplan plot of IFNG in cervical cancer (D) IDO1 expression in normal cervix and primary cervical cancer (E) IDO1 expression in normal cervix and primary cervical cancer based on the cancer stage (F) Kaplan plot of IDO1 in cervical cancer (G) ratio of IDO1 positive patients in cervical cancer, data from The Human Protein Atlas online database (https://www.proteinatlas.org/) (H) IDO1 expression in normal cervical epithelium and cervical cancer tissue. Tissue microarray was from The Human Protein Atlas (I) The correlation analysis of IFNG and IDO1 in cervical cancer. Data from TCGA database (http://ualcan.path.uab). * means P<0.05, and **** means P<0.0001.
Figure 8

Schema diagram of the effect of IFN-γ and IDO1 in cervical cancer. IFN-γ promotes the IDO1 expression in cervical cancer cells, which could catalyze tryptophan into kynurenine. The accumulation of kynurenine and assumption of tryptophan in cancer cells induce the autophagy activity. The phagocytosis ability of macrophages towards tumor cells with active autophagy is stronger, and autophagy in cervical cancer cells promotes the activation of macrophage, which could be related to the phagocytosis towards cancer cells. As a result, IFN-γ could restrict the tumor growth through IDO1-kynurenine-autophagy pathway.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SM.doc
- SF1.tif