Dendritic cells with an increased PD-L1 by TGF-β induce T cell anergy for the cytotoxicity of hepatocellular carcinoma cells

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A B S T R A C T
The effects of TGF-β on dendritic cells (DCs) in the tumor microenvironment are not well-understood. In this study, we investigated the effect of TGF-β on the induction of programmed death ligand-1 (PD-L1) expression in DCs and the underlying mechanism, and we further investigated the influence of the DCs with PD-L1 expression altered by TGF-β on T-cell immunity. We determined that TGF-β increased the expression of PD-L1 and signal transducers and activators of transcription 3 (STAT3) in DCs in both a time- and dose-dependent manner, and the expression of PD-L1 was decreased significantly after STAT3 blockade. In addition, TGF-β-treated DCs induced the apoptosis of T cells and increased the percentage of CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Tregs). Furthermore, the cytotoxicity of T cells against mice hepatocellular carcinoma cells (Hepa) was obviously suppressed. These results suggest that PD-L1 may play an important role in TGF-β-induced immune dysfunction, which finally results in a failure in the anti-tumor responses, and the TGF-β–STAT3–PD-L1 signaling pathway may contribute to novel therapeutic targets for the tumor based on DCs.

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1. Introduction
Dendritic cells (DCs) are the most powerful antigen-presenting cells (APCs) and are able to recognize tumor antigens and activate a tumor-specific T-cell response [1,2]. However, this response does not usually occur for most types of tumors, indicating that the dysfunction or immunosuppression of the host immune system might be the primary mechanism by which tumor cells escape host immunosurveillance [3–5].

T-cell abnormalities, including the imbalance between proliferation and apoptosis and the increased percentage of regulatory T cells (Tregs), are proposed to play important roles in tumor-associated immunosuppression [6,7]. However, the mechanisms of T-cell abnormalities in the tumor microenvironment remain unclear. Programmed death ligand-1 (PD-L1), also known as B7-H1, expressed in DCs, has been reported as a candidate immune regulatory molecule [8]. PD-L1 is mainly described as a negative regulatory molecule, and it has been frequently demonstrated that the expression of PD-L1 is correlated with the ability of DCs to induce tolerance [9,10]. Further studies have demonstrated that the PD-L1/PD-1 pathway is an important element resulting in T-cell anergy [11,12]. Transforming growth factor-β (TGF-β), a soluble cytokine produced and secreted mainly by inflammatory cells and tumor cells in a tumor microenvironment, is one of the key cytokines for immune tolerance [13,14]. It has been reported that TGF-β can regulate the expression of co-stimulatory molecules on the surface of DCs, which results in T-cell anergy [15].

Based on these findings, the present study was designed to investigate the influence of TGF-β on inducing PD-L1 expression in DCs and the underlying mechanism, in addition to further investigating the effect of the DCs with PD-L1 expression alteration from TGF-β and the subsequent effect on T-cell immunity. We found that TGF-β was able to up-regulate PD-L1 expression in DCs via signal transducers and activators of transcription 3 (STAT3) signaling, and the DCs with increased PD-L1 expression induced by TGF-β suppressed T-cell immunity. As a result, the cytotoxicity of T-cell to mice hepatocellular carcinoma cells (Hepa) was suppressed. These results suggest that PD-L1 may play an important role in TGF-β-induced immune dysfunction, which eventually results in a failure in anti-tumor responses, and the TGF-β–STAT3–PD-L1 signaling pathway may provide novel therapeutic targets for DC-related tumor treatments.
2. Material and methods

2.1. Animals

Pathogen-free BALB/c mice (male, 20 ± 2 g) were obtained from the Experimental Animal Center of Anhui Medical University (certificate No. SCXK-Wan-2005-001). At the start of the experiments, the mice were housed under controlled conditions with a temperature of 25 ± 2 °C, relative humidity of 60 ± 10%, room air changes 12–18 times/h, and a 12-h light/dark cycle. All animal studies were approved by the Institutional Animal Care and Use Committee of Anhui Medical University.

2.2. Reagents

Recombinant murine IL-4 and GM-CSF were purchased from Peprotech (USA). Recombinant murine TGF-β1 was purchased from R&D (Minneapolis, MN, USA). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT, USA). Fluorescence-conjugated mAbs to PD-L1, CD4, CD25, Foxp3, and mouse IgG isotype controls were purchased from BioLegend (USA). Monoclonal antibodies against STAT3, phosphorylated-STAT3 (ph-STAT3), PD-L1 and β-actin were from Santa Cruz Biotechnology (San Cruz, CA). Horse-radish peroxidase (HRP)-labeled goat-anti-rabbit and goat-anti-mouse antibodies were from Santa Cruz Biotechnology (San Cruz, CA). The STAT3 inhibitor NSC74859 was purchased from Selleck (USA). Cell Apoptosis PI Detection Kits were purchased from Nanjing KeyGen Biotech Co. Ltd (Nanjing, China). RNase A and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenytriazolium bromide (MTT) were acquired from Sigma (St, Louis, MO, USA).

2.3. Cell culture

2.3.1. Preparation of DCs [16]

Bone marrow cells were collected from both the tibia and femur of BALB/c mice by flushing the bones. Cells were pipetted vigorously up and down several times to obtain single cell suspensions, which was then passed through a nylon cell strainer to remove small pieces of the bones and debris. The cells were cultured in RPMI-1640 medium containing 10% FBS at a density of 5 × 10^6 cells/ml in 6-well plates. Three hours later, floating cells were discarded, and new medium was added with IL-4 (20 ng/ml) and GM-CSF (20 ng/ml). The cultures were fed with fresh medium and cytokines every 3 days. On day 4, TGF-β (5, 10 and 20 ng/ml) was added, and the control group received the same medium without TGF-β1. Loosely adherent clusters were harvested on day 6 of culturing and used for experiments, forming control DCs and TGF-β1-treated DCs (TGF-β1-DCs) groups. In addition, we defined these DCs with CD11c+ using flow cytometry.

2.3.2. Hepa cell line culturing

Mice hepatocellular carcinoma cell line Hepa, obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences, was grown in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere containing 5% CO₂ at 37 °C. Cells in the exponential growth phase were used in the experiments.

2.4. Flow cytometry

Cells were harvested and washed with FCM buffer (PBS with 5% FBS and 0.1% NaN₃) twice. Then, fluorescence-conjugated antibodies were added to the cell suspension (100 μl) according the manufacturer’s instructions. After gently mixing, the samples were incubated in the dark for 30 min at 4 °C and analyzed using flow cytometry. Data analysis was performed using FlowJo analysis software (Tree Star, Ashland, OR, USA), and the results reported as the mean fluorescence intensity (MFI).

2.5. Western blot analysis

DCs were prepared as described above and then lysed in cell lysis buffer with 1 mM PMSF for 4 °C for 30 min with ice vortexing every 10 min, followed by centrifugation at 12000 rpm for 10 min at 4 °C and the supernatants obtained were diluted to 4 mg protein/ml and kept frozen at −80 °C until use. A total of 50 μg of denatured protein was separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (PVDF membrane, Millipore, USA) in an ice-water environment. The membranes were blocked with blocking buffer (0.05% Tween 20-PBS with 5% non-fat milk) for 2 h at room temperature and then incubated with primary antibodies of rabbit monoclonal anti-STAT3 (1:400), ph-STAT3 (1:400), PD-L1 (1:400) and mouse monoclonal anti-β-actin (1:500) at 4 °C overnight. After washing with 0.05% Tween 20-PBS, the membranes were incubated with anti-rabbit or anti-mouse secondary antibodies conjugated with HRP (1:40000) for 2 h at 37 °C. The membranes were washed, and then, detection was achieved by measuring the chemiluminescence of the blotting agent after exposure of the filters on films. Finally, the densities of the bands were quantified with a computerized densitometer (ImageJ Launcher, Broken Symmetry Software). Equivalent protein loading and transfer efficiency were verified by staining for β-actin.

2.6. Analysis of PD-L1 expression after STAT3 inhibited with NSC74859

DCs prepared as described above were seeded at a density of 2 × 10⁶ per well in 6-well plates. STAT3 inhibitor (NSC74859, 100 μM) and TGF-β (10 ng/ml) were added on day 4 of DC culture. The expression of PD-L1 was analyzed with flow cytometry and western blot after incubation for 48 h.

2.7. Analysis of T-cell apoptosis

The extent of apoptosis in T cells induced by TGF-β1-DCs was assessed by flow cytometry. Mice splenic T lymphocytes (2 × 10⁶ cells/well) were collected with nylon wool and co-cultured with different DCs, including control DCs and TGF-β1-DCs (10 and 20 ng/ml TGF-β1) in 6-well plates at a ratio of 10:1 for 48 h at 37 °C. Then, 1 × 10⁶ T cells were collected and washed twice with cold PBS and centrifuged. The cell pellet was resuspended in 1 ml of cold PBS and fixed in 9 ml of 70% ethanol at −20 °C for at least 12 h. The cells were centrifuged and resuspended in 500 μl of PBS. RNase A was added, and the cells were incubated at 37 °C for 30 min. Then, PI staining buffer was added according to the protocol of the Cell Apoptosis PI Detection Kit. After being mixed gently, the samples were incubated in the dark for 30 min and analyzed using flow cytometry.

2.8. Detection of CD4⁺CD25⁺Foxp3⁺ Tregs

The elevated percentage of Tregs induced by TGF-β1-DCs was assessed by flow cytometry. Mice splenic T lymphocytes (2 × 10⁶ cells/well) were collected with nylon wool and co-cultured with different DCs, including control DCs and TGF-β1-DCs (5, 10 and 20 ng/ml TGF-β1) in 6-well plates at a ratio of 10:1 for 48 h at 37 °C. Then, the cells were prepared as single-cell suspensions and stained with the following antibodies: FITC-labeled anti-CD4, PE-labeled anti-CD25, and PECy5-labeled anti-Foxp3 as described previously [17] before being analyzed using flow cytometry.

2.9. Cytotoxicity assay

Mice splenic T lymphocytes were collected with nylon wool and placed in Transwell chambers (8.0 μm, Corning) at a density of 2 × 10⁶ cells per 200 μl. Then, the control DCs and TGF-β1-DCs (10 ng/ml TGF-β1) were added into separate 24-well plates. The two types of cells were co-cultured at a ratio of 10:1 for 48 h at 37 °C. Forty-
eight hours later, the T cells were harvested and cultured in 96-well plates at a density of $1 \times 10^6$ cells/well as effector cells. At the same time, Hepa cells were added at ratios of 20:1, 50:1 and 100:1 as target cells. Forty-eight hours later, the activity of Hepa cells was measured using an MTT assay, and the absorbance at 595 nm was determined using a Multi-Well Plate Reader. The percentage of lysis of each group was calculated according to the formula of the tumor specific cytolytic rate ($\%$) = $[1 - (\text{experimental} - \text{effector}) / \text{target well}] \times 100\%$. The experiments were conducted in triplicate for each condition.

2.10. Statistical analysis

Data are presented as the means ± standard deviation (SD). Statistical analysis was performed using the statistics software SPSS 13.0. Unpaired 2-tailed Student’s $t$ test was used to determine the statistical significance of the difference between two groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. TGF-β up-regulated the expression of PD-L1 in DCs

We first assessed the effects of TGF-β on PD-L1 expression in DCs with flow cytometry. Over the time course, the expression of PD-L1 in DCs was greatly associated with time. PD-L1 expression was increased in DCs treated by TGF-β (10 ng/ml) on days 3, 4 and 5 ($P < 0.05$) and achieved peak level on day 4 (Fig. 1A and B). To further investigate the influence of TGF-β in inducing PD-L1 expression in DCs, we then conducted a dose–response trial, and the results revealed that the expression of PD-L1 was up-regulated in DCs treated by TGF-β (5, 10 and 20 ng/ml) on day 4 ($P < 0.05$), especially with 20 ng/ml TGF-β ($P < 0.01$) (Fig. 1C and D). These results suggest that TGF-β up-regulates PD-L1 expression in DCs in both a time- and dose-dependent manner.

3.2. TGF-β up-regulated the expression and phosphorylation of STAT3 in DCs

STAT3 is widely expressed in various cell lines and tissues from humans and mice, and it may be activated by growth factors. We assessed the effect of TGF-β on the expression and phosphorylation of STAT3 in DCs. Over the time course, the expression and phosphorylation of STAT3 was obviously increased in DCs treated by TGF-β (10 ng/ml) on days 3, 4 and 5 ($P < 0.05$) (Fig. 2A, B and C). We then examined the dose responses of TGF-β-induced STAT3 expression and activation. The expression and phosphorylation of STAT3 was obviously increased in DCs treated by TGF-β (5, 10 and 20 ng/ml) on day 4 ($P < 0.05$) (Fig. 2D, E and F). These results suggest that TGF-β produces an up-regulation in the expression and activation of STAT3 in DCs in a time- and dose-dependent manner.

3.3. PD-L1 expression in DCs was decreased after STAT3 blockade

To understand whether TGF-β up-regulated the PD-L1 expression in DCs via STAT3 signaling, we conducted blocking experiments. We first observed the effect of STAT3 inhibition on the expression and phosphorylation of STAT3 in DCs. As shown in Fig. 3A, the expression and phosphorylation of STAT3 was decreased significantly by the inhibitor NSC74859. These results indicated that we obtained an efficient and stable STAT3 blockade in the DCs.

Then, we tested PD-L1 expression in STAT3-blockaded DCs by flow cytometry and western blot (Fig. 3B and D). The results indicated that compared with control DCs, the expression of PD-L1 in TGF-β-DCs was significantly increased. After the addition of NSC74859, the elevated PD-L1 expression by TGF-β was obviously inhibited ($P < 0.01$) (Fig. 3C and E).

3.4. TGF-β-DCs induced T-cell apoptosis

The above data demonstrated that TGF-β could up-regulate PD-L1 expression in DCs. It has been reported that PD-L1 is a co-inhibitory molecule, and its receptor PD-1 is expressed mainly on the surface of T cells. In addition, PD-L1/PD1 signaling has negative effects on T cells. Therefore, we further investigated the effect of TGF-β-DCs on T-cell apoptosis using flow cytometry (Fig. 4A). The results revealed that TGF-β-DCs (10 and 20 ng/ml TGF-β) obviously induced the apoptosis of T cells compared with control DCs ($P < 0.01$) (Fig. 4B). Importantly, TGF-β-DCs induced T-cell apoptosis, and there was a positive correlation with TGF-β-induced PD-L1 expression in DCs ($R^2 = 0.903$, $P = 0.042$) (Fig. 4C).

![Fig. 1. TGF-β induces PD-L1 expression in DCs in a time-dependent manner. DCs were seeded at a density of $2 \times 10^6$ per well in 6-well plates. TGF-β (10 ng/ml) was added on days 3, 4, and 5 of DC culture. The cells were harvested on day 6, and the expression of PD-L1 was analyzed by flow cytometry. (A) Histogram presentation of PD-L1 expression on DCs. (B) MFI analysis from the above histograms is shown as a bar chart. Data are shown as the means ± SD from 3 replicate experiments. *$P < 0.05$, **$P < 0.01$ vs. control DCs. TGF-β induces PD-L1 expression in DCs in a dose-dependent manner. DCs were seeded at a density of $2 \times 10^6$ per well in 6-well plates. Then, TGF-β (5, 10 and 20 ng/ml) was added on day 4 of DC culture. Cells were harvested on day 6, and PD-L1 expression was analyzed by flow cytometry. (C) Histogram presentation of PD-L1 expression on DCs. (D) MFI analysis from the above histograms is shown as a bar chart. Data are shown as the means ± SD from 3 replicate experiments. *$P < 0.05$, **$P < 0.01$ vs. control DCs.](image-url)
3.5. TGF-β-DCs led to an increased percentage of CD4⁺CD25⁺Foxp3⁺ Tregs

Tregs play an important role in tumor-associated immunosuppression. Thus, we examined the effect of TGF-β-DCs on Treg generation. To address this, CD4⁺CD25⁺Foxp3⁺ Tregs were enumerated by flow cytometry (Fig. 5A). The results indicated that DCs, after treatment with TGF-β (5, 10 and 20 ng/ml), displayed an increased percentage of CD4⁺CD25⁺Foxp3⁺ Tregs, compared with that of DCs without TGF-β treatment (P < 0.05), and in the treated groups, 20 ng/ml TGF-β-DCs was the most effective at increasing the percentage (P < 0.01) (Fig. 5B). Importantly, the increased percentage of CD4⁺CD25⁺Foxp3⁺ Tregs in TGF-β-DCs and the increased PD-L1 expression in DCs from

Fig. 2. TGF-β induces STAT3 expression and phosphorylation in DCs in a time-dependent manner. DCs were seeded at a density of 2 x 10⁶ per well in 6-well plates. TGF-β (10 ng/ml) was added on days 3, 4, and 5 of DC culture. Cells were harvested on day 6, and total protein was analyzed. (A) The expression and phosphorylation of STAT3 was measured by western blot. (B, C) Densitometric analysis from the above immunoblots is shown as a bar chart. Data are shown as the means ± SD from 3 replicate experiments. *P < 0.05, **P < 0.01 vs. control DCs. TGF-β induces STAT3 expression and phosphorylation in DCs in a dose-dependent manner. DCs were seeded at a density of 2 x 10⁶ per well in 6-well plates. Then, TGF-β (5, 10 and 20 ng/ml) was added on day 4 of DC culture. Cells were harvested on day 6, and total protein was analyzed. (D) The expression and phosphorylation of STAT3 was measured by western blot. (E, F) Densitometric analysis from the above immunoblots is shown as a bar chart. Data are shown as the means ± SD from 3 replicate experiments. *P < 0.05, **P < 0.01 vs. control DCs.

Fig. 3. Generation of stable STAT3-blockade DCs. (A) The expression and phosphorylation of STAT3 in DCs after inhibition by NSC74859 was measured by western blot. β-Actin was measured as an internal control. The results are representative of three independent experiments. The expression of PD-L1 in DCs after STAT3 inhibition by NSC74859. DCs were seeded at a density of 2 x 10⁶ per well in 6-well plates. NSC74859 (100 μM) and TGF-β (10 ng/ml) were added on day 4 of DC culture. Cells were harvested on day 6, and the expression of PD-L1 was analyzed by western blot and flow cytometry. (B) The expression of PD-L1 was measured by western blot. (C) Densitometric analysis from the above immunoblots is shown as a bar chart. (D) Histogram presentation of PD-L1 expression on DCs. (E) MFI analysis from the above histograms is shown as a bar chart. Data are shown as the means ± SD from 3 replicate experiments. **P < 0.01 vs. control DCs; ##P < 0.01 vs. stimulated with TGF-β only.
Fig. 4. Effect of TGF-β-DCs on apoptosis of T cells. AR, apoptosis rate. Mice splenic T lymphocytes (2 × 10^6 cells/well) were collected with nylon wool and co-cultured with different DCs, including control DCs and TGF-β-DCs (10 and 20 ng/ml TGF-β) in 6-well plates at a ratio of 10:1 for 48 h at 37 °C. Forty-eight hours later, cells were harvested and analyzed by flow cytometry. (A) Apoptotic cells were determined by flow cytometry. (B) The semi-quantitative analysis showed the changes in the apoptosis rate of T cells. (C) A scatter plot of the correlation between TGF-β-DC-induced T-cell apoptosis and TGF-β-induced PD-L1 expression in DCs. Data are shown as the means ± SD from 3 replicate experiments. **P < 0.01 vs. control DCs.

Fig. 5. Effect of TGF-β-DCs on expansion of CD4^+CD25^+Foxp3^+ Tregs. Mice splenic T lymphocytes (2 × 10^6 cells/well) were collected with nylon wool and co-cultured with different DCs, including control DCs and TGF-β-DCs (5, 10 and 20 ng/ml TGF-β) in 6-well plates at a ratio of 10:1 for 48 h at 37 °C. The cells were harvested, then stained with antibodies to CD4, CD25 and Foxp3 and analyzed by flow cytometry. Dot plot in the upper right quadrant represents positive cells (CD4^+CD25^+Foxp3^+ Tregs). (A) Dot blot presentation of Treg generation induced by TGF-β-DCs. (B) Histogram presentation of CD4^+CD25^+Foxp3^+ Treg percentages induced by TGF-β-DCs. (C) A scatter plot of the correlation between the increased percentage of CD4^+CD25^+Foxp3^+ Tregs by TGF-β-DCs and the increased PD-L1 expression in DCs by TGF-β. Data are shown as the means ± SD from 3 replicate experiments. *P < 0.05, **P < 0.01 vs. control DCs.
TGF-β treatment displayed a positive correlation ($R^2 = 0.845$, $P = 0.039$) (Fig. 5C).

3.6. The cytotoxicity of T cells for Hepa cells was suppressed by TGF-β–DCs

DCs play an important role in regulating T-cell-mediated anti-tumor immunity. The above data demonstrated that DC treatment with TGF-β results in T-cell incompetence. Thus, we further investigated the effect of TGF-β–DCs on the cytotoxicity of T cells. To address this, we tested the tumor-specific cytolytic rate by MTT assay, and the results indicated that T cells after being co-cultured with TGF-β–DCs had a lower tumor-specific cytolytic rate compared with that of T cells co-cultured with control DCs ($P < 0.01$) (Fig. 6).

4. Discussion

DCs, as professional APCs, play an important role in tumor-specific immune responses. Immunotherapy based on DCs is considered one of the most promising ways to treat cancers [18,19]. However, there are many challenges in inducing and maintaining the best immune state of DCs in a tumor microenvironment. TGF-β, a soluble cytokine widely present in the tumor microenvironment, is considered a key factor in inducing immune tolerance [14]. It has been reported that TGF-β may promote tumor progression by regulating immune cells [13,20]. The effect of TGF-β on DCs is very complicated. In the present study, we demonstrated that TGF-β was able to up-regulate PD-L1 expression in DCs in both a time- and dose-dependent manner in vitro.

Recently, it has been reported that the JAK/STAT and MAPK signaling pathways are involved in PD-L1 expression in APCs [21,22]. The activation pattern of STATs critically determines the phenotype and function of DCs, and STAT3 activation is often associated with tolerogenic functions [23]. Thus, in the present study, we focused on STAT3. STAT3 is a key cytoplasmic transcription factor that is activated by phosphorylation of a conserved tyrosine residue in response to extracellular signals, such as growth factors [24]. It is a molecular center for diverse signaling pathways such as cell cycle progression, apoptosis and immune evasion [25]. In the present study, we detected the expression and phosphorylation of STAT3 in DCs and found that the expression and phosphorylation of STAT3 in DCs was obviously increased by TGF-β in a time- and dose-dependent manner. Associated with the result that PD-L1 expression in DCs was significantly increased by TGF-β with a time- and dose-dependent manner, we deduced that PD-L1 expression in DCs induced by TGF-β might be controlled by STAT3. To further investigate the regulation of PD-L1 expression in DCs, we then conducted blocking experiments. We used the STAT3 chemical inhibitor NSC74859 (also known as S3I-20), which has been reported to be effective in hepatocellular cancers with disrupted STAT3 signaling [26], following stimulation with TGF-β, and analyzed the PD-L1 expression by flow cytometry and western blot. The expression of PD-L1 in DCs was significantly down-regulated after inhibition by NSC74859 following stimulation with TGF-β. This finding indicated that STAT3 signaling plays an important role in the expression of PD-L1 in DCs as induced by TGF-β.

PD-L1 is an interesting molecule for the manipulation of immune responses. It is a key co-inhibitory molecule expressed on DCs [27], and it has been reported that PD-L1 on DCs is involved in the induction and maintenance of immune tolerance. The interaction of PD-L1 expressed on DCs and PD-1 expressed on T cells has been demonstrated to suppress T-cell immune activities [28]. Several studies have linked PD-L1 expression to the development and function of Tregs [29,30]. In this study, to further investigate the effect of the DCs with an increased PD-L1 by TGF-β on T-cell immunity, we co-cultured TGF-β–DCs and T cells for 48 h. The results indicated that TGF-β–DCs significantly induced the apoptosis of T cells and the generation of CD4+ CD25+ Foxp3+ Tregs. We also conducted some correlation analysis, and the results indicated that TGF-β–DCs induced T-cell apoptosis, and TGF-β induced PD-L1 expression in DCs. In addition, TGF-β–DCs induced Treg generation, and TGF-β induced PD-L1 expression in DCs, which is a positive correlation between the Treg percentage and PD-L1 expression. These results indicate that the PD-L1 played an important role in the observed TGF-β–DC-induced T-cell abnormalities. It has been reported that increased PD-L1 expression in APCs or cancer cells contributes to tumor evasion of the host T cells [31]. In the present study, we found that TGF-β treatment down-regulated the ability of DCs to activate a tumor antigenic-specific CTL response.

In summary, in this study, we demonstrated that TGF-β could up-regulate PD-L1 expression in DCs via STAT3 signaling, and the DCs with increased PD-L1 by TGF-β suppressed T cell immunity, which led to T-cell anergy and diminished anti-tumor effects. Our study might help to provide a theoretical basis and novel therapeutic targets for DC-based anti-tumor immunotherapy.

Conflict of interest

All the authors declared that there was no conflict of interest in this paper.

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