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Transforming Growth Factor-β_1 Remodels the Cytoskeleton Organization of Mature Dendritic Cells *via* Smad2/3 Signaling Pathway

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Abstract: Dendritic cells (DCs) are the most potent professional antigen presenting cells as now known, which play critical roles in the initiation, programming and regulation of the immune response. Transforming growth factor-β_1 (TGF-β_1), one of the major suppressive cytokines in tumor microenvironment, can deteriorate the biomechanical characteristics and motility of mature dendritic cells (mDCs)，but the underlying molecular mechanisms are not well defined. In this study, the effects of TGF-β_1 on the motilities and T cell priming capabilities of mDCs as well as the molecular regulatory mechanisms were investigated. The results showed that the cytoskeleton (F-actin) organizations of mDCs were abnormally remodeled by TGF-β_1. Simultaneously, the migration and immune priming capabilities of mDCs were impaired by TGF-β_1 *via* Smad2/3 signaling pathway. It's significant for further understanding the interaction of DCs and TGF-β_1 in tumor host, as well as the immune escape mechanism of cancer, which may be important for enhancing the clinical efficiency of DCs-based immunotherapy against cancer.

Keywords: Mature dendritic cells, transforming growth factor β_1, immune function, motility, Smad sigling pathway.

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1 Introduction

Physiologically, with the recognition of foreign antigens, immature dendritic cells (imDCs) migrate to the secondary lymphoid tissues and become mature dendritic cells (mDCs), which up-regulate the expressions of peptide-MHC complexes and accessory molecules (such as CD11c, CD80, CD83, CD86, CCR7, et al.) on their surfaces, convert naive T cells into responding cytotoxic $CD8^+$ and helper $CD4^+$ T cells to eliminate the antigens [Carmi, Prestwood and Engleman (2015); Steinman (2012); Steinman, and Cohn (1973)]. Accordingly, the strategy of DCs-based cancer immunotherapy is tantamount to load DCs with a wide array of tumor antigens and induce maturation *in vitro* before inject back into tumor-bearing patients to induce specific anti-tumor immune responses *in vivo* [Chiang, Balint, Coukos et al. (2015)]. But the increased immunosuppressive cytokines in tumor microenvironment (TME) can impair the immune functions of DCs by lots of ways, which is one of the main backers for helping tumor cells to escape from immune attack. Several researches have demonstrated that only less than 1% *ex vivo* injected DCs can migrate to the secondary lymphoid tissue when performing DCs-based immunotherapy against cancer, suggesting that the motility of DCs is impaired by TM-derived factors [Garg, Coulie, Van den Eynde et al. (2017)].

Our previous study has showed that malignant cells can secrete high level suppressive transformed growth factor-β_1 (TGF-β_1) [Zeng, Xu, Zhang et al. (2017)]. Correspondingly, Garg et al. have demonstrated that the mRNA expression level of TGF-β_1 is up-regulated in the TME infiltrated DCs [Garg, Coulie, Van den Eynde et al. (2017)]. High level of TGF-β_1 in cancer patients (such as hepatocellular carcinoma, malignant melanomas, non-small cell lung cancer, breast cancer, et al.) usually associate with tumor invasion and poor prognosis [Peng, Yuan, Zhang et al. (2017); Westphal, Mauch, Florin et al. (2015); Jakubowska, Naumnik, Niklińska et al. (2015)]. We also demonstrated that the biomechanical characteristics and motility of $CD14^+$ monocyte-derived mDCs are deteriorated by TGF-β_1 in concentration-dependent fashion. The F-actin cytoskeleton of mDCs are abnormally reorganized by TGF-β_1, their length and density of the protrusion become shorter and fewer, the expression levels of several F-actin-binding proteins are changed [Zheng, Long, Ji et al. (2014)], but the underlying molecular mechanisms are still elusive.

It has been well established that Smad family including three members is a unique group for transduction the signal induced by TGF-β into the nucleus [Esebanmen and Langridge (2017)]. Inhibitory Smad (I-Smad), Smad7, is a kind of Smad molecules to interact with receptor-regulated Smads (R-Smads) in the purpose of inhibiting TGF-β/Smad signaling [Yan, Liao, Cheng et al. (2016)]. Smad2 and Smad3 are R-Smads which can be phosphorylated upon the activation of TGF-β receptor and then translocate into the nucleus with the help of common mediator Smad (Co-Smad) protein, Smad4 [Esebanmen and Langridge (2017); Yan, Liao, Cheng et al. (2016); Witkowska and Smolewski (2013)]. Smad2 and Smad3 are crucial in the regulation of many physiological and pathological processes (e.g. cell development, epithelial mesenchymal transition, fibrosis, et al.) [Seeger, Musso and Sozzani (2015); Muthusamy, Budi, Katsuno et al. (2015); Xu, Liu, Zhou et al. (2016)], which also be involved in the regulation of some cytoskeleton-binding proteins. Lee et al. [Lee, Moon, Lee et al. (2010)] have revealed that TGF-β_1 can regulate

cytoskeleton reorganization and down-regulate cofilin activity via Smad2/3 in human retinal pigment epithelial cells. Besides, Smad2 and Smad3 are essential for TGF-β_1 mediated regulation of Th_1 immune response [Takimoto, Wakabayashi, Sekiya et al. (2010)]. However, the precise mechanism of TGF-β_1 signaling in DCs remains unclear. To validate whether TGF-β_1 can affect the cytoskeletons of mDCs through smad2/3 signaling pathway, mDCs were treated with TGF-β_1 *in vitro*, and the cytoskeleton structure, motility, costimulatory capabilities of mDCs were investigated with or without TGF-β_1/Smad2/3 signaling pathway blockage. The results showed that the F-actin cytoskeleton structures, motilities and immune functions of mDCs were influenced by TGF-β_1 *via* Smad2/3 signaling pathway. It is significant for further understanding DCs immune function and immune escape mechanism in malignant disease, as well as enhancing the clinical efficacy of DCs-based immunotherapy against cancer.

2 Materials and methods

2.1 Isolation of monocytes and generation of mDCs

Fresh human vein blood of healthy donors was provided by the Red Cross Blood Center of Guizhou province (Guizhou, China). Donors had been given informed consents to this experimental study, approved by the ethics committee of Guizhou Medical University. Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll density gradient centrifugation. $CD14^+$ monocytes were isolated from PBMCs by cocktail immunomagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to previous protocols [Mayer, Lee, Lendlein et al. (2011); Zeng, Yao, Xu et al. (2009)]. The monocytes were cultured in RPMI1640 medium (Gibco, CA, USA) with 15% fetal bovine serum (FBS) (Gibco, Waltham, MA, USA), 1% glutamine, 1% penicillin/streptomycin, 1% Hepes, plus 150 ng/ml recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) and 100 ng/ml recombinant human intereukin-4 (rhIL-4) (Peprotech, UK). After 5 days, 10 ng/ml recombinant human tumor necrosis factor (rhTNF-α) (Peprotech, UK) was added and the cells were further cultured for another 3 days to obtain mDCs (8^{th} day). The phenotypes of DCs were analyzed by staining the cell surface with FITC or PE-conjugated mouse antibodies to human CD11c, CD40, CCR7, CD80, CD83, CD86 and HLA-DR (Sigma, Saint Louis, MO, USA) using flow cytometer (FACScan, Becton and Dickinson, San Jose, CA, USA). The cells' viabilities were detected by trypan blue staining.

2.2 Treatments of mDCs with TGF-β_1 and SB431542

The mDCs were treated with 5 ng/ml of TGF-β_1 for 15, 30, 60, 120, 360, 720, 1440 min in 6-well plate. Each group of dishes contained 1×10^6 mDCs. The treatment concentration and time of TGF-β_1 was determined by the preliminary experiments [Zheng, Long, Jia et al. (2014)]. Treatment with PBS served as control group. As a competitive ATP binding site kinase inhibitor, SB-431542 is a selective inhibitor for ALK4, 5, and 7, induces the phosphorylation of Smad protein. In order to block TGF-β_1/Smad pathway, 10 μM SB431542 was used for 30 min pre-treatment before TGF-β_1 administration. Working concentration and treatment time of SB431542 were determined according to the literature [Tanaka, Shinto, Yashiro et al. (2010)] and preliminary experiments.

2.3 Western blotting

Western blot assay was performed to determine the levels of Smad, P-Smad，actin, cofilin1 and profilin1. Cells cultured with or without TGF-β_1 for different times were collected into 200 μl of ice-cold lysis buffer (Beyotime, China) with 1 mM PMSF (Solarbio, China). After 5 min on ice, the lysate was centrifuged at 12, 000 r/min for 5 min at 4°C. Equivalent protein amounts (42 μg) were subjected to SDS-PAGE and the protein bands were transferred onto a polyvinylidene difluoride membrane (Invitrogen, USA). After blocking with 5% bovine serum bumin (BSA, Gibco, Carlsbad, CA, USA) in TBS-T, membranes were hybridized with primary antibodies specific to Smad2/3, P-Smad2/3, cofilin1, profilin1 (Bioworld, Pittsburgh, PA, USA) at 4°C overnight. After probed with HRP-labeled second antibodies (Bioworld, Pittsburgh, PA, USA), the blots were visualized by the ECL chemiluminescence system (Amersham Bioscience).

2.4 Confocal laser scanning microscopy

Sterilized cover slips were coated with Poly-L-Lysine (PLL, Sciencell, Carlsbad, CA, USA) for 30 minutes at 37°C. The mDCs were dropped on the cover slips and fixed with 4% paraformaldehyde (FlukaChemie, Switzerland) for 30 minutes at room temperature (RT) before permeabilized by 0.1% Triton X-100 (Sciencell, USA). After blocked with 1% BSA for 1 h at RT, primary antibodies specific to Smad2/3 and P-Smad2/3 (Bioworld, USA) were added and incubated at 4°C overnight. Then, cells were stained with second antibodies with FITC fluorescence (Santa Crus, CA, USA) and Alexa® Flour 647 (Abcam, UK) at RT in the dark for 2 h. For phalloidin staining, cells were incubated for 30 min with 5U rhodamine-labeled phalloidin (Invitrogen, Carlsbad, CA, USA) at RT in the dark, and therewith stained with DAPI (Cell signaling, Danvers, MA, USA) at room temperature in the dark for 5 min. Confocal laser scanning microscopy (CLSM) (Olympus, Tokgo, Japan) was used to reconstruct 3-dimensional images of mDCs. The mean fluorescent intensities of different proteins were analyzed *via* Flouview 1000 software. Twenty cells each group were selected for analyses.

2.5 Migration capability

Transwell with 5.0 μm pores (Corning, NY, USA) was used to determine the motility of mDCs under different conditioned media. 600 μl RPMI1640 medium supplemented with 5 ng/ml CCL19 was added into the lower chamber as chemokine for CCR7 on mDCs. Meanwhile, the upper chamber was added with 400 μl mDCs suspension (1×10^6) and incubated at 37 °C. After 8 hours, the cells migrated through the membranes were collected and counted. Each experiment was performed at least three times.

2.6 Mixed lymphocytes reaction

Mixed lymphocytes reaction (MLR) assay was performed to measure the antigen stimulatory capabilities of mDCs. Prior to the assay, T cells were separated from PBMCs by nylon wool fiber and resuspended to 1×10^6/ml. Then, 5×10^5, 2.5×10^5, 1×10^5, 5×10^4, 2.5×10^4 mDCs were respectively pipetted into 96-well plate and mixed with 1×10^6 T cells. After incubation at 37°C with 5% CO_2 for 48 h, each well was added 20 μl CCK8 (Dojindo,

Shanghai, China) and continually cultured for 4 h. The absorbance was measured spectrophotometrically at 450 nm. Each experiment was performed three times.

2.7 Statistical analyses

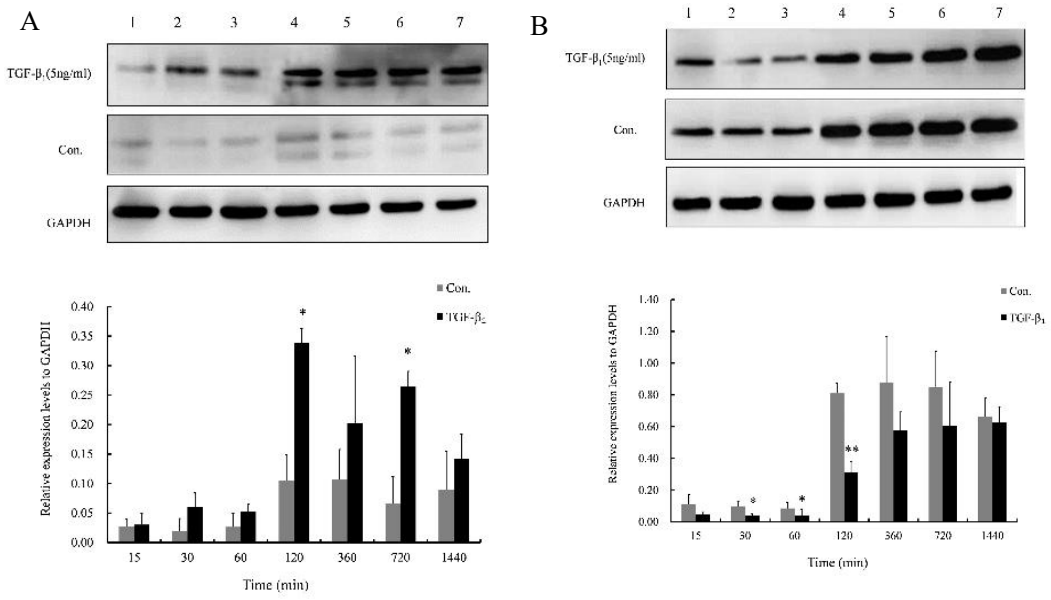
All measurements were identified using the two-tailed student's *t*-test (SPSS 19). Results are presented as the mean±standard deviations (SD). Differences with $p<0.05$ were regarded as statistically significant.

3 Results

3.1 The expression levels of Smad2/3, P-Smad2/3, cofilin1 and profilin1 in mDCs

In order to investigate the activation induced by TGF-β_1 in mDCs, the expression levels of Smad2/3 and P-Smad2/3 were measured. As shown in Fig. 1A, the expression levels of Smad2/3 in mDCs were obviously up-regulated after treated with 5 ng/ml TGF-β_1 for 120 minutes ($p<0.05$), suggesting that TGF-β_1 led to the activation of Smad2/3 in mDCs. Simutaneously, the expression levels of P-Smad2/3 (Fig. 1B) were down-regulated in mDCs ($p<0.01$).

The F-actin cytoskeleton organizations of cells are regulated by their binding proteins, among which cofilin1 and profilin1 are key players. As shown in Fig. 1-C and 1-D, compare with those of control, the expression levels of cofilin1 and profilin1 were both down-regulated after treatment with TGF-β_1 ($p<0.05$). Other binding proteins such as P-cofilin1 (phosphorylated form of cofilin1) and fascin1 were also detected, but the differential expression was not found (Data not shown).



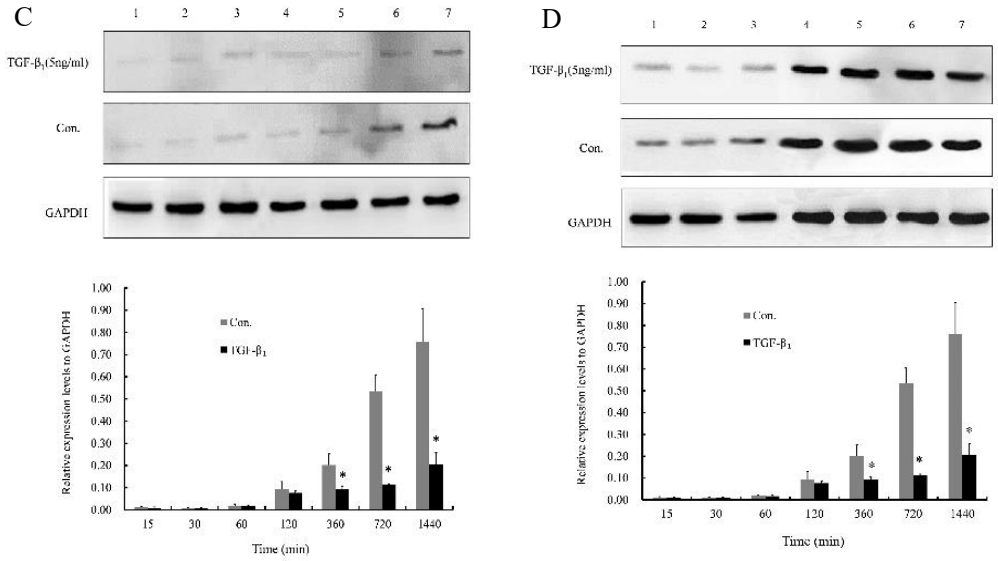


Figure 1: The expression levels of Smad2/3 (A), P-Smad2/3 (B), cofilin1 (C) and profilin1 (D) in mDCs. Lanes 1-7 represent that DCs were treated with 5 ng/ml TGF-β_1 for 15, 30, 60, 120, 360, 720, 1440 min. $^{*}p<0.05$ *vs*. control

3.2 The localizations of Smad2/3, P-Smad2/3 in mDCs

The localizations of Smad2/3 and P-Smad2/3 are crucial for their performing biological functions, which were displayed by immunofluorescence. Besides, the P-Smad2/3 specific inhibitor SB431542 was introduced for blocking TGF-β_1/Smad2/3 signaling pathway. As shown in Fig. 2 and 3, the fluorescence intensities of Smad2/3 and P-Smad2/3 in mDCs were increased ($p<0.05$), in accordance with those of western blotting assays. Smad2/3 in mDCs were mainly distributed in the cytoplasm, while P-Smad2/3 were translocated into the cell nucleus. Meanwhile, compared with those of TGF-β_1 treated group, the expression levels of Smad2/3 returned to normal levels and P-Smad2/3 completely blocked after treatment with SB-431542, inferring that the TGF-β_1/Smad signaling pathway were activated.

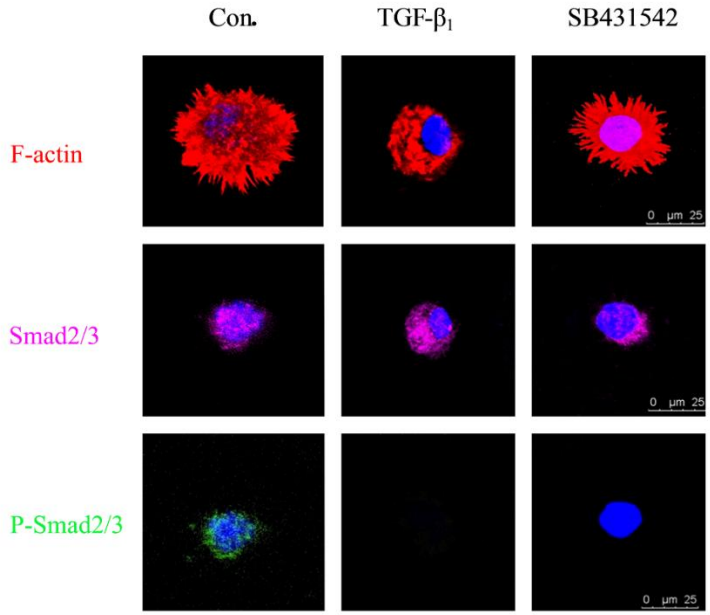


Figure 2: The organizations of cytoskeleton (F-actin) and the localizations of Smad2/3, P-Smad2/3 in mDCs cultured with 5ng/ml of TGF-β_1 and 10 μM SB431542. Cells were incubated with Smad and P-Smad and photographed by confocal microscopy. The F-actin, nucleus, Smad2/3 and P-Smad2/3 were stained with rhodmine phalloidin (red), DAPI (bugle), Alexa® Flour 647 (pink) and FITC (green), respectively. Then photographed by confocal microscope (600×)

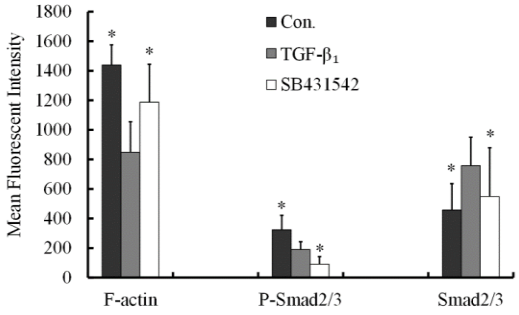


Figure 3: The mean fluorescence intensity of F-actin, P-Smad2/3, Smad2/3 in mDCs with different treatment. At least twenty cells of each group were selected for image analysis The mean fluorescent intensities of mDCs were analyzed via Flouview 1000 software. $^{*}p<0.05$ *vs*. control.

3.3 Cytoskeleton organization of mDCs

The cytoskeleton organizations of cells are their bases of structures and functions. The lengths and densities of filamentous protrusions on the surface of mDCs are closely

correlated with their motility and immune functions [Zeng, Xu and Chen (2015)]. As shown in Fig. 2-4, the F-actin structure in mDCs were markedly reorganized, the protrusions of cells became shorter and sparser after treatment with TGF-β_1, coinciding with our previous data [Zheng, Long, Jia et al. (2014)]. Furthermore, these phenomena could be reverted by SB431542 treatment.

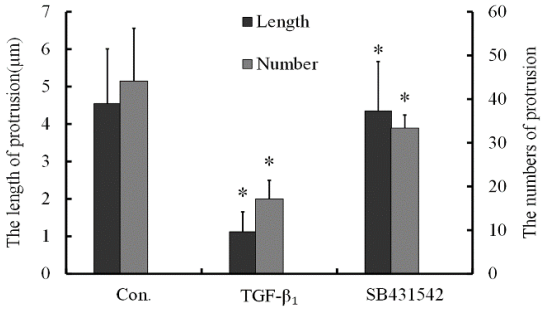


Figure 4: The changes of filopodia of surface on the mDCs in different treatment group. Each group selected twenty cells for analyses. Black and gray columns respectively represent the length and number of protrusion. $^{*}p<0.05$ *vs*. control

3.4 Migration capabilities

The motilities of DCs are crucial for their performing immune functions, e.g. antigen uptake in peripheral tissues and antigen presentation in secondary LNs. Therefore, the migration capabilities of mDCs were detected by transwell assay. SB431542 was added to block TGF-β_1/Smad2/3 singling pathway. As shown in Fig. 5, the migration capabilities of mDCs were impaired by TGF-β_1, which could be partly converted by blocking the TGF-β_1/Smad2/3 signaling pathway with SB431542.

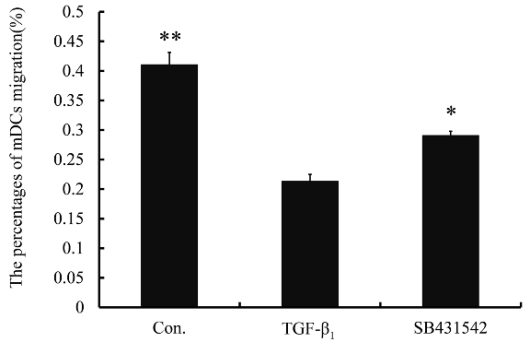


Figure 5: The migration capabilities of mDCs under different conditioned media. The percentages of mDCs migration were counted using a cell counter after 8 h. MDCs were treated with TGF-ß$_1$ (5 ng/ml) for 120 min in the presence or absence of SB-431542 (10 μM), which was added 30 min before ligand stimulation. These measurements were performed in triplicates under the same condition. $^{*}p<0.05$, $^{**}p<0.01$ *vs*. control

3.5 Immune costimulatory capabilities

To validate whether the immune function of mDCs are associated with TGF-β_1 impaired motilities, the mixed lymphocytes reaction assays were performed. As shown in Fig. 6, the costimulatory capabilities of mDCs were inhibited by TGF-β_1, which were also partly converted by blocking the TGF-β_1/Smad2 signaling pathway with SB431542.

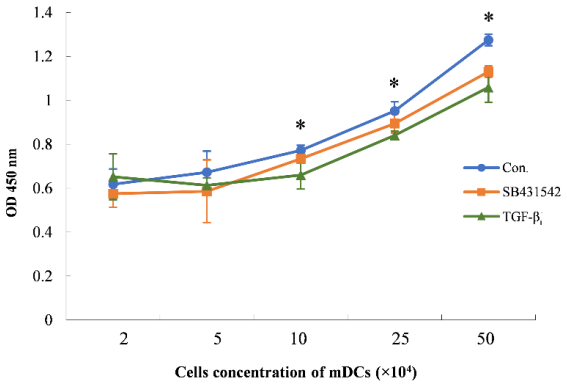


Figure 6: The stimulatory capabilities of mDCs cultured with TGF-β_1 and SB431542. Each concentration of mDCs was performed in triplicates under the same condition. $^{*}p<0.05$ *vs*. control

4 Discussion

For the significant capability in adaptive immune responses, DCs-based vaccination was approved by FDA in 2010 for castration-resistant prostate cancer immunotherapy, but the limited success in clinical trials highlights the need to investigate the complex mechanisms of immunosuppression in TME [Sabado, Meseck and Bhardwaj (2016)]. Lots of evidences indicate that high level of TGF-β_1 in the serum of malignant patients are not only associated with poor prognosis, but also reduces the efficacy of DCs-based cancer immunotherapy vaccines [Lukas, Yogev, Kel et al. (2017); Chou, Huang, Shay, et al. (2015); Lin, Shao, Chan et al. (2015); Kao, Gong, Chen et al. (2003); Peng, Zhao, Song et al. (2017)]. Our previous research revealed that 5 ng/ml TGF-β_1 obviously suppresses the length and number of protrusions on mDCs surface, which are essential for their antigen presentation, and markedly regulated by some cytoskeleton-binding proteins, such as fascin1, cofilin1, P-cofilin1 and profilin1 in mDCs [Zheng, Long, Jia et al. (2014)]. But the potential molecular mechanism is not well defined.

It is well known that TGF-β_1/Smad intracellular signaling pathway is initiated *via* two types of serine/threonine kinase transmembrane receptors on the cells surface: TGF-β type I receptor (TβRI) and TGF-β type II receptor (TβRII). TβRII directly combines with TGF-β_1, transphosphorylates and activates TβRI. Smad2 and Smad3 can receive the signaling of phosphorylation which is transmitted by TβRI. Then, P-Smad2/3 can be translocated into nuclear *via* combine with Smad4, therefore, modulates the expression of downstream target genes [Landskron, De la Fuente, Thuwajit et al. (2014); Frick, Yarka, Nunns et al. (2017); Yoon, Sudo, Kuroda et al. (2015)]. The most concerned symbol of the activation of TGF-

β_1 signaling pathway is the increasing expression level and translocation of P-Smad2/3. In this study, Smad2/3 protein was up-regulated but remain separated in the cytoplasm of mDCs, while P-Smad2/3 down-regulated but shift into nucleus after cultured with TGF-β_1. The expression levels of both Smad2/3 and P-Smad2/3 proteins could be reduced by SB431542 (Figs. 1-4), which may be responsible to two reasons. Firstly, phosphorylation signal conducted by TβRI is not only transmited to Smad2/3 but also activates various intracellular protein kinases, e.g. the small GTPases (such as Rho A, Rac1, and Cdc42, et al.), glycogen synthase kinase 3b (GSK3b), the mitogen-activated protein kinases (MAPKs) (such as extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun N-terminal kinases (JNK) et al.). These signaling molecules are known to interact with Smad family and might suppress Smad2/3 phosphorylation and lead to the accumulation of Smad2/3 in the cytoplasm of mDCs at the same time [Seeger, Musso and Sozzani (2015); Kamaraju (2005); Kim, Kim, Moon et al. (2014); Strand, Liang and Yang (2014); Zhang (2017); Bobr, Igyarto, Haley et al. (2012)]. Secondly, the "utilization" rate of P-Smad2/3 is too quick to detect. To address these doubts, more specific and sensitive experiments should be further performed.

The motilities of mDCs are important for their travelling in body and performing antigen presentation. Imai et al. have found that the number of DCs in tumor-draining lymph nodes (TDLNs) was reduced under the action of tumor derived TGF-β_1 [Imai, Minamiya, Koyota et al. (2012)]. A key reason is the down-regulation of DCs motility, which makes DCs fail to load antigen and migrate to the lymph nodes (LNs) [Kitano, Yamazaki, Takumi et al. (2016)]. The costimulatory capabilities of mDCs are their key immune function. In the process of immune response initiation, naive T cells receive antigen information and costimulatory signals by physically interacting with mDCs in secondary LNs. Several groups and our studies have found that the motilities of mDCs are closely associated with their immune function [Zeng, Xu and Chen (2015)]. In the present studies, cytoskeleton of mDCs was obviously remodeled after treatment with 5 ng/ml TGF-β_1 for 120 min. Meanwhile, their motilities and antigen stimulatory capabilities were impaired by TGF-β_1 (Figs. 8 and 9), which were partly converted by inhibitor SB431542, indicating that motilities and antigen stimulatory capabilities of mDCs were deteriorated by TGF-β_1 via Smad2/3 signaling pathway. The reasons could be explained from two aspects. One possible explanation is that, physically, the length and density of the dendrites are associated with the cell surface proportion. Long and thick protrusion, which is primarily composed of actin protein, means more contact area with T cells available, leading to the increasing efficiency of antigen presentation [Hivroz, Chemin, Tourret et al. (2012)]. Generally, the poly (F-actin) and mono (G-actin) states of actin proteins are regulated by a huge number of actin bingding protein (ABP). G-actin is ADP-rich monomer, while F-actin is ATP-rich filaments. Actin depolymerizing factor (ADF) / cofilin family almost expresses in all kinds of eukaryotic cells and are associated with the rapid recycling of actin monomers, enhancing the disintegration of ADP-actin subunits from the pointed end. Cofilin1 can decrease the rate of nucleotide exchange on actin monomers, while profilin1 maintains actin monomers in a state. Cofilin and profilin enhances the turnover of the actin filament at the opposite ends, accelerating ATP-actin added to the barbed end where they retain a high affinity for the growing barbed end of filaments. Thus, the assembly and disassembly of actin filament are highly relying on the influence of cofilin and profilin

[Yokota (2017); Spracklen and Peifer (2015); Bosch, Castro and Saneyoshi (2014); Wasnik and Mukhopadhyay (2014)]. Certainly, there should be a balance between the expression levels of cofilin and profilin in the purpose of maintaining the dynamics of mDCs [Kardos, Nevalainen, Nyitrai et al. (2013)]. In the present study, both levels of cofilin1 and profilin1 were down-regulated in mDCs after treated with 5 ng/ml TGF-β_1 (Fig. 3 and 4). The disequilibrium of cofilin1 and profilin1 is a possible reason for the abnormal remodeling of the cytoskeleton organization in mDCs. On the other hand, it is reported that TGF-β_1 could inhibit the expression of C-C Motif Chemokine Receptor 7 (CCR7) in Langerhans cells and Smad proteins have already been proved to be participated in this pathway [Worbs, Hammerschmidt and Förster (2017)]. CCR7 is the ligand of CCL19, which regulates chemotaxis, endocytosis, survival, migratory speed, and cytoarchitecture in mDCs [Clatworthy, Aronin, Mathews et al. (2014)]. In this study, TGF-β_1 impaired the migration of mDCs to CCL19 (Fig. 5), and this effect could be partly converted by blocking the TGF-β_1/Smad2/3 signaling pathway with SB431542, implying that Smad2/3 might be involved in the regulation of CCR7 *via* TGF-β_1. Several other studies found that CCR7 in DCs could be modified by various functional proteins such as FOXO1, AMP-dependent kinase (AMPK), mammalian sterile 20-like 1 kinase and so on [López-Cotarelo, Escribano-Díaz, González-Bethencourt et al. (2015); Dong, Wang, Xiao et al. (2015); Torres-Bacete, Delgado-Martín, Gómez-Moreira et al. (2015)]. Therefore, the important role of Smad protein in the regulation of CCR7 in mDCs also worth our consideration for potential target. In addition, high dose of SB431542 (>5 μM) could reduce T cell proliferation [Tanaka, Shinto, Yashiro et al. (2010)]. That might be the reason that SB431542 could not fully recover the costimulation capbility of mDCs, which inhibited by TGF-β_1.

5 Conclusion

The present research demonstrated that TGF-β_1 could abnormally remodel the cytoskeleton organization of mDCs *via* smad2/3 signaling pathway leading to unbalance some ABP expression levels and impaired motilities and antigen presentation capabilioties. As shown in Fig. 7, thereinto, regulation of cofilin and profilin might be an important target for the dysfunction caused by TGF-β_1 in mDCs. Blocking the TGF-smad2/3 signaling pathway in appropriate fashion might be a clue to improve the clinical efficiency of DCs-based cancer immunotherapy. It is significant for further understanding the DCs biological behaviour and immune escape mechanism of cancer.

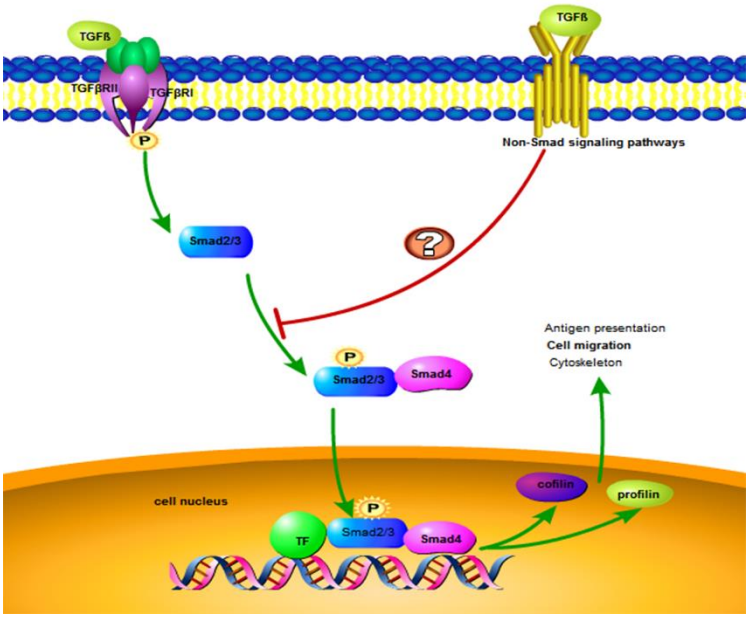


Figure 7: Mechanisms of TGF-β_1 remodels the cytoskeleton organization of mDCs. Upon stimulation with TGF-β_1, P-smad2/3 in mDCs can be translocated into nucleous and then lead to the abnormal reorganization of cytoskeleton

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