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ORIGINAL ARTICLE

Paclitaxel inhibits transforming growth factor-β-increased urokinase-type plasminogen activator expression through p38 MAPK and RAW 264.7 macrophage migration

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Summary

Purpose: *Transforming growth factor-β (TGF-β) induces alternative macrophage activation that favors tumor progression and immunosuppression. Meanwhile, paclitaxel (PTx) induces macrophage (Mφ) polarization towards antitumor phenotype. TGF-β also increases tumor stroma macrophage recruitment by mechanisms that include cell motility enhancement and extracellular matrix degradation. In this study, we aimed to determine whether PTx regulates macrophage migration and urokinase-type plasminogen activator (uPA) expression induced by TGF-β.*

Methods: *We used mouse macrophage RAW 264.7 cells treated with PTx and TGF-β combinations. Proliferation was analyzed by MTT and cell cycle assays. Immunofluorescence was performed to determine tubulin cytoskeleton and Smad3 nuclear localization. Western blot and transcriptional luciferase reporters were used to measure signal transduction activation. Migration was determined by wound healing assay. uPA activity was determined by zymography assay.*

Results: *PTx decreased RAW 264.7 cell proliferation by inducing G2/M cell cycle arrest and profoundly modified the tubulin cytoskeleton. Also, PTx inhibited TGF-β-induced Smad3 activation. Furthermore, PTx decreased cell migration and uPA expression stimulated by TGF-β. Remarkably, p38 MAPK mediated PTx inhibition of uPA activity induced by TGF-β but it was not implicated on cell migration inhibition.*

Conclusions: *PTx inhibits TGF-β induction of mouse Mφ migration and uPA expression, suggesting that PTx, as TGF-β targeting therapy, may enhance Mφ anticancer action within tumors.*

Key words: *macrophages, migration, paclitaxel, proliferation, transforming growth factor-β, urokinase-type plasminogen activator*

Introduction

Macrophages (Mφ) play important roles in the maintenance of tissue homeostasis and inflammatory immune responses. Transforming growth factor-β (TGF-β regulates Mφ activation and function that profoundly affect Mφ antitumor action within tumor stroma [1]. TGF-β exerts its function through activation of intracellular effectors such as Smad2/3 that further form a protein complex with Smad4, then this complex is translocated into the nucleus to regulate the transcription of target genes [1].

In tumor microenvironment, TGF-β is highly abundant and plays a profound role in regulating

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Mφ function and tumor growth [1]. Tumor associated Mφ (TAM) express a wide variety of phenotypes, ranging from pro-inflammatory, antitumor classical activated phenotype (M1) to immunosuppressive, anti-inflammatory, and pro-tumorigenic activated phenotype (M2) [2]. Namely, TGF-β induces M2c phenotype that is involved in adaptive immune system suppression, which support cancer cells to escape immune surveillance [3].

Paclitaxel (PTx), a natural diterpene alkaloid originally isolated from the bark of Taxus brevifolia is a widely used anticancer drug with immune-activating activities. Actually, PTx was shown to reduce Mφ tumor infiltration and induce Mφ M1 polarization, indicating its additional therapeutic effect in immunotherapy [4]. The main PTx antineoplastic activity resides in its capacity to irreversibly enhance microtubules (MT) polymerization. Thereby, PTx disrupts tubulin cytoskeleton dynamics through stabilizing the microtubule polymer and preventing MT from disassembly. This results in cell migration inhibition, mitotic spindle assembly abnormalities, G2/M cell cycle arrest and cell death in cancer cells [5,6]. Specifically in Mφ, PTx mimics lipopolysaccharide (LPS) response leads to nitric oxide (NO) and tumor necrosis factor (TNF)-β production that may enhance Mφ tumor cytotoxicity. Moreover, PTx enhances IL-1β and ROS production and induces NFκB and p38 MAPK activation [7]. Actually, p38 MAPK is activated by inflammatory insults and plays essential role in Mφ expression of pro-inflammatory mediators [8].

Mφ migration to inflamed and damaged tissue, in response to specific signals, is accompanied by a dynamic MT rearrangement, which also play a role in Mφ antigen presentation and phagocytosis [9,10]. M2 polarized Mφ expresses extracellular matrix (ECM) proteinases that may contribute to the invasion of cancer cells. In fact, urokinase-type plasminogen activator (uPA) regulates Mφ migration and infiltration into tumor microenvironment [11]. Moreover, uPA also induces M2 phenotype polarization [12].

TGF-β enhances Mφ uPA expression by Smad3 and ERK MAPK activation [13]. Interestingly, MT integrity is crucial for TGF-β intracellular signaling. For instance, PTx inhibits cell fibrosis by reducing TGF-β-Smad2/3 axis activation [4, 14]. This suggests that PTx may be useful for targeting MT cytoskeleton and regulating TGF-β signaling and functions in Mφ. In the present study, we determined PTx effects on TGF-β-induced Mφ cell migration and uPA expression by using murine RAW 264.7 Mφ cell line as a model. We found that PTx induced RAW 264.7 cells G2/M cell cycle arrest and inhibited cell proliferation, concomitantly with inhibition of TGF-β-Smad3 activation and cell migration. Furthermore, p38 MAPK mediated PTx inhibited uPA expression induced by TGF-β. Thus PTx, by targeting Mφ cytoskeleton, may influence a cancer cell supporting role of TGF-β/Mφ within tumors.

Methods

Cell culture and reagents

RAW 264.7 (ATCC TIB-71) cells were cultured in RPMI medium (Sigma- Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) in a 5% CO_2 atmosphere at 37°C and in humidified incubator. Recombinant TGF-β was provided from R&D Systems (Minneapolis, MN, USA). p38 inhibitor SB203580 and paclitaxel (PTx) were obtained from Calbiochem (Darmstadt, Germany). 4',6-diamidino-2-phenylindole (DAPI), monoclonal anti-alpha-tubulin, secondary FITC-anti-mouse or anti-rabbit and Horseradish peroxidase coupled-antibodies were from Sigma-Aldrich (St. Louis, MO, USA). Anti-phospho-Smad3 rabbit antibody was obtained from Calbiochem (Darmstadt, Germany). Anti-Smad2/3 (sc-8332) rabbit polyclonal antibody was purchased from Santa Cruz Biotechnology Inc (CA,USA). Anti-phospho-p38 and -p38 rabbit antibodies were purchased from Cell Signaling Technology Inc (Massachusetts, USA). Lipofectamine 2000 was provided by Thermofisher Sci (Waltham, MA, USA). Passive lysis reagent and Firefly luciferase activity assay were from Promega (Madison, WI, USA).

Proliferation assay

5×10^3 cells were seeded in 96-well plates and treated for 24 or 72 h. Cell viability was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) assay as is reported in [15]. After treatments, MTT (0.5 mg/ml final concentration) was added to each well and incubated for 2 h. Formazan crystals were dissolved with DMSO: isopropanol (2:3) and the absorbance determined at 630 nm in a plate reader.

Cell cycle analysis

Cells (3×10^5) were seeded in 6-well plates and treated as indicated. Then, cells were harvested, 2× phosphate buffered saline (PBS) washed and fixed in 70% ethanol for 30 min on ice. Afterwards, cells were resuspended in PBS and 50 μl of RNase A solution (100 μg/ml) and 400 μl of propidium iodide (PI) solution were added. DNA contents were examined using a BD FACS Calibur (BD Bioscience, San Diego, CA, USA). Flow cytometer data was analyzed using ModFit LT 5 program (Verity Software House, USA).

Immunofluorescence

4×10^4 cells were seeded over rounded cover slips, grown overnight and treated as indicated. Cell monolayers were fixed for 10 min with 4% formaldehyde in PBS and subsequently permeabilized with 0.5% Triton X-100 for 5 min. Cells were immunostained with either anti-tubulin or p-Smad3 antibodies followed by incubation with either

anti-mouse-FITC or anti-rabbit-FITC secondary antibody. Nuclei were stained with 1 ug/ml DAPI. Images were taken with a microscope equipped with epi-fluorescence.

Western blot assay

Western blot analyses were performed as described by Kocic et al [15]. Briefly, cell lysates in RIPA buffer, with proteinases and phosphatase inhibitors, were subjected to 10% SDS-PAGE and electrotransferred onto a nitrocellulose membrane and blocked with 4% bovine serum albumin (BSA) in 0.5% Tween-20 in TBS. Then, the membranes were incubated with correspondent primary antibodies. Secondary antibodies conjugated with HRP (Sigma-Aldrich) were used to detect the immune complexes by enhanced chemiluminescence reagent system from Applichem (Darmstadt, Germany). Protein bands were quantified by densitometric scanning, using ImageMaster TotalLab Version 1.11 software (Amersham Biotech, USA).

Plasmids, transient transfection and reporter assay

The p-4.8 uPA–Luc reporter plasmid (–4.8 kb of murine uPA promoter) was provided by Dr. Munoz-Canoves (CRG, Spain). p38 activity was monitored by PathDetect CHOP Trans-Reporting System p38 Kinase pathway (Stratagene, La Jolla, CA). PCMV-β-galactosidase expression vector was kindly provided by Dr. C. Bernabeu (CIB, Spain). Briefly, cells seeded in T24 plates (2×10^5 cells/well) were transfected with different plasmids using Lipofectamine 2000 transfection reagent according to the manufacturer's protocol. After 24 h cells were lysed with 50 µl of passive lysis reagents and Firefly luciferase activity was determined. β-galactosidase activity (Tropix, Bedford, MA, USA) was measured as an internal control for transfection efficiency.

Wound healing assay

Cells seeded in T24 well plates were grown to reach confluence and then a wound was performed by scratching monolayer cells with p200 µl tip as is describe in [16]. Cell monolayers were triple-washed with PBS and cultured in complete medium for an additional 24 h with indicated treatments. Afterwards, cells were fixed with ice-cold methanol and stained with 0.1% crystal violet for 15 min. Migration of the cells into the scratch area was photographed by inverted light microscopy and quantified by TScratch software (Computational Science and Engineering Laboratory, Swiss Federal Institute of Technology, Zurich, Switzerland).

Zymography assay

uPA activity was determined by caseinolysis zymography as previously reported [16]. Briefly, cell monolayers were treated as indicated in serum-free medium for 24 h. Then, conditioned serum-free media were subjected to 10% SDS-PAGE under non-reducing conditions. Af-

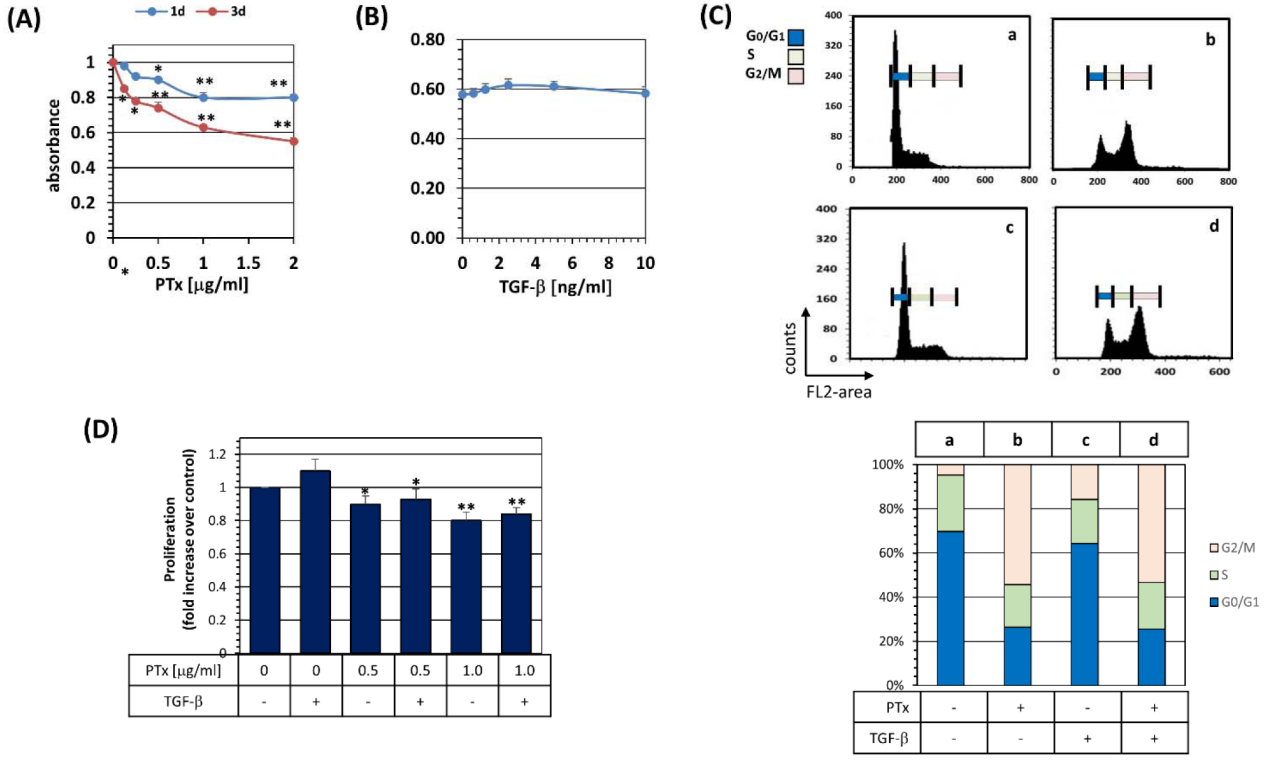

Figure 1. Ptx inhibits RAW 264.7 cell proliferation. MTT assay of RAW 264.7 cells treated with **(A)** increasing PTx concentrations for 1 day (blue) and 3 days (red) or **B:** indicated TGF-b concentrations for 3 days. **C:** Flow cytometric analysis for RAW 264.7 cells treated with TGF-β (5 ng/ml) and/or PTx (0.5 µg/ml) for 1 day. Histograms show cell cycle distribution based on DNA content, **a**, **b**, **c**, **d** and **e** represent experimental conditions indicated in the graphic bar. **D:** MTT assay after TGF-β (5 ng/ml) and PTx (0.5 µg/ml) treatment for 1 day. Representative results from three independent experiments are shown. Significant difference between treatments by t-test: *p<0.05 and **p<0.005.

ter running, gels were double-washed with 2.5% Triton X-100, for 30 min at room temperature. Acrylamide gels were placed on 1% agarose gels containing 0.5% casein and 1 μg ml^{-1} plasminogen and incubated at 37°C for 24 h. uPA-dependent proteolysis was detected as a clear band on the agarose gel (Chemidoc, BioRad, CA, USA). Quantifications of degradation bands were performed by densitometry analysis (Total Lab software).

Statistics

The results shown are representative of at least three independent experiments. Data were analyzed using Microsoft excel software and are given as means±SEM. Statistical significance was evaluated using the Students' t-test. Differences were considered to be significant at a value of (*) $p<0.05$ or (**) for $p<0.005$.

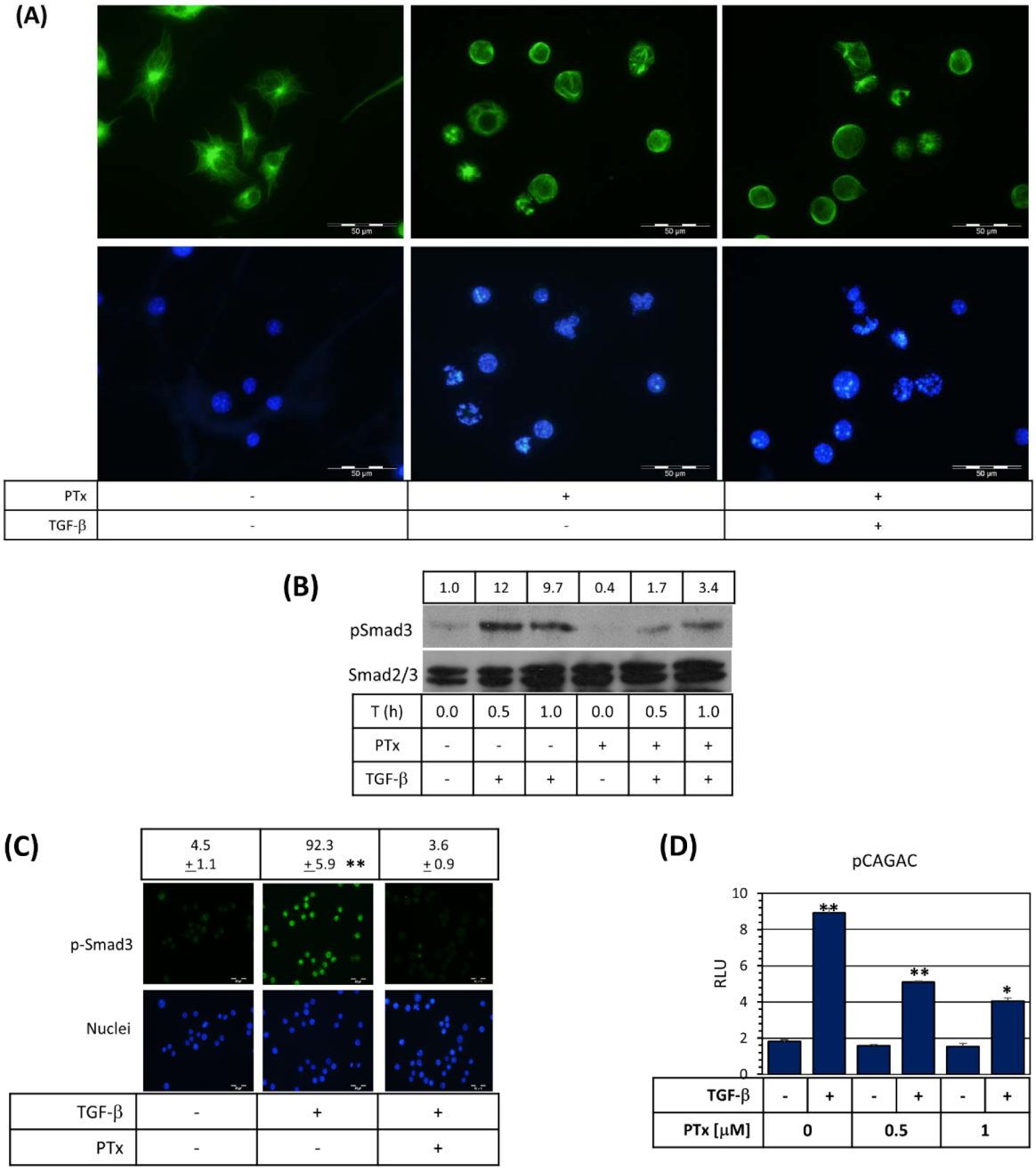

Figure 2. PTx inhibits TGF-β-induced Smad3 activation. **A:** Immunofluorescence staining for of RAW 264.7 tubulin cytoskeleton after 1 day of 0.5 μg/ml PTx treatment, in the presence or absence of 5 ng/ml TGF-β. Tubulin cytoskeleton is stained red, while blue represents DAPI counterstained nuclei (magnification 400x, bars 50 μM). **B:** Western blot analysis of Smad3 phosphorylation and **C:** Immunofluorescence showing nuclear phospho-Smad3 (green), after 1h treatment with 5 ng/ml TGF-β- in the presence or absence of 0.5 ng/ml PTx. Nuclei were counterstained by DAPI (blue) (magnification 200x, bars 30 μM). **D:** PTx inhibits TGF-β-induced Smad3 responsive pCAGAC-Luc construct transactivation. Transfected cells were treated with 5 ng/ml TGF-β with or without 0.5 μg/ml PTx for 24 h. Luciferase activity was determined and expressed as relative luciferase units (RLU). Representative results from three independent experiments are shown. Significant difference between treatments by t-test: *$p<0.05$ and **$p<0.005$.

Results

Paclitaxel inhibits RAW 264.7 cell proliferation and is TGF-β independent

To evaluate PTx cytostatic and/or cytotoxic effects, we examined either one or three days RAW 264.7 cell proliferation treated with increased drug concentrations (Figure 1A). Increased PTx reduced cell proliferation, which was evident at 0.25 μg/ml for 1 day and 0.125 μg/ml for 3 days treatments respectively. In both conditions cell proliferation was inhibited in all subsequent drug concentrations as well. Next, proliferation assay was performed to determine TGF-β effects on RAW 264.7 cell growth. All tested TGF-β concentrations did not influence cell proliferation compared to untreated cells (Figure 1B). PTx induced G2/M cell cycle arrest as expected, and led to a decrease of G0/G1 cell distribution (Figure 1C). TGF-β treatment, however, did not affect cell cycle distribution alone or in combination with PTx. Moreover, TGF-β did not modify PTx-induced G2/M cell cycle arrest, which was accompained by reduction of G0/G1 (Figure 1C). Similarly, TGF-β did not modify PTx-mediated inhibition of cell growth (Figure 1D).

Paclitaxel inhibits TGF-β activation of Smad3 signaling

Cytoskeleton integrity is necessary for the proper intracellular signaling transduction propagation and others cellular functions [17]. Next, we analyzed PTx-induced changes of microtubule cytoskeleton in RAW 264.7 cells. Control cells displayed MT network uniformly distributed within the cytoplasm, while PTx provoked MT cortical distribution and the formation of characteristic MT bundles that were not modified by TGF-β co-treatment (Figure 2A). In addition, PTx treated cells showed chromosome missaggregation, probably due to chromosome segregation defects as indicated by DAPI-stained nuclei [18]. Also, PTx produced a reduction of TGF-β intracellular signal transduction response noticed by inhibition of TGF-β-induced Smad3 phosphorylation (Figure 2B), phospho-Smad3 nuclear localization (Figure 2C) and inhibition of Smad3 transcriptional activity determined by pCAGAC smad3 reporter (Figure 3D).

Paclitaxel inhibits TGF-β-induced cell migration and secreted uPA activity

Microtubules finely control directional cell migration while perturbations in MT dynamics may

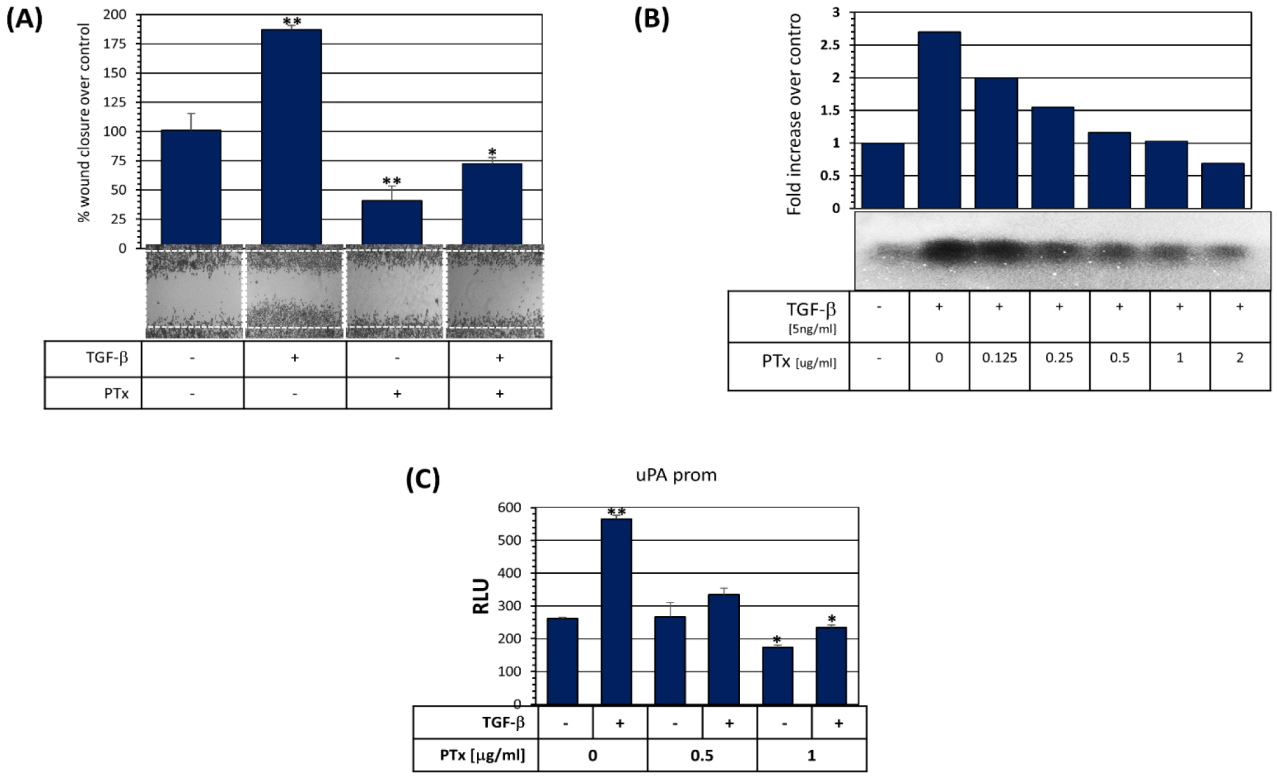

Figure 3. PTx inhibits TGF-β-induced RAW 264.7 cell migration and uPA production. **A:** RAW 264.7 monolayer cells were subjected to wound healing assay under indicated treatments. After 24 h migrated cells were fixed and photographed. **B:** uPA caseinolysis assay. Cells were 24 h treated with indicated TGF-β and PTx combinations. uPA activity was observed as dark degradation bands. **C:** RAW 264.7 cells were transfected with a p-4.8 murine uPA–Luc luciferase reporter plasmid and subjected to 24-h TGF-β 5ng/ml treatment in the presence or absence of 0.5 μg/ml PTx. RLU: relative luciferase units. *p<0.05, **p<0.05.

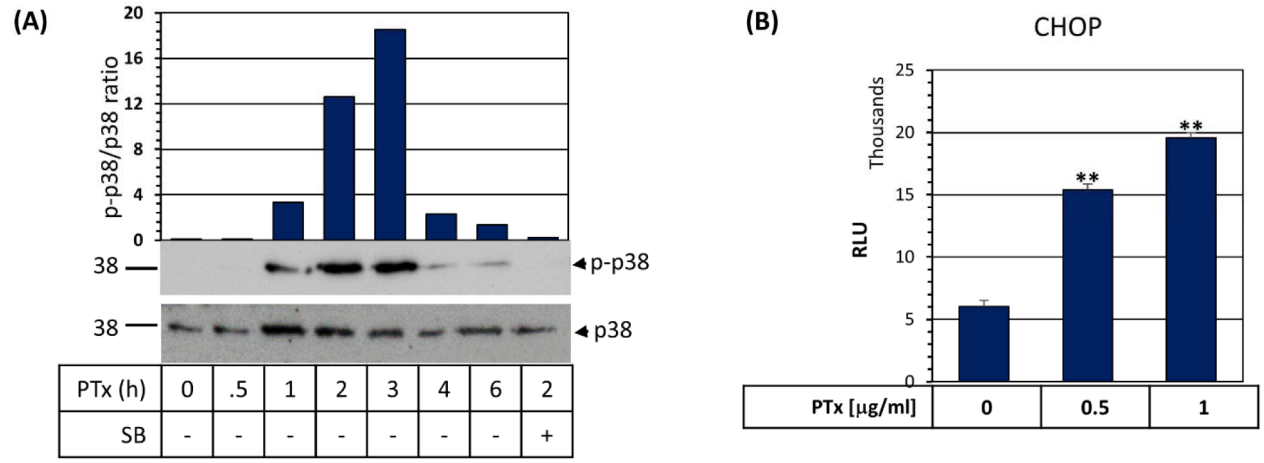

Figure 4. PTx activates p38 MAPK intracellular signaling. **A:** PTx p38 activation was determined by Western blot. Cells were cultured in serum-free medium with 0.5 μg/ml PTx during indicated time points. The inhibition of p38 phosphorylation was achieved by pre-incubating cells with 10 μM SB203580 for 30 min, followed by 2-h PTX incubation. **B:** Functional assay of p38 MAPK activation using the GAL4-CHOP trans-reporting system. RAW 264.7 cells transiently transfected with GAL4-CHOP system were treated for 24 h with indicated PTx concentrations and relative luciferase activity (RLU) was determined. Representative results from three independent experiments are shown. Significant difference between treatments by t-test: **p<0.005.

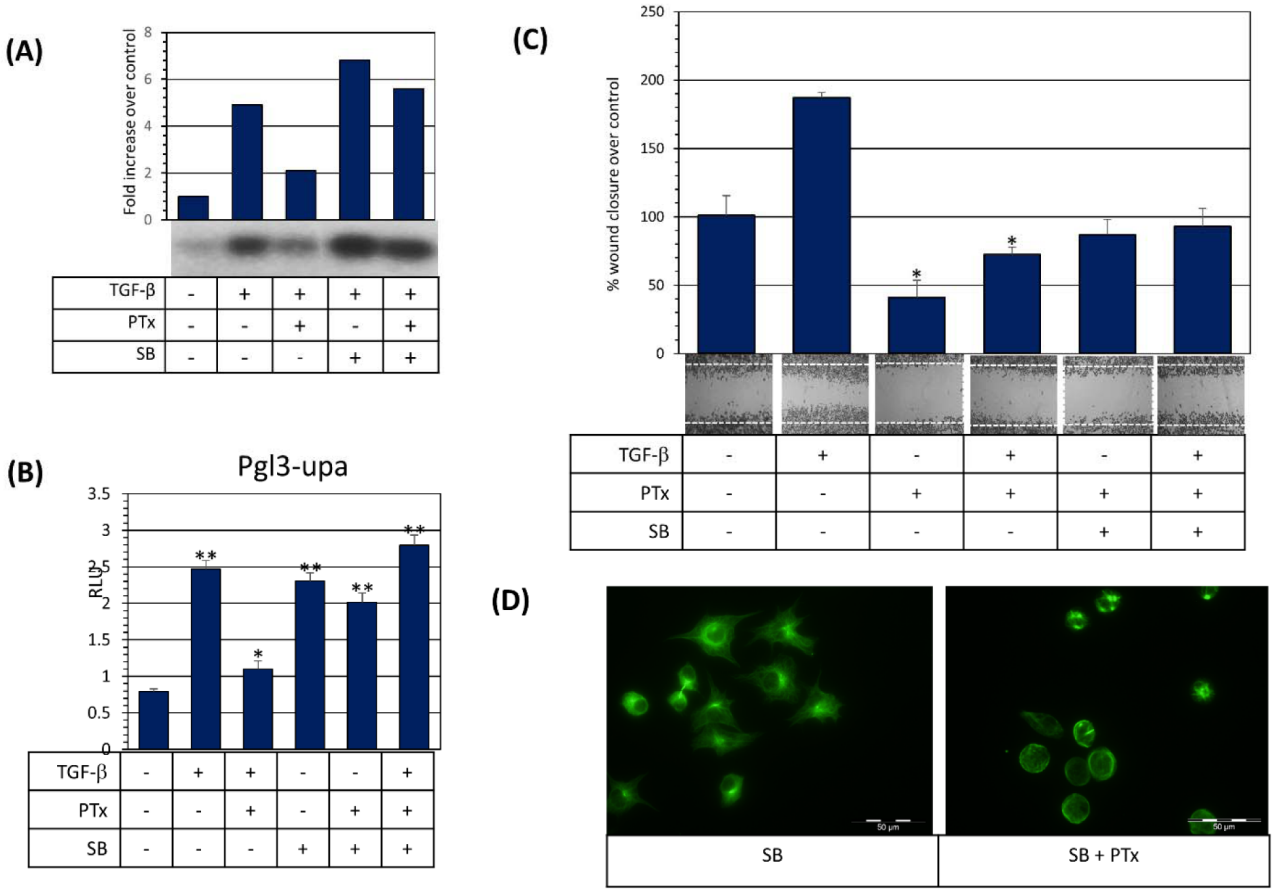

Figure 5. P38 inhibitor rescues TGF-β-induced uPA from PTx inhibition but not cell migration. **A:** uPA zymography assay after 24 h treatment with combinations of 5 ng/ml TGF-β, 0.5 mg/ml PTx and 10 μM p38 inhibitor SB203589. **B:** RAW 264.7 cells were transfected with a p-4.8 murine uPA–Luc luciferase reporter plasmid and subjected to 24-h treatment with TGF-β 5ng/ml in the presence or absence of 0.5 μg/ml PTx or 10 μM p38 inhibitor SB203589. RLU: relative luciferase units. **C:** RAW 264.7 cell monolayers were subjected to 24-h wound-healing assay after treatments indicated in **(B)**. **D:** Cells were 24-h treated with 0.5 μg/ml PTx in the presence or absence 10 μM p38 inhibitor and subjected to tubulin (green) immunofluorescence assay (magnification 400x, bars 50 μM). Representative results from three independent experiments are shown. Significant difference between treatments by t-test: *p<0.05 and **p<0.005.

highly affect cell motility [19]. We, therefore, investigated the effect of PTx and TGF-β on RAW 264.7 cells migration. TGF-β increased RAW 264.7 cell migration in a wound healing based assay, while PTx decreased both basal and TGF-β-induced migration (Figure 3A). By degrading extracellular matrix, uPA enables Mφ migration and tissue infiltration thus facilitating tissue repair and regeneration in controlled inflammation [20]. We next wanted to test if PTx-mediated decrease in migration affects the activity of Mφ-secreted uPA by performing zymography assay. TGF-β induced uPA secreted activity, which was reduced by co-treatment with increased PTx amounts (Figure 3B). Also, PTx inhibited TGF-β-induced uPA promoter transactivation (Figure 3C).

Paclitaxel activates p38 signaling

PTx may increase activation of several intracellular signal transduction pathways [21]. Thus, we determined PTx capacity to activate p38 MAPK. PTx Mφ treatment transiently induced p38 phosphorylation, with a maximum at 3 h, while the presence of p38 inhibitor (SB203080) suppressed PTx induction of p38 phosphorylation after 2-h treatment (Figure 4A). Furthermore, PTx induced pCHOP reporter transactivation used to monitor p38 downstream signaling (Figure 4B).

p38 protects paclitaxel inhibition of TGF-β-induced uPA production but not cell migration

Next, we were wondering whether p38 pathways may mediate PTx effects on RAW 264.7 cells migration and uPA production. uPA production analysis indicated that p38 inhibitor was able to rescue PTx-mediated inhibition of TGF-β-increased uPA expression (Figure 5A) and uPA promoter transactivation (Figure 5B). Meanwhile, p38 inhibition did not protect PTx capacity to inhibit TGF-β induction of cell migration (Figure 5C). Furthermore, p38 inhibitor did not modify PTx-induced MT structural rearrangement (Figure 5D).

Discussion

Innate immune system affects tumor development, progression and chemotherapy response, while tumor microenvironment infiltration by immune cells is a hallmark of tumor malignancy [22]. Upon activations TAM release a diversity of factors, cytokines and proteolytic enzymes that promote cancer cell growth and metastasis [23]. TGF-β strongly modulates Mφ functions, promotes monocyte to macrophage differentiation and regulates monocytes recruitment within tumor stroma [1,24]. Moreover, within tumor stroma TGF-β induces TAM polarization towards M2c phenotype that also increase tumor immunoregulation [25]. Here, we determined PTx capacity to interfere with TGF-β-mediated induction of uPA and migration of RAW 264.7 murine macrophage cell line.

PTx is widely used as first-line chemotherapy for a broad spectrum of cancers such as pancreatic cancer, breast cancer and cervical cancer, among others [1]. Namely, PTx belongs to microtubules interfering agents, and its primary mechanism of action is based on MT stabilization and prevention of MT disassembly. PTx induces mitotic spindle assembly and chromosome segregation abnormalities resulting in G2/M cell cycle phase arrests, and consequently cell division defects and cell death [4,5,26]. PTx treatment-induced cell inhibition by arresting RAW 264.7 cells at G2/M that was not modified by the presence of TGF-β (Figure 1). Furthermore, at clinically relevant concentrations PTx strongly induces the formation of MT bundles and RAW 264.7 morphological cells changes (Figure 2A).

Cytoskeleton is considered as a highway of vesicle transport and intracellular signaling activity [19]. TGF-β signaling has been demonstrated to depend on cytoskeleton dynamic. Smads may bound to MT and be subjected to a negative regulation [14]. In fact, changes in Mφ MT induced by PTx strongly attenuate TGF-β-induced Smad3 phosphorylation, nuclear localization and transcriptional activity (Figure 2).

TGF-β has been demonstrated to enhance Mφ motility concomitantly with uPA expression that contributes to ECM reorganization. Paradoxically, this is beneficial for wound, tissue repair and regeneration, but detrimental for inflammatory diseases and cancer [13,14]. Our data indicated that PTx inhibited both Mφ migration and uPA production (Figure 3). Cytoskeleton dynamics and ECM breakdown are the main mechanisms involved in cell migration [19,28]. In Mφ, uPA contributes to enhancing 2D and 3D migration and TGF-β transcriptionally induces uPA expression, uPA mRNA stability and uPA-secreted activity [13,29,30].

Although we previously demonstrated that Smad3 is essential for TGF-β induction of uPA in Mφ [13,31], we were interested in exploring the potential implications of intracellular signal transductions, which can be activated by PTx and their involvement on inhibition of TGF-β functions in RAW 264.7 cells. Interestingly, in Mφ PTx induced activation of NFκB and P38 MAPK that have been implicated in its capacity to mimic LPS-induced M1 polarization and production of pro-inflammatory and inflammatory cytokines [4,7]. In our experimental conditions, PTx was able to activate p38

MAPK in RAW 264.7 cells (Figure 4) and, by using the chemical p38 inhibitor SB203580, the implication of this signaling on TGF-β-induced uPA expression was also demonstrated. While p38 seems to contribute to the inhibition of TGF-β-induced uPA by PTx treatment, the underlying molecular mechanism is not well elucidated so far. We previously demonstrated that p38 mediates uPA expression inhibition in murine myoblasts [16], and we speculated that two mechanism may be operating. First, there may be a crosstalk of p38 with ERK1,2, so that ERK1,2 signaling is activated, and compensates for p38 inhibition [32] by enhancing uPA expression. Second, p38 inhibition might restore the capacity of TGF-β to activate Smad3 and recovery uPA induction by TGF-β. Nevertheless, both of these mechanisms need further analysis in order to elucidate if they operate during p38-mediated inhibition of TGF-β-induced uPA expression in RAW 264.7 cells.

PTx, by displaying LPS-like activities on Mφ, is able to activate p38 intracellular signaling. Specifically, PTx intracellularly binds to MD2, a protein that enables TLR4 to respond to LPS [33]. Then, activated TLR4 is internalized into endosomes and activates p38 MAPK downstream signaling [7]. Thereby, similar to LPS, PTx promotes Mφ polarization toward M1 phenotype. Moreover, tumor samples from PTx-treated patients exhibited increased expression of genes linked to an M1-like Mφ phenotype [7].

Although p38 inhibition rescues the capacity of TGF-β to increase uPA, it hardly protects RAW 264.7 cells from PTx inhibition of cell migration (Figure 5). The proper cytoskeleton dynamic is critical for cell migration [19] and MT interacts with actin cytoskeleton to coordinately regulate cell motility. For instance, MT polymerization induces Rac1 activation that mediates actin-driven cell protrusions and facilitates cell motility [34]. p38 inhibition did not counteract PTx-induced MT modification, and it seems that a separation between uPA expression and cell migration of RAW 264.7 is triggered. PTx has opened new possibilities for cancer therapy by reactivation of immune response against tumors. PTx is not only able to reprogram TAM toward M1 antitumor phenotype [7], but it also enhances natural killer cytotoxicity and increases dendritic cells viability [35]. Furthermore, PTx inhibits the expansion of myeloid-derived suppressor cells and induces their differentiation to dendritic cells [36].

Conclusion

PTx inhibits the capacity of TGF-β to increase RAW 264.7 cells migration via its MT targeting capacity, concomitantly with inhibition of uPA expression mediated through p38 MAPK. This suggests that PTx, by modulating Mφ responses to TGF-β, may enhance the anti-tumor effect of the immune system.

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Conflict of interests

The authors declare no conflict of interests.

References

1. Gratchev A. TGF-β signalling in tumour associated macrophages. Immunobiology 2017;222:75-81.
2. Brown JM, Recht L, Strober S. The Promise of Targeting Macrophages in Cancer Therapy. Clin Cancer Res 2017;23:3241-50.
3. Zhang F, Wang H, Wang X et al. TGF-β induces M2-like macrophage polarization via SNAIL-mediated suppression of a pro-inflammatory phenotype. Oncotarget 2016;7:52294-306.
4. Zhang D, Yang R, Wang S, Dong Z. Paclitaxel: new uses for an old drug. Drug Des Devel Ther 2014;8:279-84.
5. La Regina G, Coluccia, Naccarato V, Silvestri R. Towards modern anticancer agents that interact with tubulin. Eur J Pharm Sci 2019;131:58-68.
6. Wang X, Decker CC, Zechner L, Krstin S, Wink M. In vitro wound healing of tumor cells: inhibition of cell migration by selected cytotoxic alkaloids. BMC Pharmacol Toxicol 2019;20:4.
7. Wanderley CW, Colón DF, Luiz JPM et al. Paclitaxel Reduces Tumor Growth by Reprogramming Tumor-Associated Macrophages to an M1 Profile in a TLR4-Dependent Manner. Cancer Res 2018;78:5891-5900.
8. Raza A, Crothers JW, McGill MM, Mawe GM, Teuscher C, Krementsov DN. Anti-inflammatory roles of p38α MAPK in macrophages are context dependent and require IL-10. J Leukoc Biol 2017;102:1219-27.
9. Ridley AJ, Schwartz MA, Burridge K et al. Cell migration: integrating signals from front to back. Science 2003;302:1704-9.

10. Harrison RE, Grinstein S. Phagocytosis and the microtubule cytoskeleton. Biochem Cell Biol 2002;80:509-15.
11. Liguori M, Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages as incessant builders and destroyers of the cancer stroma. Cancers (Basel) 2011;3:3740-61.
12. Meznarich J, Malchodi L, Helterline D et al. A Urokinase plasminogen activator induces pro-fibrotic/m2 phenotype in murine cardiac macrophages. PLoS One 2013;e57837.
13. Mojsilović SS, Santibanez JF. Transforming growth factor-beta differently regulates urokinase type plasminogen activator and matrix metalloproteinase-9 expression in mouse macrophages; analysis of intracellular signal transduction. Cell Biol Int 2015;39:619-28.
14. Dong C, Li Z, Alvarez R Jr, Feng XH, Goldschmidt-Clermont PJ. Microtubule binding to Smads may regulate TGF beta activity. Mol Cell 2000;5:27-34.
15. Kocić J, Santibañez JF, Krstić A et al. Interleukin 17 inhibits myogenic and promotes osteogenic differentiation of C2C12 myoblasts by activating ERK1,2. Biochim Biophys Acta 2012;1823:838-49.
16. Kocić J, Santibañez JF, Krstić A, Mojsilović S, Ilić V, Bugarski D. Interleukin-17 modulates myoblast cell migration by inhibiting urokinase type plasminogen activator expression through p38 mitogen-activated protein kinase. Int J Biochem Cell Biol 2013;45:464-75.
17. Gundersen GG, Cook TA. Microtubules and signal transduction. Curr Opin Cell Biol 1999;11:81-94.
18. Forth S, Kapoor TM. The mechanics of microtubule networks in cell division. J Cell Biol 2017;216:1525-31.
19. Garcin C, Straube A. Microtubules in cell migration. Essays Biochem 2019;pii:EBC20190016.
20. Fleetwood AJ, Achuthan A, Schultz H et al. Urokinase plasminogen activator is a central regulator of macrophage three-dimensional invasion, matrix degradation, and adhesion. J Immunol 2014;192:3540-7.
21. Li Y, Zhang H, Kosturakis AK et al. MAPK signaling downstream to TLR4 contributes to paclitaxel-induced peripheral neuropathy. Brain Behav Immun 2015;49:255-66.
22. Ostuni R, Kratochvill F, Murray PJ, Natoli G. Macrophages and cancer: from mechanisms to therapeutic implications. Trends Immunol 2015;36:229-39.
23. Guan X. Cancer metastases: challenges and opportunities. Acta Pharm Sin B 2015;5:402-18.
24. Bierie B, Moses HL. Tumour microenvironment: TGF-beta: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 2006;6:506-20.
25. Gong D, Shi W, Yi SJ, Chen H, Groffen J, Heisterkamp N.TGFβ signaling plays a critical role in promoting alternative macrophage activation. BMC Immunol 2012;13:31.
26. Qi F, Zhou J, Liu M. Microtubule-interfering agents, spindle defects, and interkinetochore tension. J Cell Physiol 2019. doi: 10.1002/jcp.28978. [Epub ahead of print].
27. Moon MY, Kim HJ, Kim JG et al. Small GTPase Rap1 regulates cell migration through regulation of small GTPase RhoA activity in response to transforming growth factor-β1. J Cell Physiol 2013;228:2119-26.
28. Wolf K, Friedl P. Extracellular matrix determinants of proteolytic and non-proteolytic cell migration. Trends Cell Biol 2011;21:736-44.
29. Fleetwood AJ, Achuthan A, Schultz H et al. Urokinase plasminogen activator is a central regulator of macrophage three-dimensional invasion, matrix degradation, and adhesion. J Immunol 2014;192:3540-47.
30. Hildenbrand R, Jansen C, Wolf G et al. Transforming growth factor-beta stimulates urokinase expression in tumor-associated macrophages of the breast. Lab Invest 1998;78:59-71.
31. Mojsilovic SS, Mojsilovic S, Bjelica S, Santibanez JF. Estramustine Phosphate Inhibits TGF-β-Induced Mouse Macrophage Migration and Urokinase-Type Plasminogen Activator Production. Anal Cell Pathol (Amst) 2018;2018:3134102.
32. Zakrzewska M, Opalinski L, Haugsten EM, Otlewski J5, Wiedlocha A. Crosstalk between p38 and Erk 1/2 in Downregulation of FGF1-Induced Signaling. Int J Mol Sci 2019;20:pii:E1826.
33. Dziarski R, Gupta D. Role of MD-2 in TLR2- and TLR4-mediated recognition of Gram-negative and Gram-positive bacteria and activation of chemokine genes. J Endotoxin Res 2000;6:401-5.
34. Waterman-Storer CM, Worthylake RA, Liu BP, Burridge K, Salmon ED. Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. Nat Cell Biol 1999;1:45-50.
35. Kim MH, Joo HG. The role of Bcl-xL and nuclear factor-kappaB in the effect of PTx on the viability of dendritic cells. J Vet Sci 2009;10:99-103.
36. Sevko A, Michels T, Vrohlings M et al. Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model. J Immunol 2013;190:2464-71.