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Platelet-Derived Growth Factor-BB Accelerates Prostate Cancer Growth by Promoting the Proliferation of Mesenchymal Stem Cells

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ABSTRACT

Mesenchymal stem cells (MSCs) favor cancer growth by facilitating immunosuppression status in tumor microenvironment. However, the function and mechanism of MSCs in initiating and developing prostate cancer remains to be fully understood. In this study, we first found that MSCs promoted prostate cancer (PCa) tumor growth in vivo and cell proliferation in vitro by using PCs cell strain RM-1. Both exogenous and endogenous MSCs could be recruited into the tumor microenvironment by using bone-marrow transplantation model. We further demonstrated that PDGF-BB produced by RM-1 cell promoted MSCs proliferation in vivo and in vitro, which was abrogated by Si-RNA specific to PDGF-BB. And inflammatory cytokines, such as interferon gamma, tumor necrosis factor alpha, and anti-inflammatory cytokine transformation growth factor alpha, further increased the ability of RM-1 to produce PDGF-BB. Overall, PCa cells produced PDGF-BB favors the proliferation of MSCs, which may elicit immunosuppressive function and enable PCa cells to escape from the immunity surveillance in tumor inflammatory microenvironment. J. Cell. Biochem. 114: 1510–1518, 2013.

KEY WORDS: PROSTATE CANCER; PLATELET-DERIVED GROWTH FACTOR-BB (PDGF-BB); MESENCHYMAL STEM CELLS (MSCs)

Prostate cancer (PCa) is the most common cancer in men in western countries and is the second leading cause of cancer death in men [Freedland and Isaacs, 2005; Jemal et al., 2010], second only to lung cancer. Overall, cancer is second only to heart disease as a cause of death in men. Growing evidence has proved that the development of prostate cancer is related to MSCs [Placencio et al., 2010] and growth factor PDGF [Mathew et al., 2011] in cancer tissue. However, the interaction between PDGF, MSCs, and prostate cancer cells is still unknown.

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells, which can be isolated from bone marrow and may differentiate into cells of the mesodermal lineage, such as adipocytes, osteocytes, and chondrocytes. In recent years, many studies have shown that MSCs are closely related to prostate cancer development, invasion, and metastasis [Placencio et al., 2010; Tang et al., 2011]. Currently, MSCs can migrate to injured tissues and induce peripheral tolerance, where they can inhibit the release of pro-inflammatory cytokines and promote the survival of damaged cells [Djouad et al., 2003; Sotiropoulou and Papamichail, 2007; Han et al., 2011]. These contribute to the growth of cancer cells, including prostate cancer cells.

Evidence has shown that cancer cells, stimulated by some inflammatory cytokines, such as IFNγ [Fang et al., 2008], TNFα [Bird and Tyler, 1995], and anti-inflammatory cytokine TGFβ [Kuno et al., 2009], can produce a variety of growth factors like IGF [Shalita-Chesner et al., 2001], VEGF [Roskoski, 2007], and PDGF

Additional supporting information may be found in the online version of this article.

Jiwen Cheng, Huamao Ye, and Zhiyong Liu contributed equally to this work.

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[Badgett et al., 1996; Ikezumi et al., 2003] in autocrine or paracrine manners to facilitate their growth and development. PDGF is a dimeric polypeptide growth factor comprised of AA, AB, and BB isoforms [Andrae et al., 2008]. It affects diverse biological events associated with tumorigenicity, invasion, and distant metastasis such as promotion of cell proliferation, cell migration, and angiogenesis. Furthermore, PDGF, especially the BB isoform, is a well-known potent growth factor that stimulates the proliferation of some stromal-like stem/progenitor cells [Lederle et al., 2006; Bocelli-Tyndall et al., 2010]. To some extent, PDGF-BB may represent the PDGF family cytokines and play the role in experimental studies. Therefore, we made the hypothesis that PDGF-BB produced by prostate cancers may exhibit significant influence on the proliferation of mesenchymal stem cells, which would help cancer cells survive by suppressing the immune system.

In the present study, we confirmed that MSCs could contribute to the growth of prostate cancer cells (RM-1) in mice. During the process of tumor growth, exogenous and endogenous MSCs could be recruited into cancer tissue to play its role in vivo. We found that RM-1 cells, stimulated by TGFα, TNFα, or INFγ, produced a large number of PDGF-BB. Furthermore, PDGF-BB significantly promoted MSCs proliferation and recruitment into tumor both in vivo and in vitro. These results suggested that PDGF-BB, generated by prostate cancer cells in tumor inflammatory microenvironment, could accelerate prostate cancer growth by promoting the proliferation of mesenchymal stem cells.

MATERIALS AND METHODS

REAGENTS

Recombinant mouse PDGF-BB, TNF-α, IFNγ and TGFα were purchased from Peprotech (La Jolla, CA); anti-mouse CD34, CD44, CD45, CD14, CD90, CD105, CD166, and Sca-1 antibodies were purchased from BioLegend (San Diego, CA).

CELLS AND ANIMAL

MSCs were generated from bone marrow flushed out of tibia and femur of 6-week-old mice. Cells were cultured in α-MEM medium supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (all from Invitrogen, Carlsbad, CA). Non-adherent cells were removed after 72 h, and adherent cells were maintained with medium replenishment every 3 days. Cells were used at 5th to 20th passage. Murine RM-1 cells were cultured at 37°C, with 5% CO_2, in DMEM with 10% FBS, supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were sub-cultured every 3 days when they reached 70–80% confluence.

Male wide-type (wt) and EGFP-transgenic C57BL/6 mice, 6–8 weeks old, were purchased from Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). Mice in this study were housed in pathogen-free conditions, and all procedures were performed in accordance with the guideline of the Committee on Animals of the Chinese Academy of Sciences.

DIFFERENTIATION OF MSCs

MSCs were induced to differentiate into adipocytes in vitro by supplementation with 60 μM indomethacin, 0.5 mM isobutylmethylxanthin, 10 nM dexamethasone, and 10 μg/ml insulin for 14 days. The presence of adipocytes was verified by staining for triglycerides with Oil red O (Sigma–Aldrich), to reveal intracellular lipid accumulation. MSCs were cultured with osteoinductive medium consisting of DMEM supplemented with 10% FBS, β-mercaptoethanol, 100 μM L-ascorbic acids, 10 nM dexamethasone, and 10 mM β-glycerophosphate for 14 days. These cells were stained with Von Kossa to identify calcium deposition characteristic of osteoblasts.

ANIMAL MODELS

The bone marrow transplantation model: C57BL/6 wt-mice, aged 8 weeks, received lethal irradiation (eight grays in a divided dose 4 h apart), and immediately received transplantation of 1×10^6 enriched EGFP-positive MSCs and 9×10^6 EGFP-negative whole bone-marrow (BM) cells by a tail vein injection. The negative whole BM cells were obtained from 6-week-old wt-mice. EGFP-positive MSCs were isolated and cultured from EGFP-transgenic mice.

Subcutaneous tumor model: (1) RM-1 prostate cancer cells and MSCs were prepared as single-cell type suspensions (1×10^6 cells in 300 μl PBS) or a mix of cells (1×10^6 RM-1 cells and 1×10^5 MSCs in 300 μl PBS). Subcutaneous administration of these cells was performed in the armpit area of C57BL/6 wt-mice. Three mice were sacrificed every 3–4 days to collect the serum. At the end of the experiment (on the 14th day), tumor growth was evaluated by measuring the length and width of tumor mass. (2) RM-1 single-cell suspensions were injected into the armpit area of C57BL/6 wt or EGFP mice. On the 11th day wt-mice received EGFP-MSCs (1×10^5 cells in 300 μl PBS) by tail vein administrating. At the end of the experiment, we collected the tumor tissue from wt and EGFP mice to make frozen-sections. All tumor-bearing mice were sacrificed on the 14th day.

FACS ANALYSIS

The fresh specimens of prostate cancer tissue were transferred to a Petri dish, where the tissue was gently minced and filtered (100 mm) to remove large aggregates, followed by incubation for 45 min in 50 ml of Hank's containing 0.05% collagenase at 37°C with continuous stirring. 0.5 mg DNAase in 1.0 ml of PBS was added 20–40 min after this incubation period. The cell suspension was filtered (40 mm) and nonparenchymal cells were separated by discontinuous density gradients of Percoll (Pharmacia Biotech) at 1.044 and 1.07 g/ml. The final cell suspension was washed twice and analyzed on a fluorescence activated cell sorting system (FACS; Becton Dickinson, San Jose, CA) after addition of propidium iodide (Sigma) to exclude dead cells. The whole bone marrow cells were derived from BMT-mice femur. They were pretreated with erythrocyte lysis buffer, and then centrifuged to collect the remaining cells. Flow cytometry analysis was conducted as previously reported.

Wells with single cell colonies were harvested and expanded into clonal cell lines. PE-conjugated mouse antibody to CD105, CD34,

and Sca-1 as well as FITC-conjugated antimouse CD45 and CD90 (eBioscience) were applied for characterization of mice MSCs.

SHORT INTERFERING RNA (siRNA) SYNTHESIS AND TRANSIENT TRANSFECTION

Three siRNA sequences of PDGF-BB (Table I) were designed by using Oligoengine software and confirmed by nucleotide BLAST searches. Transfections were performed with a Lipofectamine 2000 kit (Invitrogen) according to the manufacturer's instructions. Cells ($1–3 \times 10^6$) grown to a confluency of 50–60% in 10 cm Petri dishes were transfected with siRNA sequence or their relative mock sequences. Then they were observed under fluorescence microscope and harvested 48 h after transfection. By verification, we confirmed that these three siRNA sequences are effect. We selected one at random to use for subsequent experiments (Supplementary Fig. S1).

REAL-TIME PCR

The RM-1 cells were incubated with IFN-γ (20 ng/ml), TNF-α (20 ng/ml), or TGF-α (20 ng/ml), respectively for 24 h. The cells and tumor tissue were collected to extract the total cellular mRNA with Trizol reagent (Invitrogen). Expression of mRNA was determined by real-time RT-PCR using SYBR Green Master Mix (Applied Biosystems, Foster City, CA). Total sample RNA was normalized to endogenous β-actin mRNA. Primers sequences for PDGF-BB were: forward: 5′-GCC GGT CCA GGT GAG AAA GAT TG-3′; reverse: 5′-GGG GCC GGC GGA TTC TCA-3′. Thermocycler conditions included an initial hold at 50°C for 2 min, and then 95°C for 10 min. This was followed by a two-step PCR program of 95°C for 15 s, and 60°C for 60 s. It was repeated for 40 cycles on an Mx4000 system (Stratagene, La Jolla, CA), on which data were collected and quantitatively analyzed. Expression level of mRNA is presented as fold change relative to an untreated control.

COLONY FORMATION ASSAY

Anchorage-independent growth was determined by colony formation in soft agar as described (20). One thousand cells in 0.5 ml of 0.4% (w/v) agarose (Sea-plaque; FMC Corp, Rockland, ME) in DMEM were overlaid onto 0.5 ml of bottom layer of 0.6% agarose in 6-well culture plates and cultured for 21 days in a humidified CO_2 incubator at 37°C. At the end of the culture, macroscopic colonies were manually counted.

TABLE I. Oligonucleotide Sequences Used in Real-Time PCR and siRNA Assays

Assay	Gene	Sequence (5′ → 3′)
Real-time PCR	PDGF-BB	F: GCCGGTCCAGGTGAGAAAGATTG R: GGGGCCGGCGGATTCTCA
	β-Actin	F: CTCCATCCTGGCCTCGCTGT R: GCTGTCACCTTCACCGTTCC
PDGF-BB siRNA	Sequence 1	Sense: GAGTCGAGTTGGAAAGCTCAT Antisense: ATGAGCTTTCCAACTCGACTC
	Sequence 2	Sense: GTTTAAGCACACCCATGACAA Antisense: TTGTCATGGGTGTGCTTAAAC
	Sequence 3	Sense: GCCTGCAAGTGTGAGACAGTA Antisense: TACTGTCTCACACTTGCAGGC
	Control	Sense: UUCUCCGAACGUGUCACGUTT Antisense: ACGUGACACGUUCGGAGAATT

WESTERN BLOTTING

Cells were washed in PBS solution, and protein was extracted according to an established protocol. Nuclear extract proteins were quantified using the Bio-Rad protein assay. Proteins were then mixed with Laemmli sample buffer, heated at 65°C for 10 min, loaded (20 μg/sample), separated by SDS–polyacrylamide gel (7.5%) electrophoresis under denaturing conditions, and electroblotted on nitrocellulose membranes. The nitrocellulose membranes were blocked by incubation in blocking buffer (1% BSA in Tris-buffered saline, 0.1% Tween 20), incubated with anti-PDGF-BB antibody (1:500 polyclonal; Bethyl), washed, and incubated with anti-rabbit peroxidase-conjugated secondary antibody (1:10,000; Sigma). Signals were visualized by chemiluminescent detection. Blots were quantified using Quantity One software from Bio-Rad, and PDGF-BB expression (peak intensity) was normalized to values in the control group. Equal loading of samples was verified by Coomassie blue staining of simultaneously run gels. Gels were run four times, and the images shown are representative.

WOUND HEALING AND TRANSWELL ASSAY

The methods for wound healing and the Transwell assay were operated as previously reported [Wu et al., 2009; Yang et al., 2009]. These experiments were performed in triplicate.

STATISTICAL ANALYSIS

Statistical analysis of the data was done by using GraphPad Prism 5. Student's *t*-test was used to compare the mean values of two groups. Final values were expressed as mean ± SEM. A difference of at least $P < 0.05$ was considered statistically significant.

RESULTS

MSCs PROMOTE THE DEVELOPMENT OF PROSTATE CANCER IN VIVO

To investigate the function of MSCs in prostate cancer, we first prepared MSCs. MSCs adhered to the plastic surface, presenting a small population of single cells which were spindle-shaped and contained one nucleus after 72 h of the primary culture. During 7–10 days after the initial plating, the cells looked like long spindle-shaped fibroblastic cells and began to form colonies. MSCs could differentiate into adipocytes and osteoblast-like cells. The surface antigen profile of mouse MSCs detected by flow cytometry was positive for CD90, CD105 CD166, CD44, and Sca-1 and negative for CD14, CD34, and CD45 (Fig. 1A,B). To investigate whether BM-derived MSCs could favor the growth of prostate cancer, the MSCs were subcutaneously co-injected with RM-1 cells in syngeneic C57BL/6 mice. We found that the growth of tumor mixed with MSCs was faster than that of RM-1 cells alone (Fig. 1C). These data suggested that MSCs could promote the development of prostate cancer in vivo.

PDGF-BB ACCELERATES THE GROWTH OF PROSTATE CANCER IN VIVO

PDGF is a key cytokine to regulate the tumor growth. To determine the role of PDGF in the development of prostate cancer in mice, we used PDGF-BB cytokine to stimulate the cancer cells in vivo and in

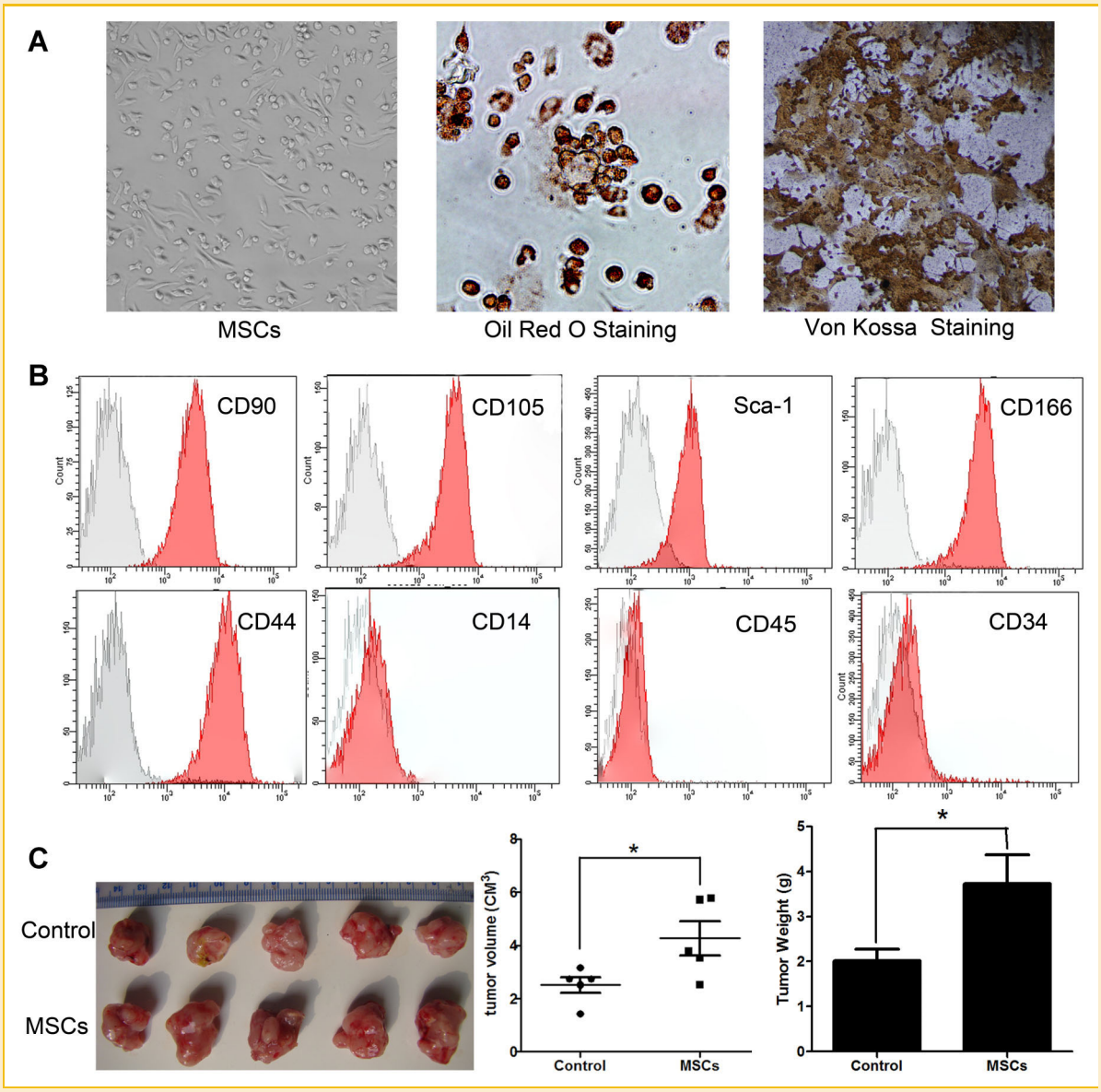

Fig. 1. MSCs accelerated the growth of prostate cancer in mice. A: MSCs at passage 6 were grown under different conditions that favored differentiation into either adipocytes (for 14 days), or osteoblasts (for 14 days), as described in Materials and Methods Section. The presence of triglycerides, characteristic of adipocytes, was revealed by staining with oil red O. Calcium deposition, indicative of osteoblasts, was stained with Von Kossa stain. B: MSCs derived from C57BL/6 mice were stained with commercially available antibodies to analyze the surface marker by flow cytometry (red). Corresponding antibodies of the same isotype were used as controls [Szczepanski et al., 2009]. C: C57BL/6 MSCs (1×105) mixed with RM-1 prostate cancer cells (1×106) to perform subcutaneous administration in the C57BL/6 mice axillary region. After 14 days of implantation, the animals were killed and tumors were dissected. The volume and weights of RM-1 tumors were measured after they were removed from the mice. D: MSCs and RM-1 cells were separately cultured in Transwell for 24 h, cell viability was dectected by CCK8. $^{*}P < 0.05$.

vitro. Tumor-bearing mice received PDGF-BB (100 μg/kg) cytokine or PBS twice a week. After 14 days of implantation, the animals were killed and tumors were dissected. The volume of RM-1 tumor was measured after been removed from the mice. We found the tumor grew faster when the mice were pretreated with PDGF-BB cytokine (Fig. 2A). In vitro the RM-1 cells were pretreated with PDGF-BB (50 ng/ml) cytokine or PBS for 12 h. Cell proliferation was detected by using CCK8 (Fig. 2B) and clone formation assay (Fig. 2C). We found that PDGF-BB had little influence on the growth of prostate cancer cells in vitro. These results suggest that PDGF-BB can accelerate the growth of prostate cancer only in vivo rather than in vitro. Therefore, there may be some factors in vivo that play an intermediary role between PDGF-BB and prostate cancer.

BOTH EXOGENOUS AND ENDOGENOUS MSCs COULD BE RECRUITED INTO CANCER SITE

To confirm the contribution of MSCs to the development of prostate cancer, we first observed whether MSCs could be recruited into

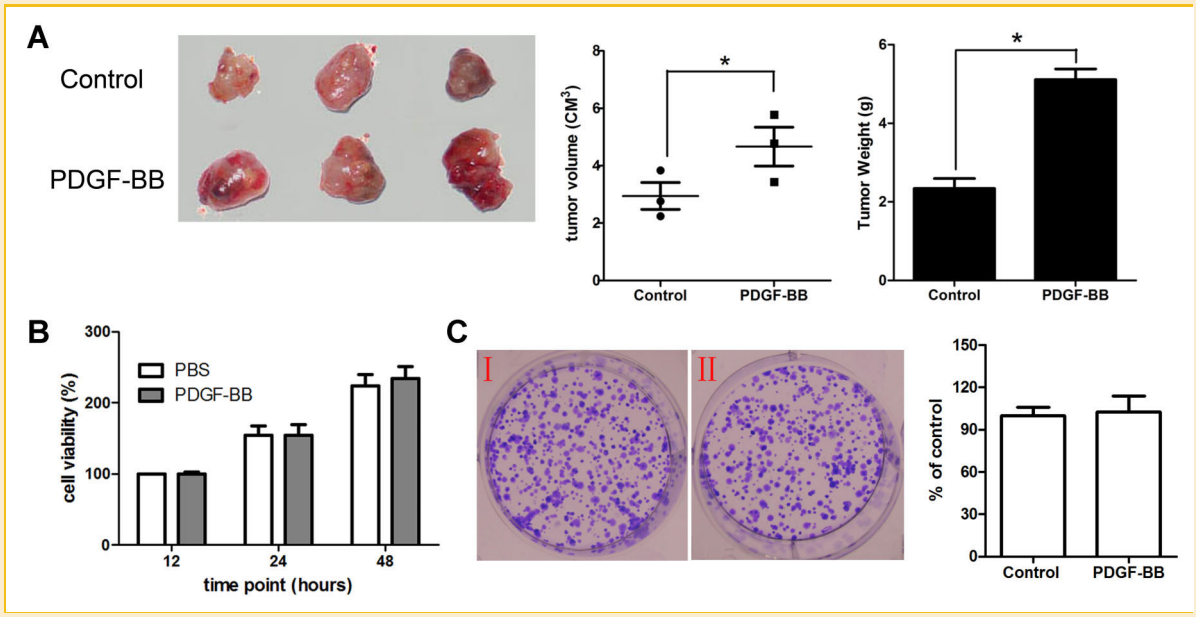

Fig. 2. PDGF-BB accelerated the growth of prostate cancer in vivo but not in vitro. A: Tumor-bearing mice received PDGF-BB (100 μg/kg) cytokine or PBS twice a week. After 14 days of implantation, the animals were killed and tumors were dissected. The volume and weights of RM-1 tumors were measured after they were removed from the mice. B: RM-1cells were pretreated with PDGF-BB cytokine (50 ng/ml) or PBS for indicated time. The proliferation of cancer cells in vitro was detected by using CCK8. C: Clone formation of RM-1 cells same with (B) was detected. Data shown are from a single experiment and are representative of three similar experiments yielding similar results. $^{*}P < 0.05$.

tumor tissue. Exogenous MSCs from EGFP-mice were administrated to tumor-bearing wt-mice by tail vein on the 11th day. Three days later, tumor tissue was dissected and traced the presence of EGFP-MSCs. The results showed that some EGFP-positive cells presented in cancer tissue (Fig. 3A, II). We also observed that endogenous MSCs, which pre-existed in bone marrow in BMT-mice and some of them expressed EGFP protein, could also be recruited into cancer tissue (Fig. 3A, III). Flow cytometry also showed that there were more

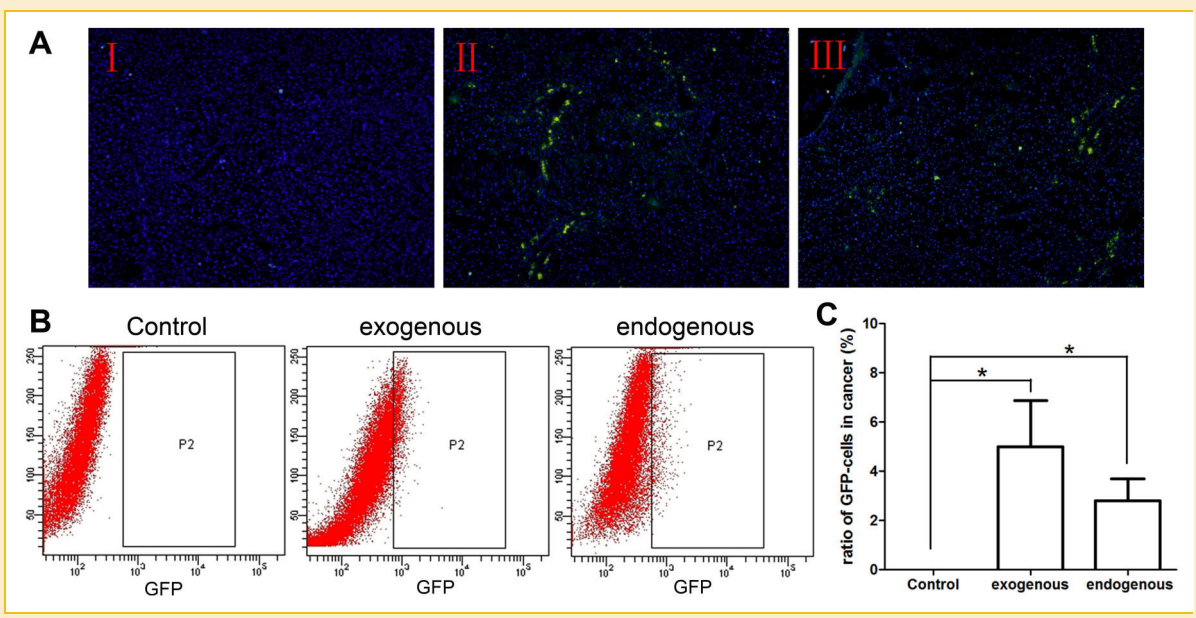

Fig. 3. Both exogenous and endogenous MSCs could be recruited into tumor site. A: RM-1 cells (1 × 106) were subcutaneously administrated in the C57BL/6 mice axillary region. After 14 days of implantation, the animals were killed and tumors were dissected to trace the recruitment of MSCs in cancer tissue on fresh frozen section. I: wt-mice as control. II: Three days before sacrifice, wt-mice were administered EGFPMSCs. III: Before implantation of RM-1 cells, mice were pretreated with bone marrow transplantation (BMT) for 1 month. BMT: wt-mice were lethally irradiated and received transplants of enriched EGFP-MSCs and EGFP-negative whole BM cells. B: The ratio of EGFP-positive cells in cancer tissue was assayed by Flow cytometry. C: The percentage of GFP-MSCs in cancer tissue. Data shown are from a single experiment and are representative of three similar experiments yielding similar results. $^{*}P < 0.05$.

exogenous EGFP-MSCs than endogenous EGFP-MSCs in cancer tissue (Fig. 3B,C), which could result from the short lifespan of MSCs. All of these data suggested that MSC could be recruited into cancer tissue to affect tumor development.

PDGF-BB PROMOTES THE PROLIFERATION OF MSCs IN VITRO

Above data indicated that both PDGF-BB and MSCs promoted PCa growth. To verify the relationship between PDGF and MSCs, we used PDGF-BB cytokine and siRNA to stimulate and inhibit PDGF-BB expression in MSCs. MSCs were seeded in complete growth medium. After 24 h the medium was replaced with fresh complete growth medium containing PBS, PDGF-BB, and/or PDGF-BB siRNA. Cell proliferation was detected by CCK8 and clone formation assay. The results showed that PDGF markedly increased MSCs proliferation in dose dependent manner (Fig. 4A). The proliferation effect of supernatant from RM-1 on MSCs could be reversed by PDGF-BB siRNA (Fig. 4B). PDGF-BB also promoted the colony formation ability of MSCs (Fig. 4C,D). Furthermore, supernatant from RM-1 cells transfected Si-RNA specific to PDFG-BB abrogated the pro-proliferative function of supernatant from RM-1 cells transfected control Si-RNA (Fig. 4E). These data indicated that PDGF-BB derived from RM-1 cells promotes the proliferation of MSCs in vitro.

PDGF-BB COULD ACCELERATE THE PROLIFERATION OF MSCs IN VIVO

To prove MSCs proliferation in vivo is difficult. To solve this problem, we extensively used the bone marrow transplantation (BMT) models. Lethal radiated C57BL/6mice accepted both wild type bone marrow and purified GFP-MSCs transplantation simultaneously 1 month before RM-1 (1×10^6) administration. Mice received PDGF-BB by tail vein injection of PDGF-BB (100 μg/kg body weight) or PBS as control daily. After 14 days of implantation, the animals were killed and the cancer tissue and bone marrow cells were analyzed by flow cytometry. We found that the ratio of GFP-MSCs in mice, which received a large number of PDGF-BB cytokine, significantly increased in comparison with control in bone marrow (Fig. 5A). Similar results were also found in cancer tissue (Fig. 5B). These results suggested that PDGF could accelerate the proliferation of MSCs in bone marrow and increase the number of MSCs in caner tissue. PDGF-BB were produced and secreted from prostate cancer cells by both inflammatory and anti-inflammatory cytokines.

Inflammatory and growth factors, like IFNγ and TNFα, and anti-inflammatory cytokine TGFα, exist in cancer tissue to regulate cancer cells function. To determine the expression and the source of PDGF, we detected the PDGF-BB by qPCR, ELISA, and Western blotting in vivo and in vitro. We found that the production of PDGF-

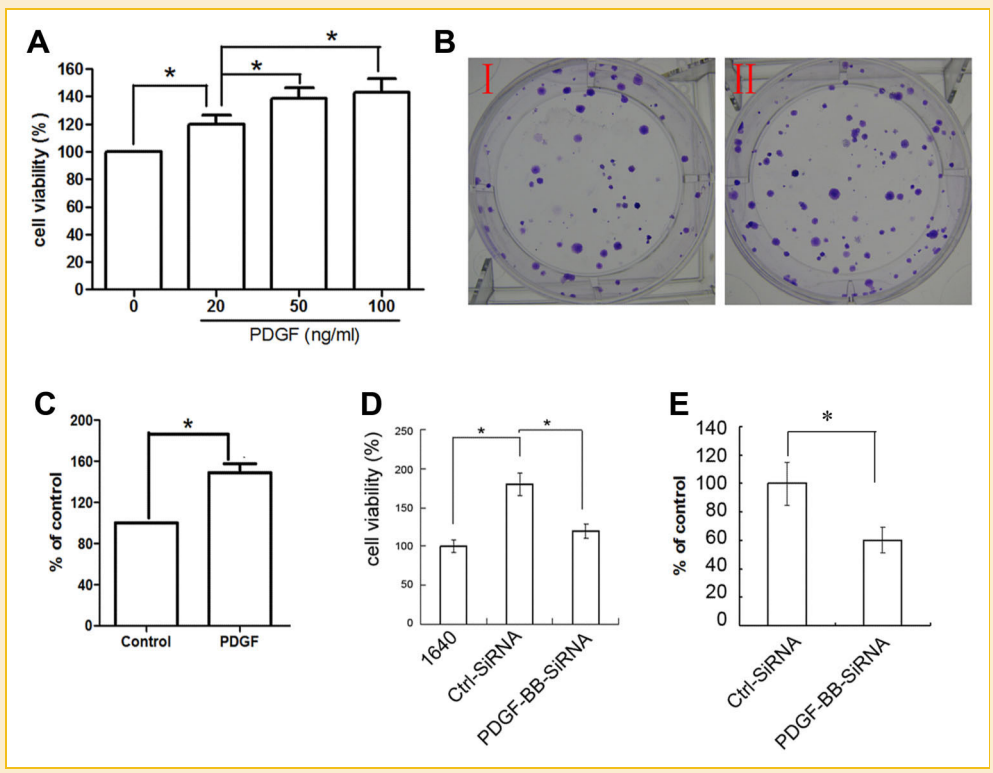

Fig. 4. PDGF-BB promoted the proliferation of MSCs in vitro. A: MSCs were seeded in complete growth medium. After 24 h the medium was replaced with fresh complete growth medium containing various dose of PDGF-BB. MSCs were pretreated with PDGF (0, 20, 50, and 100 ng/ml). Three days later the cell viability was detected by CCK8 assay. B: MSCs were seeded at a density of 1,000 per plate, then pretreated with (I) PBS, (II) PDGF-BB. Colony formation ability of MSCs was assessed after 21 days. C: Percentage of colony formation in three groups. D: MSCs were pretreated with normal medium, supernatant from RM-1 cells transfected with control or specific to PDGF-BB SiRNA and replaced for every 24 h. Three days later the cell viability was detected by CCK8 assay. E: MSCs were treated with supernatant from RM-1 cells transfected with control or specific to PDGF-BB SiRNA and replaced for every 24 h. Colony formation ability of MSCs was assessed after 21 days. Data shown are from a single experiment and are representative of three similar experiments yielding similar results. $^*P < 0.05$.

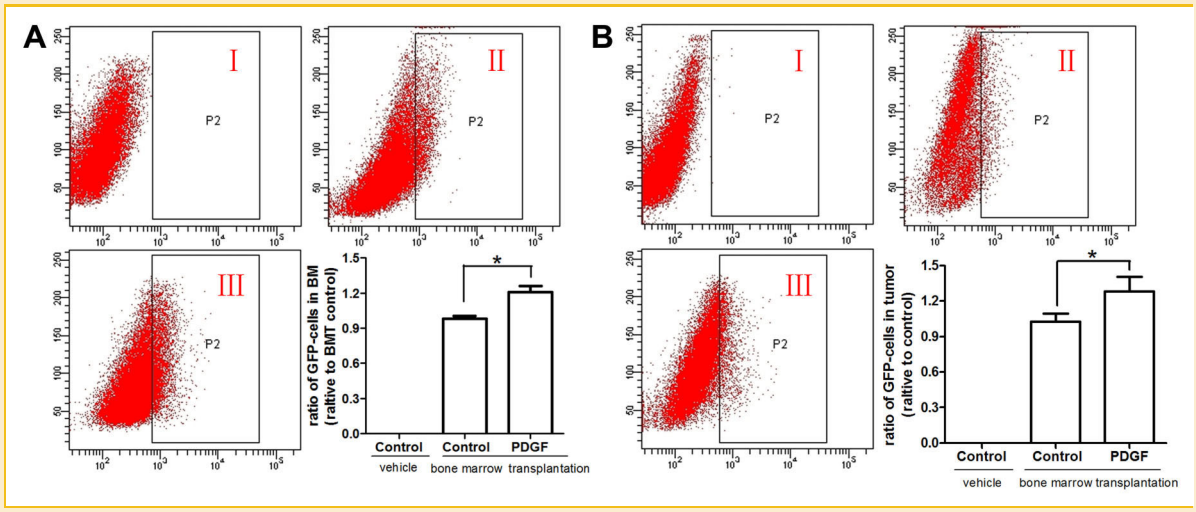

Fig. 5. PDGF-BB accelerated the proliferation of MSCs in vivo. RM-1 cells (1×106) were administrated subcutaneously in the C57BL/6 mice axillary region. After 14 days of implantation, the animals were killed. Tumors and bone marrow cells were analyzed by flow cytometry. I: wt mice; II: BMT mice same with (Fig. 3A, III); III: BMT-mice were administrated PDGF-BB (50 ng/ml). A: The ratio of GFPMSCs in bone marrow. B: The ratio of GFP-MSCs in tumor tissue. Groups as described above. Data shown are from a single experiment and are representative of three similar experiments yielding similar results. $^{*}P < 0.05$.

BB gradually increased with the development of tumor growth in mice (Fig. 6A). Then we pretreated RM-1 cells with cytokines IFNγ, TNFα, or TGFα and detected the PDGF-BB expression. The expression of PDGF-BB mRNA (Fig. 6C) and protein (Fig. 6B,D) was significantly upregulated by pretreatments of cytokines. These data suggested that PDGF-BB (at least part of the PDGF-BB) could be produced and secreted from prostate cancer cells in inflammatory microenvironment.

DISCUSSION

Tumor microenvironment is created by the tumor to escape immune surveillance and promote the tumor development. Although various immune cells, such as T cells, macrophages and dendritic cells, are recruited to the tumor site, the immune surveillance and anti-tumor function are downregulated seriously [Di Nicola et al., 2002; Corcione et al., 2006]. Many researchers have confirmed that MSCs are potent immunoregulators and have shown clinical utility in suppressing immunity in a variety of cancers, like breast cancer, hepatic carcinoma, melanoma, pancreatic carcinoma, prostate cancer, etc. [Djouad et al., 2003; Uccelli et al., 2007; Patel et al., 2010]. The immunosuppression status in tumor is closely related to the number of MSCs in cancer tissue. In this study, we demonstrate that both exogenous and endogenous MSCs could be recruited to prostate cancer site. Moreover, increasing the number of MSC could accelerate the cancer growth. Therefore, we believe that MSCs contribute to the development of prostatic carcinoma by suppressing the immune function as described in other articles [Ramasamy et al., 2007].

We first found that MSCs could promote mice PCa cells RM-1 growth in vivo, which was consistent with our previous report. To exclude the possibility that MSCs growth accounts for tumor volume and weight difference, we further found that MSCs could also promote RM-1 cell proliferation in Transwell culture system. Thus we believed that MSCs promoted RM-1 cell proliferation in vivo and in vitro.

Several lines of evidence indicated that PDGF is a strong candidate mediator of mechanisms of prostate cancer disease progression [Cheng et al., 2009; Bocelli-Tyndall et al., 2010]. PDGF family mainly consists of three proteins—PDGF-AA, PDGF-AB, and PDGF-BB—encoded by two genes, PDGF-A and PDGF-B. Currently, studies have shown that PDGF-BB and its receptors are closely correlated with the growth, the migration, and the invasion of many cancers, including prostate cancer [Yi et al., 2002; Hata et al., 2010]. However, there is little information about the relationship between PDGF-BB, MSCs, and prostate cancer. In this study, we verified that PDGB-BB could promote cancer growth in vivo but not in vitro. This suggests that the promoting effect of PDGF-BB on PCa cancer cells growth is indirect rather than direct. Taking it into account that MSCs is an important intermediate regulator, we found that PDGF-BB promoted the proliferation of MSCs in vitro, and this effect could be reversed by PDGF-BB siRNA. The promoting effect of PDGF-BB cytokine in vivo on MSCs proliferation was also confirmed by daily injection of PDGF-BB. The results show that administration of PDGF-BB in mice can increase the number and ratio of MSCs not only in cancer tissue but also in bone marrow. At the same time, we also found that PDGF-BB slightly contribute to the migration of MSCs in vitro, which may account partially for PDGF-BB function. Our findings that pretreatment of RM-1 cells with IFNγ, TNFα, and TGFα significantly increased PDGF-BB production further demonstrate the important relations between MSCs and PCa cells, the inflammatory status and tumor growth. All of the data suggest that PDGF-BB derived from prostate cancer cells contribute to the MSCs proliferation and even migration in PCa model.

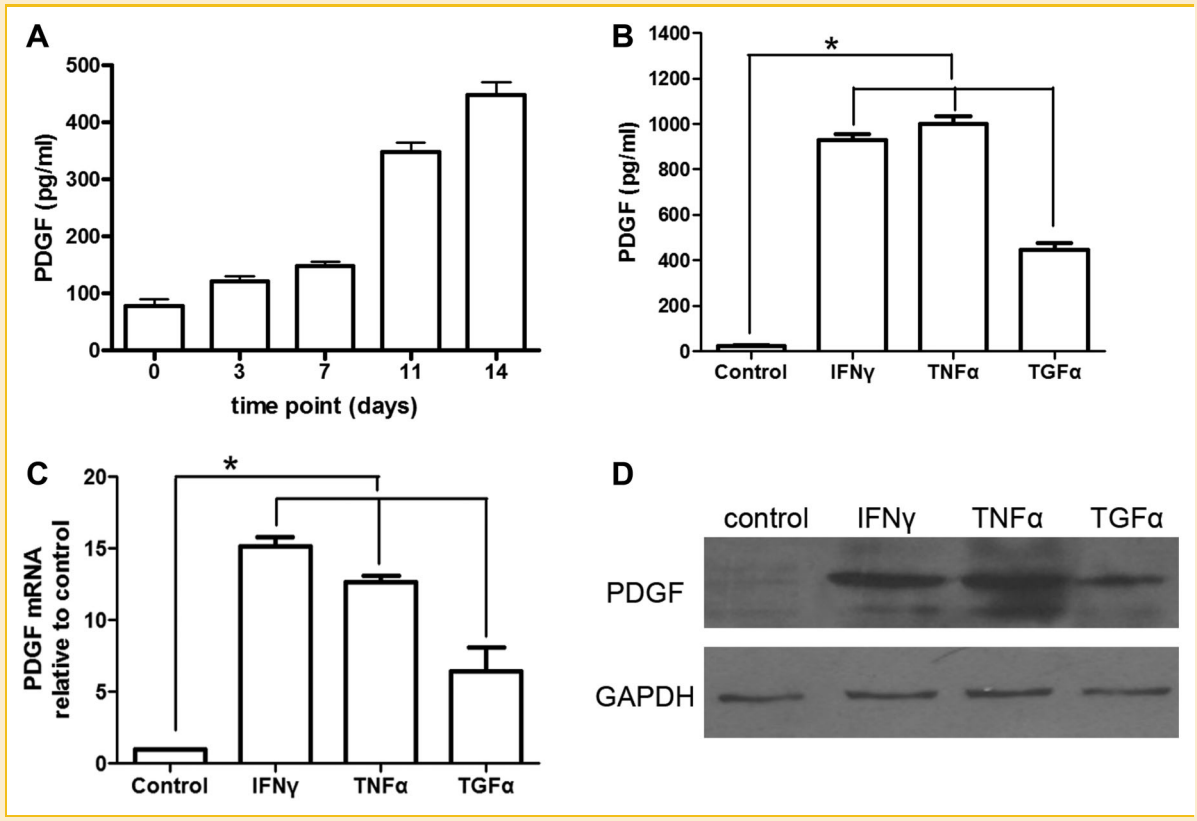

Fig. 6. PDGF-BB were produced and secreted from prostate cancer cells in inflammatory microenvironment. A: The production of PDGF-BB in serum of mice collected at indicated time after tumor implantation was detected by ELISA. B: RM-1 cells were pretreated with IFNγ, TNFα, or TGFα for 24 h. PDGF-BB production in culture medium was detected by ELISA. C: PDGF-BB mRNA expression in RM-1 cell same with (B) was detected by real-time PCR. D: PDGF-BB protein expression in RM-1 cell same with (B) was detected by Western blotting. Data shown are from a single experiment and are representative of three similar experiments yielding similar results. $^{*}P < 0.05$.

In conclusion, our results illuminate the relationship between MSCs, PDGF, and prostate cancer cells, and suggest that PDGF-BB accelerate prostate cancer growth by promoting the proliferation and migration of MSCs. We expect that our findings will offer insights into further exploring the mechanism of prostate caner development and provide a theoretical basis for finding new therapies for prostate cancer.

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