

Prostaglandin E2 promotes human CD34⁺ cells homing through EP2 and EP4 *in vitro*

YAQUN WANG*, SHUPING LAI*, JING TANG, CHUN FENG, FANGJIE LIU,
CHANG SU, WAIYI ZOU, HUIZHEN CHEN and DUORONG XU

Department of Hematology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong 510080, P.R. China

Received April 19, 2016; Accepted March 31, 2017

DOI: 10.3892/mmr.2017.6649

Abstract. Recently, certain studies have demonstrated *in vitro* that prostaglandin E2 (PGE2) promotes human cluster of differentiation (CD)34⁺ cell homing. However, the sub-type receptors activated by PGE2 are unknown, as the PGE2 receptor EP1-4 subtypes (EP1-4) are expressed on the membrane of human CD34⁺ cells. Based on the above, the present study aimed to screen the receptor subtype activity by PGE2 to promote human CD34⁺ cell homing. It was observed that human CD34⁺ cells expressed the four PGE2 sub-receptors, particularly EP2 and 4. PGE2 increased EP2 and 4 mRNA expression significantly, while EP1 and 3 mRNA exhibited no significant alteration. PGE2, EP2 agonist (EP2A), and EP4A upregulated C-X-C chemokine receptor 4 mRNA and protein expression in human CD34⁺ cells, and promoted stromal cell-derived factor 1 α (SDF-1 α) expression in bone marrow mesenchymal stem cells (BMMSCs). These phenomena were inhibited by the associated receptor antagonists. PGE2, EP2A, and EP4A facilitated human CD34⁺ cell migration towards SDF-1 α and BMMSCs. The results of the present study suggested that PGE2 promoted human CD34⁺ cell homing through EP2 and 4 receptors *in vitro*.

Introduction

Hematopoietic stem cells (HSCs) have the ability of self-renewal and multilineage differentiation potential. They are capable of regenerating hematopoietic and immunological systems following injury of bone marrow and thus have potential as therapies for bone marrow failure, hematological malignancies and immunodeficiencies (1). Hematopoietic stem cell transplantation (HSCT) has been developed rapidly in recent years. However, the limited number of HSCs and homing failure hinder the further development of transplantation. Promoting donor HSC homing and proliferation is one of the key measures to prevent implantation dysfunction (2,3).

The chemokine stromal cell-derived factor 1 α (SDF-1 α) is secreted by bone marrow mesenchymal stem cells (BMMSCs) and only binds to C-X-C chemokine receptor 4 (CXCR4), which is expressed on cluster of differentiation (CD) 34⁺ cells. The CXCR4/SDF-1 α (4-6) signaling axis is considered to serve a pivotal role in homing. Peled *et al* (4) demonstrated that exposing human cord blood-derived CD34⁺ cells to anti-CXCR4 antibody reduces homing to bone marrow (BM) in non-obese diabetic/severe-combined immunodeficient mice and indicated that CXCR4 is important for HSC homing. Improving CXCR4 or SDF-1 α expression may promote CD34⁺ cell homing.

Prostaglandin E2 (PGE2) is a metabolic product of arachidonic acid (AA). Cyclooxygenase (Cox)-1 or -2 converts AA into prostaglandin H2 (PGH2) and PGE synthase subsequently converts PGH2 into PGE2. PGE2 is involved in numerous physiological and pathological systems (7). PGE2 inhibits T cell receptor signaling and may induce inflammation (8), and also stimulates bone resorption of osteoclasts (9). In addition to this, PGE2 serves an important role in stem cells. A previous study demonstrated that PGE2 promotes progenitor proliferation in *in vitro* cell culture and in colony forming unit-spleen assays following transplantation (10). North *et al* (11), using a Zebrafish embryo model, confirmed that embryos exposed to exogenous 16,16-dimethyl-PGE2 (dmPGE2) exhibit a notable increase in HSC numbers. The increase in PGE2 synthesis improved HSC numbers, while the inhibition of PGE2 synthesis decreased the numbers of HSCs. Current studies suggested that PGE2 promotes human CD34⁺ cells homing *in vitro* (12,13). However, PGE2 has four specific G protein coupled E prostanoid (EP) receptors, PGE2

Correspondence to: Dr Duorong Xu, Department of Hematology, The First Affiliated Hospital, Sun Yat-sen University, 58 Zhongshan Second Road, Guangzhou, Guangdong 510080, P.R. China
E-mail: xudr@hotmail.com

*Contributed equally

Abbreviations: PGE2, prostaglandin E2; EP1, PGE2 receptor EP2 subtype; EP4, prostaglandin E2 receptor EP4 subtype; EP2A, EP2 agonist; EP2AA, EP2 antagonist; EP4A, EP4 agonist; EP4AA, EP4 antagonist; BMMSC, bone marrow mesenchymal stem cell; HSC, hematopoietic stem cell; CXCR4, C-X-C chemokine receptor type 4; SDF-1 α , stromal cell-derived factor 1 α

Key words: allogeneic hematopoietic stem cell transplantation, implantation dysfunction, PGE2 receptor, PGE2 receptor agonist, homing

receptor EP1-4 subtypes (EP1-4). Sugimoto *et al.* (14), using a knock-out mice model for each EP subtype receptor, identified that PGE2 is mediated by each EP sub-type receptor, which generates differences in signal transduction and physiological effects. EP1, through the activation of phospholipase C, regulates intracellular Ca²⁺ levels, and EP2 and 4 increase the levels of cyclic (c) AMP by binding with stimulatory G proteins. In contrast with EP2 and 4, EP3 inhibits cAMP production via inhibitory G proteins. Applying PGE2 directly to patients may produce a variety of serious adverse events. In previous years, studies on EP agonists (EPAs) and EP antagonists (EPAAAs) have increased exponentially. EP1AA induces apoptosis in breast cancer cells, inhibits the development of breast cancer (15) and suppresses colon cancer development in rats (16). EP2AA possesses the potential for treatment of glaucoma (17). PGE2, via EP4, stimulates anti-inflammation in the lung and provides a novel clinical perspective for chronic airway inflammatory conditions (18). However, studies on the effects of EPAs and EPAAAs on HSCs have not been reported. Therefore, the present study aimed to investigate the specific subtype receptor mediating PGE2 promotion of human CD34⁺ cell homing. More importantly, the results of the present study provide evidence to develop a novel targeted treatment to prevent human HSC implantation dysfunction in the future.

In the present study, it was demonstrated that EP2A and EP4A upregulated CXCR4 and SDF-1a expression, and increased the migratory ability of CD34⁺ cells towards SDF-1a and BMMSCs.

Materials and methods

Reagents. Healthy donors were selected from the Department of hematology, the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). The specimen handling was performed in accordance with the requirements of the hospital ethics committee. The present study was approved by the ethics committee of The First Affiliated Hospital of Sun Yat-sen University and written informed consent was obtained from all patients. Bone marrow was obtained from healthy donors directly. Peripheral blood was collected after the healthy donors received granulocyte colony stimulating factor (G-CSF) mobilization. dmPGE2 was bought from the Cayman Chemical Company (Ann Arbor, MI, USA). The EP2AA AH-6809 was bought from the Cayman Chemical Company. The EP2A ONO-AE1-259, EP4A ONO-AE1-329 and EP4AA ONO-AE3-208 were provided by Japanese ONO Pharmaceutical Co., Ltd. (Osaka, Japan).

Human CD34⁺ cell magnetic sorting. A total of 3-5 ml peripheral blood was extracted from allogeneic hematopoietic stem cell transplantation healthy donor following G-CSF mobilization. Following 3-5-fold dilution with PBS, the blood was treated with Ficoll-Paque fluid (1.077 g/ml; MP Biomedicals, Santa Ana, CA, USA) and centrifuged at 400 x g at 20°C for 30 min to separate mononuclear cells. Human CD34⁺ cells were sorted using CD34⁺ immunomagnetic bead kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), according to the manufacturer's protocol. Part of the CD34⁺ cells were collected for purity testing by flow cytometry (FCM; Beckman Coulter,

Fulterton, CA, USA) and the other part were used for the rest experiments.

BMMSC culture and identification. A total of 10 ml bone marrow fluid was collected and bone marrow mononuclear cells isolated using Ficoll-Paque liquid. The cells were subsequently resuspended in low glucose Dulbecco's modified Eagle's media (L-DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and cultured at 37°C containing 5% CO₂ of the atmosphere in a 10-cm dish. When adherent cells were >80% confluent, they were detached with 0.25% trypsin and 0.01% EDTA (Gibco; Thermo Fisher Scientific, Inc.) and subsequently replated at a dilution of 1:3. Passage 3 cells were used for immunophenotypic analysis, and osteogenic and adipogenic induction. FCM was applied to evaluate the expression of BMMSC surface antigens CD73, CD90, CD105, CD14, CD34, and CD45.

BMMSC suspension (1x10⁵; 100 μl) was added to each of 6 1.5 ml Eppendorf tubes. Each tube then received 10 μl phycoerythrin (PE) -conjugated anti-human CD73 antibody (PE-CD73; 550257; BD Biosciences, San Diego, CA, USA), 10 μl phycoerythrin cyanin 5 (PC5) -conjugated anti-human CD90 antibody (PC5-CD90; 561972; BD Biosciences), 10 μl PE-conjugated anti-human CD105 antibody (PE-CD105; 560839; BD Biosciences), 10 μl PE-conjugated anti-human CD34 antibody (PE-CD34; 550761; BD Biosciences), 10 μl fluorescein isothiocyanate (FITC)-conjugated anti-human CD14 antibody (FITC-CD14; 555397; BD Biosciences) and 10 μl PC5-conjugated anti-human CD45 antibody (PC5-CD45; 555484; BD Biosciences) respectively. The tubes were incubated in the dark and at room temperature for 30 min. The stained cells were washed twice with PBS and analyzed by FCM (CellQuest Pro; Beckman Coulter, Fulterton, CA, USA) at 488 nm. Formulated osteogenic and adipogenic induction medium (Cyagen, Santa Clara, CA, USA) was used, according to the manufacturer's protocol. The detached third passage cells were washed twice with PBS, and resuspended with osteogenic and adipogenic induction medium. The medium was changed every 3 days. After 21 days, the supernatant was discarded, and cells were stained for 30 min at room temperature with alizarin red for osteogenic evaluation and oil red O for adipogenic evaluation, and observed under an inverted microscope (magnifications, x4 and x10).

Human CD34⁺ cell treatment. The sorted human CD34⁺ cells were resuspended in RPMI 1640 (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) medium containing 10% FBS at a density of 5x10⁵/ml and divided into 6 groups, including the control [dimethyl sulfoxide (DMSO); 0.1%], the PGE2 group (1 μmol/l), the EP2A group (100 nmol/l), the EP2A (100 nmol/l) + EP2AA group (100 nmol/l), the EP4A group (100 nmol/l), and the EP4A (100 nmol/l) + EP4AA group (100 nmol/l). All the groups received 100 ng/ml recombinant human stem cell factor (rhSCF; PeproTech, Inc., Rocky Hill, NJ, USA). The cells were incubated on ice for 2 h, and then cultured at 37°C in an atmosphere containing 5% CO₂ for 22 h.

Western blotting of CXCR4. Human CD34⁺ cells were lysed on ice in radioimmunoprecipitation assay buffer [RIPA Lysis

Table I. Primer sequences used in the reverse transcription-polymerase chain reaction.

Gene	Primer sequence	
	Forward	Reverse
CXCR4	5'-CCTATGCAAGGCAGTCCATGT-3'	5'-GGTAGCGGTCCAGACTGATGA-3'
SDF-1a	5'-ACTGTGCCCTTCAGATTG-3'	5'-TTGGCTGTTGTGCTTACT-3'
GAPDH	5'-AAGGTGAAGGTCCGAGTCAAC-3'	5'-GGGGTCATTGATGGCAACAATA-3'

CXCR4, C-X-C chemokine receptor type 4; SDF-1a, stromal cell-derived factor 1 α .

Buffer (Medium; Beyotime Institute of Biotechnology, Shanghai, China) following the above treatment. The protein concentration was determined using the Bradford method. Equal amounts (30 μ g) of protein were subjected to SDS-PAGE on a 10% gel (the molecular weight CXCR4 is 40-47 kD so a 10% gel was selected) and transferred to a nitrocellulose membrane (EMD Millipore, Billerica, MA, USA). Membranes were incubated overnight at 4°C with primary antibody against CXCR4 (1:1,000; SC-9046; Santa Cruz Biotechnology, Inc.) and GAPDH (1:2,000; SC-47724; Santa Cruz Biotechnology, Inc.). Membranes were incubated at room temperature for 1 h with the horseradish peroxidase-conjugated immunoglobulin G secondary antibody (1:5,000; A32723; Pierce; Thermo Fisher Scientific, Inc.). Proteins were visualized using the Super Signal West Pico chemiluminescence kit (Pierce; Thermo Fisher Scientific, Inc.).

ELISA detection of SDF-1a levels secreted by BMMSCs. The 3rd generation BMMSCs were resuspended in L-DMEM containing 10% FBS at a density of 1×10^6 cells/ml and divided into six groups: Control group (DMSO; 0.1%); PGE2 group (1 μ mol/l); EP2A group (100 nmol/l); EP2A (100 nmol/l) + EP2AA group (100 nmol/l); EP4A group (100 nmol/l); and EP4A (100 nmol/l) + EP4AA group (100 nmol/l). All the above groups received rhSCF (100 ng/ml) and were cultured at 37°C in an atmosphere containing 5% CO₂ for 24 h. The supernatant was collected at 24 h, and the concentration of SDF-1a was detected using an ELISA kit (rhSDF-1a ELISA kit; DSA00; R&D Systems, Inc., Minneapolis, MN, USA), according to manufacturer's protocol.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) detection of CXCR4 and SDF-1a expression. Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed to cDNA using the Superscript[®] III Reverse Transcriptase (Thermo Fisher Scientific, Inc.) and oligo-dT primer. qPCR was performed on a Bio-Rad fluorescent quantitative PCR instrument using the Platinum[®] SYBR[®] Green qPCR SuperMix (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The thermocycling conditions were as follows: Initial denaturation at 95°C for 5 min; 45 cycles of denaturation at 95°C for 15 sec, annealing at 62°C for 30 sec and extension at 65°C for 5 sec. Fluorescence intensity in each cycle was monitored in real time. GAPDH was used as the endogenous control. The results

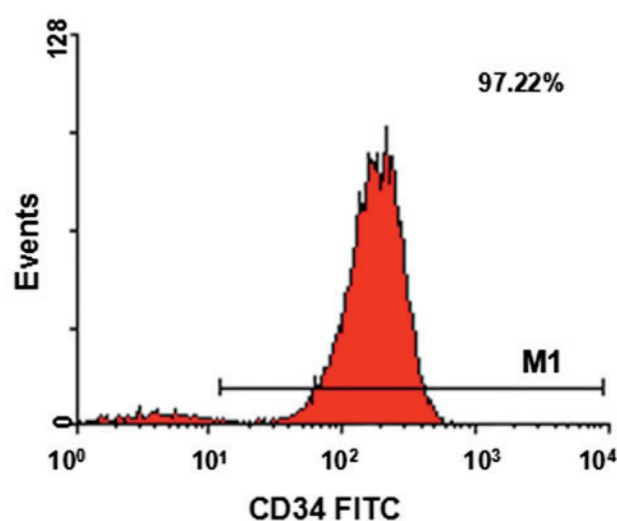


Figure 1. CD34⁺ cell purity flow cytometry assay. CD, cluster of differentiation; FITC, fluorescein isothiocyanate.

were analyzed using the 2^{- $\Delta\Delta$ C_q} method (19). The primer sequences are listed in Table I.

Chemotactic effect of rhSDF-1a on human CD34⁺ cells. The transwell assay was performed using transwell plates (3422; Corning Incorporated, Corning, NY, USA) to evaluate human CD34⁺ cell migration. A total of 400 μ l FBS-free medium containing 100 ng/ml rhSDF-1a (PeproTech) was added to the lower chamber. A total of 100 μ l medium containing 2×10^5 CD34⁺ cells/ml were added to the upper chamber following drugs treatment and twice washing in serum free medium. Plates were incubated for 4 h. The cells in the lower chamber were collected and fixed at room temperature with 4% paraformaldehyde for 1 h. FCM (CellQuest Pro software; Beckman Coulter, Fullerton, CA, USA) was used to count the CD34⁺ cells and thus calculate the migratory rate.

Chemotactic effect of BMMSCs on human CD34⁺ cells. 1×10^5 BMMSCs were seeded into the lower chamber, and treated with DMSO (control; concentration, 0.1%), PGE2 (1 μ mol/l), EP2A (100 nmol/l), EP2A (100 nmol/l) + EP2AA (100 nmol/l), EP4A (100 nmol/l) and EP4A (100 nmol/l) + EP4AA (100 nmol/l) for 24 h. Equal amounts (1×10^5) of CD34⁺ cells from the six treatment groups were added to the upper chamber, receiving the same treatment as the BMMSCs in lower chamber. Following

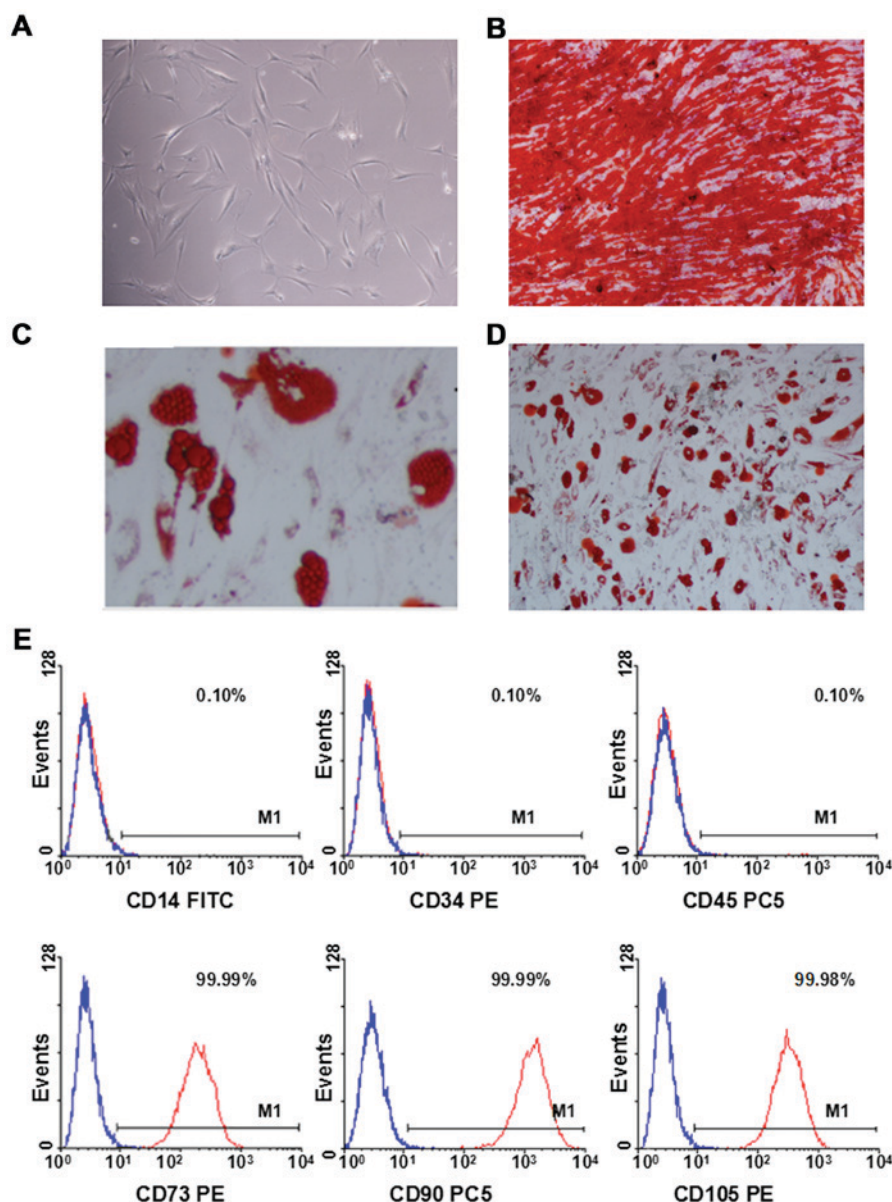


Figure 2. *In vitro* morphology, osteogenic and adipogenic differentiation, and immunophenotypic characterization of BMMSCs. (A) The morphology of BMMSCs (magnification, x10). (B) Osteogenic differentiation of BMMSCs (magnification, x4). (C) Adipogenic differentiation of BMMSCs (magnification, x10). (D) Adipogenic differentiation of BMMSCs (magnification, x4). (E) Immunophenotypic characterization of BMMSCs using flow cytometry. The expression of CD73, CD90 and CD105 is high in BMMSCs, while expression of CD14, CD34 and CD45 is absent. BMMSC, bone marrow mesenchymal stem cell; CD, cluster of differentiation; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PC5, phycoerythrin cyanin 5.

a 6 h incubation, the cells in the lower chamber were collected and fixed with 4% paraformaldehyde for 1 h. FCM was used to count the CD34⁺ cells to calculate migratory rate.

Statistical analysis. All statistical analysis was performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). Values are presented as the mean \pm standard deviation. The Paired t-test was used for data comparison. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Human CD34⁺ cell sorting and identification. Human CD34⁺ cells were isolated by magnetic beads and identified by FCM for purity, their purity was $>90\%$ (Fig. 1).

BMMSCs cultivation and identification. BMMSCs were isolated from healthy volunteers for cultivation. BMMSCs presented as fusiformis, spindle-shape, or polygon after 1 week (Fig. 2A). Subsequently, the cells were passaged for further purified cultivation. Osteogenic differentiation ability following treatment with osteogenesis-induction medium for 3 weeks was identified by staining with alizarin red. Red calcium nodules observed under the inverted microscope (Fig. 2B). These results suggested that the BMMSCs exhibited osteogenic-differentiation capabilities. The cells in the 3rd generation were treated with adipogenic-induction medium for 3 weeks and stained with oil red O. Red lipid droplets in grape-like clusters were observed under the inverted microscope (Fig. 2C and D). These results indicated that those cells exhibited the ability of adipogenic differentiation. The 3rd

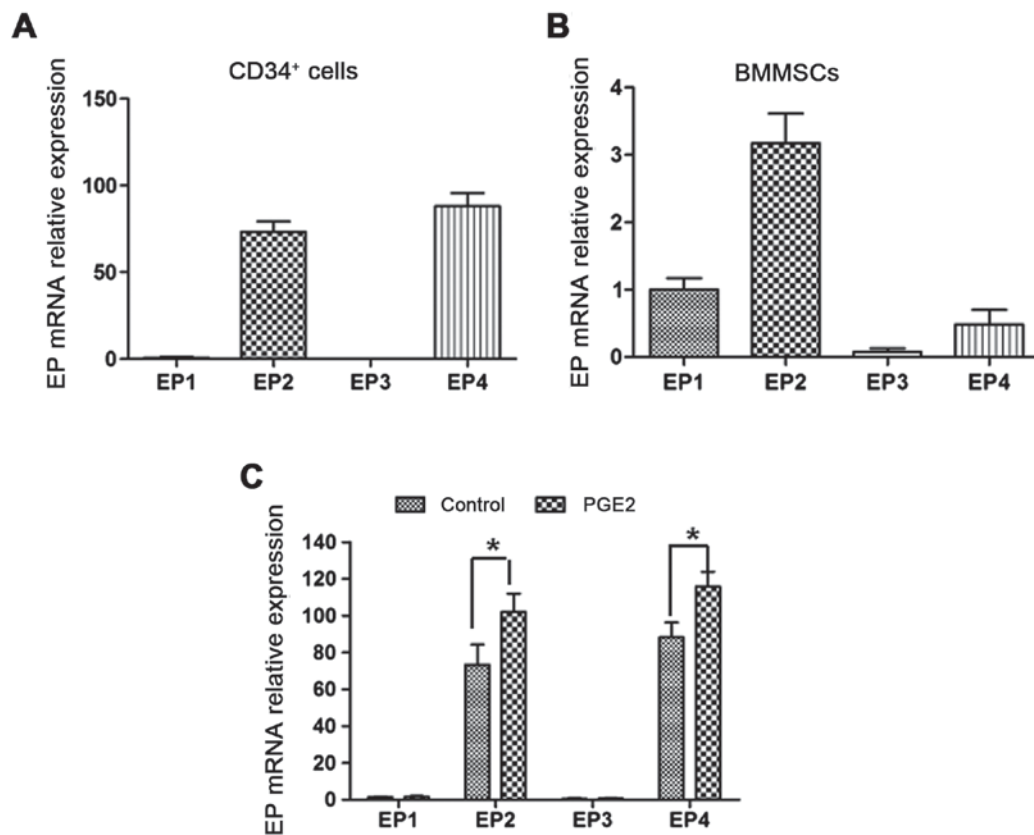


Figure 3. Expression of EP mRNA in human CD34⁺ cells and BMMSCs. (A) The expression of EP mRNA in human CD34⁺ cells. (B) The expression of EP mRNA in human BMMSCs. (C) The CD34⁺ cells were treated with PGE2 and EP mRNA expression was evaluated. Data are from three independent experiments *P<0.05. CD, cluster of differentiation; BMMSC, bone marrow mesenchymal stem cell; EP1, prostaglandin E2 receptor EP1 subtype; PGE2, prostaglandin E2.

generation cells were collected for FCM. Those cells highly expressed CD73, CD90 and CD105, while negative expressing CD14, CD34 and CD45 (Fig. 2E). These results revealed that BMMSCs present stem cell surface antigens and not hematopoietic cell surface antigen.

PGE2 receptors EP1-4 are expressed in human CD34⁺ cells and BMMSCs. RT-qPCR was applied to detect the four PGE2 receptor EP1-4 mRNA expression in human CD34⁺ cells and BMMSCs. The results demonstrated that human CD34⁺ cells and BMMSCs expressed mRNA of the four PGE2 receptors. CD34⁺ cells particularly expressed EP2 and 4 (Fig. 3A), while BMMSCs primarily expressed EP1, 2 and 4 (Fig. 3B).

PGE2 elevates EP2 and 4 mRNA expression in human CD34⁺ cells. Following treatment with PGE2, the level of EP2 and 4 mRNA in CD34⁺ cells was increased 1.44- (P=0.035) and 1.3-fold (P=0.029) compared with the control, respectively. EP1 and 3 mRNA levels did not exhibit a significant alteration (Fig. 3C). Therefore, PGE2 may serve a critical role in human CD34⁺ cells through EP2 and 4. Subsequently, EP2 and 4 were selected as the targets to investigate the effect of EPAs on human CD34⁺ cells.

PGE2 promotes CXCR4 mRNA expression in human CD34⁺ cells through EP2 and 4. RT-qPCR was performed to evaluate CXCR4 mRNA expression in human CD34⁺ cells. It was observed that compared with the control, CXCR4 mRNA

expression levels in the PGE2, EP2A and EP4A groups, were elevated 2.51- (P=0.016), 7.3- (P=0.0012) and 8.17-fold (P=0.0005), respectively. Following treatment with EP2AA (P=0.0022) or EP4AA (P=0.04), CXCR4 mRNA levels decreased significantly (Fig. 4A).

PGE2 promotes CXCR4 protein expression in human CD34⁺ cells through EP2 and 4. Western blotting was performed to test CXCR4 protein expression in human CD34⁺ cells. It was revealed that treatment of human CD34⁺ cells with PGE2, EP2A and EP4A increased the expression of CXCR4 protein. Following treatment with EP2AA or EP4AA, CXCR4 protein levels reduced markedly (Fig. 4B).

PGE2 promotes SDF-1a mRNA expression in BMMSCs through EP2 and 4. RT-qPCR was performed to test SDF-1a mRNA expression in human CD34⁺ cells. Compared with the control, the SDF-1a mRNA expression levels in the PGE2, EP2A and EP4A groups were increased 4.4- (P=0.0003), 1.72- (P=0.0158), and 2.32-fold (P=0.0003), respectively. Following treatment with EP2AA (P=0.0073) or EP4AA (P=0.0002), SDF-1a mRNA levels declined significantly (Fig. 5A).

PGE2 promotes the secretion of SDF-1a by BMMSCs through EP2 and 4. ELISA was applied to detect SDF-1a secretion in BMMSCs. The results demonstrated that treatment with PGE2, EP2A and EP4A increased SDF-1a protein secretion by 1.72- (P=0.021), 1.32- (P=0.03) and 1.43-fold (P=0.018),

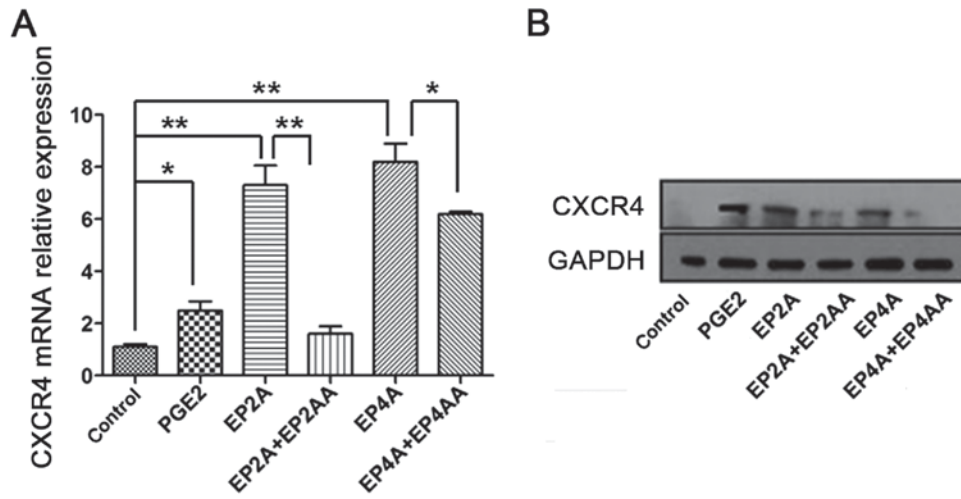


Figure 4. Expression of CXCR4 following treatment with EP2A and EP4A in CD34⁺ cells. (A) CXCR4 mRNA expression was evaluated in the six groups of CD34⁺ cells. (B) Western blotting of CXCR4 protein expression in the six groups of CD34⁺ cells. Data are from three independent experiments *P<0.05, **P<0.01. CXCR4, C-X-C chemokine receptor type 4; EP2A, prostaglandin E2 receptor EP2 subtype agonist; CD, cluster of differentiation; PGE2, prostaglandin E2; EP2AA, prostaglandin E2 receptor EP2 subtype antagonist.

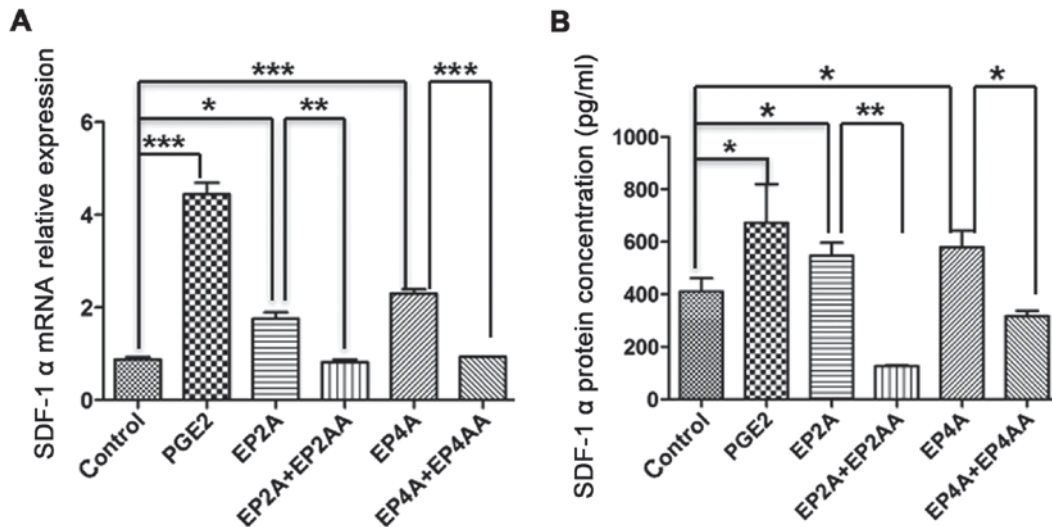


Figure 5. Expression of SDF-1α following treatment with EP2A and EP4A treatment in BMMSCs. (A) SDF-1α mRNA expression was evaluated in the six groups of human BMMSCs. (B) SDF-1α protein expression was evaluated in the six groups of human BMMSCs. Data are from three independent experiments. *P<0.05, **P<0.01, ***P<0.001. SDF-1α, stromal cell-derived factor 1α; EP2A, prostaglandin E2 receptor EP2 subtype agonist; BMMSC, bone marrow mesenchymal stem cell; PGE2, prostaglandin E2; EP2AA, prostaglandin E2 receptor EP2 subtype antagonist.

respectively. Conversely, EP2AA (P=0.009) and EP4AA (P=0.01) led to significantly decreased SDF-1α secretion (Fig. 5B).

PGE2 promotes human CD34⁺ cell migration towards high SDF-1α concentrations through EP2 and 4. Transwell assays demonstrated that treatment with PGE2, EP2A and EP4A enhanced human CD34⁺ cell migratory ability towards high SDF-1α concentrations. The migratory rate reached 71.9±3.52 (P=0.009), 62±0.58 (P=0.0178) and 64.2±2.77% (P=0.0234) in the PGE2, EP2A and EP4A groups, respectively, which was significantly increased compared with the control (48.4±3.45%). Following treatment with EP2AA or EP4AA, migratory ability decreased significantly to 51±2.94% (P=0.0228) and 52±2.02% (P=0.0236), respectively (Fig. 6A).

PGE2 promotes human CD34⁺ cell migration towards BMMSCs through EP2 and 4. Transwell assays demonstrated that treatment with PGE2, EP2A and EP4A increased human CD34⁺ cell migration towards BMMSCs. The migration rate reached 17.9±0.64, 20.3±2.367, and 21.2±1.27% in the PGE2 (P=0.0054), EP2A (P=0.041) and EP4A (P=0.0038) groups, respectively, which was significantly increased compared with the control. Following treatment with EP2AA or EP4AA, the migratory ability decreased significantly to 12.3±2.13 (P=0.0361) and 12.4±0.81% (P=0.0043), respectively (Fig. 6B).

Discussion

PGE2, a subset of the eicosanoid family, is involved in numerous physiological process, including stem cell development, inflammation and cancer (20). PGE2 exhibits a stable

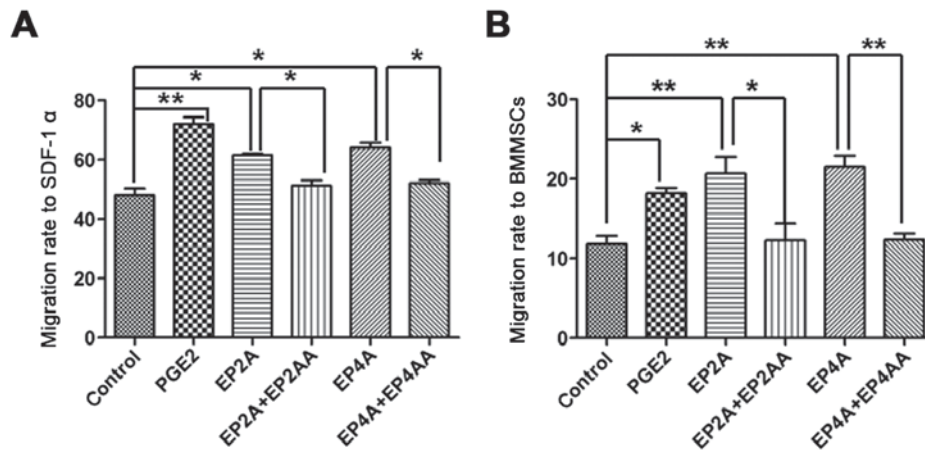


Figure 6. Migratory rate of the six groups of human cluster of differentiation 34⁺ cells towards (A) SDF-1 α and (B) BMMSCs. Data are from three independent experiments. *P<0.05, **P<0.01. SDF-1 α , stromal cell-derived factor 1 α ; EP2A, prostaglandin E2 receptor EP2 subtype agonist; BMMSC, bone marrow mesenchymal stem cell; PGE2, prostaglandin E2; EP2AA, prostaglandin E2 receptor EP2 subtype antagonist.

and direct hematopoietic regulatory ability (1,11,21-25). PGE2 promotes HSC homing following transplantation and prevents its dysfunction with clinical application prospects. PGE2 serves a role through four G-protein coupled receptors (EP1-4) on the cell surface (26,27). Different receptors mediated different physiological effects, and their expression levels varied between different tissues and organs. Aside from promoting human CD34⁺ cell homing, PGE2 may also induce other biological effects through other sub-receptors (28-34). Thus, the promoting effect of PGE2 on human CD34⁺ cells is nonspecific, and its direct application to patients may result in serious side effects. Therefore, it is necessary to investigate the sub-type of receptor that mediates the promoting effect of PGE2 on CD34⁺ cell homing.

PGE2 regulates rat hematopoietic stem/progenitor cells directly through the EP4 receptor and indirectly through stromal progenitor cells (35). PGE2 stimulates bone formation and prevents bone loss mediated by EP4 (36). Dendritic cells develop from hematopoietic progenitor cells through EP1 and 3 (37). However, few studies have investigated the sub-receptor which mediates PGE2-induced human HSC homing. In the present study, it was discovered by RT-qPCR that human peripheral blood CD34⁺ cells expressed the four receptors of PGE2, of which EP2 and 4 mRNA expression was significantly increased compared with that of EP1 and 3. Following treatment with PGE2 for 24 h, EP2 and 4 mRNA expression levels increased, while EP1 and EP3 mRNA exhibited no significant alteration. These results suggested that PGE2 may mediate its effect on human HSC through EP2 and 4. Specific EP2 and 4 agonists (EP2A and EP4A) and specific antagonists (EP2AA and EP4AA), were used to investigate the roles of EP2 and human CD34⁺ cell homing.

The CXCR4/SDF-1 α signaling axis serves a critical role in the process of HSC homing (38-40). CXCR4 is located on the surface of HSCs. Hoggatt and Pelus (21) reported that PGE2 markedly increased CXCR4 expression on the surface of mouse and human HSCs. The results of the present study also confirmed that PGE2 promotes CXCR4 mRNA and protein overexpression. It was observed that treatment with EP2A and EP4A upregulated CXCR4 expression in human CD34⁺ cells, while

treatment with EP2AA and EP4AA reduced CXCR4 expression. SDF-1 α is primarily synthesized and secreted by BMMSCs. It serves an important role in HSC proliferation, mobilization and hematopoietic reconstruction following HSCT (41-43), and provides directional power for HSCs migration. The present study revealed that, similar to treatment with PGE2, EP2A and EP4A also enhanced the chemotactic ability of human CD34⁺ cells towards SDF-1 α . PGE2, EP2A and EP4A enhanced human CD34⁺ cell migration towards BMMSCs, while EP2AA and EP4AA reduced human CD34⁺ cell migration towards BMMSCs. Further investigation demonstrated that PGE2, EP2A and EP4A promoted BMMSC secretion of SDF-1 α , whereas EP2AA and EP4AA treatment reduced SDF-1 α secretion.

In conclusion, PGE2 promoted human CD34⁺ cell homing through EP2 and 4, which was primarily associated with the effect of EP2 and 4 on CXCR4 expression on the surface of human CD34⁺ cells and on the ability of BMMSCs to secrete SDF-1 α . The results of the present study highlighted the importance of EP2 and 4 in HSC homing, and may provide novel evidence for the clinical application of HSCT.

Acknowledgements

The authors of the present study would like to thank the members of the Quentin Qiang Liu Laboratory (Guangzhou, China) for their technical support and critical comments. The authors of the present study would also like to thank Japanese ONO Pharmaceutical Co., Ltd. (Osaka, Japan) for providing the ONO-AE1-259, ONO-AE1-329 and ONO-AE3-208. The present study was supported by the National Natural Science Foundation of China (grant no. 81370663; to D. X.), and Science and Technology Planning Project of Guangdong Province, China (grant no. 2013B021800239; to D. X.).

References

1. Lord AM, North TE and Zon LI: Prostaglandin E2: Making more of your marrow. *Cell Cycle* 6: 3054-3057, 2007.
2. Ratajczak MZ and Suszynska M: Emerging strategies to enhance homing and engraftment of hematopoietic stem cells. *Stem Cell Rev* 12: 121-128, 2016.

3. Sharma M, Afrin F, Tripathi RP and Gangenahalli G: Transgene expression study of CXCR4 active mutants. Potential prospects in up-modulation of homing and engraftment efficiency of hematopoietic stem/progenitor cells. *Cell Adh Migr* 8: 384-388, 2014.
4. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, *et al*: Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 283: 845-848, 1999.
5. Bao L and Huang XJ: SDF-1/CXCR4 and multiple myeloma osteolytic bone lesions-review. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 16: 442-446, 2008 (In Chinese).
6. Okada K, Kawao N, Yano M, Tamura Y, Kurashimo S, Okumoto K, Kojima K and Kaji H: Stromal cell-derived factor-1 mediates changes of bone marrow stem cells during bone repair process. *Am J Physiol Endocrinol Metab* 310: E15-E23, 2016.
7. Miller SB: Prostaglandins in health and disease: An overview. *Semin Arthritis Rheum* 36: 37-49, 2006.
8. Wiemer AJ, Hegde S, Gumperz JE and Huttenlocher A: A live imaging cell motility screen identifies prostaglandin E2 as a T cell stop signal antagonist. *J Immunol* 187: 3663-3670, 2011.
9. Tsutsumi R, Xie C, Wei X, Zhang M, Zhang X, Flick LM, Schwarz EM and O'Keefe RJ: PGE2 signaling through the EP4 receptor on fibroblasts upregulates RANKL and stimulates osteolysis. *J Bone Miner Res* 24: 1753-1762, 2009.
10. Fehér I and Gidáli J: Prostaglandin E2 as stimulator of haemopoietic stem cell proliferation. *Nature* 247: 550-551, 1974.
11. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, *et al*: Prostaglandin E2 regulates vertebrate hematopoietic stem cell homeostasis. *Nature* 447: 1007-1011, 2007.
12. Pelus LM and Hoggatt J: Pleiotropic effects of prostaglandin E2 in hematopoiesis; prostaglandin E2 and other eicosanoids regulate hematopoietic stem and progenitor cell function. *Prostaglandins Other Lipid Mediat* 96: 3-9, 2011.
13. Hoggatt J, Singh P, Sampath J and Pelus LM: Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 113: 5444-5455, 2009.
14. Sugimoto Y and Narumiya S: Prostaglandin E receptors. *J Biol Chem* 282: 11613-11617, 2007.
15. Kawamori T, Uchiya N, Nakatsugi S, Watanabe K, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Sugimura T and Wakabayashi K: Chemopreventive effects of ONO-8711, a selective prostaglandin E receptor EP(1), on breast cancer development. *Carcinogenesis* 22: 2001-2004, 2001.
16. Kawamori T, Uchiya N, Kitamura T, Ohuchida S, Yamamoto H, Maruyama T, Sugimura T and Wakabayashi K: Evaluation of a selective prostaglandin E receptor EP1 antagonist for potential properties in colon carcinogenesis. *Anticancer Res* 21: 3865-3869, 2001.
17. Yanochko GM, Affolter T, Eighmy JJ, Evans MG, Khoh-Reiter S, Lee D, Miller PE, Shiue MH, Trajkovic D and Jessen BA: Investigation of ocular events associated with taprenepag isopropyl, a topical EP2 agonist in development for treatment of glaucoma. *J Ocul Pharmacol Ther* 30: 429-439, 2014.
18. Birrell MA, Maher SA, Dekkak B, Jones V, Wong S, Brook P and Belvisi MG: Anti-inflammatory effects of PGE2 in the lung: Role of the EP4 receptor subtype. *Thorax* 70: 740-747, 2015.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
20. Logan CM, Giordano A, Puca A and Cassone M: Prostaglandin E2: At the crossroads between stem cell development, inflammation and cancer. *Cancer Biol Ther* 6: 1517-1520, 2007.
21. Hoggatt J and Pelus LM: Eicosanoid regulation of hematopoiesis and hematopoietic stem and progenitor trafficking. *Leukemia* 24: 1993-2002, 2010.
22. Gori JL, Chandrasekaran D, Kowalski JP, Adair JE, Beard BC, D'Souza SL and Kiem HP: Efficient generation, purification, and expansion of CD34(+) hematopoietic progenitor cells from nonhuman primate-induced pluripotent stem cells. *Blood* 120: e35-e44, 2012.
23. Goessling W, Allen RS, Guan X, Jin P, Uchida N, Dovey M, Harris JM, Metzger ME, Bonifacino AC, Stroncek D, *et al*: Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. *Cell Stem Cell* 8: 445-458, 2011.
24. Durand EM and Zon LI: Newly emerging roles for prostaglandin E2 regulation of hematopoiesis and hematopoietic stem cell engraftment. *Curr Opin Hematol* 17: 308-312, 2010.
25. Inman MD, Denburg JA, Ellis R, Dahlbäck M and O'Byrne PM: The effect of treatment with budesonide or PGE2 in vitro on allergen-induced increases in canine bone marrow progenitors. *Am J Respir Cell Mol Biol* 17: 634-641, 1997.
26. Ruan D and So SP: Prostaglandin E2 produced by inducible COX-2 and mPGES-1 promoting cancer cell proliferation in vitro and in vivo. *Life Sci* 116: 43-50, 2014.
27. Kawahara K, Hohjoh H, Inazumi T, Tsuchiya S and Sugimoto Y: Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. *Biochim Biophys Acta* 1851: 414-421, 2015.
28. Kosuge Y, Miyagishi H, Shinomiya T, Nishiyama K, Suzuki S, Osada N, Ishige K, Okubo M, Kawaguchi M and Ito Y: Characterization of motor neuron prostaglandin E2 EP3 receptor isoform in a mouse model of amyotrophic lateral sclerosis. *Biol Pharm Bull* 38: 1964-1968, 2015.
29. O'Callaghan G and Houston A: Prostaglandin E2 and the EP receptors in malignancy: Possible therapeutic targets? *Br J Pharmacol* 172: 5239-5250, 2015.
30. Nasrallah R, Hassouneh R, Zimpelmann J, Karam AJ, Thibodeau JF, Burger D, Burns KD, Kennedy CR and Hébert RL: Prostaglandin E2 increases proximal tubule fluid reabsorption, and modulates cultured proximal tubule cell responses via EP1 and EP4 receptors. *Lab Invest* 95: 1044-1055, 2015.
31. Säfholm J, Manson ML, Bood J, Delin I, Orre AC, Bergman P, Al-Ameri M, Dahlén SE and Adner M: Prostaglandin E2 inhibits mast cell-dependent bronchoconstriction in human small airways through the E prostanoid subtype 2 receptor. *J Allergy Clin Immunol* 136: 1232-1239.e1, 2015.
32. Mo C, Zhao R, Vallejo J, Igwe O, Bonewald L, Wetmore L and Brotto M: Prostaglandin E2 promotes proliferation of skeletal muscle myoblasts via EP4 receptor activation. *Cell Cycle* 14: 1507-1516, 2015.
33. von der Emde L, Goltz D, Latz S, Müller SC, Kristiansen G, Ellinger J and Syring I: Prostaglandin receptors EP1-4 as a potential marker for clinical outcome in urothelial bladder cancer. *Am J Cancer Res* 4: 952-962, 2014.
34. Maślanka T, Spodniewska A, Barski D, Jasička A, Zuśka-Prot M, Ziółkowski H, Markiewicz W and Jaroszewski JJ: Prostaglandin E2 down-regulates the expression of CD25 on bovine T cells, and this effect is mediated through the EP4 receptor. *Vet Immunol Immunopathol* 160: 192-200, 2014.
35. kushima YM, Arai F, Hosokawa K, Toyama H, Takubo K, Furuyashiki T, Narumiya S and Suda T: Prostaglandin E(2) regulates murine hematopoietic stem/progenitor cells directly via EP4 receptor and indirectly through mesenchymal progenitor cells. *Blood* 121: 1995-2007, 2013.
36. Yoshida K, Oida H, Kobayashi T, Maruyama T, Tanaka M, Katayama T, Yamaguchi K, Segi E, Tsuboyama T, Matsushita M, *et al*: Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. *Proc Natl Acad Sci USA* 99: 4580-4585, 2002.
37. Singh P, Hoggatt J, Hu P, Speth JM, Fukuda S, Breyer RM and Pelus LM: Blockade of prostaglandin E2 signaling through EP1 and EP3 receptors attenuates Flt3L-dependent dendritic cell development from hematopoietic progenitor cells. *Blood* 119: 1671-1682, 2012.
38. Lapidot T, Dar A and Kollet O: How do stem cells find their way home? *Blood* 106: 1901-1910, 2005.
39. Marquez-Curtis LA, Turner AR, Larratt LM, Letcher B, Lee SF and Janowska-Wieczorek A: CD34+ cell responsiveness to stromal cell-derived factor-1alpha underlies rate of engraftment after peripheral blood stem cell transplantation. *Transfusion* 49: 161-169, 2009.
40. Jung Y, Wang J, Schneider A, Sun YX, Koh-Paige AJ, Osman NI, McCauley LK and Taichman RS: Regulation of SDF-1 (CXCL12) production by osteoblasts; a possible mechanism for stem cell homing. *Bone* 38: 497-508, 2006.
41. Goessling W, North TE, Loewer S, Lord AM, Lee S, Stoick-Cooper CL, Weidinger G, Puder M, Daley GQ, Moon RT and Zon LI: Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. *Cell* 136: 1136-1147, 2009.
42. Regan JW: EP2 and EP4 prostanoid receptor signaling. *Life Sci* 74: 143-153, 2003.
43. Wang D, Mann JR and DuBois RN: WNT and cyclooxygenase-2 cross-talk accelerates adenoma growth. *Cell Cycle* 3: 1512-1515, 2004.