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PDGFBB promotes PDGFRα-positive cell migration into artificial bone *in vivo*

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ABSTRACT

Bone defects caused by traumatic bone loss or tumor dissection are now treated with auto- or allo-bone graft, and also occasionally by artificial bone transplantation, particularly in the case of large bone defects. However, artificial bones often exhibit poor affinity to host bones followed by bony union failure. Thus therapies combining artificial bones with growth factors have been sought. Here we report that platelet derived growth factor bb (PDGFBB) promotes a significant increase in migration of PDGF receptor α (PDGFRα)-positive mesenchymal stem cells/pre-osteoblastic cells into artificial bone *in vivo*. Growth factors such as transforming growth factor beta (TGFβ) and hepatocyte growth factor (HGF) reportedly inhibit osteoblast differentiation; however, PDGFBB did not exhibit such inhibitory effects and in fact stimulated osteoblast differentiation *in vitro*, suggesting that combining artificial bones with PDGFBB treatment could promote host cell migration into artificial bones without inhibiting osteoblastogenesis.

1. Introduction

Large bone defects are seen in patients with high-impact traumas such as traffic accidents or falls from high places, or who have undergone dissection surgery for malignant bone tumors. Large bone defects are usually treated with bone auto- or allo-grafts or by transplantation with artificial bone materials including hydroxyapatite and beta tricalcium phosphate (βTCP) [1,2]. Auto-graft bones are usually provided from iliac crests of patients themselves. Auto-bone grafts have advantages compared to other methods in preventing known/unknown infection and show increased bony fusion because they are prepared from fresh bone without sterilization [3]. Meanwhile, limited bone volume, additional surgery required for bone collection, and occasional trauma to iliac crests are considered disadvantages to use of auto-bone grafts compared with allo-grafts or artificial bone transplantation methods [4]. However, pre-collection, sterilization and freeze storage are required for bone allo-grafts, such that these materials are not always supplied on time and several hospitals cannot perform this type of transplantation. In artificial bones, graft bone volume can be increased as required without a specific preparation technique; however, union of transplanted artificial bones with host bones occasionally fails since artificial bones do not contain cells.

To overcome poor affinity of transplanted artificial bones with hosts, growth factors such as transforming growth factor beta (TGFβ) and hepatocyte growth factor (HGF) have been tested [5]. Osteoblasts abundantly express TGFβ, which is deposited to bone extracellular matrix protein and reportedly functions in osteoblastogenesis followed by bone resorption by osteoclasts [6]. HGF was originally identified as a protein secreted from hepatocytes [7,8], but it is now known to be produced by tissues such as liver, kidney and lung [9–11]. HGF is reportedly produced at the site of bone fracture repair [12]. Thus both TGFβ and HGF support bone formation, but both strongly inhibit osteoblast differentiation [5,13,14]. Thus factors that enhance transplanted artificial bone affinity to hosts without inhibiting osteoblastogenesis have been sought.

PDGFBB is mainly produced by platelets and is implicated in tissue repair such as fracture repair [15]. PDGF consists A, B, C and D isoforms and forms homo or hetero dimers such as PDGFAA or

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PDGFAB [15]. Among them, PDGFBB exhibits the strongest activity [15] and has been approved by the FDA to treat bone patients with bone defects in oral and maxillofacial regions [16–19]. The PDGF receptor (PDGFR) consists two isoforms α and β and also forms homo or hetero dimers, such as PDGFRα/α, α/β and β/β [15]. PDGFRα is reportedly expressed in mesenchymal stem cells and osteoblast progenitor cells, and PDGFRα-positive cells exhibit high osteoblast differentiation capacity [20].

In this study we demonstrate that PDGFBB effectively promotes PDGFRα-positive cell migration into transplanted artificial bones *in vivo*. PDGFBB did not inhibit *in vitro* osteoblastogenesis, suggesting that it could serve as a useful factor to increase artificial bone affinity to hosts without inhibiting osteoblast differentiation.

2. Materials and methods

2.1. Recombinant proteins and antibodies

Recombinant mouse (rm) HGF, rmFGF2, recombinant human (rh) TGF-b1 and rhPDGFBB were purchased from R&D Systems (Minneapolis, MN, USA). RhBMP-2 was purchased from Pepro tech ltd (Rocky Hill, NJ USA).

Antibodies detecting phospho-ERK1/2, total-ERK1/2, phospho-p38 (Thr180/Tyr182), total-p38, phospho-SAPK/JNK (Thr183/Tyr185) and total-SAPK/JNK were purchased from Cell Signaling Technology (Beverly, MA, USA). Goat anti-rabbit IgG antibody conjugated with horseradish peroxidase (HRP) was purchased from Invitrogen (Carlsbad, CA, USA). Anti-actin antibody and anti-PDGFR-α polyclonal antibody was purchased from Sigma–Aldrich (St. Louis, Missouri, USA), and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), respectively. Alexa Fluor 488-conjugated anti-rabbit IgG antibody and 4′,6-Diamidino-2-phenylindole, dihydrochloride (DAPI) were purchased from Invitrogen and Dojindo (Tokyo, Japan), respectively.

2.2. In vitro MC3T3E1 cell culture

The mouse osteoblastic line MC3T3-E1 was maintained in α-MEM (Sigma–Aldrich) containing 10% heat-inactivated fetal bovine serum (FBS) (JRH Biosciences). Cells were seeded in 96-well tissue culture plates at a density of 1×10^4 cells/well. On achieving confluence, cells were cultured in α-MEM containing 10% FBS in the presence or absence of BMP-2 (300 ng/ml) (Pepro tech) and with or without PDGFBB (10 ng/ml), HGF (10 ng/ml), FGF2 (10 ng/ml) and TGF-β1 (10 ng/ml) for 72 h. Cells were then subjected to real-time PCR.

2.3. RNA isolation and real-time RT-PCR

Total RNA was isolated from MC3T3E1 cells and freshly isolated mouse peripheral blood cells using the RNeasy mini kit (Qiagen GmbH, Hilden, Germany). First strand cDNA was prepared using a Prime Script RT reagent Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instruction. Realtime PCR was performed with a Thermal Cycler Dice Real-Time System using SYBR Premix Ex Taq (Takara Bio). Duplicate reactions were performed for each sample, and relative mRNA expression levels were normalized to β-*actin* mRNA expression. Gene-specific forward and reverse primers were as follows:

ALP-forward; 5′-ACACCTTGACTGTGGTTACTGCTGA-3′
ALP-reverse; 3′-CCTTGTAGCCAGGCCCGTTA-5′
Runx2-forward; 5′-GACGTGCCCAGGCGTATTTC-3′
Runx2-reverse; 3′-AAGGTGGCTGGGTAGTGCATTC-5′
Osteocalcin (OCN)-forward; 5′-CTTGGGTTCTGACTGGGTGT-3′
Osteocalcin (OCN)-reverse; 3′-TGGCCACTTACCCAAGGTAG-5′
Osterix (OSX)-forward; 5′-ACTGGCTAGGTGGTGGTCAG-3′
Osterix (OSX)-reverse; 5′-GGTAGGGAGCTGGGTTAAGG-3′
PDGFbb-forward; 5′-CCTGAGGAACTGTATGAAATGCT-3′
PDGFbb-reverse; 5′-GTCATGTTCAAGTCCAGCTCAG-3′
β-actin-forward; 5′-TGAGAGGGAAATCGTGCGTGAC-3′
β-actin-reverse; 5′- AAGAAGGAAGGCTGGAAAAGAG -3′

2.4. Proliferation assay

MC3T3-E1 cell proliferation was analyzed using a cell counting kit-8 (CCK-8; Dojindo, Tokyo, Japan). MC3T3-E1 cells were cultured in 96-well plates in α-MEM containing 10% heat-inactivated FBS with or without PDGFBB (25 ng/ml) for 0, 6, 12, 24, and 48 h. The CCK-8 reagent was added at each time point and absorbance at 450 nm was determined using a microplate reader. α-MEM containing 10% heat-inactivated FBS without PDGFBB was used as a control.

2.5. Western blot

MC3T3E1 cells were cultured in 35 mm dishes in α-MEM containing 10% FBS. On achieving confluence, cells were cultured in serum free medium (Opti-MEM, Invitrogen) for 5 h to initiate starvation. Cells were then cultured in Opti-MEM with or without 10 ng/ml PDGFBB for 0, 5, 10, 30 or 60 min. Cell lysates were made in RIPA buffer (50 mM Tris–HCl, pH7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, 1 mM Na3VO4, and a protease inhibitor cocktail; Sigma–Aldrich). Cell lysates mixed with loading buffer [tris–Glycine SDS Sample Buffer (Invitrogen) with 5% 2-mercaptoethanol (2-ME)] were loaded onto 15% polyacrylamide gels and electrophoresed in SDS–PAGE. Proteins were transferred to PVDF membranes (Nihon Millipore, Yonezawa, Japan) and blotted with antibodies against phospho ERK1/2, total ERK1/2, pp38, total p38, pJNK, and total JNK followed by HRP-conjugated goat antirabbit IgG. Proteins were visualized using ECL Western Blotting Detection Reagents (GE Healthcare, Uppsala, Sweden).

2.6. βTCP implantation into mouse hind paw muscle

Eight-week-old female C57BL/6 J mice were purchased from Japan SLC (Shizuoka, Japan). All experiments were performed according to protocols approved by the Laboratory Animal Care and Use Committee of Keio University. Mice under general anesthesia with xylazine and pentobarbital were administered beta-Tricalcium phosphate (βTCP) (OSferion, 3 mm in diameter, 5 mm in thickness provided by Olympus Terumo Biomaterials Corporation (Tokyo, Japan). βTCP material was impregnated with or without 2 ug PDGFBB, followed by implantation into hamstring muscles of mouse hind paws as described [5]. At 3 and 7 days post-implantation, mice were euthanized by cervical dislocation, and βTCP cubes were removed by dissection and examined histologically. To do so, samples were fixed in 10% formalin, embedded in paraffin, cut in 5-μm-thick serial paraffin, and stained with hematoxylin and eosin (HE) or anti-PDGFRα antibody plus DAPI as described below.

2.7. Immunohistochemical analysis

Implanted βTCP that had been decalcified was stained with goat anti–PDGFRα antibody (C-20; Santa Cruz Biotechnology, INC) followed by Alexa Fluor 488-conjugated anti-rabbit IgG antibody (Invitrogen) and DAPI (Dojindo) to detect nuclei. Samples were observed under a fluorescence microscope (BZ-9000; Keyence Co., Japan). PDGFRα-positive cells on the βTCP surface were measured by OsteoMeasure XP ver 1.01 (OsteoMetrics INC, Decatur, GA USA).

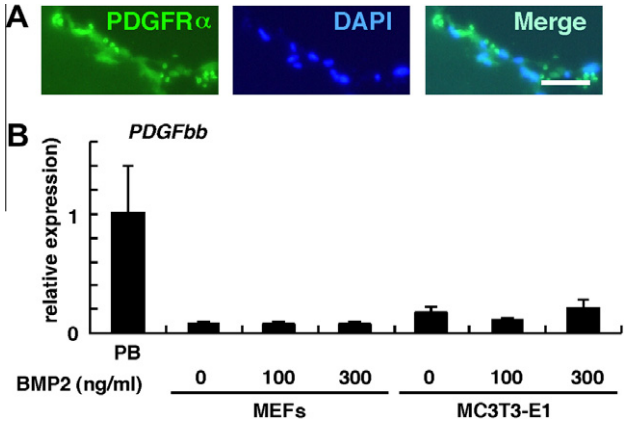

Fig. 1. PDGFRα-positive cell migration into βTCP. (A) A 3 mm diameter x 5 mm height cylindrical βTCP cube was implanted in hamstring muscles of mouse hind paws. Seven days later, the cube was removed and cells that had migrated into the βTCP were stained with anti-PDGFRα-antibody and DAPI. DAPI served as a nuclear stain. Bar = 50 μm. (B) Mouse embryonic fibroblasts (MEFs) or MC3T3-E1 cells were treated with or without BMP2 (100 or 300 ng/ml) for 3 days. RNA was extracted from cultured cells and freshly isolated mouse peripheral blood cells (PBs), and *PDGFbb* expression was analyzed. Data are means of *PDGFbb*/β-*actin* expression ± SD in MEFs or MC3T3-E1 cells relative to that seen in PB.

2.8. Statistical analysis

All data are provided as means ± standard deviation (SD). Statistical analyses were performed using Student's *t*-test (for paired samples). Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. PDGFRα-positive cells migrate into βTCP

An artificial bone material, βTCP, was transplanted into the femoral hamstring in mice, and one week later transplanted βTCP was collected and examined histologically to determine whether cells had migrated into the material (Fig. 1A). Cells that had migrated into βTCP expressed high levels of PDGFRα (Fig. 1B), a marker of mesenchymal stem cells and osteoblast progenitor cells [20]. Its ligand PDGFBB was highly expressed in peripheral blood cells, but PDGFBB expression in osteoblasts was significantly lower than that seen in peripheral blood cells (Fig. 1C). This results suggest that PDGFBB is not produced by osteoblasts themselves during cell migration into βTCP.

3.2. PDGFBB treatment stimulates PDGFα-positive cell migration into βTCP in vivo

Since PDGFBB is not produced by osteoblasts themselves, we added recombinant PDGFBB in βTCP to enhance PDGFα-positive cell migration into βTCP. PDGFBB reportedly promotes chemotaxis of vascular smooth muscle cells and osteoblasts *in vitro* [21,22]; however, its effect on osteoblast migration *in vivo* has not been demonstrated. To determine whether PDGFBB stimulates PDGFα-positive cell migration into βTCP, βTCP was transplanted with PDGFBB or vehicle (Fig. 2). PDGFBB treatment increased PDGFα-positive cell migration into βTCP at an early stage of transplantation compared with vehicle-containing βTCP (Fig. 2A). Increased cell migration was also detected by H/E staining of βTCP (Fig. 2B).

3.3. PDGFBB activates ERK but does not enhance osteoblast proliferation

The receptor tyrosine kinase PDGFRα signals to mitogen activated protein kinase (MAPK). Indeed, extracellular signal-regulated kinase (ERK), a member of the MAPK family, was strongly activated in osteoblasts following PDGFBB stimulation (Fig. 3A). In contrast, p38 and JNK were weakly and not activated by PDGFBB, respectively, in osteoblasts (Fig. 3). MAPK activation, particularly that of ERK, is known to promote cell proliferation; however, osteoblast proliferation was not stimulated by PDGFBB treatment (Fig. 3B),

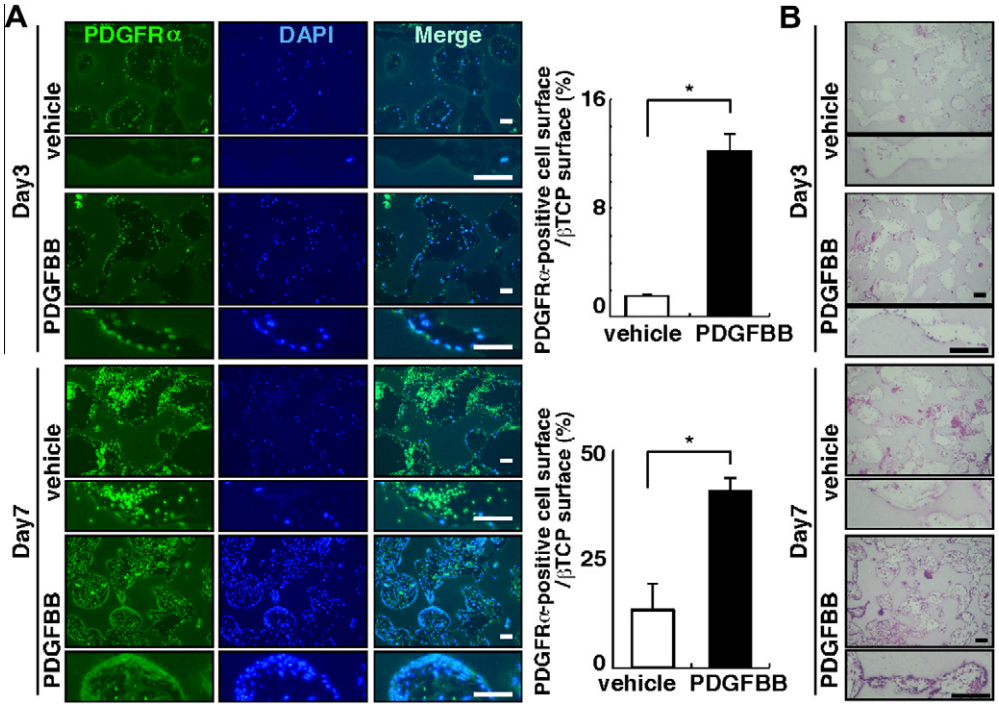

Fig. 2. PDGFRα-positive cell migration in hamstring muscles of mouse hind paws is stimulated by PDGFbb. (A) Migration of PDGFRα-positive cells was analyzed by immunofluorescence for PDGFRα at 3 and 7 days after implantation of βTCP material treated with or without PDGFBB. Nuclei were visualized using DAPI. Data are means of PDGFRα-positive cell surface per βTCP (%) ± SD (*$p < 0.01$). Bar = 50 μm. (B) Cell migration was histologically analyzed by hematoxylin and eosin staining at 3 and 7 days after implantation of cylindrical βTCP. Bar = 100 μm.

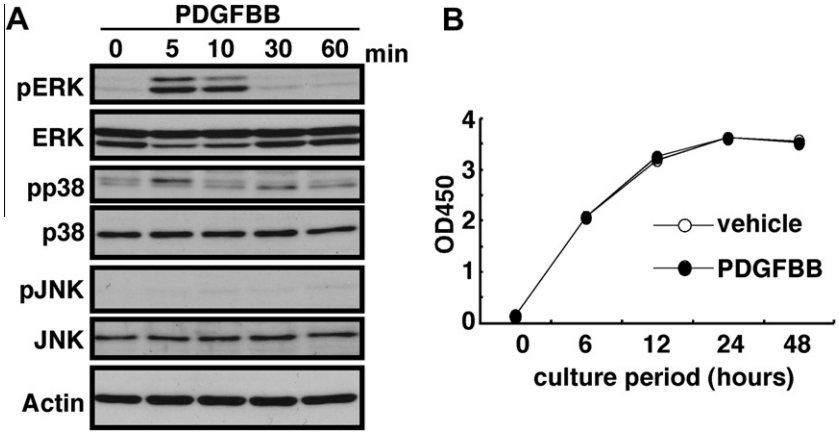

Fig. 3. Among MAPKs, ERK is specifically activated in osteoblasts but does not function in osteoblast proliferation. (A) Whole-cell lysates from MC3T3E1 cells treated with or without 10 ng/ml PDGFBB for 0, 5, 10, 30, and 60 min were analyzed by immunoblotting to detect phospho-ERK (pERK, total ERK, phospho-p38 (pp38), total p38, phospho-JNK and total JNK. Actin served as internal control. (B) MC3T3E1 cells were cultured with or without 10 ng/ml of PDGFBB for 0, 6, 12, 24 or 48 h, and cellular proliferation at each time point was measured using a CCK-8 cell counting kit.

suggesting that the increased number of PDGFRα–positive cells in βTCP seen following PDGFBB treatment is likely due to increased cell migration rather than proliferation.

3.4. PDGFBB does not inhibit osteoblast differentiation

Elevated ERK activity in osteoblasts reportedly inhibits osteoblastogenesis [5]. Thus we analyzed osteoblast differentiation induced by BMP2 in the presence or absence of PDGFBB (Fig. 4). Expression of alkaline phosphatase (ALP) and osteoclacin (OCN), both markers of osteoblast differentiation, and runt-related transcription factor 2 (Runx2) and osterix (Osx), both of which are transcription factors essential for osteoblast differentiation [23–25], induced by bone morphogenetic protein 2 (BMP2), was analyzed to evaluate osteoblast differentiation. We found that the expression of these factors was not inhibited but rather upregulated by PDGFBB treatment (Fig. 4), suggesting that PDGFBB promotes PDGFα-positive cell migration without inhibiting osteoblastogenesis. TGFβ, HGF and FGF2 have been implicated as growth factors promoting osteoblast differentiation or increasing bone mass; however, treatment of osteoblasts with these factors had very mild or even inhibitory effects osteoblastogenesis compared with PDGFBB (Fig. 4).

Bone grafting is now carried out in about 200,000 cases per year in the USA [26], and grafting efficacy greatly impacts patients' daily lives. Thus increasing graft efficacy is a critical issue for patients. BMP2 is a powerful bone-forming factor first identified from decalcified bone matrix protein [27,28]. However, BMP2 is reportedly less effective in promoting bone formation in humans than in rodents: high levels of BMP2 protein are required for effective bone formation in human patients [29]. High doses of BMP2 reportedly promote both osteoclast differentiation directly and vascular permeabilization due to local inflammation [29]. Thus, BMP2 treatment is currently disadvantageous due to cost and vascular problems. In the USA PDGFBB was approved by the FDA in 2005 to treat maxillofacial bone defects and reportedly increases radiographic bone formation in patients [16–19]. However, histological and mechanistic analysis of bone formation promoted by PDGFBB *in vivo* has not been fully characterized. In this study, we provide evidence that PDGFBB promotes PDGFα-positive cell migration into artificial bones without inhibiting osteoblastogenesis. PDGFBB is not expressed in osteoblasts themselves, and thus combining artificial bones with PDGFBB could prove useful to promote infiltration of host cells into artificial bones without inhibiting osteoblast differentiation.

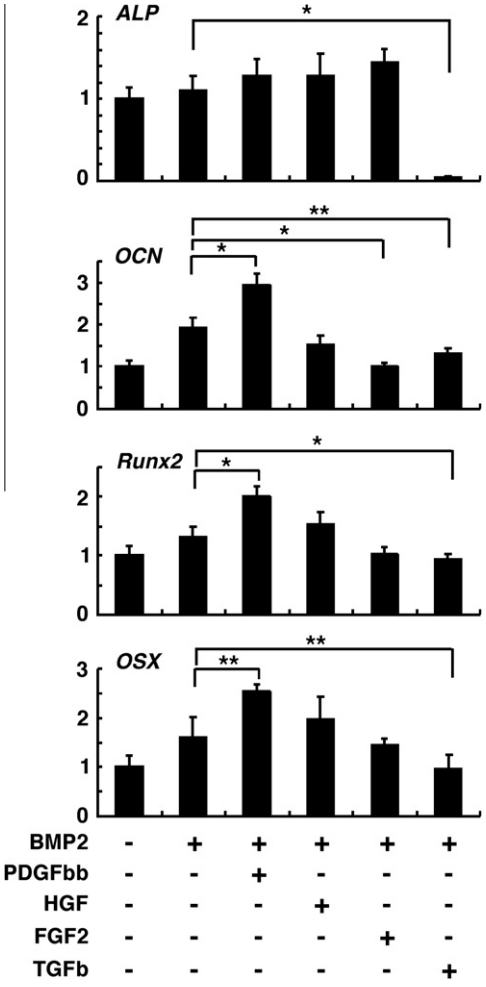

Fig. 4. PDGFBB does not inhibit osteoblast differentiation. MC3T3E1 cells were cultured in the presence or absence of 300 ng/ml BMP2 with or without PDGFBB (10 ng/ml), HGF (10 ng/ml), FGF2 (10 ng/ml) or TGF-β1 (10 ng/ml) for 2 days. Total RNA was then prepared from cultured cells and analyzed using quantitative real-time PCR. Data are normalized to *β-actin* mRNA expression in each sample and represent means ± SD of *ALP*/*β-actin*, *OCN*/*β-actin*, *Runx2*/*β-actin* or *OSX*/*β-actin* levels ($^{*}p < 0.01$, $^{**}p < 0.05$).

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References

[1] R. Petit, The use of hydroxyapatite in orthopaedic surgery: a ten-year review, Eur. J. Orthop. Surg. Traumatol. 9 (1999) 71–74.
[2] M. Hirata, H. Murata, T. Kubo, et al., Use of purified beta-tricalcium phosphate for filling defects after curettage of benign bone tumours, Int. Orthop. (SICOT) 30 (2006) 510–513.
[3] C. Myeroff, M. Archdeacon, Autogenous bone graft: donor sites and techniques, J. Bone Joint Surg. Am. 93 (2011) 2227–2236.
[4] H.S. An, K. Lynch, J. Toth, Prospective comparison of autograft vs. allograft for adult posterolateral lumber spine fusion: differences among freeze-dried, frozen, and mixed grafts, J. Spine Disord. 8 (1995) 131–135.
[5] T. Kawasaki, Y. Niki, T. Miyamoto, et al., The effect of timing in the administration of hepatocyte growth factor to modulate BMP-2-induced osteoblast differentiation, Biomaterials 31 (2010) 1191–1198.
[6] K. Janssens, P. Ten Dijke, W. Van Hul, et al., Transforming growth factor-1 to the bone, Endocr. Rev. 26 (2005) 743–774.
[7] T. Nakamura, K. Nawa, A. Ichihara, Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats, Biochem. Biophys. Res. Commun. 122 (1984) 1450–1459.
[8] T. Nakamura, T. Nishizawa, S. Shimizu, et al., Molecular cloning and expression of human hepatocyte growth factor, Nature 342 (1989) 440–443.
[9] G.K. Michalopoulos, M.C. DeFrances, Liver regeneration, Science 276 (1997) 60–66.
[10] K. Matsumoto, T. Nakamura, Hepatocyte growth factor: renotropic role and potential therapeutics for renal diseases, Kidney Int. 59 (2001) 2023–2038.
[11] K. Matsumoto, K. Date, T. Nakamura, et al., Hepatocyte growth factor in lung morphogenesis and tumor invasion: role as a mediator in epithelium–mesenchyme and tumor–stroma interactions, Cancer Chemother. Pharmacol. 38 (1996) S42–S47.
[12] Y. Imai, H. Terai, K. Takaoka, et al., Hepatocyte growth factor contributes to fracture repair by upregulating the expression of bmp receptors, J. Bone Miner. Res. 20 (2005) 1723–1730.
[13] S. Maeda, M. Hayashi, K. Miyazono, et al., Endogenous TGF-b signaling suppresses maturation of osteoblastic mesenchymal cells, EMBO J. 23 (2004) 552–563.
[14] T. Standal, N. Abildgaard, Anders Sundan, et al., HGF inhibits BMP-induced osteoblastogenesis: possible implications for the bone disease of multiple myeloma, Blood 109 (2007) 3024–3030.
[15] J.O. Hollinger, C.E. Hart, G.E. Friedlaender, et al., Recombinant human platelet-derived growth factor: biology and clinical applications, J. Bone Joint Surg. Am. 90 (2008) 48–54.
[16] A. Jayakumar, P. Rajababu, S. Pavan Kumar, et al., Multi-centre, randomized clinical trial on the efficacy and safety of recombinant human platelet-derived growth factor with β-tricalcium phosphate in human intra-osseous periodontal defects, J. Clin. Periodontol. 38 (2011) 163–172.
[17] H.K. Ridgway, J.T. Mellonig, D.L. Cochran, Human histologic and clinical evaluation of recombinant human platelet-derived growth factor and beta-tricalcium phosphate for the treatment of periodontal intraosseous defects, Int. J. Periodontics Restorative Dent. 28 (2008) 171–179.
[18] M. Nevins, W.V. Giannobile, S.E. Lynch, et al., Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial, J. Periodontol. 76 (2005) 2205–2215.
[19] M.K. McGuire, R.T. Kao, S.E. Lynch, et al., rhPDGF-BB promotes healing of periodontal defects: 24-month clinical and radiographic observations, Int. J. Periodontics Restorative Dent. 26 (2006) 223–231.
[20] S. Morikawa, Y. Mabuchi, Y. Matsuzaki, et al., Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow, J. Exp. Med. 206 (2009) 2483–2496.
[21] L.E. Benjamin, I. Hemo, E. Keshet, A plasticity window for blood vessel remodeling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF, Development 125 (1998) 1591–1598.
[22] H. Nakanishi, K. Yamanouchi, M. Nagayama, et al., The association of platelet-derived growth factor (PDGF) receptor tyrosine phosphorylation to mitogenic response of human osteoblastic cells in vitro, Oral Dis. 3 (1997) 236–242.
[23] T. Komori, H. Yagi, T. Kishimoto, et al., Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts, Cell 89 (1997) 755–764.
[24] F. Otto, A.P. Thornell, M.J. Owen, et al., Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development, Cell 89 (1997) 765–771.
[25] K. Nakashima, X. Zhou, B de. Crombrugghe, et al., The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation, Cell 108 (2002) 17–29.
[26] G.H. Westrich, D.S. Geller, D.L. Helfet, et al., Anterior iliac crest bone graft harvesting using the corticocancellous reamer system, Journal of Orthopaedic Trauma 15 (2001) 500–506.
[27] M.R. Urist, Bone: formation by autoinduction, Science 150 (1965) 893–899.
[28] M.R. Urist, B.S. Strates, Bone morphogenetic protein, J. Dent. Res. 50 (1971) 1392–1406.
[29] M.F. Termaat, F.C. Den Boer, H.J.TH.M. Haarman, et al., Bone morphogenetic proteins: development and clinical efficacy in the treatment of fractures and bone defects, J. Bone Joint Surg. 87 (2005) 1367–1378.