Journal of Hard Tissue Biology 25[3] (2016) xxx-xxx

CODEN-JHTBFF, ISSN 1341-7649

Original

Gene expression of Semaphorin 7A during osteogenic differentiation in human dental follicle cells

Ryoichi Kato[1)] and Kosuke Takahashi[1,2)]

[1)] *Department of Maxillofacial Surgery, Nihon University School of Dentistry at Matsudo, Matsudo, Japan*
[2)] *Research Institute of Oral Science, Matsudo, Japan*
(Accepted for publication, April 22, 2016)

Abstract: Semaphorin (Sema) 7A, also known as CDw108, is the only glycophosphatidylinositol (GPI)-linked member of the semaphorin family. It was recently suggested that Sema 7A regulates osteoblast and osteoclast differentiation, and is important for bone homeostasis. The dental follicle is an ectomesenchymal tissue that surrounds developing tooth germ, which contains osteoblastic/cementoblastic lineage committed stem/progenitor cells. The purpose of this study was to examine the gene expression of Sema 7A and its receptors, plexinC1 and β-integrin, in human dental follicle cells (hDFCs) during osteogenic differentiation. We analyzed gene expression profiles between hDFCs cultures with GM and OIM on day 17. Sema 7A was the most up-regulated among the semaphorin family members in OIM culture when compared to GM culture. We confirmed the gene expression of Sema 7A in three hDFCs samples isolated from three donors using real-time PCR. Sema 7A was up-regulated in OIM culture in three hDFC samples. In addition, Sema 7A mRNA showed high levels on days 0 and 17 in hDFCs cultured in OIM, as compared to days 3 and 10, exhibiting bimodal expression during osteoblast differentiation. The expression of plexin C1 and β-integrin was also up-regulated in OIM culture when compared to GM culture. These findings suggest that Sema 7A and its receptors are associated with the osteogenic differentiation of hDFCs.

Key words: Human dental follicle cells, Semaphorin 7A, Osteogenic differentiation, Stem cells.

Introduction

The semaphorins are a large family of phylogenetically similar secreted and membrane-bound proteins, and are divided into eight classes based on sequence similarities and distinct structural features[1, 2)]. Semaphorins influence cellular processes such as cell proliferation, differentiation and survival. As a consequence, they are involved in a variety of biological processes, such as axonal guidance[3)], cardiogenesis[4)], angiogenesis[5)], oncogenesis[6)], immune cell regulation[7)] and bone homeostasis[8)].

Semaphorin (Sema) 7A, also known as CDw108, was identified as a 78-80-kDa cell surface protein on red blood cells[9,10)], and was subsequently characterized as a member of the semaphorin family[11,12)]. Sema7A is the only glycophosphatidylinositol (GPI) - linked member of the semaphorin family[2)], and its effects are believed to be mediated via two receptors, Plexin C1[13)] and β1-integrin subunit[14)]. Sema 7A is broadly expressed in lymphoid cells[15)], the nervous system[13)], endothelial cells of blood vessels[16)], and bone cells[17)]. Recent studies have shown that Sema 7A and its receptors regulate osteoblast and osteoclast differentiation, both important for bone homeostasis[17, 18)].

The dental follicle is an ectomesenchymally derived connective tissue surrounding the tooth germ that contains stem cells and/or progenitor cells[19)] Human dental follicle cells (hDFCs) also have the capacity to commit to multiple cell types, not only to cells of the osteoblastic lineage, but also to cells of adipogenic and neurogenic lineages[20, 21)]. We previously reported that hDFCs can differentiate into osteogenic lineage cells in osteogenic induction medium (OIM) without dexamethasone (DEX)[22,23)], which has various biological effects, including anti-inflammatory properties. In addition, the expression of stem cell markers and growth factor receptors is similar in hDFCs and mesenchymal stem cells (hMSCs) from bone marrow, while compared to hMSCs, hDFCs strongly express LIM homeobox 8, which is associated with the development of the palatal mesenchyme and tooth germ[24)]. Based on these findings, we suggested that hDFCs are useful for therapy and in basic research of the maxillofacial region bone.

In this study, we examined the gene expression of Sema 7A and its receptors in hDFCs during osteogenic differentiation and mineralization.

Materials and Methods

Correspondence to: Dr. Ryoichi Kato, Department of Maxillofacial Surgery, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba, 271-8587 Japan; Tel/Fax: +81-47-360-9394; E-mail: fwgf4857@mb.infoweb.ne.jp

Cell cultures

hDFCs were obtained from patients aged 18 years after obtaining informed consent, as reported previously[1]. Briefly, normal human impacted third molars were surgically removed and collected. Dental follicle tissues were digested in a solution of 0.1 U/ml collagenase and 1 U/ml dispase (Roche, Mannheim, Germany) for 1 h at 37 ℃. Attached hDFCs were cultured at 37 ° C in 100-mm dishes using MSC growth medium (GM; consisting of MSC basal medium supplemented with fetal bovine serum, L-glutamine and penicillin/streptomycin; Lonza, Walkersville, MD) in a humidified incubator (CO_2 incubator MCO-175M; Sanyo, Osaka, Japan) in the presence of 5% CO_2 in air at 37 ℃. Experiments using hDFCs were performed according to the guidelines established by the ethics committee of Nihon University School of Dentistry at Matsudo (Recognition number: 10-036).

Osteogenic differentiation

For induction of osteogenic differentiation, hDFCs from the 5th to 6th passage were seeded at 3.1×10^3 cells/cm^2 in GM. After 24 h (day 0), media were replaced with MSC osteoinduction medium without DEX (OIM; consisting of osteogenic basal medium supplemented with fetal bovine serum, L-glutamine, penicillin/streptomycin, ascorbate and β-glycerophosphate; Lonza). On day 1 and every 3 days thereafter, medium was replaced with fresh medium.

Total RNA isolation

Total RNA from hDFCs was isolated using miRNeasy Mini Kits (QIAGEN, Valencia, CA**)** according to the manufacturer's instructions.

Microarray analysis

Gene expression profiling was performed using SurePrint G3 Human GE microarray 8 × 60 k (Agilent, Santa Clara, CA) according to the manufacturer's protocol. Briefly, cRNA labeled with Gy3 was generated from 100 ng of total RNA using Low Input Quick Amp Labeling kit, one-color (Agilent), and was fragmented and hybridized to the array using a Gene Expression Hybridization kit. The array was scanned (Agilent DNA Microarray Scanner), and raw data from the microarray was loaded into Gene Spring GX software (Version 11.5; Agilent). Data were normalized according to the manufacturer's protocols. Regulated genes were recognized as having more than a two-fold change in OIM culture when compared with GM culture on culture day 17.

RT-PCR and real-time PCR

cDNA was synthesized using a GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA). The PCR mixture (20 μl) containing DyNAmo SYBR Green qPCR kit (Finnzymes, Espoo, Finland), 20 pmol forward and reverse primers and 2 μl cDNA was subjected to amplification with a DNA Engine Opticon 1 (BioRad, Hercules, CA). RT-PCR under the following conditions; preheating at 95°C for 10 min, followed by 27 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 30 s. PCR fragments were electrophoresed on a 1.5 % agarose gel and subsequently stained with ethidium bromide. For real-time PCR, amplicons were directly detected by measuring the increase in fluorescence caused by binding of SYBR Green using a DNA Engine Opticon 1, with pre-heating at 95 °C for 10 min, followed by 40 cycles as follows: 94°C for 15 s, 57 °C for 30 s, and 72°C for 30 s. Gene expression levels were calculated using the ΔΔcT method[24]. Primers used for RT-PCR and real time-PCR analysis were as follows: 5'-GACCTTTCATGTGCTTTACC-3' (forward primer for Sema 7A); 5'-GATCTCCATGATGTTGAAGG-3'(reverse primer for Sema 7A); 5'-GTCAACGTGATGTT CTCCTT-3'(forward primer for Plexin C1);5' CCTTCTTGCAC TTTTACACC-3' (reverse primer for Plexin C1); 5'-TC CTTCTATTGCTCACCTTG-3'(forward primer for β1 integrin); 5'-ACTGCTGACTTAGGGATCAA-3'(reverse primer for β1 integrin); 5'-ATCACCATCTTCCAGGAG-3' (forward primer for GAPDH); and 5'-ATCGACTGTGGTCATGAG-3' (reverse primer for GAPDH).

Statistics

Data are expressed as means ± SD of three samples. Significance of differences between culture samples was determined using Student's *t*-test and a value of $p<0.005$ was considered to be statistically significant.

Results

Microarray analysis

We performed microarray analysis to determine the genes regulated in hDFCs by osteogenic induction. The expression of 42,545 genes on the SurePrint G3 Human GE microarray 8 × 60 k were compared between hDFCs cultures with GM and that with OIM on the day 17. We used a two-fold change in the expression density as the cut-off point to determine differentiation. A total of 3,988 genes showed differences in expression in culture with OIM compared to with GM; 2,174 were up-regulated and 1,814 were down-regulated (Fig. 1).

Recently, increasing evidence has pointed to a role for semaphorins during bone development, as well as in the control of normal bone homeostasis. Expression of several semaphorins was up-regulated in hDFCs by osteogenic induction (Table 1). Among these, semaphorin7A was the most up-regulated in hDFCs cultured with OIM as compared to GM. Sema3C, Sema4D and Sema6D were slightly up-regulated in OIM culture, although the expression of Sema4D and Sema6D was week in hDFCs. In contrast, Sema3 group members other than Sema3C were down-regulated in OIM culture.

Expression of semaphorin7a during osteogenic differentiation

Table 1. Gene Expressions of Semaphorin Family Members

Gene Symbol	Fold (OIM/GM)	GM culture		OIM culture		Genebank ID
		Raw intensity	Flag	Raw intensity	Flag	
Sema3A	0.5	362.3	Detected	198.6	Detected	NM_006080
Sema3B	0.2	5652.8	Detected	1054.2	Detected	NM_004636
Sema3C	2.3	253.1	Detected	586.6	Detected	NM_006379
Sema3D	0.1	898.1	Detected	102.8	Detected	NM_152754
Sema3E	0.2	18.9	Detected	2.9	Not Detected	NM_012431
Sema3F	0.5	131.6	Detected	68.6	Detected	NM_004186
Sema3G	0.6	3.8	Not Detected	2.1	Not Detected	NM_020163
Sema4A	1.1	86.9	Detected	97.8	Detected	NM_022367
Sema4B	1.4	116.6	Detected	159.5	Detected	NM_020210
Sema4C	1.7	3196.4	Detected	5360.7	Detected	NM_017789
Sema4D	3.0	4.9	Not Detected	14.6	Detected	NM_006378
Sema4F	0.8	103.9	Detected	83.2	Detected	NM_004263
Sema4G	1.5	2.9	Not Detected	4.3	Not Detected	XR_428710
Sema5A	1.2	6932.9	Detected	8356.3	Detected	NM_003966
Sema5B	0.5	4.8	Not Detected	2.4	Not Detected	NM_001256346
Sema5B	1.0	2.9	Not Detected	2.8	Not Detected	NM_001031702
Sema6A	1.2	38.2	Detected	46.9	Detected	NM_020796
Sema6B	0.9	147.0	Detected	137.0	Detected	NM_032108
Sema6C	1.4	367.6	Detected	517.8	Detected	NM_001178061
Sema6C	1.3	62.3	Detected	81.5	Detected	NM_030913
Sema6D	4.4	8.3	Not Detected	37.1	Detected	NM_024966
Sema7A	13.3	34.2	Detected	456.5	Detected	NM_003612

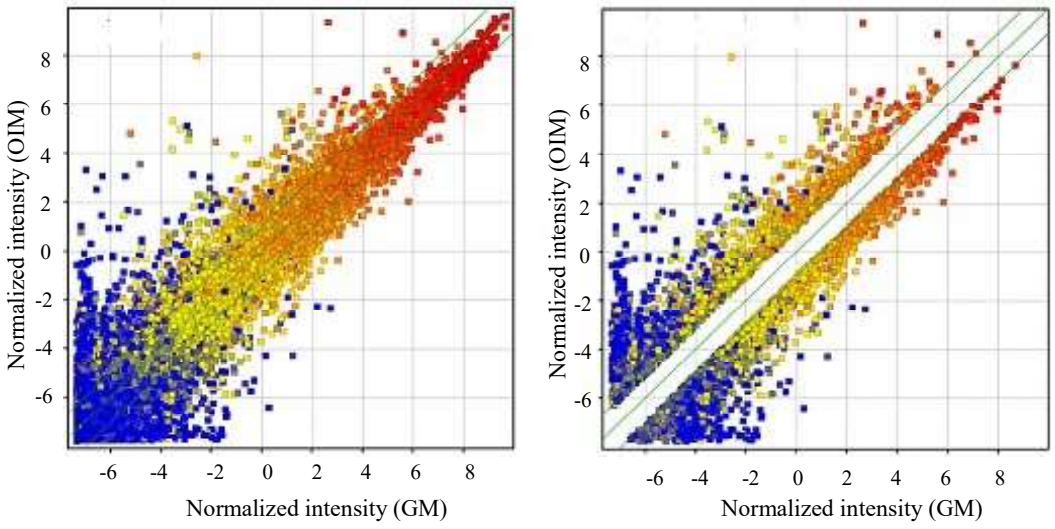

Figure 1. Scatter plots for gene expressions in hDFCs were created with GeneSpring software. Gene expression levels of 42,545 genes were compared between GM culture and OIM culture on day 17. Regulated genes were recognized as having more than a two-fold change.

Because Sema 7A was the most up-regulated among semaphorin family members by osteogenic induction using microarray, we next confirmed the gene expression of Sema 7A in hDFC during osteogenic differentiation using RT-PCR and Real time-PCR. As shown in Fig. 2, there was increased mRNA levels for Sema 7A in OIM culture when compared to GM culture on day 17, although no differences were seen between OIM and GM on day 3 using RT-PCR. We then confirmed Sema 7A gene expression in three hDFC samples obtained from three donors during osteogenic differentiation. Sema 7A was significantly up-

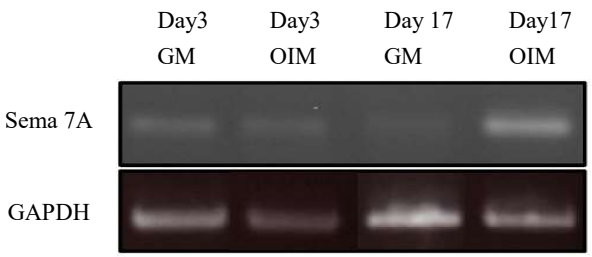

Figure 2. Sema7A mRNA levels in hDFC using RT-PCR. hDFCs were cultured in growth medium (GM) or osteogenic induction medium (OIM).

OIM when compared to GM culture on day 17.

Discussion

Several semaphorins have recently been suggested to be involved in the development and homeostasis of bone[2, 8]. However, the mechanisms and actions of semaphorins are typically investigated by histological study using mouse models, and it is unclear to what extent semaphorins and their signaling pathways are conserved between mice and humans. In this study, we analyzed the gene expression profile in hDFCs between GM culture and

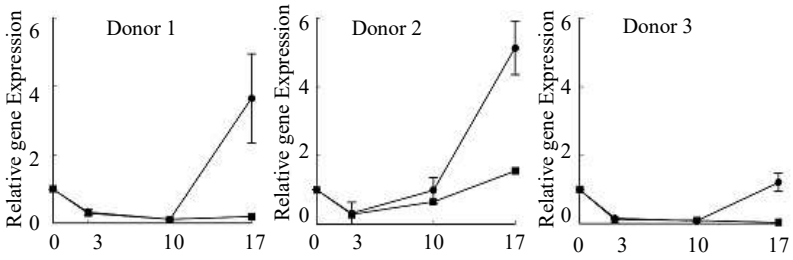

Figure 3. Gene expression of Sema 7A in hDFCs during osteogenic induction using real-time PCR. Cells were cultured with each medium for the indicated time period. hDFCs were cultured in growth medium (GM: ■) or osteogenic induction medium (OIM: ●). Values represent means ± SD of the results (n=4). **P*<0.005; OIM vs GM at indicated time point, analyzed by using Student's *t*-test.

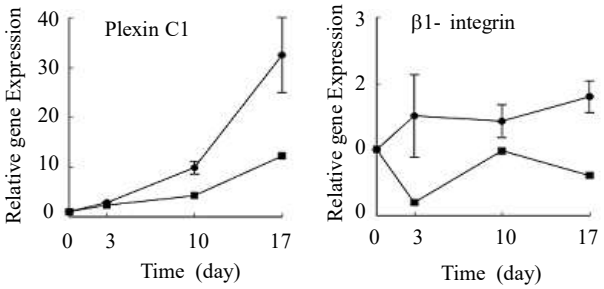

Figure 4. Gene expression of PlexinC1 and -integrin in hDFCs during osteogenic induction using real-time PCR. Cells were cultured with each medium for the indicated time period. hDFCs were cultured in growth medium (GM: ■) or osteogenic induction medium (OIM: ●). Values represent means ± SD of the results (n=4). **P*<0.005; OIM vs GM at indicated time point, analyzed by using Student's *t*-test.

regulated by OIM in comparison to GM on day 17 in three hDFC samples (Fig. 3). In addition, Sema 7A expression was decreased in hDFC cultured with both GM and OIM on day 3 when compared to day 0 (Fig. 3). The levels of Sema 7A gene expression and up-regulation varied among the three hDFC samples.

Expression of semaphorin 7A receptors during osteogenic differentiation

We next evaluated the gene expression of the semaphorin receptors plexicin C1 and β1-integrin. Expression of plexicin C1 increased on culture with OIM when compared with GM on day 17. In contrast, β1-integrin showed little increase on culture with OIM culture on day 17, when hDFCs cultured with OIM are in the mineralization stage. We found that several semaphorin family members were up-regulated in hDFCs by OIM. Sema 7A was the most up-regulated gene among semaphorin family members in hDFCs (Table 1). Previous reports have also confirmed the expression of Sema 7A mRNA in mouse osteoblast cell lines and in primary calvarial osteoblasts[17,26].

We confirmed the gene expression of Sema 7A in hDFCs using RT-PCR and real-time PCR. The gene expression of Sema 7A was elevated in hDFCs cultured with OIM when compared to GM on day 17 in three hDFC samples from three donors (Fig. 3). In addition, the gene expression of Sema 7A is down-regulated on day 3 (confluent stage) and on day 10 (early mineralization stage), as compared to day 0 (proliferation/non-differentiation stage) in all hDFC samples. The MC3T3 cell line and osteoblasts isolated from mouse calvaria expressed Sema 7A mRNA throughout the culture period from the proliferation stage to the nodule mineralization stage. In addition, Sema 7A was significantly decreased during the early nodule stage, and then increased again in the mature mineralized nodule stage in[17]. Sema 7A mRNA thus has a bimodal expression during osteoblast differentiation.

Recently, it has been investigated whether Sema 7A is associated with cell growth, as Sema 7A knockdown cells showed decreased cellular proliferation via cell-cycle arrest at the G1/S phase[27]. On the other hand, Sema 7A was suggested to be a mesenchymal stem cell marker, as it showed significantly increased

expression in mesenchymal stem cell culture when compared to non-stem cell mesenchymal cell culture[28]. We therefore believe that Sema 7A is associated with cell growth of mesenchymal stem cells/osteogenic lineage cells, including hDFCs, on culture day 0.

Sema 7A also plays an important role in the differentiation and attraction of osteoclasts. Expression of Sema 7A was low at the start of osteoclast culture from spleen-derived cells, and was high during immature osteoclast fusion[17,26]. Sema7A increases cell fusion, as exposure to exogenous Sema 7A during differentiation enhanced the number of nuclei per osteoclast and reduced the number of individual osteoclasts[17]. It has also been suggested that Sema 7A triggers osteoclast formation. The increase in Sema 7A expression by osteoblasts in the mature mineralization stage may be related to the induction of osteoclast differentiation.

The gene expression of plexin C1 and β1-integrin, which are two putative Sema 7A receptors, were examined in hDFCs during osteogenic induction. The expression of plexicin C1 was significantly increased in hDFCs cultured with OIM when compared with GM on day 17, whereas b1-integrin was slightly increased on culture in OIM. Plexin C1 belongs to the plexin family of cell surface receptors. Plexin C1 was expressed in both MC3T3-E1 and calvarial osteoblasts at all stages during osteoblast differentiation[17]. The plexin C1 cytoplasmic domain contains a Rho-GTPase binding domain region flanked by two GAP domains, which show RasGAP activity in vitro[29,30]. However, the contribution of Sema-7A-plexin C1 interactions to osteoblast differentiation is largely unknown.

β1-integrin was slightly increased in hDFCs cultured with OIM when compared with GM at all stages during osteogenic differentiation. An interaction between Sema 7A and b1-integrin was predicted based on the presence of an arginine-glycine-aspartate amino acid sequence, known as an RGD-motif, in the Sema 7A sema domain[15]. Integrin are heterodimers composed of the a- and b-subunits. In intestinal macrophages, interaction between avb1-integrin and Sema 7A produces IL-10[31]. Sema 7A initiates T-cell-mediated inflammatory responses through α1β1-integrin via FAK and MAPK activation[32]. When MC3T3 cells were treated with Sema-7A, ERK-1 and ERK-2 phosphorylation levels increased transiently[17]. This suggests that Sema-7A is involved in osteoblast migration through the MAPK pathway[17]. However a-subunit involvement remains to be confirmed in osteoblasts.

In addition, an association study among postmenopausal Korean women associated Sema 7A poly-morphisms with decreased bone mineral density and increased vertebral fracture risk[33]. Malfunction of Sema 7A might favor osteoclast clustering and increased bone resorption, resulting in osteoporosis.

In this study, we showed that expression of Sema 7A and its receptors was elevated in hDFCs by osteogenic induction. Based on these results, Sema 7A and its receptors may regulate the migration and differentiation of osteoblasts and osteoclasts, and play important roles in bone development and homeostasis.

Conflict of Interest

The author declares no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

1. Zhou Y, Gunput RAF and Pasterkamp RJ. Semaphorin signaling: progress made and promises ahead. Trends Biochem Sci 33:161-170, 2008
2. Yazdani U and Terman JR. The semaphorins. Genome Biol 7:211, 2006
3. Culotti JG and Kolodkin AL. Functions of netrins and semaphorins in axon guidance. Curr Opin Neurobiol 6: 81-88, 1996
4. Compernollea V, Brusselmansa K, Francob D, Moormanc A, Dewerchina M, Collena D and Carmeliet P. Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor-1. Cardiovasc Res 60: 569-579, 2003
5. Neufeld G, Cohen T, Shraga N, Lange T, Kessler O and Herzog Y. The Neuropilins: multifunctional semaphorin and VEGF receptors that modulate axon guidance and angiogenesis. Trends Cardiovasc Med 12: 13-19, 2002
6. Kuroki T, Trapasso F, Yendamuri S, Matsuyama A, Alder H, Williams NN, Kaiser LR and Croce CM. Allelic loss on chromosome 3p21.3 and promoter hypermethylation of *semaphorin 3B* in non-small cell lung cancer. Cancer Res 63: 3352-3355, 2003
7. Hall KT, Boumsell L, Schutze JL, Boussiotis VA, Dorfman DM, Cardoso AA, Bensussan A, Nadler LM and Freeman GJ. Human CD100, a novel leukocyte semaphorin that promotes B-cell aggregation and differentiation. Proc Natl Acad Sci USA 93: 11780-11785, 1996
8. Negishi-Koga T and Takayanagi H. Bone cell communication factors and Semaphorins. Bonekey Rep 1: 183, 2012
9. Mudad R, Rao N, Angelisova P, Horejsi V and Telen MJ. Evidence that CDw108 membrane protein bears the JMH blood group antigen. Transfusion 35: 566-70, 1995
10. Mudad R, Rao N, Issitt PD, Roy RB, Combs MR and Telen MJ. JMH variants: serologic, clinical, and biochemical analyses in two cases. Transfusion 35: 925-930, 1995
11. Sato Y and Takahashi H. Molecular cloning and expression of murine homologue of semaphorin K1 gene. Biochim Biophys Acta 1443: 419-422, 1998
12. Xu X, Ng Sh, Wu ZL, Nguyen D, Homburger S, Seidel-Dugan C, Ebens A and Luo Y. Human semaphorin K1 is glycosylphosphatidylinositol-linked and defines a new subfamily of viral-related semaphorins. J Biol Chem 273: 22428-22434, 1998

13. Tamagnone Luca, Artigiani S, Chen H, He Z, Ming G, Song H, Chedotal A, Winberg ML, Goodman CS, Poo M, Tessier-Lavigne M and Comogli PM. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell 99: 71-80, 1999

14. Suzuki K, Okuno T, Yamamoto M, Pasterkamp RJ, Takegahara N, Takamatsu H, Kitao T, Takagi J, Rennert PD, Kolodkin AL, Kumanogoh A and Kikutani H. Semaphorin 7A initiates T-cell-mediated inflammatory responses through 11 integrin. Nature 446: 680-684, 2007

15. Yamada A, Kubo K, Takeshita T, Harashima N, Kawano K, Mine T, Sagawa K, Sugamura K and Itoh K. Molecular cloning of a glycosylphosphatidylinositol-anchored Molecule CDw108. J Immunol 162: 4094-4100, 1999

16. Morote-Garcia JC, Napiwotzky D, Köhler D and Rosenberger P. Endothelial semaphorin 7A promotes neutrophil migration during hypoxia. Proc Natl Acad Sci USA 109: 4146-14151, 2012

17. Delorme G, Saltel F, Bonnelye E, Jurdic P and Machuca-GayetI. Expression and function of semaphorin 7A in bone cells. Biol. Cell 97: 589-597, 2005

18. Jongbloets BC, Ramakers GMJ and Pasterkamp RJ. Semaphorin7A and its receptors: Pleiotropic regulators of immune cell function, bone homeostasis, and neural development. Semin Cell Dev Biol 24: 129-138, 2013

19. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C and Hoffmann KH. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24: 155-165, 2005

20. Yao S, Pan F, Prpic V and Wise GE. Differentiation of stem cells in the dental follicle. J Dent Res 87: 767-771, 2008

21. Völlner F, Ernst W, Driemel O and Morsczeck C. A two-step strategy for neuronal differentiation in vitro of human dental follicle cells. Differentiation 77 433-441, 2009

22. Takahashi K, Ogura N, Aonuma H, Ito K, Ishigami D, Kamino Y and Kondoh T. Bone morphogenetic protein 6 stimulates mineralization in human dental follicle cells without dexamethasone. Arch Oral Biol 58: 690-698, 2013

23. Aonuma H, Ogura N, Kamino Y, Ito K and Kondoh T. Gene expression of osteomodulin in human dental follicle cells. IJOMS 7: 91-97, 2008

24. Aonuma H, Ogura N, Takahashi K, Fujimoto Y, Iwai S, Hashimoto H, Ito K, Kamino Y and Kondoh T. Characteristics and osteogenic differentiation of stem/progenitor cells in the human dental follicle analyzed by gene expression profiling. Cell Tissue Res 350: 317-331, 2012

25. 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

26. Baniwal SK, Shah PK, Shi Y, Haduong JH, DeClerck YA, Gabet Y and Frenkel B. Runx2 promotes both osteoblastogenesis and novel osteoclastogenic signals in ST2 mesenchymal progenitor cells. Osteoporos Int 23: 1399-1413, 2012

27. Saito T, Kasamatsu A, Ogawara K, Miyamoto I, Saito K, Iyoda M, Suzuki T, Endo-Sakamoto Y, Shiiba M, Tanzawa H and Uzawa K. PLoS One 10: e0137923, 2015

28. Wetzig A, Alaiya A, Al-Alwan M, Pradez CB, Pulicat MS, A.l-Mazrou A, Shinwari Z, Sleiman GM, Ghebeh H, A.l-Humaidan H, Gaafar A, Kanaan I and Adra C. Differential marker expression by cultures rich in mesenchymal stem cells. BMC Cell Biol 14: 54, 2013

29. Uesugi K, Oinuma I, Katoh H and Negishi M. Different requirement for Rnd GTPases of R-Ras GAP activityof plexin-C1 and plexin-D1. J Biol Chem 284: 6743-6751, 2009

30. Wang H, Hota PK, Tong Y, Li B, Shen L, Nedyalkova L, Borthakur S, Kim S, Tempel W, Buck M and Park HW. Structural basis of Rnd1 binding to plexin Rho GTPase binding domains (RBDs). J Biol Chem 286: 26093-26106, 2011

31. Kang S, Okuno T, Takegahara N, Takamatsu H, Nojima S, Kimura T, Yoshida Y, Ito D, Ohmae S, You D-J, Toyofuku T, Jang MH and Kumanogoh A. Intestinal epithelial cell-derived semaphorin 7A negatively regulates development of colitis via αvβ1 integrin. J Immunol 188: 1108-1116, 2012

32. Koh JM, Oh B, Lee JY, Lee JK, Kimm K, Kim GS, Park BL, Cheong HS, Shin HD, Hong JM, Kim TH, Park EK and Kim SY. Association study of semaphorin 7a (sema7a) polymorphisms with bone mineral density and fracture risk in postmenopausal Korean women. J Hum Genet 51: 112-117, 2006