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Original

Effects of Placental Extract on Cell Proliferation, Type I Collagen Production, and ALP Secretion in Human Osteosarcoma Cell Line Saos-2

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Abstract: Porcine placenta extract (P-placenta) is widely applied in medicine and cosmetics. However, few studies have examined the effect of the extract on the cellular behavior of the osteoblastic cell line Saos-2. Here, we demonstrated that P-placenta enhances the proliferation, collagen type I production, and alkaline phosphatase (ALP) secretion of Saos-2 *in vitro*. Proliferation of Saos-2 was assessed by MTT and DNA synthesis assays. Type I collagen production and ALP secretion were evaluated using enzyme-linked immunosorbent assay and ALP assays. The cells were treated with/without 20, 200 and 2000 μg/ml of P-placenta for 24 h. We found that 200 μg/ml P-placenta significantly induced the proliferation of Saos-2 and enhanced type I collagen production and ALP secretion. The results indicate that P-placenta controls the cellular behavior of osteoblasts, resulting in the secretion of early bone-related biomarkers.

Key words: ALP, Collagen, Osteoblast, Placenta, Saos-2

Introduction

The placenta is a temporary organ that supports fetal growth by supplying oxygen and nutrients[1]. The organ stores diverse molecules such as vitamins, bioactive peptides, minerals, and growth factors[2,3]. The placental extract has attracted attention worldwide owing to its potential as drugs for clinical treatments[4] and as supplements in cosmetics[3,4]. Previous studies showed that placental extract has various therapeutic effects, such as wound-healing[5], anti-inflammatory[6], and immune-modulating potential[7]. More recently, we reported that porcine placenta extract (P-placenta) controls type I collagen production, cell proliferation, and anti-inflammatory cytokine production of human gingival fibroblasts (HGFs)[8]. However, its pharmaceutical mechanism in other cells, such as bone-related cells, has not been thoroughly examined.

Bone metabolism is systematically regulated by the complex iteration of bone formation and resorption. Osteoblasts mainly engage in bone formation by secreting extracellular matrix, such as type I collagen and other organic proteins. Alkaline phosphatase (ALP) secreted from osteoblasts degrades inorganic pyrophosphate to phosphate, which is associated with the mineralization of bone[9]. Therefore, type I collagen and ALP are important indicators of bone metabolism[10,11]. However, little information is available regarding the effect of placenta on the cellular metabolism of osteoblasts.

The aim of this study was to evaluate the effect of P-placenta on osteoblastic cells, particularly cell proliferation, type I collagen production, and ALP secretion. To evaluate these issues, we used the osteoblastic cell line Saos-2, which was first established from osteosarcoma in 1987[12]. ALP secretion, type I collagen production, and mineralized matrix production have been evaluated in Saos-2 cells and osteoblasts[12]. These cells present mature osteoblastic characteristics[13], and thus have been extensively investigated in oncology[14] and bone biology studies[13]. Additionally, biomaterial function was previously evaluated using Saos-2 cells[15,16].

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Materials and Methods

Reagents

P-placenta was purchased from Nippon Meat Packers, Inc. (Osaka, Japan). The powder-like extract was composed of natural components from the porcine placenta without any additives. Therefore, we used these reagents for the following experiments.

Cell maintenance

Saos-2 cells (HTB85) were provided by the Riken BRC Cell

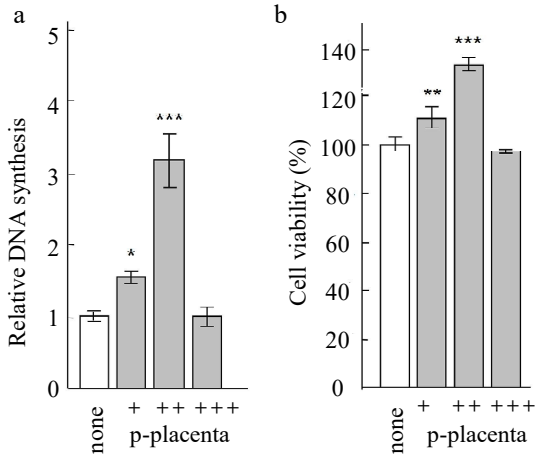

Figure 1. Effect of P-placenta on the proliferation of Saos-2 cells. (a) DNA synthesis assay. (b) MTT assay. Saos-2 seeded at a volume of 3.1 × 10^4/cm^2 were exposed to media containing 20, 200, or 2000 μg/ml P-placenta (designated as +, ++, and +++) for 24 h. All data were compared with those for cells treated with control medium without P-placenta (none). Data are shown as the mean ± standard deviation (n = 3). *P < 0.05, **P < 0.01 and ***P < 0.001, analyzed with analysis of variance with a Dunnett's test (vs. none).

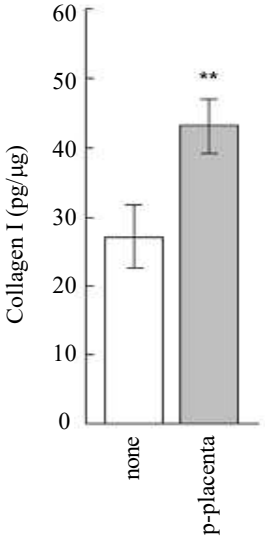

Figure 2. Type I collagen production in Saos-2 cells treated with P-placenta. Saos-2 cells were seeded at a volume of 3.1 × 10^4/cm^2 and exposed to media containing 200 μg/ml P-placenta for 24 h. Data are shown as the mean ± standard deviation (n = 3). **P < 0.01, analyzed by Student's *t*-test (vs. none).

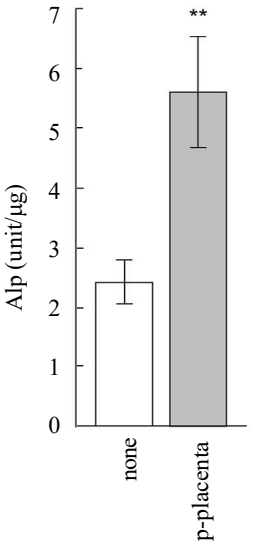

Figure 3. ALP secretion in Saos-2 cells treated with P-placenta. Saos-2 cells were seeded at 3.1 × 10^4/cm^2, and exposed to media containing 200 μg/ml P-placenta for 24 h. Data are shown as the mean ± standard deviation (n = 3). **P < 0.01, analyzed by Student's *t*-test (vs. none)

Bank (Tsukuba, Japan). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% fetal bovine serum (FBS) at 5% CO_2 and 37°C.

3-(4, 5-Dimethylthial-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and DNA synthesis assay

For DNA synthesis, Saos-2 seeded at 3.1 x 10^4/cm^2 were cultured in DMEM containing 0.5% FBS with/without P-placenta at doses of 20, 200, and 2000 μg/ml for 24 h. The level of DNA synthesis in cells was determined by measuring BrdU-incorporation using the Frontier BrdU Cell Proliferation assay kit (Millipore, Billerica, MA, USA). For the MTT assay, cells were cultured in DMEM containing 10% FBS with P-placenta at the above concentrations for 24 h. The MTT assay was conducted as reported previously[17].

Type I collagen production

Cells were seeded at the 3.1 x 10^4/cm^2 in DMEM with 10% FBS, followed by treatment with DMEM containing 1% FBS with/without P-placenta (200 μg/ml) for 24 h. The levels of collagen type I in the media were measured by an enzyme-linked immunosorbent assay (ELISA), using a biotinylated anti-collagen type I antibody (0.2 mg/ml, Rockland Immunochemicals, Limerick, PA, USA). Total protein levels were quantified in the cell lysate isolated with 0.5% Triton X-100 by using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Collagen production was normalized to the protein content in the cell lysates.

ALP assay

Cells were seeded at a density of 3.1 x 10^4/cm^2 in DMEM containing 10% FBS. Next, the cells were treated with DMEM containing 1% FBS with/without P-placenta (200 μg/ml) for 24 h. The cells were lysed with 0.05% TritonX-100 (200 μl), followed by centrifugation at 14,000 xg for 1 min, and the supernatants were collected. ALP activity in the lysates (20 μl) was measured using a LabAssay ALP kit (Wako Pure Chemicals Industries, Ltd., Osaka, Japan). Protein levels in the cell lysates were also measured using the BCA protein assay kit (Thermo Fisher Scientific). ALP activity was normalized to the total protein level in the cell lysates.

Statistical analysis

Statistical analysis was carried out using StatMate software (ATMS, Tokyo, Japan). Statistical significance was evaluated by one-way analysis of variance with a Dunnett's test or by student's

t-test. Levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered to indicate statistical significance.

Results

Effect of P-placenta on Saos-2 cell proliferation

After 24 h of treatment, 200 μg/ml P-placenta increased the level of DNA synthesis compared to the effect of other concentrations in Saos-2, whereas no obvious change was observed at 2000 μg/ml P-placenta treatment (Fig.1a). The MTT assay showed that 200 μg/ml P-placenta significantly elevated the cell viability of Saos-2 cells (Fig.1b). These results suggest that there is optimal concentration of P-placenta that facilitates Saos-2 cell proliferation.

Effect of P-placenta on type I collagen production and ALP secretion from Saos-2 cells

Based on the proliferation assay results, we further evaluated the effect of the P-placenta on type I collagen production and ALP secretion from Saos-2 cells. Addition of P-placenta to the media significantly increased the levels of type I collagen production (Fig. 2) and ALP secretion (Fig. 3) from Saos-2 cells compared to that in media without P-placenta.

Discussion

Although P-placenta may have therapeutic potential not only in medicinal drugs but also in cosmetic supplements[3], its detailed function in the cellular metabolism of osteoblasts remains unclear. In the present study, we demonstrated P-placenta enhances the cell proliferation and regulates the synthesis and secretion of bone-related proteins in osteoblastic Saos-2 cells.

Under the P-placenta stimulation, 200 μg/ml condition significantly enhanced the proliferation of Saos-2 *in vitro*. In contrast, 2000 μg/ml P-placenta showed a negligible effect on cell proliferation (Fig. 1). We previously reported that HGFs shows a similar cell proliferation tendency under the same P-placenta concentration[8]. Yoshikawa *et al*. reported that 0–100 μg/ml P-placenta elevated the proliferation of human skin fibroblasts[18]. These results suggest that concentrations near 100 and 200 μg/ml are sufficient for inducing the proliferation of human somatic cells originating from mesenchymal stem cells by using P-placenta *in vitro*.

Using 200 μg/ml P-placenta, we found that the extract induced type I collagen production and ALP secretion from Saos-2 cells *in vitro*. The mechanism by which P-placenta elevated this secretion remains unclear. However, P-placenta is produced from porcine placenta extract without any impurities. P-placenta and human placenta extract[4,19] are known to contain various minerals, vitamins and growth factors. Including insulin-like growth factor-1, a key regulator of fetal development, are synthesized in placenta[20]. Type I collagen synthesis in Saos-2 cells is reportedly upregulated by insulin-like growth factor-1[10]. Moreover, Saos-2 cells respond to extracellular calcium[21], hormones[12], microRNAs[22], and growth factors[10]. These results support that the multiple molecules in P-placenta synergistically, rather than solely, promote type I collagen production and ALP secretion.

A study conducted in Italy indicated that placenta treatment improved periodontal disease, a major cause of tooth loss[23]. However, few studies have evaluated how placenta extract controls the condition of inflammation and bone loss in periodontal disease. More recently, we reported that P-placenta has an anti-inflammatory effect on HGFs *in vitro*[8]. The current study indicates that P-placenta can elevate the secretion of bone-related protein (type I collagen and ALP), which are pivotal in bone formation (Figs. 2 and 3). Although there is a wide gap between *in vitro* and *in vivo* results, additional studies may reveal the mechanism underlying these effects of placenta on bone formation via protein production of osteoblastic cells, which may be useful for treating periodontal diseases. Peri-implant diseases lead to implant failure due to bone loss and loss of osseointegration[24]. Therefore, preventing or treating these diseases is important in dental implant treatment. Regeneration therapy has been advocated as an approach for curing peri-implant diseases[25]. Given the function of P-placenta in *in vitro* culture of Saos-2 cells in the present study, this extract might be effective for the treatment of not only periodontitis, but also peri-implant diseases, and in bone regenerative therapy. However, because the present study was performed under limited conditions and cell lines, additional studies of primary osteoblasts are imperative. Further detailed *in vivo* experiments are also required to support these results.

In conclusion, we demonstrated that P-placenta enhanced the cell proliferation, type I collagen production, and ALP secretion of Saos-2 cells *in vitro*. Although it remains unknown how placenta extract modulates the secretion of other bone-related proteins, our results suggest that the extract modulates the cellular functions of osteoblastic cells.

Acknowledgements

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Conflict of Interest

The authors have declared that no COI exists.

References

1. Jash A, Kwon HK, Sahoo A, Lee CG, So JS, Kim J, Oh YK, Kim YB and Im SH. Topical application of porcine placenta extract inhibits the progression of experimental contact hypersensitivity. J Ethnopharmacol 133: 654-662, 2011
2. Cho H, Ryou J, Lee J and Lee M. The effects of placental extract on fibroblast proliferation. J Cosmet Sci 59: 195-

202, 2008

3. Intrama V, Karnchanatat A, Bunaprasert T and Vadhanasindhu P. Study of regulation approach of growth factor product from human placenta in thailand. The First International Conference on Interdisciplinary Research and Development 19: 17.1-17.4, 2011
4. Lee KK, Choi WS, Yum KS, Song SW, Ock SM, Park SB and Kim MJ. Efficacy and safety of human placental extract solution on fatigue: a double-blind, randomized, placebo-controlled study. eCAM 2012: 130875, 2012
5. Navadiya S, Patel M and Vaghani Y. Study of topical placental extract versus povidone iodine and saline dressing in various diabetic wounds. National J Med Res 2: 411-413, 2012
6. Kawakatsu M, Y U, Goto S, Ono Y and Li TS. Placental extract protects bone marrow-derived stem/progenitor cells against radiation injury through anti-inflammatory activity. J Rad Res 54: 268-276, 2013
7. Ansari KU, Gupta N and Bapat SK. An experimental and clinical evaluation of immuno-modulating potential of human placenta extract. Indian J Pharmacol 26: 130-132, 1994
8. Honda Y, Imamura Y, Fukui T, Masuno K and Wang P. Effect of placenta on collagen type-1and inflammatory cytokine production in human gingival fibroblasts. Oral Therap Pharmacol 34: 94-99, 2015
9. Golub E and Boesze-Battaglia K. The role of alkaline phosphatase in mineralization. Curr Opin Orthop 18: 444-448, 2007
10. Kudo Y, Iwashita M, Iguchi T and Takeda Y. The regulation of type-I collagen synthesis by insulin-like growth factor-I in human osteoblast-like SaOS-2 cells. Pflugers Arch 433: 123-128, 1996
11. Orimo H and Shimada T. Regulation of the human tissue-nonspecific alkaline phosphatase gene expression by all-trans-retinoic acid in SaOS-2 osteosarcoma cell line. Bone 36: 866-876, 2005
12. Rodan SB, Imai Y, Thiede MA, Wesolowski G, Thompson D, Bar-Shavit Z, Shull S, Mann K and Rodan GA. Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. Cancer Res 47: 4961-4966, 1987
13. Pautke C, Schieker M, Tischer T, Kolk A, Neth P, Mutschler W and Milz S. Characterization of osteosarcoma cell lines MG-63, Saos-2 and U-2 OS in comparison to human osteoblasts. Anticancer Res 24: 3743-3748, 2004
14. Rasola A, Sciacovelli M, Chiara F, Pantic B, Brusilow WS and Bernardi P. Activation of mitochondrial ERK protects cancer cells from death through inhibition of the permeability transition. Proc Natl Acad Sci U S A 107: 726-731, 2010
15. Postiglione L, Di Domenico G, Ramaglia L, di Lauro AE, Di Meglio F and Montagnani S. Different titanium surfaces modulate the bone phenotype of SaOS-2 osteoblast-like cells. Eur J Histochem 48: 213-222, 2004
16. Alcaide M, Serrano MC, Pagani R, Sanchez-Salcedo S, Nieto A, Vallet-Regi M and Portoles MT. L929 fibroblast and Saos-2 osteoblast response to hydroxyapatite-betaTCP/agarose biomaterial. J Biomed Mater Res A 89: 539-549, 2009
17. Imamura Y, Fujigaki Y, Oomori Y, Usui S and Wang P. Cooperation of salivary protein histatin 3 with heat shock cognate protein 70 relative to the G1/S transition in human gingival fibroblasts. J Biol Chem 284: 14316-14325, 2009
18. Yoshikawa C. Effect of porcine placental extract on collagen production in human skin fibroblasts in vitro. Gynecol Obstetr 3: 1-4, 2013
19. Chakraborty P and Bhattacharyya D. Aqueous extract of human placenta as a therapeutic agent, In: Recent Advances in Research on the Human Placenta, ed by Zheng J, In Tech Europe Rijeka, 2012, pp77-92
20. Hiden U, Glitzner E, Hartmann M and Desoye G. Insulin and the IGF system in the human placenta of normal and diabetic pregnancies. J Anat 215: 60-68, 2009
21. Chang W, Tu C, Chen TH, Komuves L, Oda Y, Pratt SA, Miller S and Shoback D. Expression and signal transduction of calcium-sensing receptors in cartilage and bone. Endocrinology 140: 5883-5893, 1999
22. Qu F, Li CB, Yuan BT, Qi W, Li HL, Shen XZ, Zhao G, Wang JT and Liu YJ. MicroRNA-26a induces osteosarcoma cell growth and metastasis via the Wnt/beta-catenin pathway. Oncol Lett 11: 1592-1596, 2016
23. Calvarano G, De Polis F and Sabatini G. Treatment with placental extract in periodontal disease. Dent Cadmos 57: 85-86, 1989
24. Atieh MA, Alsabeeha NH, Faggion CM, Jr. and Duncan WJ. The frequency of peri-implant diseases: a systematic review and meta-analysis. J Periodontol 84: 1586-1598, 2013
25. Larsson L, Decker AM, Nibali L, Pilipchuk SP, Berglundh T and Giannobile WV. Regenerative medicine for periodontal and peri-implant diseases. J Dent Res 95: 255-266, 2016