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Evaluation of fibroblast growth factor activity exerted by placental extract used as a cosmetic ingredient

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Summary

Background: The biological activities claimed for placental extract (PE) in its medical and cosmetic applications are largely assumed to be the combined effects of its various signaling molecules and nutritional constituents. But there are considerable uncertainties about this assumption.

Aims: To determine the specific biological activity of PE at a molecular level.

Methods: Fibroblast growth factor (FGF) activity was assessed based on the ability to induce proliferation of FGF receptor (FGFR)-overexpressing BaF3 cells.

Results: Porcine PE (PPE), an ingredient in numerous cosmetics, activated proliferation of BaF3 cells overexpressing FGFR subtypes 1c, 2c, 2b, 3c, or 4, that is, all the major FGFR subtypes. The effect was suppressed largely or partially when the cells were treated with a FGFR inhibitor PD173074, and the FGFR-negative BaF3 parent cells exhibited minimal growth promotion as compared to the FGFR-expressing BaF3 cells. The high (>10 kDa) and low (<3 kDa) molecular weight fractions of PPE were effective activators of FGFR signaling. PPE was found to contain sulfated glycosaminoglycans, including heparin/heparan sulfate and chondroitin sulfate, which serve as both structural stabilizers of FGFs and indispensable cofactors for FGF-FGFR signaling.

Conclusions: These results indicate that PPE is capable of evoking FGF signaling in cells via FGFRs. Given that recombinant FGFs have proven useful for medical/cosmetic purposes, our results suggest that the medical/cosmetic utility of PPE is provided at least partly through the activation of FGF signaling in epidermal, dermal, and subdermal tissues.

KEYWORDS
fibroblast growth factor, glycosaminoglycan, placental extract, receptor, signaling

1 | INTRODUCTION

Over the course of human history, placental tissue has been consumed or placental extract (PE) has been administered orally, topically, or through injection for nutritional, pharmaceutical, and cosmetic purposes. For nearly a half-century, industrialized manufacturing of PE has provided numerous benefits, or so it appears, to individuals suffering from such symptoms as skin aging,[1] nonhealing wounds,[2] contact hypersensitivity,[3] impaired liver function,[4] steatohepatitis,[5] and menopausal disorder.[6] Although the effectiveness of PE in some of these applications/indications may simply result from supplementation of nonspecific components such as small peptides,[7] nutritional amino acids, vitamins, and minerals, some may also result from specific biological signaling. However, the latter possibility has largely been unaddressed at a molecular level, in part because the processes for manufacturing PE involve very harsh conditions such

Ito and Yamada are equally contributed to this study.

as protease digestion and heat treatment that would not be expected to allow survival of polypeptide factors. These harsh manufacturing processes are considered important and are endorsed for their potential to eliminate biohazardous substances in the placental tissue.

Fibroblast growth factors (FGFs) are a large family of polypeptide signaling molecules encoded in mammals by 22 different genes,[8] many of which are expressed in placenta.[9] Most FGFs exert effects at a cellular level by activating FGF receptor (FGFR) tyrosine kinases in the cell membrane. Once activated, the receptor tyrosine kinases affect numerous crucial processes at whole-body level, including development, morphogenesis, angiogenesis, wound healing, neural and brain function, and metabolism. Recently, recombinant FGF proteins have been used as cosmetic ingredients and as drugs in cosmetic plastic surgery. The FGFs promote wound healing by stimulating proliferation of cells in the cutaneous system, increase collagen biosynthesis in dermal fibroblasts, and regulate the hair growth cycle by affecting the activity of various hair follicle cells.

In earlier reports, we showed that our cell-based system for assaying FGF signaling is both specific and sensitive.[10-12] This system utilizes mouse proB BaF3 host cells, which do not endogenously express any type of FGFR. Once transfected with an FGFR expression vector, the cells stably overexpressing FGFR (FGFR/BaF3 cells) can be stimulated to proliferate via an endogenous signaling pathway by treatment with cognate FGF ligands specific for the corresponding FGFR subtype expressed by the cells.[13]

2 | MATERIALS AND METHODS

2.1 | Reagents

Placental extract from porcine placenta (PPE) was obtained from Japan Bio Products Co., Ltd. (Tokyo, Japan). Recombinant FGF1, which is active with all FGFR subtypes, was prepared as described previously.[10] Heparin was obtained from Sigma-Aldrich Japan (Tokyo, Japan). The FGFR inhibitors PD173074, carbazole and sulfuric acid were purchased from Sigma-Aldrich Japan.

2.2 | Culture of BaF3 cells and cell proliferation assay

BaF3 cells were originally obtained from the RIKEN BioResource Center. Construction of stable BaF3 cell transfectants, each overexpressing one of the FGFR subtypes, and analysis of FGF-induced DNA synthesis were performed as previously described.[10] Untreated and FGFR-overexpressing BaF3 cells were routinely cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 10% WEHI-3-conditioned medium, 500 μg/mL G418, and 60 μg/mL kanamycin (growth medium) at 37°C under 5% CO_2. For experiments, cells were washed once with RPMI1640 supplemented with 10% FBS (assay medium) and plated into 96-well plates at a density of 2×10^4 cells/100 μL of assay medium/well. PPE or FGF1 samples diluted in 2 μL of assay medium were added to each well and cultured for 45 hours at 37°C. When FGF1 was examined as a sample, heparin was included at a final concentration of 5 μg/mL. Thereafter, 10 μL of WST-8 reagent (Cell Counting Kit-8; Chemical Dojin, Kumamoto, Japan) was added to each well. After incubation for an additional 3 hours, absorbance at 450 nm was measured using a microplate reader.

2.3 | Cellulose acetate membrane electrophoresis of glycosaminoglycans (GAGs)

Porcine PE was pretreated with 25 units of benzonase endonuclease in 10 mmol/L Tris-HCl (pH 7.4) or in PBS (pH 7.4) before electrophoresis. Both authentic GAG and the benzonase-digested PPE were resolved on cellulose acetate membrane electrophoresis using buffer prepared by mixing 3 volumes of 0.2 mol/L calcium acetate (pH 2.0) with 2 volumes of ethylene glycol. At pH 2.0, there was efficient separation of sulfated GAGs such as heparin/heparan sulfate and chondroitin sulfate from hyaluronic acid, which is not sulfated. The samples were blotted onto cellulose acetate membranes, and electrophoresis was conducted at 250 V/5 cm for 3.5 hours. After electrophoresis, the membranes were stained for 5 minutes with 1% alcian blue in 5% acetate, and the developed GAG signals were photographed.

2.4 | Quantitation of sulfated glycosaminoglycans

For direct quantitation of GAGs, PPE samples were analyzed for their uronic acid content, which is a common component of GAGs. Briefly, PPE was treated with heparinase and/or chondroitinase ABC for 24 hours or left untreated, after which the medium-to-high MW (MHMW) fraction (cutoff MW: 3 kDa) was isolated. Three milliliters of sulfuric acid was then added to each 500-μL MHMW fraction, which was then heated in boiling water for 20 minutes. To the resultant mixture was added 200 μL of 0.1% carbazole in ethanol, and the mixture was incubated for 2 hours at 30°C. Finally, the reaction product was measured based on its optical absorbance at 530 nm. Authentic bovine intestinal heparin was measured as the reference to obtain a standard curve. In this way, the uronic acid contents corresponding to that in heparin/heparan sulfate and chondroitin sulfate were determined.

2.5 | SDS-PAGE and western blotting

Laemmli SDS-PAGE was performed using 15% polyacrylamide gel as previously described.[14] Western blotting of FGF1 was performed using a highly specific anti-FGF1 monoclonal antibody (mAb) as previously described.[14]

2.6 | Assay for protection of FGF from trypsin digestion by PPE or heparin

Recombinant FGF1 was mixed with a test sample, authentic heparin, or PPE and then treated with trypsin for 30 minutes at 37°C, as previously described.[10] The resulting reaction mixture was analyzed for the presence of intact FGF1 by Western blotting using the aforementioned anti-FGF1 mAb.

3 | RESULTS

3.1 | PE activates cellular signaling through FGFRs

Although the growth-promoting activity of PE has been reported for several cell types,[15,16] specific FGF signaling through the FGFR has not been directly demonstrated. We showed in our earlier studies that a modified BaF3 cell system, originally established to determine the receptor specificity of physiological FGF ligands,[13] is useful for studying cellular signaling through FGFRs.[10-12] Here, we used this system to examine the biological activity of PPE in comparison with the activity of FGF1, a universal FGF ligand for every subtype of FGFR. The parent BaF3 cells only proliferate in response to their cognate growth factor, interleukin-3, which is present in WEHI-3 cell-conditioned medium. However, transfecting the cells with a FGFR enables them to proliferate in response to FGF ligands able to bind to the particular FGFR subtype expressed on the cell surface.[13] This reflects activation of an endogenous signaling pathway for BaF3 cell proliferation, which is triggered by the activation of the FGFR tyrosine kinase.[11]

We therefore examined the growth-promoting activity of PPE or FGF1 toward BaF3 cells overexpressing FGFR1c (FGFR1c/BaF3), FGFR2c (FGFR2c/BaF3), FGFR2b (FGFR2b/BaF3), FGFR3c (FGFR3c/BaF3), or FGFR4 (FGFR4/BaF3). When we measured cell numbers using WST-8 reagent, based on absorbance at 450 nm, we found that PPE strongly increased numbers of FGFR1c/BaF3 cells (Figure 1A, filled circles), but not the wild-type BaF3 parent cells, which do not express FGFRs (Figure 1A, open circles). The activity toward FGFR1c/BaF3 cells of PPE was then compared with that of FGF1 (Figure 1B, left panel and right panel, respectively; filled circles) by conducting parallel experiments. It was shown that the maximum activity of PPE toward these cells was approximately 30% of that of FGF1 (shown in the vertical axis of Figure 1B, left panel). In the presence of the FGFR inhibitor PD173074, the PPE-induced increase in cell number (Figure 1B, left panel, filled circles) was significantly reduced (Figure 1B, left panel, open circles). The FGF1-induced increase in cell number (Figure 1B, right panel, filled circles) was totally inhibited in the presence of PD173074 (Figure 1B, right panel, open circles).

Similarly, PPE increased numbers of FGFR2c/BaF3 (Figure 1C), FGFR2b/BaF3 (Figure 1D), FGFR3c/BaF3 (Figure 1E), and FGFR4/BaF3 cells (Figure 1F). The optimum concentration of PPE ranged from 0.3 to 1 mg/mL. The maximum activity of PPE toward these cells varied between approximately 30% and 50% of that of FGF1 (Figure 1C–F, left panels, filled circles). The inhibitory effect of PD173074 on the PPE-induced increase in cell number was not complete for these cells (Figure 1C–F, left panels, open circles). In contrast, the FGF1-induced increase in cell number was totally inhibited (Figure 1B–F, right panels, open circles).

3.2 | FGF ligand activity is contained in the high, medium, and low MW fractions of PPE

To determine the component of PPE that exerts the FGF effect, we used MW cutoff membranes to fractionate PPE into high (HMW: 10 kDa < MW), medium (MMW: 3 kDa < MW < 10 kDa), and low MW (LMW: MW < 3 kDa) fractions. Analysis of growth-promoting activity of these PPE fractions revealed that the HMW fraction contained strong activity toward FGFR1c/BaF3 cells (Figure 2A, filled circles). Unexpectedly, the LMW fraction also strongly activated FGFR1c/BaF3 cells (Figure 2C, filled circles). The MMW fraction was the least strong toward FGFR1c/BaF3 cells (Figure 2B, filled circles). The FGFR inhibitor PD173074 effectively inhibited the activities of HMW and MMW fractions, whereas it inhibited the activity of LMW fraction only partially (Figure 2, open circles).

Similarly, FGFR2b/BaF3 (Figure 3A), FGFR3c/BaF3 (Figure 3B), and FGFR4/BaF3 cell (Figure 3C) proliferation was stimulated by the HMW, MMW, and LMW fractions (circles, triangles, and squares, respectively). By contrast, none of these fractions induced proliferation of FGFR-free BaF3 parent cells (Figure 3D).

3.3 | PE contains sulfated GAG

We next examined whether PPE contains sulfated GAG. Preliminary experiments analyzing toluidine blue dye metachromasia suggested that PPE does contain GAGs. Therefore, the GAGs in PPE were resolved using cellulose acetate membrane electrophoresis at pH 2.0. Because preliminary experiments suggested that GAGs are present in PPE as in the form of aggregates with nucleic acids, PPE was predigested with benzonase endonuclease prior to the electrophoresis. As shown in Figure 4, PPE (lanes 1 and 2; in different buffers with various efficiencies of endonuclease digestion) contains sulfated GAGs that migrate closely to chondroitin sulfate C (lane 4) and heparin (lane 5). Hyaluronic acid, a nonsulfated GAG, did not migrate at pH 2.0. Although alcian blue (pH 1.0) does not efficiently stain hyaluronic acid (lane 3 represents inefficient staining of a large amount of hyaluronic acid standard), PPE also appeared to contain hyaluronic acid when electrophoresis and staining were conducted at a pH that allowed its efficient migration and staining.

To measure levels of specific types of GAG, PPE was left untreated or digested with enzymes that specifically degrade heparin/heparan sulfate and/or chondroitin sulfate, followed by isolation of medium-to-high MW (MHMW: MW > 3 kDa) fractions after each treatment. The isolated MHMW fractions were then analyzed for their uronic acid content using the carbazole-sulfuric acid method (Table 1). Bovine intestinal mucosa heparin was used as a reference for uronic acid quantitation. As shown, the MHMW fraction obtained from 250 μg of input PPE contained 14.65 μg of heparin-equivalent uronic acid. After heparinase digestion, which breaks down both heparin and heparan sulfate at highly sulfated positions, the uronic acid remaining in the MHMW fraction had decreased to 10.43 μg. A separate PPE sample digested with chondroitinase ABC, which breaks down all types of chondroitin sulfate, yielded 6.12 μg of GAG in the MHMW fraction, and combined digestion with heparinase and chondroitinase ABC yielded only 4.11 μg of uronic acid in the MHMW fraction. Thus, the results of both cellulose acetate membrane electrophoresis and digestion with specific GAG-digesting enzymes strongly suggest that PPE contains both heparin/heparan sulfate and chondroitin sulfate.

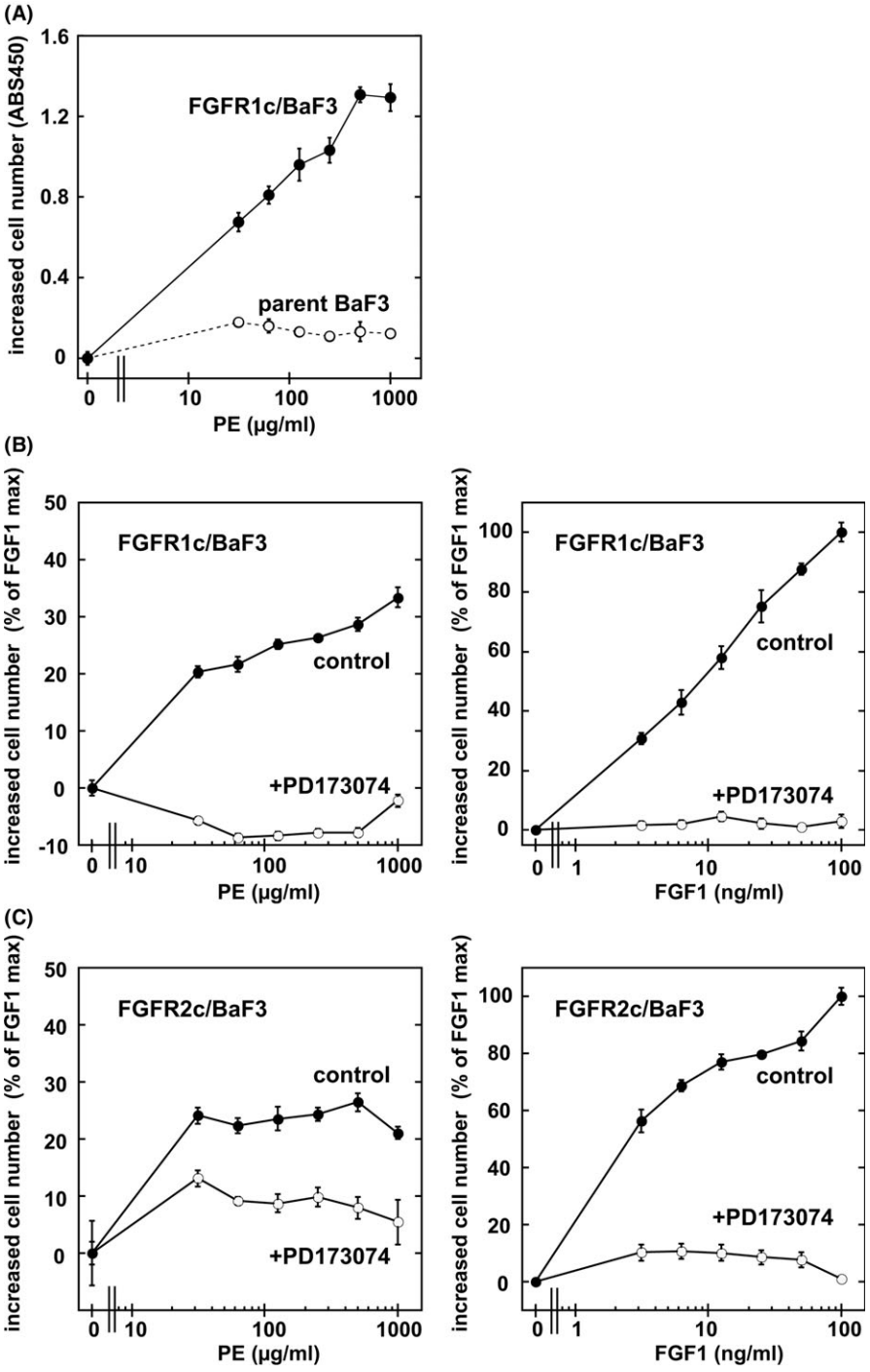

FIGURE 1 Stimulation of FGFR-dependent BaF3 cell proliferation with porcine placental extract (PPE) and FGF1. (A), Effects of PPE on proliferation of FGFR1c/BaF3 cells (filled circles) and FGFR-negative BaF3 cells (open circles). The induced increase in cell number is presented as absorbance at 450 nm. B–F, Effects of PPE (left panels) and FGF1 (right panels) on proliferation of FGFR1c/BaF3 cells (B), FGFR2c/BaF3 cells (C), FGFR2b/BaF3 cells (D), FGFR3c/BaF3 cells (E), and FGFR4/BaF3 cells (F) in the absence (filled circles) and presence of the FGFR inhibitor PD173074 at 150 nM (open circles). The induced increase in cell number is presented as percentage of FGF1-induced maximum increase in cell number in the parallel experiments. Values are means ± SE of hextuplicate samples

3.4 | Placental GAGs contained in PPE protect FGF from trypsin digestion, just as pure heparin does

Size-exclusion chromatography revealed that the HMW (MW > 10 kDa) fraction of PPE comprised 5% of the total substances with optical absorbance at 220 nm in PPE, while the rest are lower MW substances (personal communication from the manufacturer). Separation of the PPE on SDS polyacrylamide gel revealed that the HMW fraction gives a smear of Coomassie Brilliant Blue-stainable bands with MWs ranging from 10 to 30 kDa (Figure 5A, lane 1). Mass spectrometry (TOF-MS) analysis of total PPE revealed numerous small molecules ranging from 0.1 to 1 kDa (data not shown).

Western blot analysis of the PPE using a highly specific anti-FGF1 mAb revealed that PPE may contain a trace amount of FGF1 antigenicity with a molecular weight close to that of the N-terminal truncated form of full-length FGF1, but it was invisible without image enhancement (Figure 5B, lane 1).

In addition to acting as a cofactor assisting formation of an active signaling complex with FGF and FGFR,[17,18] heparin and

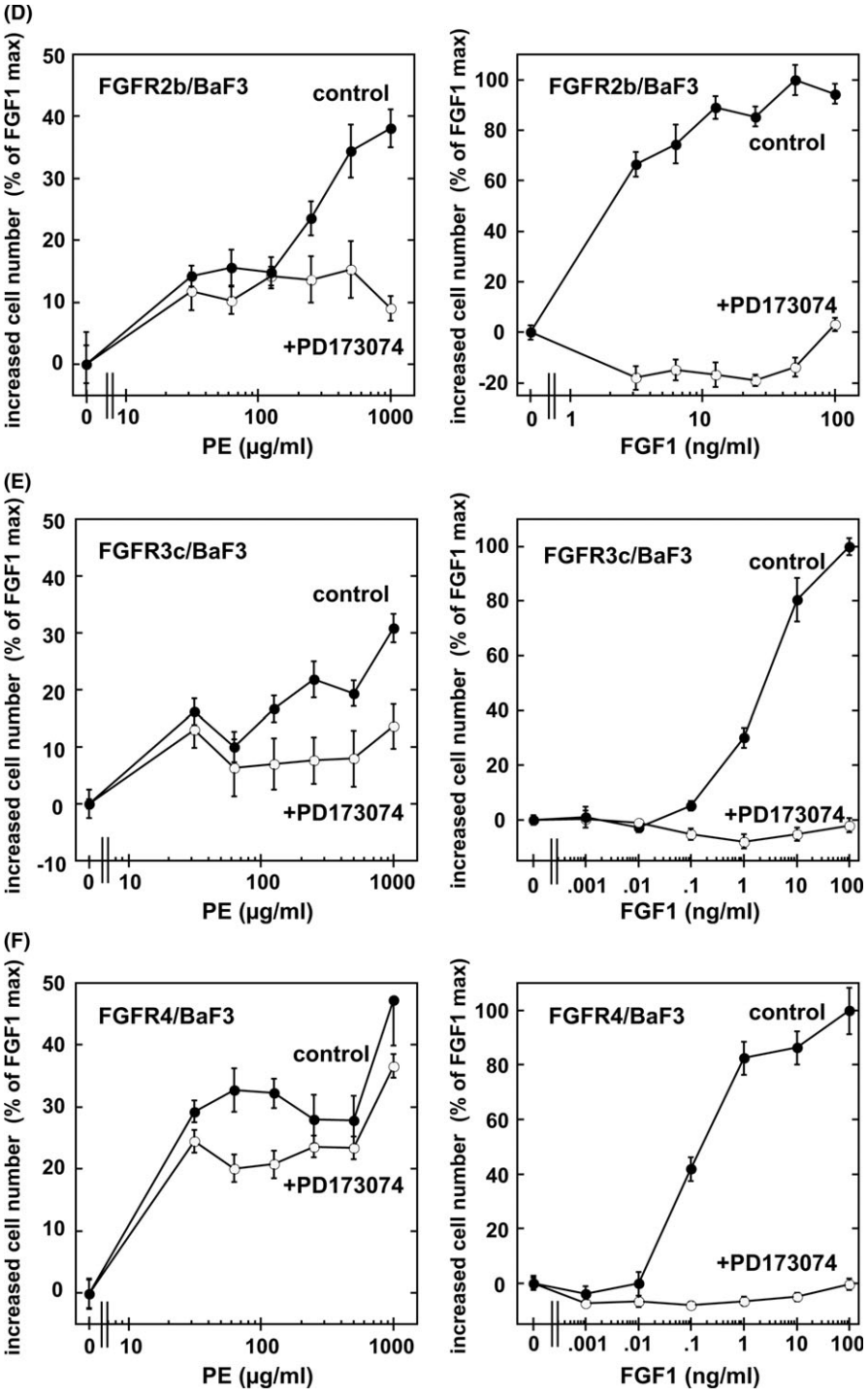

FIGURE 1 (continued)

heparan sulfate binding to FGF protects it from proteolytic degradation. We therefore tested whether PPE could protect recombinant FGF1 (N-terminal truncated form) from tryptic digestion. Recombinant FGF1 was incubated with trypsin in the absence and presence of PPE or heparin, and the resulting reaction mixture was analyzed for FGF1 antigenicity by Western blotting (Figure 5B). Although recombinant FGF1 (1 μg, lane 2; in addition to monomeric FGF1 indicated by the arrow, the dimer and other oligomers are also visible) was easily digested and degraded by 0.025% trypsin (lane 6), it was effectively protected from digestion in the presence of 5 μg heparin (lane 3), but not 50 ng of heparin (lane 4). Similarly, untreated PPE protected recombinant FGF1 from tryptic digestion (lane 5). Moreover, following treatment with either heparinase (lane 7) or chondroitinase ABC (lane 8), PPE was still able to protect recombinant FGF1 from tryptic digestion. This strongly suggests that both heparin/heparan sulfate and chondroitin sulfate in PPE contribute to the protection of FGF from tryptic digestion. Indeed, many members of the FGF ligand family bind both heparin and chondroitin sulfate.[19]

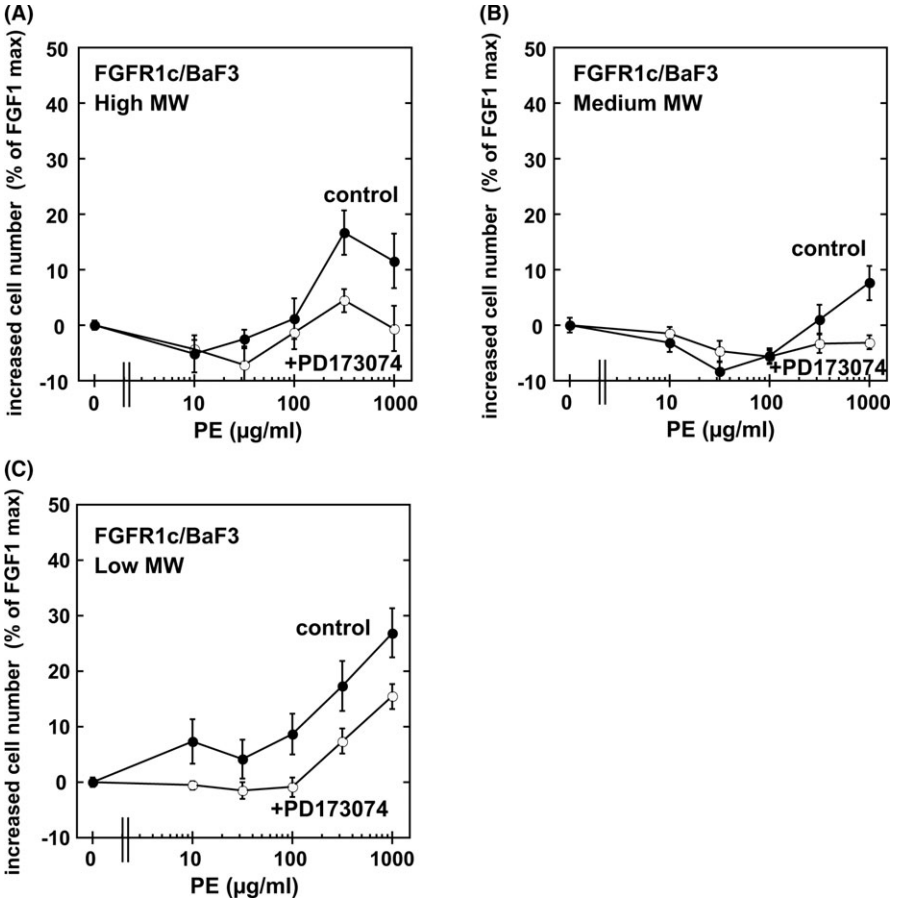

FIGURE 2 Stimulation of FGFR1c/BaF3 cell proliferation by molecular weight fractionated PPE in the absence and presence of the FGFR inhibitor PD173074. Effects of PPE separated into HMW (A, 10 kDa < MW), MMW (B, 3 kDa < MW < 10 kDa), and LMW (C, MW < 3 kDa) fractions on FGFR1c/BaF3 cell proliferation in the absence (filled circles) and presence of the FGFR inhibitor PD173074 at 150 nM (open circles). The indicated concentrations correspond to those of the original PPE solution before fractionation. The induced increase in cell number is presented as percentage of FGF1-induced maximum increase in cell number determined in the parallel experiments. Values are means ± standard error of hextuplicate samples

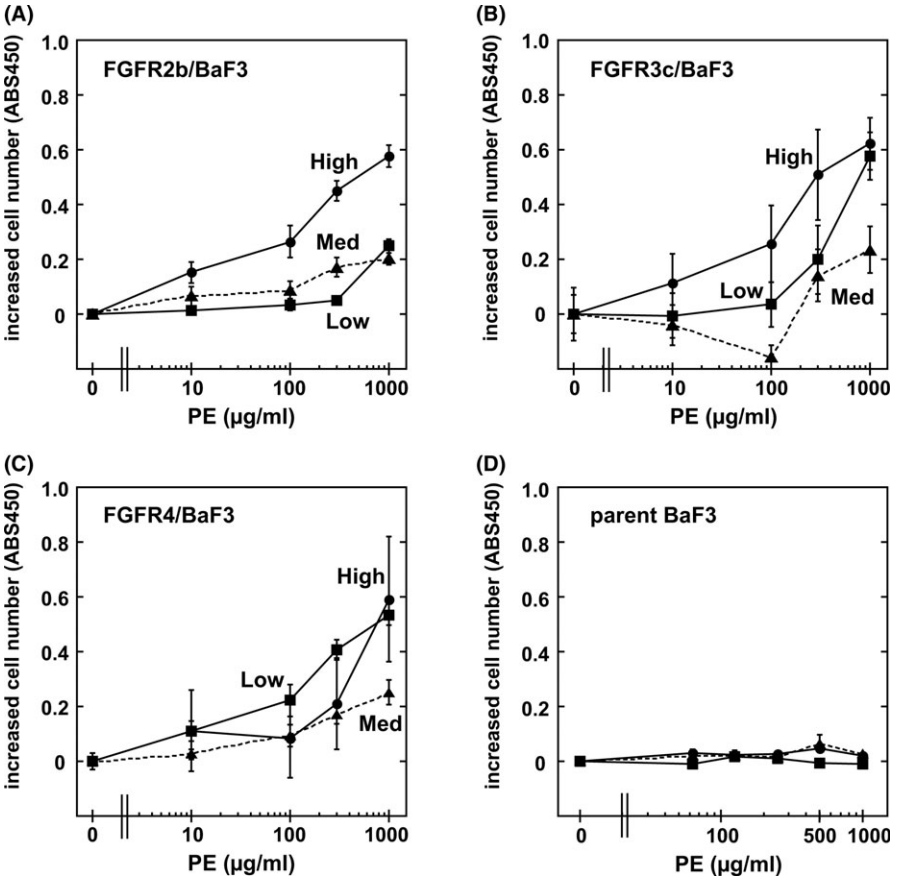

FIGURE 3 Proliferation stimulation of FGFR2b/BaF3 cells, FGFR3c/BaF3 cells, FGFR4/BaF3 cells, and parent BaF3 cells by molecular weight fractionated PPE. Effects of PPE separated into HMW (10 kDa < MW; circles), MMW (3 kDa < MW < 10 kDa; triangles), and LMW (MW < 3 kDa; squares) fractions on proliferation of FGFR2b/BaF3 cells (A), FGFR3c/BaF3 cells (B), FGFR4/BaF3 cells (C), and parent BaF3 cells (D). The indicated concentrations correspond to those of the original PPE solution before fractionation. Values are means ± SE of quadruplicate samples

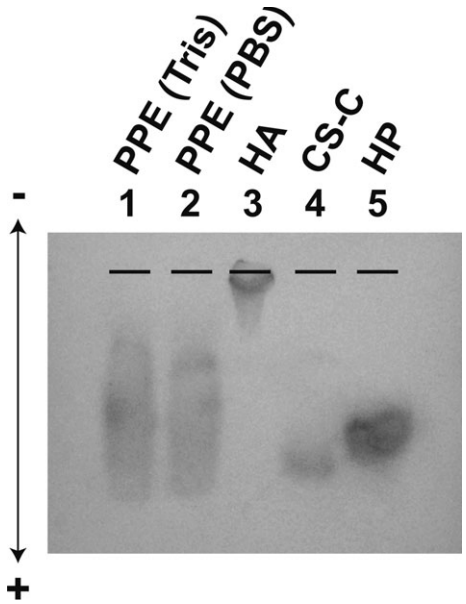

FIGURE 4 PPE contains sulfated glycosaminoglycans (GAGs). GAGs were analyzed using cellulose acetate membrane electrophoresis. Before electrophoresis, 125 μg PPE was pretreated with 25 units benzonase endonuclease in 10 mmol/L Tris-HCl, pH 7.4 (lane 1), or in PBS, pH 7.4 (lane 2). The other samples were 1.25 μg hyaluronic acid (lane 3), 1.25 μg chondroitin sulfate C (lane 4), and 1.25 μg heparin (lane 5). The samples were loaded onto a cellulose acetate membrane at the positions marked by the horizontal lines, and electrophoresis was conducted using a pH 2.0 buffer, which enables efficient separation of sulfated GAGs from nonsulfated hyaluronic acid. The anode and cathode directions were marked by + and −, respectively. After electrophoresis, the membrane was stained with alcian blue solution (pH 1.0), which efficiently stains sulfated GAGs, but stains hyaluronic acid less efficiently

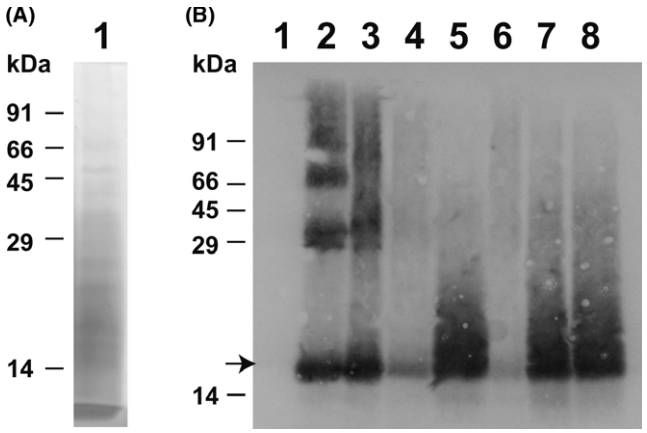

FIGURE 5 PPE contains a variety of polypeptide species, and PPE-GAG protects FGF from tryptic digestion. (A), SDS-PAGE and Coomassie Blue staining of PPE proteins. Lane 1 shows the single PPE lot examined in this paper. PPE contains peptides/polypeptides with a broad range of MWs, most of which are too small to be resolved by SDS-PAGE (see text). (B), Western blot analysis using a highly specific anti-FGF1 mAb. Lane 1: untreated PPE sample resolved on SDS-PAGE. Lane 2: 1 μg untreated recombinant FGF1 (biologically active N-terminal truncated form). Lanes 3, 4, 6: 1 μg recombinant FGF1 digested with trypsin in the absence (lane 6) or presence of 5 μg heparin (lane 3) or 50 ng heparin (lane 4). Lanes 5, 7, 8: 1 μg recombinant FGF1 digested by trypsin in the presence of untreated PPE (lane 5) or PPE digested with heparinase (lane 7) or chondroitinase ABC (lane 8). Note that untreated PPE, heparinase-digested PPE, and chondroitinase ABC-digested PPE all protected recombinant FGF1 from tryptic digestion. The arrow indicates the position of the recombinant FGF1 protein used in this experiment

TABLE 1 Uronic acid content in the medium-to-high molecular weight (MHMW) fraction of PPE before and after digestion with GAGases

GAGase used for PPE digestion	MHMW uronic acid content (μg) (as heparin equivalent)
None (input PPE: 250 μg)	14.65
Heparinase	10.43
Chondroitinase ABC	6.12
Heparinase and chondroitinase ABC	4.11

4 | DISCUSSION

The PPE product used for this experiment was manufactured according to the standard procedure for such production: Porcine placentas collected in Japan are treated with protease and heat sterilization and then spray-dried (information disclosed by the manufacturer). Through this procedure, most polypeptide signaling molecules are likely lost. However, the present results clearly indicate that PPE mediates signaling through activation of FGFR tyrosine kinase and that the active entities are present as HMW (>10 kDa), MMW (3 kDa < MW < 10 kDa), and LMW (<3 kDa) substances.

These results suggest that a portion of endogenously expressed FGF ligands (17-25 kDa) in placenta may have survived the manufacturing process and storage as powder at room temperature as partially truncated, yet active, HMW substances. This interpretation is supported by the observation that PPE contains GAGs that protect recombinant FGF from protease digestion, just as pure heparin protects FGF. Indeed, we detected trace amounts of endogenous placental FGF1 with a MW close to that of the active N-terminal truncated form of FGF1.[20] Our results also suggest that small amounts of other FGF ligands in placenta may have survived the manufacturing process as partially truncated active forms. Although the FGF activity contained in PPE was strongly mediated through FGFR1c, it was also mediated through FGFR2c, FGFR2b, FGFR3c, and FGFR4 (Figure 1). In full-thickness skin, physiological expression levels of FGFR1 and FGFR3 are higher than those of FGFR2 and FGFR4.[21,22] Both FGFR1c and FGFR2b are important for homeostasis of the cutaneous system: Activation of FGFR1c stimulates proliferation of many cell types, while FGFR2b activation stimulates proliferation of epithelial cells, including keratinocytes. On the other hand, signaling through FGFR3c and FGFR4 often results in both growth and nongrowth responses, such as quiescence in hair follicles and synthesis of bile acid in liver. This means that the overall

biological activity of PPE through the FGF system may differ depending on the context, including the spatiotemporal expression pattern of FGFRs.

Our findings indicate that while the activity of PPE toward FGFR/BaF3 cells was strongly inhibited by the FGFR inhibitor PD173074, partial activity persisted in the presence of the inhibitor (Figure 1C–F). In some cells, the remaining activity appeared to be stronger than the activity toward wild-type BaF3 cells, which lack FGFRs (compare Figure 1A and Figure 1C–F). The reason is not yet clear.

The results also indicate that portions of the FGF signaling activity of PPE are present as LMW and MMW substances. Given that synthetic partial peptides derived from the primary structures of FGF ligands have been shown to induce biological activities,[23] the activity of LMW and MMW substances is noteworthy and warrants further analysis of the nature and mechanism of these active substances.

Glycosaminoglycans are abundant in the extracellular matrix of numerous tissues, which is consistent with the present finding that PPE contains GAGs. In physiological settings, GAGs are present as varied molecular forms in the extracellular matrix and as cell surface proteoglycans.[24] GAGs serve both as a reservoir and structural stabilizer/protector for heparin-binding growth factors, such as FGF ligands.[17,18] It is also known that some GAGs, particularly heparan sulfate/heparin and some types of chondroitin sulfate, bind to FGF and serve as indispensable cofactors for the formation of active FGF-FGFR signaling complexes.[18,19] FGFs are unstable by themselves, but once bound to a sulfated GAG, they are structurally stable and resistant to protease digestion.[25] Our results clearly indicate that PPE is able to protect recombinant FGF from trypsin digestion. This suggests that the sulfated GAGs present in porcine placenta are sufficient to preserve small amounts of biologically active FGFs throughout the harsh PPE manufacturing process, even if they are not the fully intact endogenously expressed FGF ligands. We also found that PPE can potentiate activity of recombinant FGF toward FGFR, just as pure heparin does (data not shown).

In sum, our results clearly indicate that PPE is capable of exerting FGFR-dependent activity in cells. Given that recombinant FGFs have proven to be useful for medical/cosmetic purposes in humans, our results suggest that the medical/cosmetic utility of PPE is provided at least in part through the activation of FGF signaling in the cutaneous system.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Yoshikawa C, Koike K, Takano F, Sugiura K, Suzuki N. Efficacy of porcine placental extract on wrinkle widths below the eye in climacteric women. *Climacteric*. 2014;17:370-376.
2. Tiwary SK, Shukla D, Tripathi AK, Agrawal S, Singh MK, Shukla VK. Effect of placental extract gel and cream on non-healing wounds. *J Wound Care*. 2006;15:325-328.
3. Kim YS, Park JJ, Sakoda Y, et al. Preventive and therapeutic potential of placental extract in contact hypersensitivity. *Int Immunopharmacol*. 2010;10:1177-1184.
4. Wu J, Yang T, Wang C, et al. Laennec protects murine from concanavalin A-induced liver injury through inhibition of inflammatory reactions and hepatocyte apoptosis. *Biol Pharm Bull*. 2008;31:2040-2044.
5. Choi JY, Lee K, Lee SM, et al. Efficacy and safety of human placental extract for alcoholic and nonalcoholic steatohepatitis: An open-label, randomized, comparative study. *Biol Pharm Bull*. 2014;37:1853-1859.
6. Lee YK, Chung HH, Kang SB. Efficacy and safety of human placental extract in alleviating climacteric symptoms: Prospective, randomized, double-blind, placebo-controlled trial. *J Obstet Gynaecol Res*. 2009;35:1096-1101.
7. Togashi S, Takahashi N, Iwama M, Watanabe S, Tamagawa K, Fukui T. Antioxidative collagen-derived peptides in human-placenta extract. *Placenta*. 2002;23:497-502.
8. Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. *Trends Genet*. 2004;20:563-569.
9. Gospodarowicz D, Cheng J, Lui GM, Fujii DK, Baird A, Böhle P. Fibroblast growth factor in the human placenta. *Biochem Biophys Res Commun*. 1985;128:554-562.
10. Motomura K, Hagiwara A, Komi-Kuramochi A, et al. An FGF1:FGF2 chimeric growth factor exhibits universal FGF receptor specificity, enhanced stability and augmented activity useful for epithelial proliferation and radioprotection. *Biochim Biophys Acta*. 2008;1780:1432-1440.
11. Suzuki M, Uehara Y, Motomura-Matsuzaka K, et al. βKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. *Mol Endocrinol*. 2008;22:1006-1014.
12. Nakamura M, Uehara Y, Asada M, et al. Sulfated glycosaminoglycans are required for specific and sensitive fibroblast growth factor (FGF) 19 signaling via FGF receptor 4 and betaKlotho. *J Biol Chem*. 2011;286:26418-26423.
13. Ornitz DM, Xu J, Colvin JS, et al. Receptor Specificity of the Fibroblast Growth Factor Family. *J Biol Chem*. 1996;271:15292-15297.
14. Imamura T, Oka S, Tanahashi T, Okita Y. Cell cycle-dependent nuclear localization of exogenously added fibroblast growth factor-1 in BALB/c 3T3 and human vascular endothelial cells. *Exp Cell Res*. 1994;215:363-372.
15. Seo T, Han I, Yoon J, et al. Growth-promoting activity of Hominis Placenta extract on regenerating sciatic nerve. *Acta Pharmacol Sin*. 2006;27:50-58.
16. Cho H, Ryou J, Lee J, Lee M. The effects of placental extract on fibroblast proliferation. *J Cosmet Sci*. 2008;59:195-202.
17. Sommer A, Rifkin DB. Interaction of heparin with human basic fibroblast growth factor: Protection of the angiogenic protein from proteolytic degradation by a glycosaminoglycan. *J Cell Physiol*. 1989;138:215-220.

18. Pellegrini L. Role of heparan sulfate in fibroblast growth factor signalling: A structural view. *Curr Opin Struct Biol*. 2001;11:629-634.
19. Asada M, Shinomiya M, Suzuki M, et al. Glycosaminoglycan affinity of the complete fibroblast growth factor family. *Biochim Biophys Acta*. 2009;1790:40-48.
20. Burgess WH, Mehlman T, Marshak DR, Fraser BA, Maciag T. Structural evidence that endothelial cell growth factor beta is the precursor of both endothelial cell growth factor alpha and acidic fibroblast growth factor. *Proc Natl Acad Sci USA*. 1986;83:7216-7220.
21. Kawano M, Komi-Kuramochi A, Asada M, et al. Comprehensive analysis of FGF and FGFR expression in skin: FGF18 is highly expressed in hair follicles and capable of inducing anagen from telogen stage hair follicles. *J Invest Dermatol*. 2005;124:877-885.
22. Komi-Kuramochi A, Kawano M, Oda Y, et al. Expression of fibroblast growth factors and their receptors during full-thickness skin wound healing in young and aged mice. *J Endocrinol*. 2005;186:273-289.
23. Ito C, Saitoh Y, Fujita Y, et al. Decapeptide with fibroblast growth factor (FGF)-5 partial sequence inhibits hair growth suppressing activity of FGF-5. *J Cell Physiol*. 2003;197:272-283.
24. Perrimon N, Bernfield M. Cellular functions of proteoglycans—an overview. *Semin Cell Dev Biol*. 2001;12:65-67.
25. Yoneda A, Asada M, Oda Y, Suzuki M, Imamura T. Engineering of an FGF-proteoglycan fusion protein with heparin-independent, mitogenic activity. *Nat Biotechnol*. 2000;18:641-644.

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