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Oversulfated fucoidan inhibits the basic fibroblast growth factor-induced tube formation by human umbilical vein endothelial cells: its possible mechanism of action

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Abstract

We have previously demonstrated that chemically oversulfated fucoidan (OSF) but not native fucoidan (NF) effectively suppresses the tube structure formation by human umbilical vein endothelial cells (HUVEC) on the basement membrane preparation, Matrigel. In this study, using more defined systems where basic fibroblast growth factor (bFGF) induces the tube formation by HUVEC on collagen gel, we investigated the mechanism responsible for the inhibition of angiogenesis by OSF in vitro. Unlike NF and desulfated fucoidan (desF), OSF potently inhibited the bFGF-induced HUVEC migration and tube formation. ELISA for tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) in the culture media indicated that OSF increased the bFGF-induced release of PAI-1 antigen, but not of t-PA antigen. Analyses of the binding of bFGF to HUVEC surfaces and the following protein tyrosine phosphorylation revealed that OSF could promote the cell binding and autophosphorylation of 140 and 160 kDa receptors. In heparitinase-treated HUVEC, contrarily, the bFGF binding and PAI-1 release were decreased by OSF. These results suggest that OSF is a highly sulfated unique polysaccharide that can promote the binding of bFGF to the heparan sulfate molecules required for binding to the high affinity receptors with tyrosine kinase activity. The resultant increase in PAI-1 release may play a key role for the prevention of cell migration accompanied by matrix proteolysis.

Keywords: Fucoidan; Basic fibroblast growth factor; Angiogenesis; Plasminogen activator inhibitor-1; Endothelial cell (human umbilical vein)

1. Introduction

Angiogenesis is a fundamental process by which microblood vessels are newly formed. Highly regulated and transient angiogenesis plays an important role in embryogenesis, wound healing and corpus luteum formation [1,2]. By contrast, uncontrolled and persistent angiogenesis is involved in cancer, diabetic retinopathy and rheumatoid [1,2]. Therefore, suppression of the pathological neovascularization

Abbreviations: NF, native fucoidan; OSF, oversulfated fucoidan; u-PA, urokinase-type plasminogen activator; HUVEC, human umbilical vein endothelial cells; PAI-1, plasminogen activator inhibitor-1; t-PA, tissue-type plasminogen activator; bFGF, basic fibroblast growth factor; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; desF, desulfated fucoidan; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; HPF, high power field

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may result in new therapeutic approaches to these disorders.

Fucoidan (NF) is a sulfated poly(L-fucopyranose) present in brown marine algae and has been reported previously to have anticoagulant activity in vitro and in vivo [3]. Our previous studies have shown that the anticoagulant activity of NF is improved by its further sulfation, and that the oversulfated fucoidan (OSF) is effective for the prevention of lipopolysaccharide-induced hepatic vein thrombosis in hyperlipemic rats [4]. OSF also inhibited the invasion of 3LL cells through the reconstituted basement membrane, Matrigel, by suppressing the laminin-induced urokinase-type plasminogen activator (u-PA) [5] and the tube formation of cultured human umbilical vein endothelial cells (HUVEC) in Matrigel by increasing the release of plasminogen activator inhibitor-1 (PAI-1) [6]. These findings suggest that OSF acts as a suppressor of cell migration that results from matrix proteolysis.

In this study, we focus on how OSF increases the release of PAI-1 from HUVEC cultured in Matrigel. Because the reconstituted membrane, derived from murine Engelbreth-Holm-Swarm sarcoma, contains tissue-type plasminogen activator (t-PA), laminin, type IV collagen and various angiogenic factors including basic fibroblast growth factor (bFGF), these factors may cooperatively affect the tube formation by HUVEC. Therefore, the present experiments were designed to test for the potential role of OSF in a defined angiogenic system, where bFGF induces the tube formation by HUVEC on collagen gel. The results demonstrate that OSF increases the bFGF-induced PAI-1 release from HUVEC by increasing the binding of bFGF to the cell surface heparan sulfate (HS) molecules, which are required for binding to the high affinity receptors with tyrosine kinase activity.

2. Materials and methods

2.1. Materials

The following materials were commercially obtained: *Fucus vesiculosus* NF, porcine intestinal heparin and murine Engelbreth-Holm-Swarm sarcoma heparan sulfate proteoglycan (HSPG) from Sigma; recombinant human bFGF from Becton Dickinson Labware, Bedford, MA, USA; human plasma fibronectin from Boehringer Ingelheim; *Flavobacterium heparinum* heparitinase I from Seikagaku Kogyo Co., Tokyo, Japan; porcine type I and type IV collagens from Nitta Gelatin Co., Osaka, Japan; and human plasminogen from American Diagnostica, Inc., Greenwich, CT, USA.

2.2. HUVEC culture

Cryo HUVEC and the culture medium EGM-2 were purchased from Sanko Junyaku Co., Ltd., Tokyo, Japan. EGM-2 is composed of modified MCDB 131 medium and the supplements that contain fetal bovine serum, epidermal growth factor, vascular endothelial growth factor, bFGF, insulin-like growth factor, heparin, ascorbic acid, hydrocortisone, amphotericin B and gentamicin. The cells were grown in heparin-removed EGM-2 at 37°C in a humidified 5% CO_2 atmosphere. Subcultures were obtained by treating the HUVEC cultures with 0.05% trypsin/0.53 mM EDTA (Gibco) at 37°C for 5 min. HUVEC in the 3rd to 4th passages were used for experiments. The culture medium used for the experiments was modified MCDB 131 supplemented with 4% TCM, a proprietary formulation of vitamins and other molecules (does not contain bFGF) from CELOX Laboratories, MN, USA (TCM/MCDB 131).

2.3. Tube formation by HUVEC

Four hundred μl of a type I collagen solution (2.4 mg/ml) containing bFGF (20 ng/ml), fibronectin and plasminogen (20 μg/ml each) were placed into each well of a 24-well culture plate at 4°C and allowed to polymerize by incubation at 37°C. HUVEC (5×10^4 cells) were seeded on the collagen gel in 1 ml of TCM/MCDB 131 supplemented with the above concentrations of bFGF, fibronectin, plasminogen and, if indicated, the definite concentrations of polysaccharide. The cells were incubated at 37°C for 24 h in a humidified 5% CO_2 atmosphere. Three different phase-contrast microscopic fields ($\times100$) per well were photographed, and the micrographic pictures were put on a computer. The total length of tube structures in each photograph was measured using Adobe Photoshop software.

2.4. Assay of HUVEC migration

Polycarbonate filter Transwell inserts (24 well size) with 8-μm pores were used for this invasion assay. Type IV collagen (50 μl from a 250 μg/ml dilution in TCM/MCDB 131 supplemented with the above concentrations of bFGF, fibronectin and plasminogen) was applied to the upper surface of each filter and dried at 25°C. To the lower chamber were added 700 μl of TCM/MCDB 131 supplemented with bFGF, fibronectin, plasminogen and, if indicated, polysaccharide. HUVEC (5×10^4 cells/ml, 200 μl) were added to the upper chamber and incubated at 37°C for 48 h. HUVEC that remained on the upper side of the filter were removed with cotton swabs. Filters were then fixed and stained with Giemsa's reagent (Merck). The stained cells were counted in three fields under a ×100 high power field (HPF).

2.5. Purification and modification of NF

NF (200 mg) was purified on a Sephadex G-100 column (2.5×36 cm) equilibrated with 0.5 M NaCl. OSF with a molecular mass of 100–130 kDa was prepared as described previously [4]. Briefly, the purified NF was further sulfated in a mixture of dimethylformamide and sulfur trioxide–trimethylamine complex at 50°C for 24 h. The product was recovered by ethanol precipitation, followed by purification on the Sephadex G-100 column. The yield of OSF was 69%; the sulfate content was estimated to be 52.4% (for NF, 31.2%). Desulfated fucoidan (desF) was prepared by treating NF with 10% (v/v) methanol in dimethyl sulfoxide at 80°C for 18 h [4].

2.6. Assay of t-PA and PAI-1 antigens

Antigen levels of t-PA and PAI-1 in the conditioned media were determined with ELISA kits, TintElize t-PA and TintElize PAI-1 (Biopool AB, Umeâ, Sweden), respectively, according to the manufacturer's instructions.

2.7. Assay of bFGF binding

HUVEC were seeded at a density of 5×10^3 cells/cm^2 into each well of a 24-well culture plate in heparin-removed EGM-2 and allowed to reach confluency. After removal of the conditioned media, the HUVEC monolayers were rinsed twice with 1 ml of Dulbecco's modified Eagle's medium (DMEM). The rinsed monolayers were treated at 37°C for 24 h in 1 ml of TCM/MCDB 131 with or without 0.02 units/ml heparitinase I. After washing with DMEM, the HUVEC monolayers were treated at 4°C for 30 min with 1 ml of DMEM containing 20 ng/ml bFGF, followed by extraction with 1 ml of PBS containing 0.5% Triton X-100. The cell lysates were centrifuged at $9000\times g$ for 10 min, and the supernatants were subjected to bFGF antigen assay. The assay was performed with ELISA kit (TiterZyme FGF basic EIA, PerSeptive Diagnostics, Inc.) according to the manufacturer's instructions.

2.8. Detection of protein tyrosine phosphorylation

Confluent cultures of HUVEC in 175-cm^2 tissue culture flasks were rinsed with DMEM and incubated at 37°C for 30 min in 20 ml of TCM/MCDB 131 containing bFGF (20 ng/ml). Each cell culture was detached by treating with 10 ml of 0.05% trypsin/0.53 mM EDTA, and pelleted by centrifugation. The pellet was washed three times with 10 ml of cold PBS, and centrifuged again. The washed cells (5×10^6) were lysed at 4°C for 30 min in 100 μl of PBS containing 2 mM EDTA/10% glycerol/1% Triton X-100. Insoluble materials were removed by centrifugation at $10\,000\times g$ for 20 min. The cell-free lysates (10 μg protein/μl, 20 μl each) were subjected to 10% SDS–polyacrylamide gel electrophoresis [7]. The separated proteins were transferred to a nitrocellulose membrane. The following detection of phosphorylated tyrosines was performed with the biotin-enhanced 4G10 Western blot kit for anti-phosphotyrosine (Upstate Biotechnology, Inc., NY, USA), according to the manufacturer's instructions.

2.9. Statistical analysis of data

All values are expressed as the mean ± S.D., and the significant levels between groups were assessed by Student's *t*-test.

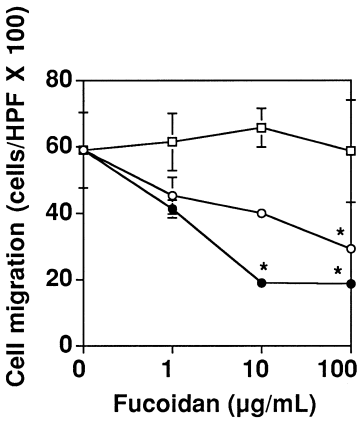

Fig. 1. Concentration-dependent effects of fucoidans on the bFGF-induced HUVEC migration through collagen-coated filter. Experimental procedures are as described in Section 2. Symbols: ○, NF; ●, OSF; □, desF. Each bar represents the mean ± S.D. ($n = 3$). *$P < 0.05$, compared to control.

3. Results

3.1. Effects of fucoidans on bFGF-induced cell migration and tube formation by HUVEC

Because the migration of endothelial cells is an important process during angiogenesis, we first examined the effects of NF, desF and OSF on the bFGF-induced migration of HUVEC in Transwell chambers. As shown in Fig. 1, HUVEC efficiently migrated into the lower side of the filter in the presence of 20 ng/ml bFGF (control, 59.0 ± 11.36 cells/HPF × 100). The migration of HUVEC were unaffected by the addition of 1–100 μg/ml desF. NF at 100 μg/ml significantly decreased the HUVEC migration (49% of the control, $P < 0.05$). The addition of 10 μg/ml OSF resulted in a 68% inhibition of the control cell migration ($P < 0.05$), but increasing its concentration to 100 μg/ml did not increase the inhibitory effect. Additionally, the migration of HUVEC was not observed in the absence of bFGF. We next assessed their abilities to suppress the bFGF-induced capillary-like network formation by HUVEC. As shown in Fig. 2, desF had no effect on the tube formation at the concentrations of ~100 μg/ml. NF at 100 μg/ml inhibited the tube formation by 27% ($P < 0.01$), while OSF at 10 μg/ml inhibited the tube formation by 31% ($P < 0.001$). In the presence of 100 μg/ml OSF, HUVEC failed to form the capillary-like networks (Fig. 3B).

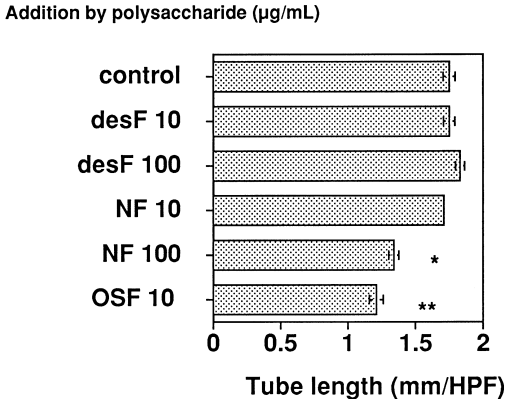

Fig. 2. Effects of fucoidans on the bFGF-induced tube formation by HUVEC. Experimental procedures are as described in Section 2. Each bar represents the mean ± S.D. ($n = 3$). *$P < 0.01$; **$P < 0.001$, compared to control.

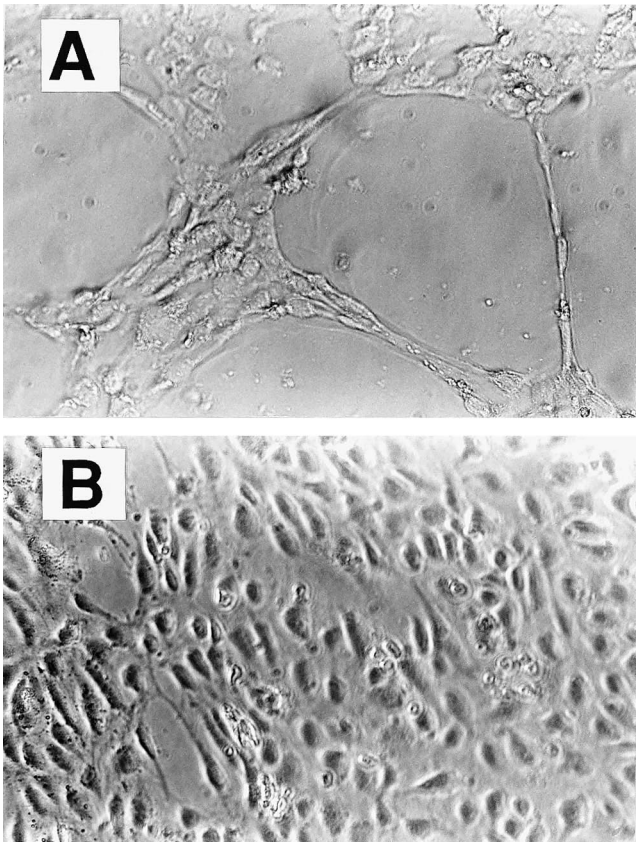

Fig. 3. Light micrographs (×200) of the bFGF-induced tube formation by HUVEC. Panel A: Control HUVEC. Panel B: OSF (100 μg/ml)-treated HUVEC.

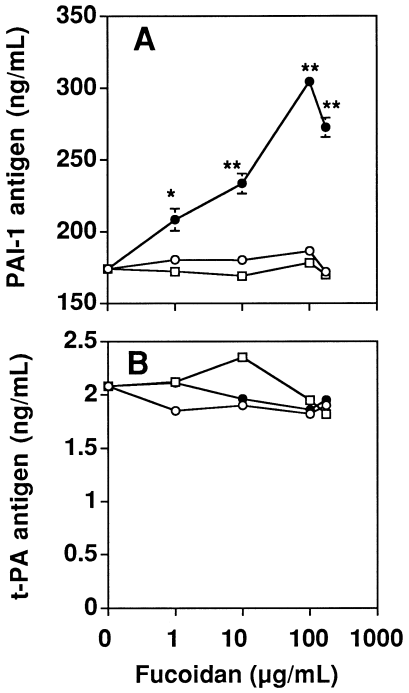

Fig. 4. Concentration-dependent effects of fucoidans on the bFGF-induced release of PAI-1 (panel A) and t-PA (panel B) antigens from HUVEC. Experimental procedures are as described in Section 2. Symbols: ○, NF; ●, OSF; □, desF. Each bar represents the mean ± S.D. ($n = 3$). *$P < 0.01$; **$P < 0.001$, compared to control.

3.2. Effects of fucoidans on bFGF-induced release of t-PA and PAI-1 from HUVEC

HUVEC were seeded on the collagen gel as described in Section 2 and cultured in the medium containing bFGF (20 ng/ml), plasminogen and fibronectin (20 μg/ml each). The HUVEC released t-PA and PAI-1 antigens into the media at the concentrations of 2.1 ± 0.13 ng/ml and 174.2 ± 4.32 ng/ml, respectively, as determined at 24 h (Fig. 4). The simultaneous presence of OSF in the medium caused an increase in the release of PAI-1 in a concentration-dependent manner from 1 to 100 μg/ml (panel A). The addition of 100 μg/ml OSF resulted in a 1.7-fold increase in the PAI-1 levels (304.5 ± 5.03 ng/ml, $P < 0.001$), compared to the control. Neither desF nor NF influenced the release of PAI-1 from HUVEC at the concentration range of 1–500 μg/ml. On the other hand, the release of t-PA antigen was unaffected by the addition of these three fucoidans (panel B).

3.3. HSPG present on cell surface and extracellular matrix is essential for the effect of OSF to increase bFGF-induced release of PAI-1 from HUVEC

HSPG present at the cell surface is an accessory molecule required for binding of bFGF to the high affinity receptors with tyrosine kinase activity [8,9]. We therefore investigated how fucoidans acted to the binding of bFGF to HSPG and the following cell signaling. As shown in Fig. 5A, the HUVEC monolayers treated at 4°C for 30 min with a saturating concentration (20 ng/ml) of bFGF bound the angiogenic factor at 47.6 ± 2.41 pg/well. The simultaneous presence of OSF (10 μg/ml) significantly increased the bFGF binding (77.4 ± 4.57 pg/well, $P < 0.01$), but that of NF did not. Heparin (10 μg/ml) also increased the bFGF binding by 1.3-fold (61.7 ± 5.58 pg/well, $P < 0.05$) compared to the control. By contrast, the addition of soluble HSPG resulted in a decrease in bound bFGF (35.2 ± 4.43 pg/well,

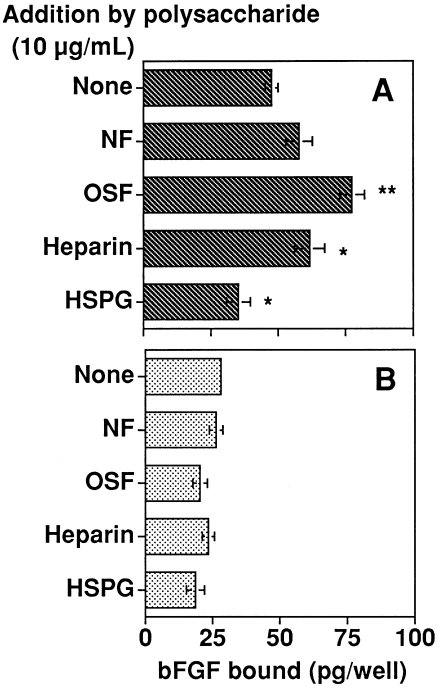

Fig. 5. Effects of fucoidans and other polysaccharides on the binding of bFGF to cell surfaces of heparitinase-untreated (panel A) and -treated (panel B) HUVEC. Experimental procedures are as described in Section 2. Each bar represents the mean ± S.D. ($n = 3$). *$P < 0.05$; **$P < 0.01$, compared to each control.

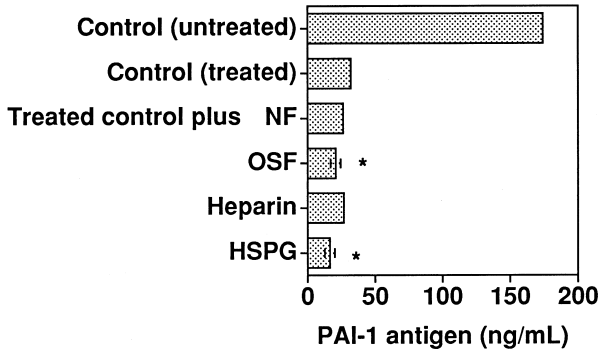

Fig. 6. Effects of fucoidans and other polysaccharides on the bFGF-induced release of PAI-1 from heparitinase-treated HUVEC. Experimental procedures are as described in Section 2. Each polysaccharide was added at the concentration of 10 μg/ml. Each bar represents the mean ± S.D. ($n = 3$). *$P < 0.05$, compared to heparitinase-treated control.

$P < 0.05$). Fig. 5B shows the effects of these polysaccharides on the binding of bFGF to HUVEC monolayers, whose HS have largely been removed by heparitinase. The heparitinase treatment (0.02 units/ml for 24 h) reduced the binding of bFGF to the cell layers (41% of untreated control). Unlike the results from intact HUVEC, these polysaccharides showed a tendency to suppress the binding of bFGF to HS-removed HUVEC monolayers. OSF and heparin (10 μg/ml each) decreased the bFGF binding from 28.1 ± 1.40 pg/well to 20.4 ± 2.67 pg/well and 23.4 ± 2.30 pg/well, respectively. Soluble HSPG also had an inhibitory effect (18.6 ± 3.31 pg/well). These results suggest that the HS molecule is an essential co-factor for the interaction of bFGF with HUVEC and that OSF especially promotes this interaction. In the HS-removed HUVEC, contrarily, these polysaccharides may act as inhibitors of the bFGF–HUVEC interaction rather than that they replace HS molecules as the co-factor for bFGF-dependent receptor activation. To confirm this possibility, we determined the effects of these polysaccharides on the bFGF-induced release of PAI-1 from heparitinase-treated HUVEC. As shown in Fig. 6, the HS-removed HUVEC reduced the bFGF-induced PAI-1 release to a level of 18% (32.1 ± 1.50 ng/ml) of the untreated control. The presence of OSF or soluble HSPG (10 μg/ml each) caused a further decrease in the PAI-1 release (OSF, 20.7 ± 3.73 ng/ml; soluble HSPG, 16.4 ± 3.78 ng/ml). NF and heparin also had a weak inhibitory effect. Fig. 7 shows the effect of OSF on the bFGF-induced protein tyrosine phosphorylation in HUVEC. The result indicates that the autophosphorylation of the receptors with molecular masses of 140 and 160 kDa is enhanced in the concomitant presence of 50 μg/ml OSF.

4. Discussion

The present results show that OSF, different from the nature of other polysaccharides tested, has the ability to promote the binding of bFGF to HS present on the HUVEC surfaces and extracellular matrix (Fig. 5A) and the following receptor tyrosine phosphorylation (Fig. 7). HS present on HSPG binds bFGF with a dissociation constant of 10^{-8} to 10^{-9} M [10–12] and may confer a stable, receptor-compatible conformational change upon bFGF [9]. The biological response of cells to bFGF is mediated through the high affinity ($K_d = 2–20 \times 10^{-11}$ M) cell surface receptors that possess intrinsic tyrosine kinase activity [13]. Yayon et al. [9] have previously demonstrated that Chinese hamster ovary cell mutants expressing high affinity bFGF receptors, but lacking HSPG, do not bind bFGF unless heparin or soluble HS is included in the binding medium, suggesting a possible role of the added polysaccharides as the counterpart for the cell surface HS that promotes the binding of bFGF to the high affinity receptors. In their experiments, the ability of heparin to restore high affinity receptor binding was detected at the concentrations as low as 10–40 ng/ml. In the present study, we also examined the restoration of bFGF binding to HS-removed HUVEC at polysaccharide concentrations as high as 10 μg/ml (Fig. 5B). The concentration range of OSF can effectively cause

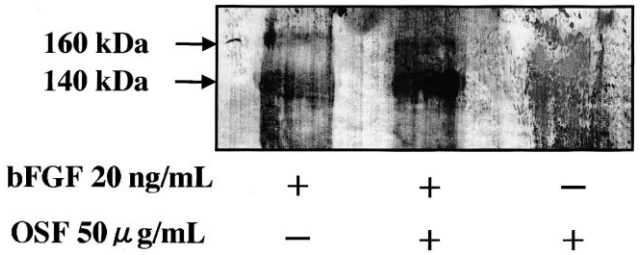

Fig. 7. Effect of OSF on the bFGF-induced receptor tyrosine phosphorylation in HUVEC. Experimental procedures are as described in Section 2.

an increase in the bFGF-induced PAI-1 release from intact HUVEC (Fig. 4) and decreases in the HUVEC migration (Fig. 1) and tube formation (Fig. 2). However, the high concentrations of heparin, OSF and also soluble HSPG failed to increase the localization of bFGF at the HS-removed HUVEC monolayers (Fig. 5B) and the following PAI-1 release (Fig. 6), indicating that they could not act as the counterpart of the removed HS at the high concentrations. On the other hand, OSF and heparin significantly increased the bFGF binding to intact HUVEC surfaces (Fig. 5A). The resultant increase in bFGF binding seems to directly contribute to the augment of bFGF cell signaling for PAI-1 release (Fig. 6). From these results, the HS present on cell surface and extracellular matrix may play an important role in the OSF-induced promotion of bFGF binding to the high affinity receptors and PAI-1 release. A central question raised by these observations is how OSF promotes the localization of bFGF to the HS molecules. We assume that OSF could first bind bFGF and simply transfer it to HS with higher affinity. Heparitinase treatment of the HUVEC monolayer largely decreases HS molecules. The resultant minority of HS molecules may fail to act as the acceptors of bFGF. On the other hand, the predominant OSF may leave bFGF in the bound form and rather prevent its transfer to the acceptor molecule HS, since OSF showed a tendency to suppress the binding of bFGF to HS-removed HUVEC monolayers (Fig. 5B) and the PAI-1 release (Fig. 6). There is evidence that OSF at 10 μg/ml, but not NF, inhibits the binding of lipopolysaccharide to HSPG on HUVEC surface [14]. Both the high degree of sulfation and the high molecular weight (100–130 kDa) may largely contribute to the unique nature of OSF.

Basic FGF is a potent mitogen that stimulates proliferation, migration, and differentiation of cells [15,16]. Another fundamental question raised by our findings is why the OSF-induced augment of bFGF cell signaling does not promote the angiogenesis (Fig. 2). In general, bFGF promotes endothelial cell migration by increasing PA production [17]. PAs such as t-PA and u-PA can convert pro-collagenase to active collagenase through converting plasminogen to plasmin. Plasmin itself is also active in degrading extracellular components such as fibronectin and laminin. Our data suggest that the increase in PAI-1 release with OSF shifts the balance of activation and inhibition of plasminogen to the inhibition and results in the suppression of cell migration following collagenolysis. Another striking feature of OSF is that it selectively stimulates bFGF-induced release of PAI-1. It is at present unknown why OSF does not stimulate the t-PA release through promotion of the bFGF binding to receptor(s). One possibility is that, although OSF promotes the binding of bFGF to HS, it also affects the binding of bFGF to the receptor(s) in a mode different from that of this cytokine alone.

The observed increase in PAI-1 release may be involved as at least one of the mechanisms by which OSF inhibits the bFGF-induced cell migration and tube formation by HUVEC in collagen gel and also in Matrigel [6], where type IV collagen, t-PA and bFGF are abundantly included. The released PAI-1 is accumulated in the cell surface and extracellular matrix [18], where it may suppress the matrix degradation required for cell migration by limiting plasmin generation. In fact, increasing PAI-1 release increased the accumulation of PAI-1 on the HUVEC monolayers (data not shown). We have a particular interest in the intracellular signaling for increasing the release of PAI-1 from HUVEC. We have previously demonstrated that exposure of cultured HUVEC to cell permeable ceramide analogs and sphingomyelinase leads to the increase in PAI-1 release [19]. This finding suggests an involvement of the intracellular ceramide signaling event in PAI-1 release. Tumor necrosis factor-α also utilizes the ceramide formed by activation of lysosomal acidic sphingomyelinase for the induction of PAI-1 release from HUVEC [20]. Additionally, Fan et al. [21] have shown that suramin, a polysulphonated naphthylurea, blocks angiogenesis. A recent report by Gill and Windebank [22] suggests that suramin induces ceramide accumulation within neurons and leads to apoptotic cell death. Therefore, further studies will be needed to determine whether OSF-induced apoptosis is an additional mechanism for its anti-angiogenic effects.

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References

[1] M. Klagsbrun, P.A. D'Amore, Annu. Rev. Physiol. 53 (1991) 217–239.
[2] J. Folkman, Y. Shing, J. Biol. Chem. 267 (1992) 10931–10934.
[3] G.F. Springer, H.A. Wurzel, G.M. McNeal, N.J. Ansell, M.F. Doughty, Proc. Soc. Exp. Biol. Bed. 94 (1957) 404–409.
[4] S. Soeda, S. Sakaguchi, H. Shimeno, A. Nagamatsu, Biochem. Pharmacol. 43 (1992) 1853–1858.
[5] S. Soeda, S. Ishida, H. Shimeno, A. Nagamatsu, Jpn. J. Cancer Res. 85 (1994) 1144–1150.
[6] S. Soeda, Y. Shibata, H. Shimeno, Biol. Pharm. Bull. 20 (1997) 1131–1135.
[7] U.K. Laemmli, Nature 227 (1970) 680–685.
[8] A.C. Rapraeger, A. Krufka, B.B. Olwin, Science 252 (1991) 1705–1708.
[9] A. Yayon, M. Klagsbrun, J.D. Esko, P. Leder, D.M. Ornitz, Cell 64 (1991) 841–848.
[10] G. Neufeld, D. Gospodarowicz, J. Biol. Chem. 261 (1986) 5631–5637.
[11] D. Moscatelli, J. Cell. Physiol. 131 (1987) 123–130.
[12] P. Bashkin, S. Doctrow, M. Klagsbrun, C.M. Svahn, J. Folkman, I. Vlodavsky, Biochemistry 28 (1989) 1737–1743.
[13] S.R. Coughlin, P.J. Barr, L.S. Cousens, L.J. Fretto, L.T. Williams, J. Biol. Chem. 263 (1988) 928–933.
[14] S. Soeda, N. Fujii, H. Shimeno, A. Nagamatsu, Biochim. Biophys. Acta 1269 (1995) 85–90.
[15] J. Folkman, M. Klagsbrun, Science 235 (1987) 442–447.
[16] D.B. Rifkin, D. Moscatelli, J. Cell Biol. 109 (1989) 1–6.
[17] Y. Sato, D.B. Rifkin, J. Cell Biol. 107 (1988) 1199–1205.
[18] J. Mimuro, R.R. Schleef, D.J. Loskutoff, Blood 70 (1987) 721–728.
[19] S. Soeda, O. Honda, H. Shimeno, A. Nagamatsu, Thromb. Res. 80 (1995) 509–518.
[20] S. Soeda, T. Tunoda, Y. Kurokawa, H. Shimeno, Biochim. Biophys. Acta 1448 (1998) 37–45.
[21] T.P. Fan, R. Jaggar, R. Bicknell, Trends Pharmacol. Sci. 16 (1995) 57–66.
[22] J.S. Gill, A.J. Windebank, Cell Death Differ. 5 (1998) 876–883.