Structural characterization and pro-angiogenic property of a polysaccharide isolated from red seaweed *Bangia fusco-purpurea*

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Abstract

BFP exhibited significant cell migration- and tube formation-promoting activities toward human umbilical vein endothelial cells (HUVECs) in a concentration-dependent manner via increasing the N-cadherin expression and decreasing the E-cadherin expression. Furthermore, ERK and p38 mitogen-activated protein kinase (MAPK) specific inhibitors exhibited potent inhibitory effects on BFP-induced cell migration but not JNK MAPK inhibitor, suggesting ERK and p38 MAPK signaling pathways were mainly involved in BFP-induced cell migration. Moreover, vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor significantly inhibited BFP-induced cell migration and tube formation in HUVECs, suggesting VEGF receptors of HUVECs were involved in the pro-angiogenesis activity of BFP.

Materials & Methods

1. Cell culture and cell proliferation assay

- The cell proliferation effect of BFP on HUVECs was determined by a colorimetric assay with thiazolyl blue tetrazolium bromide (MTT) as described previously;
- After BFP treatment, MTT solution was added to each treated well to the final concentration of 0.05%. After 1 h incubation at 37℃, the medium of each well was aspirated and 100 μLof dimethyl sulfoxide (DMSO) was added into each well to dissolve MTT formazan. The optical density of DMSO in each treated well was measured at the wavelength of 570 nm using a Cytation™ 5 Cell Imaging Multi-Mode Reader .

2. Wound healing assay

- After each well was rinsed thrice with the growth medium carefully, varying final concentrations of BFP in growth medium (0, 20, 100, and 500 μg/mL) were added to the wells of HUVECs and incubated under the normal culture condition. The images of cell monolayers in treated-wells were taken at 0, 6, 12, and 24 h using a Nikon 50i eclipse microscope equipped with a CCD camera. The open area of scraping at 0 h was used as the control group. The wound healing rate analysis was done using Image J software.

3. Western blot analysis

- Bands were visualized with the ECL Plus western blotting detection reagents and imaged using agel imaging system . To standardize the loaded protein levels, blotting with anti-GAPDH antibody was also conducted at the same time.

4. Tube formation assay

- Briefly, each well of 96-well plate was coated with 50 μL of cold Matrigel , and further incubated at 37°C in a 5% CO2 incubator for 30 min to solidify the Matrigel. 100μ L of HUVECs suspensions (3 × 10^5 cells/mL) mixed with or without BFP (final 100 μg/mL) were seeded in the upper surface of the Matrigel-coated wells, followed by incubating for another 8 h at 37°C. The enclosed capillary networks of tube formation were photographed using a Nikon 50i eclipse microscope equipped with a CCD camera.

5. Tube formation assay

- The adherent HUVECs monolayers with scratches in 6-well plates were pre-incubated with each MAPK inhibitor at the final concentration of 20 μM or VEGF receptor inhibitor at the final concentration of 1 μM for 1 h at 37°C in the growth medium, and then BFP wasadded into each treated well.After 24 h incubation 37°C, the wound healing rate of each treated monolayer was determined as described above.

Conclusion

- *In vitro* biological activity analyses demonstrated that BFP exerted significant cell migration- and tube formation-promoting activities toward HUVECs in a concentration-dependent manner. BFP significantly increased the expression of N-cadherin and simultaneously decreased the expression of E-cadherin.
- The analysis using specific MAPK inhibitors suggested that ERK and p38 MAPK signaling pathways were mainly involved in BFP-induced cell migration. Since VEGF receptor tyrosine kinase inhibitor significantly suppressed BFP-induced cell migration and tube formation in HUVECs, VEGF receptors were suggested to be involved in the pro-angiogenesis activity of BFP.
- Our results verified the hypothesis of that BFP promotes angiogenesis through acting on the specific cell membrane receptors and intracellular signaling pathways in HUVECs model.
- The findings obtained in this study may shed light on the potential of practical use of natural polysaccharide like BFP to be developed as a biological dressing and pro-angiogenesis agent.

Results

1.1 Effects of BFPon the proliferation and migration of HUVECs

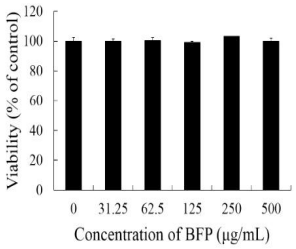

Fig.1 Effects of BFP on the viability of HUVECs determined by MTT assay. Adherent HUVECs (1 × 10^4 cells per well in 96-well plates) were incubated with the indicated concentrations of BFP in the growth medium at 37°C. After 24 h, the viabilities of the cells were examined by MTT assay as described in the text. Data were presented as mean ± SD, n =3

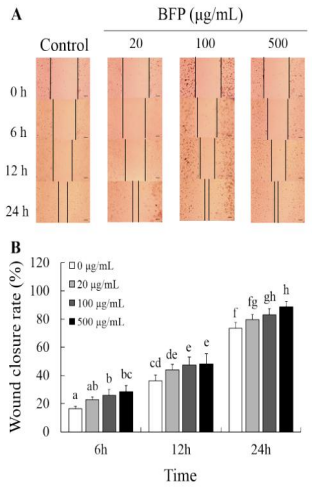

Fig.2 Effect of BFP on the migration of HUVECs. Representative images (A) and quantification (B) (three regions of three biological triplicates) of wound-healing assay results of HUVECs treated without BFP (Control) or with different concentrations of BFP (20, 100, and 500 μg/mL) for 6 h, 12 h, and 24 h. Scale bar, 20 μm. Different letters suggest significant differences, $P< 0.05$

1.2 Impact of BFPon the protein levels of E-cadherin and N-cadherin in HUVECs

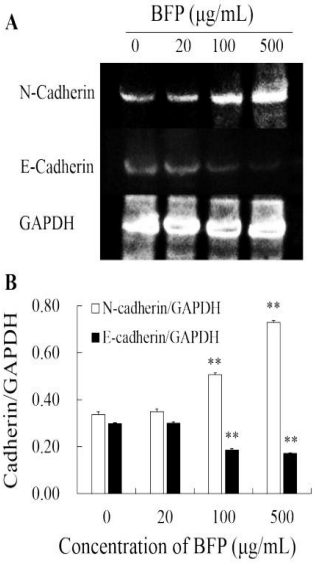

Fig.3 (A) Representative images of protein expression levels of N-cadherin and E-cadherin in HUVECs treated with different concentrations of BFP by Western blotting.(B) Quantification (three regions of three biological triplicates) of western blotting results of HUVECs treated with different concentrations of BFP. Asterisks suggest significant differences, $P < 0.05$.

1.3 Effect of BFPon the tube formation capability of HUVECs

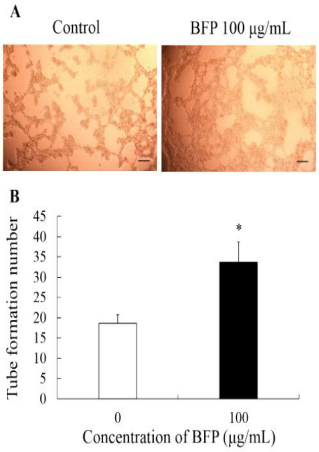

Fig.4 Representative images (A) and quantification (B) (three regions of three biological triplicates) of the capillary-network formation in HUVECs treated without BFP (Control or 0 μg/mL) or with BFP (100 μg/mL). Scale bar, 20 μm. Different letters suggest significant differences, $P < 0.05$.

1.4 Effects of MAP kinase specific inhibitors on BFP-induced cell migration in HUVECs

Inhibitors	Wound healing rate (% of control [a])
U0126	32.37 ± 3.37**[b]
SB203580	40.74 ± 8.66**
SP600125	66.10 ± 3.99

1.5 Effects of VEGF receptor tyrosine kinase inhibitor on BFP-induced pro-angiogenic potential in HUVECs

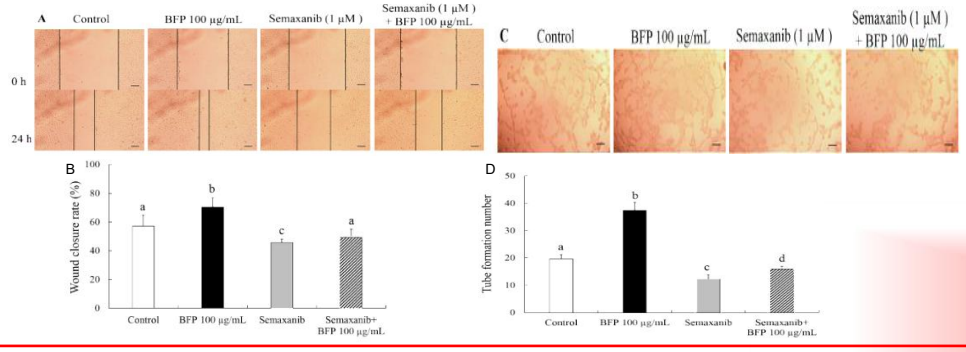

Fig.5 Effects of VEGF receptor tyrosine kinase inhibitor on BFP-induced cell migration and the capillary-network formation in HUVECs. Representative images (A) and quantification (B) (three regions of three biological triplicates) of wound-healing assay results of HUVECs after a 24 h incubation with the following treatments: no treatment (Control), BFP (100 μg/mL), Semaxanib (1 μM), and Semaxanib (1 μM) + BFP (100 μg/mL). Representative images (C) and quantification (D) (three regions of three biological triplicates) of the capillary-network formation in HUVECs after a 24 h incubation with the following treatments: no treatment (Control), BFP (100 μg/mL),Semaxanib (1 μM), and Semaxanib (1 μM) + BFP (100 μg/mL). Scale bar, 20 μm.Different letters suggest significant differences, $P < 0.05$.