Supplemental Data

Supplemental file 1.

The array layout of the Oligo GEArray® human angiogenesis array and the list of genes with expression changed after WSS25 treatment.

Table 1 shows the array layout of the Oligo GEArray® human angiogenesis array.

Table 2 is the list of gene with expression alteration after WSS25 treatment.

Table 1 Array layout of Oligo GEArray® human angiogenesis microarray

RPS27A	AKT1	ANGPT1	ANGPT2	ANGPTL3	ANGPTL4	ANPEP	BAI1
1	2	3	4	5	6	7	8
CCL11	CCL2	CDH5	COL18A1	COL4A3	CSF3	CXCL1	CXCL10
9	10	11	12	13	14	15	16
CXCL11	CXCL2	CXCL3	CXCL5	CXCL6	CXCL9	ECGF1	EDG1
17	18	19	20	21	22	23	24
EFNA1	EFNA2	EFNA3	EFNA5	EFNB2	EGF	ENG	EPAS1
25	26	27	28	29	30	31	32
EPHB4	EREG	F2	FGF1	FGF2	FGF6	FGFR3	FIGF
33	34	35	36	37	38	39	40
FLT1	HAND2	HGF	HIF1A	HPSE	ID1	ID3	IFNA1
41	42	43	44	45	46	47	48
IFNB1	IFNG	IGF1	IL10	IL12A	IL18	IL1B	IL6
49	50	51	52	53	54	55	56
IL8	ITGAV	ITGB3	JAG1	KDR	LAMA5	LECT1	LEP
57	58	59	60	61	62	63	64
MDK	MMP19	MMP2	MMP9	NOTCH4	NPPB	NPR1	NRP1
65	66	67	68	69	70	71	72
NRP2	NUDT6	PDGFA	PDGFB	PECAM1	PF4	PGF	PLAU
73	74	75	76	77	78	79	80
PLG	PLXDC1	PROK2	PTEN	PTGS1	PTGS2	PTN	RNASE4
81	82	83	84	85	86	87	88
SERPINF1	SH2D2A	SPHK1	STAB1	STAB2	TEK	TGFA	TGFB1
89	90	91	92	93	94	95	96
TGFB2	TGFB3	TGFBR1	THBS1	THBS2	TIE	TIMP1	TIMP2
97	98	99	100	101	102	103	104
TIMP3	TNF	TNFAIP2	TNFRSF12A	TNFSF15	TNNT1	VEGF	VEGFB
105	106	107	108	109	110	111	112
VEGFC	VG5Q	PUC18	Blank	Blank	AS1R2	AS1R1	AS1

113	114	115	116	117	118	119	120
GAPD	B2M	HSPCB	HSPCB	ACTB	ACTB	BAS2C	BAS2C
121	122	123	124	125	126	127	128

Table 2

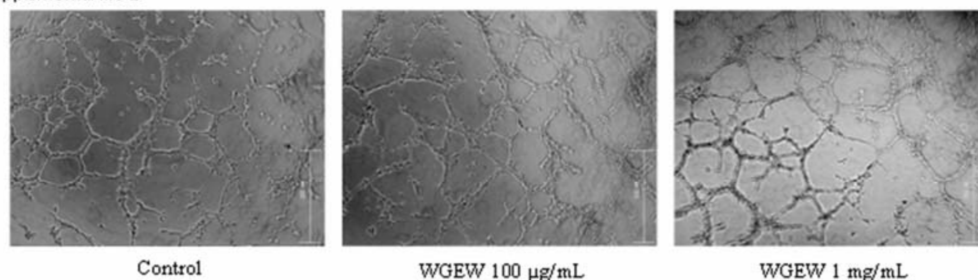
Position	GeneBank	Symbol	Description	WS25/Control
18	NM_002089	CXCL2	Chemokine (C-X-C motif) ligand 2	3.8764E+1
20	NM_002994	CXCL5	Chemokine (C-X-C motif) ligand 5	3.8651E+1
24	NM_001400	EDG1	Endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	3.6403E+0
29	NM_004093	EFNB2	Ephrin-B2	2.1313E+1
45	NM_006665	HPSE	Heparanase	2.7227E+0
53	NM_000882	IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	2.4543E+0
60	NM_000214	JAG1	Jagged 1 (Alagille syndrome)	4.8677E+0
71	NM_000906	NPR1	Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	4.7958E+0
76	NM_002608	PDGFB	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	2.1241E+1
84	NM_000314	PTEN	Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	4.7935E+0
85	NM_000962	PTGS1	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	1.7052E+1
46	NM_002165	ID1	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	5.1298E-4

69	NM_004557	NOTCH4	Notch homolog 4 (Drosophila)	4.0464E-1
80	NM_002658	PLAU	Plasminogen activator, urokinase	4.7772E-2
88	NM_001145	ANG	Angiogenin, ribonuclease, RNase A family, 5	1.4712E-1
94	NM_000459	TEK	TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal)	5.7841E-2
110	NM_003283	TNNT1	Troponin T type 1 (skeletal, slow)	2.1087E-1

Note: The upregulated genes were selected based on the value of WSS25/control >2, while the downregulated genes were selected based on the value of WSS25/control <0.5.

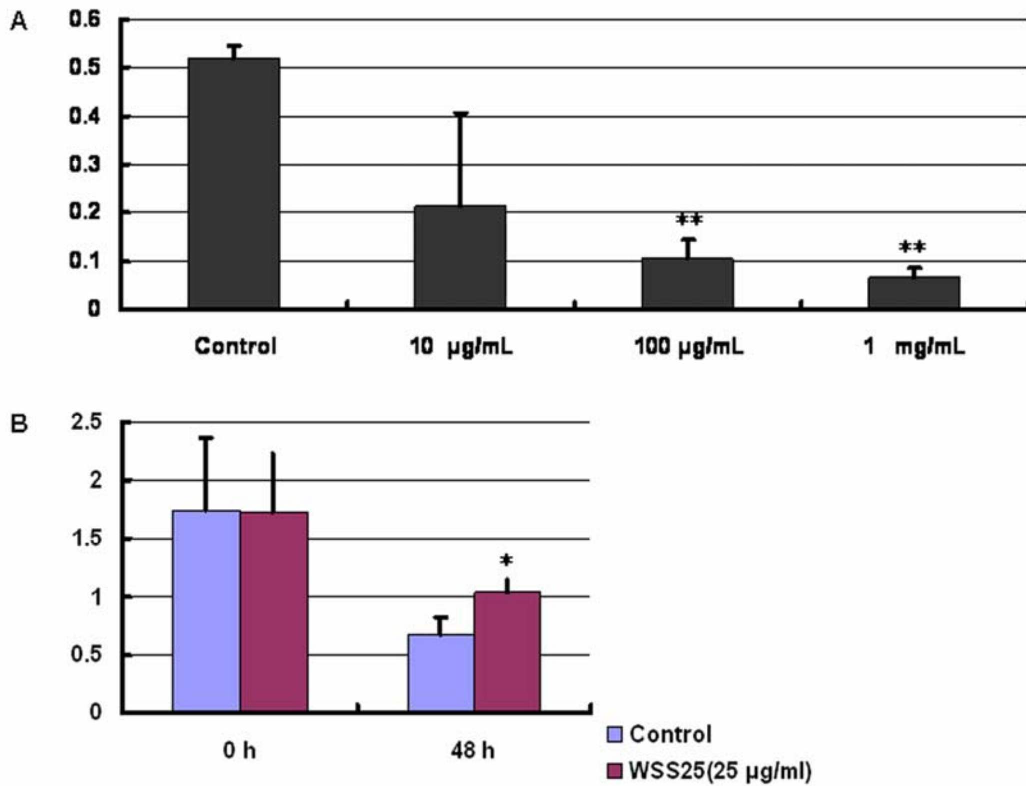
Supplemental file 2

WGEW has no effect on the tube formation of HMEC-1 cells on Matrigel. HMEC-1 cells (90 μL) combined with WGEW (10 μL) in different final concentration (100 μg/mL, 1 mg/mL) or vehicle were seeded into the 96 well plate pre-coated with 50 μL Matrigel for 10 h.



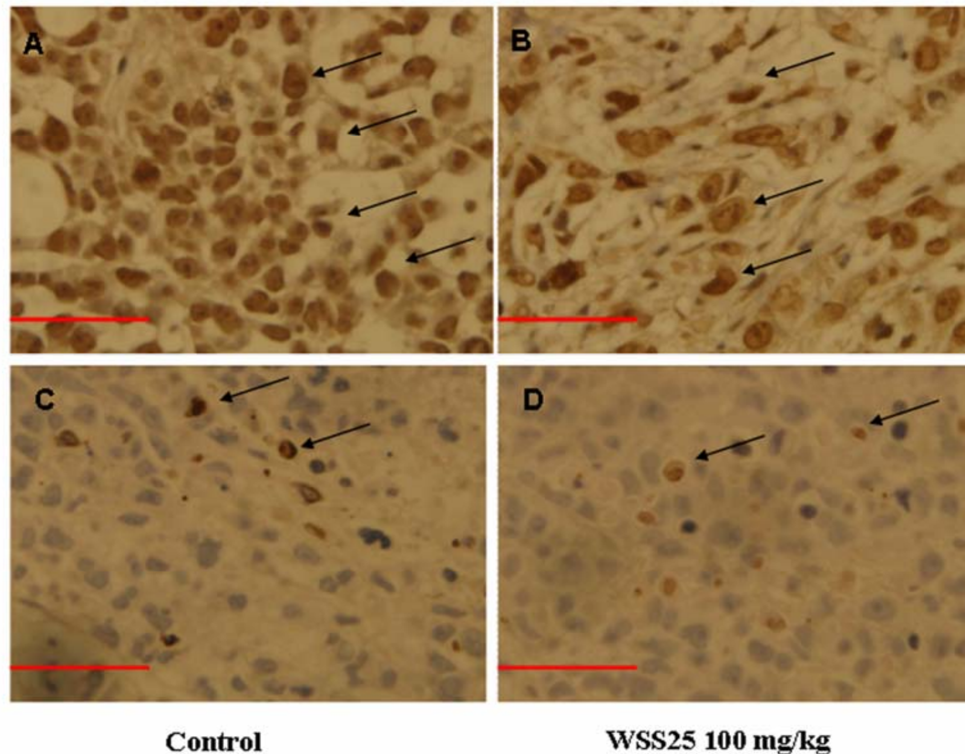
Supplemental file 3

The results of inhibitory effect of WSS25 on migration of HMEC-1 cells were statistically analyzed. A, Results based on transwell migration assay; B, Results based on wound healing assay.



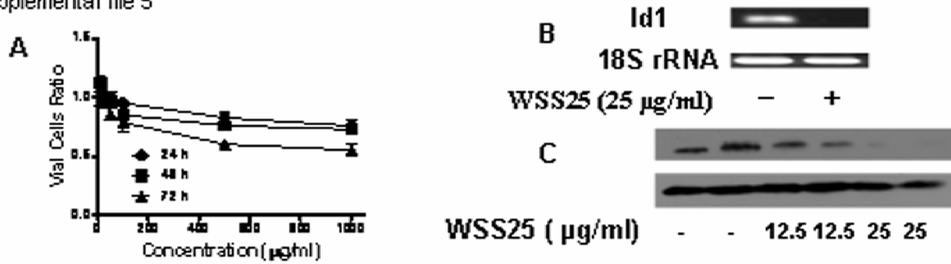
Supplemental file 4.

Ki67 (Abcam) immunohistochemistry and TUNEL (R&D) immunohistochemistry were used to analyze the proliferation and apoptosis of the tumor tissue. Compared to the control (A), the expression of Ki67 in the WSS25 treated tissue (B) was reduced. TUNEL assay results showed that there is no significant difference in apoptosis between the control (C) and WSS25 treated tissue (D).



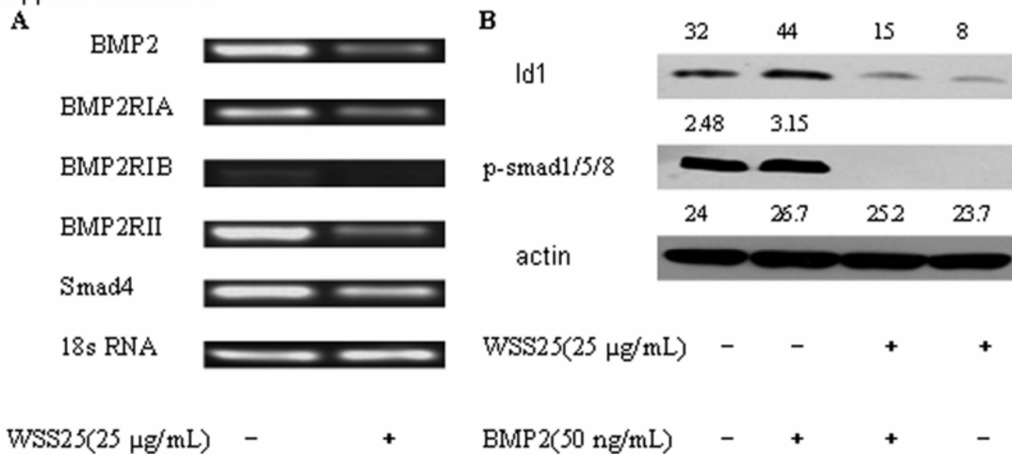
Supplemental file 5.

WSS25 inhibits the Id1 expression in Bel7402 cells and growth of the cells. A, Bel7402 cells were seeded into the 96-well plate. After 24 h incubation, WSS25 was added with final concentration at 1 μg/mL, 10 μg/mL, 100 μg/mL, 500 μg/mL and 1 mg/mL, respectively. The cells were subjected to MTT assay at 24 h (-◆-), 48 h (-■-), or 72 h (-▲-) later. All experiments were performed according to the Materials and Methods. The results are representatives of triplicate experiments. B, WSS25 (25 μg/mL) nearly completely inhibited the Id1 mRNA expression in Bel7402 cells with 18S rRNA as the internal control. C, WSS25 down-regulated the Id1 protein expression in Bel7402 cells in a dose dependent manner. The β-actin was used as a control for protein loading.



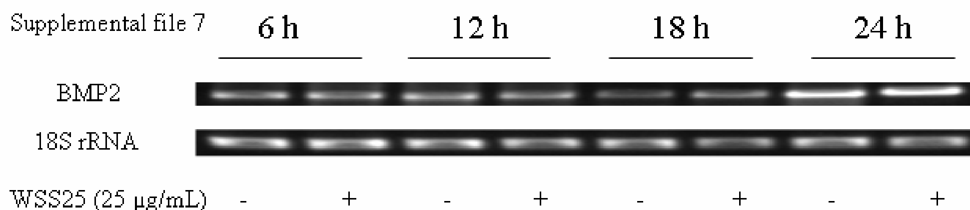
Supplemental file 6.

WSS25 blocks BMP/Smad signaling in Bel7402 cells. A, The total RNA in the cells treated by WSS25 (25 µg/mL) for 18 h was extracted and reverse-transcribed into cDNA. The cDNA was then subject to PCR analysis for the detection of BMP2, BMPRIA, BMPRIB, BMPRII, and smad4. B, Cells were pre-treated by 25 µg/mL of WSS25 or vehicle treatment for 23 h. The cells were then treated with 50 ng/mL of BMP2 for 1 h more. The extracted proteins were subjected to Western blotting analysis probed with Id1 and pSmad1/5/8 antibodies. The β-actin was used as a control for protein loading. The experiments were repeated twice.



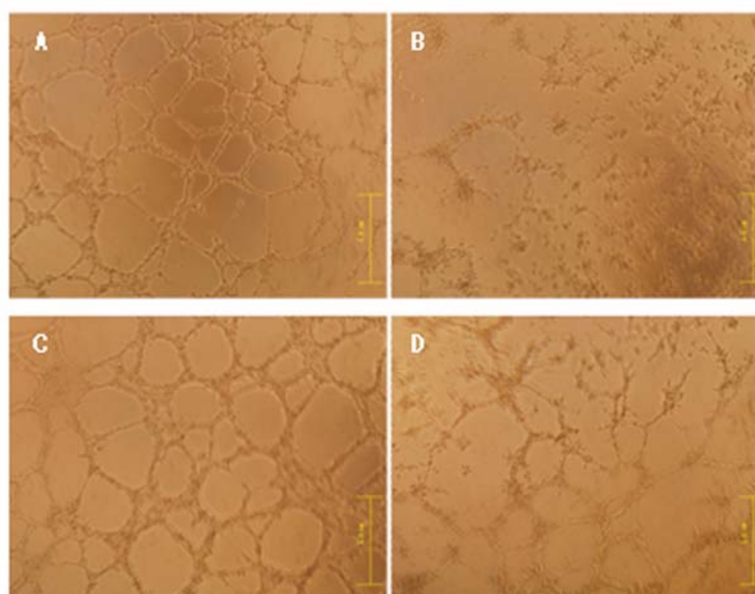
Supplemental file 7.

BMP2 expression in HMEC-1 cells with WSS25 treatment in different time point. HMEC-1 cells (2×10^5 cells/well) were seeded into 6-well plates. After 24 h incubation, the cells were cultured with WSS25 (25 µg/mL) for 6 h, 12 h, 18 h, and 24h, respectively. The total RNA of the cells was then extracted and reverse-transcribed into cDNA. RT-PCR was performed as described in materials and methods to detect the expression of BMP2, 18S rRNA as the internal control.



Supplemental file 8.

High expression of BMPRIB in HMEC-1 cells could partially rescue the inhibitory effect of WSS25 on the tube formation of HMEC-1 cells on Matrigel. Human full-length BMPRIB (RC210218) was purchased from OriGene, U.S.A. After the cells were seeded in the 6-well plate for 24 h, the vector pCMV6-Entry (A, B) and the pCMV-Entry-BMPRIB (C, D) plasmid were transferred into HMEC-1 cells, respectively. The cells were subject to tube formation analysis 24 h after transfection with (B, D) or without 20μg/mL WSS25 (A, C). Photos were taken 10 h later using the Olympus microscope IX51.



Supplemental file 9.

BMP2 could bind to BMP2 receptors. The WSS25 biosensor surface was prepared as described in the part of *Quartz crystal microbalance (QCM) analysis* in the *Experimental Procedures* section. Biotinylated BMP2 receptors (rhBMPRIA, rhBMPRIB, rhBMPRII) and rhIgGFc (as control) were synthesized according to the following procedure: NHS-PEG12-Biotin (0.6 μL, 5 mM) in Hepes buffer (10 mM Hepes, 150 mM NaCl, pH 7.4) was added to receptor (39.4 μL, 0.508 mg/mL) in the same buffer, and the mixture was stirred

at room temperature for 30 min. The biotinylated receptors and rhIgGFc can be purified from non-reacted biotin reagent by using desalting column. The biosensor experiments were carried out on Attana A100 QCM instrument (Attana AB, Stockholm, Sweden). The Attana biotin sensor surfaces were mounted in the QCM system and equilibrated with buffer solution (10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 7.4) at a flow rate 25 µL/min, and the samples were prepared in the same buffer. Subsequently the streptavidin solution (100 µg/ml) was injected, and the biotinylated BMP2 receptors and rhIgGFc were immobilized on the streptavidin surface to produce BMP2 receptors and rhIgGFc biosensor surfaces, respectively. The interaction between BMP2 receptors, rhIgGFc and WSS25, respectively were then measured by injecting WSS25 (500 µg/mL, 50 µL) in the running buffer (10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 7.4) onto the BMP2 receptors and rhIgGFc biosensor surface, and frequency data were collected. A continuous flow of running buffer at a flow rate of 25µL/min was used throughout. The frequency responses produced from the interactions were monitored by frequency logging with Attester 1.1 (Attana), where the mass changes from the bound or released ligands were recorded as the resulting frequency shifts (Δf) (Zhichao Pei, et al. Study of Real-time Lectin -carbohydrate Interactions on the Surface of a Quartz Crystal Microbalance. *Biosens. Bioelectron.* 2005, 21, 60-66).

In supplemental file 9, as a control, the frequency responses by the WSS25 interaction with the rhIgGFc (bottom curve) and streptavidin (the second curve from bottom) surface were measured, yielding a small response, respectively. Definitely, the frequency shifts produced from biotinylated rhBMPRII (The third curve from bottom), rhBMPIA (The fourth curve from bottom), and rhBMPIB (The fifth curve from bottom) binding to WSS25 indicated that this polysaccharide could bind to BMP2 receptors in different intensity.

Supplemental file 9