FEBS Letters 587 (2013) 2179–2185

FEBS Letters

journal homepage: www.FEBSLetters.org

VEGF-A induces its negative regulator, soluble form of VEGFR-1, by modulating its alternative splicing

Tetsuya Saito [a], Norihiko Takeda [a,b,*], Eisuke Amiya [a], Tomoko Nakao [a], Hajime Abe [a], Hiroaki Semba [a], Katsura Soma [a], Katsuhiro Koyama [a], Yumiko Hosoya [a], Yasushi Imai [a], Takayuki Isagawa [a], Masafumi Watanabe [a], Ichiro Manabe [a], Issei Komuro [a,c], Ryozo Nagai [a,d], Koji Maemura [a,e,*]

[a] Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Japan
[b] PRESTO, JST, Japan
[c] Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Japan
[d] Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan
[e] Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Japan

ARTICLE INFO

Article history:
Received 25 March 2013
Revised 28 April 2013
Accepted 9 May 2013
Available online 24 May 2013

Edited by Veli-Pekka Lehto

Keywords:
Angiogenesis
VEGF-A
VEGFR-1
sVEGFR-1
Alternative splicing

ABSTRACT

Vascular endothelial growth factor-A (VEGF-A) is one of the major angiogenic factors, and its actions are primarily mediated through its two membrane receptors, VEGFR-1 and VEGFR-2. A soluble form of VEGFR-1 (sVEGFR-1) sequesters the free form of VEGF-A, and acts as a potent anti-angiogenic factor. While sVEGFR-1 is synthesized as a splice variant of VEGF-R1 gene, the interactions between VEGF-A and sVEGFR-1 remain largely unknown. Here, we show that VEGF-A upregulates sVEGF-R1 expression in human vascular endothelial cells but leaves full-length VEGF-R1 expression unchanged, and that this induction was dependent on the VEGFR-2-protein kinase C-MEK signaling pathway. The VEGF-A-induced sVEGFR-1 upregulation can operate as a negative feedback system, which if modulated can become a novel therapeutic target for regulating pathological angiogenesis.

1. Introduction

Vascular endothelial growth factor-A (VEGF-A) is a potent vascular endothelial cell (EC)-specific mitogen that stimulates EC proliferation, migration and angiogenesis, and it has also been shown to improve EC function and survival in vitro as well as vascular reactivity in vivo [1–3]. The actions of VEGF-A are primarily mediated through its two major tyrosine kinase receptors, namely VEGF receptor-1 (VEGFR-1, also known as fms-like tyrosine kinase; Flt1) and VEGF receptor-2 (VEGFR-2, also known as kinase insert domain receptor; KDR) [4]. Of these two receptors, VEGFR-2 possesses stronger tyrosine kinase activity and thus mediates most of the mitogenic signals of VEGF-A [1,5]. However, recent gene-targeting studies indicated VEGFR-1-mediated signalings have also significant roles in pathological angiogenesis [6]. Indeed, we previously reported that HIF-2α, a member of hypoxia-inducible transcription factors dominantly expresses in vascular endothelial cells, transactivates VEGFR-1 and enhances mature angiogenesis [7].

The soluble form of VEGFR-1 (sVEGFR-1) is transcribed as a splice variant of full-length VEGFR-1 by a premature polyadenylation of the VEGFR-1 transcript at the intron 13 [8]. Although soluble VEGFR-1 strongly binds to VEGF-A and placental growth factor (PlGF), it actually functions as a potent angiogenic inhibitor and causes endothelial dysfunction since it lacks the seventh immunoglobulin-like domain as well as the transmembrane and tyrosine kinase regions [9–12].

In cases of pathological angiogenesis such as preeclampsia and malignancies, sVEGFR-1 can potentially be used as a therapeutic target or as a prognostic biomarker. For example, hypoxic conditions of the placenta in preeclampsia not only increase VEGF-A expression, but also induces increased sVEGFR-1 secretion. The resultant net effect is a reduced bioavailability of free VEGF-A and PlGF, thereby leading to hypertension, glomerular dysfunction and proteinuria [13–16]. Indeed, increased levels of sVEGFR-1 and reduced levels of PlGF have been demonstrated to be predictors of the subsequent development of preeclampsia [17]. Moreover, a

* .Corresponding authors Address: Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Japan.
E-mail address: ntakeda-tky@umin.ac.jp (N. Takeda).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.05.038

lower sVEGFR-1/VEGF-A or sVEGFR-1/PlGF ratio correlates significantly with a more favorable prognosis and better responsiveness to anti-cancer therapy in gliomas, breast cancer and pancreatic cancer patients [18–21]. These results indicate that angiogenesis is strictly regulated by the balance between pro- and anti-angiogenic factors, and that any imbalances between them have the potential to elicit pathological angiogenic disorders.

The phenomenon of feedback interactions between a ligand and its endogenous inhibitor, such as seen with Wnt glycoproteins and its antagonists during processes of cell proliferation and differentiation, has been extensively reported elsewhere. Wnt signaling is negatively regulated by soluble antagonists such as secreted Frizzled-related protein-1 (sFRP-1) or DICKKOPF-1 [22], the expressions of which are in fact induced by canonical Wnt signaling and thereby creates a negative feedback loop to antagonize persistent Wnt signal activation under physiological conditions [23,24].

In a similar way, we therefore hypothesized that sVEGFR-1 may act as a negative regulator in VEGF-A signaling. In this study, we identified that VEGF-A, but not PlGF, affects the splicing processes of the VEGFR-1 gene via the VEGFR-2-protein kinase C (PKC)-MEK pathway, leading to the induction of sVEGFR-1 expression. The induction of sVEGFR-1 splicing is dependent on the premature polyadenylation of its intron 13 [8]. We also confirmed that a VEGF-A responsive element exists within intron 13 of the VEGFR-1 gene.

Our findings give insights into a novel negative feedback system in physiological or pathological angiogenesis that, if modulated, may be a novel therapeutic target in the treatment of numerous pathological angiogenic disorders.

2. Materials and methods

2.1. Materials

Human recombinant VEGF-A, human recombinant PlGF, and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) were obtained from R&D Systems (Minneapolis, MN). CalphostinC, u0126, LY294002 and SB203580 were obtained from Calbiochem (La Jolla, CA).

2.2. Cell culture

Human aortic endothelial cells (HAECs), human umbilical vein endothelial cells (HUVECs), and human aortic smooth muscle cells (hASMCs) were obtained from BioWhittaker (Walkersville, MD). Human aortic endothelial cells and HUVECs were maintained in endothelial growth medium 2 (EGM-2, Cambrex) while HASMCs were maintained in smooth muscle growth medium 2 (SMGM-2, Cambrex). AD293 cells (human embryonic kidney cells), bEnd.3 cells (murine brain endothelial cells), EOMA cells (murine hemangioendothelioma endothelial cells) and RAW cells (murine macrophage cells) were purchased from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma–Aldrich JAPAN, Tokyo, Japan) containing 10% FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin. All cells were cultured at 37 °C, 5% CO_2 and cells from passages 5 to 10 were used for the experiments.

2.3. Northern blot analysis

Total cellular RNA was extracted by the RNeasy Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. Northern blot analysis using probe 1 was performed as described previously [7]. The 350 bp (AL138712 bp157156–157505) fragment of the proximal end of intron 13, the 465 bp (AL138712 bp153527–153991) fragment of the distal end of intron 13, and the 436 bp (AL138712 bp152304–152739) fragment of exon 14 of the human VEGFR-1 gene were amplified by PCR with, respectively, VEGFR-1 probes 2, 3, and 4 (see Fig. 1A). To correct for differences in RNA loading, the membranes were rehybridized with radiolabeled 18S oligonucleotide.

2.4. Western blot analysis

Total cellular protein of HAECs was extracted using extraction buffer (25 mM Tris, pH 7.4, 50 mM NaCl, 0.5% Na deoxycholate, 2% NP-40, and 0.2% SDS). Equal amounts of protein (10 μg) were subjected to SDS–PAGE as described previously [7]. Full-length VEGFR-1 and sVEGFR-1 were detected using monoclonal anti-hVEGFR-1 mouse IgG antibody (Sigma–Aldrich, 1:1000) and anti-mouse horseradish peroxidase-conjugated IgG (Cell Signaling, 1:5000) as a second antibody, followed by enhanced chemiluminescence plus detection (GE healthcare Japan, Tokyo, Japan).

2.5. siRNA duplex and transfection

The siRNA directed against full-length *VEGFR-1* was designed to target a sequence in Exon 27 of the VEGFR-1 gene, and the sequences of the siRNA duplex were 5′-UUGGGAUGUAGUCUUUACCdTdT-3′ (sense strand) and 5′-GGUAAAGACUACAUCCCAAdTdT-3′ (antisense strand). The scrambled control duplex did not target any gene, and the sequences were 5′-CUUACUAACAUGGUGUGUUdTdT-3′ (sense strand) and 5′-AACACACCAUGUUAGUAAGdTdT-3′ (antisense strand). The siRNA duplexes were synthesized using the Silencer siRNA Construction Kit (Ambion) according to the manufacturer's instructions. The siRNA directed against *VEGFR-2* was purchased from Dharmacon (Target sequences: GGGCAUGUACUGACGAUUA, CUACAUUGUUCUUCCGAUA, GGAAAUCUCUUGCAAGCUA and GCGAUGGCCUCUUCUGUAA).

HAECs were plated onto 6-cm cell culture dishes and grown to 70% confluence before transfection by adding a total of 10 μl of Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) and 200 pmol siRNA duplex.

2.6. Construction of reporter plasmids

The pGL3-Basic plasmid was purchased from Promega (Madison, WI). To generate the VEGFR-1 reporter plasmid (Fig. 4-A-(1)), the promoter region of the VEGFR-1 gene from position −1160 to +305 was amplified by PCR and subcloned into the pGL3-Basic plasmid between *Sac*I and *Hind*III restriction sites. To generate the reporter plasmid with a modulated poly(A) signal, the intron 13 fragment (AL138712 bp152676–157553) of the VEGFR-1 gene was amplified by PCR and inserted into the VEGFR-1 reporter plasmid between *Sal*I and *Xba*I restriction sites in place of the SV40 late poly(A) signal (Fig. 4-A-(2)).

2.7. Transient transfection assay

HAECs were cultured in 12-well culture plates and transfected with 2.0 μg of the reporter construct using the Neon Transfection system (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. To correct for variations in transfection efficiency, we cotransfected 0.1 μg of pCMV-βgal in all experiments. Cell extracts were prepared by a detergent lysis method (Promega KK, Tokyo, Japan). The ratio of luciferase activity to β-galactosidase activity in each sample served as a measure of normalized luciferase activity. Each construct was transfected at least 3 times, and each transfection was done in triplicate.

2.8. Statistical analysis

Results are expressed as mean ± S.E. Two-tailed Student's *t* test for unpaired samples or one-way ANOVA was used for parameter comparisons, and a $P < 0.05$ was considered significant.

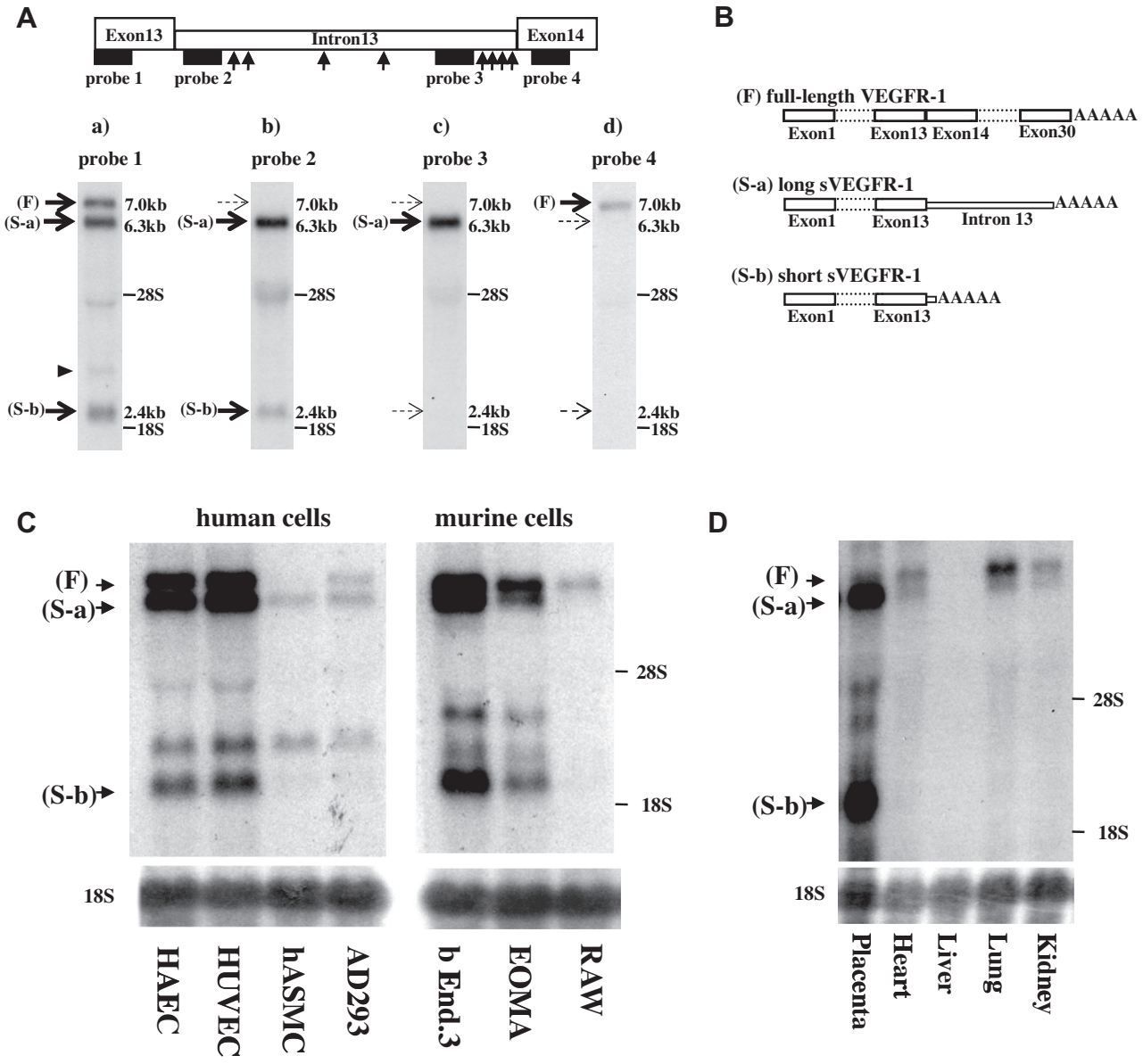

Fig. 1. Identification of full-length VEGFR-1 and several splice variants of sVEGFR-1 mRNA in Northern blot analysis and expression profile in different cell types and organs. (A) Top, Binding locations of probes 1–4 for Northern blot analysis. Probe 1 was located at the N-terminus site of the human VEGFR-1 cDNA. Probe 2 and 3 was located near the 5′- and 3′-ends of intron 13, respectively. Probe 4 was located in exon 14. Arrows represent sites of the canonical cleavage-polyadenylation signal AAUAAA or the signal variant AUUAAA within intron 13. Bottom, Representative results of Northern blot analysis using the 4 probes. Probe 1 hybridized with 3 major transcripts (arrows), but some transcripts were not present when other probes were used (dotted arrows). Black arrowhead marks another sVEGFR-1 splice variant (approx. 3.0 kb) which lacks intron 13. (B) Identification of 3 major bands shown in Northern blot analysis: (F) VEGFR-1 (approx. 7.0 kb), (S-a) long sVEGFR-1 (approx. 6.3 kb), and (S-b) short sVEGFR-1 (approx. 2.4 kb). (C, D) Total RNA was isolated from several different types of human/murine cells (C) and murine organs (D), and equal amounts (10 μg) of RNAs were analyzed by Northern blot using probe 1 or murine VEGFR-1 cDNA as a probe.

3. Results

3.1. Identification of full-length VEGFR-1 and several splice variants of sVEGFR-1 mRNA in Northern blot analysis

Both full-length and soluble forms of VEGFR-1 transcripts are synthesized as splice isoforms from the VEGFR-1 gene. However, the expression profiles of each transcript have not been fully elucidated. To investigate the expression of these transcripts, we performed Northern blotting using 4 probes around intron 13 of the VEGFR-1 gene. Probe 1 was located at the N-terminus site of the VEGFR-1 cDNA and was used to detect both full-length VEGFR-1 and sVEGFR-1 mRNAs (Fig. 1A). Probe 1 detected 3 bands that were approximately 7.0, 6.3 and 2.4 kb in length (Fig. 1A-a; (F), (S-a) and (S-b)). Probe 2 was located near the 5′-end of intron 13 and was expected to detect only sVEGFR-1. It detected 2 bands of 6.3 and 2.4 kb in length, indicating the presence of two isoforms of sVEGFR-1 (Fig. 1A-b; (S-a) and (S-b)). Probe 3, located near the 3′-end of intron 13, detected only one band (Fig. 1A-c; (S-a)), suggesting that the longer form (6.3 kb) of sVEGFR-1 contains almost all of the sequence of intron 13. When we used probe 4, which corresponds to exon 14, it detected one 7.0 kb band but not the longer (6.3 kb) form of sVEGFR-1 (Fig. 1A-d; (F)).

Taken together, 3 major bands were identified from our Northern blot analyses, namely (F) full-length VEGFR-1 (approx. 7.0 kb), (S-a) long sVEGFR-1 (approx. 6.3 kb), and (S-b) short sVEGFR-1 (approx. 2.4 kb) (Fig. 1B).

We could also detect another faint band between long and short sVEGFR-1 (Fig. 1A, black arrowhead). We speculate this corresponds to another sVEGFR-1 splice variant (approx. 3.0 kb)

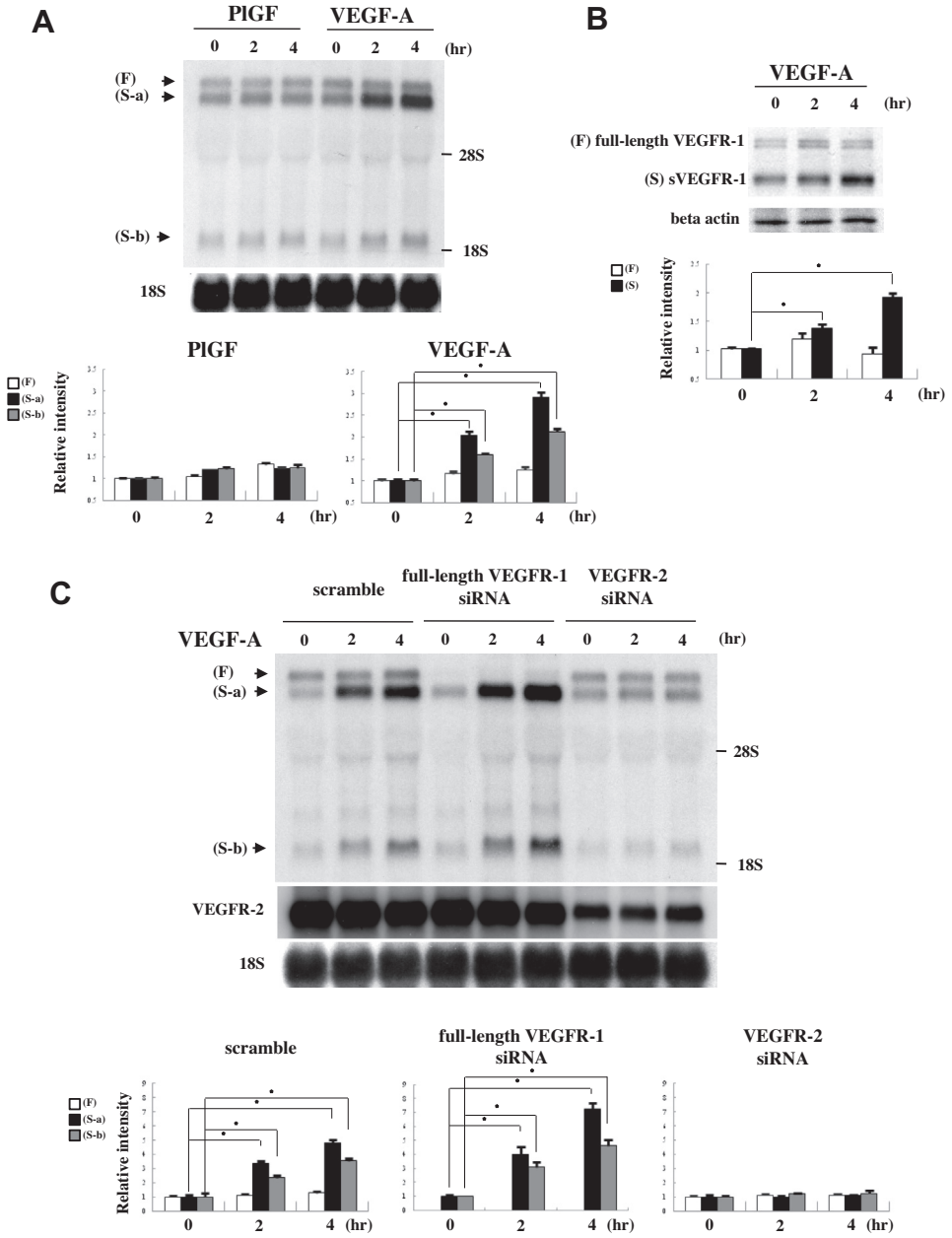

Fig. 2. VEGF-A induces sVEGFR-1 expression through VEGFR-2 signaling. (A) HAECs were incubated with PlGF (50 ng/ml) or VEGF-A (50 ng/ml). Northern blot analysis was performed using probe 1. Quantitative analysis is shown below the figure. (B) Western blot analysis using whole HAECs. Equal amounts of total cellular proteins (10 μg) were subjected to SDS–PAGE, and then VEGFR-1 and sVEGFR-1 were detected using monoclonal anti-hVEGFR-1 antibody. Quantitative analysis is shown below the figure. (C) HAECs were transfected with siRNA duplexes that targeted full-length VEGFR-1 or VEGFR-2, or scrambled siRNA, for 24 h, followed by treatment with VEGF-A (50 ng/ml) for 3 h. Northern blot analysis was performed using probe 1. Quantitative analysis is shown below the figure.

revealed by Sela et al. which is preferentially expressed in smooth muscle cells [25].

3.2. Variable expression of full-length VEGFR-1 and sVEGFR-1 in different cell types and organs

We next investigated the expression profiles of full-length VEGFR-1 and sVEGFR-1 mRNA in several different cell types and organs. The major form of VEGFR-1 expressed in smooth muscle cells and placental tissue was sVEGFR-1, whereas full length VEGFR-1 was the predominant form expressed in macrophages and lung tissue (Fig. 1C and D). In contrast, various types of endothelial cells express both full-length and sVEGFR-1 abundantly (Fig. 1C). These indicate that the ratio of full-length/sVEGFR-1 dynamically changes depending on the cell type or organ.

3.3. VEGF-A induces the expression of sVEGFR-1

The ratio of sVEGFR-1/VEGF-A or sVEGFR-1/PlGF significantly correlates with the clinical course of patients with cancer [18]. In this study, we hypothesized that there may be a link between VEGF-A or PlGF and their endogenous inhibitor, sVEGFR-1, and therefore investigated the interactions between VEGF-A signaling and sVEGFR-1 expression using HAECs.

We found that sVEGFR-1 mRNA expression was upregulated by VEGF-A, whereas PlGF had no effect. Since the abundance of

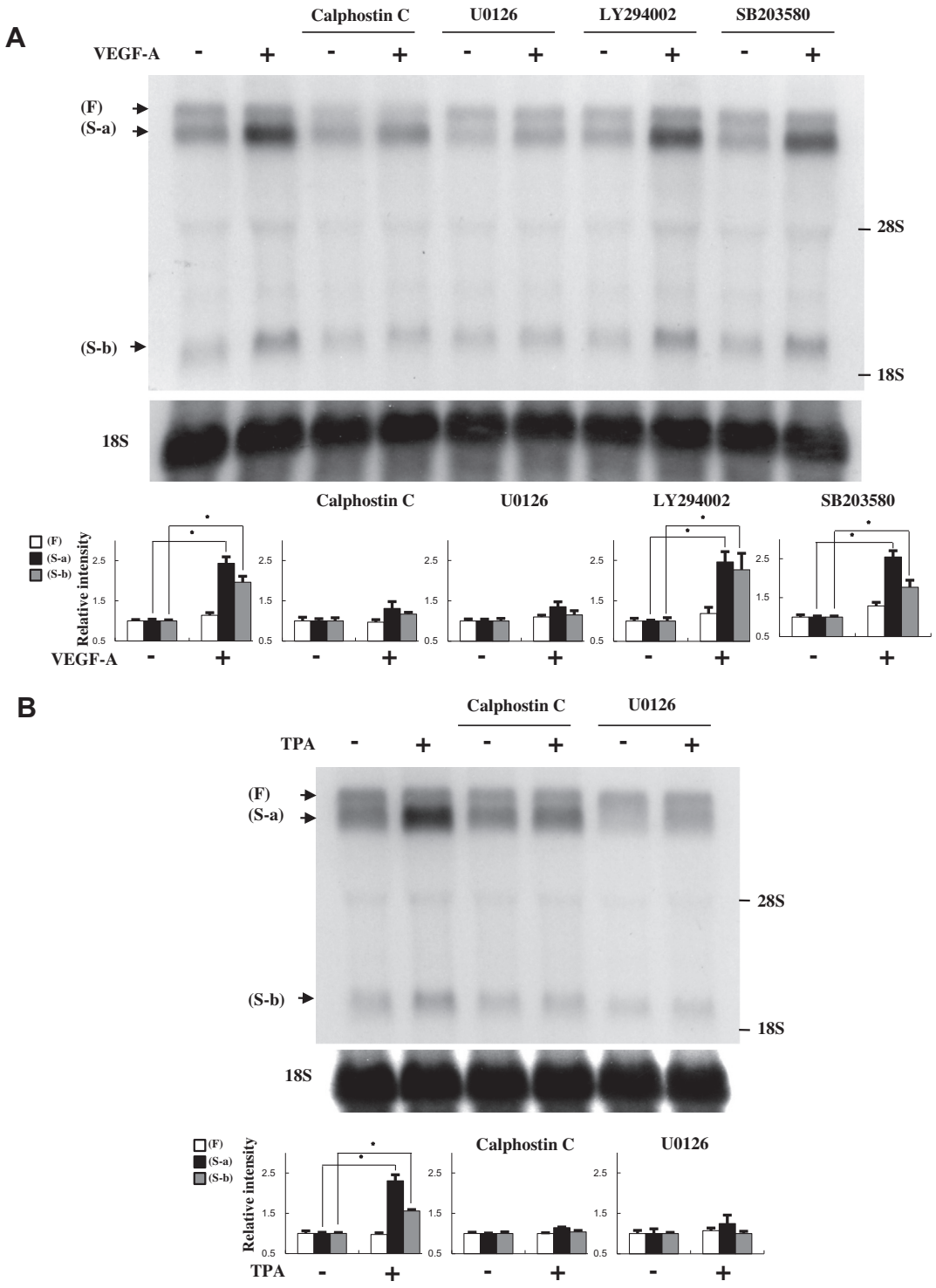

Fig. 3. VEGF-A-induced expression of sVEGFR-1 was mediated via PKC-MEK signaling. (A) HAECs were pretreated with protein kinase C (PKC) inhibitor (calphostin C (1 μM)), MEK inhibitor (u0126 (10 μM)), P13K inhibitor (LY294002 (10 μM)), or p38 MAPK inhibitor (SB203580 (10 μM)) for 30 min, followed by treatment with VEGF-A (50 ng/ml) for 3 h. Northern blot analysis was performed using probe 1. Quantitative analysis is shown below the figure. (B) HAECs were pretreated with either PKC inhibitor (calphostinC (1 μM)) or MEK inhibitor (u0126 (10 μM)) for 30 min, followed by treatment with TPA (100 pg/ml) for 3 h. Northern blot analysis was performed using probe 1. Quantitative analysis is shown below the figure.

full-length VEGFR-1 was unchanged, this suggested that VEGF-A affects the splicing process of the VEGFR-1 gene (Fig. 2A). Consistent with this finding, Western blot analysis also showed that VEGF-A induced an increase in the protein levels of sVEGFR-1 in HAECs as well (Fig. 2B).

3.4. VEGF-A induces sVEGFR-1 expression through VEGFR-2 signaling

We next examined the signaling pathways that regulate VEGF-A-mediated sVEGFR-1 induction. Knockdown of full-length VEGFR-1 with siRNA significantly enhanced the VEGF-A-induced

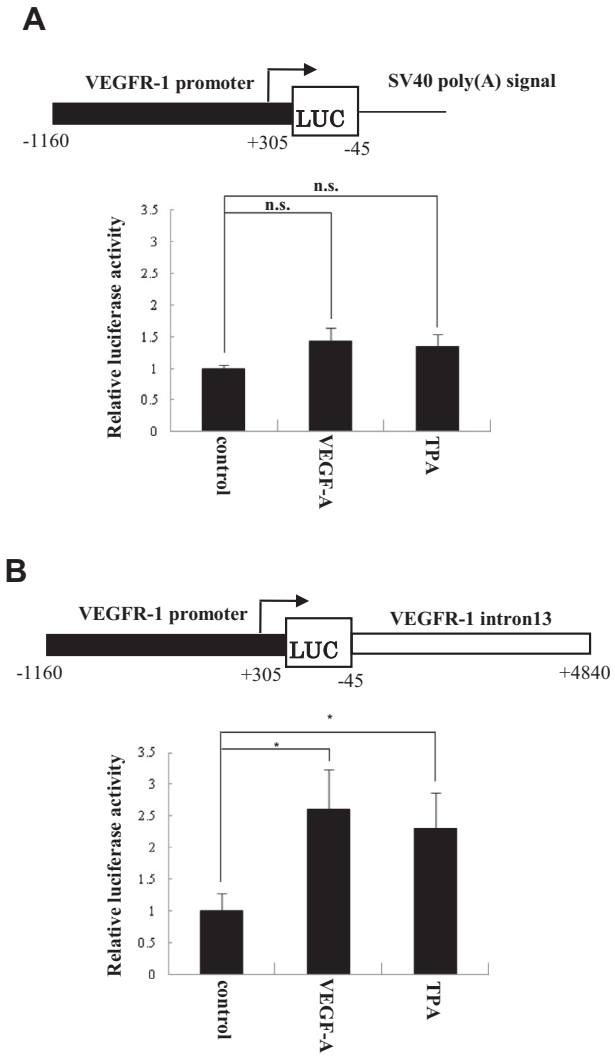

Fig. 4. PKC signaling upregulated the polyadenylation efficiency of intron 13. Different manipulation of the poly(A) additional signal in two luciferase reporter constructs. (A) A reporter construct driven by the VEGFR-1 promoter with the poly(A) additional signal of SV40 intact. (B) A reporter construct also driven by the VEGFR-1 promoter but with the poly(A) additional signal replaced by intron 13 of the VEGFR-1 gene. The two constructs were transfected into HAECs, followed by treatment with VEGF-A (50 ng/ml) or TPA (100 pg/ml) for 20 h. Luciferase reporter assay was then performed.

expression of sVEGFR-1 mRNA. In contrast, knockdown of VEGFR-2 abrogated the induction of sVEGFR-1, indicating that VEGF-A upregulates sVEGFR-1 through VEGFR-2-mediated pathways (Fig. 2C).

3.5. VEGF-A-induced expression of sVEGFR-1 was mediated via PKC-MEK signaling

VEGFR-2 is known to activate the protein kinase C (PKC)-MEK-ERK pathway, the PI3K-Akt pathway, and the p38MAPK pathway [26]. To further elucidate the signaling pathways underlying VEGF-A-mediated sVEGFR-1 induction, we used inhibitors for each of these pathways. HAECs were pretreated with protein kinase inhibitors and then exposed to VEGF-A. VEGF-A-elicited induction of sVEGFR-1 was significantly attenuated by PKC (calphostin C) or MEK (U0126) inhibitors, whereas PI3K (LY294002) or p38MAPK (SB203580) inhibitors did not have any effect (Fig. 3A). Furthermore, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (100 pg/ml), an activator of PKC, significantly induced the expression of sVEGFR-1 mRNA without affecting that of full-length VEGFR-1, and this induction was totally abrogated by PKC (calphostin C) or MEK (U0126) inhibitors (Fig. 3B). Pretreatment with higher concentrations (20–100 ng/ml) of TPA partially restored sVEGF-R1 induction in the presence of PKC inhibitor (data not shown). Collectively, these results indicate that VEGF-A induces sVEGFR-1 expression through the VEGFR-2-PKC-MEK pathway.

3.6. VEGF-A responsive elements exist in intron 13 of the VEGFR-1 gene

It is well known that intron 13 is critically important for the alternative splicing of VEGFR-1 gene. Therefore, we examined the contribution of intron 13 during the process of VEGF-A-elicited sVEGFR-1 induction using luciferase reporter assay. We generated a luciferase reporter construct that was driven by the VEGFR-1 promoter (Fig. 4A), and a second reporter construct that was also driven by the VEGFR-1 promoter but with the SV40 poly(A) additional signal of this plasmid replaced by intron 13 of the VEGFR-1 gene (Fig. 4B). Neither VEGF-A nor TPA affected VEGFR-1 promoter activity (Fig. 4A), whereas both VEGF-A and TPA significantly increased the luciferase activity of the reporter plasmid containing intron 13 of VEGFR-1 (Fig. 4B). These results suggest that VEGF-A or TPA responsive elements exist in intron 13 of the VEGFR-1 gene, which is critically important in its alternative splicing process.

4. Discussion

While both full-length and soluble forms of VEGFR-1 are synthesized as splice variants from VEGFR-1 gene [8], how this alternative splicing is regulated has remained largely unknown. In this study, we showed that VEGF-A regulates alternative splicing of the VEGFR-1 gene and induces the expression of sVEGFR-1 mRNA without affecting full-length VEGFR-1 expression. We also demonstrated that the induction was mediated via VEGFR-2 signaling pathways. This result correlates well with the observation that PlGF, a specific ligand for VEGFR-1, had no effect on sVEGFR-1 expression. Interestingly, specific knockdown of full-length VEGFR-1 significantly enhanced the induction of sVEGFR-1 by VEGF-A, a result that may be explained by an enhanced bioavailability of the VEGFR-2 pathway owing to the reduction in levels of VEGFR-1 that would otherwise act as a "decoy" receptor [5].

By using inhibitors for the various pathways that are involved in signaling through VEGFR-2, we further revealed that the induction of the expression of sVEGFR-1 by VEGF-A is mediated through the PKC-MEK pathway. Since it is well known that the major angiogenic signals of VEGF-A is largely transduced through VEGFR-2 and its downstream PKC-MEK-ERK pathways [27], these findings lend further support to the concept that VEGF-A-induced sVEGFR-1 expression can indeed operate as a negative feedback system to regulate angiogenic processes.

Lastly, we investigated the molecular mechanisms by which VEGF-A upregulates sVEGFR-1 expression. The critical region element for sVEGFR-1 production exists in intron 13 of the VEGFR-1 gene, where premature polyadenylation serves as a signal for alternative splicing [8,28,29]. While we demonstrated that there is a VEGF-A responsive element within intron 13 of the VEGFR-1 gene with the use of luciferase reporter assays, the precise molecular mechanisms of how VEGF-A regulates the alternative splicing at this intron of the VEGFR-1 gene still remains to be elucidated.

Angiogenesis is an important process in reproduction [30], embryonic development [31], and wound repair [32], but excessive angiogenesis can also result in numerous diseases such as atherosclerosis, arthritis, diabetic retinopathy and cancer progression [32,33]. It follows that tight spatial and temporal regulation of angiogenesis is vital and necessary, yet surprisingly little is known

about the negative feedback system in VEGF-A mediated angiogenic processes except for vasohibin [34]. Vasohibin has recently been identified as an endothelium-specific inhibitor of angiogenesis that is upregulated by VEGF-A and FGF-2. Therefore, the specific induction of sVEGFR-1 by VEGF-A revealed by this study may operate in another additional negative feedback system in VEGF-A-induced angiogenic processes.

In this study, we demonstrated that the expression profiles of the full-length and soluble VEGFR-1 genes significantly differ between tissues and cell types. The molecular mechanisms underlying such cell type-specific differences need to be elucidated in order to fully understand the precise mechanisms of angiogenesis. Moreover, the functional difference between the long form and short form sVEGFR-1 also has to be further investigated. One possibility, for example, is that the long 3′UTR of the long form of sVEGFR-1 may perhaps affect the post-transcriptional regulation of sVEGFR-1 by modulating its mRNA stability or translational efficiency.

In conclusion, we found that VEGF-A induces sVEGFR-1 in vascular endothelial cells through regulating the alternative splicing of the VEGFR-1 gene. Our findings further indicate that there is a clear interaction between VEGF-A and its negative regulator, sVEGFR-1, and therefore the modulation of this system can be a novel target for future therapeutic applications.

Acknowledgements

N.T. was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS, Japan) (to N.T. 24591109), JST PRESTO, the Banyu Life Science Foundation International and the Takeda Science Foundation. This research is also granted by the Japan Society for the Promotion of Science (JSPS) through the "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)," initiated by the Council for Science and Technology Policy (CSTP).

References

[1] Shibuya, M. (2001) Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. Cell Struct. Funct. 26, 25–35.
[2] Drake, C.J., LaRue, A., Ferrara, N. and Little, C.D. (2000) VEGF regulates cell behavior during vasculogenesis. Dev. Biol. 224, 178–188.
[3] Siemeister, G., Martiny-Baron, G. and Marme, D. (1998) The pivotal role of VEGF in tumor angiogenesis: molecular facts and therapeutic opportunities. Cancer Metastasis Rev. 17, 241–248.
[4] Neufeld, G., Cohen, T., Gengrinovitch, S. and Poltorak, Z. (1999) Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 13, 9–22.
[5] Kliche, S. and Waltenberger, J. (2001) VEGF receptor signaling and endothelial function. IUBMB Life 52, 61–66.
[6] Luttun, A., Tjwa, M., Moons, L., Wu, Y., Angelillo-Scherrer, A., Liao, F., Nagy, J.A., Hooper, A., Priller, J., De Klerck, B., Compernolle, V., Daci, E., Bohlen, P., Dewerchin, M., Herbert, J.M., Fava, R., Matthys, P., Carmeliet, G., Collen, D., Dvorak, H.F., Hicklin, D.J. and Carmeliet, P. (2002) Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nat. Med. 8, 831–840.
[7] Takeda, N., Maemura, K., Imai, Y., Harada, T., Kawanami, D., Nojiri, T., Manabe, I. and Nagai, R. (2004) Endothelial PAS domain protein 1 gene promotes angiogenesis through the transactivation of both vascular endothelial growth factor and its receptor, Flt-1. Circ. Res. 95, 146–153.
[8] Kendall, R.L., Wang, G. and Thomas, K.A. (1996) Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. Biochem. Biophys. Res. Commun. 226, 324–328.
[9] Park, J.E., Chen, H.H., Winer, J., Houck, K.A. and Ferrara, N. (1994) Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. J. Biol. Chem. 269, 25646–25654.
[10] Roeckl, W., Hecht, D., Sztajer, H., Waltenberger, J., Yayon, A. and Weich, H.A. (1998) Differential binding characteristics and cellular inhibition by soluble VEGF receptors 1 and 2. Exp. Cell Res. 241, 161–170.
[11] Hoshida, T., Sunamura, M., Duda, D.G., Egawa, S., Miyazaki, S., Shineha, R., Hamada, H., Ohtani, H., Satomi, S. and Matsuno, S. (2002) Gene therapy for pancreatic cancer using an adenovirus vector encoding soluble flt-1 vascular endothelial growth factor receptor. Pancreas 25, 111–121.
[12] Hasumi, Y., Mizukami, H., Urabe, M., Kohno, T., Takeuchi, K., Kume, A., Momoeda, M., Yoshikawa, H., Tsuruo, T., Shibuya, M., Taketani, Y. and Ozawa, K. (2002) Soluble FLT-1 expression suppresses carcinomatous ascites in nude mice bearing ovarian cancer. Cancer Res. 62, 2019–2023.
[13] Maynard, S.E., Min, J.Y., Merchan, J., Lim, K.H., Li, J., Mondal, S., Libermann, T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E., Epstein, F.H., Sukhatme, V.P. and Karumanchi, S.A. (2003) Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J. Clin. Investig. 111, 649–658.
[14] Ahmad, S. and Ahmed, A. (2004) Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. Circ. Res. 95, 884–891.
[15] Vuorela, P., Helske, S., Hornig, C., Alitalo, K., Weich, H. and Halmesmaki, E. (2000) Amniotic fluid-soluble vascular endothelial growth factor receptor-1 in preeclampsia. Obstet. Gynecol. 95, 353–357.
[16] Lee, E.S., Oh, M.J., Jung, J.W., Lim, J.E., Seol, H.J., Lee, K.J. and Kim, H.J. (2007) The levels of circulating vascular endothelial growth factor and soluble Flt-1 in pregnancies complicated by preeclampsia. J. Korean Med. Sci. 22, 94–98.
[17] Levine, R.J., Maynard, S.E., Qian, C., Lim, K.H., England, L.J., Yu, K.F., Schisterman, E.F., Thadhani, R., Sachs, B.P., Epstein, F.H., Sibai, B.M., Sukhatme, V.P. and Karumanchi, S.A. (2004) Circulating angiogenic factors and the risk of preeclampsia. N. Engl. J. Med. 350, 672–683.
[18] Yang, F., Jin, C., Jiang, Y.J., Li, J., Di, Y. and Fu, D.L. (2011) Potential role of soluble VEGFR-1 in antiangiogenesis therapy for cancer. Expert Rev. Anticancer Ther. 11, 541–549.
[19] Lamszus, K., Ulbricht, U., Matschke, J., Brockmann, M.A., Fillbrandt, R. and Westphal, M. (2003) Levels of soluble vascular endothelial growth factor (VEGF) receptor 1 in astrocytic tumors and its relation to malignancy, vascularity, and VEGF-A. Clin. Cancer Res. 9, 1399–1405.
[20] Chang, Y.T., Chang, M.C., Wei, S.C., Tien, Y.W., Hsu, C., Liang, P.C., Tsao, P.N., Jan, I.S. and Wong, J.M. (2008) Serum vascular endothelial growth factor/soluble vascular endothelial growth factor receptor 1 ratio is an independent prognostic marker in pancreatic cancer. Pancreas 37, 145–150.
[21] Toi, M., Bando, H., Ogawa, T., Muta, M., Hornig, C. and Weich, H.A. (2002) Significance of vascular endothelial growth factor (VEGF)/soluble VEGF receptor-1 relationship in breast cancer. Int. J. Cancer 98, 14–18.
[22] Kawano, Y. and Kypta, R. (2003) Secreted antagonists of the Wnt signalling pathway. J. Cell Sci. 116, 2627–2634.
[23] Caldwell, G.M., Jones, C.E., Taniere, P., Warrack, R., Soon, Y., Matthews, G.M. and Morton, D.G. (2006) The Wnt antagonist sFRP1 is downregulated in premalignant large bowel adenomas. Br. J. Cancer 94, 922–927.
[24] Gonzalez-Sancho, J.M., Aguilera, O., Garcia, J.M., Pendas-Franco, N., Pena, C., Cal, S., Garcia de Herreros, A., Bonilla, F. and Munoz, A. (2005) The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. Oncogene 24, 1098–1103.
[25] Sela, S., Itin, A., Natanson-Yaron, S., Greenfield, C., Goldman-Wohl, D., Yagel, S. and Keshet, E. (2008) A novel human-specific soluble vascular endothelial growth factor receptor 1: cell-type-specific splicing and implications to vascular endothelial growth factor homeostasis and preeclampsia. Circ. Res. 102, 1566–1574.
[26] Koch, S. and Claesson-Welsh, L. (2012) Signal transduction by vascular endothelial growth factor receptors. Cold Spring Harbor Perspect. Med. 2, a006502.
[27] Ferrara, N., Gerber, H.P. and LeCouter, J. (2003) The biology of VEGF and its receptors. Nat. Med. 9, 669–676.
[28] Huckle, W.R. and Roche, R.I. (2004) Post-transcriptional control of expression of sFlt-1, an endogenous inhibitor of vascular endothelial growth factor. J. Cell. Biochem. 93, 120–132.
[29] Thomas, C.P., Andrews, J.I. and Liu, K.Z. (2007) Intronic polyadenylation signal sequences and alternate splicing generate human soluble Flt1 variants and regulate the abundance of soluble Flt1 in the placenta. FASEB J. 21, 3885–3895.
[30] Jaffe, R.B. (2000) Importance of angiogenesis in reproductive physiology. Semin. Perinatol. 24, 79–81.
[31] Breier, G., Damert, A., Plate, K.H. and Risau, W. (1997) Angiogenesis in embryos and ischemic diseases. Thromb. Haemost. 78, 678–683.
[32] Folkman, J. and Shing, Y. (1992) Angiogenesis. J. Biol. Chem. 267, 10931–10934.
[33] Folkman, J. (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat. Med. 1, 27–31.
[34] Watanabe, K., Hasegawa, Y., Yamashita, H., Shimizu, K., Ding, Y., Abe, M., Ohta, H., Imagawa, K., Hojo, K., Maki, H., Sonoda, H. and Sato, Y. (2004) Vasohibin as an endothelium-derived negative feedback regulator of angiogenesis. J. Clin. Investig. 114, 898–907.