Urotensin II Stimulates Vascular Endothelial Growth Factor Secretion From Adventitial Fibroblasts in Synergy With Angiotensin II

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Background: The adventitia plays an important role in and is considered to be the initiating site for vascular remodeling. Urotensin II (UII) and angiotensin II (Ang II) are the two most important vascular peptides involved in vascular remodeling in the adventitia. Nevertheless, little is known about their effect on the expression of vascular endothelial growth factor (VEGF). It was hypothesized that both UII and Ang II could induce VEGF expression in adventitial fibroblasts and VEGF may play a role in cell proliferation and collagen I synthesis induced by UII or Ang II.

Methods and Results: Growth-arrested adventitial fibroblasts were incubated in serum-free medium with UII and/or Ang II and inhibitors of the mitogen-activated protein kinase (MAPK) pathway or VEGF-neutralizing antibodies. The VEGF expression was evaluated using enzyme-linked immunosorbent assay (ELISA), while the proliferation and collagen I synthesis were detected using methyl thiazol tetrazolium (MTT) assay and ELISA. It was found that: (1) both UII and Ang II could stimulate VEGF expression in adventitial fibroblasts and they had a synergistic effect; (2) MAPK pathway inhibitors could inhibit VEGF secretion induced by UII and/or Ang II; and (3) VEGF-neutralizing antibodies could inhibit UII/Ang II-induced cell proliferation and collagen synthesis in adventitial fibroblasts.

Conclusions: Induction of VEGF expression may be a new mechanism involved in vascular remodeling for UII and Ang II. (*Circ J* 2012; **76**: 1267–1273)

Key Words: Adventitial fibroblasts; Angiotensin II; Urotensin II; Vascular endothelial growth factor; Vascular remodeling

Vascular remodeling is an important field of cardiovascular physiology and has the highest potential for useful translation from basic research to clinical medicine. It is a keystone of many cardiovascular diseases including hypertension, atherosclerosis and restenosis. The adventitia plays a critical role in vascular remodeling.¹ Following injury, adventitial fibroblasts are activated and differentiate into myofibroblasts, which have contractile properties and significant proliferative and synthetic activities, migrating to the intima and contributing to neointima formation.^{1,2} The differentiation, migration and proliferation of adventitial fibroblasts and collagen synthesis in these cells are involved in vascular remodeling.³ However, the mechanisms behind vascular remodeling in the adventitia are still not completely understood.

Urotensin II (UII) and angiotensin II (Ang II) are two of the most important and most studied peptides involved in vascular

remodeling. They have been recognized as potent vascular constrictors that, along with their receptors, are up-regulated in patients with hypertension, atherosclerosis and restenosis following balloon angioplasty.^{4–6} They can induce the proliferation of vascular endothelial cells and vascular smooth muscle cells.^{7–9} Recent studies have shown that UII and Ang II may induce vascular remodeling in the adventitia by inducing the differentiation, migration and proliferation and collagen synthesis in adventitial fibroblasts.^{3,10} The above effects are mainly achieved through the binding of UII and Ang II with their respective receptors to activate downstream pathways such as mitogen-activated protein kinase (MAPK), protein kinase C (PKC) and calcium channels.³ However, the exact mechanisms by which UII and Ang II mediate vascular remodeling in the adventitia are still largely unclear.

VEGF is one of the most important angiogenic cytokines involved in vascular remodeling.^{11–16} In vivo experiments have

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shown that VEGF can induce intimal hyperplasia in rabbit carotid arteries. Researchers have also demonstrated that VEGF can stimulate the proliferation of vascular cells, including vascular endothelial cells and smooth muscle cells, and can induce the proliferation and migration of adventitial fibroblasts.^{10,17} Previous studies have shown that VEGF can be produced by vascular wall cells that express the VEGF receptor Flt-1, such as endothelial cells and vascular smooth muscle cells.¹⁰ Jin et al found that Flt-1 was expressed in adventitial fibroblasts; however, little is known about whether adventitial fibroblasts serve as a novel source of VEGF.¹⁷

The effects of Ang II and UII on VEGF expression have been mostly studied in vascular endothelial cells and smooth muscle cells. Ang II has been reported to induce VEGF expression in human endothelial cells and smooth muscle cells.¹⁰ Furthermore, researchers have shown that VEGF is involved in Ang II-induced cell proliferation and collagen synthesis.^{9,18} However, Albertin et al found that UII did not affect VEGF expression in human endothelial cells.¹⁹ In adventitial fibroblasts, Ang II was shown to up-regulate the expression of Flt-1, a receptor of VEGF. However, the true effect of Ang II and UII on VEGF expression in adventitial fibroblasts is currently unknown. Furthermore, it is still unclear whether VEGF is involved in cell proliferation and collagen synthesis induced by Ang II and UII.

In the present study, we hypothesized that both Ang II and UII could induce VEGF expression in adventitial fibroblasts and that VEGF was involved in Ang II- and UII-induced cell proliferation and collagen synthesis.

Methods

Animals

Male Sprague-Dawley rats weighing 120–180 g were supplied by the Animal Center, Peking University, Beijing, China. Experimental protocols were in compliance with the Animal Management Rules of China (Documentation 55, 2001, Ministry of Health, China).

Reagents

Rat UII and Ang II were purchased from Phoenix Pharmaceuticals Inc (Belmont, CA, USA). Fetal bovine serum (FBS) was from Hyclone (Logan, UT, USA). Dulbecco's modified Eagle's medium (DMEM), methyl thiazol tetrazolium (MTT) assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and DMSO (dimethyl sulfoxide) were from Sigma (St. Louis, MO, USA). PD123319, PD98059 and SB203580 were bought from Promega (USA). Urapidine and losartan were supplied by Peptides International, Inc (Louisville, KY, USA). Rat VEGF enzyme-linked immunosorbent assay (ELISA) kits and rat collagen Type I ELISA kits were from BIOPCE (Beijing, China). The VEGF antibody used for western blot was from Santa Cruz. The neutralizing antibody against VEGF and IgG was from R&D Systems (Minneapolis, MN, USA).

Adventitial Fibroblast Cell Culture

Aortic adventitial fibroblasts were prepared according to the method used by Tsuruda et al, with some modifications.²⁰ Briefly, rats were anesthetized with isoflurane and rapidly decapitated. The full length of the thoraco-abdominal aorta was isolated in a pool of 4°C phosphate-buffered saline (PBS) after gently opening the parietal pleura, separating loose connective tissue and collateral vessels and transecting the segmental arteries at their origins. Under magnification, the adventitial and medial layers could be distinguished at both ends

of the aorta. Endothelial cells were removed by gently rubbing the lumen with the blunt side of dissecting scissors, and the medial layer was peeled off using two forceps. The remaining tissues, predominantly the adventitia, were cut rapidly into small pieces and placed in a 25 cm² tissue culture flask with DMEM medium containing 20% FBS. The explants were cultivated until large colonies of cells formed surrounding the explants. Fibroblasts grew from these explants in approximately 4–5 days. After achieving confluence, they were harvested with trypsin, used for experiments at passage 3–5 and cultured with DMEM containing 10% FBS. The morphology and growth characteristics of the cells were typical of fibroblasts and were distinguishable from smooth muscle cells by the absence of a “hill-and-valley” growth pattern and the lack of α -smooth muscle actin staining.

Study Design

To examine the induction of VEGF expression through UII and Ang II in adventitial fibroblasts, cells were subcultured in 96-well plates and incubated with serum-free medium (containing 0.5% FBS) for 24 h. After synchronization, the medium was changed to DMEM without any serum. Thereafter, the adventitial fibroblasts were incubated in the presence or absence of UII and/or Ang II. To explore the molecular mechanisms of these proteins, inhibitors such as urapidine (an antagonist of the UII receptor [GPR14]), losartan (an antagonist of the Ang II type I receptor [AT₁R]), PD123319 (an antagonist of the Ang II type II receptors [AT₂R]), and the MAPK inhibitors PD98059 and SB203580 were used.

Based on the experimental design, cells were divided into the following groups: (1) control group, cells were only incubated with serum-free DMEM; (2) UII group, 10⁻¹⁰ to 10⁻⁶ mol/L of UII was added to the medium; (3) Ang II group, 10⁻¹⁰ to 10⁻⁶ mol/L of Ang II was added to the medium; (4) time-dependent research group, 10⁻⁸ mol/L of UII or 10⁻⁶ mol/L of Ang II was added to the medium for 6 h to 72 h; (5) UII and Ang II group, 10⁻¹⁰ mol/L of UII and 10⁻¹⁰ mol/L of Ang II or 10⁻⁸ mol/L of UII and 10⁻⁷ mol/L of Ang II were added, respectively; and (6) UII and/or Ang II and inhibitors group, cells were pre-treated in serum-free DMEM with urapidine (10⁻⁶ mol/L), losartan (10⁻⁶ mol/L), PD123319 (10⁻⁶ mol/L), the MAPK inhibitor PD98059 (10⁻⁶ mol/L) or SB203580 (10⁻⁶ mol/L), VEGF-neutralizing antibody (20 μ g/ml) or Ig G (which was used as negative control for VEGF-neutralizing antibody), and incubated with UII (10⁻⁸ mol/L) and/or Ang II (10⁻⁶ mol/L). Serum was collected from the cells after 24 h of incubation except for the time-dependent research group.

Enzyme-Linked Immunosorbent Assay for VEGF and Collagen Type I

The medium was collected from cultured adventitial fibroblasts with or without the treatments mentioned above and centrifuged immediately. The supernatant was measured using rat VEGF and Collagen Type I ELISA kits according to the manufacturer's instructions. The absorbance of colored products was determined using a microplate reader set to 450 nm.

Western Blot Assay for VEGF

After incubation, the culture medium was removed, and adventitial fibroblasts were lysed directly in the dish at 4°C for 30 min with lysis buffer (20 mmol/L Tris, 150 mmol/L NaCl, 1 mol/L ethylenediaminetetraacetic acid, 1% Nonidet P-40, 2 mol/L Na₃VO₄) supplemented with 1 mol/L phenylmethylsulphonyl fluoride, 5 mg/ml leupeptin. Lysates were cleared by centrifugation at 12,000 g for 15 min at 4°C. Protein con-

Figure 1. Angiotensin II stimulates VEGF expression in adventitial fibroblasts. **(A)** Time course of Ang II-mediated induction of VEGF secretion (n=6). Rat adventitial fibroblasts were treated with Ang II (10⁻⁶ mol/L) for the time indicated. **(B)** Effect of different concentrations of Ang II on VEGF secretion in cultured adventitial fibroblasts in vitro (n=6). Cells were stimulated with different concentrations of Ang II (10⁻¹⁰ to 10⁻⁶ mol/L) for 24 h. **(C)** Effect of different concentrations of Ang II on VEGF expression in cultured adventitial fibroblasts in vitro assayed by western blot (n=6). **(D)** Effect of different inhibitors on VEGF secretion induced by Ang II (10⁻⁶ mol/L) (n=6). PD, PD98059; SB, SB203580; LO, Losartan. **P<0.01 vs. control; ###P<0.01 vs. Ang II group.

tent in the lysate was analyzed by the Bradford assay. Equal amounts of protein were resolved by electrophoresis in a 12% sodium dodecyl sulfate polyacrylamide gel (12% running gel, 5% stacking gel) and transferred onto nitrocellulose membranes with a 0.2-mm-pore size. The membrane was incubated in blocking buffer [Tris-buffered saline (TBS), containing 0.1% Tween 20 and 5% non-fat milk] for 1 h at room temperature followed by the addition of the primary antibody monoclonal anti VEGF (1:1,000 in 5% nonfat dry milk in TBS-T) for 2 h at room temperature. Then, the membrane was rinsed three times with TBS/Tween 20 and incubated with goat-anti-mouse IgG-peroxidase conjugate (1:2,000 in 5% nonfat dry milk in TBS-T). Immunocomplexes were visualized using renaissance chemiluminescence reagents (Santa Cruz, CA, USA) and exposure to radiograph film (Kodak, Rochester, NY, USA). The optical density of immunoblots was determined by scanning densitometry and the result was presented in densitometric units. Translation was calculated by dividing the normalized VEGF density by the normalized β-actin density (VEGF/β-actin ratio).

Assessment of Cell Proliferation—MTT Assay

The effect of Ang II or U1I and a VEGF antagonist on cell proliferation was determined using the MTT assay. Briefly, adventitial fibroblasts were subcultured in 96-well plates and

incubated with serum-free medium for 24 h. Quiescent cultures were treated with U1I (10⁻⁸ mol/L) or Ang II (10⁻⁷ mol/L) with or without the specific VEGF-neutralizing antibody for another 24 h. IgG was used for negative control. Twenty microliters of MTT (15 mg/ml) was added to each well and incubated for 4 h at 37°C. The culture supernatant was discarded by aspiration, and 150 μl of DMSO was added to each well and incubated for 10 min. Light absorbance was detected at 570 nm using an ELISA reader.

Statistical Analysis

The data were analyzed with the statistical software package SPSS 16.0 (SPSS, Chicago, IL, USA) and were displayed as the mean ± standard deviation. Comparisons were done using the one-way ANOVA test, and post-hoc multiple comparisons were done with the least significant difference test. A P value of <0.05 was considered to be statistically significant.

Results

Ang II Up-Regulates the Expression of VEGF in Adventitial Fibroblasts

In the present investigation, we first explored whether adventitial fibroblasts were a novel source of VEGF and whether Ang II could stimulate VEGF secretion and synthesis in ad-

Figure 2. UII induces VEGF expression in adventitial fibroblasts. (A) Time course of UII-mediated induction of VEGF secretion ($n=6$). Rat adventitial fibroblasts were treated with UII (10^{-8} mol/L) for the time indicated. (B) Effect of different concentrations of UII on VEGF secretion in cultured adventitial fibroblasts in vitro ($n=6$). Cells were stimulated with different concentrations of UII (10^{-10} to 10^{-6} mol/L) for 24 h. (C) Effect of different concentrations of UII on VEGF expression in cultured adventitial fibroblasts in vitro assayed by western blot ($n=6$). (D) Effect of different inhibitors on VEGF secretion in adventitial fibroblasts induced by UII (10^{-8} mol/L) ($n=6$). PD, PD98059; SB, SB203580; UR, Urantide. ** $P<0.01$ vs. control; ## $P<0.01$ vs. UII group.

ventitial fibroblasts. Adventitial fibroblasts were treated with different concentrations of Ang II (10^{-10} to 10^{-6} mol/L) for 24 h or with Ang II (10^{-6} mol/L) for 0 to 72 h. We determined the baseline level of VEGF secretion and demonstrated that Ang II could induce VEGF secretion in adventitial fibroblasts in a time- and dose-dependent manner. As shown in Figure 1A, when adventitial fibroblasts were treated with Ang II (10^{-6} mol/L), VEGF secretion kept increasing from 6 h to 72 h, with a maximal effect at 24 h, when it was approximately 1.95-fold higher than in the control group (121.27 ± 3.49 pg/ml vs. 62.13 ± 1.75 pg/ml, $P<0.01$). VEGF secretion in adventitial fibroblasts was affected at all doses of Ang II used in our study, with the maximal effect reached at 10^{-6} mol/L ($P<0.01$, Figure 1B). Also, we confirmed the same trend in VEGF synthesis using western blot analysis (Figure 1C). To evaluate the role of two specific Ang II receptors (AT₁R and AT₂R) in Ang II-mediated induction of VEGF expression, adventitial fibroblasts were pre-treated with antagonists of these receptors, losartan and PD123319. Our results showed that the AT₁R antagonist losartan caused a significant decrease in Ang II-induced VEGF secretion, whereas the AT₂R antagonist PD123319 had no effect (Figure 1D), suggesting that Ang II-induced up-regulation of VEGF is mediated by the AT₁R receptor. When adventitial fibroblasts were pre-treated with MAPK inhibitors (PD98059 and SB203580), we found that VEGF secretion was inhibited, suggesting that

the MAPK pathway is also involved in the induction of VEGF expression by Ang II.

UII Increases the Expression of VEGF in Adventitial Fibroblasts

After confirming that Ang II induces VEGF secretion in adventitial fibroblasts, we studied the effect of UII on VEGF secretion. Adventitial fibroblasts were treated with different concentrations of UII (10^{-10} to 10^{-6} mol/L) for 24 h or with UII (10^{-8} mol/L) for different amounts of time. As with Ang II, a time-dependent and dose-dependent induction of VEGF expression was observed. Our results showed that, after 6 h of treatment, 10^{-8} mol/L of UII induced VEGF secretion above baseline levels, and the induction peaked at 24 h (102.02 ± 2.28 pg/ml vs. 50.47 ± 2.24 pg/ml, $P<0.01$, Figure 2A). Additionally, we discovered that the maximal effect was reached at 10^{-8} mol/L of UII, and induction of VEGF secretion was less effective at other concentrations (Figure 2B). Also, we confirmed the same trend in VEGF synthesis through western blot analysis (Figure 2C). Nevertheless, pre-treatment with urantide or MAPK inhibitors (PD98059 and SB203580) could completely or partly inhibit UII-induced VEGF secretion in adventitial fibroblasts (Figure 2D), suggesting that the binding of UII to GPR14 and MAPK pathway receptors may play an important role in the induction of VEGF secretion by UII.

U1I and Ang II Have Synergistic Effects on VEGF Secretion in Adventitial Fibroblasts

We found that low doses of Ang II or U1I (10⁻¹⁰mol/L) slightly induced VEGF secretion in adventitial fibroblasts when used separately. However, when adventitial fibroblasts were incubated with both together, VEGF secretion was up-regulated significantly (Figure 3A). Although 10⁻⁶mol/L of Ang II and 10⁻⁸mol/L of U1I were found to exert maximal effects on VEGF secretion, they still had synergistic effects when used together (Figure 3B). Furthermore, our results demonstrated that inhibitors of the MAPK pathway inhibited the synergetic effects described above (Figures 3A,3B).

Involvement of VEGF in U1I- or Ang II-Induced Proliferation and Collagen Synthesis in Adventitial Fibroblasts

We used a specific neutralizing antibody against VEGF (Ab-VEGF) to explore whether VEGF was involved in the proliferation and collagen synthesis in adventitial fibroblasts induced by U1I or Ang II. Adventitial fibroblasts were pre-treated with Ab-VEGF for 0.5 h and then incubated with U1I (10⁻⁸mol/L) or Ang II (10⁻⁶mol/L). The results of the MTT and ELISA assays showed that Ab-VEGF significantly inhibited U1I- or Ang II-induced proliferation and collagen I synthesis, suggesting that VEGF plays an important role in the above processes (Figures 4A–D). We also observed that the neutralizing antibody against VEGF had an inhibitive effect

on the basal proliferation of adventitial fibroblasts and collagen synthesis in these cells.

Discussion

Recent studies have shown that adventitia is the initiating site for vascular remodeling. There are few studies about the effect of U1I and Ang II on VEGF expression in adventitial fibroblasts that play an important role in vascular remodeling. Although it was shown that VEGF could induce and was important for vascular remodeling induced by U1I and Ang II was still unclear, which was characterized by the proliferation and collagen synthesis of adventitial fibroblasts. As vascular remodeling was a complex course in vivo, to clarify the role of VEGF in vascular remodeling induced by U1I and Ang II more clearly, we performed an in vitro experiment in order to provide evidence for the above.

In the present study, we provide evidence for the first time that U1I and Ang II can induce VEGF expression in adventitial fibroblasts and that VEGF is involved in U1I- and Ang II-induced cell proliferation and collagen synthesis.

Previously, little was known regarding VEGF expression in adventitial fibroblasts. We demonstrate for the first time that VEGF is secreted at a baseline level in adventitial fibroblasts and show that U1I and Ang II stimulate VEGF secretion in

Figure 4. Involvement of VEGF in UII-induced and Ang II-induced proliferation and collagen synthesis in adventitial fibroblasts. The results of ELISA (**A,B**) and MTT (**C,D**) assays show a significant inhibition of Ab-VEGF on adventitial proliferation and collagen synthesis induced by Ang II (10^{-7} mol/L) and UII (10^{-8} mol/L). All $n=6$. * $P<0.05$ vs. control; ** $P<0.01$ vs. control; ## $P<0.01$ vs. UII group or Ang II group.

adventitial fibroblasts. Our results are similar to what has been observed in human endothelial cells, vascular endothelial cells and smooth muscle cells, where Ang II has been shown to induce VEGF expression.^{10,21} The time- and concentration-dependent properties observed in our study are consistent with a study done by Jin et al, which demonstrated that Ang II up-regulates Flt, a receptor for VEGF, in a time- and dose-dependent manner.¹⁷ The only difference is that, in our study, the maximal effect of Ang II was seen at a concentration of 10^{-6} mol/L, while in theirs, it was seen at a concentration of 10^{-7} mol/L. This difference can be explained by receptor desensitization of Flt and the fixed half-life of VEGF. In contrast to Albertin et al, who did not observe the induction of VEGF expression by UII in human endothelial cells, we discovered that UII induces VEGF secretion in adventitial fibroblasts.¹⁹ The different cell types used may be a major cause for these conflicting results.

The mechanisms underlying the regulation of VEGF expression have been the subject of intensive investigation. Using a pharmacological approach, we demonstrated that stimulation of VEGF through UII in adventitial fibroblasts was mediated by the receptor GPR14 and pathways downstream of GPR14, including ERK and p38 signaling. These pathways were also shown to be involved in Ang II-induced VEGF expression in adventitial fibroblasts. It should be mentioned that it is AT₁R and not AT₂R that is involved in Ang II-mediated induction of VEGF expression, which is similar to observations in endothelial cells and cardiac myofibroblasts.^{21,22} Previous studies have suggested that there are different pathways responsible for the regulation of VEGF expression, depending on cell types and stimuli. Many studies have demonstrated that

the MAPK pathway (especially ERK and p38) plays an important role in the regulation of VEGF expression.^{23–26} However, Wu et al reported that inhibition of ERK had no effect on VEGF expression in retinal microvascular endothelial cells.²⁷ The reason for these contradictory results may be the differences in cell types and stimuli used in the studies. Our results demonstrate for the first time that the ERK and p38 pathways play an important role in the induction of VEGF expression through Ang II and UII in adventitial fibroblasts.

Many peptides interact with each other, and this appears to be the case based on our result that Ang II induces VEGF secretion in synergy with UII. Our results are similar to those of Wang and Lamarre, who showed that UII and Ang II have a synergistic effect on vasoconstriction in the endothelium-denuded aorta and aortic rings with intact adventitia and endothelium.^{28,29} We further demonstrated that the ERK and p38 pathways were involved in the synergistic action of UII and Ang II. However, inhibitors of ERK and p38 could not fully block the synergistic action, suggesting that other pathways are also involved in the synergistic effects described above. Many studies have shown that PKC pathways are involved in the regulation of VEGF. Thus, it is possible that the PKC pathway, which plays an important role in the synergistic effect of UII and Ang II on vasoconstriction, might also be involved in the synergistic effect observed in our study. Additionally, it is possible that pathways such as JNK, STAT/Smad and PKA, which can all regulate VEGF expression, might also be at work.

In this study, we demonstrate that VEGF-neutralizing antibodies can significantly inhibit Ang II- and UII-induced proliferation and collagen synthesis in adventitial fibroblasts. This

suggests that VEGF may be a down-stream angiogenic mediator of Ang II and U11. However, VEGF-neutralizing antibodies could not completely inhibit the cell proliferation and collagen synthesis induced by U11 and Ang II, suggesting that other factors that regulate cell proliferation and collagen synthesis, such as TGF- β and PDGF, might also be involved. The induction of VEGF expression by Ang II and U11 may be of considerable clinical significance, especially in diseases with defective vascular remodeling such as hypertension, atherosclerosis and restenosis. Pro-inflammatory cytokines play a crucial role in vascular remodeling in atherosclerosis and hypertension. VEGF produced by adventitial fibroblasts in response to U11 and Ang II can activate neutrophils and monocytes, resulting in their adherence to endothelial cells. This might trigger pathophysiological changes in vascular remodeling. In addition, VEGF can induce the proliferation vascular endothelial cells and smooth muscle cells and the proliferation and migration of and collagen synthesis in adventitial fibroblasts, thus inducing vascular remodeling. The finding that the VEGF-neutralizing antibody inhibits Ang II- and U11-induced proliferation and collagen synthesis provides new support for the use of drugs against VEGF in the treatment of cardiovascular diseases with vascular remodeling. However, it should be noted that the VEGF-neutralizing antibody also inhibited the baseline proliferation and collagen synthesis in adventitial fibroblasts. Therefore, application of a VEGF-neutralizing antibody in clinical practice should be applied with great caution.

In conclusion, U11 and Ang II can synergistically stimulate VEGF secretion in adventitial fibroblasts. Several pathways, such as ERK and p38, are involved in the induction of adventitial fibroblast-mediated VEGF secretion by U11 and/or Ang II. VEGF is involved in U11- and Ang II-mediated induction of adventitial fibroblast proliferation and collagen synthesis. Thus, our results clarify new mechanisms involved in vascular remodeling in the adventitia induced by U11 and Ang II, and provide new evidence supporting the application of VEGF-neutralizing antibodies in clinical practice.

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