Biochemical and Biophysical Research Communications 434 (2013) 552–558

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ang II induces capillary formation from endothelial cells via the AT1R-dependent inositol requiring enzyme 1 pathway

Xiang Wang [a,1], Yong-Ping Bai [b,1], Dan Hong [a], Hai-Chao Gao [a], Ling-Fang Li [a], Chuan-Chang Li [b], Ling-Ping Zhu [b], Quan Sun [b], Guo-Gang Zhang [a,*]

[a] Department of Cardiovascular Medicine, Xiangya Hospital, Central South University, Changsha 410078, China
[b] Department of Geriatric Medicine, Xiangya Hospital, Central South University, Changsha 410078, China

ARTICLE INFO

Article history:
Received 16 March 2013
Available online 10 April 2013

Keywords:
Angiotension II
GRP78
IRE1
MAPKs
AT1 receptor
Angiogenesis

ABSTRACT

Previous studies have demonstrated an important interaction between angiotension II type 1 receptor (AT1R) and angiotension II (Ang II) -induced capillary formation from endothelial cells and vascular endothelial growth factor (VEGF). However, the underlying mechanism remains elusive. Recent studies revealed that the unfolded protein response regulates an angiogenic response by the kidney epithelium during ischemic stress. Therefore, in the present study, we investigated the effects of Ang II on AT1R-mediated capillary formation from endothelial cells and the possible involvement of the IRE1/JNK/p38 MAPK pathway. Our results show that Ang II (1 nmol/L) induced the expression of VEGF and enhanced capillary formation from endothelial cells in the Matrigel assay. This effect was significantly depressed by the AT1R blocker losartan and different inhibitors (irestatin, IRE1 specific inhibitor; SP600125, JNK specific inhibitor; SB203580, p38 MAPK specific inhibitor) but not by the AT2R blocker PD123319. Next, we investigated the effect of Ang II on the IRE1/JNK/p38 MAPK pathway and the 78 kDA glucose regulated protein 78 (GRP78) activity in HUVECs and the role of the AT1 Receptor. The results show that Ang II activated both the IRE1/JNK/p38 MAPK pathway and GRP78 binding activity. These effects were markedly inhibited by the AT1R blocker losartan. The IRE1 specific inhibitor irestatin, the JNK specific inhibitor SP600125, and the p38 MAPK specific inhibitor SB203580 significantly inhibited Ang II-induced capillary formation from endothelial cells and VEGF expression but had no effect on GRP78. Collectively, these findings suggest for the first time that Ang II promotes capillary formation by inducing the expression of VEGF via Ang II type 1 receptor-mediated stimulation of the IRE1/JNK/p38 MAPK pathway.

1. Introduction

Angiogenesis, defined as the formation of new capillaries, is a physiological process necessary for embryonic development and wound repair, as well as in various pathological events, such as tissue ischemia and atherosclerosis [1]. More importantly, angiogenesis is a vital feature of the complications of the atherogenic process, such as an acute coronary event and stroke, due to the observation of intraplaque hemorrhage in the microvasculature that may cause the atherosclerotic plaque to rupture [2]. Vascular endothelial growth factor (VEGF) is a key angiogenic growth factor that stimulates proliferation, migration, and capillary tube formation [3]. Due to its varied functions, VEGF may be considered a "Swiss army knife" of growth factors, being able to initiate all of the angiogenic processes [4].

Angiotensin II (Ang II) has been implicated in both angiogenesis and pathological vascular growth [5]. The contributory role of Ang II in angiogenesis is supported by several lines of evidence [6–7]. Firstly, coronary capillary angiogenesis is mediated by AT1R-mediated upregulation of VEGF at the insulin-resistant stage in the noninsulin-dependent diabetes mellitus rat model. Secondly, hypoxia and ischemia-induced angiogenesis are promoted through AT1R-mediated upregulation of VEGF and its receptors. Thirdly, Ang II can induce capillary formation directly from endothelial cells by increasing VEGF production via the LOX-1 dependent redox-sensitive pathway [8]. Finally, AT1R antagonists and angiotensin-converting enzyme inhibitors block angiogenesis both in vivo and in endothelial cell models. However, the details of the mechanisms regulating Ang II induced angiogenesis are incompletely understood.

The endoplasmic reticulum (ER) is an organelle that plays an essential role in multiple cellular processes, such as the folding of secretory and membrane proteins, including VEGF, calcium homeostasis, and lipid biosynthesis [9]. A variety of insults

* Corresponding author. Fax: +86 086 731 84327691.
E-mail address: xyzgg2006@sina.com (G.-G. Zhang).
[1] These two authors contributed equally to this work.

0006-291X/$ - see front matter Crown Copyright © 2013 Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.113

(ischemia, Ang II, and ox-LDL et al.) can interfere with ER function, triggering the unfolded protein response (UPR) to cope with the resulting ER stress [10]. The UPR is triggered by three upstream proteins, inositol requiring enzyme (IRE) 1, activating transcription factor (ATF) 6, and PERK (RNA-dependent protein kinase, such as endoplasmic reticulum kinase). Several recent studies have revealed that the UPR, especially IRE1 signaling, may mediate angiogenesis. Benjamin Drogat et al. [11] demonstrated that IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. Additionally, it was reported that IRE1-dependent signaling is a key regulatory pathway for angiogenesis and tumor progression in malignant glioma [12]. More recently, Nicolas Bouvier et al. identified that the unfolded protein response regulates an angiogenic response by the kidney epithelium during ischemic stress [13]. Therefore, we postulated that the UPR, especially IRE1 signaling, mediates the angiogenic response induced by Ang II and further investigated this details of the mechanism.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were obtained from Hyclone. The following reagents and antibodies were purchased: Matrigel with reduced growth factors (BD Biosciences); the AT1R blocker losartan (Sigma), the Ang II type 2 receptor (AT2R) blocker PD123319 (Sigma), the p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 (Santa Cruz Biotechnology), the JNK inhibitor SP600125 (Sigma) and the IRE1 inhibitor irestatin (Axon) and all of the primary antibodies for western blot analysis (abcam). The PCR primer and SYBR green RT PCR master mix kit was purchased from Takara; all other biochemicals used were of the highest purity available.

2.2. Cell culture

Human umbilical vein endothelial cells (HUVECs) were originally purchased from ATCC. The cells were seeded at a density of 3×10^5 per 100-mm dish in DMEM, supplemented with 20 mM HEPES and 10% FBS. The cultures were maintained at 37 °C with a gas mixture of 5% CO_2/95% air. The medium was supplemented with 5 U/ml heparin, 100 IU/ml penicillin and 100 μg/ml streptomycin. Endothelial cells of the forth to sixth passages in the actively growing condition were used for the experiments.

2.3. Experimental protocol

Firstly, to investigate the effect of Ang II on ER stress and capillary tube formation from endothelial cells, the endothelial cells were exposed to Ang II (0, 0.1, 1, 10 and 30 nmol/L) for 24 h, and the expression of GRP78, IRE1, and JNK or capillary tube formation was subsequently determined.

Secondly, in the following parallel studies, to further examine the role of the AT1R/ER stress pathway in Ang II induced capillary tube formation, the endothelial cells were pretreated for 30 min with losartan (1, 3 and 10 μmol/L), PD123319 (10 μmol/L), irestatin 9389 (2.5 μmol/L), SP600125 (10 μmol/L) and SB203580 (10 μmol/L) before exposure to Ang II. All of the concentrations and durations of incubation listed above were chosen on the basis of published data and modified based on pilot experiments.

2.4. Capillary tube formation

The Matrigel was thawed on ice overnight and spread evenly over each well (30 μL) of a 24-well plate. The plates were incubated for 1 h at 37 °C to allow the Matrigel to polymerize. HCAECs were seeded at 3×10^4 per well and grown in 500 μL of endothelial cell basal medium-2 supplemented with 5% FBS and without endothelial cell growth supplement for 24 h in a humidified 37 °C, 5%-CO_2 incubator. In certain experiments, the endothelial cells were cultured in the presence or absence of different chemicals or antibodies. After washing, the plates were fixed using 70% ice-cold ethanol. Capillary formation was visualized by staining with hematoxylin and eosin and assessed as previously described [14].

2.5. Western blot analysis

The cells were lysed for 30 min at 4 °C in a lysis buffer. Total cell protein concentration was determined using the bicinchoninic acid reagent. Total protein (50–100 μg) was resolved by SDS–polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and subjected to immunoblot analysis. The primary antibodies for GRP78 (1:500), IRE-1 (1:1000), JNK (1:1000), VEGF (1:1000), p-38 MAPKs (1:2000), β-actin (1:5000) and horseradish peroxidase-conjugated secondary antibodies (Santa Cruz) were used. The bands were visualized using enhanced chemiluminescence reagents and analyzed with a gel documentation system (Bio-Rad Gel Doc1000 and Multi-Analyst version 1.1). All of the results are representative of at least three independent experiments.

2.6. Reverse transcription PCR

Total RNA was isolated from the cells of each group using Trizol (Invitrogen) according to the manufacturer's protocol. The reverse transcription reaction was performed with 4 μg of total RNA according to the RevertAid cDNA synthesis kit (Fermentas) protocol. RT-PCR was performed using a PCR cycler (Eppendorf). RT-PCR analysis was performed with the primer sequences shown in Table 1. RT-PCR was performed in a volume of 25 μL containing 50 ng/mL cDNA, 10 μM primers, 12.5 μL PCR mix (BioTeke). The cycler conditions were a denaturation of 5 min at 95 °C followed by 30–40 cycles of amplification (30 s at 95 °C, 30 s at 58 °C, and 1 min at 72 °C), and a final incubation for 10 min at 72 °C. Next, the PCR products were separated by 1.5% agarose gel electrophoresis and visualized by gelview on a UV transilluminator (Kodak). The intensity values were normalized with GAPDH reference genes.

2.7. Statistical analysis

The results are expressed as the mean ± SEM. The data were analyzed by ANOVA followed by a Newmann–Student–Keuls test for multiple comparisons. The significance level was chosen as $P < 0.05$.

Table 1
the primer sequences for RT-PCR analysis.

Name	Sequence (5′–3′)
GRP78	P+: 5′-GTCTACTATGAAGCCCGTCCAG-3′ P−: 5′-GATTGTCTTTTGTCAGGGGTCT -3′
IRE1	P+: 5′-ACAGGCTCAATCAAATGGACTT-3′ P−: 5′-GTTCGCCCAAGATACAGAAGAG-3′
JNK	P+: 5′-GCCACAAAATCCTCTTTCCA-3′ P−: 5′-GATAACAAATCCCTTGCCTGA-3′
GAPDH	P+: 5′-CTGCACCACCAACTGCTTAG-3′ P−: 5′-AGGTCCACCACTGACACGTT-3′

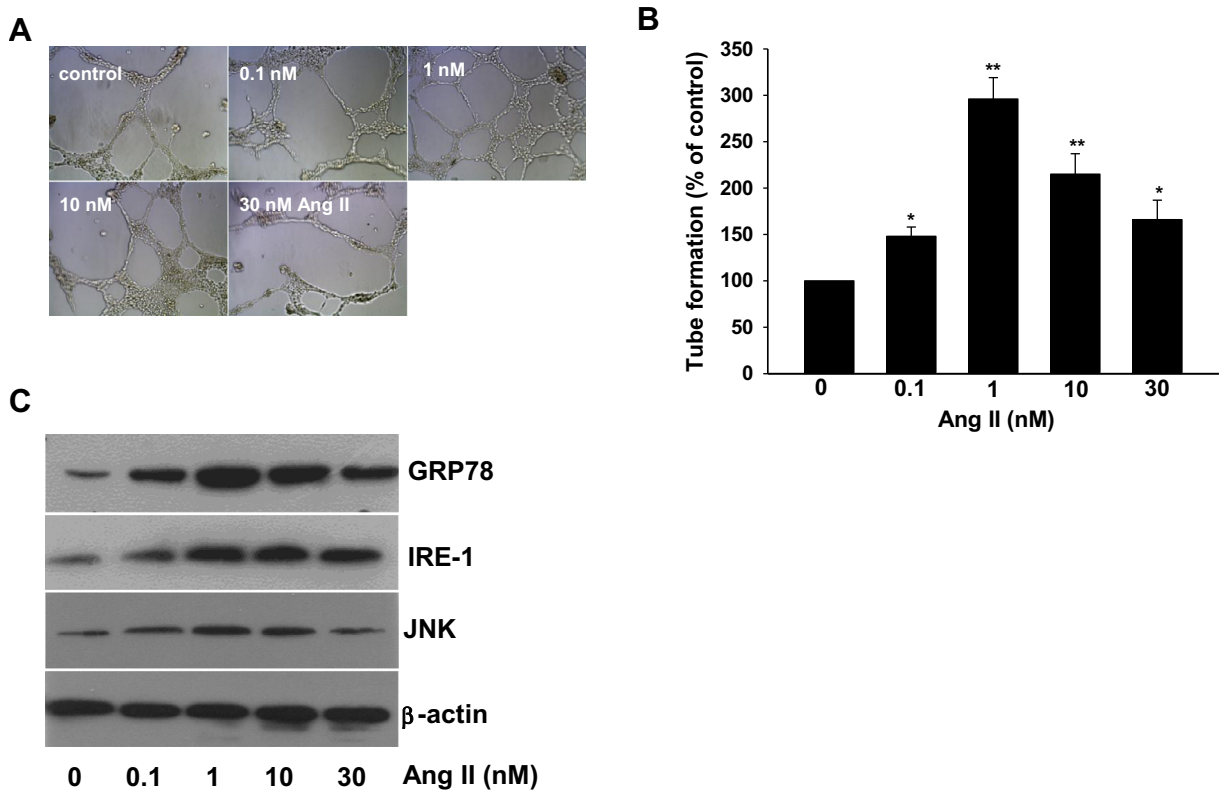


Fig. 1. Ang II triggers ER stress activation and capillary formation from endothelial cells. (A) The endothelial cells were exposed to Ang II at different concentrations (0, 0.1, 1, 10 and 30 nM) for 24 h. Ang II at concentrations of 0.1 and 1 nM increased capillary formation in a dose-dependent manner. In contract, Ang II lost its ability to induce capillary formation at concentrations >10 nM; higher concentrations were actually injurious to the cells. (B) A summary of the data on capillary formation from the endothelial cells. (C) In keeping with panel A, Ang II can induce ER stress, including GRP78, IRE1 and JNK protein expression. However, higher concentrations of Ang II (10 or 30 nmol/L) were noted to cause less protein expression. The data are expressed as the mean ± SEM, $n = 6$ each and were performed in triplicate. Compared with control (0 nM Ang II), $^{*}P < 0.05$; $^{**}P < 0.01$.

3. Results

3.1. Ang II induces capillary tube formation from endothelial cells and the activation of ER stress

As shown in Fig. 1A and B, low concentrations of Ang II (0.1, 1 and 10 nmol/L) led to the formation of capillary-like structures. The maximum capillary formation occurred in response to 1 nmol/L concentration of Ang II. In contrast, a higher concentration of Ang II (30 nmol/L) was observed to cause less tube formation.

In keeping with the above capillary tube formation results, as shown in Fig. 1C, the treatment of HUVECs with Ang II (0, 0.1 and 1 nmol/L) for 24 h induced the expression of the ER stress sensors GRP78, IRE1 and JNK in a concentration-dependent manner. However, the higher concentration of Ang II (10 or 30 nmol/L) was noted to cause less protein expression. Based on these findings, we selected the most proangiogenic concentration of Ang II (1 nmol/L) in the subsequent experiments.

3.2. Ang II induces capillary tube formation from endothelial cells via the AT1 receptor

The biological effects of Ang II are mediated by the activation of Ang II receptors, of which the two major subtypes (AT1R and AT2R), have been identified. Most of the cardiovascular actions of Ang II have been attributed to AT1R activation. We observed that the pretreatment of HUVECs with the AT1R blocker losartan (1, 3 and 10 μmol/L) suppressed Ang II-induced capillary tube formation in a dose-dependent manner (Fig. 2). In contrast, pretreatment with the AT2R blocker PD123319 (10 μmol/L) had no effect on Ang II-induced capillary tube formation. Losartan (10 μmol/L) and PD123319 (10 μmol/L) alone also had no effect on capillary formation.

3.3. The role of the AT1R/ER stress pathway in Ang II induced capillary tube formation from endothelial cells

To investigate the role of the AT1R/ER stress pathway in Ang II-induced capillary tube formation from endothelial cells, we used specific inhibitors. Endothelial cells were pretreated for 30 min with the AT1 blocker losartan (10 μmol/L), the IRE1 specific inhibitor irestatin 9389 (2.5 μmol/L), the JNK specific inhibitor SP600125 (10 μmol/L) and the p38 MAPKs specific inhibitor SB203580 (10 μmol/L) before exposure to Ang II. As shown in Fig. 3A and B, losartan (10 μmol/L), irestatin 9389 (2.5 μmol/L), SP600125 (10 μmol/L) and SB203580 (10 μmol/L) markedly inhibited Ang II-induced capillary formation. In keeping with the above-mentioned capillary formation studies, losartan (10 μmol/L), irestatin 9389 (2.5 μmol/L), SP600125 (10 μmol/L) and SB203580 (10 μmol/L) also markedly suppressed VEGF protein expression induced by Ang II (Fig. 3C and D).

3.4. Ang II and the AT1R/IRE1/JNK/p38 MAPKs pathway

As shown in Fig. 4, pretreatment with the AT1R blocker losartan (10 μmol/L) successfully suppressed Ang II-induced GRP78 expression in endothelial cells (both at the protein expression and the

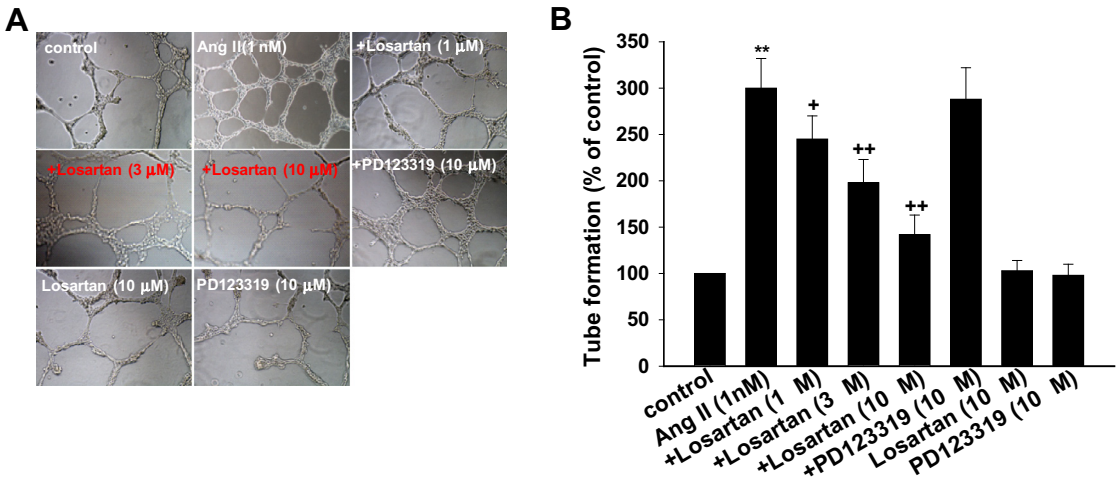


Fig. 2. The inhibitory effect of losartan on Ang II-induced capillary formation from endothelial cells. Ang II-mediated capillary formation was inhibited by the AT1R blocker losartan in a concentration-dependent manner but not by the AT2R blocker PD123319. Losartan or PD123319 alone had no effect on capillary formation. (A) Capillary formation from endothelial cells. (B) A summary of the data on capillary formation from endothelial cells. The data are expressed as the mean ± SEM, $n = 6$ each and were performed in triplicate. Compared with control (0 nM Ang II), $^{*}P < 0.05$; $^{**}P < 0.01$.

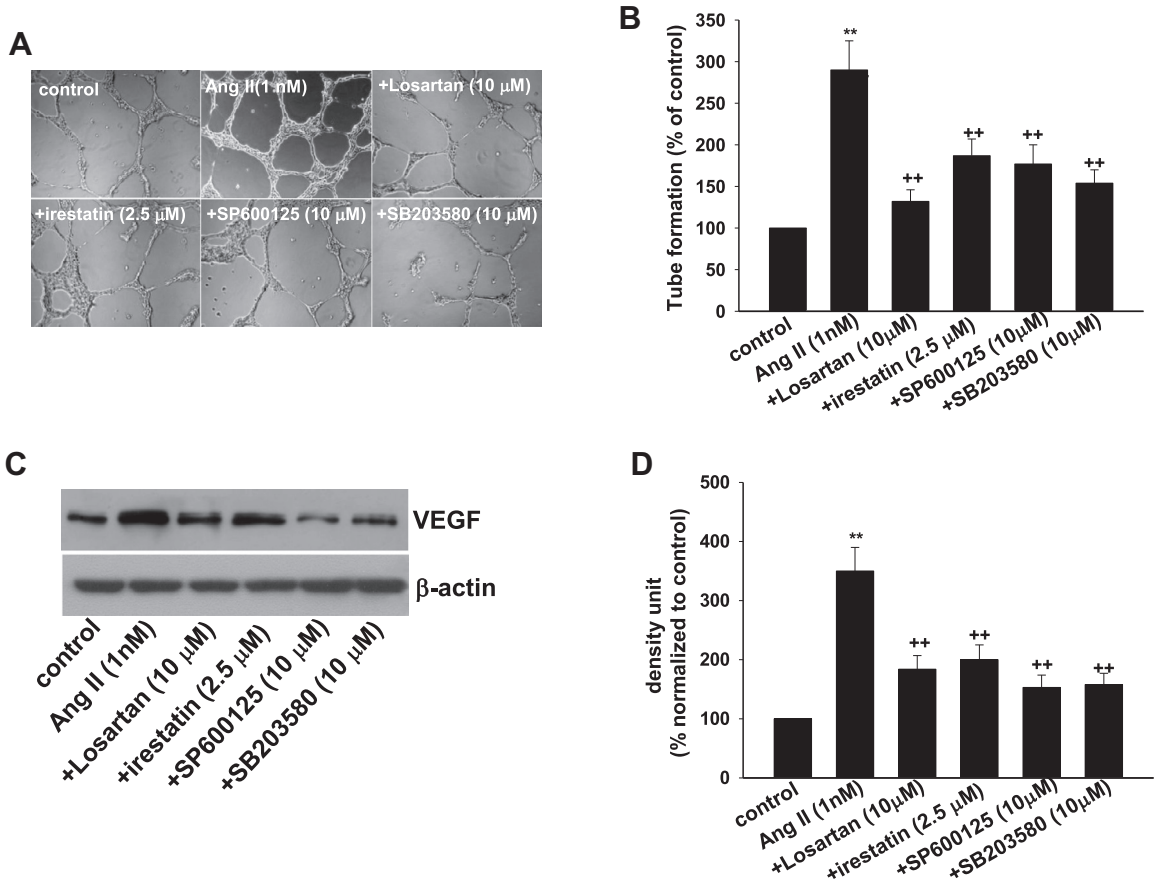


Fig. 3. The role of the AT1R/ER stress pathway in Ang II-induced capillary tube formation from endothelial cells. A representative inhibitory effect of losartan (AT1R blocker), irestatin (IRE1 specific inhibitor), SP600125 (JNK specific inhibitor) and SB203580 (p38 MAPK specific inhibitor) on Ang II-induced capillary tube formation (A and B), as well as VEGF expression (C and D). The data are expressed as the mean ± SEM, $n = 6$ each and were performed in triplicate. Compared with control (0 nM Ang II), $^{*}P < 0.05$; $^{**}P < 0.01$.

mRNA level). To verify the activity of this signaling pathway, specific inhibitors were used. In contrast, pretreatment with the IRE1 specific inhibitor irestatin 9389 (2.5 μmol/L), the JNK specific inhibitor SP600125 (10 μmol/L) and the p38 MAPKs specific inhibitor SB203580 (10 μmol/L) had no effect on Ang II-induced GRP78 expression in endothelial cells. These data indicated that Ang II induces GRP78 activation via the AT1 receptor, and IRE1, JNK, and p38 MAPKs are downstream of GRP78. Secondly, the AT1R blocker

losartan (10 μmol/L) and the IRE1 specific inhibitor irestatin 9389 (2.5 μmol/L) markedly inhibited Ang II-induced IRE1 protein expression and mRNA up-regulation, and pretreatment with the JNK specific inhibitor SP600125 (10 μmol/L) and the p38 MAPKs specific inhibitor SB203580 (10 μmol/L) had no effect. Thirdly, the AT1R blocker losartan (10 μmol/L), the IRE1 specific inhibitor irestatin 9389 (2.5 μmol/L) and the JNK specific inhibitor SP600125 (10 μmol/L) significantly suppressed Ang II-induced JNK protein expression and mRNA up-regulation. However, pretreatment with the p38 MAPKs specific inhibitor SB203580 (10 μmol/L) had no effect. Finally, all of above specific inhibitors blocked p38 MAPKs expression when exposed to Ang II. All of these data indicated that Ang II activates ER stress via the GRP78/IRE1/JNK/p38 MAPKs pathway.

4. Discussion

Pathological angiogenesis is associated with the abnormal rapid proliferation of blood vessels and is involved in various diseases, especially atherosclerosis [15]. The atherosclerotic regions, particularly in humans, are rich in microvasculature. The formation of new capillaries may well be proatherogenic by providing nutrition to the growing plaque and facilitating the migration of macrophages while the plaque is in the growth phase [16]. Meanwhile, exploring the signals regulating angiogenesis has attracted increasing levels of attention worldwide. Numerous previous studies have showed that Ang II induces to both angiogenesis and pathological vascular growth [17]. However, the mechanisms underlying Ang II-induced capillary tube formation from endothelial cells are not thoroughly understood.

The results obtained from the present study have demonstrated that Ang II stimulates capillary tube formation from endothelial cells and VEGF expression, and this stimulatory effect is mediated by the activation of endoplasmic reticulum stress especially IRE1 signaling; the JNK/p38 MAPK pathway may participate in the downstream signal transduction of IRE1 activation; the AT1 R blocker losartan suppressed Ang II-induced capillary tube formation via inhibiting the IRE/JNK/p38 MAPK pathway and later attenuated VEGF up-regulation.

Recently, several studies have demonstrated that ER stress is a potent inducer of VEGF expression [18–19]. GRP78, is a highly abundant endoplasmic reticulum chaperone [20]. The existence of ER stress in atherosclerosis animal models and human lesions was observed by the detection of GRP78, which could represent the levels of the ER stress. Along with its role in protein folding, GRP78 is also known to be an important component in modulating the unfolded protein response (UPR). GRP78 constitutively binds to and maintains the three UPR transmembrane sensors, ATF6, PERK, and IRE1, in an inactive form. Under conditions of ER stress, when unfolded proteins accumulate in the lumen of the ER, GRP78 is released from the UPR sensors, leading to their activation [21]. A recent study revealed that the exposure of GRP78 on the surface of stimulated endothelial cells was found to be implicated in the angiogenic process [22]. Otherwise, IRE1 is an endoplasmic reticulum (ER)-resident transmembrane protein acting as a proximal sensor of the unfolded protein response (UPR). As such, IRE1 participates in the early cellular response to the accumulation of misfolded proteins in the ER occurring under both physiological and pathological situations. Recent studies demonstrated that IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo [11]. Different stimuli trigger distinct UPR pathways; for example, hypoxia/ischemia and glucose deprivation activate VEGF via the IRE pathway [23]. The results from the present study showed that exogenous Ang II can activate GRP78 and IRE1 protein expression in a dose-dependent manner in the endothelial cells. Additionally, we demonstrated that the activation of IRE1 is necessary for both VEGF production and capillary tube formation from endothelial cells induced by Ang II because irestatin, an IRE1 specific inhibitor, significantly reduced VEGF production and attenuated capillary tube formation from endothelial cells induced by Ang II.

MAPKs represent a family of eukaryotic protein kinases involved in various cellular processes. Three parallel cascades (p38 MAPK, ERK, and JNK) are now commonly described. Recent studies [24] have demonstrated that excessive ER stress and oxidative stress can trigger the activation of multiple signaling pathways, such as the phosphorylation of p38 MAPK and JNK, which are closely associated with angiogenesis. Hu CP et al. revealed that activated p38 MAPKs is critical for Ang II induced angiogenesis [8]. More recently, Kaikai Shen et al. [25] demonstrated that JNK regulates VEGFA-induced VEGFR2 sustained phosphorylation, which

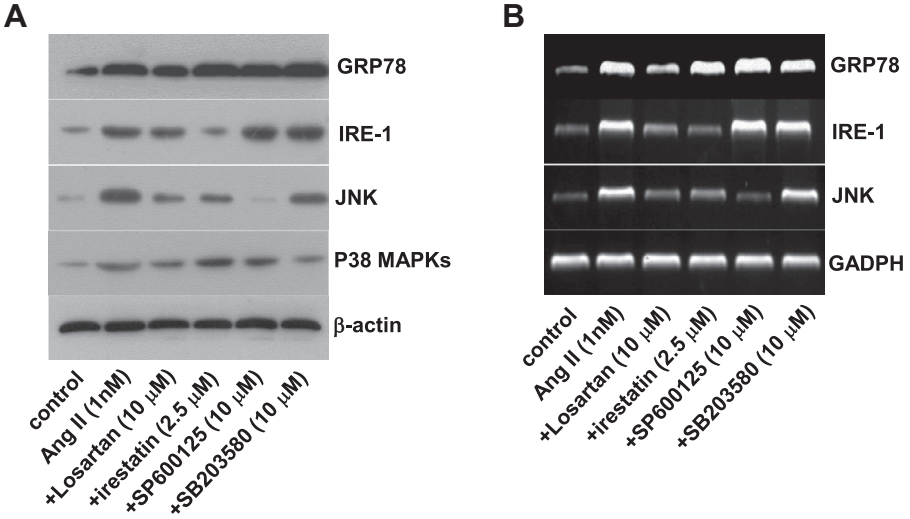
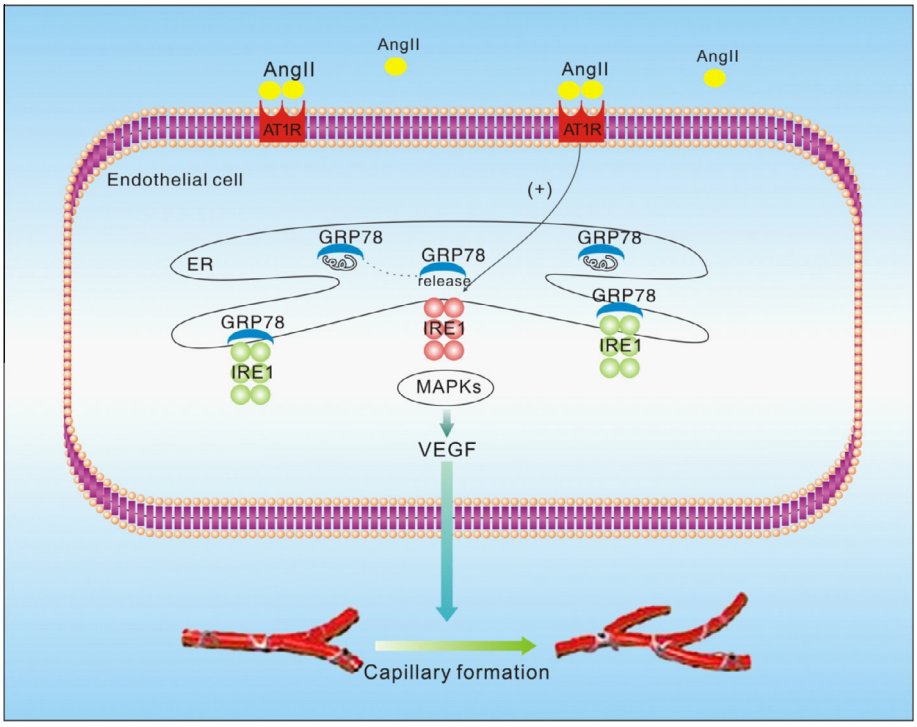


Fig. 4. Ang II-triggered ER stress is AT1R dependent. (A) Protein expression of GPR78, IRE1, JNK and p38 MAPK by western blot. (B) mRNA levels of GPR78, IRE1, and JNK by RT-PCR. Control: wild-type cells. ox-LDL: wild-type cells treated with 1 nM Ang II for 24 h; + losartan (10 μM): cells were pretreated with 10 μM of losartan (AT1R blocker) for 1 h prior to Ang II exposure; + irestatin (2.5 μM): cells were pretreated with 2.5 μM of cells were pretreated with 10 μM of SP600125 (JNK specific inhibitor) for 1 h prior to Ang II exposure;+SB203580 (10 μM): cells were pretreated with 10 μM of SB203580 (p38 MAPK specific inhibitor) for 1 h prior to Ang II exposure; These data are representative of six separate experiments.



Scheme 1. The potential mechanisms of Ang II-induced capillary formation from endothelial cells.

plays an important role in VEGFA-induced angiogenesis in HUVECs. Moreover, MAPKs are important mediators of the intracellular signal transduction pathways that are responsible for cell growth and differentiation [26]. As an upstream signaling molecule, the activation of IRE1 may mediate the activation of MAPKs, such as p38 MAPK and JNK. Thus, we investigated specific downstream signaling molecules that could potentially be important targets of IRE1. Our results have demonstrated that MAPKs activation is induced by Ang II at the maximal level at 24 h. To determine whether JNK/p38 MAPK is activated via the IRE1 pathway, we used the IRE1 specific inhibitor (irestatin), which markedly reduced MAPKs activation, suggesting that Ang II-induced MAPKs activation is IRE1-dependent. We also found that SP600125 (JNK specific inhibitor) and SB203580 (p38 MAPK inhibitor) significantly decreased VEGF production and markedly suppressed capillary tube formation from endothelial cells induced by Ang II. Moreover, we found that the JNK-specific inhibitor was capable of inhibiting p38-MAPK activation in endothelial cells treated with Ang II, but a p38-specific inhibitor did not have a similar effect on JNK activation. This result provides, to the best of our knowledge, the first evidence that the IRE1/JNK/p38-MAPK pathway mediates Ang II-induced VEGF production and capillary tube formation from endothelial cells. It is necessary to note that all of the specific inhibitors of IRE1, JNK and p38-MAPK fail to block GRP78 protein expression induced by Ang II. Possible explanation is that ER transmembrane sensors such as IRE1 are maintained in an inactive state through the interaction of their N-terminus with GRP78. When unfolded proteins accumulate in the ER, GRP78 releases these sensors to allow their oligomerization and thereby initiates the UPR [10]. And the finding that over-expression of GRP78 attenuates the UPR implies a role for GRP78 as a negative regulator of IRE1 [27]. However, by which mechanism does GRP78 dissociate from IRE1? It deserves to be further investigated in the future.

This study confirms previous observations that Ang II, in small amounts, induces capillary tube formation from vascular endothelial cells, and this effect is mediated by AT1R activation. This conclusion is based on our observation that the pretreatment of HUVECs with the AT1R blocker losartan (1, 3, and 10 μmol/L) suppressed Ang II-induced capillary tube formation in a dose-dependent manner (Fig. 2). In contrast, pretreatment with the AT2R blocker PD123319 (10 μmol/L) had no effect on Ang II-induced capillary tube formation. Moreover, we postulated that Ang II may trigger ER stress via the AT1 R pathway. Indeed, our study provides evidence of this triggering. In the present study, we demonstrated that the AT1R blocker losartan can markedly inhibit GRP78, IRE1 and JNK expression at both the mRNA and protein levels.

In conclusion, the present study indicates that small concentrations of Ang II promote capillary formation by inducing the expression of VEGF via AT1R-mediated stimulation of the ER stress-related pathway. The schematic illustration of the propose pathway is as shown in Scheme 1. These findings may provide an important mechanism of how Ang II causes angiogenesis and provide a novel approach for the prevention of pathological angiogenesis.

References

[1] D.C. Felmeden, A.D. Blann, G.Y. Lip, Angiogenesis: basic pathophysiology and implications for disease, Eur. Heart J. 24 (2003) 586–603.
[2] B. Doyle, N. Caplice, Plaque neovascularization and antiangiogenic therapy for atherosclerosis, J. Am. Coll. Cardiol. 49 (2007) 2073–2080.
[3] F.T. Wu, M.O. Stefanini, F. Mac Gabhann, C.D. Kontos, B.H. Annex, A.S. Popel, A systems biology perspective on sVEGFR1: its biological function, pathogenic role and therapeutic use, J. Cell. Mol .Med. 14 (2010) 528–552.
[4] M. Shibuya, Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis, BMB Rep. 41 (2008) 278–286.
[5] J. Carbajo-Lozoya, S. Lutz, Y. Feng, J. Kroll, H.P. Hammes, T. Wieland, Angiotensin II modulates VEGF-driven angiogenesis by opposing effects of type 1 and type 2 receptor stimulation in the microvascular endothelium, Cell. Signal. 24 (2012) 1261–1269.
[6] P.K. Mehta, K.K. Griendling, Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system, Am. J. Physiol. Cell Physiol. 292 (2007) C82–C97.
[7] L.M. Willis, A.B. El-Remessy, P.R. Somanath, D.L. Deremer, S.C. Fagan, Angiotensin receptor blockers and angiogenesis: clinical and experimental evidence, Clin. Sci. (Lond.) 120 (2011) 307–319.
[8] C. Hu, A. Dandapat, J.L. Mehta, Angiotensin II induces capillary formation from endothelial cells via the LOX-1 dependent redox-sensitive pathway, Hypertension 50 (2007) 952–957.

[9] Yien Che Tsai, Allan M. Weissman, The unfolded protein response degradation from the endoplasmic reticulum and cancer, Genes Cancer 1 (2010) 764–778.
[10] T. Minamino, M. Kitakaze, ER stress in cardiovascular disease, J. Mol. Cell. Cardiol. 48 (2010) 1105–1110.
[11] B. Drogat, P. Auguste, P. Beaune, D.T. Nguyen, N. Pallet, M. Bouchecareilh, R. Pineau, J. Nalbantoglu, R.J. Kaufman, E. Chevet, A. Bikfalvi, M. Moenner, IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo, Cancer Res. 67 (2007) 6700–6707.
[12] Gregor Aufa, Arnaud. Jabouille, Sylvaine Guérit, Raphaël Pineau, Maylis Delugin, Bouchecareilh Marion, Noël Magnin, Alexandre Favereaux, Marlène Maitre, Timo Gaiser, Andreas von Deimling, Marcus Czabank, Peter Vajkoczy, Eric Chevet, Andreas Bikfalvi, Michel Moenne, Inositol-requiring enzyme 1α is a key regulator of angiogenesis and invasion in malignant glioma, Proc. Natl. Acad. Sci. USA 107 (2010) 15553–15558.
[13] N. Bouvier, S. Fougeray, P. Beaune, E. Thervet, N. Pallet, The unfolded protein response regulates an angiogenic response by the kidney epithelium during ischemic stress, J. Biol. Chem. 287 (2012) 14557–14568.
[14] S. Miura, M. Fujino, Y. Matsuo, A. Kawamura, H. Tanigawa, H. Nishikawa, K. Saku, High density lipoprotein-induced angiogenesis requires the activation of Ras/MAP kinase in human coronary artery endothelial cells, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 802–808.
[15] Yihai Cao, Renhai Cao, Ebba Bråkenhielm, Antiangiogenic mechanisms of diet-derived polyphenols, J. Nutr. Biochem. 13 (2002) 380–390.
[16] J. Jiang, M. Yan, J.L. Mehta, C. Hu, Angiogenesis is a link between atherosclerosis and tumorigenesis: role of LOX-1, Cardiovasc. Drugs Ther. 25 (2011) 461–468.
[17] Y. Numaguchi, T. Murohara, RAAS and angiogenesis, Nihon Rinsho 67 (2009) 723–728.
[18] O.V. Oskolkova, T. Afonyushkin, A. Leitner, E. von Schlieffen, P.S. Gargalovic, A.J. Lusis, B.R. Binder, V.N. Bochkov, VN.ATF4-dependent transcription is a key mechanism in VEGF up-regulation by oxidized phospholipids: critical role of oxidized sn-2 residues in activation of unfolded protein response, Blood 112 (2008) 330–339.
[19] C.N. Roybal, S. Yang, C.W. Sun, D. Hurtado, D.L. Vander Jagt, T.M. Townes, S.F. Abcouwer, Homocysteine increases the expression of vascular endothelial growth factor by a mechanism involving endoplasmic reticulum stress and transcription factor ATF4, J. Biol. Chem. 279 (2004) 14844–14852.
[20] M. Ni, A.S. Lee, ER chaperones in mammalian development and human diseases, FEBS Lett. 581 (2007) 3641–3651.
[21] J.D. Malhotra, R.J. Kaufman, The endoplasmic reticulum and the unfolded protein response, Semin. Cell Dev. Biol. 18 (2007) 716–731.
[22] Annat Raiter, Chana Weiss, Zafrir Bechor, Itzik Ben-Dor, Alexander Battler, Boris Kaplan, Britta Hardy, Activation of GRP78 on endothelial cell membranes by an ADAM15-derived peptide induces angiogenesis, J. Vasc. Res. 47 (2010) 399–411.
[23] A. Salminen, A. Kauppinen, J.M. Hyttinen, E. Toropainen, K. Kaarniranta, Endoplasmic reticulum stress in age-related macular degeneration: trigger for neovascularization, Mol. Med. 16 (2010) 535–542.
[24] Jyoti D. Malhotra, Randal J. Kaufman, Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword?, Antioxid Redox Signal. 9 (2007) 2277–2294.
[25] K. Shen, L. Ji, B. Lu, Z. Wang, c-Jun N-terminal kinase mediated VEGFR2 sustained phosphorylation is critical for VEGFA-induced angiogenesis *in vitro* and *in vivo*, Cell Biochem. Biophys. 64 (2012) 17–27.
[26] E.K. Kim, E.J. Choi, Pathological roles of MAPK signaling pathways in human diseases, Biochim. Biophys. Acta 2010 (1802) 396–405.
[27] Y. Kimata, D. Oikawa, Y. Shimizu, Y. Ishiwata-Kimata, K. Kohno, A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1, J. Cell Biol. 167 (2004) 445–456.