日本財団補助金による
２０００年度日中医学学術交流促進事業

⑤. 在留中国人研究者研究助成

(1) ラット胎児平滑筋細胞に於けるアンジオテンシンＩＩ２型受容体刺激によるチロシンホスファターゼ、SHP-1活性化の意義

ラット胎児平滑筋細胞におけるアンジオテンシンII２型受容体刺激によるチロシンホスファターゼ、SHP-1活性化の意義

崔　泰興
中国吉林省延辺大学医学院附属病院　医師
愛媛大学医学部医化学第一

研究要旨
本研究では、アンジオテンシン２型受容体刺激が細胞内情報伝達系においてもアンジオテンシン１型受容体からの刺激に拮抗するか否かを初代培養系を用いて検討し、さらにそのメカニズムとしてホスファターゼに着目した研究を行った。ラット胎児より平滑筋細胞を調整することで、AT1R および AT2R の両方を発現する初代培養系を確立し、特異的な受容体拮抗剤を用いた検討を行った。AT2R 選択的刺激により caspase の活性化を伴うアポトーシスの亢進が認められたが、AT1R 選択的刺激によりその作用は抑制された。また、細胞内情報伝達系の Extracellular signal-regulated kinase (ERK)の解析では、AT1R 選択的刺激による活性化が AT2R 選択的刺激により抑制された。チロシンホスファターゼの SHP-1 が AT2R 選択的刺激により活性化されることに着目し、その優性抑制型変異体遺伝子を過剰発現させた系で詳細な検討を行ったところ、AT2R 選択的刺激によるアポトーシスの亢進および ERK の活性化抑制作用は減弱した。つまり、AT2R 選択的刺激はチロシンホスファターゼ、SHP-1 を介した作用を有することが確認された。　キーワード：　アンジオテンシン II、ホスファターゼ、ERK、アポトーシス

1. Introduction

Angiotensin (Ang) II is classically considered as a vasoconstricting, progrowth molecule acting via stimulation of the G protein-coupled seven membrane-spanning Ang II type 1 (AT1) receptor. In addition to the "classical" AT1 receptor, it is now conceivable that Ang II activates another receptor subtype (type 2 or AT2 receptor). Although both AT1 and AT2 receptors belong to the seven-transmembrane, G protein-coupled receptor family, recent evidence reveals that the functions of AT1 and AT2 receptors are mutually antagonistic. The AT2 receptor is abundantly and widely expressed in fetal tissues, but present only at low levels in adult tissues and re-expressed in certain pathological conditions such as ovarian atresia, wound injury, myocardial infarction, and vascular injury [1]. The highly abundant expression of this receptor during embryonic and neonatal growth and its quick disappearance after birth has led us to the suggestion that this receptor may be involved in growth, development and/or differentiation. In many organs, tissue remodeling and degeneration involve an active process, termed apoptosis (programmed cell death), which is a ubiquitous, evolutionally conserved, physiological mechanism of cell death that regulates mass and architecture in many tissues. The genetic program for apoptosis may be activated at a defined time during embryogenesis or in the maturation of adult cells, by a wide range of physiological stimuli. These results provide us with the possibility that AT2 may induce apoptosis. Actually proapoptotic effects of AT2 receptor have been demonstrated in PC12W cells [2-5], R3T3 mouse fibroblasts [2,4], rat ovarian granulosa cells [6], human umbilical venous endothelial cells [7] and AT2 receptor-transfected adult rat VSMCs [8].

The growth inhibitory effects of the AT2 receptor have been shown to associate with the activation of a series of phosphatases including the protein tyrosine phosphatase SHP-1 [9,10] or the mitogen-activated protein kinase phosphatase-1 (MKP-1) [2,4], and the serine/thronine phosphatase 2A (PP2A) [11], which results in the inactivation of AT1 receptor- and/or growth factor-activated extracellular signal-regulated kinase (ERK). SHP-1[formerly termed PTP1C, SH-PTP-1, SHP, and HCP], a two Src homology 2 (SH2) domain-containing cytosolic tyrosine phosphatase, has been implicated in the negative regulation of a broad spectrum of growth-promoting receptors, including receptor tyrosine kinases such as c-Kit [12,13], TrK A [14], and epidermal growth factor (EGF) [15] receptors; cytokine receptors such as interleukin-3 (IL-3) [12], interferon-α/β [16] and erythropoietin [17] receptors; and B cell FcγRIIB [18] receptor. Given the breadth of its interaction with multiple growth factor receptors, we hypothesize that SHP-1 is involved in AT2 receptor intracellular pathway which negatively crosstalks with AT1 receptor signaling. Moreover, we have

recently observed that SHP-1 is one of the most proximal effector in AT2 receptor-mediated ERK inhibition and apoptosis in PC12 cells [10].

Accordingly, we used fetal VSMC, which endogenously express both AT1 and AT2 receptors, and examined the possibilities that AT2 receptor stimulation exerts proapoptotic effects by antagonizing the function of AT1 receptor and that SHP-1 is pivotal in the proapototic effect of AT2 receptor stimulation.

2. Methods

2.1. Cell Culture

Fetal VSMCs were prepared from the thoracic aortae of Sprague-Dawley rat fetuses (embryonic day 20) and cultured in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies Inc., Rockville, Maryland, USA) supplemented with 10% fetal bovine serum (FBS) as previously described [19]. In addition to the morphological observation, characterization of VSMC was examined by indirect immunofluoresence using a monoclonal antibody specific for α-smooth muscle actin (clone 1A4; Sigma Chemical Co.) [20]. Our culture of fetal VSMCs exhibited the "hill-and-valley" appearance which is characteristic of adult VSMC. The cells were uniformly positive for α-smooth muscle actin by immunofluorescence. These results indicate that the cells are characteristic of VSMC although the possible contamination of fibroblasts and endothelial cells cannot be avoided. All the experiments were performed using the cells at the second or third passage. AT1 and AT2 receptor binding was measured as previously described [19].

2.2. Plasmid Constructs and Transfection

The SHP-1(C453/S) mutant and SHP-2(C463/S) mutant cDNA were inserted into the pcDNA3 vector [21]. Transient transfection was performed with 0.5 μg of plasmid DNA per well of a 6-well plate and LipofectAMINE PLUS (GIBCO-BRL) according to the manufacturer's instructions (DNA:LipofectAMINE:PLUS ratio, 1:10:10μl). After transient transfection, the transfected cells were fed with complete growth medium for 48 h to allow protein expression. Transfected cells were identified by β-galactosidase staining. The transfection efficiency was 25 ± 5%, a similar level as previously reported [22]. Overexpression of SHP-1(C453/S) mutant and SHP-2(C463/S) mutant was confirmed by immunoblot with an anti-SHP-1 or an anti-SHP-2 antibody (Santa Cruz Biotechnology, Inc.).

2.3. Determination of Cell Death

Rat fetal VSMCs were grown in 10% FBS DMEM to a subconfluent state in 10-cm dishes and incubated for an additional 48 h in serum-free DMEM. Nuclear chromatin morphology analysis and caspase-3 activity assay were used as quantitative indices of apoptosis. Nuclear chromatin morphology was analyzed by staining with Hoechst33342 (Molecular Probes, Inc., Eugene, Oregon, USA) and viewed under fluorescence microscopy, as previously described [2]. The caspase-3 activity assay was performed using an adaptation of a previously described protocol [23]. Results were normalized to basal activity in viable cells maintained in serum-containing medium.

2.4. Determination of ERK Activity

Subconfluent VSMCs were incubated in serum-free DMEM for 24 h and were treated with vehicle or Ang II (0.3 μmol/L) with or without PD123319 (10 μmol/L) or CV11974 (10 μmol/L) for 15 min. The activation of ERK was assessed as previously described [19]. Briefly, the cells were washed quickly with HEPES-buffered saline, frozen in liquid nitrogen, scraped and lysed. The lysate was centrifuged for 20 min at 6000 g at 4°C. Protein concentration was measured in the cleared supernatant by Bio-Rad protein
assay. The supernatant (20 μg) was subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and electrotransferred onto Hybond-ECL nitrocellulose membrane. Phosphorylated or total ERK was detected with a phospho-ERK antibody or a total ERK antibody (New England Biolabs Inc., Beverly, Massachusetts, USA), respectively, visualized by enzyme-linked chemiluminescence (Amersham). Densitometric analysis was performed using an image scanner (EPSON GT-8000) and National Institutes of Health image software. Densities of the bands corresponding to ERK-1/2 were added to represent total ERK.

2.5. Measurement of SHP-1 Tyrosine Phosphatase Activity

Subconfluent VSMCs were incubated in serum-free DMEM for 24 h and were treated with vehicle or Ang II (0.3 μmol/L) with or without PD123319 (10 μmol/L) or CV11974 (10 μmol/L) for 3 min. SHP-1 tyrosine phosphatase activity was measured as previously described [9,10].

2.6. *Data Analysis*

All values are expressed as mean ± SEM. The data were evaluated by analysis of variance followed by a Newman-Keuls' test for multiple comparisons. The difference was considered to be significant if $p < 0.05$.

3. Results

3.1. Cross-talk of AT1 and AT2 Receptors Regulate Cell Death in fetal VSMCs

To examine the role of endogenous AT2 receptor in Ang II regulated apoptosis, we employed VSMC prepared from rat fetal aorta. VSMCs were cultured from the aorta of fetal rat at embryonic day 20, and shown to express both AT1 and AT2 receptors (AT1 receptor 11.7 ± 0.65 fmol/10^6 cells; AT2 receptor, 5.27 ± 2.28 fmol/10^6 cells, n= 6, mean ± SE). Upon serum starvation for 24 h, chromatin dye staining showed that VSMCs underwent morphological changes of typical apoptosis, such as fragmented nuclei with condensed chromatin (data not shown). We characterized the time course of caspase-3 activation after serum deprivation in our system and we examined the effects of Ang II, since caspase-3 plays a central role in serum or trophic factor deprivation-induced apoptosis. Serum starvation induced a significant increase in caspase-3 activity, reaching a maximum at 12 h and declining to basal level within 48 h. However, the addition of Ang II (0.3 μmol/L) significantly inhibited caspase-3 activation measured at 12 h after serum depletion. Stimulation with Ang II (0.3 μmol/L) in the presence of AT2 receptor-specific antagonist PD123319 (10 μmol/L) further inhibited caspase-3 activity compared with stimulation with Ang II alone. On the contrary, addition of the AT1 receptor-specific antagonist CV11974 (10 μmol/L), Ang II stimulation further increased the serum starvation-induced caspase-3 activation. Neither CV11974 nor PD123319 alone influenced the caspase 3 activity.

3.2. Ang II Activates ERK via the AT1 Receptor and Inhibits ERK via the AT2 Receptor in Fetal VSMCs

To explore the molecular mechanism involved in the cross-talk of AT1 and AT2 receptors in regulating cell death in rat fetal VSMCs, we examined ERK activation in response to the stimulation of AT1 or/and AT2 receptors. Immunoblot using an anti-phospho-ERK antibody demonstrates that Ang II (0.3 μmol/L) stimulation significantly increased ERK phosphorylation. CV11974 (10 μmol/L) strongly attenuated the Ang II-induced ERK phosphorylation. In contrast, PD123319 (10 μmol/L) significantly enhanced the Ang II-induced ERK phosphorylation. ERK phosphorylation was not affected by treatment with CV11974 (10 μmol/L) and PD123319 (10 μmol/L) alone.

3.3. Activation of AT2 Receptor Increases SHP-1 Activity in Fetal VSMCs

We first examined the protein level of SHP-1 in rat fetal VSMCs and found that SHP-1 is expressed in fetal VSMC. Next we examined the SHP-1 activity and observed that Ang II (0.3 μmol/L) stimulation for 3 min significantly increased SHP-1 activity and this Ang II-mediated SHP-1 activation was blocked with PD123319 (10 μmol/L) but not by CV11974 (10 μmol/L).

3.4. Role of SHP-1 in AT2 Receptor-induced Apoptosis in Fetal VSMCs

To investigate whether SHP-1 plays a role in the proapoptotic effect of AT2 receptor, we transfected rat fetal VSMCs with a dominant negative (dn) SHP-1 mutant in which the active site cysteine 453 was mutated to serine (C453/S) [21]. Overexpression of dn SHP-1 mutant was confirmed by immunoblot showing 6-fold increase in SHP-1 immunoreactivity compared with the control vector pcDNA3 transfected cells. Basal SHP-1 activity was low in VSMCs transfected with dnSHP-1 mutant and we could not observe further significant increase in SHP-1 activity in response to Ang II. We then examined the functional significance of SHP-1 activation on AT2 receptor-mediated ERK inhibition and apoptosis. Since the SHP-2 phosphatase [formerly termed SHPTP-2, SHPTP-3, Syp, PTP2C, and PTP1D], in contrast to SHP-1, frequently functions as a positive effector of growth-stimulatory signaling pathway, we also examined the possible involvement of SHP-2 in the regulation of ERK activation and cell death by Ang II in rat fetal VSMCs. After identification of endogenous expression of SHP-2 in rat fetal VSMCs by immunoblot, we transfected rat fetal VSMCs with a dnSHP-2 mutant in which the active site cysteine 463 was mutated to serine (C463/S). Overexpression of dn SHP-2 mutant was confirmed by immunoblot showing 3-fold increase in SHP-2 immunoreactivity compared with the control vector pcDNA3 transfected cells. We first examined the ERK activation in cells overexpressing dnSHP-1 or dnSHP-2. In dnSHP-1 transfected VSMCs, increase in ERK activity by Ang II was further significantly enhanced compared to that in pcDNA3 transfected VSMCs and ERK activation by Ang II was not influenced with PD123319. In contrast, dnSHP-2 transfection did not modify the effect of Ang II on ERK

activation. Moreover, we observed that transfection of dn SHP-1 or dn SHP-2 did not influence the protein levels of total ERK. Next we examined the effect of Ang II treatment on apoptotic changes in dnSHP-1 or dnSHP-2 transfected rat fetal VSMCs. Upon serum starvation for 12 h, a near 4-fold increase in caspase-3 activity was observed in each transfectant. In control vector pcDNA3 transfected VSMCs, Ang II (0.3 μmol/L) stimulation led to inhibition of the caspase-3 activation, which was enhanced by addition of PD123319 (10 μmol/L), and selective activation of AT2 receptor by Ang II in presence of CV11974 (10 μmol/L) further increased the caspase-3 activation induced by serum-starvation, as that observed in non-transfected VSMCs. However, the enhancement of Ang II stimulation-induced inhibition of the caspase-3 activation by PD123319 or the aggravation of serum starvation-induced caspase-3 activation by Ang II in presence of CV11974 were blocked by overexpression of dnSHP-1 in VSMCs. Transfection of dnSHP-2 did not modulate the effects of Ang II on caspase-3 activation caused by serum depletion in VSMCs.

4. Discussion

Increasing evidence reveals that AT2 receptor acts as an antagonist against the classical AT1 receptor, i.e., the AT2 receptor exerts antigrowth, antihypertrophic, and proapoptotic effects and promotes differentiation [1]. Expression of the AT2 receptor in rat embryonic blood vessels is " turned on" at late gestation (embryonic days 16-21) and early neonatal period, but decreases rapidly to very low levels in the adult vessel [24,25]. The spatial and temporal patterns of rat vascular AT2 receptor expression, together with its growth modulatory action, have led us to hypothesize that this receptor plays an important role in vascular development and remodeling in late gestation. Indeed, pharmacological blockade of the AT2 receptor using the specific antagonist PD123319 during rat embryonic days 16-21 results in increased aortic DNA synthesis [24]. Our previous results using AT2 receptor null mice suggest that AT2 receptor promotes vascular differentiation and contributes to vasculogenesis [26]. Moreover, we observed that VSMCs prepared from the aorta of fetal or newborn AT2 receptor null mice showed enhanced proliferative activity [19]. Here we demonstrate that Ang II regulates apoptosis in fetal VSMCs, i.e., stimulation of AT1 receptor inhibits apoptosis, whereas activation of AT2 receptor potentiates it. These results provide
further support to the hypothesis that Ang II-regulated programmed cell death is involved in vascular development or vasculogenesis.

To explore the molecular mechanism of Ang II-regulated apoptosis in fetal VSMCs, we focused on ERK activation since the ERK cascade is crucial in proliferation, differentiation, and hypertrophy or apoptosis of VSMC [27], and is likely involved in AT2 receptor-mediated antigrowth and proapoptotic actions on VSMC [8,24,28]. In cultured VSMCs prepared from embryonic day 20 rat, we found that activation of AT1 increases ERK phosphorylation and activation of AT2 receptor shows an inhibitory effect on the AT1 receptor-mediated ERK phosphorylation. Such opposite modulation of ERK by AT1 and AT2 receptors was also observed in rat cultured neurons, which contained both AT1 and AT2 receptors [29]. Our results suggest that the facilitating effect of AT2 receptor stimulation on serum deprivation-induced cell death in fetal VSMCs is at least partly due to the inhibitory effect of AT2 receptor activation on ERK phosphorylation.

SHP-1 is a soluble tyrosine phosphatase that participates in the negative regulation of receptor tyrosine kinase pathway [12-15]. Our previous results have shown that stimulation of AT2 receptor rapidly activates SHP-1 in N1E-115 and AT2 receptor-transfected Chinese hamster ovary (CHO) cells [9], and that SHP-1 is a proximal effector of the AT2 receptor, implicated in the apoptotic effect of AT2 receptor in PC12 cells [10]. Therefore we speculated that SHP-1 may be a key molecule in AT2 receptor signaling in rat fetal VSMCs. We observed that stimulation of AT2 receptor also rapidly activates SHP-1 in rat fetal VSMCs, where AT1 activation does not. To establish a functional link between AT2 receptor-mediated activation of SHP-1 and inactivation of ERK, we used a catalytically inactive, dominant negative (dn) mutant SHP-1 and demonstrated that preventing SHP-1 activation abrogates AT2 receptor stimulation-induced ERK inhibition in rat fetal VSMCs. Moreover, the ERK inhibition is due to activation/phosphorylation, not to translation of ERK, since total protein level of ERK was not changed by overexpression.of dnSHP-1 in rat fetal VSMCs.

It has already been demonstrated that for a number of membrane receptors such as EGF receptor, SHP-1 terminated these receptors' signal by direct interaction with the receptors [12,15,17,18]. Tyrosine kinase activation by the AT1 receptor is at least partly attributed to transactivation of a tyrosine kinase receptor such as the epidermal growth factor (EGF) receptor or the platelet derived growth factor (PDGF)
receptor [30-32]. In VSMC, Ang II stimulation has been shown to activate the EGF receptor by tyrosine phosphorylation [31]. Very recent evidence showed that activation of AT2 receptor results in functional transinactivation of insulin receptor kinase in CHO cells, leading to inhibition of ERK phosphorylation and cell proliferation [33]. Therefore, it is likely that ERKs may not be direct cellular targets for AT2 receptor-activated SHP-1, but rather that AT2 receptor-activated SHP-1 affects upstream intermediates of AT1 receptor-induced ERK pathway such as EGF receptor.

The result of inhibition of AT2 receptor-meediated apoptosis by overexpression of dnSHP-1 suggests that the proapoptotic effect of AT2 receptor is associated with the inactivation of ERK via a signaling pathway involving the activation of SHP-1 in rat fetal VSMCs. In addition, sst2 somatostatin receptor-mediated activation of SHP-1 has been shown to act as an initial transducter of the antiproliferating aignaling mediated by this receptor [21]. It is interesting that SHP-1 has been reported to be required for the delivery of the Fas apoptosis signal in lymphoid cell [34]. These observation suggest that SHP-1 is a transducer of the antimitogenic and/or proapoptotic signals mediated by different membrane receptors, including AT2 receptor, probably through negative regulation of signaling of growth factor receptors, such as AT1 receptor.

In contrast, SHP-2 has been shown as a positive effector of tyrosine kinase growth factor receptors including those of PDGF, EGF, insulin-like growth factor, insulin and nerve growth factor [35-37]. In addition, it has also been suggested that SHP-2 functions as an adaptor protein for Janus kinase 2 (JAK2) association with AT1 receptor, thereby facilitating JAK2 phosphorylation and activation [38]. However, our data showed that preventing SHP-2 activation with transfection of dnSHP-2 into fetal VSMCs did not affect either AT1 receptor-mediated ERK phosphorylation and cell survival nor AT2 receptor-mediated ERK inhibition and cell death. These results indicate that SHP-2 may not be involved in the crosstalk between AT1 and AT2 receptors in regulating ERK activation and cell death in fetal VSMCs.

The results of this study may be relevant to vascular pathobiology. It has been shown that neointimal VSMCs in vascular lesions such as atherosclerosis and restenosis after balloon angioplasty resemble fetal VSMCs, rather than adult medial VSMCs [39,40]. AT2 receptor is re-expressed in injured blood vessels and attenuates neointima formation [1]. Furthermore, a recent study clearly showed that selective stimulation of AT2 receptor induces vascular mass regression by stimulating VSMC apoptosis in spontaneously hypertensive rats in vivo [41]. Thus, SHP-1 might play an important role in AT2 receptor-mediated apoptosis, which contributes to the pathogensis and remodeling of cardiovascular diseases.

In summary, these results provide us with direct evidences that crosstalk between AT1 and AT2 receptor controls the fate of fetal VSMCs. It is also possible that this antagonistic interaction may contribute to the fetal vascular development or vasculogenesis. We also demonstrated that SHP-1 is a pivotal effector in the signal transduction pathway of AT2 receptor in fetal VSMCs.

References

[1] Horiuchi M, Akishita M, Dzau VJ. Recent progress in angiotensin II type 2 receptor research in cardiovascular system. Hypertension. 1999;33:613-621.

[2] Yamada T, Horiuchi M, Dzau VJ. The novel angiotensin II type 2 receptor mediates programmed cell death. Proc Natl Acad Sci USA. 1996;93:156-160.

[3] Horiuchi M, Yamada T, Hayashida W, Dzau VJ. Interferon regulatory factor-1 upregulates angiotensin type 2 receptor and induces apoptosis. J Biol Chem. 1997;272:11952-11958.

[4] Horiuchi M, Hayashida W, Kambe T, Yamada T, Dzau VJ. Angiotensin type 2 receptor dephosphorylates Bcl-2 by activating mitogen-activated protein kinase phophatase-1 and induces apoptosis. J Biol Chem. 1997;272:19022-19026.

[5] Lehtonen JYA, Horiuchi M, Daviet L, Akishita M, Dzau VJ. Activation of the de novo biosynthesis of sphingolipids mediates angiotesin II type 2 receptor-induced apoptosis. J Biol Chem. 1999;274:16901-16906.

[6] Tanaka M, Ohnishi J, Ozawa Y, Sugimoto M, Usuki S, Naruse M, Murakami K, Miyazaki H. Charaterization of angiotensin II receptor type 2 during differentiation and apoptosis of rat ovarian cultured granulosa cells. Biochem Biophys Res Commun. 1995;207:593-598.

[7] Dimmeler S, Rippmann V, Weiland U, Haendeler J, Zeiher AM. Angiotensin II induces apoptosis of human endothelial cells: protective effect of nitric oxide. Circ Res. 1997;81:970-976.

[8] Yamada T, Akishita M, Pollman M, Gibbons GH, Dzau VJ, Horiuchi M. Angiotensin II type 2 receptor mediates vascular smooth muscle cell apoptosis and antagonizes angiotensin II type 1 receptor action: an in vitro gene transfer study. Life Sci. 1998;63:289-295.

[9] Bedecs K, Elbaz N, Sutren M, Masson M, Susini C, Strosberg AD, Nahmias C. Angiotensin II type 2 receptors mediate inhibition of mitogen-activated protein kinase cascade and functional activation of SHP-1 tyrosine phophatase. Biochem J. 1997;325;449-454.

[10] Lehtonen JYA, Daviet L, Nahmias C, Horiuchi M, Dzau VJ. Analysis of functional domains of angiotensin II type 2 receptor involved in apoptosis. Mol Endocrinol. 1999;13:1051-1060.

[11] Shenoy UV, Richards EM, Hang XC, Sumners C. Angiotensin II type 2 receptor-mediated apoptosis of cultured neurons from newborn rat brain. Endocrinology. 1999;140:500-509.

[12] Yi T, Ihle J. Association of hematopoietic cell phosphatase with c-Kit after stimulation with c-Kit ligand. Mol Cell Biol. 1993;13:3350-3358.

[13] Paulson RF, Vesely S, Siminovitch KA, Bernstein A. Signaling by the W/Kit receptor tyrosine kinase is negatively regulated in vivo by the protein tyrosine phosphatase Shp1. Nat Genet. 1996;13:309-315.

[14] Vambutas V, Kaplan DR, Sells MA, Chernoff J. Nerve growth factor stimulates tyrosine phosphorylation and activation of Src homology-containing protein-tyrosine phosphatase 1 in PC12 cells. J Biol Chem. 1995;270:25629-25633.

[15] Tomic S, Greise RU, Lammers R, Kharitonenkov A, Imyanitov E, Ullrich A, Bohmer F. Association of SH2 domain protein tyrosine phophatases with the epidermal growth factor receptor in human tumor cells:phosphatidic acid activates receptor dephosphorylation by PTP1C. J Biol Chem. 1995;270:21277-21284.

[16] David M, Chen HE, Goelz S, Larner AC, Neel BG. Differential regulation of the α/β interferon-stimulated Jak/Stat pathway by the SH2 domain-containing tyrosine phosphatase SHPTP1. Mol Cell Biol. 1995;15:7050-7058.

[17] Klingmüller U, Lorenz U, Cantley LC, Neel BG, Lodish HF. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. Cell. 1995;80:729-738.

[18] Nadler MJB, Chen B, Anderson JS, Wortis HH, Neel BG. Protein tyrosine phosphatase SHP-1 is dispensable for FcγRIIB-mediated inhibition of B cell antigen receptor activation. J Biol Chem. 1997;272:20038-20043.

[19] Akishita M, Ito M, Lehtonen JYA, Daviet L, Dzau VJ, Horiuchi M. Expression of the AT2 receptor developmentally programs extracellular signal-regulated kinase activity and influences fetal vascular growth. J Clin Invest. 1999;103:63-71.

[20] Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G. A monoclonal antibody against a-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol. 1986;103:2787-2796.

[21] Lopez F, Estéve JP, Buscail L, Delesque N, Saint-Laurent N, Thévenian M, Nahmias C, Vaysse N, Susini C. The tyrosine phosphatase SHP-1 associates with the sst2 somatoststin receptor and is an essential component of sst2-mediated inhibitory growth signaling. J Biol Chem. 1997;272:24448-24454.

[22] Bai HZ, Pollman MJ, Inishi Y, Gibbons GH. Regulation of vascular smooth muscle cell apoptosis: Modulation of bad by a phosphatidylinositol 3-kinase-dependent pathway. Circ Res. 1999;85:229-237.

[23] Shimizu S, Eguchi Y, Kamiike W, Matsuda H, Tsujimoto Y. Bcl-2 expression prevents activation of the ICE protease cascade. Oncogene. 1996;12:2251-2257.

[24] Nakajima M, Hutchinson H, Fujinaga M, Hayashida W, Morishita R, Zhang L, Horiuchi M, Pratt RE, Dzau VJ. The angiotensin II type 2 (AT2) receptor antagonizes the growth effects of AT1 receptor: gain-of-function study using gene transfer. Proc Natl Acad Sci USA. 1995;92:10663-10667.

[25] Shanmugan S, Corvol P, Gasc JM. Angiotensin II type 2 receptor mRNA expression in the developing cardiopulmonary system of the rat. Hypertension. 1996;28:91-97.

[26] Yamada H, Akishita M, Ito M, Tamura K, Daviet L, Lehtonen JYA, Dzau VJ, Horiuchi M. AT2 receptor and vascular smooth muscle cell differentiation in vascular development. Hyertension. 1999;33:1414-1419.

[27] Force T, Bonventre JV. Growth factor and mitogen-activated protein kinase.Hypertension. 1998;31:152-161.

[28] Hayashida W, Horiuchi M, Dzau VJ. Intracellular third loop domain of angiotensin II type 2 receptor: role in mediating signal transduction and cellular function. J Biol Chem. 1996;271:21985-21992.

[29] Huang XC, Richards EM, Sumners C. Mitogen-activated protein kinase in rat brain neuronal cultures are activated by angiotensin II type 1 receptors and inhibited by angiotensin II type 2 receptors. J Biol Chem. 1996;271:15635-15641.

[30] Li X, Lee JW, Graves LM, Earp HS. Angiotensin II stimulates ERK via two pathways in epithelial cells: protein kinase C suppresses a G-protein coupled receptor-EGF receptor transactivation pathway. *EMBO J.* 1998;17:2574-2583.

[31] Eguchi S, Numaguchi K, Iwasaki H, Matsumoto T, Yamakawa T, Utsunomiya H, Motley ED, Kawakatsu H, Owada K, Hirata Y, Marumo F, Inagami T. Calcium-dependent epidermal growth factor receptor transactivation mediates the angiotensin II-induced mitogen-activated protein kinase activation in vascular smooth muscle cell. J Biol Chem. 1998;273:8890-8896.

[32] Murasawa S, Mori Y, Nozawa Y, Gotoh N, Shibuya M, Masaki H, Maruyama K, Tsutsumi Y, Moriguchi Y, Shibazaki Y, Tanaka Y, Iwasaka T, Inada M, Matsubara H. Angiotensin II type 1 receptor-induced extracellular signal-regulated protein kinase activation is mediated by Ca2+/calmodulin-dependent transactivation of epidermal growth factor receptor. *Circ Res.* 1998;82;1338-1348.

[33] Elbaz N, Bedecs K, Masson M, Sutren M, Strosberg ADS, Nahmias C. Functional transinactivation of insulin receptor kinase by growth-inhibitory angiotensin II AT2 receptor. Mol Endocrinol. 2000;14:795-804.

[34] Su X, Zhou T, Wang Z, Yang P, Jope RS, Mountz JD. Defective expression of hematopoietic cell protein tyrosine phosphatase (HCP) in lymphoid cells blocks Fas-mediated apoptosis. Immunity 1995;2:353-362.

[35] Xiao S, Rose DW, Sasaoka T, Maegawa H, Burke TR, Roller JPP, Shoelson SE, Olefsky JM. Syp (SH-PTP2) is a positive mediator of growth factor-stimulated mitogenic signal transduction. J Biol Chem. 1994;269:21244-21248.

[36] Milarski KL, Saltiel AR. Expression of catalytically inactive Syp phosphatase in 3T3 cells blocks stimulation of mitogen-activated protein kinase by insulin. J Biol Chem. 1994;269:21239-21243.

[37] Wright JH, Drueckes P, Bartoe J, Zhao Z, Shen SH, Krebs EG. A role of the SHP-2 tyrosine phosphatase in nerve growth factor-induced PC12 cell differentiation. Mol Biol Cell. 1997;8:1575-1585.

[38] Marrero MB, Venema VJ, Ju H, Eaton DC, Venema RC. Regulation of angiotensin II-induced JAK2 tyrosine phosphorylation: roles of SHP-1 and SHP-2. Am J Physiol. 1998;275:C1216-C1223.

[39] Majesky MW, Giachelli CM, Reidy MA, Schwartz SM. Rat carotid neointimal smooth muscle cells reexpress a developmentally regulated mRNA phenotype during repair of arterial injury. Circ Res. 1992;71:759-768.

[40] Schwartz SM, Campbell GR, Campbell JH. Replication of smooth muscle cells in vascular disease. Circ Res. 1986;58:427-444.

[41] Tea BS, Sarkissian SD, Touyz RM, Hamet P, DeBlois D. Proapoptotic and growth-inhibitory role angiotensin II type 2 receptor in vascular smooth muscle cells of spontaneously hypertensive rats in vivo. Hypertension. 2000;35:1069-1073.