The angiotensin-converting enzyme 2 in tumor growth and tumor-associated angiogenesis in non-small cell lung cancer

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Received October 19, 2009; accepted December 15, 2009

Abstract. Angiotensin II (AngII) is a multifunctional bioactive peptide and previous studies have shown that the renin-angiotensin system (RAS) of both host and tumor are important in tumor growth and angiogenesis in lung cancer. Angiotensin-converting enzyme 2 (ACE2) is a newly identified component of RAS, with 42% amino acid homology to ACE. However, the expression and function of ACE2 in non-small cell lung cancer (NSCLC) are still unclear. In the present study, we analyzed ACE2 expression in NSCLC tissue by Western blot analysis and immunohistochemistry. AngII concentrations in the tissue homogenate were also detected using radioimmunoassay. We also examined the function of ACE2 by transducing A549 cells with MSCV-ACE2. We have shown for the first time that ACE2 expression decreased in NSCLC tissue in which AngII was higher than the matching non-malignant tissues. A concentration of 10⁻⁶ mol/l of AngII significantly increased expression of vascular endothelial growth factor a (VEGF a) and AT1-R and decreased ACE2 expression. We also found that overexpression of ACE2 may have a protective effect by inhibiting cell growth and VEGFa production in vitro. ACE2 may become a target of novel strategies to treat NSCLC.

Introduction

Lung cancer is the leading cause of cancer deaths worldwide (1) and 85% of lung cancers are non-small cell lung cancer (NSCLC). Despite improvements in treatment modalities, the 5-year survival rate for NSCLC has improved to only 14% in the past 30 years. This grim prognosis indicates a continued need for novel therapeutic approaches to reduce lung cancer mortality.

The renin-angiotensin system (RAS) is important in regulating cardiovascular homeostasis and blood pressure (2). Angiotensin II (AngII), a biologically active octapeptide in RAS, mediates its biologic effect by binding to two subtypes of receptors: type 1 (AT1-R) and type 2 (AT2-R), which belong to the G-protein-coupled receptor superfamily (3). Emerging data suggest that, in addition to systemically produced angiotensin, the tumor environment contains all RAS components necessary to produce angiotensin locally and local RAS system contributes importantly to tumor angiogenesis and tumor progression (4-6). A large scale clinical trial for hypertension demonstrated that inhibitors of angiotensin-converting enzyme (ACE) reduced mortality rates not only in cardiovascular diseases but also for malignant tumors (7). The relative risk was lowest in patients with lung or gender-specific cancers. Recent studies have associated ACE inhibitor use with a lower likelihood of history of cancer in patients with diabetes (8). Indeed, many studies show that captopril and other ACE inhibitors reduce NSCLC growth and angiogenesis (9,10,28). These findings suggest that the ACE-AngII-AT1-R pathway may be associated with NSCLC progression.

Angiotensin-converting enzyme 2 (ACE2) is a newly identified component of RAS, with 42% amino acid homology to ACE. Studies demonstrate moderate ACE2 expression in lungs of both humans (11) and mice (12), with high levels of ACE2 in kidney, heart, testis and small intestine in both species (12-14). In the human lung, immunostaining has localized ACE2 to endothelial and smooth muscle cells of large and small blood vessels, as well as to types I and II alveolar epithelial cells and bronchial epithelial cells (15,16). ACE2 catalyzes conversion of AngI to Ang-(1-9) (17,18). Moreover, ACE2 converts AngII to Ang-(1-7) (19), a peptide with vasodilator and anti-proliferative properties. Various in vivo studies strongly suggest that a major role of ACE2 is indeed the generation of Ang-(1-9) from AngII, and that its conversion of AngI to Ang-(1-9) is not normally of physiological importance (20-22). Gallagher and Tallant (23) showed...
that treatment with Ang-(1-7) resulted in dose- and time-
dependent reductions in serum-stimulated DNA synthesis in
NSCLC cell lines. Recent studies show that Ang-(1-7) can
inhibit lung tumor growth in vivo, using a human lung tumor
 xenograft model (24). ACE2 thus provides an apparent
mechanism to directly balance levels of AngII and Ang-(1-7)
to modulate the pressor/mitogenic and depressor/growth
inhibitory arms of RAS. However, the expression and role of
ACE2 in NSCLC is still unclear.

Based on previous studies, we hypothesized that ACE2
plays an important role in tumor growth and angiogenesis
and that its expression may decrease in lung cancer tissues
compared to non-malignant tissues. In this study we clarified
the expression of ACE2 and AngII in NSCLC. We also
investigated the effects of AngII on lung cancer cells and
examined the impact of overexpression of ACE2 on VEGF
expression and cell proliferation in vitro. The results reported
here may provide the basis for a novel and effective strategy to
treat lung cancer.

Materials and methods

Tissue acquisition. Histologically confirmed NSCLC samples
and matching non-malignant tissues (n=19) were obtained from
patients who underwent surgical resection at Ruijin Hospital
of Shanghai Jiaotong University School of Medicine between
2005 and 2007. Matched pairs of NSCLC specimens were
dissected from tumors and from adjacent tumor-free (TF)
tissues. Four of the patients were excluded for history of
hypertension, renal disease or protein degradation. Tissue
samples were frozen in liquid nitrogen for Western blotting
and AngII assays. In addition, formalin-fixed paraffin-
embedded tissue blocks containing 64 NSCLC samples,
obtained from patients who underwent surgical resection
between 2003 and 2004, were subjected to immunohisto-
chemistry. All patients signed appropriate consent for tissue
acquisition and study. The study was approved by the Hospital
Ethics Committee.

Immunohistochemistry. ACE2 were detected in NSCLC
sections and normal lung tissues. Briefly, samples were
deparaffinized in xylene and graded alcohols and rehydrated
in Tris-buffered saline (TBS, pH 7.5). Following antigen
retrieval in citrate buffer (0.01 M, pH 6.0) for 45 min at 120˚C,
sections were rinsed in TBS, treated with 3% hydrogen
peroxide in TBS, rinsed and incubated with 10% normal rabbit
serum for 30 min. Sections were incubated in anti-ACE2
dilution) IgG antibody (1:200) overnight at 4˚C, rinsed with
TBS, then incubated in anti-goat biotinylated IgG antibody
plasmid, which expresses human-ACE2. 293T cells were transfected
with DNA (4 μg pMD-gag-pol, 4 μg pMD-VSVG and 4 μg
retroviral vector pMSCV-ACE2 or pMSCV) using Lipo-
fectamine 2000 reagent (Invitrogen Corporation, Carlsbad,
CA) and VSV-G-pseudotyped viral supernatant fractions
collected after 48 h were transduced into A549 cells using
polybrene (Sigma Corporation, Cream Ridge, NJ) and centri-
fugation. Transduced cells were selected in the presence of
4 μg/ml puromycin. The stable transfectants were maintained
in 2 mg/ml puromycin medium.

AngII concentration detection. NSCLC tissues were weighed
and cut into small pieces after washing. Homogenates (10% w/v)
were prepared in a solution containing 50 mM phosphate
buffer (pH 7.3). AngII concentration in the culture homogenate
was measured using radioimmunoassay, carried out at the
Ruijin Hypertension Institute Laboratory.

Protein isolation. NSCLC tissues and matching non-
malignant tissues were mechanically homogenized in RIPA
buffer containing 100 mg/ml phenylmethylsulfonyl fluoride.
Confluent cell plates were washed three times with ice cold
phosphate-buffered saline, then cells were scraped into 1 ml
RIPA buffer. The RIPA buffer cell lysate suspension was
then centrifuged at 13,000 rpm for 30 min. The supernatant
was collected and protein concentration determined spectro-
photometrically using the Bradford Protein assay (Bio-Rad
Laboratories, Hercules, CA).

Cell culture and cell growth assay. A549 lung cancer cells
(Shanghai Institute of Cells) were maintained in Ham's F12
medium with 10% FBS, 100 mg/ml penicillin and 100 U/ml
streptomyacin using media and growth reagents from Gibco
(Gibco BRL, Grand Island, NY). Cell preparation was done
in a room kept at 37˚C with a humidified atmosphere of 5% CO2
and 95% room-air. To measure the influence of different
concentrations of AngII and overexpression of ACE2 on cell
growth, A549 cells (3x104) were seeded into a 96-well plate
and then half were treated with AngII (10^-7^-10^-6 mol/l) for 72 h.
A549 cells alone and those transfected with vector or MSCV-
ACE2 were also plated in a 96-well plate. After 24 h
incubation with 10% FBS, the cells were serum-starved for
24 h, then incubated in serum-free medium for different
lengths of time, as indicated. Afterward, 10 μl of cell count
kit-8 (CCK-8, Dojindo, Kumamoto, Japan) were added into
each well and incubated for 3 h. The plates were read at
450 nm with a Safire 2 spectrophotometer (Tecan Group, Ltd,
Männedorf, Switzerland). Experiments were carried out in
triple and repeated at least three times.

Retroviral vector construct and transduction. The pcDNA3.1
vector containing human-ACE2 cDNA was kindly provided by
Dr Paul McCray (University of Iowa, Iowa City, IA). They
were ligated into the BglII/Xhol site of the pMSCV plasmid,
which expresses human-ACE2. 293T cells were transfected
with DNA (4 μg pMD-gag-pol, 4 μg pMD-VSVG and 4 μg
retroviral vector pMSCV-ACE2 or pMSCV) using Lipo-
fectamine 2000 reagent (Invitrogen Corporation, Carlsbad,
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fugation. Transduced cells were selected in the presence of
4 μg/ml puromycin. The stable transfectants were maintained
in 2 mg/ml puromycin medium.
Cell cycle analysis using flow cytometry. A549 cells (5 x 10^5) were seeded into a 100-mm culture dish and pre-cultured for 24 h. A549 cells overexpressing ACE2 and control cells were serum starved for 24 h. The cells were then incubated in Ham's F12 medium with 10% FBS for 72 h. The cells were trypsinized, washed twice with PBS and suspended in 500 μl PBS containing 0.1% FBS on ice. The cell suspension was mixed with 5 ml ice cold 70% ethanol and stored at -4°C until analysis. On the day of analysis, cells were washed twice and re-suspended in 1 ml PBS containing 0.1% FBS. After incubation with RNase A (250 μg/ml) for 30 min and staining with propidium iodide (PI, 10 μg/ml) for 10 min, cell cycle analysis was conducted using the FACS system (BD Biosciences, San Jose, CA). Histograms were generated and cell cycle analysis was conducted using WinMDI 2.8 software (Joe Trotter, Scripps Research Institute, La Jolla, CA).

Enzyme-linked immunosorbent assay (ELISA). VEGFa levels were measured in the supernatants of A549 cells. After 24 h incubation with 10% FBS, A549 cells overexpressing ACE2 and control cells were serum-starved for 24 h. Cells were then incubated in serum-free medium for 24 h, the supernatant recovered and VEGFa levels determined by human VEGFa enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN).

Real-time quantitative reverse transcription analysis. ACE, ACE2, AT1-R and VEGFa mRNA expression were examined by real-time quantitative reverse transcription-based polymerase chain reaction (qRT-PCR) in A549 cells and those stimulated with AngII (10^-7, 10^-6 mol/l) for 15 min. ACE, ACE2 and VEGFa mRNA expression were also examined in A549 cells overexpressing ACE2 compared with control cells. Total RNAs were extracted from cells by TRIzol reagent. RNA was treated with DNase (Promega, Madison, WI) and complementary DNA was synthesized using a cDNA synthesis kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Fluorescence qRT-PCR was performed with the double-stranded DNA dye SYBR-Green (PCR Core Reagents, PE Biosystems, Warrington, UK) using the ABI PRISM 7300 system (Applied Biosystems). The SYBR-Green assay contained 1 μl 10X SYBR-Green PCR buffer, 0.8 μl deoxynucleoside triphosphate (dNTP) mixture, 0.1 μl AmpliEnaseUNG (1 U/μl), 0.05 μl AmpliTaq Gold DNA Polymerase (5 U/μl), 1.2 μl MgCl2 (25 mM), 0.1 μl forward and reverse primer (20 μM), 1 μl cDNA and 5.65 μl double distilled H2O. PCR was begun with one cycle at 50°C for 2 min and 95°C for 10 min and preceded by 45 cycles with denaturing at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec. All data were analyzed using ABI PRISM SDS 2.0 software (PerkinElmer, Wellesley, MA). Using the ΔCt method, GAPDH was coamplified to normalize the amount of RNA added to the reaction and the data were subjected to cycling threshold analysis. PCR was repeated at least three times. The primers used in this study were as follows: ACE forward 5'-CCGGATCTGGCAGACACTTC-3' and reverse 5'-GTTGTCGAGATGCACACTTT-3'; ACE2 forward 5'-CCCCACGTGCTCAACTACCT-3' and reverse 5'-CTATCTCAGCTTTTGGATGT-3'; AT1-R forward 5'-ATTGCCAGAGATCGCAGA-3' and reverse 5'-GAGATCATTGGCCTCAGGAGA-3'.

Western blot analysis. ACE2 protein expression in tumor tissues was studied by Western blot analysis. AT1 receptor and VEGFa protein levels in A549 cells were studied after treatment with or without AngII (10^-7, 10^-6 mol/l) for 24 h. Expression of ACE2, AT1 receptor and VEGFa were also measured in A549 cells overexpressing ACE2 compared with control cells. After being treated with protein assay reagent (Bio-Rad Laboratories), soluble protein was separated on 10% SDS-polyacrylamide gels and transferred to PVDF membranes (Millipore, Billerica, MA). ACE2 antibody (AP933, R&D Systems), AT1 receptor antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), VEGFa antibody (1:1000, Santa Cruz Biotechnology) and β-actin monoclonal antibody (1:10000, Sigma Corporation) were used to detect ACE2, AT1 receptor protein, VEGFa protein and β-actin protein respectively. The immunoreactive bands were visualized by ECL plus reagent (Amersham Biosciences, Piscataway, NJ). Expression of VEGFa protein in A549 cells was analyzed with Scion Image (Scion Corporation, Frederick, MD).

Statistical analysis. Data are presented as means ± SD. Student's t-test was used to compare the differences between two groups. The correlation between ACE2 expression and clinicopathological data was examined using Chi-squared analysis. Differences were considered statistically significant at P<0.05 (two-tailed). All statistical analyses were conducted using SPSS version 11.0 (SPSS Inc., Chicago, IL).

Results

Expression of ACE2 and AngII concentration in NSCLC and adjacent TF tissues. We carried out Western blot analysis to determine the expression of ACE2. Equal concentrations of the isolated protein were electrophoresed on sodium dodecyl sulfate polyacrylamide gels. Blots were stripped and reprobed with β-actin antibody to control for loading errors. As shown in Fig. 1A, ACE2 protein expression was examined in all 15 comparison samples, 11 of which (~73%) were lower in NSCLC tissues than in adjacent TF tissues. We also found that AngII concentration in the tissue homogenate of NSCLC was higher than in matching non-malignant tissues (Fig. 1B).

Localization and immunohistochemical expression of ACE2 in NSCLC tissues. As shown in Fig. 2, ACE2 was localized to the membrane and cytoplasm. Among 64 samples of NSCLC tissues, 19 (29.6%) showed high ACE2 expression (Fig. 2A and C) and 45 (70.3%) showed low expression (Fig. 2B and D). The relationship between ACE2 expression in NSCLC and clinicopathological factors is summarized in Table I. Statistical analysis indicated that the level of ACE2 protein expression correlated with clinical stage (P=0.011) and smoking status.
Expression groups did not obviously differ in gender, age, differentiation or histological type (SCC vs. AD).

**Effect of AngII on VEGFa and RAS components in vitro.** Analysis of (qRT-PCR) data of ACE, ACE2, AT1-R and VEGF mRNA, corrected with GAPDH as an internal control, showed that 10^-6 mol/l of AngII significantly increased expression of VEGFa (Fig. 3A) and AT1-R mRNA (Fig. 3B, P<0.01) and decreased expression of ACE2 mRNA (Fig. 3C, P<0.01). In contrast, no change in ACE mRNA was observed in A549 cells treated with AngII (Fig. 3D), demonstrating the differential regulation of the two enzymes. In Western blot analysis, expression of VEGFa and AT1-R protein in 10^-6 mol/l AngII-treated A549 cells were significantly higher than in 10^-7 mol/l AngII and control cells (Fig. 3E and F).

**Effect of ACE2 gene transfusion in A549 cells.** Our objective was to establish the efficacy of MSCV-ACE2 transfusion. A549 cells were infected with MSCV-ACE2 and selected in the presence of puromycin. Infection of A549 cells with MSCV-ACE2 resulted in robust ACE2 expression by Western blot analysis at 72 h. No significant ACE2 protein expression was observed in A549 cells infected with the vector by the same way or in the normal lung tissue used as a positive control (Fig. 4A). As expected, expression of ACE2 mRNA (Fig. 4B, P<0.01) was notably higher in the MSCV-ACE2 group compared to vector. Moreover, AT1-R protein products (Fig. 4C) of A549 cells also decreased in the MSCV-ACE2 group compared to vector.

**Effects of AngII and overexpression of ACE2 on cell growth of cultured A549 cells.** Cell growth was determined by CCK-8 assay in A549 cells. As shown in Fig. 5A, treatment with

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**Table I. Relationship between ACE2 protein expression and clinicopathological factors of patients with NSCLC.**

Scc, squamous cell carcinoma and Ad, adenocarcinoma.
AngII (10^{-9}-10^{-6}) for 72 h did not affect viability of A549 cells. However, we found that ACE2 overexpression significantly decreased cell viability compared with vector (Fig. 5B). Furthermore, DNA staining by propidium iodide was done to discriminate cells in G_{0}/G_{1} or G_{2}/M phase by FACS (Fig. 5C). Overexpression of ACE2 induced a significant increase in cells in the G_{0}/G_{1} phase and a decrease in cells in the G_{2}/M phase compared to vector. A549 cells infected with MSCV-ACE2 notably increased compared with vector in G_{0}/G_{1} phase at 72 h (69.52±0.90% vs. 76.17±1.20%, P<0.01) and decreased in G_{2}/M phase at 72 h (9.33±0.87% vs. 5.62±0.58%, P<0.05) (Table II).

ACE2 inhibits AngII-induced VEGFa mRNA accumulation and protein production in cultured A549 cells. We sought to investigate whether overexpression of ACE2 can decrease VEGFa mRNA accumulation and protein secretion in A549 cells. Gene expression analysis by (qRT-PCR) showed that the VEGFa mRNA level decreased in A549 cells infected with MSCV-ACE2 compared with vector (Fig. 6A). VEGFa expression in A549 cells was detected by Western blot analysis. VEGFa protein levels in the supernatants were also determined by ELISA. As shown in Fig. 6B and C, VEGFa protein levels decreased in ACE2 infected A549 cells and supernatants compared with vector.

**Discussion**

The RAS system is important in regulating vascular homeostasis and AngII plays a role in proliferation, migration, and...
Figure 5. Overexpression of ACE2 reduced cell growth in A549 cells. (A) Treated with or without AngII (10^-9-10^-6) for 72 h did not affect cell viability in A549 cells. (B) Overexpression of ACE2 caused significantly decreased cell viability. Cell growth was determined by the CCK-8 assay in A549 cells. Data are means ± SD values obtained from four culture wells per experiment, determined in three independent experiments. *P<0.05, #P<0.01, compared with the vector. (C) The FACS scans of cell cycle analysis of A549 cells infected with MSCV-ACE2. The A549 cells infected with MSCV-ACE2 and vector were incubated for 72 h. The cell cycle were analysed with flow cytometer using propidium iodide for DNA staining. This experiment was repeated separately three times. The results are expressed in the histogram.

Figure 6. Overexpression of ACE2 inhibits AngII-induced VEGFa production. VEGFa production and mRNA accumulation decreased in A549 cells infected with MSCV-ACE2 compared with vector by Western blot analysis (A) and real-time PCR (B) (means ± SD, n=3) *P<0.05. (C) VEGFa protein levels in the supernatants were determined by ELISA. (Means ± SD, n=3) *P<0.05.
growth factor synthesis in several types of vascular cells. Expression of several components of RAS in various cancers, including brain, lung, breast, prostate, skin, cervix, and glioblastoma, has been previously demonstrated (25). Some studies show that the host stromal AT1 receptor pathway is important in tumor growth and tumor-associated angiogenesis (26,27). However, current evidence supports the idea that both host and tumor RAS are important in tumor growth and angiogenesis in lung cancer. Imai et al (28) found tumor size and VEGFa reduced significantly in AT1a-/- mice treated with TCV-116 (an AT1 receptor antagonist) compared with untreated mice. As a recently reported homologue of ACE, ACE2 is a new component of the updated RAS shown to be critical in the balance between AngII and Ang-(1-7) and to play a protective role in many diseases (29-31). However, its expression and effect in NSCLC remain unclear.

In this study, we demonstrated that ACE2 protein expression was lower in NSCLC tissues than in adjacent TF tissues by Western blot analysis. We also found higher AngII concentration in the tissue homogenate of NSCLC than in matching non-malignant tissues. In addition, immunohistochemical analysis showed that many NSCLC cells exhibited low to high ACE2 staining, mainly in the membrane and cytoplasm. Statistical analysis of ACE2 expression and the clinical features of patients with NSCLC showed that ACE2 expression was closely correlated to clinical stage and smoking status. This study is the first to investigate the expression of ACE2 and AngII in NSCLC. These findings suggest that the AngII-AT1-R system may play a significant role in the localized RAS within these NSCLC tumor tissues. Many situations influence RAS activity, including hypertension, renal disease and having taken ACEI or ARB. In our study, the 15 NSCLC tissue samples subjected to Western blot analysis were from patients with no history of hypertension, renal disease or taking ACEI or ARB.

Next, we examined whether AngII and overexpression of ACE2 could change the expression of other components of RAS. Consistent with a previous study (28), the main components of RAS were found to express in lung cancer cells. We found that expression of AT1-R protein in 10^-6 mol/l AngII-treated A549 cells were significantly higher than in 10^-7 mol/l AngII and control cells. Consistent with Western blot analysis results, (qRT-PCR) demonstrated an increase in mRNA expression of AT1-R. We also found that incubation of A549 cells with 10^-6 mol/l AngII reduced ACE2 mRNA expression by (qRT-PCR). The AT1-R-mediated ERK/P38 MAP kinase signaling pathway may be a key mechanism by which AngII downregulates ACE2 expression (32). In contrast, AngII had no effect on ACE mRNA in A549 cells, which AngII downregulates ACE2 expression (32). In this study, we demonstrated that ACE2 protein expression was lower in NSCLC tissues than in adjacent TF tissues by Western blot analysis. We also found higher AngII concentration in the tissue homogenate of NSCLC than in matching non-malignant tissues. In addition, immunohistochemical analysis showed that many NSCLC cells exhibited low to high ACE2 staining, mainly in the membrane and cytoplasm. Statistical analysis of ACE2 expression and the clinical features of patients with NSCLC showed that ACE2 expression was closely correlated to clinical stage and smoking status. This study is the first to investigate the expression of ACE2 and AngII in NSCLC. These findings suggest that the AngII-AT1-R system may play a significant role in the localized RAS within these NSCLC tumor tissues. Many situations influence RAS activity, including hypertension, renal disease and having taken ACEI or ARB. In our study, the 15 NSCLC tissue samples subjected to Western blot analysis were from patients with no history of hypertension, renal disease or taking ACEI or ARB.

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The roles of the AngII and AT1-R pathways in tumor progression, through effects on cellular proliferation, are unclear. AngII has been shown to stimulate proliferative and hypertrophic growth in vascular smooth muscle cells (34) and neonatal bladder stromal cells (35) through binding to AT1-R. However, it did not affect cell viability of murine Lewis lung carcinoma (LLC) cells (28) and pancreatic ductal adenocarcinoma (PDA) cells (36). We found that treatment with AngII (10^-6-10^-4) did not affect cell viability in A549 cells. Furthermore, consistent with the enhanced proliferative effect of ACE2 in PDA cells by RNA interference (37), we demonstrated overexpression of ACE2 inhibited cell growth compared to vector, even on the first day of the experiment. These data indicate that endogenously generated AngII is implicated in NSCLC cellular proliferation and viability. They also suggest that Ang-(1-7) may not inhibit early overexpression of ACE2, because Ang-(1-7) inhibits lung cancer A549 cell growth after five days (23). In the present study, cells in the G_0/G_1 phase significantly increased and those in the G_0/M phase decreased in MSCV-ACE2 compared to vector, indicating that ACE2 overexpression arrested growth in A549 cells.

As others have pointed out, VEGFa is an important mediator of angiogenesis (38). Consistent with previous studies in LLC cells, we found that VEGFa protein expression and VEGFa mRNA production were increased by stimulation with 10^-6 AngII in A549 cells. Our results suggest that the tumor RAS promotes tumor angiogenesis through VEGFa induction. Kang et al (39) showed that AngII stimulated VEGFa synthesis in podocytes and that VEGFa production induced by AngII was mediated, in part, through activation of the p38 mitogen activated protein kinase pathway. Consistent with Western blot analysis and (qRT-PCR) results, we found VEGFa expression decreased in the supernatants of A549 cells infected with MSCV-ACE2 compared with vector. These findings reflect the fact that ACE2 inhibits tumor angiogenesis by decreasing VEGFa expression. Further studies will be required to discover the effect of ACE2 in tumor RAS using animal models.

In conclusion, the present study is the first to demonstrate lower expression of ACE2 in NSCLC tissues than in adjacent TF tissues and higher AngII concentrations in the tissue homogenate of NSCLC than in matching non-malignant tissues. We also demonstrated that ACE2 overexpression can decrease AT1-R protein production. Moreover, we showed that ACE2 may have a protective effect by inhibiting cell growth and VEGFa production in vitro. Combined with these findings, our data suggest that local RAS plays an important role in NSCLC development and progression. ACE2 may become the basis of a novel and effective strategy to treat NSCLC.

Acknowledgements

This study was supported by a grant from the Shanghai Medical Key Discipline (06JC14054).

References


