Therapeutic effects of lenvatinib in combination with rAd-p53 for the treatment of non-small cell lung cancer

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Abstract. The aim of the present study was to analyze the effects of the combined treatment of lenvatinib and adenoviral delivered p53 gene (rAd-p53) on non-small cell lung cancer (NSCLC) cells and a total of 120 patients with NSCLC. The therapeutic effects of gene therapy of rAd-p53 and target therapy of Lenvatinib were investigated in NSCLC patients. The anti-tumor effects of combined treatment of llenvatinib and rAd-p53 was administered orally once-daily in NSCLC patients. Patients with NSCLC were divided into three groups and received lenvatinib (n=40), rAd-p53 (n=40) or combined treatment of lenvatinib and rAd-p53 (n=40) for a total of 30 days. Results showed that p53 was down-regulated and VEGFR, FGFR and PDGFR-β were up-regulated in NSCLC tissues compared to adjacent normal tissues. Combined treatment of Lenvatinib and rAd-p53 markedly inhibited NSCLC cell growth, migration and invasion, and promoted apoptosis compared to either lenvatinib or rAd-p53 alone. The most common treatment-related adverse events included hypertension, diarrhea, nausea, proteinuria and body weight loss. Outcomes indicated that combined treatment of lenvatinib and rAd-p53 markedly inhibited tumor growth compared to lenvatinib and rAd-p53 alone for NSCLC patients. Combined treatment of lenvatinib and rAd-p53 did not exhibit drug accumulation after 30-day treatment. In conclusion, these outcomes indicate that combined treatment of lenvatinib and rAd-p53 may be an efficient therapeutic schedule for the treatment of NSCLC patients.

Introduction

Lung cancer is one of the most common human cancer in the world (1). The morbidity and mortality rate of lung cancer in increasing since 2000 in the world (2). Non-small cell lung cancer (NSCLC) is the most common lung cancer, which includes adenocarcinoma, large cell carcinoma and squamous cell carcinoma (3-5). NSCLC is generally resistant to chemotherapy and radiotherapy (6). Although various treatments (chemoradiotherapy and targeted therapy) have been developed for the treatments of patients with NSCLC, the survival rate of patients remains properly poor (7-9). Therefore, it is essential to explore the novel clinical treatments for to improve the therapeutic effects for patients with NSCLC in clinic.

Gene therapy drug of rAd-p53 is the first generation gene drug and has been approved for human cancer therapy (10). Previous trials have indicated that the no serious adverse effect related to rAd-p53 has been reported in the majority of large intrathoracic malignant and gastric cancer cases, which is a safe anti-cancer agent (11,12). In addition, rAd-p53 could enhance the sensitivity of human gastric cancer cells to chemotherapy, which decreased anti-apoptosis Bcl-2 expression and increased proapoptosis Bax expression in gastric cancer cells (13). Furthermore, the combination of recombinant rAd-p53 and adriamycin presented more efficacy that single treatment and improved drug resistance in chemotherapy of lung squamous cell cancer (14). However, single treatment of rAd-p53 is not enough to improve survival of cancer patients (10,11,15).

Currently, target therapy has been widely applied for the treatment of human cancer, such as lung cancer, liver cancer and breast cancer (16-18). Lenvatinib is a target therapy drug, which targets for vascular endothelial growth factor receptor 1-3 (VEGFR1-3), fibroblast growth factor receptor 1-4 (FGFR1-4), platelet-derived growth factor receptor-β (PDGFR-β), RET, and kinase insert domain receptor (KIT) (19). Study has indicated that Lenvatinib is beneficial for the treatment of patients with renal cell carcinoma (RCC) (20). Phase 1 study of Lenvatinib combined with carboplatin and paclitaxel showed antitumor activity in patients with NSCLC (21). Nagashima *et al* (22), found that Lenvatinib treatment is effectiveness for thyroid cancer with lung metastases. However, combined therapeutic effects of Lenvatinib and rAd-p53 have not investigated for patients with NSCLC.

In the present study, we investigate the therapeutic effects of Lenvatinib and rAd-p53 in patients with NSCLC. We intended to determine the pharmacokinetic (PK) profile of Lenvatinib and rAd-p53 for NSCLC patients. This study also analyzed the progression-free survival (PFS) after treated by Lenvatinib and rAd-p53 in NSCLC patients.

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Materials and methods

Ethic statement. The present study was approved by Ethical Committee of Mudanjiang medical University affiliated HongQi Hospital.

Patients. The phase-I study was performed in Mudanjiang medical University affiliated HongQi Hospital from May 2011 to October 2016. All patients provided written informed consent before any study-related procedures were performed. Eligibility criteria included age ≥18 years, with a Karnofsky performance status ≥80%; adequate haematological (platelet count of ≥100x10^9/l; absolute neutrophil count of ≥1.5x10^9/l; and haemoglobin ≥8.5 g/dl), hepatic (serum alanine amino-transferase; bilirubin ≤25 μmol/l and aspartate transaminase ≤3 x the upper limit of normal) and renal function (a creatinine clearance ≥60 ml/min or serum creatinine ≤1.5 x the upper limit of normal by Cockcroft-Gault formula). Patients with cancer history were excluded from this study.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). NSCLC cells were isolated from NSCLC tissues as described previously (23) and cultured in DMEM medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total RNA was extracted from cells (1x10^6) using RNAeasy Mini Kit (Qiagen, Inc., Valencia, CA, USA) and RNA (1 μg) was transcribed to cDNA by using an RT kit (Qiagen, Inc.) and quality was confirmed by electrophoresis. The cDNA (10 ng) was subjected to qPCR (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SYBR-Green Master Mix system (cat. no. 4304886; Applied Biosystems; Thermo Fisher Scientific, Inc.). All the forward and reverse primers were synthesized by Invitrogen (P53, forward, 5'-CTATCTTATCTATCTTCTCTATCTTC-3'; reverse, 5'-CTATCTTATCTTCTCTCATCTCTAC-3', VEGFR, forward, 5'-TTCAGAGCGGAGAAAGCAT-3'; reverse, 5'-TAGTTCCCGAAACCCTGAG-3'; FGFR, forward, 5'-CGTGGAAAAGAACGGCAGTAAATA-3'; reverse, 5'-GAACTATTTATCCCCGAGTGCTTG-3'; PDGFR-β, forward, 5'-CCATTCCCGAGGAGCTTTATC-3', reverse, 5'-GGTCATGTTCAGGTCCAACTC-3'; GAPDH, forward, 5'-AGTGCCAGCCTCGTCTCATAG-3'; reverse, 5'-CGTTGA ACTTGCCGTGGGTAG-3'). The reaction conditions were performed as follows: 95°C for 2 min and 45 cycles of 95°C for 20 sec and 54°C for 1 min and 72°C for 30 sec. Relative mRNA expression changes were calculated by $2^{-\Delta\Delta Cq}$ (24). The results were presented as the n-fold change compared with β-actin using Quantiscan2.1 (software demo of AB QuantStudio™ 12 K Flex System, Thermo Fisher Scientific, Inc.).

MTT assay. NSCLC cells were cultured in 96-well plates and incubated with Lenvatinib (2 mg/ml) and/or rAd-p53 (10^{11} pfu) for 48 h at 37°C. A total of 20 μl MTT (5 mg/ml) in PBS solution was added to each well and the cells were cultured for 4 h at 37°C. The medium was removed and 100 μl dimethyl sulfoxide (DMSO) was added into the wells to solubilize the crystals. The optical density was measured by a Bio-Rad (ELISA) reader (Bio-Rad Laboratories, Inc.) at 450 nm.

Apoptosis of NSCLC cells. NSCLC cells (A549) were grown at 37°C until 90% confluences were reached. Cells (1x10^8) were

Table I. Patients' characteristics.

Variables	No. of patients	Percentage, %
Total patients with NSCLC	120	100.0
Sex		
Female	72	60.0
Male	58	40.0
Age (years)	48.5±14.8	
Performance status (Karnofsky)		
100	60	40.1
90	36	22.5
80	24	37.5
Drugs treatment		
Lenvatinib	40	33.3
rAd-p53	40	33.3
Combination	40	33.3

then incubated with Lenvatinib (2 mg/ml; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and/or rAd-p53 (10^{11} pfu) for 48 h at 37°C. After incubation, the tumor cells were tryp-sinized and collected. The cells were then washed in cold PBS, adjusted to 1x10^6 cells/ml with PBS, labeled with Annexin V-FITC and PI (Annexin V-FITC kit; BD Biosciences, Franklin Lakes, NJ, USA), and analyzed with a FACScan flow cytometer (BD Biosciences). The treatments were performed in triplicate, and apoptosis was analyzed with a FACScan flow cytometer (BD Biosciences). using CellQuest Pro solfware (v.5.1, BD Biosciences).

Cells invasion and migration assays. NSCLC cells were cultured in six-well plates with chamber inserts (BD Biosciences) and incubated with Lenvatinib (2 mg/ml) and/or rAd-p53 (10^{11} pfu) for 48 h at 37°C. Mg63 cells were cultured in a 24-well culture plate with chamber inserts (BD Biosciences). For migration assays, 1x10^6 cells/well were cultured in DMEM medium (Thermo Fisher Scientific, Inc.) supplemented with 5% heat-inactivated FBS (Gibco; Thermo Fisher Scientific, Inc.) and placed into the upper chamber with the non-coated membrane. For invasion assays, cells (1x10^6 cells/well) were placed into the upper chamber with a Matrigel-coated membrane. Procedures were performed according to the manufacturer's instructions. Cells were fixed in 4% paraformaldehyde (Sigma-Aldrich; Merck KGaA) and stained with 0.1% crystal violet (Sigma) to quantify cell migra-tion and invasion for 15 min at 37°C. The tumor cells invasion and migration were counted in at least three randomly stained microscope fields (Olympus BX51; Olympus Corp., Tokyo, Japan) for every membrane.

Western blot analysis. NSCLC cells (1x10^6) were incubated with Lenvatinib (2 mg/ml) and/or rAd-p53 (10^{11} pfu) for 48 h at 37°C and harvested by scraping and lysed in radioimmu-noprecipitation assay buffer (Sigma-Aldrich; Merck KGaA) followed by homogenization at 4°C for 10 min. Protein
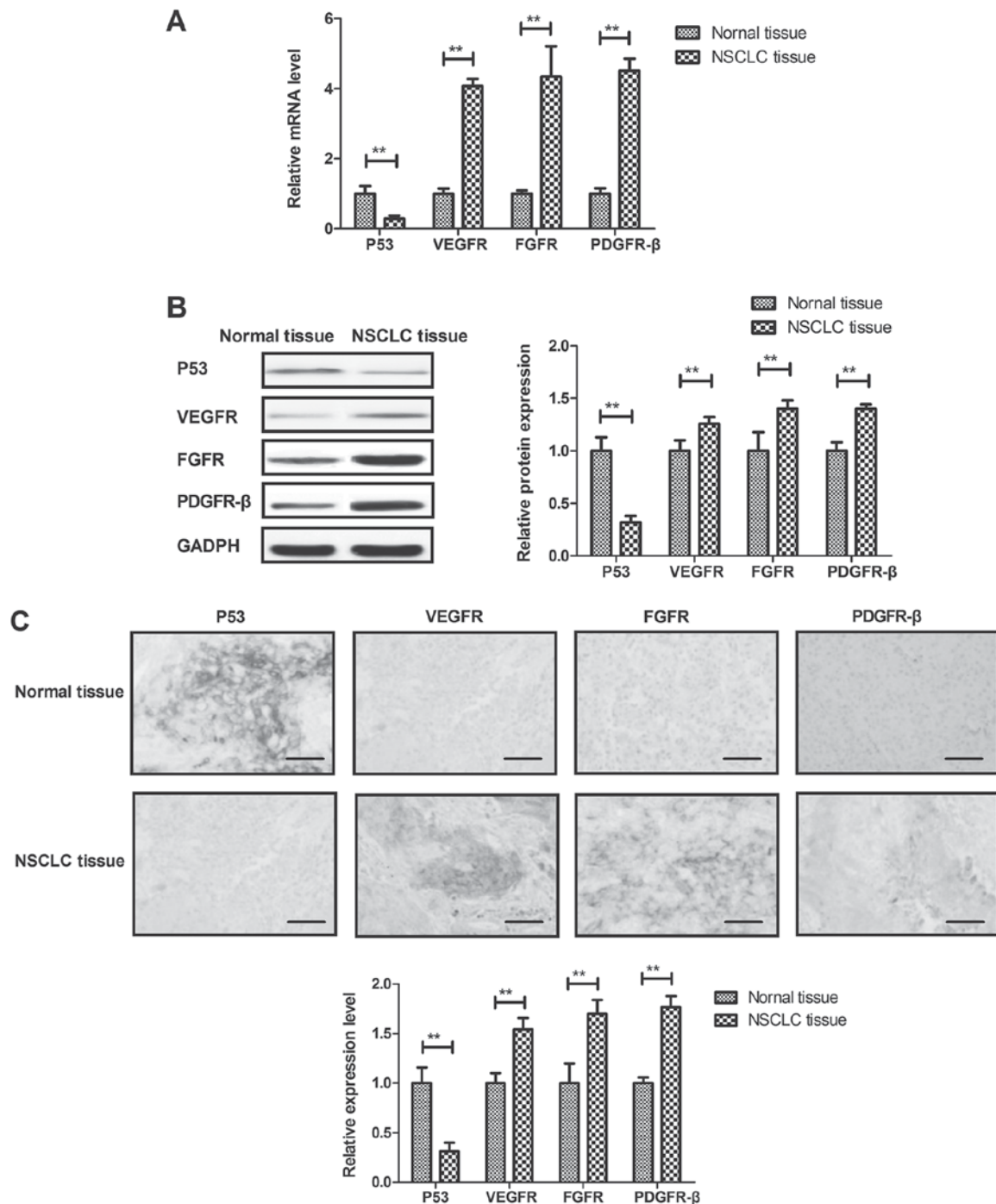
Figure 1. Expression levels of p53, VEGFR, FGFR and PDGFR-β in NSCLC cells and tissues. (A and B) Gene and protein expression levels of p53, VEGFR, FGFR and PDGFR-β in NSCLC cells and adjacent normal cells. (C) Immunohistochemistry assay analyzes p53, VEGFR, FGFR and PDGFR-β levels in NSCLC tissues and adjacent normal tissues (Scale bar=100 µm). **P<0.01. VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; PDGFR-β, platelet-derived growth factor receptor- β; NSCLC, non-small cell lung cancer.

concentration was measured by a BCA protein assay kit (Thermo Fisher Scientific, Inc.). Protein (10 µg) was separated by 15% SDS-PAGE followed transfer to PVDF membranes (EMD Millipore, Billerica, MA, USA). Proteins were blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) for 2 h at 37°C and incubation with primary rabbit anti-human antibodies: P53 (ab131442), VEGFR (ab36844), FGFR (ab10646), PDGFR-β (ab220745) and GAPDH (ab9485) (all 1:500 dilutions; Abcam, Shanghai, China) for 12 h at 4°C. Subsequently, proteins were incubated with the corresponding

rabbit horseradish peroxidase-labeled IgG (1:5,000; Vector Laboratories, Inc., Burlingame, CA, USA) for 2 h at 37°C. The proteins expression levels were detected using a chemi-luminescence detection system (v.3.0; Sigma-Aldrich; Merck KGaA). The density of the bands was analyzed by Quantity one software v.4.62 (Bio-Rad Laboratories, Inc.).

Immunohistochemical staining. NSCLC tissues from patients were fixed using 10% formaldehyde for 30 at 37°C, washed with PBS (0.01 mmol/l, pH 7.4) and followed with embedding
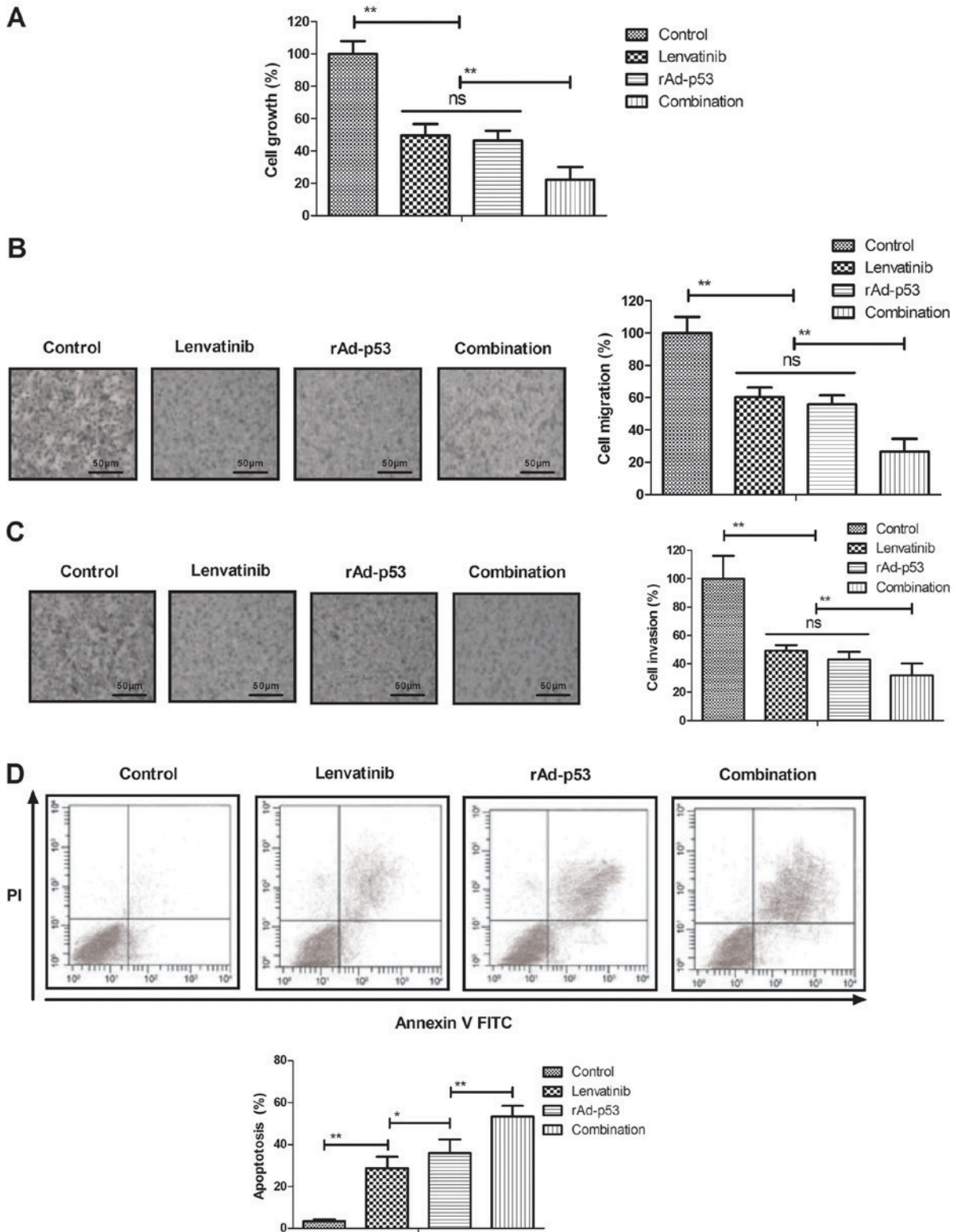
Figure 2. The inhibitory effects of combined treatment of lenvatinib and rAd-p53 for NSCLC cells. (A) Effects of treatment of lenvatinib and/or rAd-p53 on NSCLC cells growth. (B and C) Effects of treatment of lenvatinib and/or rAd-p53 on migration and invasion of NSCLC cells. (D) Effects of combined treatment of lenvatinib and/or rAd-p53 on apoptosis of NSCLC cells induced by tunicamycin. *P<0.05, **P<0.01. NSCLC, non-small cell lung cancer; ns, not significant.

in paraffin wax. Tissues were deparaffinized in xylene and rehydrated in grade alcohols. Tissues were cut into 4-μm thick sections and antigen retrieval was performed using Antigen Retrieval Reagents (cat. no. CTS015; Bio-Rad Laboratories, Inc.). The sections were washed with PBS for 10-15 min at 37˚C and subsequently blocked using 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) for 2 h at 37˚C. Tumor sections were incubated with CD4 (1:1,000 dilutions, Clone 4B12; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) and CD8 (1:1,000 dilutions, Clone C8/144B; Dako;
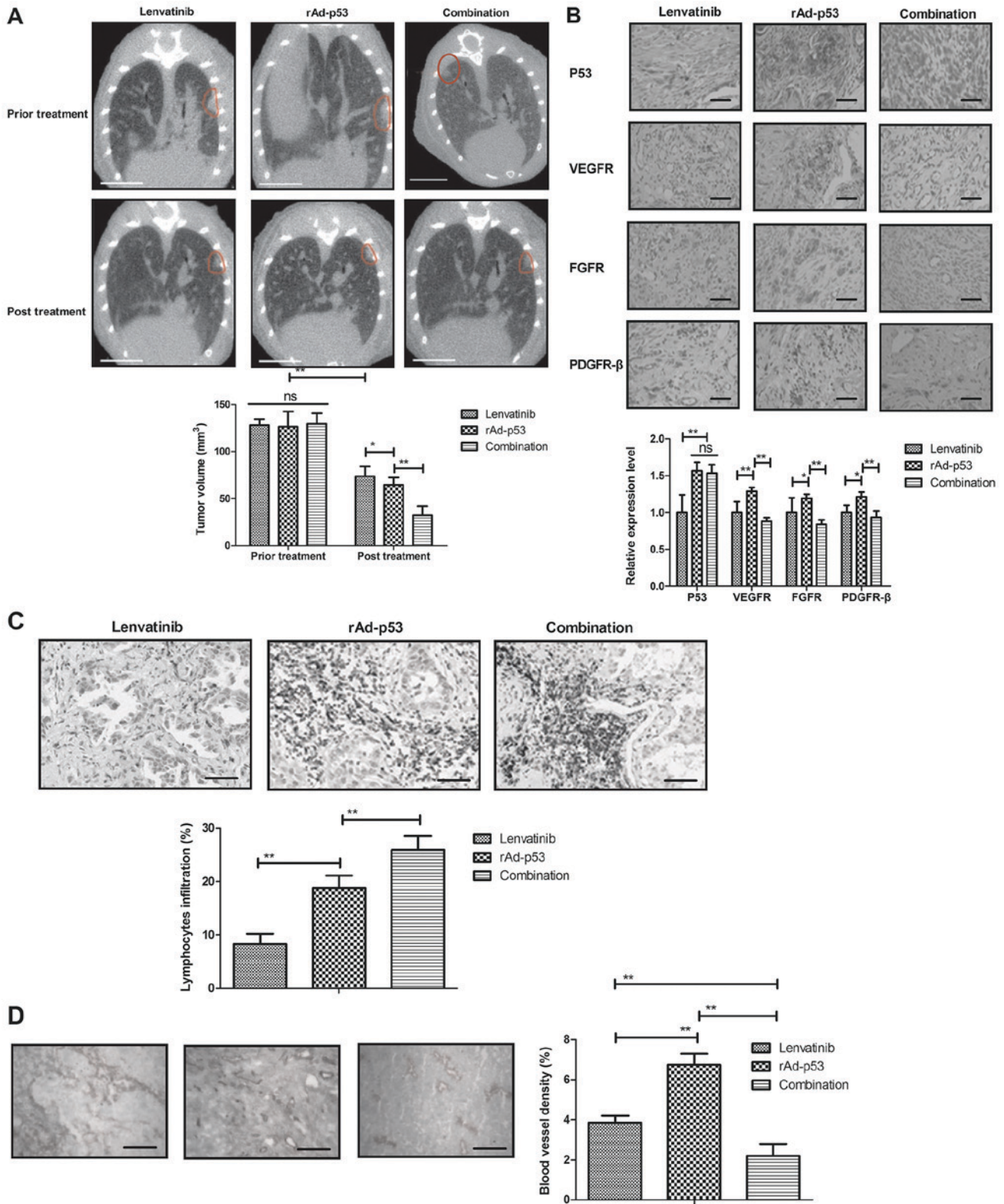
Figure 3. Anti-tumor activity of combined treatment of lenvatinib and rAd-p53 for NSCLC patients. (A) Effects of combined treatment of lenvatinib and/or rAd-p53 on tumor growth for NSCLC patients (Scale bar=5 mm). (B) Effects of combined treatment of lenvatinib and/or rAd-p53 on expression levels of p53, VEGFR, FGFR and PDGFR-β in NSCLC tissues and adjacent normal tissues (Scale bar=50 µm). (C) Effects of combined treatment of lenvatinib and/or rAd-p53 on lymphocytes infiltration in NSCLC tissue (Scale bar=100 µm). (D) Blood vessel density was lower in combined treatment of lenvatinib and rAd-p53group than rAd-p53 and lenvatinib groups. These results indicate that combined treatment of lenvatinib and rAd-p53 can efficiently inhibit NSCLC growth (Scale bar=50 µm). *P<0.05, **P<0.01. VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; PDGFR-β, platelet-derived growth factor receptor- β; NSCLC, non-small cell lung cancer; ns, not significant.

Agilent Technologies, Inc.), P53 (1:500 dilutions; ab32049), VEGFR (1:500 dilutions; ab36844), FGFR (1:500 dilutions; ab10646), PDGFR-β (1:500 dilutions, ab220745; all from Abcam) for 12 h at 4°C. The sections were washed three times

Table II. Treatment-related adverse event of Lenvatinib and/or rAd-p53 for NSCLC patients.

Adverse event	Total (n=120) (%)	Lenvatinib (n=40) (%)	rAd-p53 (n=40) (%)	Combination (n=40) (%)	P-value
Hypertension	16 (13)	4 (10)	5 (13)	7 (18)	0.035[a], 0.045[b]
Diarrhea	14 (12)	3 (8)	4 (10)	7 (18)	0.027[a], 0.035[b]
Nausea	8 (7)	1 (1)	2 (1)	5 (13)	0.002[a], 0.021[b]
Proteinuria	18 (15)	5 (13)	3 (8)	10 (25)	0.003[a], 0.006[b]
Body weight loss	15 (13)	5 (13)	3 (8)	7 (18)	
Stomatitis	3 (3)	0 (0)	0 (0)	3 (8)	<0.0001[a], <0.0001[b]
Vomiting	4 (4)	0 (0)	0 (0)	4 (10)	<0.0001[a], <0.0001[b]

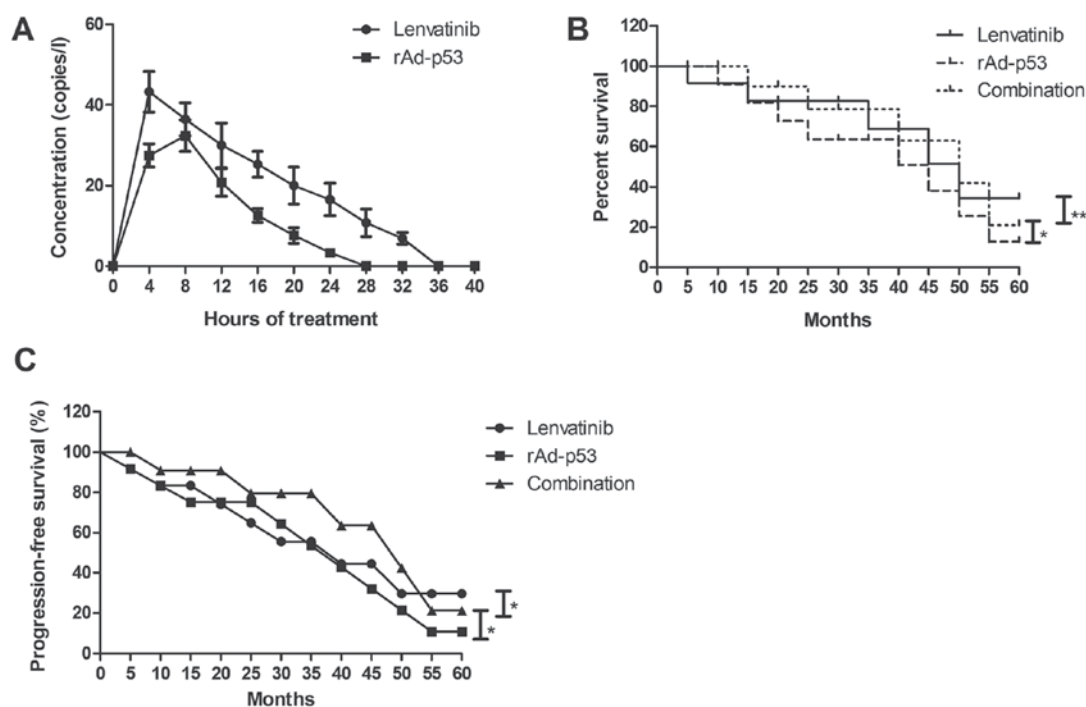Symbol, [a]Combination vs. Lenvatinib; [b]Combination vs. rAd-p53. NSCLC, non-small cell lung cancer.

Figure 4. PK profile of Lenvatinib and rAd-p53 for NSCLC patients. (A) Metabolism of Lenvatinib and rAd-p53 in blood in NSCLC patients. (B and C) Combined treatment of Lenvatinib and rAd-p53 improves survival rate (B) and PFS (C) compared to either Lenvatinib or rAd-p53 for NSCLC patients. *P<0.05, **P<0.01. Data were analyzed by one-way ANOVA followed by Tukey's test. PK, pharmacokinetic; NSCLC, non-small cell lung cancer.

with PBS for 3 min at room temperature and were incubated with HRP-labeled secondary goat anti-rabbit antibodies (1:2,000, ab150077; Abcam). Sections were visualized using ZEISS LSM 510 confocal microscope at x40 magnification.

Treatment administration. Lenvatinib (twice-daily, 32 mg/day) (21) and rAd-p53 (twice-daily, 1x10^{11} viral units/day) (25) were administered orally and intratumor injection, respectively. The treatments were continued for a 30-day administration schedule.

Evaluation of toxicity. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria (v3.0). Physical examination, full blood count, biochemical profile measurement of blood pressure and urinalysis were performed every two days during combined therapy. Electrocardiograms and biochemical detection were performed every three days (data not shown).

ELISA. Concentration levels of P53 and Lenvatinib were analyzed in patients with NSCLC by using commercialized human p53 ELISA kit (DYC1043-2; Bio-Rad Laboratories, Inc.) and human VEGFR ELASA kit (FAB357P) according to the manufacturer's instructions. Results were measured at 450 nm in an ELISA reader (Bio-Rad Laboratories, Inc.).

CT scan protocol. The CT diagnosis system was used to analyze tumor volume using preprogrammed setting in clinical trials. The preprogrammed setting was optimized to reach the best image formation. NSCLC patients were underwent CT according to instrument of the manufacture (Philips Medical Systems, Inc., Bothell, WA, USA). The details of principles

and settings of CT were described in previous study (26). Data of CT images were analyzed by computerized tomography system (Murphy-M2; Cook Medical, Bloomington, IN, USA).

Statistical methods. All data were reported as means and SEM and analyzed using SPSS Statistics v.19.0 (IBM Corp., Armonk, NY, USA). Statistical significance of differences between mean values was assessed by Student's t test for unpaired data. Comparisons of data between multiple groups were performed with analysis of variance (ANOVA) followed by Tukey's honest significant difference test. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 120 NSCLC patients were enrolled and the mean age of patients was 48.5 years old (average, 33.7-63.3). In the present study, the numbers of men (n=72) were more than women (n=48). None of the patients have received any anti-cancer treatments before this study. All characteristics of NSCLC patients were summarized in Table I.

Expression of p53, VEGFR, FGFR and PDGFR-β in NSCLC cells and tissues. We investigated expression of p53, VEGFR, FGFR and PDGFR-β in NSCLC cells and tissues. As shown in Fig. 1A and B, gene and protein expression levels of p53 were down-regulated and VEGFR, FGFR and PDGFR-β were up-regulated in NSCLC cells compared to adjacent normal cells. Immunohistochemistry demonstrated that p53 were decreased and VEGFR, FGFR and PDGFR-β were increased in NSCLC tissues compared to adjacent normal tissues (Fig. 1C). These results indicate that changes of p53, VEGFR, FGFR and PDGFR-β expression levels may be associated with NSCLC cells growth and metastasis.

Inhibitory effects of combined treatment of Lenvatinib and rAd-p53 for NSCLC cells. The inhibitory effects of combined treatment of Lenvatinib and rAd-p53 on NSCLC cells were analyzed *in vitro*. We showed that combined treatment of Lenvatinib and rAd-p53 significantly inhibited NSCLC cells growth compared to Lenvatinib and rAd-p53 (Fig. 2A). As shown in Fig. 2B and C, combined treatment of Lenvatinib and rAd-p53 inhibited migration and invasion of NSCLC cells compared either Lenvatinib or rAd-p53. We also demonstrated that combined treatment of Lenvatinib and rAd-p53 increased apoptosis of NSCLC cells induced by tunicamycin (Fig. 2D). These results indicate that combined treatment of Lenvatinib and rAd-p53 can inhibit NSCLC cells growth and aggressiveness.

Anti-tumor activity of combined treatment of Lenvatinib and rAd-p53 for NSCLC patients. The therapeutic effects of combined treatment of Lenvatinib and rAd-p53 were performed in NSCLC patients. We showed that combined treatment of Lenvatinib and rAd-p53 markedly inhibited tumor growth compared to Lenvatinib and rAd-p53 for NSCLC patients (Fig. 3A). Results found that expression levels of p53 were increased and VEGFR, FGFR and PDGFR-β were decreased in NSCLC tissues compared to adjacent normal tissues after combined treatment of Lenvatinib and rAd-p53 (Fig. 3B). Surgical removal of the NSCLC tumor tissues presented more lymphocytes infiltration in rAd-p53 and combined treatment of Lenvatinib and rAd-p53 groups than Lenvatinib group (Fig. 3C). Blood vessel density was lower in combined treatment of Lenvatinib and rAd-p53group than rAd-p53 and Lenvatinib groups (Fig. 3D). These results indicate that combined treatment of Lenvatinib and rAd-p53 can efficiently inhibit NSCLC growth.

Side effects and pharmacokinetic (PK) profile of Lenvatinib and rAd-p53 for NSCLC patients. The side effects and PK profile of Lenvatinib and rAd-p53 were investigated in NSCLC patients. We demonstrated that the most common treatment-related treatment-emergent adverse events were hypertension, diarrhea, nausea, proteinuria and body weight loss in NSCLC patients after treatment with Lenvatinib and/or rAd-p53 (Table II). Combined treatment of Lenvatinib and rAd-p53 had more side effects including stomatitis and vomiting. We observed that Lenvatinib and rAd-p53 could metabolize in blood after 24 h and 36 h, respectively, after received drugs and rAd-p53 (Fig. 4A). Notably, outcomes found that combined treatment of Lenvatinib and rAd-p53 improved survival rate of and progression-free survival (PFS) compared to either Lenvatinib or rAd-p53 for NSCLC patients (Fig. 4B and C). These results indicate that combined treatment of Lenvatinib and rAd-p53 could improve survival of NSCLC patients.

Discussion

Tumor gene therapy and target therapy has been widely used for human cancer treatment (27). Evidences have indicated that rAd-p53 is safe, well tolerated, and study has showed that rAd-p53 combined with platinum-based chemotherapy can be associated with a significant reduction of ovarian tumor (11). Study has found that Lenvatinib can improve thyroid cancer patients' survival in clinical trials (28). In the present study, we investigated the anti-tumor efficacy of combined treatment of Lenvatinib and rAd-p53 for NSCLC patients. We reported that combined treatment of Lenvatinib and rAd-p53 significantly inhibited NSCLC tumor growth and improved survival rate of NSCLC patients.

Treatment of rAd-p53 has become a leading candidate for clinical cancer treatment, such as renal cell carcinoma, hepatic carcinoma and melanomas (11,29,30). Study has found that expression of P53 is decreased and the potential chemo-preventive effect of pterostilbene dependents on p53-positive cells during early carcinogenesis (31). We confirmed previous outcomes (30) and we observed that rAd-p53 enhanced apoptosis of NSCLC cells. Promoting the infiltration and function of CD8(+) T cells could enhance the antigrowth effects of cisplatin on lung cancer, which provided new evidence for lung cancer therapy (32). We showed that rAd-p53 increased lymphocytic infiltration in NSCLC tissues compared to Lenvatinib. The combination of rAd-p53 and adriamycin increased the efficacy of chemotherapy for NSCLC patients, which overcome resistance of lung squamous cell cancer for chemotherapy (14). In the present study, we reported that rAd-p53 treatment inhibited tumor growth and decreased tumor volume for NSCLC patients.

Currently, target therapy for VEGF has presented anti-cancer efficacy for human cancers (33-35). We observed that VEGF is higher expressed in human NSCLC tissues and cells than normal lung tissue and cells. Lenvatinib is a multi-targeted tyrosine kinase inhibitor of multi receptors-mediated angiogenesis, which has been regarded as a potential drug for human cancer therapy (36). In this study, we observed that Lenvatinib inhibited NSCLC cells growth and aggressiveness *in vitro* and in NSCLC patients. Study has found that surgery combined with adenoviral p53 gene therapy showed efficacious effects in preventing recurrence or metastasis and improving progression free survival and overall survival after a radical surgery in patients with NSCLC in a phase II study (25). Our outcomes have indicated that Lenvatinib presented more efficient in inhibiting NSCLC growth than rAd-p53. In this study, we analyzed the therapeutic effects of combined treatment of Lenvatinib and rAd-p53 for NSCLC patients. Outcomes found that the most common treatment-related treatment-emergent adverse events were hypertension, diarrhea, nausea, proteinuria and body weight less in NSCLC patients after treatment with Lenvatinib and/or rAd-p53, which could metabolize after 48 h drug taken. Notably, combined treatment of Lenvatinib and rAd-p53 improved survival rate of and progression-free survival (PFS) compared to either Lenvatinib or rAd-p53 for NSCLC patients.

In conclusion, the present study indicates that combined treatment of Lenvatinib and rAd-p53 are well tolerated when administered to patients with NSCLC. Encouraging anti-tumor effects were observed for patients with NSCLC after combined treatment of Lenvatinib and rAd-p53. However, further clinical trials should be performed in large number NSCLC patients and in other cancer patients.

Acknowledgements

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Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

RY performed the experiments. HY designed the experiments. MW, XZ, ZS and AJ prepared the investigations and analyzed data.

Ethics approval and consent to participate

The protocols were approved by the Ethics Committee of Mudanjiang medical University affiliated HongQi Hospital (Mudanjiang, China). Informed consent was obtained from patients before any study-related procedures were performed.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Isobe H, Mori K, Minato K, Katsura H, Taniguchi K, Arunachalam A, Kothari S, Cao X and Kato T: Real-world practice patterns for patients with advancednon-small cell lung cancer: Multicenter retrospective cohort study in Japan. Lung Cancer (Auckl) 8: 191-206, 2017.
2. Prince RM, Atenafu EG and Krzyzanowska MK: Hospitalizations during systemic therapy for metastatic lung cancer: A systematic review of real world vs clinical trial outcomes. JAMA Oncol 1: 1333-1339, 2015.
3. Brody H: Lung cancer. Nature 513: S1, 2014.
4. Moro-Sibilot D, Smit E, de Castro Carpeño J, Lesniewski-Kmak K, Aerts JG, Villatoro R, Kraaij K, Nacerddine K, Dyachkova Y, Smith KT, *et al*: Non-small cell lung cancer patients with brain metastases treated with first-line platinum-doublet chemotherapy: Analysis from the European FRAME study. Lung Cancer 90: 427-432, 2015.
5. Barnett SA, Downey RJ, Zheng J, Plourde G, Shen R, Chaft J, Akhurst T, Park BJ and Rusch VW: Utility of routine PET imaging to predict response and survival after induction therapy for non-small cell lung cancer. Ann Thorac Surg 101: 1052-1059, 2016.
6. Cadranel J, Park K, Arrieta O, Pless M, Bendaly E, Patel D, Sasane M, Nosal A, Swallow E, Galebach P, *et al*: Characteristics, treatment patterns and survival among ALK+ non-small cell lung cancer (NSCLC) patients treated with crizotinib: A chart review study. Lung Cancer 98: 9-14, 2016.
7. Saadeddin A: Radiotherapy for NSCLC: Review of conventional and new treatment techniques. J Infect Public Health 5 (Suppl 1): S45-S49, 2012.
8. van Meerbeeck JP and Surmont VF: Stage IIIA-N2 NSCLC: A review of its treatment approaches and future developments. Lung Cancer 65: 257-267, 2009.
9. Bianco A, Tridico F, Rebecchi F, Contessa L, Fusca M, Calello G, Monticone C, De Rocca C, Panier Suffat L, Giaccone C and Panier Suffat P: Adrenal synchronous metastasis from non small cell lung carcinoma (NSCLC): Combined surgical treatment? Case report and review of the literature. Minerva chir 56: 535-537, 2001 (In Italian).
10. Li Y, Li LJ, Wang LJ, Zhang Z, Gao N, Liang CY, Huang YD and Han B: Selective intra-arterial infusion of rAd-p53 with chemotherapy for advanced oral cancer: a randomized clinical trial. BMC Med 12: 16, 2014.
11. Buller RE, Runnebaum IB, Karlan BY, Horowitz JA, Shahin M, Buekers T, Petrauskas S, Kreienberg R, Slamon D and Pegram M: A phase I/II trial of rAd/p53 (SCH 58500) gene replacement in recurrent ovarian cancer. Cancer Gene Ther 9: 553-566, 2002.
12. Liu K, Zhao J, Jiang H, Ma J, Tan J, Pei Y and Chen J: A patient with a large intrathoracic malignant schwannoma who showed a complete clinical response to rAd-p53-combined with radiotherapy. Anticancer Drugs 26: 902-906, 2015.
13. Chen GX, Zheng LH, Liu SY and He XH: rAd-p53 enhances the sensitivity of human gastric cancer cells to chemotherapy. World J Gastroenterol 17: 4289-4297, 2011.
14. Du CH, Wu Z and Xu J: The combination of recombinant rAd-p53 and adriamycin for management of primary drug resistance in chemotherapy of lung squamous cell cancer. Zhonghua Jie He He Hu Xi Za Zhi 29: 622-624, 2006 (In Chinese).
15. Guan YS, Liu Y, Zou Q, He Q, La Z, Yang L and Hu Y: Adenovirus-mediated wild-type p53 gene transfer in combination with bronchial arterial infusion for treatment of advanced non-small-cell lung cancer, one year follow-up. J Zhejiang Univ Sci B 10: 331-340, 2009.
16. Takasu S, Mutoh M, Takahashi M and Nakagama H: Lipoprotein lipase as a candidate target for cancer prevention/therapy. Biochem Res Int 2012: 398697, 2012.
17. Miyazono K: Ectodomain shedding of HB-EGF: A potential target for cancer therapy. J Biochem 151: 1-3, 2012.

18. Sebens S and Schafer H: The tumor stroma as mediator of drug resistance-a potential target to improve cancer therapy? Curr Pharm Biotechnol 13: 2259-2272, 2012.
19. O'Reilly A and Larkin J: Lenvatinib for use in combination with everolimus for the treatment of patients with advanced renal cell carcinoma following one prior anti-angiogenic therapy. Expert Rev Clin Pharmacol 10: 251-262, 2017.
20. Kuznar W: Lenvatinib extends survival in metastatic renal-cell carcinoma. Am Health Drug Benefits 8: 18, 2015.
21. Nishio M, Horai T, Horiike A, Nokihara H, Yamamoto N, Takahashi T, Murakami H, Yamamoto N, Koizumi F, Nishio K, et al: Phase 1 study of lenvatinib combined with carboplatin and paclitaxel in patients with non-small-cell lung cancer. Br J Cancer 109: 538-544, 2013.
22. Nagashima S, Matsuo S, Takahashi M, Umemoto Y, Hirano T, Enomoto K, Sakurai K and Amano S: Effectiveness of lenvatinib for thyroid cancer with lung Metastases -report of a case. Gan To Kagaku Ryoho 43: 2121-2123, 2016 (In Japanese).
23. Halim NHA, Zakaria N, Satar NA and Yahaya BH: Isolation and characterization of cancer stem cells of the non-small-cell lung cancer (A549) cell line. Methods Mol Biol 1516: 371-388, 2016.
24. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
25. Deng B, Sun T, Tang B, Tao S, Kang P, Qian K, Jiang B, Li K, Li K, Zhou J, et al: Surgery combined with adenoviral p53 gene therapy for treatment of non-small cell lung cancer: A phase II study. Oncotarget 8: 107089-107095, 2017.
26. Ghantous Y, Naddaf R, Barak M, Abd-Elraziq M and AbuEln-Naaj I: The role of fine needle aspiration in the diagnosis of parotid gland tumors: Correlation with preoperative computerized tomography tumor size. J Craniofac Surg 27: e192-e196, 2016.
27. Solbach C, Roller M, Peters S, Nicoletti M, Kaufmann M and Knecht R: Pituitary tumor-transforming gene (PTTG): A novel target for anti-tumor therapy. Anticancer Res 25: 121-125, 2005.
28. Mayor S: Lenvatinib improves survival in refractory thyroid cancer. Lancet Oncol 16: e110, 2015.
29. Xie Q, Liang BL, Wu YH, Zhang J, Chen MW, Liu HY, Gu XF and Xu J: Synergistic anticancer effect of rAd/P53 combined with 5-fluorouracil or iodized oil in the early therapeutic response of human colon cancer in vivo. Gene 499: 303-308, 2012.
30. Luo SH, Zheng CS, Feng GS, Sun XM, Zhou GF, Liang HM, Xia XW and Fang JL: Experimental studies of rAd-p53 injection by interventional approach for the treatment of rabbit VX2 liver cancer. Zhonghua Gan Zang Bing Za Zhi 18: 502-505, 2010 (In Chinese).
31. Lee H, Kim Y, Jeong JH, Ryu JH and Kim WY: ATM/CHK/p53 pathway dependent chemopreventive and therapeutic activity on lung cancer by pterostilbene. PLoS One 11: e0162335, 2016.
32. Li W, Yang Y, Ouyang Z, Zhang Q, Wang L, Tao F, Shu Y, Gu Y, Xu Q and Sun Y: Xiao-Ai-Ping, a TCM injection, enhances the antigrowth effects of cisplatin on lewis lung cancer cells through promoting the infiltration and function of CD8(+) T lymphocytes. Evid Based Complement Alternat Med 2013: 879512, 2013.
33. Jia J, Dellinger AE, Weiss ES, Bulusu A, Rushing C, Li H, Howard LA, Kaplan N, Pang H, Hurwitz HI and Nixon AB: Direct Evidence of Target Inhibition with Anti-VEGF, EGFR and mTOR Therapies in a Clinical Model of Wound Healing. Clin Cancer Res 21: 3442-3452, 2015.
34. Shibuya M: Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: A crucial target for anti- and pro-angiogenic therapies. Genes Cancer 2: 1097-1105, 2011.
35. Canavese M, Altruda F, Ruzicka T and Schauber J: Vascular endothelial growth factor (VEGF) in the pathogenesis of psoriasis-a possible target for novel therapies? J Dermatol Sci 58: 171-176, 2010.
36. Tohyama O, Matsui J, Kodama K, Hata-Sugi N, Kimura T, Okamoto K, Minoshima Y, Iwata M and Funahashi Y: Antitumor activity of lenvatinib (e7080): An angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. J Thyroid Res 2014: 638747, 2014.