Int J Clin Exp Pathol 2013;6(3):421-430
www.ijcep.com /ISSN:1936-2625/IJCEP1211041

Original Article

Therapeutic effect of 188Re-MAG3-depreotide on non-small cell lung cancer in vivo and in vitro

Fei Yu, Mingli Lv, Haidong Cai, Dong Li, Xueyu Yuan, Zhongwei Lv

Department of Nuclear Medicine, Tenth People's Hospital of Tongji University, Shanghai 200072, China

Received November 29, 2012; Accepted January 9, 2013; Epub February 15, 2013; Published March 1, 2013

Abstract: Objective: To investigate the in vivo and in vitro therapeutic effect of 188Re -MAG3-depreotide on non-small cell lung cancer (NSCLC). Methods: MTT was done to measure the cell proliferation; flow cytometry to detect cell apoptosis; Transwell invasion assay to determine the invasiveness of NSCLC. In addition, HE staining, TUNEL staining and immunohistochemistry for CD34 were employed to investigate the influence of 188Re -MAG3-depreotide on the growth of NSCLC. Results: 1) Within 2-6 days, the inhibitory effect of 188Re -MAG3-depreotide on the proliferation of A549 cells and SPC-A1 cells increased over time. 2) At 48 h after treatment with 188Re -MAG3-depreotide, the apoptosis rate of A549 cells and SPC-A1 cells was 23.1% and 22.6%, respectively. 3) After 188Re -MAG3-depreotide treatment, the number of invasive A549 cells and SPC-A1 cells was reduced by about 3 times when compared with control group. 4) The cancer in the control group presented with unlimited growth. The cancer growth continued after treatment with 188Re or MAG3-depreotide alone, while the cancer growth was markedly inhibited after 188Re -MAG3-depreotide treatment when compared with control group. Conclusion: 188Re-MAG3-depreotide can inhibit the proliferation and invasion of A549 cells and SPC-A1 cells. Treatment with 7.4MBq 188Re-MAG3-depreotide via tail vein can significantly suppress the in vivo cancer growth and induce the apoptosis of cancer cells. These findings demonstrate that 188Re-MAG3-depreotide can induce the apoptosis of NSCLC cells and directly kill the NSCLC cells, which provide evidence for the radiotherapy of NSCLC.

Keywords: 188Re, depreotide, non-small cell lung cancer, in vitro killing effect, cancer bearing nude mice

Introduction

In a majority of patients, NSCLC is usually diagnosed at advanced stage. Under this condition, surgical intervention is usually infeasible. There is evidence showing that numerous patients with NSCLC can not be treated with surgery at first hospital visit, the surgical intervention is feasible in 20% of NSCLC patients and the incidence of post-operative recurrence and metastasis is higher than 50% [1]. Platinum based chemotherapy as first-line treatment has limited efficacy in NSCLC patients, has poor specificity and may prolong the survival time of NSCLC patients to a limited extent. In addition, few second-line chemotherapeutics have been approved by the Federal Drug Administration. The platinum based chemotherapy has been regarded as standardized chemotherapy, but the therapeutic efficacy is as low as 30%, and patients receiving chemotherapy finally fail to respond to treatment [2]. Although the chemotherapy and radiotherapy are performed simultaneously, the long-term survival is still poor [3]. Implantation of radioactive particles based comprehensive therapy is only palliative not curative strategy, and usually has complications such as pneumothorax, hemorrhage, pain, low fever and radiation injury (acute radiation pneumonitis and radiation pulmonary fibrosis) [4]. Thus, it is imperative to develop new strategies targeting the abnormal signaling pathways in cancer cells which can reduce the side effects and increase the therapeutic efficacy.

In recent years, great progress has been achieved in the targeted therapy with radionuclide labeled molecules which include monoclonal antibody, polypeptide (receptor and ligand) and others (such as AS-ODN). The most common ligands in the receptor targeted therapy

Table 1. Inhibition rate of A549 cells and SPC-A1 cells at different time points (%)

	2d	3d	4d	5d	6d
A549	13.62±4.75	23.48±7.13	31.05±6.43	42.17±7.88	50.32±9.20
SPC-A1	15.29±4.87	27.26±5.18	39.03±8.65	50.01±6.39	59.44±7.92

are somatostatin analogs. Somatostatin (SST) is a group of cyclic peptide hormones secreted by D cells. The somatostatin receptor (SSTR) has been regarded as one of markers for genotype of cancers to which increasing attention has been paid. The bioeffects of SST are mediated by the SSTR on cell membrane. Studies have confirmed that lung cancer of all types has high expression of SSTR, and the expression of SSTR and its affinity to SST are markedly increased when compared with normal tissues. In addition, semi-quantitative analysis showed the SSTR expression in NSCLC was higher than that in SCLC ($P<0.01$). Thus, SSTR can be used as a target for the diagnosis and treatment of NSCLC. The endogenous SST is instable and susceptible to degradation. In recent years, investigators have developed a series of somatostatin analogs (SSTA) by altering the non-essential amino acids in SST. These SSTAs have characteristics similar to SST but are not susceptible to degradation and easily to be labeled with radionuclides. Depreotide, a new SSTA, is a disulfide-bond-free cyclic SSRA which avoids the fracture of disulfide bonds due to reduction during the labeling. Thus, depreotide has been a hot topic in researches.

Recently, increasing studies have been conducted to investigate the anti-tumor effect of radionuclide labeled SSTA, for which the rationales include: 1) SSTA can bind to specific SSTR to exert direct anti-tumor effect, inhibit the production of hormones and cytokines which are essential for the cancer growth or angiogenesis in cancers or induce the apoptosis of cancer cells exerting anti-tumor effect; 2) the cytotoxic radionuclides may enter cells via the binding between SSTA and SSTR, and the α ray, β ray, Auger electron and internal-conversion electron produced during the disintegration of radionuclides may exert direct killing effect on cancer cells. Thus, radionuclide mediated therapy has been a novel and promising strategy for the treatment of cancers.

A variety of investigators have investigated the expression of SST of different subtypes in different cancers with different methods [5-8]. Although there is limitation in the methodology of these studies and the findings are inconsistent, several consensuses have been achieved from these studies: one cancer may express SST of different types; different cancers also present difference in the type of expressed SST. In 2002, Rogers et al [9] for the first time confirmed in the NSCLC mice that receptor targeted radionuclide therapy with polypeptides was effective for NSCLC. NSCLC has high expression of SSTR which has high affinity to SST and SSTA. This provides evidence for the application of SST and SSA in the diagnosis and treatment of cancers and presents reference for the SSR targeted therapy of NSCLC. Some investigators investigated the binding of RC-160 (a conjugate of SSA and 131I) to the specific ligand, and results revealed that SSA could bind to the SSTR on cancer cells with a high affinity. In addition, the conjugate of SSA and DOX (AN-238) has been applied in the treatment of cancer-bearing mice achieving favorable outcome.

In the present study, the anti-tumor effect of 188Re-MAG3-depreotide on NSCLC was investigated in vitro and in vivo. Our findings may provide evidence for the receptor mediated therapy of NSCLC.

Materials and methods

Main reagents and manufacturers

188Re was generated by the 188W/188Re generator (Shanghai Kexing Pharmaceutical company). RPMI 1640 was purchased from Hyelone (USA). Matrigel was purchased from the Vigorous Biotech (Beijing) Co., Ltd. 24-well Transwell was purchased from the Corning (USA). DAB Peroxidase Substrate Kit was purchased from the Boster Biotech. Xinhua I chromatography paper was from Xi'an Reagent Company. MTT solution was from Sigma (USA). DMSO was from Duchefa Biochemie (Netherland). 25-cm2 flasks and 6-well plates were from Corning (USA).

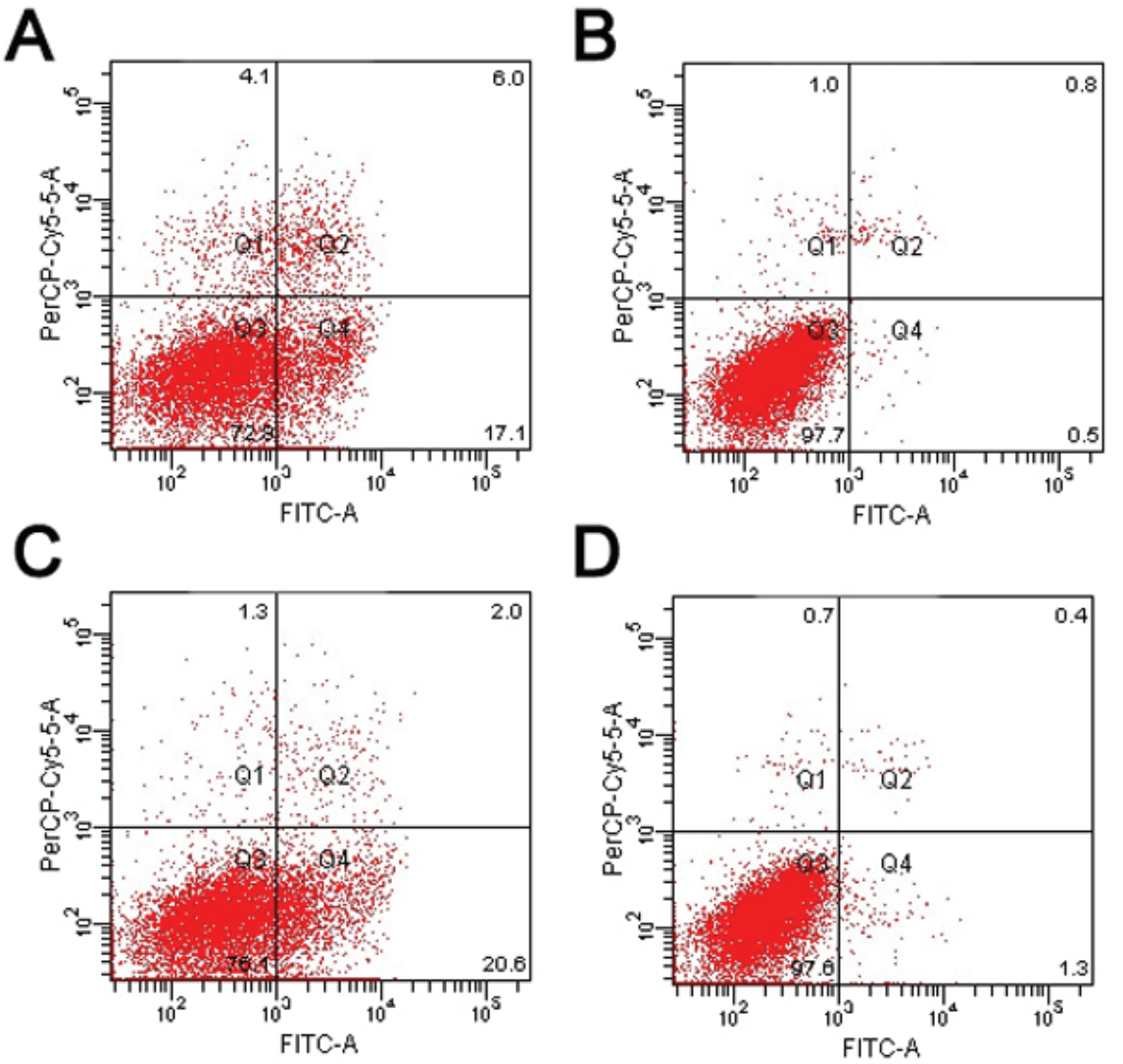

Figure 1. Detection of apoptosis with Annexinv-FITC/PI by flow cytometry. A. A549 cells in control group; B. A549 cells after 188Re-MAG3-depreotide treatment; C. SPC-A1 cells in control group; D. SPC-A1 cells after 188Re-MAG3-depreotide treatment.

Cell lines and animals

Human lung cancer cells (A549 cells and SPC-A1 cells) were kindly provided by the Institute of Biochemistry and Cell Biology in Research Institute of Life Science of Chinese Academy of Sciences. Nude mice (n=20) aged 3-4 weeks and weighing 20-25 g were purchased from the Shanghai Experimental Center and used for preparation of NSCLC animal model.

In vitro killing effect of 188Re- MAG3-depreotide on NSCLC cells

Labeling of MAG3-depreotide with 188Re, labeling rate and radiochemical purity: MAG3-depreotide was dissolved in ammonium acetate solution followed by addition of tartrate solution and stannous chloride solution. Finally, 200 μL of 740 MBq 188Re04- eluant was added followed by incubation at room temperature for 15-30 min. The labeling rate was measured. The labeled products were subjected to purification with Sephadex G25 column and collection was done step by step. The eluting peak was determined and the radiochemical purity measured.

Culture of A549 cells and SPC-A1 cells: A549 cells were digested with 0.25% trypsin and 0.02% EDTA followed by passaging. One week later, cells were harvested for experiment. The

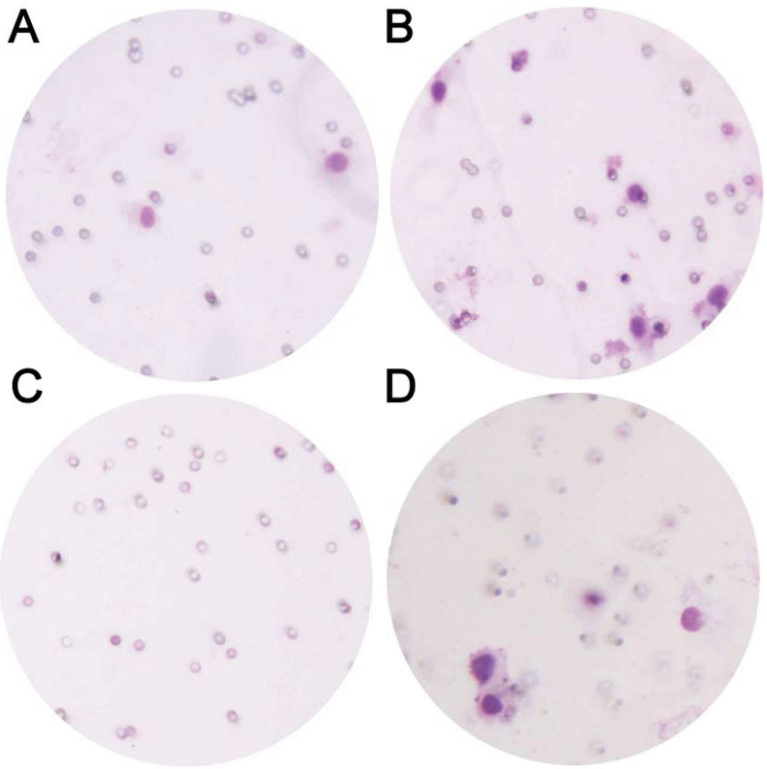

Figure 2. Invasiveness of A549 cells and SPC-A1 cells. A. A549 cells in control group; B. A549 cells after 188Re-MAG3-depreotide treatment; C. SPC-A1 cells in control group; D. SPC-A1 cells after 188Re-MAG3-depreotide treatment.

SPC-A1 cells in logarithmic growth phase were maintained in complete RPMI-1640 containing 15% fetal bovine serum at 37°C in an environment with 5% CO2. When the cell confluence reached 80-90%, the medium was removed and cells were washed in PBS twice. After digestion in 0.25% trypsin, single cell suspension was prepared and cells were the seeded into 48-well plates (2×105/well). One day later, monolayer cells were washed in PBS for used following removal of medium.

Killing effect of 188Re-MAG3-depreotide on A549 cells and SPC-A1 cells: The A549 cells and SPC-A1 cells in logarithmic growth phase were harvested and then maintained in 200 μl of RPMI-1640 or 100 μl of 37MBq/L 188Re-MAG3-depreotide (final concentration: 12.3MBq/L) for 60 min at 37°C. Eight wells were included in each well. From day 2 to day 6, MTT assay was performed to detect the cell growth. In brief, 5 mg/ml MTT solution (20 μl) was added to each well followed by incubation for 4 h. Then, the medium was removed and 150 μl of DMEM was added followed by incubation under continuous shaking. The absorbance (A) was measured at 570 nm. The cell growth inhibition rate (IR) was calculated as follow: IR = [(Acontrol – Aexperiment)/ Acontrol] ×100%

Detection of apoptosis by flow cytometry: A549 cells and SPC-A1 cells were maintained in complete medium and digested with 0.25% trypsin. The cell density was adjusted to 5×105cells/mL with complete RPMI 1640 and cells were seeded into flasks. On the second day, 100 μl of 37MBq/L 188Re-MAG3-depreotide (final concentration: 12.3 MBq/L) was added. In the blank control group, 188Re-MAG3-depreotide was not added. Twelve hours later, the medium was removed and cells were washed twice with complete medium. Then, cells were maintained in complete medium for 48 h. Following digestion with 0.25% trypsin, single cell suspension was prepared and cells were washed in PBS thrice. After centrifugation, cells were re-suspended in 200 μL of binding buffer. For detection of apoptosis, cells were incubated with 10 μL of Annexin V-FITC in dark at room temperature for 30 min and then with 5 μL of PI at room temperature for 5 min. After addition of 400 μL of binding buffer, cells were subjected to flow cytometry.

Transwell invasion assay: The transwell was placed in 24-well plate. Then, 50 μl of above solution was added to the upper chamber. The 188Re-MAG3-depreotide treated cells were washed in PBS at 4°C twice to remove 188Re-MAG3-depreotide. Then, these 188Re-MAG3-depreotide (37 MBq/L) treated A549 cells and SPC-A1 cells and cells in the control group were re-suspended in serum free DMEM for preparation of single cell suspension. The cell density was 1×106/ml. The upper chamber was obtained and non-invasive cells were removed with a swab. The invasive cells

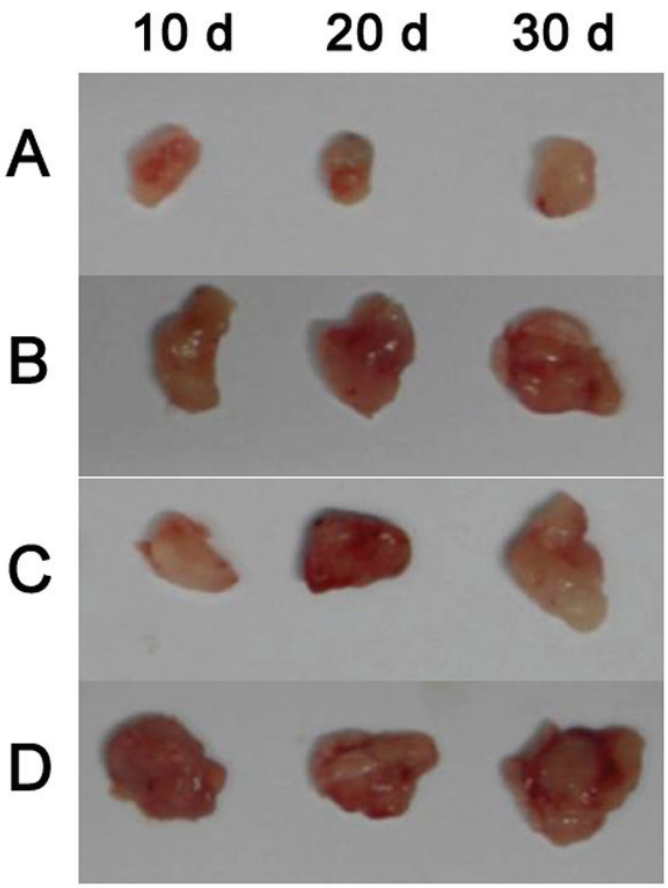

Figure 3. Cancer growth in nude mice after different treatments. A: 188Re-MAG3-depreotide group; B: depreotide group; C: 188Re group; D: Normal saline group.

were then fixed in formaldehyde for 30 min followed by HE staining. Observation was done under a light microscope at a magnification of 200. Five fields were randomly selected and the invasive cells were counted followed by averaging.

In vivo anti-tumor effect of 188Re-MAG3-depreotide on NSCLC in nude mice

Inhibitory effect of 188Re-MAG3-depreotide on cancer growth in nude mice: The NSCLC bearing mice were prepared. When the cancer tumor was about 0.5 cm3, 20 nude mice were randomly assigned into 4 groups (n=5 per group): 7.4 MBq 188Re-MAG3-depreotide group, 10 μg of depreotide group, 7.4 MBq 188Re group and normal saline group. Depreotide, 188Re and 188Re-MAG3-depreotide were injected via tail vein. The tumor size was measured before and once every 10 days after treatment. The tumor volume was calculated as follow: $V = \pi/6$ (d1 × d2 × d3), where d1, d2 and d3 represent the length, width and height, respectively. During the study, the growth and body weight of nude mice were closely monitored. When the body weight was reduced by 10%, the cancer developed evident necrosis (tumor volume was no larger than 0.5 cm3), or the tumor volume was larger than 20 cm3, mice were sacrificed.

H&E staining: The cancers were obtained and fixed in 10% formaldehyde followed by preparation of 0.3-μm paraffin-embedded sections and subsequent HE staining.

TUNEL staining: The above paraffin-embedded sections were processed for TUNEL staining, and the apoptotic cells were observed.

Immunohistochemsitry for CD34: Sections were incubated with streptavidin – peroxidase at room temperature for 10 min and visualization was done with DAB. Counterstaining was performed with hematoxylin followed by mounting with neutral gum. The isolated or cluster-like structure which was formed by endothelial cells and cord-like or space-like and had lumen. This structure with less than 8 red blood cells in the lumen was regarded as a blood vessel, and the separated endothelial cells were determined as a blood vessel. The structure with more than 8 red blood cells or smooth muscle in wall was not counted.

Detection of toxicity and pyrogenicity of 188Re-MAG3-depreotide: The labeled solution was filtered via a sterilized 0.22-μm filter (Millex-GP, Millipore, USA) and the aerobic bacteria, anaerobic bacteria and mildews were detected. The pyrogenicity measurement was done with limulus test according to the instructions in Pharmacopoeia of People's Republic of China.

Statistical analysis

Data were expressed as mean ± standard deviation. Statistical analysis was done with SPSS

version 13.0. One way analysis of variance was employed for comparisons of means among groups. A value of P<0.05 was considered statistically significant.

Results

In vitro anti-tumor effect of 188Re-MAG3-depreotide on NSCLC cells

Labeling rate and radiochemical purity of 188Re-MAG3-depreotide: In the developing agent I, the colloid and marker were in the original point in the normal saline group, but the free 188Re was ahead of the developing agent I. In 85% acidified ethanol (pH=3.5), the colloid was in the original point but the free 188Re and marker were ahead of the developing agent. Paper chromatography showed the labeling rate of 188Re MAG3-depreotide was higher than 80%, the specific activity was 200 GBq/mmol and radiochemical purity was as high as 90%. These suggest that the 188Re MAG3-depreotide meets the requirement for experiment.

Detection of cell proliferation by MTT assay after 188Re-MAG3-depreotide treatment: The inhibition rate of cell growth within 2-6 days is shown in **Table 1**. Results showed the inhibition rate increased over time and reached a maximal level on day 6 (50-60%). In addition, the influence of 188Re-MAG3-depreotide on the proliferation was similar between A549 cells and SPC-A1 cells (P>0.05). This finding suggests that 188Re-MAG3-depreotide can significantly inhibit the in vitro proliferation of NSCLC cells.

Detection of apoptosis by flow cytometry: After treatment with 188Re-MAG3-depreotide for 48 h, A549 cells and SPC-A1 cells (5×105-106 cells) were collected and double staining was done with Annexin V/PI according to the manufacturer's instructions. The apoptotic cells were measured by flow cytometry (**Figure 1**). Results showed the normal cells could be differentiated from the necrotic cells and apoptotic cells which were represented by different quadrants: Q1: AnnexinV-FITC$^-$ and PI+ cells (mechanically injured cells); Q2: AmiexinV-FITC+ and PI$^-$ cells (early apoptotic cells); Q3: AnnexinV-FITC$^-$ and PI$^-$ cells (viable cells); Q4: AnnexinV-FITC+ and PI+ cells (late apoptotic cells or necrotic cells). Results showed treatment with 188Re-MAG3-depreotide for 48 h could significantly increase the apoptosis of A549 cells and SPC-A1 cells (apoptosis rate: 23.1% and 22.6%, respectively).

Detection of invasiveness by transwell assay: After treatment with 188Re-MAG3-depreotide, the invasiveness of A549 cells and SPC-A1 cells was measured. Results showed the 188Re-MAG3-depreotide markedly reduced the invasiveness of both cell lines and the number of invasive cells was reduced by 3 times when compared with the control group (P<0.05) (**Figure 2**).

In vivo anti-tumor effect of 188Re-MAG3-depreotide in mice with NSCLC

Inhibitory effect on cancer growth: In the normal saline group, the growth of cancer was unlimited. After treatment with 188Re or depreotide, continuous growth of cancer was still observed. No marked difference was observed in the cancer growth among normal saline group, 188Re group and depreotide group (P>0.05). However, 188Re-MAG3-depreotide significantly inhibit the cancer growth in nude mice when compared with above three groups (P<0.05). Thirty days after treatment, the tumor volume was 0.828±0.058 cm3 in 188Re-MAG3-depreotide group, 1.831±0.178 cm3 in depreotide group, 1.432±0.281 cm3 in 188Re group and 1.921±0.260 cm3 in normal saline group (**Figure 3**).

Histological examination by H&E staining: H&E staining showed the necrotic area in the 188Re-MAG3-depreotide group was the largest and massive patchy necrosis was observed. In the 188Re, only spotty necrosis was found. However, after treatment with depreotide or normal saline, no evident necrosis was found (**Figure 4**). The apoptotic cells were round and had darkly stained nucleus and condensed cytoplasm, the chromosomes were mass-like and "sprouting" phenomenon was noted on the cells.

TUNEL staining: TUNEL staining was performed to investigate the apoptotic cells. Results showed a large number of apoptotic cells were found in the 188Re-MAG3-depreotide group. After treatment with 188Re, only a few apoptotic were presented. In the depreotide group and normal saline group, no obvious apoptotic cells were observed (**Figure 5**).

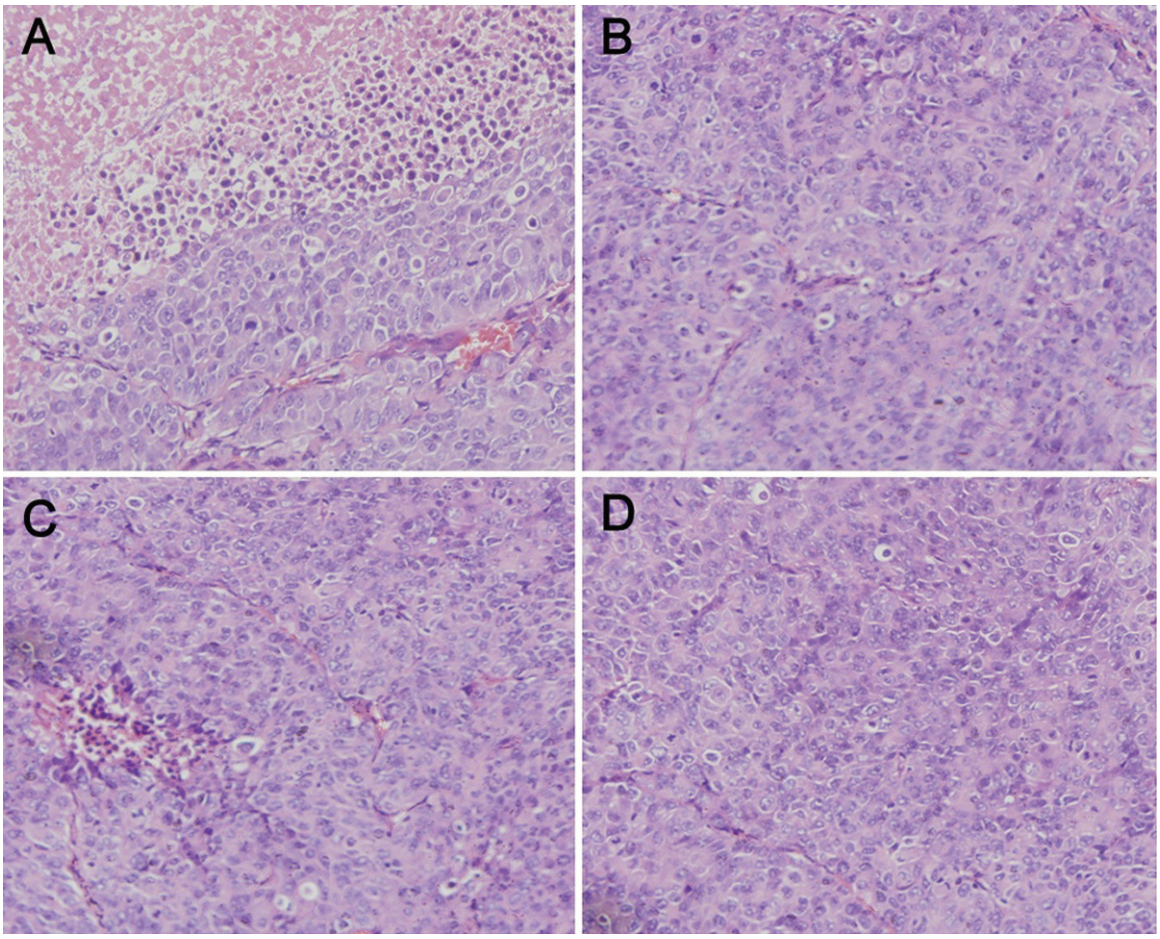

Figure 4. HE staining of cancers of nude mice in different groups (200×). A: 188Re-MAG3-depreotide group; B: depreotide group; C: 188Re group; D: Normal saline group.

Immunohistochemistry for CD34: CD34 is a transmembrane glycoprotein on cells and can mediate the adherence and promote the aggregation of endothelial progenitor cells to induce angiogenesis. The expression of CD34 can be used to evaluate the extent of angiogenesis which reflects the biological behaviors of cancers and is helpful for determination of risk for recurrence and prognosis. As shown in **Figure 6**, the CD34 positive cells were the vascular endothelial cells which were yellow-brown or brown. In the 188Re group, depreotide group and normal saline group, the number of newly generated blood vessels increased markedly and the invasiveness of cancer cells was obvious. After treatment with 188Re-MAG3-depreotide, the newly generated blood vessels significantly reduced when compared with the other three groups, suggesting that 188Re-MAG3-depreotide effectively inhibits the invasion and metastasis of cancer cells.

Discussion

188Re and 99mTc are two elements in group VII and have similar chemical and physical features. Thus, they have possibility to be used to label drugs and present with wide application. The 188Re labeled specific molecules can be used for internal radiation therapy of cancers, which may elevate the targeting of anti-tumor therapy. 188Re is an ideal radionuclide for therapeutic purposes. Its half-life is 16.7 h and the maximal energy of beta ray emitted by 188Re is 2.12MeV. The beta ray emitted by 188Re has moderate energy and has short distance of internal radiation (maximum: 11 mm), and 95% beta ray is absorbed within 4 mm. Thus, the beta ray emitted by 188Re causes little injury to surrounding tissues and applicable in internal radiation therapy. In addition, 188Re can emit γ ray with energy of 0.155 MeV for imaging which can be used in studies on the biological

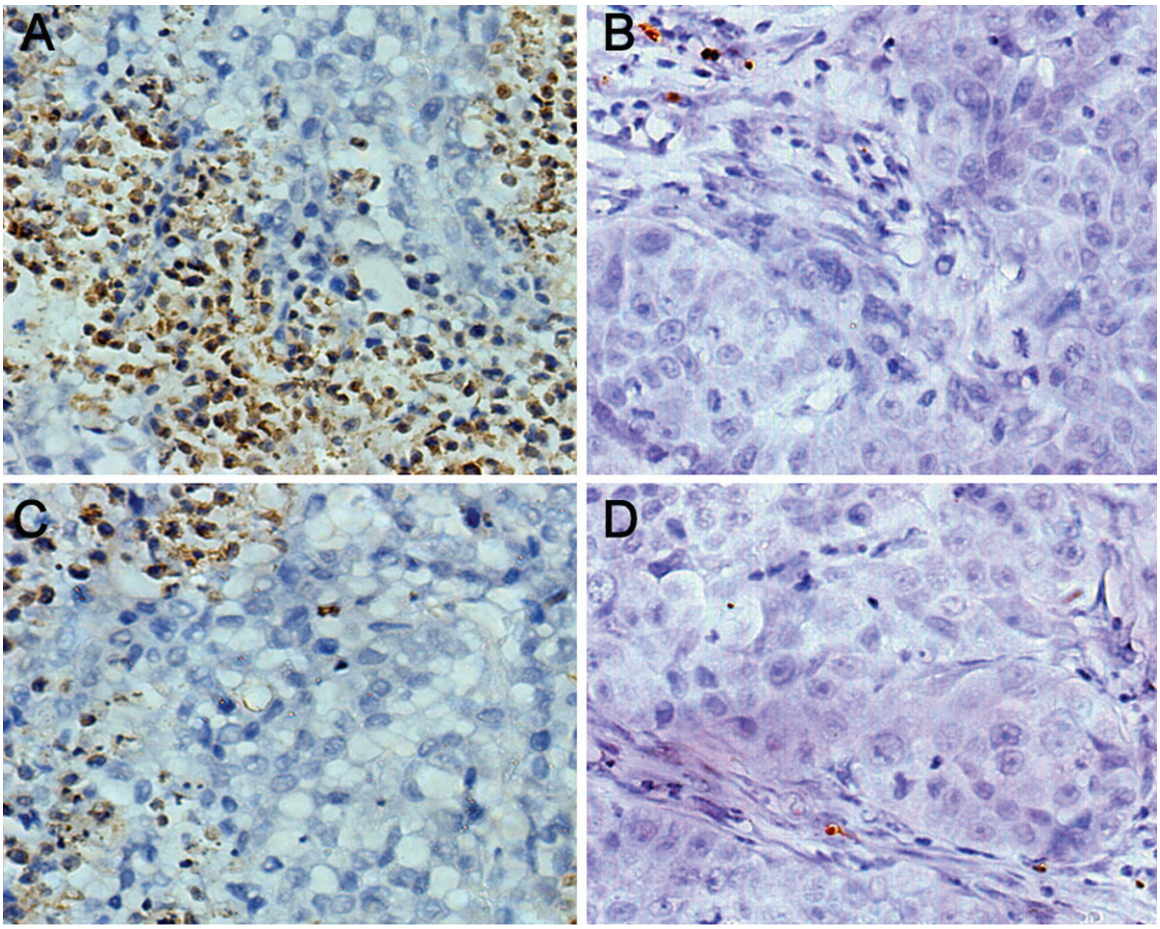

Figure 5. TUNEL staining of cancers of nude mice in different groups (200×). A: 188Re-MAG3-depreotide group; B: depreotide group; C: 188Re group; D: Normal saline group.

distribution, radiation dose and pharmacokinetics of radionuclide. The beta ray emitted by 188Re has potent ionizing radiation, high surface dose and evident gradient dose. Thus, beta ray emitted by 188Re can directly cause damage to biomacromolecules and induce apoptosis. The damage to cancer cells may compromise the proliferation, disturb the metabolism and induce aging or death in cancer cells. In addition, the beta ray induced ionizing radiation may produce some reactive oxygen species which indirectly promote the apoptosis exerting therapeutic efficacy. On the basis of above physical and biological characteristics of 188Re, 188Re and 188Re labeled molecules have been widely applied in the internal radiation therapy of cancers [10].

It has been found that 188Re can induce the apoptosis of breast cancer ER-75-30 cells and prostate PC-3 cells which are demonstrated by morphology and biochemical examination [11]. Bian et al [12] investigate the effect of internal radiation therapy with 188Re on the cell cycle and apoptosis of liver cancer ells. Results revealed that the dead cells after 188Re treatment presented with typical features of apoptosis. In animal studies, intratumor or intrapleural injection of 7.4MBq 188Re labeled SSTA (RC-160) was performed to treat cancers achieving favorable outcome [13]. In another study, 11~33 MBq 188Re labeled SSTA (188Re-P2045) was injected via vein and the cancer growth was found to be markedly inhibited [14]. These findings demonstrate that 188Re has the ability to induce the apoptosis of cancer cells and directly kill the cancer cells, which provides evidence for the clinical application of 188Re.

There are two key points in the SSTA receptor mediated radionuclide therapy: 1) synthesis of SSTA coupled to bifunctional chelating agent; 2) labeling with radionuclide. Solid phase syn-

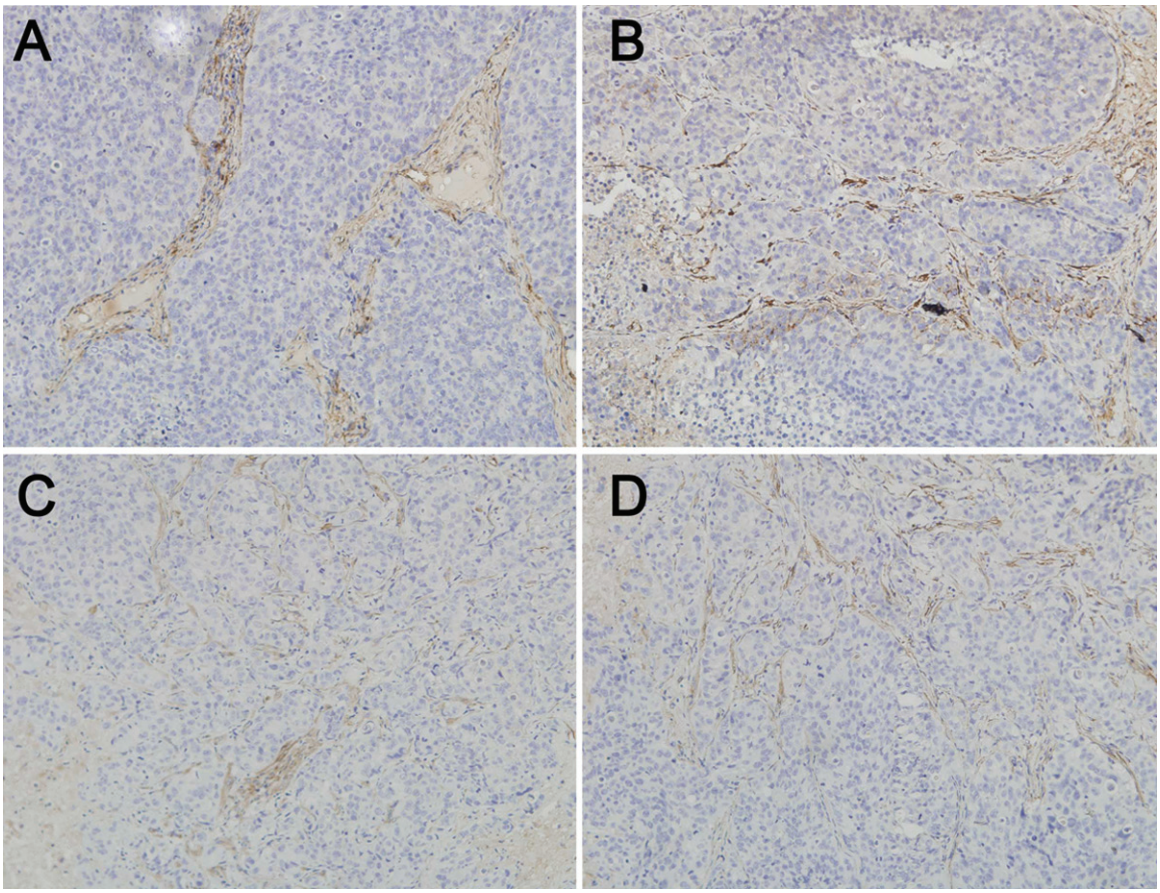

Figure 6. Immunohistochemistry for CD34 in cancers of nude mice in different groups (200×). A: 188Re-MAG3-depreotide group; B: depreotide group; C: 188Re group; D: Normal saline group.

thesis method and liquid phase segment connecting method were used to acquire depreotide with high purity, which is a basis for the synthesis of peptide. In respect of labeling with radionuclide, there are direct and indirect methods. In previous studies, VIIB element 99mTc was used in the direct and indirect labeling. Results showed the labeling with direct method was relatively violent and may cause damage to polypeptide. Moreover, the bonding is not potent and susceptible to shedding, and the labeling rate is at a low level. With the indirect method, bifunctional chelating agent is used to couple the radionuclide to the target, which may be performed in general condition and not alter the characteristic of the target. In addition, the bonding is stable and the damage to the target is avoided. Studies have compared three bifunctional chelating agents (HYNIC, NHS-MAG3 and SHNH) for labeling with 99mTc. Results showed the NHS-MAG3 labeled target was more stable in the serum and plasma at room temperature. In vitro study was conducted to investigate the clearance of 99mTc-depreotide in A549 cells as target cells. Results revealed that 99mTc-depreotide was rapidly removed in A549 cells and had specific internalization. Both 188Re and 99mTc are elements in the group VIIB, and have similar chemical and physical characteristics.

Thus, 188Re-MAG3-depreotide was used to treat the NSCLC cells and NSCLC in nude mice. Results showed 188Re-MAG3-depreotide could inhibit the proliferation and invasion of A549 cells and SPC-A1 cells, two NSCLC cell lines. After injection of 7.4 MBq 188Re-MAG3-depreotide via the tail vein, the cancer growth was markedly inhibited, and immunohistochemistry showed a large number of apoptotic cells. These findings demonstrate that 188Re-MAG3-depreotide can induce the apoptosis of NSCLC cells and directly kill the cancer cells, which provide evidence for the radiotherapy of NSCLC.

Conclusion

The labeling rate and radiochemical purity of 188Re-MAG3-depreotide was 80% and 90%, respectively, which meets the requirement for experiment. Our results reveal that 188Re-MAG3-depreotide can significantly inhibit the in vitro proliferation of A549 cells and SPC-A1 cells (50-60% on day 6). The inhibition rate of A549 cells and SPC-A1 cells is comparable. Flow cytometry indicate that 188Re-MAG3-depreotide treatment for 48 h markedly increases the apoptosis of A549 cells and SPC-A1 cells (apoptosis rate: 23.1% and 22.6%, respectively). Transwell invasion assay show the 188Re-MAG3-depreotide can dramatically inhibit the invasiveness of both cell lines. In NSCLC bearing nude mice, results demonstrate that 7.4 MBq 188Re-MAG3-depreotide can effectively inhibit the cancer growth. HE staining and TUNEL staining indicate massive apoptosis of cancer cells after 188Re-MAG3-depreotide treatment, and immunohistochemistry for CD34 show the suppression of angiogenesis following 188Re-MAG3-depreotide treatment. The inhibitory effects on the proliferation and invasion of NSCLC and promotive effect on the apoptosis demonstrate the in vitro and in vivo anti-tumor effects of 188Re-MAG3-depreotide.

Address correspondence to: Dr. Zhongwei Lv, Department of Nuclear Medicine, Tenth People's Hospital of Tongji University, Shanghai 200072, China. Phone: +8621-66301051; E-mail: yufei@tongji.edu.cn; doczhong@yeah.net

References

[1] Milleron B. [Editorial. NSCLC and personalized medicine: yesterday, today and tomorrow]. Rev Pneumol Clin 2011; 67 Suppl 1: S1-2.

[2] Pallis AG and Georgoulias V. Is there a standard regimen for first-line treatment of advanced/metastatic Non-Small-Cell Lung Cancer? What has meta-analyses contributed to today's standard of care. Lung Cancer 2012; 75: 269-274.

[3] O'Rourke N, Roque IFM, Farre Bernado N and Macbeth F. Concurrent chemoradiotherapy in non-small cell lung cancer. Cochrane Database Syst Rev 2010; CD002140.

[4] Zhang S, Zheng Y, Yu P, Yu F, Zhang Q, Lv Y, Xie X and Gao Y. The combined treatment of CT-guided percutaneous 125I seed implantation and chemotherapy for non-small-cell lung cancer. J Cancer Res Clin Oncol 2011; 137: 1813-1822.

[5] Forssell-Aronsson EB, Nilsson O, Bejegard SA, Kolby L, Bernhardt P, Molne J, Hashemi SH, Wangberg B, Tisell LE and Ahlman H. 111In-DTPA-D-Phe1-octreotide binding and somatostatin receptor subtypes in thyroid tumors. J Nucl Med 2000; 41: 636-642.

[6] Reubi JC, Schaer JC, Waser B and Mengod G. Expression and localization of somatostatin receptor SSTR1, SSTR2, and SSTR3 messenger RNAs in primary human tumors using in situ hybridization. Cancer Res 1994; 54: 3455-3459.

[7] Buscail L, Saint-Laurent N, Chastre E, Vaillant JC, Gespach C, Capella G, Kalthoff H, Lluis F, Vaysse N and Susini C. Loss of sst2 somatostatin receptor gene expression in human pancreatic and colorectal cancer. Cancer Res 1996; 56: 1823-1827.

[8] Reubi JC, Kappeler A, Waser B, Laissue J, Hipkin RW and Schonbrunn A. Immunohistochemical localization of somatostatin receptors sst2A in human tumors. Am J Pathol 1998; 153: 233-245.

[9] Rogers BE, Zinn KR, Lin CY, Chaudhuri TR and Buchsbaum DJ. Targeted radiotherapy with [(90)Y]-SMT 487 in mice bearing human nonsmall cell lung tumor xenografts induced to express human somatostatin receptor subtype 2 with an adenoviral vector. Cancer 2002; 94: 1298-1305.

[10] Wang XQ, Liu XD, Dong F and Zhang XW. Application of 188Re intervention in the treatment of tumor. J Lanzhou Univ (Med Sci) 2008; 34: 79-82.

[11] Sun X. Application of 188Re in tumor treatment. Foreign Medical Sciences (Section of Radiation Medicine and Nuclear Medicine) 2003; 27: 151-153.

[12] Bian HJ, Chen ZN and Lou C. Cell cycle block and apoptosis in hepatocellular carcinoma cells induced by radionuclide ~(188)Re. Chin J Radiol Med Protect 2001; 21: 185-187.

[13] Zamora PO, Gulhke S, Bender H, Diekmann D, Rhodes BA, Biersack HJ and Knapp FF Jr. Experimental radiotherapy of receptor-positive human prostate adenocarcinoma with 188Re-RC-160, a directly-radiolabeled somatostatin analogue. Int J Cancer 1996; 65: 214-220.

[14] Bujag JE, Friebe M and Cyr JE. Radiotherapeutic efficacy of 188Re-P2045 in small cell lung cancer and non-small cell lung cancer mouse models by specific SSTR-targeting. Chem Nucl Med 2002; 21: 357-362.