Int J Clin Exp Pathol 2015;8(12):16064-16072
www.ijcep.com /ISSN:1936-2625/IJCEP0017833

Original Article

^{99m}Tc-3P-RGD$_2$ SPECT to monitor early response to bevacizumab therapy in patients with advanced non-small cell lung cancer

Bin Chen[1*], Guoqing Zhao[1*], Qingjie Ma[1], Bin Ji[1], Tiefeng Ji[1], Hua Xin[2], Shi Gao[1]

[1]Department of Nuclear Medicine, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China; [2]Department of Thoracic Surgery, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China. [*]Equal contributors.

Received October 14, 2015; Accepted November 28, 2015; Epub December 1, 2015; Published December 15, 2015

Abstract: The present study assessed the predictive value of ^{99m}Tc-3(poly-(ethylene glycol), PEG)$_4$-arginine-glycine-aspartic (^{99m}Tc-3P-RGD$_2$) single photon emission computed tomography (SPECT) for the early identification of response to antiangiogenic treatment with bevacizumab in patients with advanced non-small cell lung cancer (NSCLC). Patients with advanced NSCLC treated with bevacizumab were prospectively studied with ^{99m}Tc-3P-RGD$_2$ SPECT before and after 2 weeks from start of treatment. The tumor response was evaluated with RECIST criteria and related to observed change in the tumor to non-tumor (T/N) ratio for the largest known lesion. Receiver operating characteristic (ROC) curve analysis was used to determine T/N ratio changes with regard to predicting response to bevacizumab therapy. Change in T/N ratio was also related to progression-free survival (PFS) and overall survival (OS). Twenty-six patients were included, and 23 were finally assessable for metabolic response evaluation with ^{99m}Tc-3P-RGD$_2$ SPECT. The cut-off value of T/N ratio change defined by ROC analysis was 24.4%. The sensitivity, specificity, and negative predictive value of ^{99m}Tc-3P-RGD$_2$ SPECT for predicting tumor response were 81.8%, 91.7%, and 84.6%, respectively. Using the cut-off value defined by ROC analysis on ^{99m}Tc-3P-RGD$_2$ SPECT, metabolic non-progressive disease patients (mNP) showed prolonged PFS (5.6 months versus 3.4 months; $P < 0.001$) and OS (17.1 months versus 8.6 months; $P < 0.001$) than metabolic progressive disease patients (mP). ^{99m}Tc-3P-RGD$_2$ SPECT scan is a promising test to predict tumor response in patients with advanced non-small cell lung cancer early in the course of bevacizumab therapy.

Keywords: ^{99m}Tc-3P-RGD$_2$, SPECT, bevacizumab, response, non-small cell lung cancer

Introduction

Lung cancer remains the leading cause of malignancy-related deaths worldwide, with over one million cases diagnosed yearly [1, 2]. The most common forms of lung cancer are non-small cell lung cancer (NSCLC) histological subtypes [3-5]. For NSCLC, surgery remains the best curative treatment in early stage disease. Unfortunately, more than 40% of the patients are diagnosed with advanced and/or metastatic disease and are not eligible for surgical treatment or curative radiotherapy [6, 7]. Chemotherapy is still recognized as the best available treatment in patients with advanced lung cancer, but progress appears to be stagnating [8, 9].

Tumor angiogenesis is an essential requirement for the development, progression, and metastasis of malignant tumors [10]. Inhibiting angiogenesis represents a promising strategy for controlling and eradicating tumor growth. Some antiangiogenic agents, such as bevacizumab [11, 12]. Ramucirumab [13] and VEGF Trap [14], have been routinely used in NSCLC therapy. However, response monitoring of antiangiogenic therapy is complicated. Tumor shrinkage usually does not occur until several cycles of therapy and is difficult to evaluate because of inter- and intraobserver variation [15]. As a result, response and progression can be underestimated when tumor size is used as an early predictive marker. Therefore, finding suitable translational biomarkers for antiangio-

Table 1. Patient Characteristics

Characteristics	N° (Total n=23)	%
Age, median (range)	58 years (27-72)	
Sex		
Male	18	78
Female	5	22
Stage		
IIIB	8	35
IV	15	65
Pathological type		
Adenocarcinomas	13	56
Large cell carcinomas	6	26
Squamous cell carcinoma	2	8
ECOG		
0	8	35
1	14	61
2	1	4
Metabolic response SPECT		
Metabolic non-progressive	10	43
Metabolic progressive	13	57
RECIST		
Complete response	0	0
Partial response	8	35
Stable disease	3	13
Progressive disease	12	52

genic modulation of the tumor vasculature has become more important.

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) could use a paramagnetic gadolinium-based contrast agent to measure tumor perfusion by a combination of microvascular flow and permeability, but this method can be technically challenging and standardization is complex [16, 17]. Previously, quantification of tumor vasculature has been assessed in surgical specimens or preclinical samples using immunohistochemistry [18, 19]. However, such assays are impractical in clinical trials not only because of potential variability in the measurement of microvessel density (MDV) but also because of the ethical and physical limitations of serial invasive procedures. Clearly, a noninvasive method for assessing tumor vascularity could obviate these technical challenges.

Both positron emission tomography (PET) and single photon emission computed tomography (SPECT) tracers have been developed to evaluate expression of certain integrins, particularly αvβ3, which are involved in angiogenesis [20-23]. However, the expense and requires more complex kit preparation of ^{18}F-labeled radiotracers in most nuclear medicine laboratories restrict its use. Therefore, there is an unmet need for radiotracers that are readily available and clinically useful for early detection of integrin αvβ3-positive tumors and metastases and for monitoring antiangiogenic therapy.

^{99m}Tc-3(poly-(ethylene glycol), PEG)$_4$-arginine-glycine-aspartic (^{99m}Tc-3P-RGD$_2$) is a well-designed, dimeric RGD peptide, which is readily prepared with very high specific activity using a kit formulation. The probe has been certified with high integrin αvβ3 binding affinity and high tumor contrast resolution in a nude mouse model [24]. Previously, our group applied this novel tracer for noninvasive differentiation of solitary pulmonary nodules (SPNs) [25]. The tracer demonstrated an impressive image quality with high sensitivity in detecting malignant SPNs. More recently, we evaluated ^{99m}Tc-3P-RGD$_2$ SPECT in 33 patients with stage II and III breast cancer treated with neoadjuvant chemotherapy suggested that early ^{99m}Tc-3P-RGD$_2$ SPECT after 2 weeks of therapy may be able to predict response [23]. This article reports on changes in relative ^{99m}Tc-3P-RGD$_2$ tumor uptake over time in patients undergoing bevacizumab treatment at baseline and at 2 weeks after treatment initiation. Imaging results are related to clinical outcomes, as assessed with response evaluation criteria in solid tumors (RECIST).

Materials and methods

Patients

Patients with advanced NSCLC who were scheduled for bevacizumab treatment were enrolled prospectively from September 2012 to May 2015 in China-Japan Union Hospital. Eligibility criteria were: histologically or cytologically proven NSCLC; unresectable stage IIIB/IV disease; age over 18 years; Eastern Cooperative Oncology Group (ECOG) performance status between 0 and 2; adequate bone marrow function, liver function, and renal function. Patients were not included if they had previous lung diseases such as interstitial pneumonitis or lung fibrosis identified by chest Computed Tomography (CT) scan. Life expectancy was pre-

Table 2. Tumor ^{99m}Tc-3P-RGD$_2$ Uptake, CT and SPECT assessments of response rates, OS and PFS (n=23)

No	Diagnosis	Sex/Age	T/N_1	T/N_2	ΔT/N (%)	Response to treatment	Metabolic response	PFS (mo)	OS (mo)
1	large cell carcinomas	F/38	5.24	2.91	0.44	PR	mNP	5.7	28.8
2	adenocarcinomas	M/74	2.51	1.19	0.53	PR	mNP	7.3	18.5
3	adenocarcinomas	M/63	2.65	1.85	0.30	PR	mNP	3.6	11.7
4	adenocarcinomas	M/36	3.07	2.14	0.30	SD	mNP	5.0	15.3
5	adenocarcinomas	M/61	3.84	2.87	0.25	PR	mNP	8.4	28.9
6	adenocarcinomas	M/60	2.93	2.19	0.25	PR	mNP	7.9	21.5
7	large cell carcinomas	M/29	2.61	2.98	-0.14	PR	mP	4.1	14.1
8	large cell carcinomas	F/33	1.54	1.42	0.08	SD	mP	3.3	9.0
9	squamous cell carcinomas	M/46	2.77	1.94	0.30	SD	mNP	5.9	9.3
10	adenocarcinomas	M/50	4.58	3.10	0.32	PR	mNP	3.6	13.2
11	adenocarcinomas	F/47	3.05	1.93	0.37	PR	mNP	4.4	14.7
12	adenocarcinomas	M/26	3.49	2.90	0.17	PD	mP	3.1	7.4
13	large cell carcinomas	M/71	4.42	4.75	-0.07	PD	mP	3.2	7.2
14	adenocarcinomas	F/65	3.79	4.65	-0.23	PD	mP	4.3	6.1
15	adenocarcinomas	M/44	4.15	3.78	0.09	PD	mP	2.4	10
16	large cell carcinomas	M/39	3.57	3.56	0.004	PD	mP	5.1	6.2
17	adenocarcinomas	M/51	3.33	2.61	0.21	PD	mP	3.6	8.3
18	squamous cell carcinomas	M/70	2.67	3.15	-0.18	PD	mP	2.2	6.1
19	adenocarcinomas	M/53	3.51	1.91	0.46	PD	mNP	4.0	9.5
20	large cell carcinomas	F/51	3.43	2.62	0.24	PD	mP	3.1	10.3
21	adenocarcinomas	M/48	3.13	4.01	-0.28	PD	mP	2.2	3.4
22	adenocarcinomas	M/37	2.33	1.93	0.17	PD	mP	4.2	14.6
23	adenocarcinomas	M/60	2.07	1.68	0.19	PD	mP	3.0	9.6

F, female; M, male; T/N, tumor to non-tumor ratio; PR, partial response; SD, stable disease; PD, progressive disease; mP, metabolic progressive; mNP, metabolic non-progressive; PFS, progression-free survival; OS, overall survival.

dicted to be longer than 12 weeks. Patient characteristics are shown in **Table 1**.

Patients received intravenous carboplatin AUC 6 plus paclitaxel PAC 200 mg/m^2 on day 1 of a 3-week cycle, continued for six cycles or until disease progression or unacceptable toxicity. Intravenous bevacizumab 15 mg/kg was administered every 3 weeks during chemotherapy and as maintenance therapy until disease progression or unacceptable toxicity. Conventional staging and follow-up were performed according to standards of care. Clinical and biochemical evaluation were performed for any patient every month until treatment discontinuation.

Informed written consent to participate in this study was obtained from all patients. The study and use of the new radiotracer ^{99m}Tc-3P-RGD$_2$ were approved by the local independent Ethics Committees and the Institutional Review Boards of China-Japan Union Hospital, Changchun, China.

SPECT study

Radiolabeling and quality control procedures for 3P-RGD$_2$ were synthesized as published previously [25]. 3P-RGD$_2$ was radiolabeled with 626 ± 71 MBq ^{99m}technetium and then administered via a single intravenous bolus injection into the arm contralateral to the affected lung, followed by a 10-mL saline flush. ^{99m}Tc-3P-RGD$_2$ SPECT was performed at 60 min after injection. Patients were in supine position with raised arms during imaging.

Two ^{99m}Tc-3P-RGD$_2$ SPECT scans were planned: SPECT$_1$ before starting therapy and SPECT$_2$ within 2 weeks after starting therapy. SPECT was performed using a double-head γ camera (Precedence, Philips Healthcare, Eindhoven,

The Netherlands), equipped with low-energy parallel hole collimators. The matrix was 128×128 pixels, and the photopeak was centered at 140 keV with a symmetrical 20% window. Imaging was performed using six angular steps with a 20-s time-frame. The distance between the chest and the detector was minimized. The Digital Imaging and Communications in Medicine image files of each patient were saved on optical disks and transferred to Extended Brilliance™ workspace (Philips Healthcare) for centralized reconstruction, reading and analysis.

Image interpretation

All ^{99m}Tc-3P-RGD$_2$ SPECT images were qualitatively and semi-quantitatively by two experienced nuclear medicine physicians blinded to all other information including patient identity, results of any other imaging modality, and final pathology results that indicated residual disease status. ^{99m}Tc-3P-RGD$_2$ uptake was quantitatively assessed using region of interest (ROI) analysis. The image of the lung that best demonstrated the greatest extent of the index lesion was selected for analysis. A tumor ROI was defined to include all pixels with intensities greater than 50% of the maximum tumor intensity. An adjacent area of normal lung tissue was selected to represent background activity. If the lung exhibited diffuse tumor uptake suggestive of multifocal or multicentre disease, the background ROI was placed within normal lung tissue of the contralateral lung. The tumor to non-tumor (T/N) ratio was calculated as (mean counts per pixel in the tumor ROI)/(mean counts per pixel in the background ROI). The resultant data were all reached by consensus. The percentage changes in T/N ratio, which was used as the measure of metabolic response, were calculated as: $\Delta T/N = (T/N_1\text{-}T/N_2)/T/N_1 \times 100\%$.

Clinical response validation

Conventional evaluation included at least clinical examination plus CT scan performed within 1 week before (CT_1) and 8 weeks (CT_2) after the bevacizumab therapy. CT data were interpreted by two experienced physicians specialists in thoracic oncology blinded to SPECT results according to the Response Evaluation Criteria in Solid Tumors (RECIST criteria) by comparison of CT_1 and CT_2. Therapeutic response evaluation was defined as: 1) complete response (CR); 2) partial response (PR); 3) progressive disease (PD); and 4) stable disease (SD). Two major groups, the progressive disease (P) group and non-progressive disease (NP) group including CR, PR and SD, were thus defined.

Patients underwent further CT scan evaluation every 2 months or in case of clinical suspect of disease progression. Patients were followed for survival every 2 months after their bevacizumab discontinuation.

Statistical analysis

Except for survival data that were expressed as the median, all data were expressed as the mean ± standard deviation (SD). The differences in the dosimetric parameters between group P and NP were evaluated by t test. To determine a threshold for percent reduction in T/N ratios, receiver-operating characteristic (ROC) curve analysis was used. The area under the ROC curve (AUC) provided the predictive power for ^{99m}Tc-3P-RGD$_2$ SPECT imaging. Using the threshold defined by ROC analysis, patients were classified again into 2 groups: metabolic progressive (mP) or metabolic non-progressive (mNP). Progression-free survival (PFS) and overall survival (OS) of group mNP and mP were determined by standard Kaplan-Meier survival analysis, and between-group comparison was performed by log-rank test. PFS was defined as the time interval from the date of enrolment in the study until the first signs of progression. OS was calculated from the date of enrolment until death from any cause. All analyses were performed using software SPSS for Windows (version 19.0). $P < 0.05$ was considered to indicate statistical significance.

Results

Population

Between September 2012 and May 2015, 26 patients were enrolled. Three of the 26 patients were excluded from analysis due to various reasons as follow: 2 patients ultimately refused the proposed chemotherapy treatment; One patient was not included in the subsequent studies because of extremely low ^{99m}Tc-3P-RGD$_2$ uptake at baseline. Consequently, 23 eligible patients with stage IIIB to IV NSCLC (15 adenocarcinomas, 6 large cell carcinomas, 2 squamous cell carcinomas), 5 women (21.7%) and 18 men (78.3%) with a mean age of 50 ±

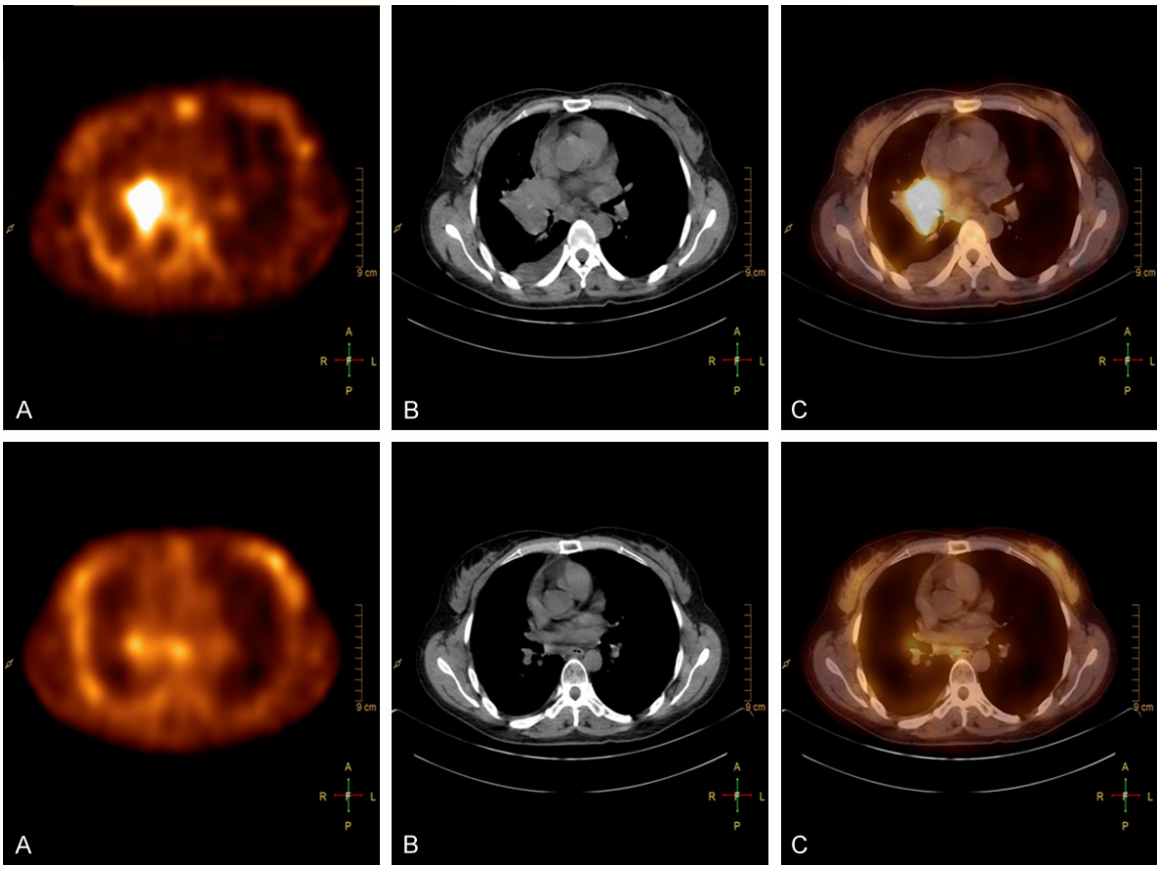

Figure 1. Example of a mNP patient. Non-progressive patient with right upper lobe NSCLC. (A) SPECT, (B) CT and (C) fusion images. The T/N ratio was 5.24 and 2.91 for $SPECT_1$ (up) and $SPECT_2$ (down), respectively. Based on a ΔT/N ratio cut-off value of 24.4%, the patient was classified as mNP, in accordance with RECIST evaluation on CT scan (performed 58 days after starting bevacizumab).

14 years (range 29-74 years), were included in the final analysis.

Tumor ^{99m}Tc-3P-RGD$_2$ uptake

Tumor T/N ratio and their changes between scans were calculated (**Table 2**). The mean tumor T/N_1 of target lesions was 3.25 ± 0.85 (range 1.54-5.24); mean T/N_2 was 2.70 ± 0.97 (range 1.18-4.74); mean change of the ΔT/N ratio in the target lesion was 16.4 ± 22.3% (range -28.0-52.6%).

^{99m}Tc-3P-RGD$_2$ SPECT response versus conventional evaluation

Evaluation of response to treatment according to RECIST criteria demonstrated 12 (52%) patients with progressive disease and 11 (48%) patients with non-progressive disease including 8 cases of PR and 3 SD.

We did not observe a significant difference in baseline ^{99m}Tc-3P-RGD2 uptake values among patients with P group or NP group (3.16 ± 1.03 vs. 3.33 ± 0.70, P>0.05).

According to ROC analyses, the ΔT/N ratios were used for the predictor of RECIST P group or NP group as the dependent variables. The AUC was 0.82 (95% CI, 0.625 to 1.0; P = 0.01), translating into maximal specificity of 81.8% and sensitivity of 91.7% for non-progression at a cut-off of 24.4% reduction in ΔT/N ratio. An example of individual metabolic non-progressive was shown in **Figure 1.**

Correlation between ^{99m}Tc-3P-RGD$_2$ SPECT response and survival outcome.

Early ^{99m}Tc-3P-RGD$_2$ SPECT response was found to predict patient outcome in terms of PFS and OS (**Figure 2A**, **2B**). Overall median PFS and OS were 4.3 months (95% CI 2.2-8.4 months) and

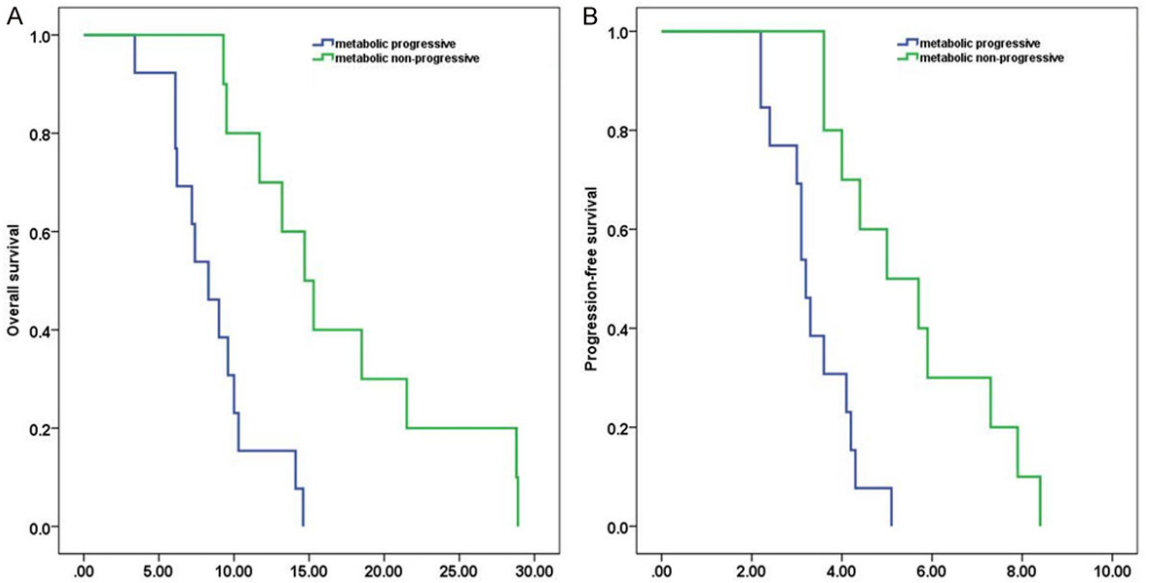

Figure 2. Overall survival (A) and progression-free survival (B) curves according to ^{99m}Tc-3P-RGD$_2$ SPECT response. (A) Patients classified as metabolic progressive on early ^{99m}Tc-3P-RGD$_2$ SPECT had a median OS of 8.6 months (95% CI 6.9-10.4 months), whereas it was 17.1 months (95% CI 12.7-21.6 months) in patients with metabolic non-progressive, respectively (P < 0.001). (B) Patients classified as metabolic progressive on early ^{99m}Tc-3P-RGD$_2$ SPECT had a median PFS of 3.4 months (95% CI 2.9-3.8 months), whereas it was 5.6 months (95% CI 4.5-6.9 months) in patients with metabolic non-progressive, respectively (P < 0.001).

12.3 months (95% CI 3.4-28.9 months), respectively. Using the cut-off value defined by ROC analysis on ^{99m}Tc-3P-RGD$_2$ SPECT, patients classified as mP group showed a significantly shorter PFS (3.4 months versus 5.6 months; P < 0.001) and OS (8.6 months versus 17.1 months; P < 0.001) than mNP group.

Discussion

Targeted antiangiogenic therapies are becoming more widespread clinically and have been shown to have promise in the treatment of various kinds' tumors including NSCLC. Bevacizumab, a recombinant humanised monoclonal antibody developed against VEGF, binds to soluble VEGF, preventing receptor binding and inhibiting endothelial cell proliferation and vessel formation. Pre-clinical and clinical studies have shown that bevacizumab alone or in combination with a cytotoxic agent decreases tumor growth and increases median survival time and time to tumor progression [26].

Integrins are cell adhesion receptors that are evolutionary old and that play important roles during developmental and pathological processes. It is known that the integration of signalling pathways initiated by receptor tyrosine kinases and integrins is essential for growth-factor-mediated biological responses [27]. Interactions between αvβ3 and vascular endothelial growth factor receptor (VEGFR)/platelet-derived growth factor receptor (PDGFR) seem to be particularly important for vascularization [28]. This relationship regulates many cellular activities during angiogenesis, including endothelial cell migration, survival, and tube formation, and hematopoietic cell functions within the vasculature. This makes αvβ3 a mechanistically relevant biomarker for assessing downstream biologic effects of bevacizumab therapy. Over the past decade, radiolabeled RGD peptides and analogues have been intensively investigated for noninvasive imaging of tumor integrin αvβ3 expression [21, 24, 25].

^{99m}Tc-3P-RGD$_2$ is a well-designed, dimeric RGD peptide, which is readily prepared with very high specific activity using a kit formulation. Moreover, it has been shown that ^{99m}Tc-3P-RGD$_2$ uptake is positively correlated with the integrin αvβ3 expression level [29]. In the present exploratory study, dynamic assessment of the change in T/N ratio provided an impressive predictive power. After 2 weeks of bevacizumab

therapy, using a cut-off value of 24.4%, ^{99m}Tc-3P-RGD$_2$ SPECT demonstrated a sensitivity of 81.8%, a specificity of 91.7% and a negative predictive value of 84.6%. Shih et al. investigated the use of ^{99m}Tc-Tetrofosmin imaging to evaluate tumor response to chemotherapy in nonresectable NSCLC [30]. Using a threshold of 1.2 in T/N ratio before chemotherapy, ^{99m}Tc-Tetrofosmin imaging predicted the response with an accuracy of 90%, a sensitivity of 90%, and a specificity of 90%. However, they used a planar data for analyzed. In our study, in several patients with a malignant lesion that was in the deeper region of the lung, planar uptake could not be detected using a conventional gamma camera. Consequently, only SPECT images were used for data analyses. Nonetheless, compared with ^{99m}Tc-Tetrofosmin, single photon techniques using ^{99m}Tc-3P-RGD$_2$ allows description of the angiogenic status, and may give more information on the tumor in lung cancer patients undergoing anti-angiogenesis therapy. Moreover, in our previous study, we classified patients as responders (CR and PR) and non-responders (SD and PD) [31], whereas in our study, patients were divided into mP group and mNP group. This patient classification seems to be more appropriate to assess response to targeted therapy that is designed to stabilize disease, rather than achieve complete response.

20/23 patients were correctly classified by applying the ΔT/N ratio cut-off determined by ROC analysis for ^{99m}Tc-3P-RGD$_2$ SPECT. There were two false progressive results in our study. A significant increase of ^{99m}Tc-3P-RGD$_2$ uptake was observed in one patient who was classified as PR by RECIST criteria at completion of therapy. This patient had a peritumoral inflammatory response was visually observed from the SPECT images acquired after the 2 weeks of bevacizumab therapy. As is known to all, inflammation was different from other benign lesions, always showed high cell density and vascularity, likely responsible for the increased uptake. Previous studies have also shown that the integrin αvβ3 can exist on neutrophils, monocytes, and vascular smooth muscle cells [32], which can be the main reason for the T/N ratio of this tumor to increase. Another patient who was classified as SD by RECIST criteria showing a T/N ratio of 1.54 at SPECT$_1$ and did not change at SPECT$_2$, the lesion with approximately 2 cm in diameter was located in the inferior lobe of right lung close to liver. According to our previous research about pharmacokinetics of ^{99m}Tc-3P-RGD$_2$, the tracer was metabolized via hepatobiliary system and the radioactivity will accumulation in the liver in the process of imaging [29]. In this patient, the results might have been flawed because of a partial-volume effect and spillover from liver tissue.

One limitation of semi-quantitative analysis of ^{99m}Tc-3P-RGD$_2$ SPECT is that it does not take into account the development of new lesions. A significant decrease of ^{99m}Tc-3P-RGD$_2$ uptake was observed in one patient who was classified as PD by RECIST criteria due to the appearance of a new lesion, despite the tumor demonstrated significant shrinkage.

With the advance of targeted therapies, which also can be associated with significant toxicity and side effects, a noninvasive and early evaluation of treatment response that is predictive of PFS and OS becomes critically important. Our results suggest that PFS and OS of bevacizumab therapy can be predicted 2 weeks after starting bevacizumab. This result confirms the results of Ma et al., who showed, in a series of 28 patients receiving chemoradiotherapy plus bevacizumab, that an early (2 cycles of chemotherapy) partial metabolic response was associated with improved OS [31]. But no data on PFS was reported in that study.

In conclusion, ^{99m}Tc-3P-RGD$_2$ SPECT assessment after 2 weeks of bevacizumab treatment could be useful to identify early resistant patients and to predict clinical outcome in patient with advanced NSCLC. An early identification of bevacizumab activity could be a better strategy of therapy tailoring, providing a solution to low availability of tumor tissue for genotype analyses in NSCLC.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NSFC) projects (Grant number: 81271606, 81571708, 81501506), Research Fund of Science and Technology Department of Jilin Province (Grant number: 20150520154JH), Hygiene Specific Subjects of Jilin Province (Grant number: SCZSY201508).

Disclosure of conflict of interest

None.

Address correspondence to: Shi Gao, Department of Nuclear Medicine, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China. Tel: +86-0431-84995124; Fax: +86-28-84651636; E-mail: gaoshi@jlu.edu.cn; Hua Xin, Department of Thoracic Surgery, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China. Tel: +86-0431-84995124; Fax: +86-28-84651636; E-mail: xinhua5124@163.com

References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.

[2] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[3] Molina-Pinelo S, Gutierrez G, Pastor MD, Hergueta M, Moreno-Bueno G, Garcia-Carbonero R, Nogal A, Suarez R, Salinas A, Pozo-Rodriguez F, Lopez-Rios F, Agullo-Ortuno MT, Ferrer I, Perpina A, Palacios J, Carnero A and Paz-Ares L. MicroRNA-dependent regulation of transcription in non-small cell lung cancer. PLoS One 2014; 9: e90524.

[4] Schwander B, Ravera S, Giuliani G, Nuijten M and Walzer S. Cost comparison of second-line treatment options for late stage non-small-cell lung cancer: cost analysis for Italy. Clinicoecon Outcomes Res 2012; 4: 237-243.

[5] Boch C, Kollmeier J, Roth A, Stephan-Falkenau S, Misch D, Gruning W, Bauer TT and Mairinger T. The frequency of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC): routine screening data for central Europe from a cohort study. BMJ Open 2013; 3.

[6] Rapp E, Pater JL, Willan A, Cormier Y, Murray N, Evans WK, Hodson DI, Clark DA, Feld R, Arnold AM, et al. Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer--report of a Canadian multicenter randomized trial. J Clin Oncol 1988; 6: 633-641.

[7] Wakelee HA, Bernardo P, Johnson DH and Schiller JH. Changes in the natural history of nonsmall cell lung cancer (NSCLC)--comparison of outcomes and characteristics in patients with advanced NSCLC entered in Eastern Cooperative Oncology Group trials before and after 1990. Cancer 2006; 106: 2208-2217.

[8] Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995; 311: 899-909.

[9] Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J and Johnson DH. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346: 92-98.

[10] Kaira K, Oriuchi N, Shimizu K, Ishikita T, Higuchi T, Imai H, Yanagitani N, Sunaga N, Hisada T, Ishizuka T, Kanai Y, Endou H, Nakajima T, Endo K and Mori M. Correlation of angiogenesis with 18F-FMT and 18F-FDG uptake in non-small cell lung cancer. Cancer Sci 2009; 100: 753-758.

[11] Keating GM. Bevacizumab: a review of its use in advanced cancer. Drugs 2014; 74: 1891-1925.

[12] Ritzwoller DP, Carroll NM, Delate T, Hornbrook MC, Kushi L, Bowles EJ, Loggers ET and Menter A. Comparative effectiveness of adjunctive bevacizumab for advanced lung cancer: the cancer research network experience. J Thorac Oncol 2014; 9: 692-701.

[13] Garon EB, Ciuleanu TE, Arrieta O, Prabhash K, Syrigos KN, Goksel T, Park K, Gorbunova V, Kowalyszyn RD, Pikiel J, Czyzewicz G, Orlov SV, Lewanski CR, Thomas M, Bidoli P, Dakhil S, Gans S, Kim JH, Grigorescu A, Karaseva N, Reck M, Cappuzzo F, Alexandris E, Sashegyi A, Yurasov S and Perol M. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomised phase 3 trial. Lancet 2014; 384: 665-673.

[14] Leighl NB, Raez LE, Besse B, Rosen PJ, Barlesi F, Massarelli E, Gabrail N, Hart LL, Albain KS, Berkowitz L, Melnyk O, Shepherd FA, Sternas L, Ackerman J, Shun Z, Miller VA and Herbst RS. A multicenter, phase 2 study of vascular endothelial growth factor trap (Aflibercept) in platinum- and erlotinib-resistant adenocarcinoma of the lung. J Thorac Oncol 2010; 5: 1054-1059.

[15] Erasmus JJ, Gladish GW, Broemeling L, Sabloff BS, Truong MT, Herbst RS and Munden RF. Interobserver and intraobserver variability in measurement of non-small-cell carcinoma lung lesions: implications for assessment of tumor response. J Clin Oncol 2003; 21: 2574-2582.

[16] Chung J, Kim JH, Lee EJ, Kim YN and Yi CA. Semiautomatic determination of arterial input functions for quantitative dynamic contrast-enhanced magnetic resonance imaging in non-small cell lung cancer patients. Invest Radiol 2015; 50: 129-134.

[17] Wang LL, Lin J, Liu K, Chen CZ, Liu H, Lv P, Fu CX and Zeng MS. Intravoxel incoherent motion diffusion-weighted MR imaging in differentiation of lung cancer from obstructive lung consolidation: comparison and correlation with pharmacokinetic analysis from dynamic con-

trast-enhanced MR imaging. Eur Radiol 2014; 24: 1914-1922.
[18] Foote RL, Weidner N, Harris J, Hammond E, Lewis JE, Vuong T, Ang KK and Fu KK. Evaluation of tumor angiogenesis measured with microvessel density (MVD) as a prognostic indicator in nasopharyngeal carcinoma: results of RTOG 9505. Int J Radiat Oncol Biol Phys 2005; 61: 745-753.
[19] El-Assal ON, Yamanoi A, Soda Y, Yamaguchi M, Igarashi M, Yamamoto A, Nabika T and Nagasue N. Clinical significance of microvessel density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: possible involvement of vascular endothelial growth factor in the angiogenesis of cirrhotic liver. Hepatology 1998; 27: 1554-1562.
[20] Mena E, Owenius R, Turkbey B, Sherry R, Bratslavsky G, Macholl S, Miller MP, Somer EJ, Lindenberg L, Adler S, Shih J, Choyke P and Kurdziel K. [(1)(8)F]fluciclatide in the in vivo evaluation of human melanoma and renal tumors expressing alphavbeta 3 and alpha vbeta 5 integrins. Eur J Nucl Med Mol Imaging 2014; 41: 1879-1888.
[21] Rylova SN, Barnucz E, Fani M, Braun F, Werner M, Lassmann S, Maecke HR and Weber WA. Does imaging alphavbeta3 integrin expression with PET detect changes in angiogenesis during bevacizumab therapy? J Nucl Med 2014; 55: 1878-1884.
[22] Shi J, Wang L, Kim YS, Zhai S, Jia B, Wang F and Liu S. 99mTcO(MAG2-3G3-dimer): a new integrin alpha(v)beta(3)-targeted SPECT radiotracer with high tumor uptake and favorable pharmacokinetics. Eur J Nucl Med Mol Imaging 2009; 36: 1874-1884.
[23] Ji B, Chen B, Wang T, Song Y, Chen M, Ji T, Wang X, Gao S and Ma Q. (9)(9)mTc-3PRGD(2) SPECT to monitor early response to neoadjuvant chemotherapy in stage II and III breast cancer. Eur J Nucl Med Mol Imaging 2015; 42: 1362-1370.
[24] Jia B, Liu Z, Zhu Z, Shi J, Jin X, Zhao H, Li F, Liu S and Wang F. Blood clearance kinetics, biodistribution, and radiation dosimetry of a kit-formulated integrin alphavbeta3-selective radiotracer 99mTc-3PRGD 2 in non-human primates. Mol Imaging Biol 2011; 13: 730-736.
[25] Ma Q, Ji B, Jia B, Gao S, Ji T, Wang X, Han Z and Zhao G. Differential diagnosis of solitary pulmonary nodules using (9)(9)mTc-3P(4)-RGD(2) scintigraphy. Eur J Nucl Med Mol Imaging 2011; 38: 2145-2152.
[26] Herbst RS, O'Neill VJ, Fehrenbacher L, Belani CP, Bonomi PD, Hart L, Melnyk O, Ramies D, Lin M and Sandler A. Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small-cell lung cancer. J Clin Oncol 2007; 25: 4743-4750.
[27] Eliceiri BP. Integrin and growth factor receptor crosstalk. Circ Res 2001; 89: 1104-1110.
[28] Taeger J, Moser C, Hellerbrand C, Mycielska ME, Glockzin G, Schlitt HJ, Geissler EK, Stoeltzing O and Lang SA. Targeting FGFR/PDGFR/VEGFR impairs tumor growth, angiogenesis, and metastasis by effects on tumor cells, endothelial cells, and pericytes in pancreatic cancer. Mol Cancer Ther 2011; 10: 2157-2167.
[29] Guanghui C, Shi G, Tiefeng J, Qingjie M, Bing J, Zuowei C and Qing W. Pharmacokinetics and radiation dosimetry of ~(99m)Tc-3PRGD_2 in healthy individuals: A pilot study. Nuclear Science and Techniques 2012; 23: 349-354.
[30] Shih CM, Shiau YC, Wang JJ, Ho ST and Kao A. Using technetium-99m tetrofosmin chest imaging to predict taxol-based chemotherapy response in non-small cell lung cancer but not related to lung resistance protein expression. Lung 2003; 181: 103-111.
[31] Ma Q, Min K, Wang T, Chen B, Wen Q, Wang F, Ji T and Gao S. (99m)Tc-3PRGD 2 SPECT/CT predicts the outcome of advanced nonsquamous non-small cell lung cancer receiving chemoradiotherapy plus bevacizumab. Ann Nucl Med 2015; 29: 519-527.
[32] Horton MA. The alpha v beta 3 integrin "vitronectin receptor". Int J Biochem Cell Biol 1997; 29: 721-725.