

## 99mTc-3P4-RGD2 Radiotracers for SPECT/CT of Esophageal Tumor Postprint

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### Abstract

In recent years, several RGD (Arg-Gly-Asp)-based radiotracers have already been successfully tested in human for the visualization of integrin  $\alpha_3\beta_3$ , demonstrating its feasibility in tumor diagnosis. In this paper, we evaluated the  $^{99m}\text{Tc}$ -3P4-RGD2 single-photon emission computed tomography/computerized tomography (SPECT/CT) in patients (58.3 years) with a suspected space-occupying lesion of the esophagus were included, thus finally obtaining their definitive diagnosis (32 benign,  $n = 8$ ). All patients underwent endoscopic, barium esophagography and SPECT/CT imaging preoperatively. The diagnosis precision, sensitivity and specificity among these methods were compared. The relationship between radioactive uptake and clinical pathological stage of esophageal carcinoma was discussed by calculating the tumor to normal esophagus (T/N). Meanwhile, the integrin  $\alpha_3\beta_3$  expression was assessed immunohistochemically in postoperative esophageal tissues. 31 patients were diagnosed as esophageal carcinoma; and 1, leiomyosarcoma; and 6, leiomyoma; and 2, esophageal tuberculosis. The accuracy, sensitivity and specificity of barium esophagography, endoscopic and SPECT/CT imaging are 92.5/93.8/87.5%, 97.5/96.9/100%, and 90/90.6/87.5%, respectively. Abnormal accumulation of radiotracer in 29 malignant lesions is observed. The SPECT/CT imaging displayed the region of radioactive uptake and lesions matched extremely with the T/N ratio from 1.31 to 2.79 (mean 2.04). A case with pulmonary metastases and a case with mediastinal lymph node metastases were found which were missed by barium esophagography and endoscopic. The  $^{99m}\text{Tc}$ -3P4-RGD2 uptake of the esophageal carcinoma masses had no relevance to the tumor pathologic classification ( $P > 0.05$ ). There was a significant positive correlation between T/N ratio and positive cell percentage of integrin  $\alpha_3\beta_3$  ( $r = 0.976$ ), demonstrating the certain clinical potential in the diagnosis of esophageal carcinoma.

## Full Text

### Preamble

#### **99mTc-3P4-RGD2 Radiotracers for SPECT/CT Imaging of Esophageal Tumors**

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### Abstract

In recent years, several RGD (Arg-Gly-Asp)-based radiotracers have been successfully tested in humans for visualizing integrin  $\alpha_3$  expression, demonstrating feasibility in tumor diagnosis. *3P4-RGD2 single photon emission computed tomography/computed tomography (SPECT/CT) in patients with occupying esophageal lesions. Forty patients (34 males and 6 females; mean age : 58.3 years) with suspected esophageal lesions were included, with final pathologic diagnoses obtained (malignant, 32; benign, n = 8). All patients underwent endoscopy, barium esophagography, and SPECT/CT imaging preoperatively. 99mTc-3P4-RGD2 at a dose of 939 ± 118 MBq. The diagnostic accuracy, sensitivity, and specificity of these methods were compared. The relationship between radioactive uptake and clinical pathological stage of esophageal carcinoma was assessed by calculating tumor-to-normal esophagus (T/N) ratios. Meanwhile, integrin  $\alpha_3$  expression was evaluated immunohistochemically in postoperative esophageal tissues. Final diagnoses included 31 esophageal carcinomas, 1 leiomyosarcoma, 6 leiomyomas, and 2 esophageal tuberculosis cases. The accuracy, sensitivity, and specificity were 92.5%/93.8%/87.5% for barium esophagography, 97.5%/96.9%/100% for endoscopy, and 90%/90.6%/87.5% for SPECT/CT, respectively. Abnormal radiotracer accumulation was observed in 29 malignant lesions. SPECT/CT imaging showed excellent correlation between regions of radioactive uptake and lesions, with T/N ratios ranging from 1.31 to 2.79 (mean 2.04). One case with pulmonary metastases and another with mediastinal lymph node metastases were detected by SPECT/CT but missed by barium esophagography and endoscopy. 99mTc-3P4-RGD2 uptake in esophageal carcinoma masses showed no correlation with tumor pathologic classification (P > 0.05). There was a significant positive correlation between T/N ratio and the percentage of integrin  $\alpha_3$ -positive cells (r = 0.976), demonstrating clinical potential for esophageal carcinoma diagnosis.*

**Keywords:** 99mTc-3P4-RGD2, Esophageal tumor, SPECT, Integrin  $\alpha_3$

### Introduction

Esophageal carcinoma has high morbidity and mortality. Currently, FDG-PET is one of the most widely applied imaging techniques in oncology. For

esophageal carcinoma, FDG-PET is used for detecting metastases and evaluating recurrence, but not for local-regional disease [1-3]. However, the high cost of FDG-PET limits its extensive clinical application.

Integrin  $\alpha_3\beta_1$  is overexpressed on tumor cells and neovasculature in various tumors, including glioblastomas, malignant melanomas, and breast tumors [4]. A close relationship between integrin  $\alpha_3\beta_1$  expression and tumor metastasis/angiogenesis has been investigated [5-8]. There is increasing interest in peptides containing the Arg-Gly-Asp (RGD) sequence for targeting integrin  $\alpha_3\beta_1$  to image malignant tumors. Thus far, most radiolabeled RGD-containing agents have been evaluated in animal models, while  $^{18}\text{F}$ -Galacto-RGD and  $^{18}\text{F}$ -AH111585 have been under clinical investigation for noninvasive visualization of integrin  $\alpha_3\beta_1$  expression [5-9]. Recently, a series of investigations were performed on  $^{99\text{m}}\text{Tc}$ -3P4-RGD2 as a novel radioactive molecular tumor probe. Studies in xenograft models confirmed that it is very promising for early detection of integrin  $\alpha_3\beta_1$ -positive tumors [10]. Additionally,  $^{99\text{m}}\text{Tc}$ -3P4-RGD2 could be easily prepared using freeze-dried kits with high labeling yield and radiochemical purity, exhibiting excellent in vivo behavior in nonhuman primates [11]. Clinical evaluations of  $^{99\text{m}}\text{Tc}$ -3P4-RGD2 scintigraphy in lung tumors showed promise for detecting lung malignancies [12,13]. In this paper, we report that esophageal carcinoma was diagnosed by SPECT/CT with 90% accuracy. Notably, one pulmonary metastasis lesion and one mediastinal lymph node metastasis lesion missed by barium esophagography and endoscopy were successfully detected by SPECT/CT, demonstrating its advantages over conventional methods.

## 2.1 Subjects

This clinical study was conducted between September 2009 and May 2012. Forty patients with suspected space-occupying esophageal lesions (34 males and 6 females; age range: 38-82 years; mean age: 58.3 years) were recruited from our hospital. None had received prior therapy including surgery, chemotherapy, or radiotherapy. Final pathologic diagnoses were obtained for all patients. The study was approved by the local independent Ethics Committee and Institutional Review Boards of China-Japan Union Hospital, Changchun, China. All patients signed informed consent forms. In addition to  $^{99\text{m}}\text{Tc}$ -3P4-RGD2 SPECT/CT imaging, supplementary examinations including endoscopy and barium esophagography were performed within two weeks. The diagnostic standard was based on pathology results from surgical operation or endoscopic biopsy.

### 2.2.1 Radiopharmaceutical Preparation

$^{99\text{m}}\text{Tc}$ -3P4-RGD2 was provided by the Medical Isotopes Research Center of Peking University and prepared using freeze-dried kits with added  $\text{Na}^{99\text{m}}\text{TcO}_4$  solution. The kit composition was primarily HYNIC-3P4-RGD2, with procedures following references [12-14]. Quality control was performed using radioac-

tive thin layer chromatography (ITLC), demonstrating a high labeling yield of approximately 95%.

### 2.2.2 Endoscopy

Endoscopy and suspicious tumor biopsy were performed by professionals from two departments respectively, with evaluation by a specialized pathologist. Based on pathological results, definite diagnoses were made, with malignant lesions designated as positive and benign lesions as negative.

### 2.2.3 Barium Esophagography

Patients were examined in the upright position and turned obliquely to the left. The esophagus was visualized in left posterior oblique, anteroposterior, and right posterior oblique projections during continuous drinking of barium suspension, demonstrating functional changes and abnormal mucosal folds. Positive imaging features included ulceration, mucosal layer interruption, esophageal wall rigidity, and localized filling defects. At least one specialized radiologist participated in each examination.

### 2.2.4 SPECT/CT Image Acquisition

Patients received  $99\text{mTc-}3\text{P4-RGD2}$  at 11.1 MBq (0.3 mCi)/kg via intravenous injection. Data acquisition was performed using an integrated SPECT/CT system (PRECEDENCE Systems, Philips, Netherlands) 4 hours post-injection. The dual-head camera scanner used low-energy high-resolution collimators with a 20% energy window centered at 140 keV. Chest CT scanning was performed with a  $256 \times 256$  matrix and 5-mm section thickness, followed by SPECT emission scanning covering the identical transverse field of view (zoom,  $1 \times 1$ , 30 s/frame/ $6^\circ$ ). Image data sets were reconstructed iteratively using CT data for attenuation correction, and coregistered images were displayed on a workstation. During scanning, patients were in the supine position with hands placed behind the head.

### 2.2.5 SPECT/CT Image Analysis

Images were assessed by a skilled radiologist and two experienced nuclear medicine physicians blinded to patient conditions. Foci with  $T/N > 1.2$  were considered positive, and  $T/N \leq 1.2$  negative. Once a positive esophageal lesion was identified, semi-quantitative analysis of tumor uptake was performed using T/N ratios measured by region of interest (ROI) over the lesion location determined by CT. Similar T/N ratios were obtained for negative endoscopy and barium meal cases.

### 2.2.6 Pathology and Immunohistochemistry of Integrin $\alpha 3$ Expression

Hematoxylin-eosin staining was first used to define pathological diagnosis. Immunohistochemical detection was then performed to examine integrin  $\alpha 3$  expression in 40 esophageal tissue samples collected by operation or biopsy. The staining intensity was scored as 1+ (faint yellow), 2+ (claybank), and 3+ (sepia). The number of positively staining tumor cells at the surface membrane was counted. Positivity was ensured when both grades were equal to or greater than 3. Paraffin-embedded esophageal tumor tissue was stained using rabbit polyclonal anti-integrin  $\alpha 3$  antibody (1:300, Abbotec, USA) following the standard ABC method [15].

### 2.2.7 Statistical Analysis

All quantitative data were expressed as mean  $\pm$  SD. Rank correlation parameters were evaluated by analyses. T and  $\chi^2$  tests were performed at a 5% level of statistical significance using SPSS (version 13.0).

## 2.4 Clinical and Pathological Data

All 40 lesions were identified by pathology, including 32 malignant cases (31 esophageal carcinomas and 1 leiomyosarcoma) and 8 benign cases (6 leiomyomas, 2 esophageal tuberculosis). Clinical and pathological information is listed in Table 1.

## 2.5 SPECT/CT Imaging Results

SPECT/CT imaging showed perfect correlation between radioactive uptake regions and lesions, with abnormal radioactivity accumulation observed in 29 malignant lesions. T/N ratios ranged from 1.31 to 2.79. Cases with multiple pulmonary and mediastinal lymph node metastases were further identified as squamous cell carcinoma by needle biopsy (Fig.2). Three esophageal carcinoma cases showed no  $^{99m}\text{Tc}$ -3P4-RGD2 uptake, representing false-negative imaging, while one tuberculosis case showed abnormal uptake, representing false-positive imaging.

## 2.6 Comparison of Endoscopy, Barium Meal, and SPECT/CT Imaging

Diagnostic performance of endoscopy, barium meal, and SPECT/CT imaging were compared according to accuracy, sensitivity, specificity, and positive and negative predictive values (PPV, NPV). Table 2 shows that SPECT/CT imaging achieved 90.6% sensitivity and 87.5% specificity, with no statistically significant difference compared to the other methods ( $P > 0.05$ ).

## 2.7 SPECT/CT Imaging and Clinicopathological Features of Esophageal Cancer

The relationship between SPECT/CT imaging and clinicopathological features of esophageal carcinoma, including invasion depth, differentiation, and lymph node metastasis, was investigated. Table 3 shows that  $^{99m}\text{Tc}$ -3P4-RGD2 uptake had no correlation with tumor pathologic classification ( $P>0.05$ ).

## 2.8 Immunohistochemistry of Integrin $\alpha 3$ Expression

Among the 32 esophageal malignant tumors, 30 showed positive integrin  $\alpha 3$  expression, confirmed by sepia granules in the cell membrane, cytoplasm of cancer cells, and tissue vessels. Two benign tumors (leiomyoma and tuberculosis samples) also showed positive integrin  $\alpha 3$  expression. There was a significant positive correlation between T/N ratios and the percentage of integrin  $\alpha 3$ -positive cells ( $r=0.976$ ).

## 3 Discussion

Despite early detection and treatment of many esophageal carcinomas, poor prognosis remains due to invasion and metastasis, which are inseparable from neovascularization. Integrin  $\alpha 3$  plays a critical role in tumor-related angiogenesis and metastasis [16 19]. We demonstrated that  $^{99m}\text{Tc}$ -3P4-RGD2 has increased receptor binding affinity and improved kinetics for in vivo SPECT imaging of integrin  $\alpha 3$  expression in esophageal tumors.

Endoscopic and pathological techniques are standard clinical methods for diagnosing esophageal tumors, providing important information about differentiation and morphological changes [20]. Conventional imaging examinations such as CT and barium esophagography can reveal tumor anatomic or morphological characteristics but not pathological features. FDG-PET offers high sensitivity and accuracy for esophageal carcinoma but is limited by high cost [21]. Recently,  $^{99m}\text{Tc}$ -3P4-RGD2 SPECT imaging was applied to solitary pulmonary nodule patients, demonstrating good differentiation between malignant and benign nodules [12].

The accuracy, sensitivity, and specificity of barium esophagography, endoscopy, and  $^{99m}\text{Tc}$ -3P4-RGD2 SPECT/CT imaging were 92.5%/93.8%/87.5%, 97.5%/96.9%/100%, and 90%/90.6%/87.5%, respectively. SPECT/CT showed no statistically significant difference compared to the other two examinations ( $P>0.05$ ). In SPECT imaging, abnormal radioactivity accumulation was observed in 29 malignant lesions. Combined SPECT/CT scans showed excellent correlation between radioactive uptake regions and lesions. Three esophageal carcinoma cases smaller than 1.5 mm were not detected, likely due to low imaging device resolution [22]. However, two cases with metastases (one pulmonary and one mediastinal lymph node) missed by barium esophagography and endoscopy were successfully detected by SPECT/CT.

In esophageal carcinoma cases, T/N ratios showed that radioactive uptake level had no correlation with clinical stage or malignancy degree. Among 31 esophageal carcinomas, 17 metastatic lymph nodes were located at one side of the esophagus and left gastric artery, but showed no uptake on SPECT imaging. This may be due to high uptake in esophageal lesions and abdomen overlaying adjacent lymph nodes, cardiac impulse, gastrointestinal motility, and imaging device limitations.

Immunohistochemistry studies showed high integrin  $\alpha_3\beta_1$  expression in malignant esophageal lesion tissues, primarily as sepia granules in cell membranes, cytoplasm, and tissue vessels. The significant positive correlation between T/N ratios and integrin  $\alpha_3\beta_1$  expression suggests that imaging can provide vital reference for assessing esophageal tumor characteristics and neovascularization.

#### 4 Conclusion

The accuracy, sensitivity, and specificity of  $^{99m}\text{Tc}$ -3P4-RGD2 SPECT/CT imaging are 90%, 90.6%, and 87.5%, respectively. There was a significant positive correlation between T/N ratio and the percentage of integrin  $\alpha_3\beta_1$ -positive cells. The imaging can provide accurate clinical information, indicating potential for diagnosing and detecting esophageal cancer. Increasing sample sizes and pathological types can further confirm the imaging feasibility for noninvasively monitoring tumor growth.

#### References

1. Deng S M, Zhang B, Wu Y W, et al. Nucl Sci Tech, 2011, 5: 293–298.
2. van Westreenen H L, Westerterp M, Bossuyt P M, et al. J Clin Oncol, 2004, 22: 3805–3812.
3. van Vliet E P, Heijnenbroek-Kal M H, Hunink M G, et al. Br J Cancer, 2008, 98: 547–557.
4. Hynes R O. Cell, 1992, 69: 11–25.
5. Haubner R, Weber W A, Beer A J, et al. P Lo S Med, 2005, 2: e70.
6. Beer A J, Haubner R, Sarbia M, et al. Clin Cancer Res, 2006, 12: 3942–3949.
7. Beer A J, Grosu A L, Carlsen J, et al. Clin Cancer Res, 2007, 13: 6610–6616.
8. Kenny L M, Coombes R C, Oulie I, et al. J Nucl Med, 2008, 49: 879–886.

9. Wang L J, Shi J Y, Kim Y S, et al. Mol Pharm, 2009, 6:
10. Jia B, Liu Z, Zhu Z, et al. Mol Imaging Biol, 2011, 13:
11. Ma Q, Ji B, Jia B, et al. Eur J Nucl Med Mol Imaging, 2011, 38: 2145–2152.
12. Scatena M, Almeida M, Chaisson ML, et al. J Cell Biol, 1998, 4: 1083–1093.
13. De S, Chen J, Narizhneva NV, et al. J Biol Chem, 2003, 40: 39044–39050.
14. Rosa Hwang, MD, Judy Varner. Hematol Oncol Clin N Am, 2004, 5: 991–1006.
15. Chin B B, Chang P P. Chang. Best Pract Res Clin Gastroenterol, 2006, 1: 3–21.
16. Liu Y, Xie T W, Liu Q. Nucl Sci Tech, 2011, 3: 165–173.

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