A pilot study on ^{99m}Tc-3PRGD2 scintigraphy in diagnosis of brain glioma

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Abstract The ^{99m}Tc-3PRGD$_2$ targeted SPECT/CT scanning was of significance in detecting differentiated glioma. In this work, the diagnostic value of ^{99m}Tc-3PRGD$_2$ scintigraphy in brain glioma was evaluated by the ten clinically verified brain glioma patients after obtaining informed consent. The patients first accepted X-ray imaging to localize the detecting regions before administrating with ^{99m}Tc-3PRGD$_2$ at a mean radioactivity of 849±115 MBq *via* single intravenous bolus injection 2 h prior to SPECT/CT imaging. Tumor samples for detecting αvβ3 were collected by surgical operations two weeks after the scintigraphy. The results of CT and SPECT scanning were merged and compared. The correlation between tumor occupation (*T*/*N* ratio) and αvβ3 expression level were analyzed. The *T*/*N* ratios in brain glioma were proportionally correlated to αvβ3 positive cell percentage (R^2=0.9253, p<0.05). This study primarily evaluated the clinical application of ^{99m}Tc-3PRGD$_2$ SPECT scintigraphy on brain glioma. The more pathological types and detecting strategies covering a large amount of samples would aid to clarify the potentials.

Key words Radioactive tracer, Tumor, SPECT, CT, Integrin

1 Introduction

After operational treatment by radiochemical therapies, the brain glioma is currently accounting for about 46% of intracranial tumors with a median survival of 8 to 11 months. The mortality data from the World Health Organization shows malignant glioma is the second death cause for tumor patients of less than 34 years old and the third for those between 35 to 54 years old[1].

The early diagnosis and classification of brain glioma could prolong patient survival period[2,3]. The brain glioma can be demonstrated by the diagnostic measurements using dimensional parameters, such as computed tomography, magnetic resonance imaging and ultrasonic imaging, but limited in presenting tumor biological information. Biopsy detection could be an alternative to compensate this limitation *via* direct histological analysis, however, this traumatic operation requirement is that the glioma is close to the cranial inner parts or distant from critically sensitive regions in brain. Radionuclide scintigraphy technique, highly sensitive, target specific and non-traumatic could collect information reflecting tumor biological features. The ^{99m}Tc-MIBI is one well-recognized tumor-targeted radioactive tracer in clinical diagnosis.

This radioactive tracer originated from nuclear medicine cardiac imaging was not specifically designed for tumor imaging. The exact mechanism of malignancy detection is still unknown, thus limiting an evaluation for the biological status of the tumor. Furthermore, the ^{99m}Tc-MIBI image quality is not optimal, and has not a reliable interpretation[4]. Integrin $\alpha_v\beta_3$ as heterodimeric glycoprotein is

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preferentially expressed on several types of cancer cells including melanoma, glioma, and ovarian, and breast cancers[5,6]. Because arginine-glycine-aspartic acid (RGD) containing peptides can bind strongly to the integrin $\alpha_v\beta_3$, many RGD peptide probes have been developed for imaging of integrin expression[7,8]. The PET imaging with positron emitting radioisotopes (mainly ^{18}F) labeled RGD peptide has high sensitivity for various types of tumors[9,10], but their high cost and lack of availability in developing countries limit its application. The SPECT is always an alternative method because of its low cost and easy preparation. For example, the ^{99m}Tc-NC100692, ^{99m}Tc-labeled RGD containing peptide, is successfully evaluated by detecting various cancers for years[11,12].

The ^{99m}Tc-3PRGD$_2$, a well-designed dimeric RGD peptide, shows high tumor uptake in mouse breast cancer xenografts[13]. Our group has applied this novel tracer as noninvasive differentiation of solitary pulmonary nodules (SPNs). The tracer demonstrating an impressive image quality shows highly sensitivity in detecting malignant SPNs[14,15]. In this pilot study, the feasibility of ^{99m}Tc-3PRGD$_2$ targeted brain glioma imaging was evaluated.

2 Materials and Methods

2.1 Patient information

Total ten brain glioma patients (6 male and 4 female, median age of 41.6±14.6 (18 to 68), body weight of 59±9 kg) verified by biopsy analysis were recruited. All participants were not in late stage of malignancies, and had no experience of any chemotherapy, brachytherapy or surgery within 3 months, nor liver and kidney associated disorders, thus excluding potential disturbing effects on biological distribution, metabolism and excretion of ^{99m}Tc-3PRGD$_2$. The written informed consent was from all participants. This pilot clinical study and application of the new radiotracer ^{99m}Tc-3PRGD$_2$ were approved by the local independent Ethics Committees and the Institutional Review Boards of China-Japan Union Hospital, Changchun, China. All patients accepted the ^{99m}Tc-3PRGD$_2$ imaging two weeks before surgical operation.

2.2 ^{99m}Tc- 3PRGD$_2$ scintigraphy

Radiolabeling and quality control procedures for 3PRGD$_2$ were performed as described previously[14]. The ^{99m}Tc-3PRGD$_2$ with a mean radioactivity of 849±115 MBq was administered by single intravenous bolus injection 2 h before imaging, followed by a 10 mL saline flush. During scintigraphy, the patients were laid in a supine position, their heads were fixed in the detection pillow, and hands were put beside the body. An X-ray scanning by Philips SPECT/CT Precedence (Philips Healthcare) was performed to show the detecting range. The following CT scanning was set at a matrix of 256×256 and a 5-mm layer thickness. After collecting CT images, the detecting bench positioned automatically for SPECT data collection at the matrix of 256×256 and magnification of 1, and last for 360° at the speed of 6° per frame (30 s for each frame).

2.3 Analysis of ^{99m}Tc- 3PRGD$_2$ scintigraphy

All images were interpreted qualitatively by two experienced nuclear medicine radiologists who were unaware of the clinical history and other test results of all patients. Visual analysis was performed on a per-lesion basis and in a blinded fashion. In merged SPECT/CT scintigraphy, abnormal nuclide accumulations in cranial region were marked as positive, malignant tumors (*T*); and less or no nuclide uptake as the negative (*NT*) to calculate *T*/*NT* ratios.

2.4 Pathological and immunohistochemical detections

The immunohistochemistry of αvβ3 expression for the brain tissue sample was performed as described previously[14]. The abnormal tissues were snap-frozen, sectioned into 3 μm, and immersed into 10% neutral buffered formalin for fixation for 24 h at 4°C. For immunohistochemical investigation, the specimens were stained using the biotinylated monoclonal anti-αvβ3 antibody LM609 (1:100; Chemicon Europe). The detection was gained by peroxidase staining using 3-amino-9-ethylcarbazole (AEC, Vector Laboratories) as the substrates. Staining was analyzed by a senior pathologist who was unaware of the results of the clinical scintigraphy. Grade 1 was used as the most differentiation degrees; and undifferentiated as Grade

4. For immunohistochemical detections, five random vision fields were selected for each slide, and the 200 cells were counted under each vision field. The ratio of positive cells in 1000 cells was calculated.

2.5 Statistical analysis

The data was expressed as $\chi\pm s$, and SPSS 13.0 was exploited as statistical analysis. The correlation was analyzed by Spearman rank correlation test ($p<0.05$).

3 Results

3.1 ^{99m}Tc-3PRGD$_2$ scintigraphy, pathological and immunohistochemical detections

Pathological detections from 10 patient samples were performed (6 cases of astrocytoma, 3 cases of glioma, and 1 case of oligodendroglioma). The average tumor size was 3.02±0.57 cm (2.2–4.0 cm). The ^{99m}Tc-3PRGD$_2$ scintigraphy revealed 9 positive cases with an average *T*/*NT* ratio of 1.93±45 (1.13–2.51) and 1 negative case. Integrin αvβ3 positive cells accounted for 19.06%±4.78% (9.31– 24.78), as shown in Table 1.

Fig.1 shows that the ^{99m}Tc-3PRGD$_2$ scintigraphy had radioactive accumulation in tumor regions and high nuclide uptakes in choroid plexus, parotid gland, thyroid gland and cranium. Immunohistochemical assay had a remarkable staining in cytoplasma and cell membrane.

Table 1 Patient information and data of related detections

No.	Pathological diagnosis	Gender	Age	Weight/kg	Size / cm	*T*/*NT*	SPECT	αvβ3 positive
1	AstrocytomaG1–G2	Female	38	51	3.0	1.43	Positive	15.28
2	Glioma G2–G3	Male	58	67	2.3	2.51	Positive	22.65
3	AstrocytomaG3–G4	Female	27	48	3.1	2.37	Positive	24.78
4	Glioma G4	Male	57	56	3.7	1.74	Positive	17.74
5	AstrocytomaG3–G4	Male	32	69	4.0	2.15	Positive	20.72
6	Glioma G2–G3	Female	18	43	3.0	1.58	Positive	15.23
7	AstrocytomaG4	Male	40	62	2.8	2.23	Positive	21.64
8	AstrocytomaG1–G2	Male	35	65	3.4	1.13	Negative	9.31
9	AstrocytomaG1–G2	Female	43	58	2.7	1.85	Positive	19.19
10	OligodendrogliomaG3–G4	Male	68	71	2.2	2.27	Positive	24.03

Note: G1 means Grade 1 about pathological and immunohistochemical detections described in the section of 2.4.

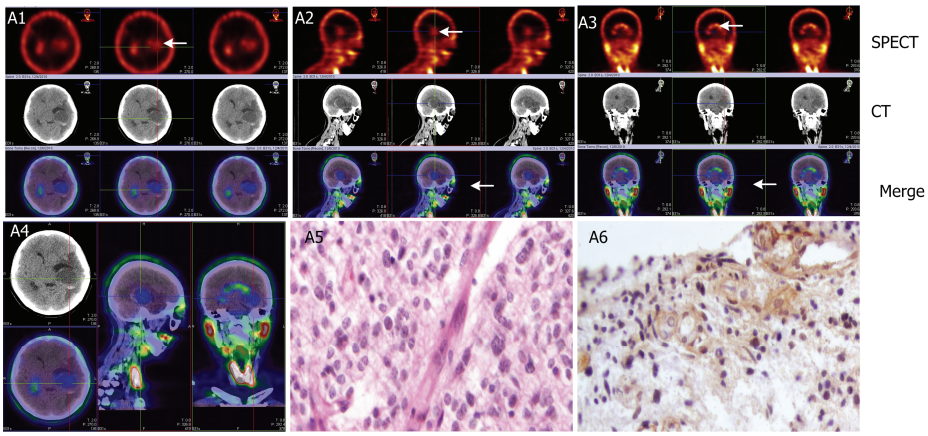

Fig.1 Representative data from patient No.3.

Panels of A1 (transverse section), A2 (longitudinal section) and A3 (horizontal section) increased nuclide uptake in left frontotemporal regions by ^{99m}Tc-3PRGD$_2$ scintigraphy at 2 h, and CT images was low density in left frontotemporal regions surrounded by high density signals, thus shifting the ventricle structures to right lateral with squeezed left lateral ventricles. The A5, HE staining at a magnification of 400 folds was less differentiation status accompanied by local necrotic regions,

supporting the diagnosis of brain glioma (G3–G4). The A6, immunohistochemical staining of αvβ3 positive cells at 400-fold showed a large amount of brown particles in cytoplasm and cell membranes. For the only oligo dendroglioma patient, brain SPECT imaging had no obvious radioactive accumulation in tumor regions, nor less staining in cell membrane *via* immunohistochemical assay (Fig.2). Panels of B1 (transverse section), B2 (longitudinal section) and B3 (horizontal section) increased nuclide uptake in bilateral choroidal branches of lateral ventricles by ^{99m}Tc-3PRGD$_2$ scintigraphy at 2 h without abnormal uptake in left thalamic regions; and CT images, low density in left thalamic regions, thus shifting the ventricle structures to right lateral with squeezed left lateral ventricles. The B5, HE staining of sample from Patient No.9 at a magnification of 400 folds was well differentiated cells accompanied by local necrotic regions, supporting the diagnosis of astrocytoma (G1–G2). The B6, immunohistochemical staining of αvβ3 positive cells at 400-fold had a small amount of brown particles in cell membranes.

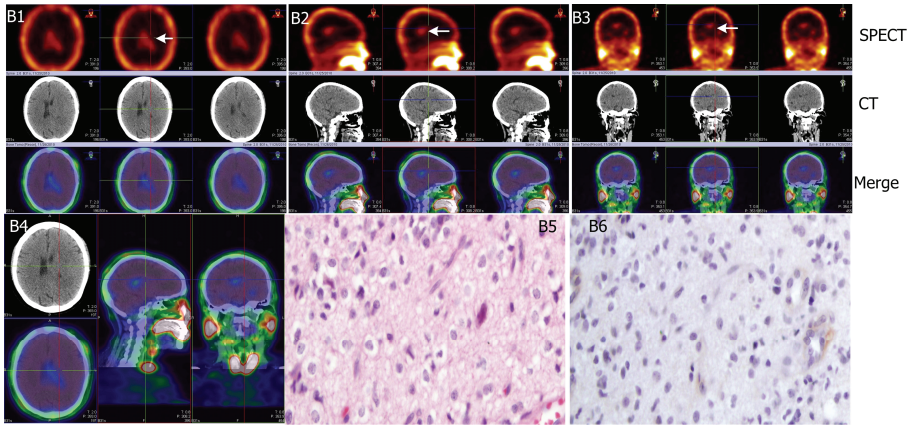

Fig.2 Representative data from patient No.8.

3.2 ^{99m}Tc -3PRGD$_2$ uptake and αvβ3 positive cell proportion in brain glioma

In Fig.3, the proportion between ^{99m}Tc-3PRGD$_2$ uptake and αvβ3 positive cell in brain glioma demonstrated a positive linear correlation (R^2=0.93, p<0.05).

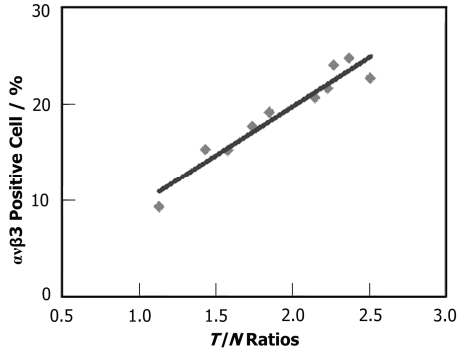

Fig.3 *T*/*N* ratios in brain glioma proportionally correlated to αvβ3 positive cells (R^2=0.9253, P< 0.05).

4 Discussion

The ^{99m}Tc-3PRGD$_2$ is a refined dimeric RGD peptide with the enhanced binding affinity and tumor uptake in preclinical experiments. We have shown that RGD-containing peptide can detect xenografted tumors using scintigraphy, and its uptake quantified by in *vitro* and in *vivo* was proportional to integrin density and tumor size[7–10]. Here, our results show that the integrin targeting imaging can be used to detect tumors in humans noninvasively, this have also been demonstrated by clinical trials with RGD-based tracers [10–12]. To our knowledge, the ^{99m}Tc-3PRGD$_2$ is first applied to patients of brain glioma.

The 10 cases of brain glioma were conducted by the ^{99m}Tc-3PRGD$_2$ scintigraphy, indicating that the 7 in the 9 positive imaging cases were well differentiated (over Grade 2), and the 1 negative case was astrocytoma (Grade 1–2). These results suggested that the tumor cell differentiation status might affect ^{99m}Tc-3PRGD$_2$ uptake in tumor regions[16,17]. The more differentiated was, the more uptakes were, so the ^{99m}Tc-3PRGD$_2$ scintigraphy may be used to determine glioma developing stages, but this needs further be studied by larger samples. Interestingly, two cases of

less malignant astrocytoma patients were positive images, this might be attributed to the temporal transition from the advanced differentiation to less differentiation that enhanced ^{99m}Tc-3PRGD$_2$ uptake[18].

The integrin αvβ3, which is a key factor in regulating tumor vascularization process, involved in tumor vascular endothelial cell activation, proliferation, apoptosis and transmigration. The integrin αvβ3 in promotes generation of nascent vascular vessels when combining with several growth factors synergistically [19]. The immunohistochemical staining showed a proportional correlation between ^{99m}Tc-3PRGD$_2$ uptake and integrin αvβ3 expression, thus supporting the notion that ^{99m}Tc-3PRGD$_2$ SPECT/CT scanning bears the promising value in evaluation of the vascularization of the brain glioma.

5 Conclusions

We applied ^{99m}Tc-3PRGD$_2$ scintigraphy to detect brain glioma in ten patients, obtaining 9 positive cases. Semi-quantitive analysis on tumor images and integrin αvβ3 expression level fitted a positive linear correlation. This research verified that the ^{99m}Tc-3PRGD$_2$ scintigraphy was a potential strategy for staging of brain malignancies and significance in clinical evaluation of target vascularization. Further, the studies should be warranted to analyze large scale cohorts for related pathological features and compare their parallel measurement

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