Evaluation of EGFR-TK Expression with a ^{99m}Tc -Labeled Complex Bearing Quinazoline Pharmacophore

Zhan Si,^{1,2,*} Pengcheng Hu,^{1,2,*} Jun Zhou,^{1,2} Qingyu Lin,^{1,2} Yan Xiu,^{1,2} and Dengfeng Cheng^{1,2}

Abstract

Objectives: The epidermal growth factor receptor (EGFR) signaling pathway is widely recognized to play an important role in development and progression of many malignant tumors, including lung cancer. The aim of this study was to produce a useful technetium-99m (^{99m}Tc) complex for early detection and staging of EGFR-positive tumors.

Methods: The authors labeled quinazoline derivative (3-iodinephenyl) quinazoline-4, 6-diamine with ^{99m}Tc using S-acetylmercaptoacetyltriglyglycinate (MAG_3) conjugated to quinazoline derivative through 4-[(2-aminoethyl)amino]-4-oxobut-2-enoic acid because it is a bifunctional chelator with an average yield of $93.9\% \pm 2.5\%$ and radiochemical purity of $>98\%$.

Results: *In vitro* studies indicate that the [^{99m}Tc]-complex₁₃ has high stability in physiological conditions and binds the HCC 827 cells and shows HCC 827 internalization. The biodistribution of the [^{99m}Tc]-complex₁₃ in healthy Institute of Cancer Research mice indicates the rather fast blood clearance through the hepatobiliary system.

Conclusion: These preliminary results pave the road for estimating the status of EGFR and monitoring the response to EGFR tyrosine kinase inhibitors as a personalized cancer therapy regimen.

Keywords: ^{99m}Tc labeling, epidermal growth factor receptor (EGFR), single photon emission computed tomography (SPECT), tyrosine kinase inhibitors

Introduction

Epidermal growth factor receptor (EGFR) is part of the human EGFR family. It contains an extracellular ligand binding domain, a transmembrane region, and an intracellular protein cytoplasmatic domain with tyrosine kinase activity.^{1,2} Ligand binding to EGFR triggers dimerization of two EGFRs, followed by phosphorylation of tyrosine residues in intracellular cytoplasmatic domain and then activation of downstream signaling that leads to cell proliferation and protection from apoptosis.³ Previous reports showed overexpression of EGFR in various cancers such as head and neck tumors, colorectal, breast, non-small-cell lung cancer, and many types of epithelial cancers.⁴ Furthermore, the crucial importance of EGFR to tumor progression and resistance to other treatments has motivated research on novel treatment strategies to target these receptors.^{5,6}

Monoclonal antibodies directed against the extracellular ligand binding domain of the receptor and small molecule

tyrosine kinase inhibitors (TKIs) directed against the intracellular adenosine triphosphate (ATP) binding site of the tyrosine kinase domain are two classes of drugs approved for clinical use for targeting EGFR.^{7–10} However, the radiolabeled antibodies clear slowly from circulation which causes dose-limiting hematologic toxicity and requires a longer wait time between injection of the radiolabeled antibody and imaging,¹¹ whereas the TKIs clear quickly from blood, offering a more flexible time window for imaging.

The superiority of the two commonly used EGFR TKIs (i.e., gefitinib and erlotinib) over conventional chemotherapy has been reported, and there has also been growing interest in using radionuclide (e.g., ^{18}F , ^{11}C , ^{99m}Tc , and ^{111}In)-labeled TKIs as biomarkers^{12–14} for targeting EGFR.^{15–18} Several classes of TKIs have been established, among which those based on the 4-anilinoquinazoline core scaffold analogues were identified as the most potent competitive inhibitors of ATP, and they are the most heavily investigated so far.^{19,20} Several EGFR TKIs have been approved as curative effective

¹Department of Nuclear Medicine, Zhongshan Hospital, Fudan University, Shanghai, China.

²Department of Nuclear Medicine, Zhongshan Hospital, Shanghai Institute of Medical Imaging, Shanghai, China.

*These authors contributed equally to this work.

Address correspondence to: Dengfeng Cheng or Yan Xiu; Department of Nuclear Medicine, Zhongshan Hospital; 180 Fenglin Road, Shanghai 200032, China

E-mail: cheng.dengfeng@zs-hospital.sh.cn or Xiu.yan@zs-hospital.sh.cn

inhibitors for targeting EGFR by the U.S. Food and Drug Administration (FDA); however, the clinical effects of these inhibitors vary in many cancers because of the different levels of expression and mutations of EGFR because of the heterogeneity of cancers.²¹

Although radiotracers with the potential ability to reflect the status of EGFR have been developed, some inadequacy was observed. For example, ¹⁸F-PEG₆-IPQA has a complicated synthesis procedure and the ¹⁸F-PEG₆ metabolic cleavage from the quinazoline core will increase the background signal.²² ¹¹C-gefitinib has substantial uptake in the intestine, and the application is hampered by nuclide ¹¹C,²³ for its short half-life. In addition, it is not convenient for PET studies because of the need for an on-site cyclotron.

The widespread availability of single-photon emission computed tomography (SPECT) scanners and ⁹⁹Mo-^{99m}Tc generators has suggested that the development of metastable technetium-99m (^{99m}Tc)-labeled molecular probes offers a distinct advantage for imaging. The traditional method of ^{99m}Tc-labeling biomolecules involves using *N*-hydroxysuccinimidyl hydrazino nicotinate hydrochloride (NHS-HYNIC)²⁴ and requires a coligand. The resulting chelate usually has poor *in vivo* and *in vitro* stability.²⁵ Here, the authors used the *N*-hydroxysuccinimidyl *S*-acetylmercaptoacetyltriglycinate (NHS-MAG₃) as a bi-functional chelator that is easily synthesized and conjugated. The conjugation product was easily radiolabeled with ^{99m}Tc, and the radiolabeled probes have shown stability suitable for diagnosis and therapeutic application in the laboratory.²⁶

According to the previous researches,^{19,20,27} 4-anilinoquinazoline quantitative structure-activity relationship could be summarized as follows: First, the nitrogen atom at 1-position forms a hydrogen bond with the key amino acid Met-769 or Met-793, which is important for the combination of 4-anilinoquinazoline to the EGFR target. For the same reason, the N-3 atom by acting as a hydrogen-bond acceptor to interact with the target is also important for its inhibitory potency. Second, the hydrophobic substituent group at 4-position stretch into a hydrophobic cavity that contains a variety of amino acids and opens at Thr790. The interaction of the hydrophobic substituent group with the hydrophobic cavity confers high avidity to the inhibitor and the target. In addition, the halogen substituted anilines at the C-4 position are essential for improving its inhibitory potency. Finally, the Michael acceptor side chain of irreversible inhibitors at 6-position forms a covalent bond with the sulfhydryl group of the Cys-773 of EGFR. Molecular modeling studies have indicated that a covalent bond confer a greater affinity than the reversible inhibitor.

Based on this information, the authors reported the synthesis and characterization of a MAG₃-conjugated quinazoline analogue and the synthesis, characterization, and biological evaluation of the corresponding ^{99m}Tc complex₁₃.

Materials and Reagents

^{99m}Tc-pertechnetate was purchased from Shanghai Atom Kexing Pharmaceutical Co., Ltd. (Shanghai, China) and eluted in saline solution. SnCl₂·2H₂O were purchased from

Sigma-Aldrich Corporation (St. Louis, MO). All other solvents and reagents were analytical grade from China National Pharmaceutical Group Corporation (Beijing, China) and used without further purification. The C18 Sep-Pak columns were purchased from Huayi Isotopes (Jiangsu, China).

Human lung cancer cell line (HCC 827) was obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Institute of Cancer Research (ICR) male mice (6–8 weeks old) were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) which were housed under specific pathogen-free conditions at the Department of Laboratory Animal Science of Fudan University (Shanghai, China). All procedures were performed in accordance with guidelines approved by the Animal Care Committee of Fudan University (Shanghai, China).

Synthesis of 12

The synthesis of **12** is shown in Figure 1a. A mixture of compound **3** (3.00 g, 14.31 mmol, 1.00 equiv.) and **4** (3.29 g, 15.03 mmol, 1.81 mL, 1.05 equiv.) in isopropanol (30.00 mL) was stirred at 50°C for 1 h. After liquid chromatography–mass spectrometry (LCMS) showed that the reaction was completed, the mixture was cooled to 15°C afterward. After filtering, the solid was washed with 100 mL isopropanol. The solid was dried in vacuo to give compound **5** (3.00 g, crude) as a yellow solid. Raney-Ni (2.00 g, 23.33 mmol) and NH₂NH₂·H₂O (3.83 g, 76.50 mmol, 3.72 mL) were added to a solution of **5** (3.00 g, 7.65 mmol) in H₂O (2.00 mL), EtOH (96.00 mL), and isopropanol (100 mL) at 80°C. After addition, the mixture was stirred at 95°C for 1 h. Then the mixture was cooled to 15°C. After filtering, the filtrate was concentrated under reduced pressure to produce **6** (2.80 g, crude) as a yellow oil.

A solution of compound **7** (2.00 g, 12.48 mmol, 1.96 mL), **8** (1.22 g, 12.48 mmol) in DCM (20.00 mL) was stirred at 15°C for 30 min. The solvent was removed under reduced pressure to produce **9** (3.30 g, crude) as a white solid. A mixture of **9** (5.60 g, 15.46 mmol), **6** (4.39 g, 17.01 mmol), HATU (O-(7-Aza-1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate) (7.64 g, 20.10 mmol), and DIEA (N,N-Diisopropylethylamine) (4.00 g, 30.92 mmol, 5.40 mL) in DMF (N,N-Dimethylformamide) (2.00 mL) was stirred at 15°C for 5 h. LCMS showed that the reaction was complete. Soon afterward, the solvent was removed under reduced pressure. The residue was purified by pre-high-performance liquid chromatography (HPLC) (acid condition, TFA) to give **10** (2.00 g, crude) as a yellow solid.

A solution of **10** (1.50 g, 2.49 mmol) in hydrochloric acid (HCl)/EtOAc (Ethyl acetate) (4M, 20.00 mL) was stirred at 15°C for 30 min. The solvent was removed under reduced pressure to produce **11** (1.40 g, crude, HCl) as a yellow solid. A mixture of **11** (1.25 g, 4.10 mmol), compound **2** (1.25 g, 4.10 mmol), HATU (1.56 g, 4.10 mmol), and DIEA (1.25 g, 9.65 mmol, 1.69 mL) in DMF (1.00 mL) was stirred at 15°C for 5 h. Then the solvent was removed under reduced pressure. The mixture was purified by pre-HPLC (acid condition, TFA) to produce final **12** (220.00 mg, 10.98% yield, 95% purity) as a yellow solid.

2 was obtained as follows: the triglycine (6.74 g, 35.63 mmol, 1.00 equiv.) was dissolved in NaOH (1M,

FIG. 1. (a) Synthesis of compound₁₂; (b) synthesis of [^{99m}Tc]-complex₁₃.

35.63 mL, 1.00 equiv.). Then this solution was added in one portion to a warm solution (60°C) of **1** (10.96 g, 47.39 mmol, 1.33 equiv.) in MeCN (Acetonitrile) (70.00 mL). The mixture was stirred at 60°C for 4 h, and then was stirred at 4°C for 16 h. After the evaporation of CH₃CN and dilution with water (100 mL), the mixture was acidified to pH=2 with 1 N HCl solution. After cooling overnight in the refrigerator, the formed precipitate was filtered off. **2** (6.90 g, 22.60 mmol, 63.43% yield) was produced as a white solid.

In vitro radiolabeling

The synthesis of [^{99m}Tc]-complex₁₃ is shown in Figure 1b. In brief, 20 μg of compound **12** was dissolved in 100 μL DMF, and then the mixture was added to a combined solution of 45 μL ammonium acetate (0.25 M) and 15 μL tartrate buffer, and subsequently <100 μL (~370 MBq) freshly generated ^{99m}TcO₄⁻ solution was added. Immediately after gentle vortexing, 5 μL freshly prepared 1 mg/mL SnCl₂·2H₂O solution was added. The combined solution

was incubated at room temperature for 1 h with occasional vortexing. After the reaction, the mixture was diluted using 30 mL sterile water and passed through a C18 Sep-Pak plus cartridge to trap the [^{99m}Tc]-complex₁₃ on the Sep-Pak, and thereafter dimethyl sulfoxide (DMSO; 0.2 mL) was used to elute the radioactivity afforded [^{99m}Tc]-complex₁₃. The residue radioactivity in the filter was no more than 19% of the reaction solution. The DMSO solution was diluted 10-fold with saline and the final pH was ~ 6.5 . The labeling rate and radiochemical purity were measured by radio-HPLC. Finally, an average decay-corrected specific activity of [^{99m}Tc]-complex₁₃ was documented. The chemical identity of the product was determined by HPLC to compare the retention time of precursor and co-injection of both precursor and [^{99m}Tc]-complex₁₃.

In vitro stability study

After purification, [^{99m}Tc]-complex₁₃ was diluted and incubated in 0.01 M phosphate-buffered saline (PBS), normal saline, and 0.1% BSA for 1, 2, 3, 6, and 9 h at 37°C. After incubation, the samples were filtered using a 0.22 μm Millipore filter and assessed by RP-HPLC (Agilent technologies 1260 Infinity with a variable wavelength UV detector and a gamma detector; HPLC column: phenomenex Gemini, 5 μm NX-C18 110 Å, 150 $\times$ 4.6 mm; mobile phase: 0.1% TFA in H₂O, 0.1% TFA in acetonitrile eluting with a gradient flow over 30 min of 1:99 H₂O/MeCN to 20:80 H₂O/MeCN, flow rate: 1 mL/min).

Cellular uptake studies

In brief, HCC 827 cells were cultured with RPMI 1640 cell culture medium supplemented with 10% fetal bovine serum and antibiotic mixture (100 U/mL penicillin and 0.1 mg/mL streptomycin), and incubated at 37°C in humidified air with 5% CO₂. Cells were kept in the log-phase of proliferative activity. For cell binding studies, HCC 827 cells (5×10^5 cells/mL per well) were seeded in 24-well plate and incubated for 48 h. To prevent EGFR activation, HCC 827 cells were washed with culture medium without fetal bovine serum three times. Then, the cells were incubated with [^{99m}Tc]-complex₁₃ (0.925 MBq/well) with or without the blocking precursor (1000-fold molar of compound 12) for different time intervals (5, 30, 60, 120, and 240 min). The total volume of each well was 0.5 mL. Cells were washed three times with PBS and the supernatants were collected, then the cells were detached with 1.0 M NaOH. The radioactivity of supernatant and cells was measured by γ -counter (CRC-15R; Capintec, Inc., Ramsey, NJ). The cell uptake of [^{99m}Tc]-complex₁₃ was expressed as a percentage of the total added radioactivity with decay corrected. All experiments were conducted in triplicate to ensure reproducibility.

Biodistribution studies

Eighteen healthy male ICR mice (6–8 weeks old; weighing 18–20 g) were randomly selected for *in vivo* biodistribution study. Then, the mice were injected through the tail vein with 150 μL of 5 MBq [^{99m}Tc]-complex₁₃. The mice were randomly grouped and killed at 5, 15, 30, 60, and 180 min with slightly overdosing anesthesia (0.1 M chloral hydrate, 0.2 mL/mouse) postinjection. Blood, heart, liver, spleen, lung, kidney, brain, muscle, stomach, bone, skin, small intestine,

large intestine, and thyroid were harvested and placed into weighed tubes. The radioactivity was measured using an automatic gamma counter and uptake was calculated as the percentage of the total injected dose of radioactivity per gram of tissue (%ID/g), and data were plotted as histograms.

Results

Chemistry

As shown in Figure 1a, **2** was prepared from **1** by reaction with triglycine in NaHCO₃ solution. The reaction between **3** and 3-iodoaniline produced **5**, which was then treated with Raney-Ni and NH₂NH₂·H₂O to afford **6**. **7** was treated with maleic anhydride in DCM to produce **9**. Then **9** was further reacted with **6** to provide **10**. The deprotection of **10** with HCl/EtOAc afford compound **11**. **12** was obtained by reacting compound **11** reacting with **2** with a yield of 10.98%. The reaction was monitored by LCMS. **12** was then fully characterized using spectroscopic methods and the ¹H NMR spectra and mass spectra were consistent with the expected structure (data not shown). **12** was stable in the solid state for months, as shown in the HPLC chromatogram (Fig. 2), with an R_t of 14.2 min.

Radiochemistry

The synthesis of [^{99m}Tc]-complex₁₃ is shown in Figure 1b. A single-step method followed the developed method with a

FIG. 2. Analytical HPLC chromatogram of [^{99m}Tc]-complex₁₃ co-injected with complex₁₃. *Black*: Radioactive detection. *Gray*: UV detection. There was a small difference in retention time because of the setup of a serial detector. HPLC, high-performance liquid chromatography.

FIG. 3. Stability analysis of $[^{99m}\text{Tc}]$ -complex₁₃ in 0.01 M PBS (left panel), 0.9% NaCl (middle panel), and 0.1% BSA (right panel) at 1, 2, 3, 6, and 9 h, respectively. PBS, phosphate-buffered saline.

few modifications.²⁶ In brief, the precursor was dissolved in labeling buffer and reacted with $^{99m}\text{TcO}_4^-$ and SnCl_2 to produce the $[^{99m}\text{Tc}]$ -complex₁₃. The radiochemical yields (RCYs) of $[^{99m}\text{Tc}]$ -complex₁₃ ranged from 90% to 96%, with an average yield of $93.9\% \pm 2.5\%$ ($n=15$). After purification by C18 Sep-Pak, the radiochemical purity was nearly 100%, as indicated by radio-HPLC analysis. An average specific activity of 5.1 MBq/nmol of $[^{99m}\text{Tc}]$ -complex₁₃ was achieved at the end of the synthesis (decay corrected).

In vitro stability

Samples of $[^{99m}\text{Tc}]$ -complex₁₃ were examined for its *in vitro* stability, and the analyses were repeated three times. As shown in the radio-HPLC results at different points in time, $[^{99m}\text{Tc}]$ -complex₁₃ demonstrated pronounced stability, and there was no decomposition observed in PBS, normal saline, or BSA incubation after incubation at different points in time from 1 to 9 h, suggesting excellent stability *in vitro* (Fig. 3).

Cellular uptake studies

The cellular uptake of $[^{99m}\text{Tc}]$ -complex₁₃ was evaluated using HCC 827 cells with a modified version of a previously described method,²⁸ and the results were plotted. As given in Figure 4, $[^{99m}\text{Tc}]$ -complex₁₃ showed a rapid uptake by the HCC 827 cells during the initial phase (the first 60 min). At the time point of 60 min, the accumulation of $[^{99m}\text{Tc}]$ -complex₁₃ in HCC 827 cells had reached at $1.36\% \pm 0.11\%$ ($n=3$). Addition of the precursor (1000-fold molar of compound 12) significantly decreased the internalization of the $[^{99m}\text{Tc}]$ -complex₁₃ in HCC 827 cells. After the first 30 min, the internalization of $[^{99m}\text{Tc}]$ -complex₁₃ leveled off at $0.282\% \pm 0.019\%$ ($n=3$).

Biodistribution studies

To examine the *in vivo* stability of $[^{99m}\text{Tc}]$ -complex₁₃ and its pharmacokinetic profile, the biodistribution in healthy female ICR mice was tested after injection at different points in time (Fig. 5). The blood concentration decreased from $11.36 \pm 0.67\% \text{ID/g}$ at 5 min post injection to $0.26 \pm 0.15\% \text{ID/g}$ at 15 min postinjection, which showed rather rapid blood clearance. High probe uptake in the liver was observed at 15 min postinjection with a value of $20.92 \pm 1.65\% \text{ID/g}$, but the probe cleared quickly to $0.99 \pm 0.52\% \text{ID/g}$ at 30 min postinjection. The accumulation of the probe within the wall of the small intestine increased to $28.04 \pm 1.34\% \text{ID/g}$ at 15 min postinjection and then decreased slowly to $3.72 \pm 1.03\% \text{ID/g}$ at 60 min postinjection. Accu-

mulation in the kidney lasted for 60 min postinjection. The activity in the two lungs was $25.49 \pm 6.28\% \text{ID/g}$ at 5 min postinjection, which was followed by a gradual clearance over time (from 15 to 60 min postinjection). The activity in other organs such as the heart, spleen, brain, muscle, stomach, skin, thyroid, and large intestine remained low throughout the study. The uptake of the probe by all the organs decreased to no more than $0.072 \pm 0.015\% \text{ID/g}$ (except for the great intestine: $0.689 \pm 0.076\% \text{ID/g}$) at 180 min postinjection.

Discussion

Over the past decades, the EGFR has emerged as an effective target for tumor therapy. Multiple molecular imaging probes have been developed to assess EGFR expression level in tumors, mainly including antibody-based and peptide-based imaging agents. However, because of the inherent limitations of these agents such as relatively large size, weak tissue penetration, and unfavorable receptor binding kinetics, imaging applications with these molecules have been limited.^{6,19} The tailored drugs have smaller size, which offer many advantages in molecule imaging, for example, more favorable pharmacokinetics, better tissue penetration, and more preferable flexibility in chemical modification.^{25,29}

To date, several EGFR-targeted probes have been reported in evaluating the EGFR expression, such as the carbon-11, fluorine-18, or iodine-125-labeled anilinoquinazoline derivatives, which have shown promise in imaging EGFR expression level in nonsmall cell lung carcinoma (NSCLC) patients.^{9,22,30} Whereas the use of ^{11}C and ^{18}F

FIG. 4. Uptake of $[^{99m}\text{Tc}]$ -complex₁₃ into HCC 827 cells were measured at the indicated points in time.

FIG. 5. Biodistribution (mean $\pm$ SD, $n=3$) of radioactivity (%ID/g) in the ICR mice after injection of [^{99m}Tc]-complex $_{13}$. ICR, Institute of Cancer Research; SD, standard deviation.

radionuclides in clinical trials have been hampered to a certain extent because of their short half-life, which require an on-site cyclotron for production, and repeated fabrication (especially ^{11}C) when several subjects need to be examined, the ^{125}I has a long half-life that would promote accumulation radiation dosimetry. Considering widespread application, in this study, an anilinoquinazoline derivative was conjugated with a bifunctional chelator and then labeled with ^{99m}Tc to establish a novel SPECT imaging probe, namely [^{99m}Tc]-complex $_{13}$. ^{99m}Tc is a commonly used radionuclide in the clinic, with appropriate half-life and excellent radiochemical properties for nuclear imaging.

The radiolabeling of compound $_{12}$ was rapid and efficient, with a high RCY rate, which was consistent with the convenient formulation of the labeling kit. In addition, the *in vitro* stability of [^{99m}Tc]-complex $_{13}$ was favorable in PBS and saline that could last at least for 9 h. Although [^{99m}Tc]-complex $_{13}$ was quickly bound by the albumin when incubated with 0.1% BSA, this physical adsorption did not affect the stability of [^{99m}Tc]-complex $_{13}$.

HCC 827 cell line has been characterized as a sensitive one to gefitinib, which is another EGFR inhibitor sharing an identical 4-anilinoquinazoline core scaffold with compound 12. To evaluate the potential utility of [^{99m}Tc]-complex $_{13}$ as a molecular probe for EGFR-TK imaging, the authors carried out *in vitro* cellular studies to obtain first insight into the internalization of the [^{99m}Tc]-complex $_{13}$ in HCC 827 cells at different points in time. As given in Figure 4, the results indicated that the probe bound specifically to the EGFR-expressing cells.

The biodistribution of [^{99m}Tc]-complex $_{13}$ was measured in healthy ICR mice at 5, 15, 30, 60, and 180 min postin-

jection. As given in Figure 5, the most striking feature of the [^{99m}Tc]-complex $_{13}$ was the fast clearance from the blood-stream and excretion from the major organs such as the liver, lung, and intestines, which substantially reduced background radioactivity. Of note, because of physical adsorption, a few radioactive particles with diameters larger than those of the capillary might generate. When the [^{99m}Tc]-complex $_{13}$ solution was injected into the vein, embolism might occur in the pulmonary microvasculature. But it would be substantially different from the ^{99m}Tc -MAA used clinically, the activity of which remained detectable for 2 d after injection, whereas the [^{99m}Tc]-complex $_{13}$ could be cleaned fast from the pulmonary with no significant activity 1 h later, similar to the result of the previous study.¹⁸ This might be because the aggregation was caused by simple physical adsorption rather than the chemical bond formation.

The main excretory route of the [^{99m}Tc]-complex $_{13}$ was the hepatobiliary system, as evidenced by decreasing activity in liver and accumulation of radioactivity in the mouse feces ($88.4 \pm 1.5\%$ ID/g at 24 h postinjection, data not shown). The activities of the stomach and thyroid were consistently low, which indicated no *in vivo* reoxidation to $^{99m}\text{TcO}_4^-$. Of interest, there was no obvious accumulation of radioactivity in the brain, which was consistent with previous reports advocating that normal brain expresses low EGFR.³¹ Therefore, low accumulation of [^{99m}Tc]-complex $_{13}$ in the normal brain may facilitate its use as a radiotracer in identifying brain tumors that express EGFR with a minimal signal-to-noise ratio. These tumors have been demonstrated to be more sensitive to erlotinib.^{32,33}

Clinical studies have shown that the high expression of EGFR kinase was associated with improved progression-free

survival and overall survival after treatment with EGFR kinase inhibitors compared with patients with wild-type EGFR. Furthermore, it is unfeasible to administrate repetitive invasive punctures of multiple tumor lesions in patients with advanced NSCLC.^{11,22} However, the whole-body noninvasive imaging based on probes specific for activated status of EGFR kinase could provide information of EGFR expression of the tumor lesions for forecasting treatment response before initiating the EGFR inhibitor-based therapy.³⁴ The results demonstrated the feasibility of using complex₁₃ as a SPECT tracer for screening EGFR TKIs sensitive patients. In addition, complex₁₃ holds promise not only for noninvasively screening sensitive patients for treatment of EGFR inhibitors but also for guiding tumor excision. This would bring remarkable benefits to patient's therapy response and improve the survival rate.

Conclusion

In this study the authors evaluated a highly efficient chemical synthesis of [^{99m}Tc]-complex₁₃, which produced the ^{99m}Tc labeling of the quinazoline scaffold in high yield and with high radiochemical purity. Animal studies indicated a high *in vivo* stability and a fast clearance from the circulation. Biodistribution studies of [^{99m}Tc]-complex₁₃ in healthy mice have been favorable. Taken together, preliminary biological studies have been carried out, and *in vivo* studies in tumor-bearing mice are warranted to prove its feasibility as an imaging probe for early detection and staging of EGFR-positive tumors. Altogether, the results suggest that the tracer has the potential to be used as a SPECT probe to identify EGFR TKI sensitive patients.

Authors' Contributions

Z.S., Methodology; P.H., Methodology; J.Z., Software; Q.L., Software; Y.X., Design; D.C., Design. All authors have reviewed the article and approved publication before submission.

Disclosure Statement

No competing financial interests exist.

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