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Integrin $\alpha_v\beta_3$–Targeted Imaging of Lung Cancer[1]

Xiaoyuan Chen[*,†], Eric Sievers*[‡], Yingping Hou*[†], Ryan Park*[*], Michel Tohme*[*], Robert Bart*[‡], Ross Bremner*[‡], James R. Bading*[*] and Peter S. Conti*[*]

*Molecular Imaging Science Center, Department of Radiology, University of Southern California, Los Angeles, CA 90033, USA; †Molecular Imaging Program at Stanford (MIPS), Stanford University School of Medicine, 300 Pasteur Drive, Edwards Building, Room 354, Stanford, CA 94305-5344, USA; ‡Department of Cardiothoracic Surgery, University of Southern California, Los Angeles, CA 90033, USA

Abstract

A series of radiolabeled cyclic arginine–glycine–aspartic acid (RGD) peptide ligands for cell adhesion molecule integrin $\alpha_v\beta_3$–targeted tumor angiogenesis targeting are being developed in our laboratory. In this study, this effort continues by applying a positron emitter ^{64}Cu-labeled PEGylated dimeric RGD peptide radiotracer ^{64}Cu-DOTA-PEG-E[c(RGDyK)]$_2$ for lung cancer imaging. The PEGylated RGD peptide indicated integrin $\alpha_v\beta_3$ avidity, but the PEGylation reduced the receptor binding affinity of this ligand compared to the unmodified RGD dimer. The radiotracer revealed rapid blood clearance and predominant renal clearance route. The minimum nonspecific activity accumulation in normal lung tissue and heart rendered high-quality orthotopic lung cancer tumor images, enabling clear demarcation of both the primary tumor at the upper lobe of the left lung, as well as metastases in the mediastinum, contralateral lung, and diaphragm. As a comparison, fluorodeoxyglucose (FDG) scans on the same mice were only able to identify the primary tumor, with the metastatic lesions masked by intense cardiac uptake and high lung background. ^{64}Cu-DOTA-PEG-E[c(RGDyK)]$_2$ is an excellent positron emission tomography (PET) tracer for integrin-positive tumor imaging. Further studies to improve the receptor binding affinity of the tracer and subsequently to increase the magnitude of tumor uptake without comprising the favorable *in vivo* kinetics are currently in progress.

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Introduction

Lung cancer is the leading cause of cancer death for both men and women, killing more people than breast, prostate, colon, and pancreas cancers combined [1]. The major histologic types of lung cancer are squamous cell carcinoma, adenocarcinoma, large cell carcinoma (referred to as non small cell lung cancer, or NSCLC) and small cell lung carcinoma (SCLC). Early detection and treatment may lead to improved survival for some types of lung cancer [2]. Even patients with the earliest surgical stage (T_1N_0) have disseminated disease between 15% and 30% of the time [3]. Chemotherapy, surgery, and radiation therapy have been shown to control symptoms and improve quality of life; there remains no standard optimal therapy regimen for NSCLC [4]. The mechanisms of resistance to drug and radiation therapy are poorly understood [5]. Despite significant progress, the molecular events underlying the development of lung cancer are largely unknown. No drug has been found useful in the prevention of lung cancer [6]. Although there are proven means of early diagnosis available for lung cancer, whether these techniques are effective in terms of improving patient survival or outcome remains unknown [7].

Lung cancer diagnosis based on histopathology requires fine-needle biopsy, bronchoscopy, or open-lung biopsy to differentiate benign from malignant lesions, before and often after surgical resection or radiation therapy of the primary tumor lesions. Bronchoscopy, including bronchial washings and brushing, has a sensitivity of 65% for malignancy, and transbronchial biopsy increases the sensitivity to 80% for lesions that are accessible. Pneumothorax and associated morbidity can occur following these invasive procedures. Conventional anatomic imaging techniques such as chest X-ray, computed tomography (CT) scans, bone scans, or magnetic resonance imaging (MRI) can neither accurately stage the disease when metastases occurs without anatomic change, nor differentiate between malignant and nonmalignant tumors. Fluorodeoxyglucose positron emission tomography (FDG-PET) imaging, which visualizes glucose utilization by lung cancer cells, is increasingly used for the management of patients with lung cancer, especially those with NSCLC. FDG-PET quantifies a specific cellular process, namely, phosphorylation of 2-deoxyglucose by hexokinase. The technique

Abbreviations: RGD, arginine–glycine–aspartic acid; PET, positron emission tomography; NSCLC, non small cell lung cancer; DOTA, 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid; PEG, poly(ethylene glycol)

Address all correspondence to: Peter S. Conti, MD, PhD, USC Department of Radiology, 1510 San Pablo Street, Suite 350, Los Angeles, CA 90033. E-mail: pconti@usc.edu

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provides highly sensitive and specific data for the diagnosis, staging, and restaging of NSCLC, without the need for a biopsy, and is now well accepted by most physicians as an effective complement to existing imaging modalities [8–10]. FDG-PET may also evolve as a predictor of response after local or systemic treatment [11].

Although assessment of tissue metabolism using the tracer FDG provides useful imaging of lung neoplasms, it is relatively nonspecific and is usually less useful for imaging tumors that have very low growth rates, or as a guide for delivery of specific molecular therapy. The development of tracers that target specific molecular or genetic abnormalities, which are the basis of lung cancer, is therefore essential for the development and utility of clinically relevant PET procedures that can be use to assess the efficacy of therapeutic drugs designed to treat those abnormalities. The goals are: to allow early detection and characterization of the disease, to provide more timely and direct assessments of treatment effects, and to obtain more fundamental understanding of the disease process. The rapidly expanding knowledge of the molecular pathogenesis of lung cancer indicates that respiratory epithelial cells require many genetic alterations to become invasive, leading to metastatic cancer. In addition, most solid tumors are angiogenesis-dependent [12]. Antiangiogenic therapy has been shown to prevent tumor growth and even to cause tumor regression in various tumor models including lung cancer [13]. NSCLC growth, angiogenesis, invasion, and metastases to specific organs are dependent on an orchestrated series of events that include: cellular transformation; establishment of a proangiogenic environment; tumor cell proliferation, invasion, and entry into the circulation; and tumor cell trafficking and metastatic tumor growth in specific organs [14]. Cell adhesion receptors of the integrin family, which are responsible for a wide range of cell–extracellular matrix (ECM) and cell–cell interactions, have been well studied in many tumor types including brain, breast, skin, and ovarian tumors. However, little is known regarding the function of integrins in lung cancer growth and metastasis [15].

Due to its high expression on tumor vasculature and tumor cells compared to resting endothelial cells and normal tissues, $\alpha_v\beta_3$ integrin is an excellent target for integrin-targeted interventions [16,17]. It has been shown that $\alpha_v\beta_3$ integrin antagonists, such as monoclonal antibodies, arginine–glycine–aspartic acid (RGD) peptides, and small molecules, block angiogenesis and tumor growth by selectively promoting apoptosis of vascular endothelial cells [16–18]. The development of noninvasive methods to visualize and quantify α_v integrin expression *in vivo* appears to be crucial for the success of antiangiogenic therapy based on integrin antagonism [17,19]. A number of molecular probes have been developed for MRI [20,21], PET [22–30], single-photon computed tomography (SPECT) [31–35], and ultrasound [36] imaging applications.

^{64}Cu-labeled RGD peptides are of particular interest because ^{64}Cu [$t_{1/2}$ = 12.8 h; 40% β^- (656 keV); 19% β^+ (600 keV); 38% EC] is an attractive radionuclide for both PET imaging and targeted radiotherapy of cancer [37]. We first coupled monomeric RGD peptide c(RGDyK) with the macrocyclic chelator, 1,4,7,10-tetraazacyclododecane-*N,N′,N″*,*N‴*-tetraacetic acid (DOTA), and labeled the RGD-DOTA conjugate with ^{64}Cu for tumor targeting. The radiotracer showed intermediate tumor uptake but also high retention in liver and kidney [23]. Introduction of a bifunctional poly(ethylene glycol) (PEG; M_W = 3,400) moiety between DOTA and RGD led to significantly improved *in vivo* kinetics of the resulting radiotracer ^{64}Cu-DOTA-PEG-RGD compared to that of ^{64}Cu-DOTA-RGD [25]. We also observed that a dimeric RGD peptide E[c(RGDyK]$_2$ with higher integrin binding affinity, when conjugated with DOTA and labeled with ^{64}Cu, yielded almost twice as much uptake in tumors, as well as significantly increased renal activity accumulation compared to the monomeric analogue [27]. In this study, we sought to extend this effort by examining integrin expression in lung cancer using noninvasive PET imaging and a novel radiotracer—^{64}Cu-labeled PEGylated dimeric RGD peptide ^{64}Cu-DOTA-PEG-E[c(RGDyK)]$_2$ (Figure 1).

Materials and Methods

Materials and Analyses

DOTA was purchased from Macrocyclics, Inc. (Dallas, TX). *N*-hydroxysulfonosuccinimide (SNHS), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), and Chelex 100 (50–100 mesh) were obtained from Aldrich (St. Louis, MO). Heterofunctional PEG (M_W 3400), *t*-Boc-NH-PEG-CO_2NHS, was obtained from Nektar, Inc. (San Carlos, CA). Cyclic RGD peptide c(RGDyK) was synthesized through solution cyclization of the fully protected linear pentapeptide, H-Gly-Asp(OtBu)-D-Tyr(OtBu)-Lys(Boc)-Arg(Pbf)-OH, followed by TFA deprotection [22–24]. Dimeric RGD peptide E[c(RGDyK)]$_2$ was prepared by coupling Boc-Glu-OH with two equivalents of monomeric RGD peptide c(RGDyK) followed by TFA cleavage [26,27]. Radio-thin layer chromatography (TLC) was performed using MKC18F plates (Whatman, Clifton, NJ), a Bioscan system 200, and Winscan (Washington, DC) software, version 2.2. Reversed-phase extraction C-18 SepPak cartridges were obtained from Waters (Milford, MA). ^{64}Cu was produced on a CS-15 biomedical cyclotron at THE Washington University School of Medicine by the ^{64}Ni(p,n)^{64}Cu nuclear reaction [37]. Chromalux HB microplates were obtained from Dynex Technologies (Chantilly, VA). [^{125}I]echistatin labeled by the lactoperoxidase method to a specific activity of 2000 Ci/mmol was from Amersham Biosciences (Piscataway, NJ). Echistatin was purchased from Sigma (St. Louis, MO).

Semipreparative reversed-phase high-performance liquid chromatography (HPLC) was accomplished on a Waters 515 chromatography system with a 486 tunable absorbance detector. Version 7.2.1 Labtech Notebook/XE software (Andover, MA) was used to record chromatograms. Purification was performed on a Vydac protein and peptide column 218TP510 (5 μm, 250 $\times$ 10 mm). The flow was 5 ml/min, with the mobile phase starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in