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Radiolabeled Multimeric Cyclic RGD Peptides as Integrin $\alpha_v\beta_3$ Targeted Radiotracers for Tumor Imaging

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Abstract: Integrin $\alpha_v\beta_3$ plays a significant role in tumor angiogenesis and is a receptor for the extracellular matrix proteins with the exposed arginine-glycine-aspartic (RGD) tripeptide sequence. These include vitronectin, fibronectin, fibrinogen, lamin, collagen, Von Willibrand's factor, osteoponin, and adenovirus particles. Integrin $\alpha_v\beta_3$ is expressed at low levels on epithelial cells and mature endothelial cells, but it is overexpressed on the activated endothelial cells of tumor neovasculature and some tumor cells. The highly restricted expression of integrin $\alpha_v\beta_3$ during tumor growth, invasion, and metastasis presents an interesting molecular target for both early detection and treatment of rapidly growing solid tumors. In the past decade, many radiolabeled linear and cyclic RGD peptide antagonists have been evaluated as the integrin $\alpha_v\beta_3$ targeted radiotracers. Significant progress has been made on their use for imaging tumors of different origin by single photon emission computed tomography (SPECT) or positron emission tomography (PET) in several tumor-bearing animal models. [^{18}F]Galacto-RGD is under clinical investigation as the first integrin $\alpha_v\beta_3$ targeted radiotracer for noninvasive visualization of the activated integrin $\alpha_v\beta_3$ in cancer patients. This review will focus on the radiolabeled multimeric cyclic RGD peptides (dimers and tetramers) useful as radiotracers to image the tumor integrin $\alpha_v\beta_3$ expression by SPECT and PET, and some fundamental aspects for the development of integrin $\alpha_v\beta_3$ targeted radiotracers. These include the choice of radionuclide and bifunctional chelators, selection of targeting biomolecules, and factors influencing the integrin $\alpha_v\beta_3$ binding affinity and tumor uptake, as well as different approaches for modification of radiotracer pharmacokinetics.

Keywords: Integrin $\alpha_v\beta_3$; radiotracers; radiopharmaceuticals; tumor imaging

Introduction

Tumors produce many angiogenic factors, which are able to activate endothelial cells in the established blood vessels and induce endothelial proliferation, migration, and new vessel formation (angiogenesis) through a series of sequential but partially overlapping steps. Angiogenesis is a requirement for both tumor growth and metastasis.[1−7] Without the for-

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mation of neovasculature to provide oxygen and nutrients, tumors cannot grow beyond 1−2 mm in size. Once vascularized, previously dormant tumors begin to grow rapidly and their volumes increase exponentially.

The angiogenic process depends on vascular endothelial cell migration and invasion, which are regulated by cell adhesion receptors. Integrins are such a family of proteins that facilitate cellular adhesion to and migration on the extracellular matrix proteins found in intercellular spaces and basement membranes, and regulate cellular entry and withdrawal from the cell cycle.[7−10] Integrin $\alpha_v\beta_3$ serves as a receptor for a variety of extracellular matrix proteins with the exposed arginine-glycine-aspartic (RGD) tripeptide sequence.[8−13] These include vitronectin, fibronectin, fibrinogen, lamin, collagen, Von Willibrand's factor, osteoponin, and adenovirus particles. Integrin $\alpha_v\beta_3$ is expressed at low levels on epithelial cells and mature endothelial cells; but it is highly expressed on the activated endothelial cells in the neovasculature of tumors, including osteosarcomas, neuroblastomas, glioblastomas, melanomas, lung carcinomas, and breast cancer.[14−20] A recent study shows that the integrin $\alpha_v\beta_3$ is overexpressed on both endothelial cells and tumor cells in human breast cancer xenografts.[21] The integrin $\alpha_v\beta_3$ expression correlates well with tumor progression and invasiveness of melanoma, glioma, and ovarian and breast cancers.[10−20] The highly restricted expression of integrin $\alpha_v\beta_3$ during tumor growth, invasion and metastasis present an interesting molecular target for early diagnosis of rapidly growing and metastatic tumors.[21−27]

Many high-affinity integrin $\alpha_v\beta_3$ antagonists (peptides and peptidomimetics) have been identified.[28−38] These anti-

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angiogenic agents are designed to block the formation of new blood vessels, thereby starving tumors and inhibiting tumor growth, and have been extensively reviewed.[2,3,39−42] Inhibition of integrin $\alpha_v\beta_3$ with cyclic RGD peptides, such as EMD 121974, has been shown to induce endothelial apoptosis,[28] inhibit angiogenesis,[29,41,42] and increase endothelial monolayer permeability.[43] The inhibition of integrin $\alpha_v\beta_3$ activity has also been associated with decreased tumor growth in breast cancer xenografts.[42,43] Synergy of EMD 121974 with radioimmunotherapy (RIT) has also resulted in increased efficacy of therapy in a murine breast cancer model.[43] Moreover, blocking of the integrin $\alpha_v\beta_3$ activity can reduce the invasiveness and spread of metastasis.[29,41−43] However, there are several challenges in antiangiogenic clinical trials: (a) selection of the right patients to enter clinical trials who will benefit most from the specific antiangiogenic trials, (b) monitoring the therapeutic efficacy of the antiangiogenic treatment, and (c) optimization of dose and schedule for antiangiogenic treatment in the cancer patient. Thus, it would be highly advantageous to develop an imaging agent that could be used to noninvasively visualize and quantify the integrin $\alpha_v\beta_3$ expression level before and during antiangiogenic therapy.

In the past decade, many radiolabeled RGD peptides have been evaluated for their potential as the integrin $\alpha_v\beta_3$ targeted radiotracers. Significant progress has been made on their use in imaging tumors by SPECT (single photon emission computed tomography) or PET (positron emission tomography) in preclinical animal models and human clinical trials. Several review articles appeared recently covering radiolabeled peptide and non-peptide integrin $\alpha_v\beta_3$ antagonists.[22−27] This review is not intended to be an exhaustive review of current literature on radiolabeled RGD peptides. Instead, it will focus on the use of radiolabeled cyclic RGD peptide dimers and tetramers as new radiotracers to image the integrin $\alpha_v\beta_3$ expression by SPECT and PET, and some fundamental aspects in the development of integrin $\alpha_v\beta_3$ targeted radiotracers. These include choice of radionuclide, selection of BM, and factors influencing the integrin $\alpha_v\beta_3$ binding affinity and tumor uptake of radiolabeled cyclic RGD peptides, as well as different approaches to modify the radiotracer pharmacokinetics.

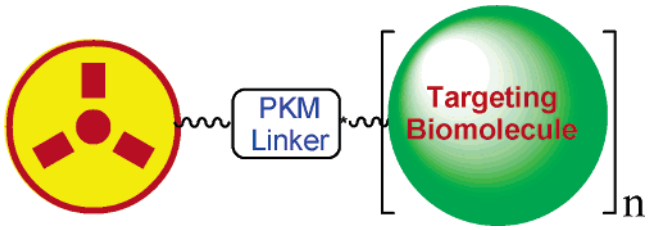

Figure 1. Schematic presentation of the radiopharmaceuticals design. The targeting biomolecule is a cyclic RGD peptide. The PKM linker is used for modification of pharmacokinetics of radiotracers. For the metal-containing radiotracers, a BFC is used to attach the metallic radionuclide to the targeting biomolecule. For ^{18}F-based radiotracers, an organic precursor or synthon is often needed to attach ^{18}F onto the cyclic RGD peptide.

Radiopharmaceutical Design

In general, an integrin $\alpha_v\beta_3$ targeted radiotracer can be divided into three parts (Figure 1): the targeting biomolecule (BM = cyclic RGD peptide), PKM (pharmacokinetic modifying) linker, and radiometal chelate or ^{18}F-containing synthon. The cyclic RGD peptide serves as a vehicle to carry radionuclide to the integrin $\alpha_v\beta_3$ overexpressed on tumor cells and the activated endothelial cells of tumor neovasculature. For the metal-containing radiotracer, a bifunctional chelator (BFC) is used to attach the metallic radionuclide to the targeting biomolecule. For ^{18}F-based radiotracers, an organic precursor or synthon is often needed to attach ^{18}F onto the cyclic RGD peptide.

Characteristics of Optimal Radiotracers. For a new integrin $\alpha_v\beta_3$ targeted radiotracer to be successful, it must show clinical indications for several high-incidence tumor types (e.g., breast, colorectal, and lung). The radiotracer should be able to have high tumor uptake with a diagnostically useful target-to-background (T/B) ratio in a short period of time. To achieve this goal, the radiotracer should have a fast blood clearance to minimize nontarget radioactivity. The time for radiotracer to reach the target should also be short. Integrin $\alpha_v\beta_3$ binding rate should be fast, and the dissociation rate should be slow. In this way, the radioactivity accumulation in the tumor can be maximized. Since most high-incidence tumors (namely, lung, colorectal, and breast cancers metastatic to the lymphatic system) occur in torso, renal excretion is necessary to avoid accumulation of

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radioactivity in the gastrointestinal tract, which may interfere with interpretation of tumor activity in the abdominal region.

It is important to note that early detection is only the first step of cancer patient management. The integrin $\alpha_v\beta_3$ targeted radiotracer should also be able (1) to distinguish between benign and malignant tumors, (2) to follow the course of a particular tumor type and its response to antiangiogenic therapy, and (3) to predict success or failure of a specific therapeutic regime for a given type of tumor in a particular cancer patient.

Choice of Radionuclide. Nearly 80% of radiopharmaceuticals used in the nuclear medicine department are ^{99m}Tc compounds for planar and SPECT imaging due to optimal nuclear properties of ^{99m}Tc and its easy availability at low cost.[44−47] The 6 h half-life is long enough to allow radiopharmacists to carry out radiosynthesis and for physicians to collect clinically useful images. At the same time, it is short enough to permit administration of 20−30 mCi of ^{99m}Tc without imposing a significant radiation dose to the patient. The monochromatic 140 keV photons are readily collimated to give high-quality images with high spatial resolution.

^{18}F is a cyclotron-produced isotope suitable for PET imaging. It has a half-life of 110 min. For the last several years, ^{18}F-FDG (FDG = 2-fluoro-2-deoxyglucose) has been widely used as an imaging tool for diagnosis of cancers and brain and cardiovascular diseases. Despite its short half-life, ^{18}F-labeled biomolecules have become much more accessible to researchers and clinicians in the medium-sized institutions. The availability of mobile trailers for PET imaging will make the development of ^{18}F-labeled tumor-specific radiotracers a reality in the near future.

^{64}Cu is another PET isotope useful for development of target-specific radiotracers. It has a half-life of 12.7 h with a low β^+ emission (18%) and maximum β^+ energy of 0.66 MeV. Despite poor nuclear properties, the long half-life makes ^{64}Cu feasible for PET imaging with radiolabeled small biomolecules. Copper radionuclides and related radiochemistry have been reviewed extensively by Blower et al.[48] Nuclear medicine applications of ^{64}Cu-labeled proteins and peptides have been reviewed by Anderson et al.[49,50]

Bifunctional Chelators. The choice of BFC depends on the radionuclide. Since ^{18}F can be incorporated into the biomolecule via a covalent bond directly or through a PKM linker, there is no need for the BFC. In contrast, the BFC is an important part of both ^{99m}Tc- and ^{64}Cu-based radiotracers. BFCs for ^{99m}Tc-labeling of small biomolecules have been reviewed extensively.[44,46,47] Among various BFCs (Figure 2), 6-hydazinonicotinic acid (HYNIC) is of great interest due to its high ^{99m}Tc-labeling efficiency (rapid radiolabeling and high radiolabeling yield), the high solution stability of its ^{99m}Tc complexes, and the easy use of different coligands for modification of biodistribution characteristic of the ^{99m}Tc-labeled small biomolecules.[51] DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and its derivatives (Figure 2) will be used as the BFCs for ^{64}Cu-labeling small biomolecules mainly due to the high solution stability of their ^{64}Cu chelates.[48−50]

Targeting Biomolecules. Selection of targeting biomolecules depends largely on their integrin $\alpha_v\beta_3$ binding affinity and selectivity. In general, the targeting biomolecule should be an antagonist since the use of an agonist may cause certain unwanted side effects even at low dose. It should have very high integrin $\alpha_v\beta_3$ binding affinity with IC_{50} values in the nanomolar range with a high selectivity for integrin $\alpha_v\beta_3$ over glycoprotein IIb/IIIa (GPIIb/IIIa). In the past decades, many high-affinity RGD peptide antagonists have been identified and have been shown to induce endothelial apoptosis[28] and to inhibit angiogenesis of tumors.[29,41,42] Figure 3 shows several examples of cyclic RGD peptides, such as EMD 121974,[32,33] that have high affinity and selectivity for the integrin $\alpha_v\beta_3$ with IC_{50} in the nanomolar range. Arrows indicate possible sites for attachment of a radioisotope or conjugation of the radiometal chelate. These cyclic RGD peptides have been successfully used as targeting biomolecules to carry the radionuclide to tumor cells and tumor neovasculature.

Why Small RGD Peptides? There are several advantages in using radiolabeled small RGD peptides as radiotracers. The RGD tripeptide sequences are natural binding units that are responsible for interactions between integrin $\alpha_v\beta_3$ and extracellular matrix proteins, such as vitronectin, fibronectin, fibrinogen, lamin, collagen, and adenovirus particles. Small RGD peptides can tolerate harsh conditions for radiolabeling and chemical modification. Unlike antibodies, they are less likely to be immunogenic. Because of their small size, they have a rapid blood clearance. The faster blood clearance results in adequate T/B ratios earlier so that it is practical to use ^{99m}Tc and ^{18}F, which are the preferred radionuclides for SPECT and PET, respectively. When labeled with a suitable radionuclide, radiotracers targeting integrin $\alpha_v\beta_3$ might be useful for tumor radiotherapy.[25,46,52]

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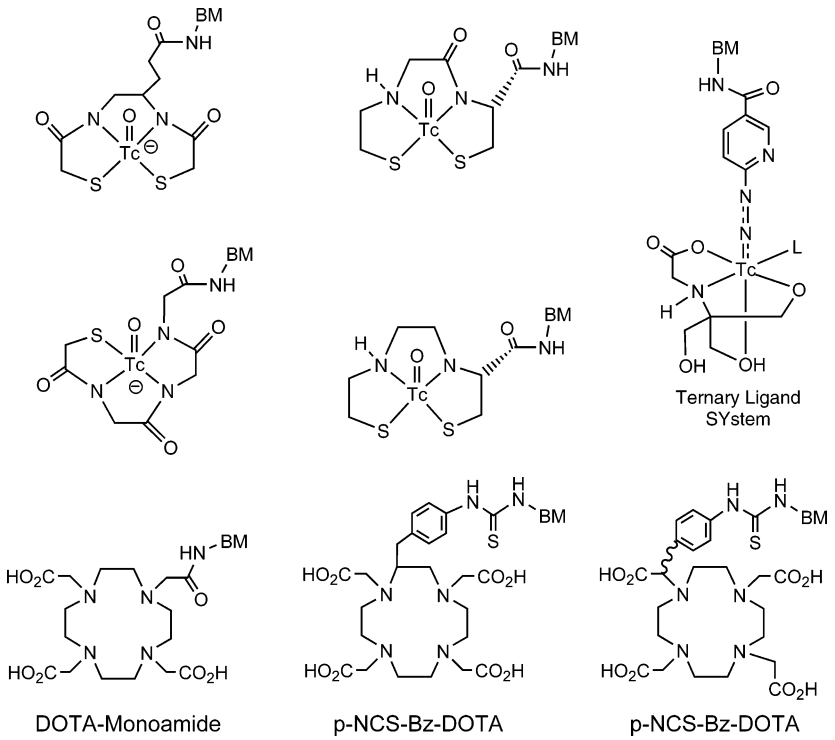

Figure 2. ChemDraw structures of bifunctional chelating systems useful for radiolabeling of biomolecules (BM) with metallic radionuclides, such as ^{99m}Tc and ^{64}Cu.

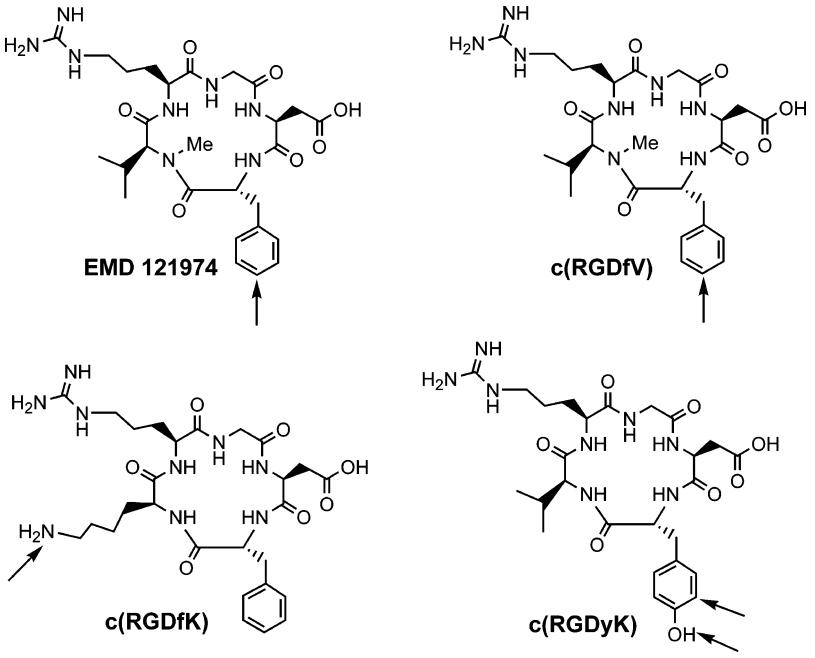

Figure 3. ChemDraw structures of cyclic RGD peptides useful as targeting biomolecules for the integrin $\alpha_v\beta_3$ targeted radiotracers. Arrows indicate possible sites for attachment of a radioisotope or conjugation of the radiometal chelate.

Factors Influencing Integrin $\alpha_v\beta_3$ Binding

Linear RGD Peptides. A major challenge in designing integrin $\alpha_v\beta_3$ antagonists is to improve the integrin $\alpha_v\beta_3$ binding affinity. In theory, targeting biomolecules can be linear or cyclic if they contain one or more RGD tripeptide sequences. A ^{99m}Tc-labeled linear decapeptide αP2 (RGD-SCRGDSY) has been reported.[53] There are two RGD motifs in the peptide αP2. In patients diagnosed with metastatic melanoma, six out of eight lymph node metastases (75%) and all 11 other tumor sites were successfully imaged using ^{99m}Tc-labeled peptide αP2.[53] An ^{18}F-labeled linear RGD peptide (KPQVTRGDFTEG-NH_2) has recently been prepared

via rapid solid-phase synthesis.[54] It has only one RGD tripeptide sequence, and the biodistribution data show a very low tumor uptake in Balb/c mice bearing colorectal tumors. A major disadvantage using linear RGD peptides is that they are often degraded rapidly in serum by proteases. The combination of low binding affinity (IC_{50} > 100 nM), lack of specificity (integrin $\alpha_v\beta_3$ versus GPIIb/IIIa), and rapid degradation makes them nonoptimal for tumor imaging.[25,45]

Cylic RGD Peptides. It has been shown that cyclization of RGD peptides via linkers, such as S−S disulfide, thioether, and rigid aromatic rings or other heterocycles, leads to the increased receptor binding affinity and selectivity.[30,31,55] However, there is little evidence to show that any particular mode of cyclization will result in high-affinity receptor binding. One thing is clear: Cyclic peptides with the conformation at the receptor-binding motif similar to that of the natural receptor ligand are likely to have higher receptor binding affinity and better selectivity.[31,55] After extensive structure−activity-relationship studies,[30,31,33,55] it was found that incorporation of the RGD sequence into a cyclic pentapeptide framework (Figure 3) significantly increases the binding affinity and selectivity for integrin $\alpha_v\beta_3$. It was also shown that the amino acid in position 5 has no significant impact on the integrin $\alpha_v\beta_3$ binding affinity. For example, the valine amino acid (V) of c(RGDfV) can be replaced by lysine (K) or glutamic acid (E) to afford cyclic pentapeptides, c(RGDfK) and c(RGDfE), respectively, without changing their integrin $\alpha_v\beta_3$ binding affinity.

Multivalency Concept. Since the natural mode of interactions between integrin $\alpha_v\beta_3$ and RGD-containing proteins, such as vitronectin, fibronectin, fibrinogen, and lamin, may involve multivalent binding sites, the idea to improve the integrin $\alpha_v\beta_3$ binding affinity with multivalent cyclic RGD peptides could provide more effective antagonists with better targeting capability and higher cellular uptake through the integrin-dependent endocytosis pathway.[56] Multivalent interactions are used in such a way that weak ligand−receptor interactions may become biologically relevant.[57] The multivalent concept has been used for development of radiotracers. For example, Goel et al. reported the ^{99m}Tc-labeled divalent and tetravalent scFv's of mAb CC49.[58] Results from biodistribution studies showed that ^{99m}Tc-[sc(Fv)$_2$]$_2$ had approximately 3-fold higher tumor localization than ^{99m}Tc-sv(Fv)$_2$.[58] Viti et al. also reported increased binding affinity and tumor targeting capability for the ^{125}I-labeled divalent recombinant antibody fragment.[59] Results from biodistribution studies in athymic nude mice bearing sc grafted F9 murine teratocarcinoma showed that ^{125}I-scFv(E1) and ^{125}I-scFv(L19) dimers had tumor uptake that was 3−5 times higher than that of ^{125}I-scFv(E1) and ^{125}I-scFv(L19) monomers.[59] Similarly, Kok et al. showed increased affinity of RGD ligands due to multivalent interactions.[60]

Cyclic RGDfK and RGDyK Dimers. Rajopadhye and co-workers were the first to use cyclic RGD dimers, such as E[c(RGDfK)]$_2$ (Figure 4), as targeting biomolecules for the development of diagnostic (^{99m}Tc and ^{111}In) and therapeutic (^{90}Y and ^{177}Lu) radiotracers.[61−67] Recently, Chen

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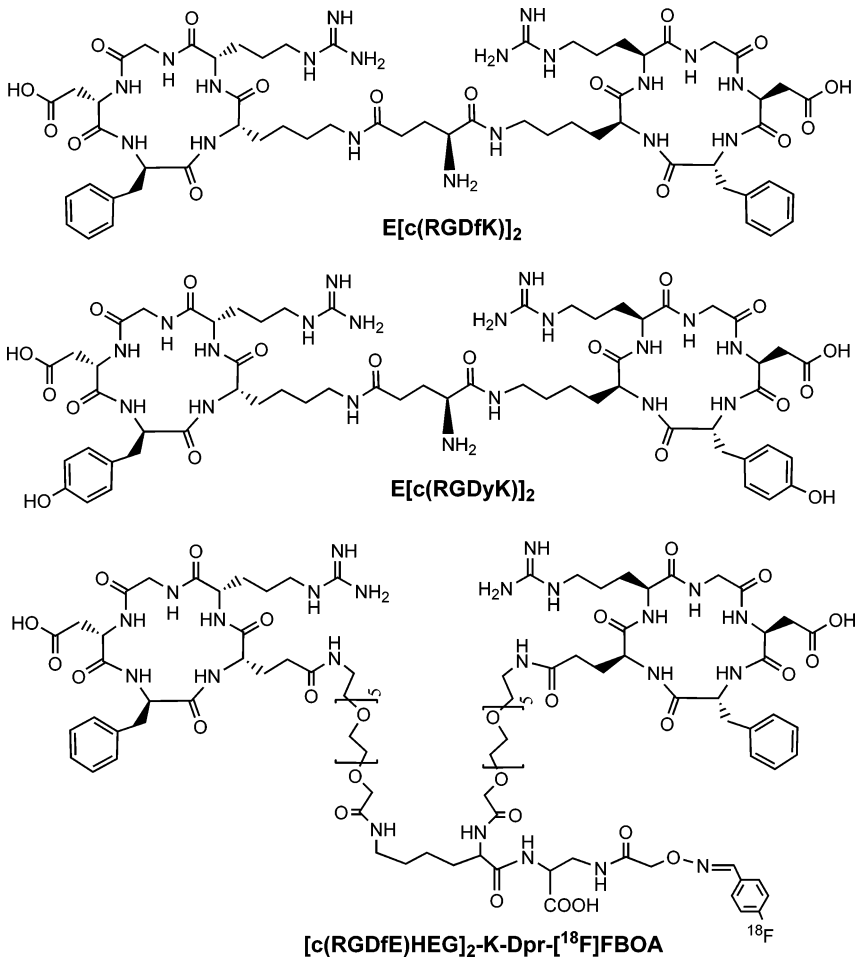

Figure 4. ChemDraw structures of cyclic RGD dimers: E[c(RGDfK)]$_2$, E[c(RGDyK)]$_2$, and [c(RGDfE)HEG]$_2$-K-Dpr-[^{18}F]FBOA.

et al.[68,69] reported the use of E[c(RGDyK)]$_2$ for preparation of ^{64}Cu- and ^{18}F-based PET radiotracers. The success of E[c(RGDfK)]$_2$ and E[c(RGDyK)]$_2$ as targeting biomolecules is very intriguing. Given the short distance (~20 bond distance) between two cyclic RGD peptides, it is unlikely that they would bind to the adjacent integrin $\alpha_v\beta_3$ receptors simultaneously. However, the binding of one RGD motif to integrin $\alpha_v\beta_3$ will significantly increase "local concentration" of the second RGD motif in the vicinity of the receptor-binding site. This might lead to the enhanced integrin $\alpha_v\beta_3$ binding rate or the reduced dissociation rate of the cyclic RGD peptide from the integrin $\alpha_v\beta_3$. The high "local RGD peptide concentration" may explain the higher tumor uptake and longer tumor retention times of the radiolabeled (^{99m}Tc, ^{111}In, ^{90}Y, ^{18}F, and ^{64}Cu) cyclic RGD dimers as compared to their monomeric analogues.[64−69]

Cyclic RGDfE Dimers. Recently, Poethko et al. reported the ^{18}F-labeled cyclic RGDfE peptide dimer (Figure 4: [c(RGDfE)HEG]$_2$-K-Dpr-[^{18}F]FBOA) and found that [c(RGDfE)HEG]$_2$-K-Dpr-[^{18}F]FBOA had much higher integrin $\alpha_v\beta_3$ binding affinity to the immobilized integrin $\alpha_v\beta_3$ than its monomeric analogue: c(RGDfE)HEG-Dpr-[^{18}F]FBOA.[70−73] Apparently, the distance between two cyclic RGDfE moieties

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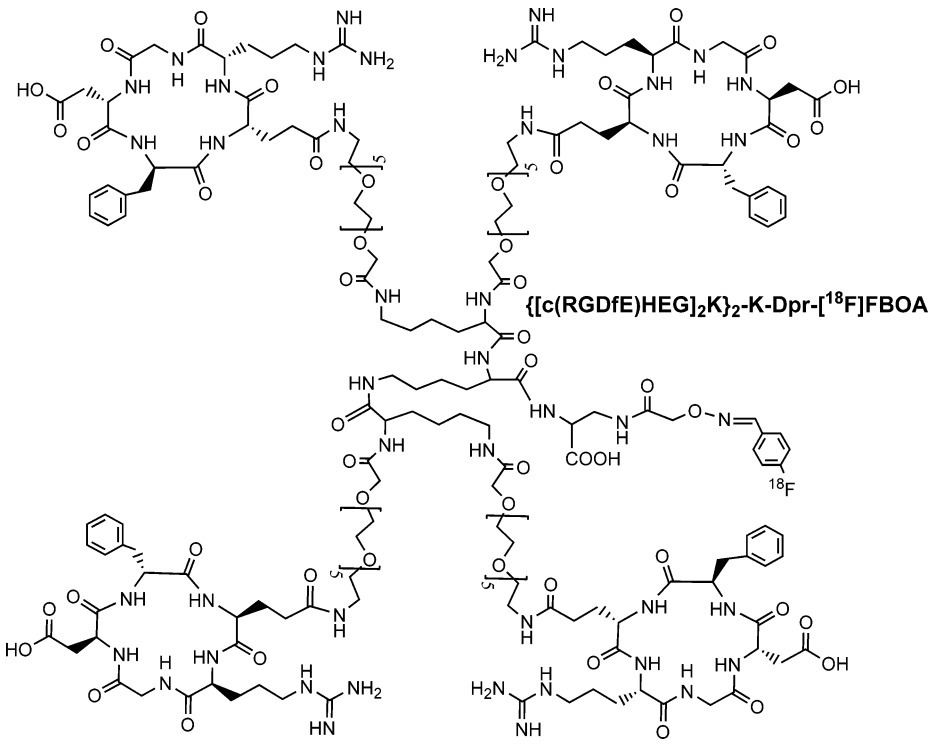

Figure 5. ChemDraw structure of an ^{18}F-labeled cyclic RGDfE tetramer, {[c(RGDfE)HEG]$_2$K}$_2$-K-Dpr-[^{18}F]FBOA.

in c(RGDfE)HEG-Dpr-[^{18}F]FBOA is long enough for them to bind to adjacent integrin $\alpha_v\beta_3$ receptors simultaneously.

Cyclic RGD Tetramers. Several research groups applied the multivalent concept to prepare cyclic RGD peptide tetramers. For example, Boturyn et al. reported a series of cyclic RGDfK tetramers[56] and found that the increase of peptide multiplicity significantly enhances the integrin $\alpha_v\beta_3$ binding affinity and internalization in the CHO3a (Chinese hamster ovary) in vitro assays. Kessler et al. reported a cyclic RGDfE peptide tetramer (Figure 5) that had better integrin $\alpha_v\beta_3$ binding affinity than its monomeric and dimeric analogues,[70−73] and they found that the minimum linker length is about 3.5 nm (~25 bond distances) for simultaneous binding of two c(RGDfE) motifs in the immobilized integrin $\alpha_v\beta_3$ assay.[70]

Recently, Liu et al. prepared a cyclic RGDfK tetramer, E{E[c(RGDfK)]$_2$}$_2$ (Figure 6), which has been used to develop integrin $\alpha_v\beta_3$ targeted radiotracers for tumor imaging by SPECT and PET.[74−76] It was found that the integrin $\alpha_v\beta_3$ binding affinity of E{E[c(RGDfK)]$_2$}$_2$ ($IC_{50} = 15.1 \pm 1.1$ nM) is higher than that of E[c(RGDfK)]$_2$ ($IC_{50} = 32.2 \pm 2.1$ nM) against ^{125}I-echistatin using the integrin $\alpha_v\beta_3$ positive U87MG human glioma cancer cells.[75] The longest distance between two RGD motifs is ~30 bond lengths, which is long enough for them to bind to adjacent integrin $\alpha_v\beta_3$ receptors simultaneously. Apparently, the increase of peptide multiplicity enhances the integrin $\alpha_v\beta_3$ binding affinity. Results from biodistribution and imaging studies also showed that the use of E{E[c(RGDfK)]$_2$}$_2$ as the targeting biomolecule enhances the tumor-targeting capability of radiotracers (^{99m}Tc, ^{111}In, and ^{64}Cu).[74−76]

Metal Chelate Effect. Table 1 lists the integrin $\alpha_v\beta_3$ binding data for selected HYNIC and DOTA conjugates (Figure 7) and their metal complexes. HYNIC-c(RGKfD) was designed as a negative control, and has a very low binding affinity for integrin $\alpha_v\beta_3$ with $IC_{50} > 10\,000$ nM, clearly indicating that the RGD sequence is responsible for the integrin $\alpha_v\beta_3$ binding. "Cold" ^{99}Tc, ^{89}Y, and ^{114}In complexes ([^{99}Tc]RP593, [^{99}Tc]RP685, [^{114}In]RP686, and [^{89}Y]RP686) were also prepared to study the impact of radiometal chelate on integrin $\alpha_v\beta_3$ affinity.[25] In the ELISA assay against biotinated vitronectin, both HYNIC and DOTA conjugates of c(RGDfK) and E[c(RGDfK)]$_2$ show high binding affinity

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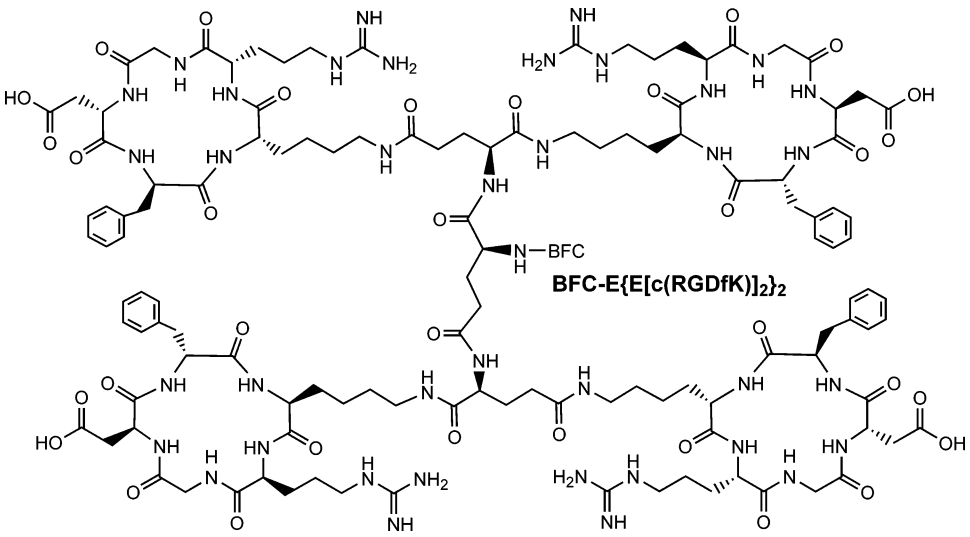

Figure 6. ChemDraw structure of a cyclic RGDfK peptide tetramer, E{E[c(RGDfK)]$_2$}$_2$. For ^{99m}Tc-labeling, the BFC is HYNIC. DOTA will be used for chelation of ^{111}In, ^{90}Y, and ^{64}Cu.

Table 1. Selected Integrin $\alpha_v\beta_3$ Binding Data for HYNIC/DOTA-Conjugated RGD Peptides and Their Metal Complexes

compound	radiometal complex	IC_{50} (nM) ELISA against biotinated Vn	IC_{50} (nM) IIb/IIIa against ^{125}I-fibrinogen	structure acronym
vitronectin (Vn)		5 ($n = 5$)	294 ($n = 1$)	vitronectin
c(RGDfV)		0.4 ($n = 2$)	15 399 ($n = 1$)	c(RGDfV)
HYNIC-c(RGDfK)	RP582	1.0 ($n = 2$)	8 842 ($n = 1$)	c(RGDfK)
HYNIC-E[c(RGDfK)]$_2$	RP593	0.6 ($n = 3$)	10 209 ($n = 1$)	HYNIC-c(RGDfK) dimer
HYNIC-E[c(RGDfK)]$_2$	[^{99}Tc]RP593	2.0 ($n = 2$)	>10 000 ($n = 2$)	^{99}Tc complex
HYNIC-c(RGKfD)	RP685	>10 000 ($n = 4$)	>20 000 ($n = 1$)	c(RGKfD)
	[^{99}Tc]RP685	>10 000 ($n = 2$)	>20 000 ($n = 1$)	^{99}Tc complex
DOTA-E[c(RGDfK)]$_2$	RP686/RP697	0.7 ($n = 4$)	8 894 ($n = 3$)	DOTA-c(RGDfK) dimer
In-DOTA-E[c(RGDfK)]$_2$	[^{111}In]RP686	1.2 ($n = 2$)	>10 000 ($n = 1$)	indium complex
Y-DOTA-E[c(RGDfK)]$_2$	[^{90}Y]RP697	1.5 ($n = 2$)	>10 000 ($n = 1$)	yttrium complex

and good selectivity for integrin $\alpha_v\beta_3$ (Table 1). There was no significant difference in the binding affinity between HYNIC-E[c(RGDfK)]$_2$ or DOTA-E[c(RGDfK)]$_2$ and their corresponding radiometal complexes, suggesting that attachment of the radiometal chelate has no significant impact on integrin $\alpha_v\beta_3$ binding affinity of the cyclic RGDfK dimer.[24] Similar results were obtained for the HYNIC- and DOTA-conjugated cyclic RGDfK tetramer, E{E[c(RGDfK)]$_2$}$_2$.[74−76]

Factors Influencing Tumor Uptake

Integrin $\alpha v\beta 3$ Receptor Population. There are many factors that can influence the tumor uptake and tumor retention of a specific radiotracer. These include the integrin $\alpha_v\beta_3$ population, integrin $\alpha_v\beta_3$ binding affinity, receptor binding and dissociation rates, and excretion kinetics. The fact that the ^{99m}Tc-labeled peptide αP2 has been successfully used for imaging tumors in patients diagnosed with metastatic melanoma strongly suggests that there is sufficient integrin $\alpha_v\beta_3$ overexpression on tumor cells and the activated endothelial cells of neovasculature for SPECT and PET imaging. This has been further confirmed by the promising results from PET imaging studies with ^{18}F-galacto-RGD in cancer patients with metastases of malignant melanoma, sarcomas, and osseous metastases.[77,78]

Integrin $\alpha v\beta 3$ Binding Affinity. For the last several years, Edwards's group at Bristol-Myers Squibb (BMS) Medical Imaging has been using HYNIC (Figure 7) as a BFC for the ^{99m}Tc-labeling of cyclic RGD peptides.[79−81] It was found that the HYNIC-RGD conjugate must have $IC_{50} \leq 10$ nM in the ELISA assay in order for its ternary ligand ^{99m}Tc complex [^{99m}Tc(HYNIC-RGD)(tricine)(TPPTS)] (TPPTS = trisodium

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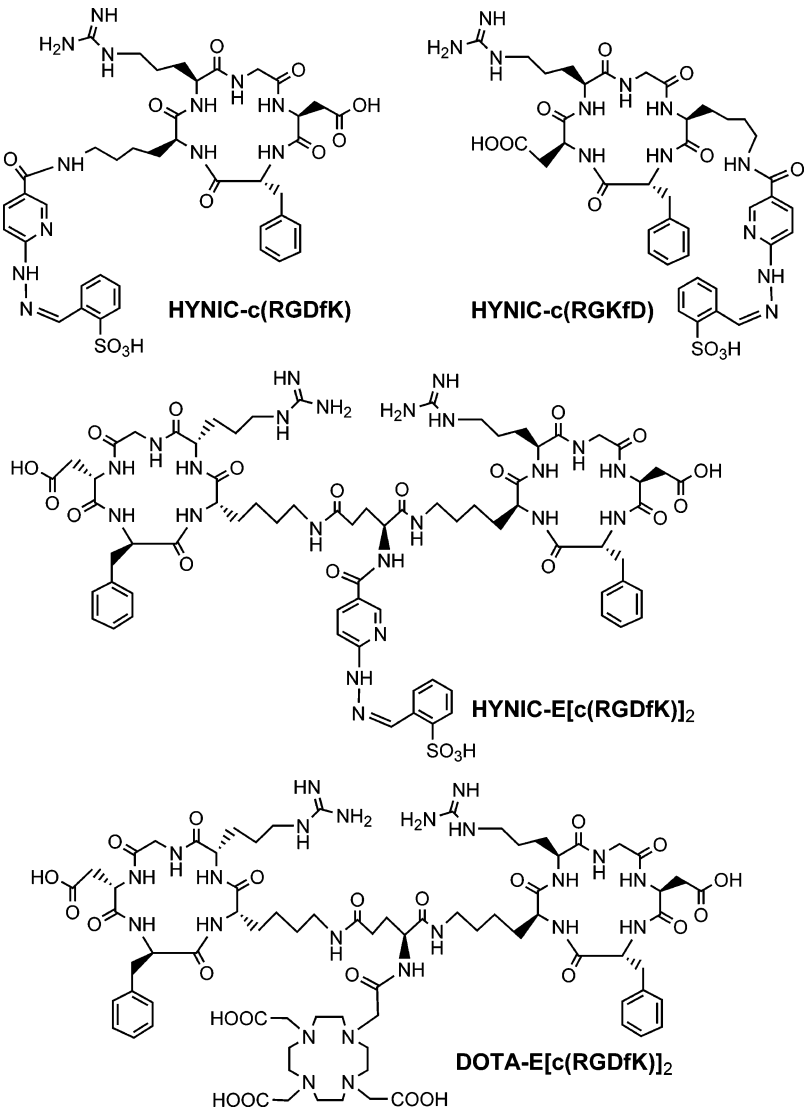

Figure 7. ChemDraw structures of the selected HYNIC- and DOTA-conjugated cyclic RGDfK peptide monomers and dimers.

triphenylphosphine-3,3′,3″-trisulfonate) to be useful as a tumor imaging agent.[24] Higher binding affinity of the HYNIC-RGD conjugate often leads to better tumor uptake for its ternary ligand ^{99m}Tc complex [^{99m}Tc(HYNIC-RGD)(tricine)(TPPTS)]. For example, HYNIC-c(RGKfD) has very low integrin $\alpha_v\beta_3$ binding affinity (IC_{50} > 10 000 nM) due to the "scrambled" peptide sequence. Its ^{99m}Tc complex [^{99m}Tc(HYNIC-c(RGKfD))(tricine)(TPPTS)] (RP685) has no significant tumor uptake (Figure 8). HYNIC-E[c(RGDfK)]$_2$ has a very high binding affinity for integrin $\alpha_v\beta_3$ (IC_{50} = 0.6 nM), and its ^{99m}Tc complex [^{99m}Tc(HYNIC-E[c(RGDfK)]$_2$)(tricine)(TPPTS)] (RP593) has a high tumor uptake (Table 2 and Figure 8) and has a long tumor retention time.

Monomer versus Dimer. More than 20 ^{99m}Tc-labeled cyclic RGD peptides have been screened in the c-neu Oncomouse model.[25] The results from biodistribution studies showed that RP593 has the best biodistribution characteristics (Table 2) in terms of tumor uptake and T/B (tumor/liver and tumor/lungs) ratios. RP593 showed superior tumor uptake and longer retention than its monomer counterpart [^{99m}Tc-(HYNIC-c(RGDfK))(tricine)(TPPTS)] (RP582). The tumor uptake of RP593 can be blocked by coinjection of excess c(RGDfV), a known integrin $\alpha_v\beta_3$ antagonist, suggesting that

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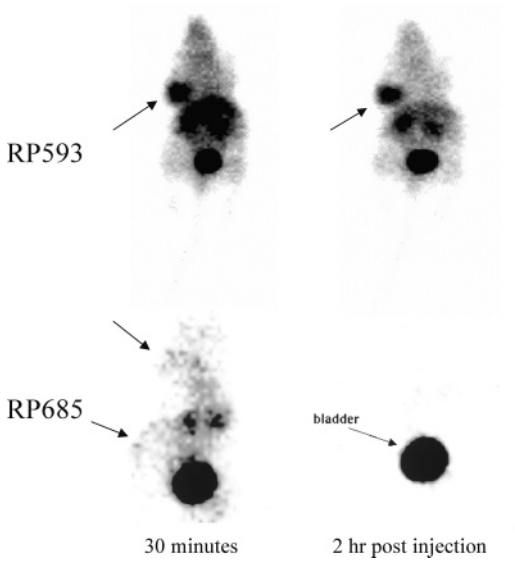

Figure 8. Representative images of RP593 (top) and RP685 (bottom) at 30 min and 2 h postinjection in the c-neu Oncomouse model. Arrows indicate the presence of radioactivity in tumors or bladder. Images have not been filtered.

Table 2. Biodistribution Data of Radiometal Complexes (2 mCi/kg, Iv) at 2 h Postinjection in the c-neu Oncomouse Tumor Model

radiotracer (radiometal complex)	tumor uptake (% ID/g)	blood activity (% ID/g)	kidney uptake (% ID/g)	liver uptake (% ID/g)
RP582	1.281 ($n = 3$)	0.029 ($n = 3$)	4.73 ($n = 3$)	3.08 ($n = 3$)
RP593	3.65 ($n = 5$)	1.02 ($n = 5$)	11.3 ($n = 5$)	5.18 ($n = 5$)
RP685	0.61 ($n = 2$)	0.91 ($n = 2$)	3.4 ($n = 2$)	0.51 ($n = 2$)
RP686	3.14 ($n = 4$)	0.25 ($n = 4$)	4.6 ($n = 4$)	3.78 ($n = 4$)
RP697	3.05 ($n = 4$)	0.20 ($n = 4$)	4.4 ($n = 4$)	3.55 ($n = 4$)

its tumor localization is indeed due to the integrin $\alpha_v\beta_3$ binding. Both RP593 and RP582 have also been evaluated in the female BALB/c mice with subcutaneously growing OVCAR-3 ovarian carcinoma xenograft.[64,65] At 1, 2, and 4 h postinjection, the tumor uptake of RP593 was significantly higher than that of RP582 (Table 2). These results strongly suggest that the cyclic RGD dimer E[c(RGDfK)]$_2$ has a significant advantage over the monomer counterpart with respect to tumor uptake and retention times. A similar conclusion was also made for the ^{18}F-labeled cyclic RGD peptide dimer.[69]

Radiometal Chelate Effect. ^{111}In-DOTA-E[c(RGDfK)]$_2$ (RP686) and ^{90}Y-DOTA-E[c(RGDfK)]$_2$ (RP697) showed much higher tumor uptake and longer tumor retention time (~3.0% ID/g at 2 h and ~1.5% ID/g at 24 h postinjection) than their monomer counterparts (~1.28% ID/g at 2 h and ~0.5% ID/g at 24 h postinjection) in the c-neu Oncomouse model.[25] Similar results were obtained for RP593, RP686, and RP697 in BALB/c mice bearing ovarian carcinoma.[64,65] Changing the chelator from a bulky ternary ligand system (HYNIC, tricine, and TPPTS) to a small DOTA did not cause a significant change in tumor uptake; but it had a significant impact on the radiotracer uptake in several major organs, particularly liver and kidneys (Table 2). For example, RP686 and RP697 have a much lower kidney uptake than RP593 in the same animal model, strongly suggesting that the biodistribution characteristics of a radiotracer can be modified by the choice of bifunctional chelator or metal chelate.

Impact of Cyclic RGD Peptides. Recently, Chen et al.[68] reported two radiotracers, ^{64}Cu-DOTA-E[c(RGDfK)]$_2$ and ^{64}Cu-DOTA-E[c(RGDyK)]$_2$, for PET imaging of breast cancer in nude mice bearing MDA-MB-435 breast cancer xenografts.[68] It was found that replacing c(RGDfK) with c(RGDyK) had little effect on the tumor uptake; but ^{64}Cu-DOTA-E[c(RGDyK)]$_2$ showed a faster liver clearance than ^{64}Cu-DOTA-E[c(RGDfK)]$_2$, probably due to the two extra hydroxyl groups in ^{64}Cu-DOTA-E[c(RGDyK)]$_2$. The tumor uptake can be blocked by coinjection of c(RGDyK), demonstrating that the high tumor uptake of ^{64}Cu-DOTA-E[c(RGDyK)]$_2$ and ^{64}Cu-DOTA-E[c(RGDfK)]$_2$ is due to the integrin $\alpha_v\beta_3$ binding.

Dimer versus Tetramer. Liu et al. prepared the HYNIC-E{E[c(RGDfK)]$_2$}$_2$ conjugate and its ternary ligand ^{99m}Tc complex [^{99m}Tc(HYNIC-E{E[c(RGDfK)]$_2$}$_2$)(tricine)(TPPTS)].[74] Biodistribution studies of [^{99m}Tc(HYNIC-E{E[c(RGDfK)]$_2$}$_2$)-(tricine)(TPPTS)]) and RP593 were performed in athymic nude mice bearing MDA-MB-435 breast cancer xenografts. It was found that the tumor uptake of [^{99m}Tc(HYNIC-E{E-[c(RGDfK)]$_2$}$_2$)(tricine)(TPPTS)] was much higher than that of RP593 at 120 min postinjection (Figure 9), most likely due to higher integrin $\alpha_v\beta_3$ binding affinity of E{E-[c(RGDfK)]$_2$}$_2$. It is very interesting to note that there is a steady increase in tumor uptake for [^{99m}Tc(HYNIC-E{E-[c(RGDfK)]$_2$}$_2$)(tricine)(TPPTS)] while the tumor uptake of RP593 remains relatively unchanged over the 2 h study period. Because of the increased tumor uptake, its T/B ratios were also significantly increased.

To further illustrate the advantage of E{E[c(RGDfK)]$_2$}$_2$, three DOTA conjugates, DOTA-E-c(RGDfK), DOTA-E[c(RGDfK)]$_2$, and DOTA-E{E[c(RGDfK)]$_2$}$_2$, and their ^{111}In complexes have been prepared.[76] Biodistribution studies on ^{111}In-DOTA-E-c(RGDfK), ^{111}In-DOTA-E-[c(RGDfK)]$_2$, and ^{111}In-DOTA-E{E[c(RGDfK)]$_2$}$_2$ were performed in athymic mice bearing sc SK-RC-52 tumors. Based on their biodistribution patterns and T/B ratios (Figure 10), it was clear that the cyclic RGDfK tetramer E{E[c(RGDfK)]$_2$}$_2$ is the best targeting biomolecule with respect to tumor uptake and T/B ratios for its radiotracers.[76]

^{64}Cu-DOTA-E{E[c(RGDfK)]$_2$}$_2$ was also evaluated as a PET radiotracer in nude mice bearing U87MG human glioma xenografts.[75] ^{64}Cu-DOTA-E{E[c(RGDfK)]$_2$}$_2$ has a high tumor uptake (9.93 ± 1.05% ID/g at 30 min postinjection) with a long tumor retention time (4.56 ± 0.51% ID/g at 24 h postinjection). It also has a rapid blood clearance (0.61 ± 0.01% ID/g at 30 min postinjection and 0.21 ± 0.01% ID/g at 4 h postinjection) predominantly via the renal system. MicroPET images (Figure 11) clearly showed the presence of glioma tumors in the tumor-bearing nude mice. The com-

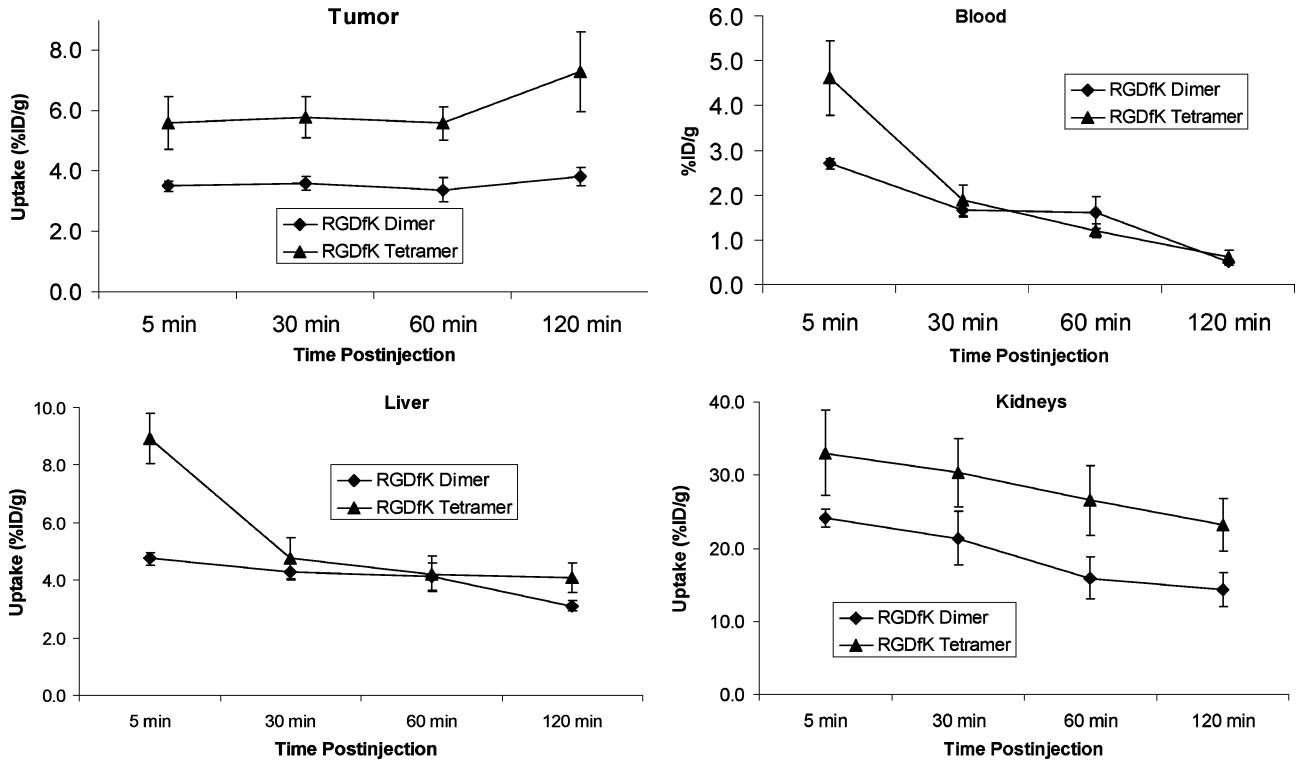

Figure 9. Direct comparison of the biodistribution characteristics of RP593 (dimer) and [^{99m}Tc(HYNIC-E{E[c(RGDfK)]$_2$}$_2$)(tricine)-(TPPTS)]) (tetramer) in the athymic nude mice bearing the MDA-MB-435 human breast cancer xenografts.

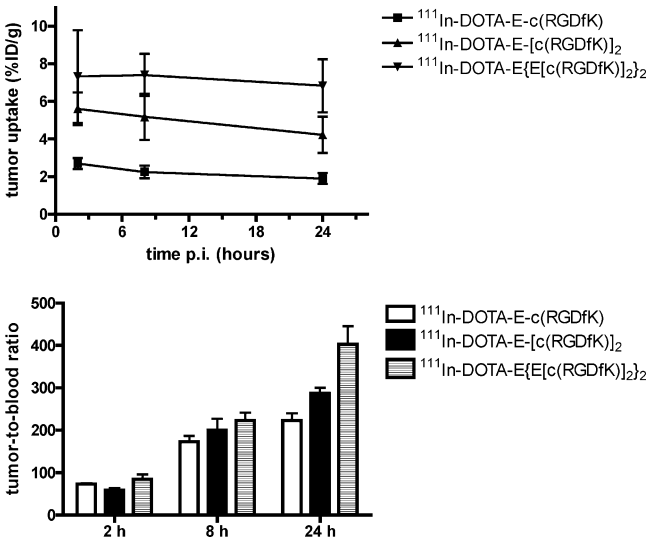

Figure 10. Direct comparison of tumor uptake (top: % ID/g) and tumor-to-blood ratios (bottom) between ^{111}In-DOTA-E-c(RGDfK), ^{111}In-DOTA-E-[c(RGDfK)]$_2$, and ^{111}In-DOTA-E{E-[c(RGDfK)]$_2$}$_2$ at 2, 8, and 24 h postinjection.

bination of high tumor uptake and rapid clearance from nontarget organs led to very high T/B ratios for ^{64}Cu-DOTA-E{E[c(RGDfK)]$_2$}$_2$ (tumor/muscle = 15.9 ± 1.2; tumor/liver = 2.32 ± 0.18; tumor/kidney = 1.54 ± 0.12). These promising results suggest that ^{64}Cu-DOTA-E{E[c(RGDfK)]$_2$}$_2$ has the potential as a PET radiotracer to image integrin $\alpha_v\beta_3$ expression in glioma.[75]

Pharmacokinetic Considerations

The main pharmacokinetic consideration for a new tumor imaging agent is that it has a high tumor uptake with diagnostically useful T/B ratio in a short period of time. The high lipophilicity often leads to more hepatobiliary excretion. High protein binding often results in longer blood retention of radioactivity. Hepatobiliary excretion and high protein binding are detrimental for improvement of the T/B ratio. Therefore, an important aspect of the research on integrin $\alpha_v\beta_3$ targeted radiotracers is to improve the T/B ratios by modifying excretion kinetics of radiolabeled cyclic RGD peptides. PKM linkers and their use for modification of radiotracer pharmacokinetics have been reviewed recently.[25,45,52] The following examples will illustrate their impact on biodistribution properties of the radiolabeled cyclic RGD peptides.

Carbohydrate-Modified Cyclic RGD Peptides. Haubner et al. were the first to use 3-^{125}I-D-Tyr^4-cyclo(RGDyV) and 3-^{125}I-D-Tyr^4-cyclo(RGDyK(SAA1)) (Figure 12: SAA = sugar amino acid) as radiotracers for tumor imaging.[82−84] It was found that substitution of leucine with the SAA-functionalized lysine resulted in improved renal excretion and better T/B ratios. A blocking study using c(RGDfV) at

(82) Haubner, R.; Wester, H. J.; Senekowitsch-Schmidtke, R.; Diefenbach, B.; Kessler, H.; Stöcklin, G.; Schwaiger, M. RGD-peptides for tumor targeting: biological evaluation of radioiodinated analogs and introduction of a novel glycosylated peptide with improved biokinetics. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 383−385.

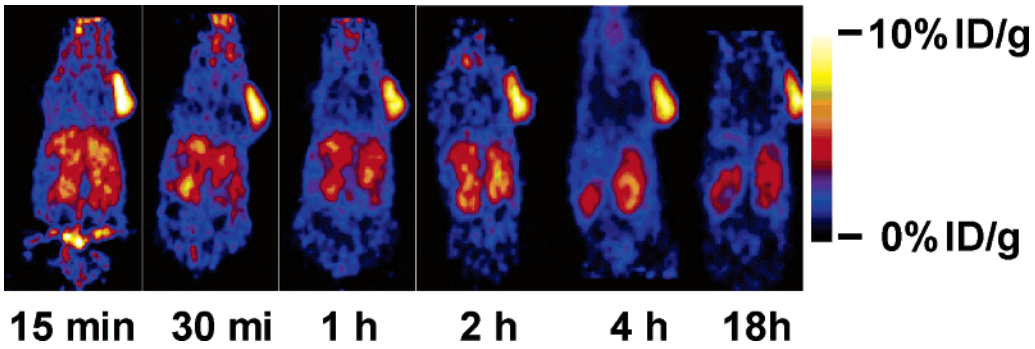

Figure 11. MicroPET images of the tumor-bearing mouse (with U87MG human glioma xenografts) administered with about 250 μCi of ^{64}Cu-DOTA-E[c(RGDyK)]$_2$.

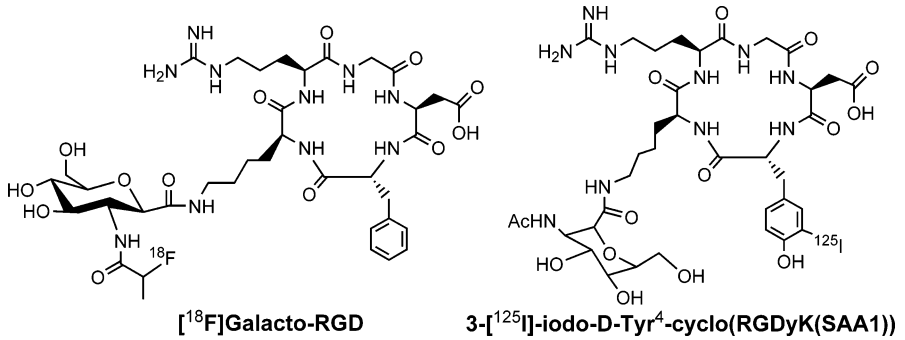

Figure 12. Examples of the carbohydrate-conjugated cyclic RGD peptides.

a dose of 3 mg/kg clearly demonstrated that the localization of radiotracer in tumor is due to integrin $\alpha_v\beta_3$ binding.[83,84] [^{18}F]Galacto-RGD (Figure 12) has also been used for PET imaging of integrin $\alpha_v\beta_3$ expression in melanoma- and osteosarcoma-bearing mice.[85] Introduction of the sugar moiety not only improves renal excretion but also enhances tumor uptake of the radiotracer. Because of the pioneering efforts from the Haubner's group, [^{18}F]galacto-RGD has been under clinical trials as the first-generation integrin $\alpha_v\beta_3$ targeted radiotracer for tumor imaging.[77,78]

Peptide PKM Linkers. Small biomolecules in the blood plasma are filtered through glomerular capillaries in kidneys and may be subsequently reabsorbed by the proximal tubular cells through carrier-mediated endocytosis. Membranes of renal tubular cells contain negatively charged sites to which the positively charged groups (such as guanidine in the RGD sequence) are expected to bind. Carboxylic and sulfonic groups will be deprotonated under physiological conditions. Therefore, negatively charged small peptide sequence and single amino acid have been proposed as PKM linkers to reduce renal uptake and kidney retention of radiolabeled small biomolecules.[24,45,46,52] The use of highly charged peptide PKM linkers may also reduce protein bonding, thereby reducing liver uptake of the radiotracer.

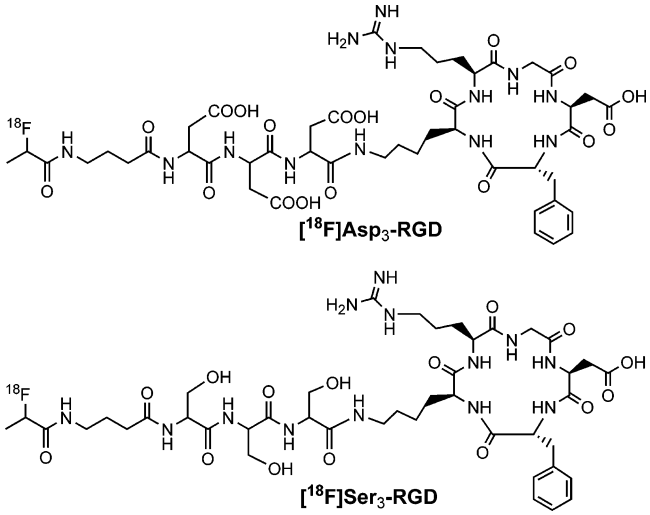

Figure 13. ChemDraw structures of [^{18}F]Asp$_3$-RGD and [^{18}F]-Ser$_3$-RGD.

(83) Haubner, R.; Wester, H.-J.; Reuning, U.; Senekowisch-Schmidtke, R.; Diefenbach, B.; Kessler, H.; Stöcklin, G.; Schwaiger, M. Radiolabeled $\alpha_v\beta_3$ integrin antagonists: a new class of tracers for tumor imaging. *J. Nucl. Med.* **1999**, *40*, 1061−1071.

(84) Haubner, R.; Wester, H. J.; Burkhart, F.; Senekowisch-Schmidtke, R.; Weber, W.; Goodman, S. L.; Kessler, H.; Schwaiger, M. Glycolated RGD-containing peptides: tracer for tumor targeting and angiogenesis imaging with improved biokinetics. *J. Nucl. Med.* **2001**, *42*, 326−336.

(85) Haubner, R.; Wester, H. J.; Weber, W. A.; Mang, C.; Ziegler, S. I.; Goodman, S. L.; Senekowisch-Schmidtke, R.; Kessler, H.; Schwaiger, M. Noninvasive imaging of $\alpha_v\beta_3$ integrin expression using ^{18}F-labeled RGD-containing glycopeptide and positron emission tomography. *Cancer Res.* **2001**, *61*, 1781−1785.

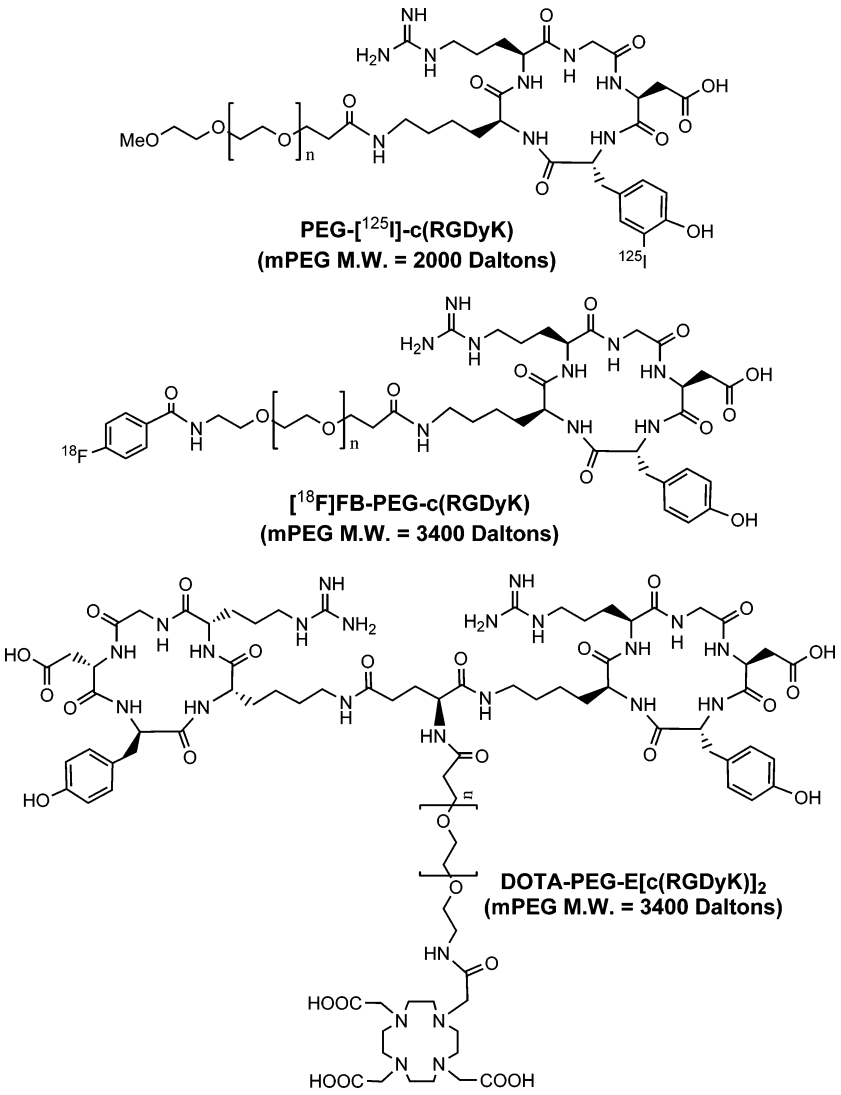

Figure 14. PEGylated RGD peptides, [^{18}F]FB-PEG-c(RGDyK) and DOTA-PEG-E[c(RGDyK)]$_2$.

The di(cysteic acid) linker has successfully been used to improve the blood clearance and minimize the liver and kidney activity of the radiolabeled nonpeptide integrin $\alpha_v\beta_3$ receptor antagonists.[25,86,87] The Asp_3 and Ser_3 tripeptide sequences (Figure 13) were also used by Poethko et al.[27,70] to modify excretion kinetics of the ^{18}F-labeled cyclic RGD peptide, c(RGDfK). It was found that [^{18}F]Asp_3-RGD and [^{18}F]Ser_3-RGD had biodistribution and excretion characteristics comparable to those of [^{18}F]galacto-RGD (Figure 12).

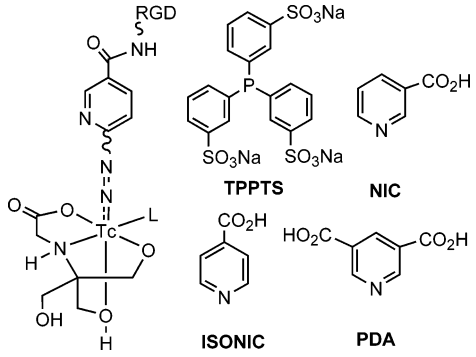

Figure 15. Structures of coligands and their ternary ligand ^{99m}Tc complexes: [^{99m}Tc(HYNIC-RGD)(tricine)(L)] (RGD = E[c(RGDfK)]$_2$; L = TPPTS, ISONIC, NIC, and PDA).

PEG PKM Linkers. The advantage of using poly(ethylene glycol) (PEG) as the PKM linker is that its molecular weight can be controlled by adjusting chain length without changing the overall molecular charge. Harris et al.

(86) Harris, T. D.; Kalogeropoulos, S.; Nguyen, T.; Liu, S.; Bartis, J.; Ellars, C. E.; Edwards, D. S.; Onthanks, D.; Yalamanchili, P.; Robinson, S. P.; Lazewatsky, J.; Barrett, J. A. Design, synthesis and evaluation of radiolabeled integrin $\alpha_v\beta_3$ antagonists for tumor imaging and radiotherapy. *Cancer Biother. Radiopharm.* **2003**, *18*, 627−641.

(87) Onthank, D. C.; Liu, S.; Silva, P. J.; Barrett, J. A.; Harris, T. D.; Simon P. Robinson, S. P.; Edwards, D. S. ^{90}Y and ^{111}In complexes of A DOTA-conjugated integrin $\alpha_v\beta_3$ receptor antagonist: different but biologically equivalent. *Bioconjugate Chem.* **2004**, *15*, 235−241.

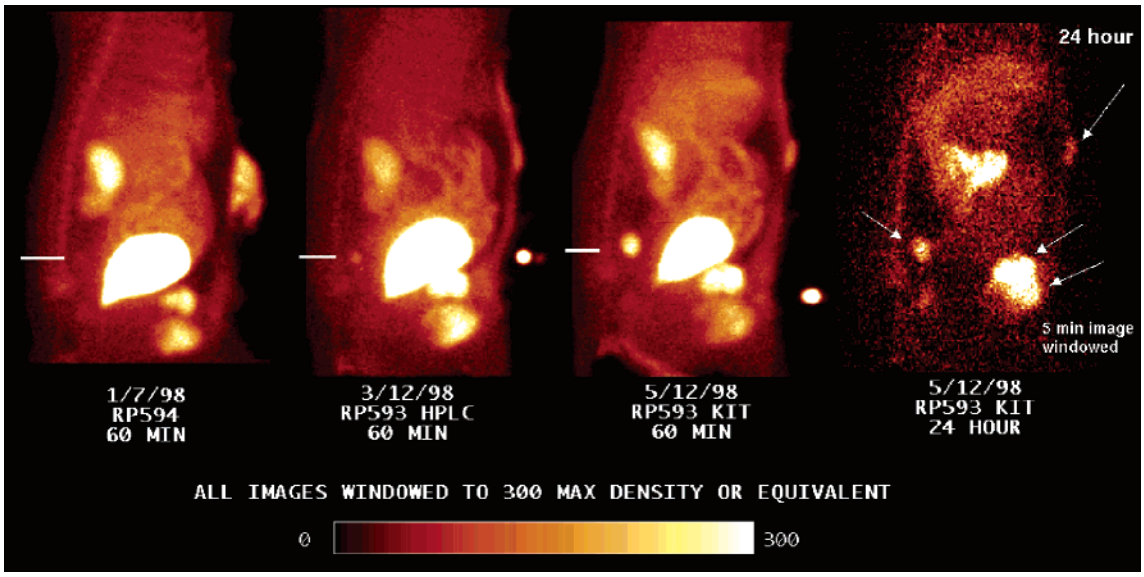

Figure 16. Representative images (no decay correction) of RP593 at 60 min postinjection in a canine with confirmed mammary adenocarcinoma. Arrows indicate presence of tumors.

have successfully used a PEG4 as the PKM linker for the ^{99m}Tc-labeled non-peptide integrin $\alpha_v\beta_3$ receptor antagonists, and found that the PEG linker can significantly improve tumor uptake and T/B ratios.[24,86,87] Kessler et al. reported the use of HEG (hexaethylene glycolic acid) as a PKM linker for the ^{18}F-labeled cyclic RGDfE dimer and tetramer.[71] The HEG linker increases the distance between the RGD motifs. Chen et al. also found that the introduction of the PEG linker (Figure 14) can improve the tumor uptake and excretion kinetics of ^{125}I- and ^{18}F-labeled c(RGDyK) and ^{64}Cu-labeled E[c(RGDyK)]$_2$.[88−90]

Coligand Effect. Liu et al. have been using the ^{99m}Tc-labeling of the cyclic RGD peptides, such as E[c(RGDfK)]$_2$, to image integrin $\alpha_v\beta_3$ expression in tumors of different origin.[62,67,91] It was found that the coligand has a significant impact on tumor uptake, metabolic stability, and excretion kinetics of [^{99m}Tc(HYNIC-E[c(RGDfK)]$_2$)(tricine)(L)] (Figure 15: L = TPPTS, nicotinic acid (NIC), isonicotinic acid (ISONIC), and 2,5-pyridinedicarboxylic acid (PDA)). Among several radiotracers, [^{99m}Tc(HYNIC-E[c(RGDfK)]$_2$)(tricine)-(TPPTS)] (RP593) is the best for its imaging quality of tumor-bearing mice.[91] RP593 is a promising radiotracer for planar and SPECT imaging of the integrin $\alpha_v\beta_3$ expression in future preclinical and clinical evaluations.

Monitoring Tumor Growth and Metastasis

As discussed in the previous section, early detection is only the first step of cancer patient management. In addition to its capability to image tumors of different origin, the integrin $\alpha_v\beta_3$ targeted radiotracer should also be able to stage the extent of tumor metastasis and to monitor the tumor growth and therapeutic response of cancer treatment. With this in mind, Edwards's group has tested RP593 (a ^{99m}Tc-labeled cyclic RGDfK dimer) in canines with confirmed mammary adenocarcinomas. Figure 16 shows representative planar images of the same dog administered with RP593 at 60 min postinjection.[24] Scintigraphic images of the dog administered with RP593 show the presence of tumors, including a rapidly growing tumor (on the left side of each image). At the time when the first imaging study was performed, the image showed no indication of any metastatic tumor. Two months later (03/12/98), the metastatic tumor is clearly seen 60 min after injection of RP593 (0.5 mCi/kg, iv). As the tumor size increases, the images show increased localization of radioactivity in the tumor. These results demonstrated that the integrin $\alpha_v\beta_3$ targeted radiotracers, such as RP593, have the capability to monitor metastasis and tumor growth. They might have the potential to monitor the therapeutic response of antiangiogenic treatment.

Conclusions

Radiolabeled RGD peptides represent a new class of radiotracers with the potential both for early detection of rapidly growing and metastatic tumors and for monitoring tumor growth, metastasis, and possibly therapeutic response of various treatment regimens. For the past several years,

(88) Chen, X.; Park, R.; Shahinian, A. H.; Tohme, M.; Khankaldyyan, V.; Bozorgzadeh, M. H.; Bading, J. R.; Moats, R.; Laug, W. E.; Conti, P. S. ^{18}F-labeled RGD peptide: initial evaluation for imaging brain tumor angiogenesis. *Nucl. Med. Biol.* **2004**, *31*, 179−189.

(89) Chen, X.; Park, R.; Shahinian, A. H.; Bading, J. R.; Conti, P. S. Pharmacokinetics and tumor retention of ^{125}I-labeled RGD peptide are improved by PEGylation. *Nucl. Med. Biol.* **2004**, *31*, 11−19.

(90) Chen, X.; Sievers, E.; Hou, Y.; Park, R.; Tohme, M.; Bart, R.; Bremner, R.; Bading, J. R.; Conti, P. S. Integrin $\alpha_v\beta_3$-targeted imaging of lung cancer. *Neoplasia* **2005**, *7*, 271−279.

(91) Jia, B.; Shi, J.; Yang, Z.; Xu, B.; Liu, Z.; Zhao, H.; Liu, S.; Wang, F. ^{99m}Tc-labeled cyclic RGDfK dimer: initial evaluation for SPECT imaging of brain tumor integrin $\alpha_v\beta_3$ expression. *Bioconjugate Chem.*, submitted.

significant progress has been made on the use of radiolabeled RGD peptides to visualize tumors by SPECT or PET in various tumor-bearing animal models. [^{18}F]Galacto-RGD has been under clinical investigations as the first integrin $\alpha_v\beta_3$ targeted radiotracer for noninvasive visualization of the activated integrin $\alpha_v\beta_3$ in cancer patients.

The tumor uptake of integrin $\alpha_v\beta_3$ targeted radiotracers is dependent on the integrin $\alpha_v\beta_3$ expression levels and the integrin $\alpha_v\beta_3$ binding affinity of cyclic RGD peptides. On the basis of experiences from imaging studies with ^{99m}Tc-labeled peptide αP2 and [^{18}F]galacto-RGD in cancer patients with metastatic melanoma, it is clear that there is a sufficient integrin $\alpha_v\beta_3$ expression for SPECT and PET imaging. The results from several research groups have demonstrated that increasing the peptide multiplicity can significantly enhance the integrin $\alpha_v\beta_3$ binding affinity of RGD peptides and improve tumor targeting capability of the radiotracer. Among several cyclic RGDfK peptides (monomer, dimer, and tetramer), E{E[c(RGDfK)]$_2$}$_2$ is the best targeting biomolecule with respect to its integrin $\alpha_v\beta_3$ binding affinity and tumor targeting capability of its radiotracers (^{99m}Tc, ^{111}In, and ^{64}Cu). From this point of view, further increase of RGD peptide multiplicity may result in formation of oligomeric or polymeric cyclic RGD peptides with the improved integrin $\alpha_v\beta_3$ binding affinity and tumor targeting efficacy. However, modification of in vivo pharmacokinetics of radiolabeled oligomeric or polymeric cyclic RGD peptides will become much more difficult.

Radiotracers targeting integrin $\alpha_v\beta_3$ are useful not only for early detection of tumors but also for staging the extent of disease (local or widespread) and monitoring the response of cancer treatment. They will have direct contact with the intravasculature, and are expected to be more specific for growing and metastatic tumors. Since most rapidly growing and metastatic cancers involve angiogenesis, radiotracers targeting integrin $\alpha_v\beta_3$ would be useful for a large population of cancer patients with integrin $\alpha_v\beta_3$ positive tumors.

It is important to note that the integrin $\alpha_v\beta_3$ overexpression is not limited to rapidly growing and metastatic tumors of different origin. The integrin $\alpha_v\beta_3$ is also overexpressed in endothelial cells during wound healing and postinfarct remodeling, in rheumatoid arthritis and psoriatic plaque.[1−4,92] Therefore, the integrin $\alpha_v\beta_3$ targeted radiotracers developed for tumor imaging have been proposed for imaging myocardial angiogenesis[93] and inflammatory diseases.[94] Recent results showed that the ^{111}In-labeled non-peptide integrin $\alpha_v\beta_3$ antagonist (RP748) was able to image the angiogenesis in the heart,[94] and the radiotracer uptake in the infarct region was associated with the integrin $\alpha_v\beta_3$ expression level. The results reported by Pichler et al.[93] suggest that [^{18}F]galacto-RGD might become a powerful tool to distinguish between acute and chronic phases of T-cell mediated immune responses. These promising results give rise to the possibility of extending applications of the integrin $\alpha_v\beta_3$ targeted radiotracers from imaging tumor angiogenesis to detection of inflammatory processes, and to monitoring outcomes of therapeutic interventions in patients with cancer, myocardial infarction, and inflammation.

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