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Review Article

Integrin $\alpha_v\beta_3$ directed radiopharmaceuticals for tumor imaging

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CONTENTS

Abstract

Accurate and rapid detection of rapidly growing and metastatic tumors is of great importance for implementation of a tailored therapeutic regimen. A number of imaging procedures are available for detection of tumor lesions. Morphological and functional imaging modalities including ultrasonography, computed tomography and magnetic resonance imaging, provide details of structural changes, variations in density anddifferences in proton content in tissues. Nuclear medicine procedures using [^{99m}Tc]-labeled small biomolecules can be used for *in vivo* characterization of cellular structure and function and for monitoring biological changes in tumor tissues at the molecular level. This article will focus on radiolabeled integrin $\alpha_v\beta_3$ receptor antagonists as radiopharmaceuticals for tumor imaging. The integrin $\alpha_v\beta_3$ is generally expressed at low levels on epithelial cells and mature endothelial cells but highly expressed on the neovasculature and tumor cells. Because integrin $\alpha_v\beta_3$ expression is highly restricted during tumor invasion and metastasis, the molecule represents an interesting target for diagnosis of rapidly growing solid tumors.

Introduction

Cancer is the leading cause of death worldwide. The most common malignancies in the U.S. and European countries are lung, colorectal, breast and prostate. There are several imaging modalities currently available for the diagnosis of cancer and these include X-ray computed tomography (CT), ultrasound (US), nuclear magnetic resonance imaging (MRI) and nuclear medicine procedures. While CT, US and MRI procedures are better suited for anatomic analysis of solid tumors, it is very difficult to use these modalities to monitor biochemical changes in tumor tissues at the molecular level. This is mainly due to the fact that MRI and CT often require the accumulation of much higher concentration of the contrast agent to achieve reasonable contrast. In addition, the specificity and sensitivity of CT and US for diagnosis of high-incidence tumors (*e.g.*, breast, colorectal, lung and prostate) are generally low. Therefore, there is an unmet need for tumor-specific radiopharmaceuticals which can be used not only for early diagnosis of cancers of high-incidence but also for staging the progression of tumor growth and monitoring the response of treatment regimens.

Radiopharmaceuticals for tumor imaging

Tumor imaging has been a challenge for the last few decades. The radiopharmaceutical search has been focused on achieving the following goals: 1) to detect the presence of tumor; 2) to distinguish between benign and malignant process or tumor types; 3) to follow the course of a particular tumor over time and its response to the therapeutic treatment; 4) to predict success or failure of a specific therapeutic regime for a given type of tumor; and 5) to access the prognosis of a particular tumor. For a new radiopharmaceutical to be successful, it has to show clinical indications for several of high-incidence tumor types (*e.g.*, breast, colorectal, lung and prostate). Localization of the radiopharmaceutical in the tumor has to be sufficient to provide a planar image density of about

2000 counts/cm^2 and exhibit rapid blood clearance so that diagnosis can be made within 4 h after i.v. injection. Since most of high-incidence tumor types occur in the torso, namely the lung and colorectal and breast cancers metastatic to the lymphatic system, renal excretion without significant renal retention is necessary. Because of the short half-life of [^{99m}Tc], a kit formulation is often required. The new radiopharmaceutical should have high radiochemical purity with high solution stability, and must be nonimmunogenic. Injection of the whole or part of the reconstituted kit should not cause any pharmacological response.

A large number of radiolabeled biomolecules have been studied for their potential as target-specific radiopharmaceuticals for tumor imaging. These include antibodies, small peptides and nonpeptide receptor ligands. Radiolabeled small peptides are of great interest because they have the potential to detect primary sites, identify occult metastatic lesions, guide surgical intervention, stage tumors and predict the efficacy of therapeutic agents. Peptide-based radiopharmaceuticals have been a subject of a number of excellent recent reviews (1-15).

Small peptides are necessary elements in many fundamental biological processes and in many cases the affinities of small peptides for their receptors are significantly greater than that of antibodies. They can also tolerate harsher chemical conditions for modification or radiolabeling. Small peptides are easy to synthesize and modify, less likely to be immunogenic, and can have rapid blood clearance. The faster blood clearance results in adequate T/B ratios earlier thus making [^{99m}Tc] practical to use; it is the preferred radionuclide for diagnostic nuclear medicine. When labeled with a suitable therapeutic radionuclides, small peptides can be used as radiopharmaceuticals for tumor therapy. Two radiolabeled small peptides ([^{111}In]-DTPA-octreotide, OctreoScan®; [^{99m}Tc]-depreotide, NeoTech™) have been approved for imaging somatostatin-positive tumors while some are still under preclinical and clinical investigation for tumor radiotherapy.

Integrin $\alpha_v\beta_3$ targeted radiopharmaceuticals

Tumors produce diffusible angiogenic factors which activate endothelial cells in nearby established capillaries or venules and, through a series of sequential but partially overlapping steps, induce endothelial proliferation, migration and new vessel formation. Once vascularized, previous dormant tumors begin to grow rapidly and their volumes increase exponentially. The formation of new blood vessels (angiogenesis) is a requirement for malignant tumor growth and metastasis (16-19). The angiogenic process depends on vascular endothelial cell migration and invasion, regulated by cell adhesion receptors.

Integrins are a family of proteins that facilitate cellular adhesion to and migration on the extracellular matrix proteins found in intercellular spaces and basement membranes. They also regulate cellular entry and withdrawal from the cell cycle. Integrin $\alpha_v\beta_3$ is a receptor for a wide variety of extracellular matrix proteins with its exposed RGD tripeptide sequence. Ligands include vitronectin, fibronectin, fibrinogen, lamin, collagen, Von Willibrand's factor, osteoponin and adenovirus particles. The expression of integrin $\alpha_v\beta_3$ enables a given cell to adhere to, migrate on or respond to almost any matrix protein it may encounter. Despite its promiscuous behavior, integrin $\alpha_v\beta_3$ is generally expressed at low levels on epithelial cells and mature endothelial cells. However, it has been reported that integrin $\alpha_v\beta_3$ is highly expressed on the neovasculature of tumors, including osteosarcomas, neuroblastomas, glioblastomas, melanomas, lung carcinomas, breast, prostate and bladder cancers (20-25). A recent study showed that integrin $\alpha_v\beta_3$ is overexpressed not only on endothelial cells but also on tumor cells in human breast cancer xenografts (26). Expression of integrin $\alpha_v\beta_3$ correlates with tumor progression in melanoma, glioma, ovarian and breast cancers (20-26). The highly restricted expression of integrin $\alpha_v\beta_3$ during tumor invasion and metastasis presents an interesting molecular target for diagnosis of rapidly growing solid tumors (18, 27, 28).

Advantages over other radiopharmaceuticals

Most modalities currently available for tumor imaging rely on features of the tumor cells (increased uptake of lipophilic cations such as Miraluma®), their metabolic rate (the basis for [^{18}F]-FDG imaging) or tumor cell specific receptors such as the somatostatin receptor ([^{111}In]-DTPA-octreotide and [^{99m}Tc]-depreotide). Radiopharmaceuticals targeting integrin $\alpha_v\beta_3$ would be used not only for early detection of tumors but also for staging the extent of the disease (local, regional or widespread) and monitoring the therapeutic response of cancer treatment. These applications would take advantage of the unique ability of nuclear medicine procedures to do simultaneous whole body imaging. The radiopharmaceutical will have direct contact with the intravasculature space. It is also possible for the radiopharmaceutical to avoid barriers associated with extravasation and penetration into tumor cells. Radiopharmaceuticals targeting integrin $\alpha_v\beta_3$ would be more specific for growing and metastatic tumors than those targeting tumor cell associated receptors. When labeled with a suitable radionuclide, radiopharmaceuticals targeting integrin $\alpha_v\beta_3$ can be used for radiotherapy of solid tumors (29, 30).

Radiopharmaceutical design

New radiopharmaceuticals targeting integrin $\alpha_v\beta_3$ can be divided into 4 parts (Fig. 1): the integrin $\alpha_v\beta_3$ targeting biomolecule, linker, bifunctional chelator (BFC) and the radionuclide. The $\alpha_v\beta_3$ targeting biomolecule serves as a vehicle to carry the radionuclide to the receptor site at the tumor neovasculature and tumor cells. The radionuclide

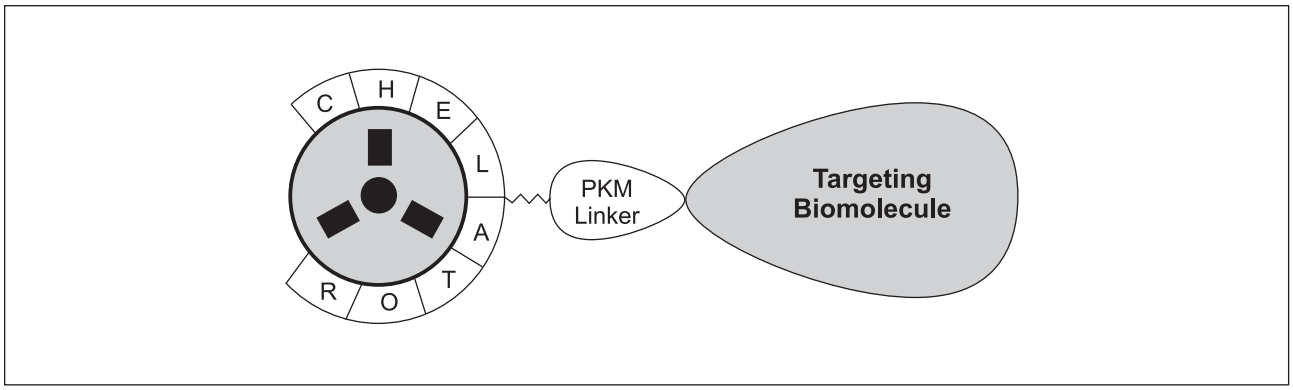

Fig. 1. Schematic presentation of the radiopharmaceuticals design. Diagnostic radionuclide: ^{99m}Tc. Bifunctional chelators: HYNIC. Pharmacokinetic linker: poly (amino acid). Targeting biomolecule: integrin $\alpha_v\beta_3$ receptor antagonists.

is the radiation source. Between the targeting biomolecule and radionuclide is the BFC, which strongly associates with the metal ion and is covalently attached to the targeting molecule either directly or through a linker. Selection of a BFC is largely determined by the nature and oxidation state of the metallic radionuclide. The linker can be a simple hydrocarbon chain or a small peptide sequence, which is often used for modification of pharmacokinetics.

Radionuclide

[^{99m}Tc] is the most widely used label for diagnostic imaging due to its optimal nuclear property, easy availability and low cost. Nearly 80% of all radiopharmaceuticals used in nuclear medicine department are [^{99m}Tc]-labeled compounds. The 6 h half-life is long enough to allow a radiochemist to carry out radiopharmaceutical synthesis and for nuclear medicine practitioners to collect useful images. At the same time, it is short enough to permit the administration of 20-30 mCi of [^{99m}Tc] without significant radiation exposure to the patient. The monochromatic 140 KeV photons are readily collimated to give images of superior spatial resolution.

[^{18}F] is a cyclotron-produced isotope suitable for positron-emission tomography (PET). For the last several years, [^{18}F]-FDG (FDG = 2-fluor-2-deoxyglucose) has been widely used as a powerful nuclear imaging tool for the diagnosis of cancer, brain and cardiovascular diseases. However, the half-life ($t_{1/2}$ = 110 min) of [^{18}F] is short, which makes it very difficult for small or medium-sized institutions to have access to [^{18}F]-FDG. Only limited medical research institutions can afford to maintain the cyclotron facility and keep the supply of [^{18}F]-FDG for various clinical applications. Despite the recent development of mobile trailers for FDG PET imaging, developing a target specific radiopharmaceutical based on [^{18}F]-labeled small biomolecules remains an elusive goal. The overriding factor limiting the success of PET imaging is a financial one. The financial burden of purchasing and operating the equipment forces the price of these PET studies to be extremely high.

Bifunctional chelators (BFCs)

BFC is an important part of the target-specific radiopharmaceutical. Various chelating systems have been developed for [^{9m}Tc]-labeling of biomolecules. These include N_3S triamidethiols, N_2S_2 diamidedithiols, N_2S_2 diaminedithiols, N_2S_2 monoamidemonoaminedithiols and 6-hydrazinonicotinamide (HYNIC). The “3+1” chelating systems have also been used for [^{99m}Tc]-labeling of small biomolecules. BFCs useful for [^{99m}Tc]-labeling of small biomolecules have been reviewed extensively (2-5, 15). HYNIC in particular offers several advantages when compared to polydentate chelators. It has extremely high [^{99m}Tc]-labeling efficiency (rapid radiolabeling and high radiolabeling yield). The higher the [^{99m}Tc]-labeling efficiency is, the smaller amount of the BFC-biomolecule conjugate will be needed to achieve high radiochemical purity for the [^{99m}Tc] complex. Thus, there would be less competition from the unlabeled BFC-biomolecule conjugate for receptor binding. The combination of HYNIC with tricine and TPPTS results in a versatile ternary ligand system that forms [^{99m}Tc] complexes with extremely high solution stability. The ternary ligand system (HYNIC, Tricine and TPPTS) has been successfully used for [^{99m}Tc]-labeling of small biomolecules, including GPIIb/IIIa receptor antagonists (31-34), a chemotactic peptide (35), LTB_4 receptor antagonists (36) and integrin $\alpha_v\beta_3$ receptor antagonists (37-39).

Targeting biomolecule

The targeting biomolecules can be small peptides or nonpeptide heterocycles. The selection of the targeting biomolecules is largely dependent on their receptor-binding affinity and selectivity for the integrin $\alpha_v\beta_3$ over GPIIb/IIIa. Selected targeting biomolecule should be

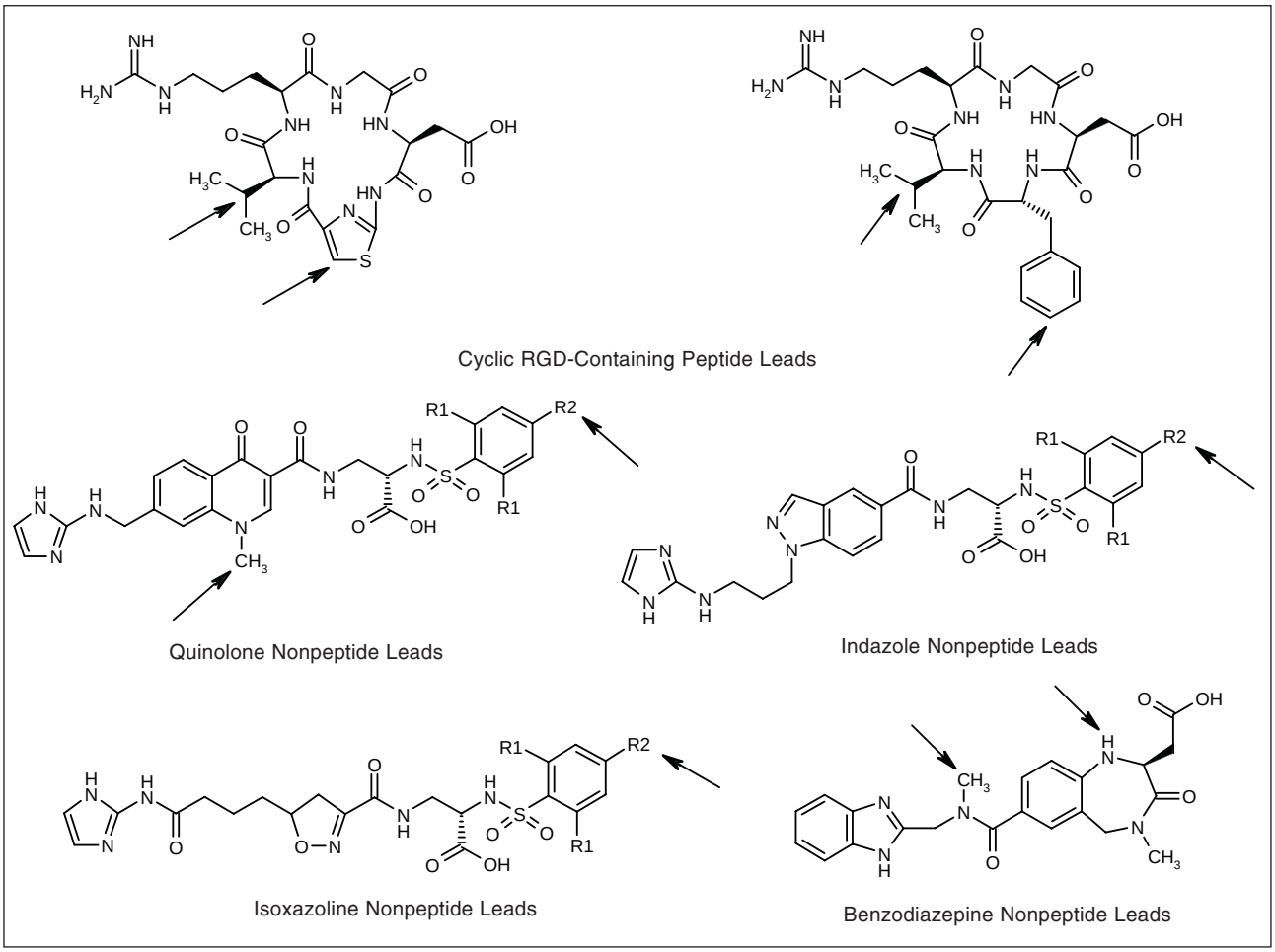

Fig. 2. Selected examples of peptide and nonpeptide leads. Arrows indicate possible sites for conjugation.

antagonists since the use of an agonist may cause certain unwanted side effects even at very low concentrations. It should have high receptor binding affinity for integrin $\alpha_v\beta_3$, with IC_{50} values preferably in the nanomolar range. It should also have high selectivity for integrin $\alpha_v\beta_3$, with high IC_{50} ratios ($\alpha_v\beta_3$/GPIIb/IIIa). The lipophilicity of the targeting molecule can be systematically modified using various water soluble linkers.

Many peptide and nonpeptide integrin $\alpha_v\beta_3$ receptor antagonists have been studied for their potential use as therapeutic drugs for the treatment of cancer (40-52) and some have demonstrated very high binding affinity and selectivity for integrin $\alpha_v\beta_3$ and have been shown to inhibit neovascularization, tumor-induced angiogenesis and tumor growth (45-47). Figure 2 shows several peptide and nonpeptide leads that have high affinity for integrin $\alpha_v\beta_3$, with IC_{50} values in the nanomolar or subnanomolar range. Arrows indicate possible sites for conjugation of the radiometal chelate. These integrin $\alpha_v\beta_3$ receptor antagonists are potential candidates as targeting biomolecules to carry the radionuclide to tumors (18, 27, 28).

Radiolabeled integrin $\alpha_v\beta_3$ receptor antagonists for tumor imaging

Linear peptides

A synthetic linear decapeptide αP2 (RGDSCRGDSY) has been radiolabeled with [^{99m}Tc] by ligand exchange with [^{99m}Tc]-glucoheptonate (53). The linear peptide contains two RGD sequences for integrin $\alpha_v\beta_3$ receptor binding. It was believed that the cysteine residue inserted in the primary structure is responsible for [^{99m}Tc]-binding. However, there is no structural information reported for the corresponding [^{99m}Tc] complex. In humans, 6 out of 8 lymph node metastases (75%) and all other neoplastic sites (11 sites) were successfully imaged using the [^{99m}Tc]-labeled peptide αP2. [^{99m}Tc]-labeled αP2 was also rapidly cleared from circulation via the renal system. An [^{18}F]-labeled linear RGD-containing peptide (KPQVTEGDFTEG-NH_2) was recently been prepared via rapid solid-phase synthesis (54). However, biodistribution data show very low tumor uptake in Balb/c mice bearing colorectal tumors.

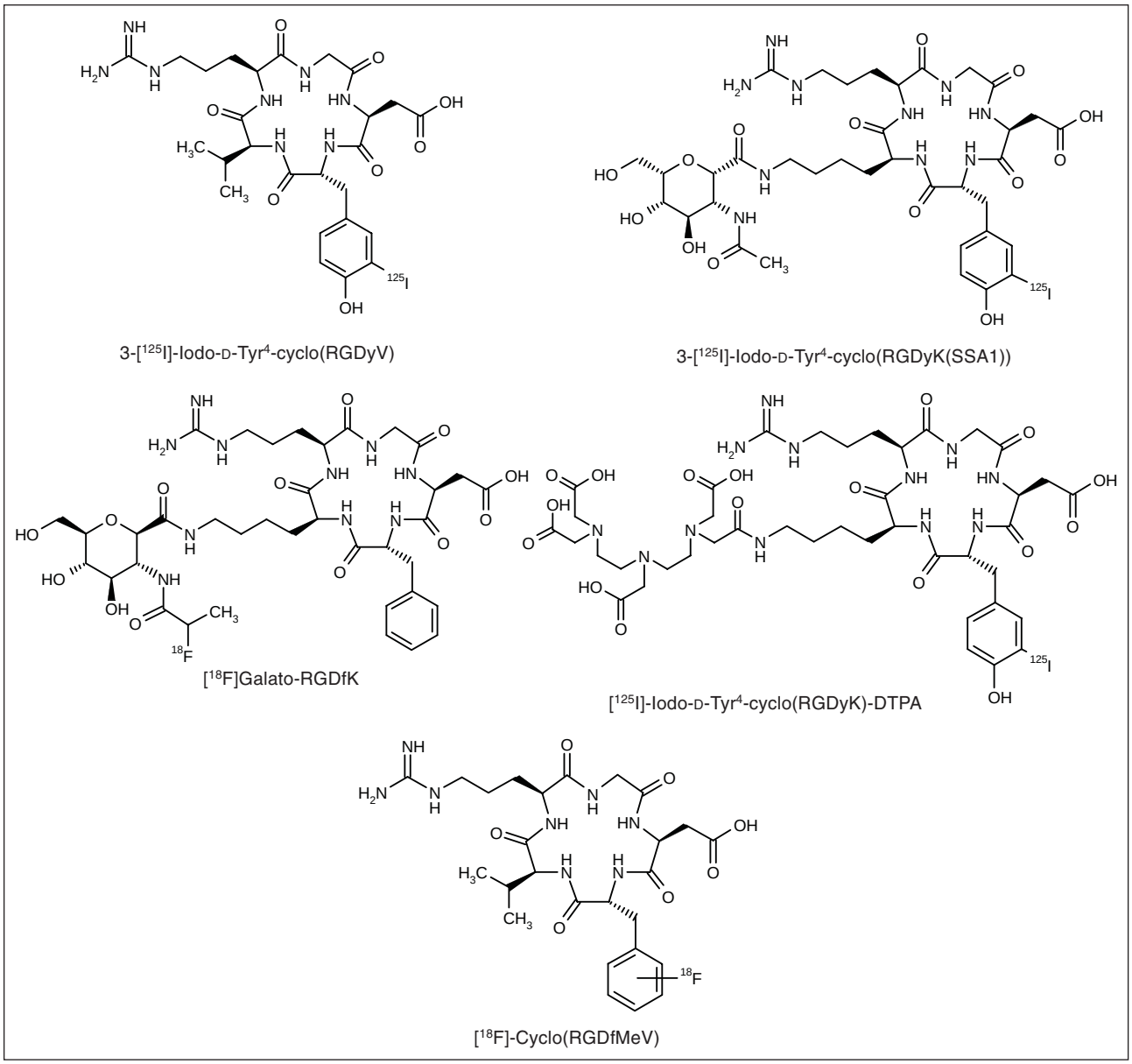

Fig. 3. Structures of [^{125}I]- and [^{18}F]-labeled RGD-containing cyclic peptides.

Linear peptides are often degraded rapidly in serum by protease. The combination of low receptor binding affinity, lack of specificity and rapid degradation makes linear peptides nonoptimal targeting biomolecules for the development of target-specific radiopharmaceuticals (55). It has been shown that cyclization of RGD-containing peptides via various linkers (S-S disulfide, thioether-S and rigid aromatic rings or other heterocycles) leads to increased receptor binding affinity and selectivity (56, 57). However, there is little evidence to show that any particular mode of cyclization will result in high affinity receptor binding. However, it is clear that cyclic peptides with a 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 (56, 57).

Cyclic peptides

Peptides containing the RGD sequence will bind to both the GPIIb/IIIa and the integrin $\alpha_v\beta_3$ receptors. A major challenge in the design of integrin $\alpha_v\beta_3$ antagonists is to impart selectivity. It has been shown that incorporation of the RGD-sequence in a cyclic pentapeptide (Fig. 3) results in improved selectivity for integrin $\alpha_v\beta_3$ (56, 57). Further SAR studies showed that the amino acid

substitution in position 5 has no influence on activity. Recently, Kessler and coworkers described two [^{125}I]-labeled cyclic pentapeptides (Fig. 3): 3-[^{125}I]-D-Tyr^4-cyclo(RGDyV) and 3-[^{125}I]-D-Tyr^4-cyclo-(RGDyK(SAA1)) (SAA = sugar amino acid). It was found that 3-[^{125}I]-D-Tyr^4-cyclo-(RGDyV) has fast hepatobiliary and renal excretion (58, 59). The tumor/muscle and tumor/blood ratios for melanoma in nude mice were 5.5 and 9.5, respectively, at 60 min postinjection. Substitution of leucine with a SAA-functionalized lysine amino acid residue resulted in improved blood retention time, renal excretion and a better target to background ratio. A blocking study using cyclo(RGDfV) at a dose of 3 mg/kg demonstrated that the localization of radioactivity in the tumor is due to the integrin $\alpha_v\beta_3$ receptor binding (59). The [^{18}F]-labeled cyclo(RGDfV) analog has also been used for PET imaging in melanoma- and osteosarcoma-bearing mice (60, 61). Introduction of the sugar moiety improves the pharmacokinetics and increases the tumor uptake (about 3% ID/g at 1-4 h postinjection) of the radiotracer.

A dual isotope ([^{125}I] and [^{111}In]) labeled cyclic peptide conjugate, c(RGDyK)-DTPA (Fig. 3), was recently reported (62). In autoradiolography and immunohistochemistry studies, the [^{125}I]-labeled analog appeared to bind specifically and with high affinity to integrin $\alpha_v\beta_3$ on neovascular blood vessel sections of different major human cancers, particularly breast and prostate cancers. The [^{125}I]-labeled analog was found to bind to and undergo internalization in human carcinoid Bon cells and rat pancreatic CA20948 tumor cells (62). The internalization is receptor-specific, and time and temperature dependent. However, biodistribution data for the [^{111}In]-labeled cyclic peptide DTPA conjugate showed very low tumor uptake and the tumor uptake is dependent on the injected dose of unlabeled DTPA conjugate.

Hnatowich and coworkers (63) recently reported the *in vitro* and *in vivo* evaluation of a [^{99m}Tc]-labeled RGD-containing cyclic peptide, RGD-4C, which contains 4 cysteine residues for cyclization. HYNIC was used as the BFC and tricine as the coligand for the [^{99m}Tc] labeling. The tumor uptake of [^{99m}Tc]-labeled RGD-4C in nude mice bearing human renal adenocarcinomas(ACHN) tumors was very low, which has been attributed to the limited numbers of integrin $\alpha_v\beta_3$ receptors per tumor cell and relatively low binding affinity of the [^{99m}Tc] complex (63).

A cyclic peptide (Fig. 3) has been labeled with [^{18}F] by direct electrophilic fluorination of the phenylalanine residue of cyclo(RGDfMeV) (64). In the integrin $\alpha_v\beta_3$ and GPIIb/IIIa ELISA assay, the cyclco(RGDfMeV) shows extremely high receptor binding affinity (IC_{50} = 2.3 nM) for integrin $\alpha_v\beta_3$ and a reasonably high selectivity over GPIIb/IIIa (IC_{50} = 120 nM). Results from biodistribution studies in tumor-bearing mice with DLD-1 (human colon adenocarcinomas) showed that the [^{18}F]-labeled peptide [^{18}F]-cyclco(RGDfMeV) has a relatively high tumor uptake (0.88 ± 0.06% IG/g) although the uptake in liver, intestine and kidney was too high to allow accurate detection of tumors (64).

Milind and coworkers at the Bristol-Myers Squibb Medical Imaging (BMS) recently reported synthesis of HYNIC conjugates of a series of RGD containing cyclic peptides (37-39). Figure 4 shows selected examples of HYNIC-peptide conjugates. Table I summarizes the integrin $\alpha_v\beta_3$ and GPIIb/IIIa receptor binding data for the HYNIC-peptide conjugates. Most of HYNIC-peptide conjugates show high receptor binding affinity and good selectivity for integrin $\alpha_v\beta_3$ with IC_{50} values in the nanomolar range. SU-013 and SU-011 are specifically designed as the negative control for *in vivo* imaging studies. Due to changes in the peptide sequence or stereochemistry of a specific amino acid, they show low binding affinity for the

Table I: The integrin $\alpha_v\beta_3$ and GPIIb/IIIa receptor binding data for HYNIC conjugates and ternary ligand [^{99}Tc] complexes.

Compound	[^{99m}Tc] Complex	IC_{50} (nM) ELISA B.Vn	IC_{50} (nM)/IIb/IIIa ^{125}I-Fibrinogen	Structure Acronym
Vn		5 (n=5)	294 (n=1)	Vitronectin
RGDFV		0.4 (n=2)	15,399 (n=1)	c(RGDFV)
SQ-152	RP-570	3.0 (n=1)	1,234 (n=1)	c(RGDy*V)
	[^{99}Tc]RP-570	1.0 (n=1)	>1000 (n=1)	[^{99}Tc] complex
SQ-096	RP-580	0.3 (n=1)	35 (n=1)	c(R"GD(ata)K*)
SQ-157	RP-582	1.0 (n=2)	8,842 (n=1)	c(RGDfK*)
SQ-159	RP-583	5.0 (n=1)	>10,000 (n=1)	C(RGDyK*)
SQ-168	RP-593	0.6 (n=3)	10,209 (n=1)	c(RGDFK*) dimer
	[^{99}Tc]RP-593	2.0 (n=2)	>10,000 (n=2)	[^{99}Tc] complex
SU-013	RP-685	>10,000 (n=4)	>20,000 (n=1)	c(RGK*fD)
	[^{99}Tc]RP-685	>10,000 (n=2)	>20,000 (n=1)	[^{99}Tc] complex
SG-386	RP-678	3 (n=2)	2,838 (n=1)	Quinolone monomer
	[^{99}Tc]RP-678	0.1 (n=2)	>647 (n=2)	[^{99}Tc] complex
SQ-102	RP-692	3 (n=1)	22,426 (n=1)	Quinolone monomer
SQ-103	RP-693	0.5 (n=1)	18,498 (n=1)	Quinolone monomer
SQ-104	RP-696	0.1 (n=1)	10,124 (n=1)	Quinolone dimer
SG-391	RP-711	0.2 (n=1)	1,857 (n=1)	Quinolone monomer

*Indicating the site for HYNIC conjugation.

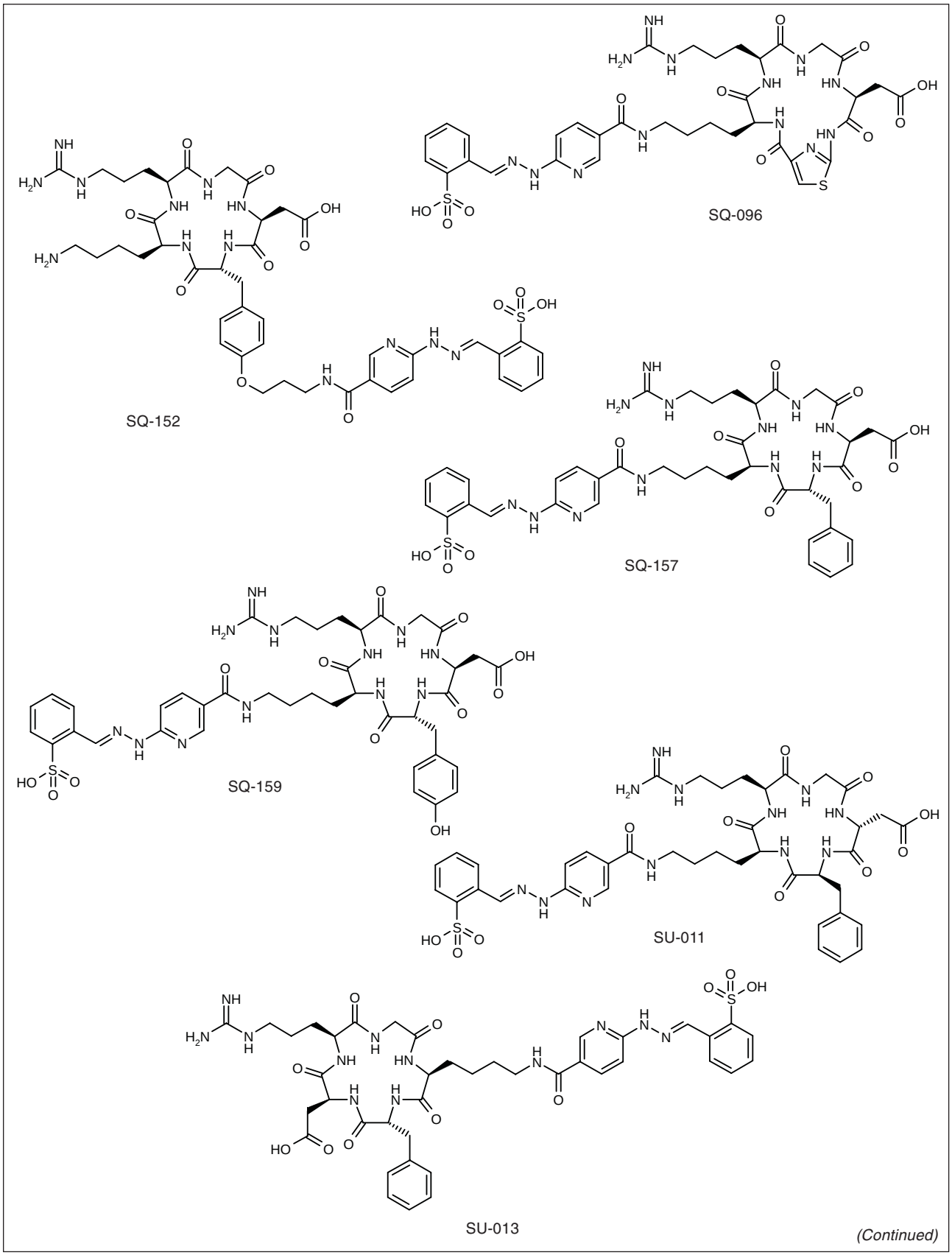

Fig. 4. Structures of selected HYNIC conjugates of cyclic peptides.

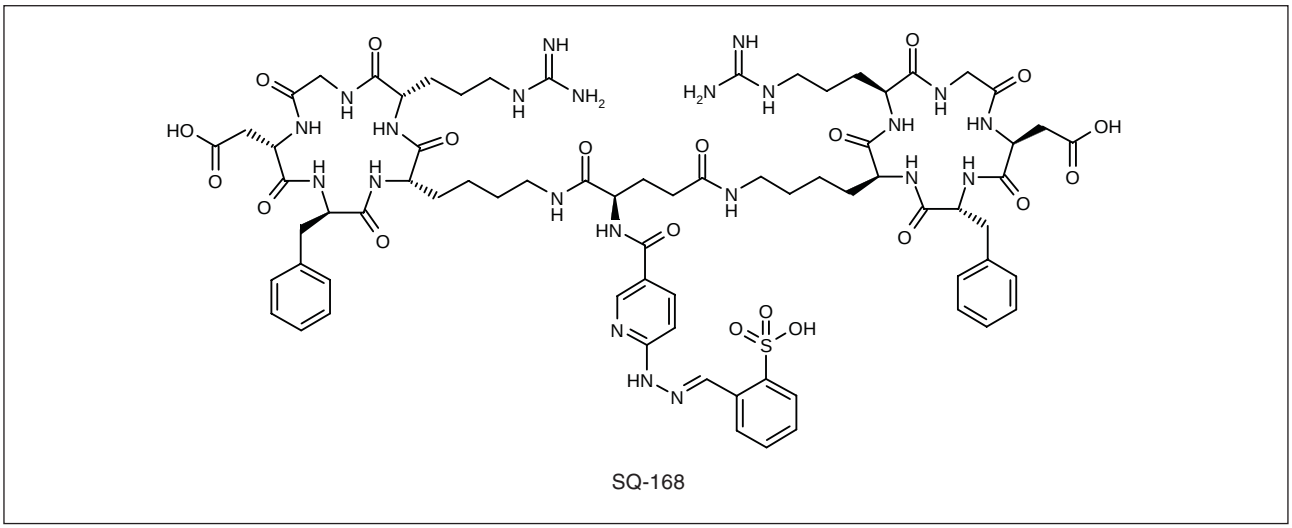

Fig. 4 (Cont.). Structures of selected HYNIC conjugates of cyclic peptides.

integrin $\alpha_v\beta_3$. These HYNIC-peptide conjugates form very stable ternary ligand technetium complexes [^{99m}Tc-(HYNIC-peptide)(tricine)(TPPTS)] when TPPTS and tricine are used as coligands. Ternary ligand [^{99}Tc] complexes ([^{99}Tc]-RP-593 and [^{99}Tc]-RP-685) were also prepared and tested for their receptor binding affinity for the integrin $\alpha_v\beta_3$ to study the impact of radiolabeling on receptor binding affinity of the corresponding HYNIC conjugate. In both cases, attachment of the technetium chelate shows no significant impact (within the experimental error of the assay) on the receptor binding.

In general, for a ternary ligand [^{99m}Tc] complex to have any potential use as a new imaging agent, the corresponding HYNIC conjugate must have a high receptor binding affinity with the IC_{50} of 10 nM or less in the ELISA assay. The higher the binding affinity for the HYNIC conjugate is, the better the tumor uptake for the corresponding [^{99m}Tc] complex. For example, SU-013 has very low affinity for integrin $\alpha_v\beta_3$ (IC_{50} > 10,000 nM) and its [^{99m}Tc] complex, RP-685, shows no significant accumulation of radioactivity in the tumors (Fig. 6). On the other hand, SQ-168 has a high receptor binding affinity for integrin $\alpha_v\beta_3$ (IC_{50} = 0.6 nM). The corresponding [^{99m}Tc] complex, RP-593, show very high tumor uptake (Fig. 6) and has a long tumor retention time. The tumor uptake of RP-593 can be blocked by coinjection of excess unlabeled SQ-168, suggesting that the tumor uptake is most likely due to integrin $\alpha_v\beta_3$ binding.

Both RP-593 and RP-582 have also been evaluated in the athymic female BALB/c mice with s.c. OVCAR-3 ovarian carcinoma xenografts (65). The tumor uptake peaked at 5.8 ± 0.7% ID/g and 5.2 ± 0.6% ID/g for RP-593 and RP-582, respectively. At 1, 2 and 4 h postinjection, the tumor uptake of RP-593 was significantly higher than that of RP-582. The tumor-to-blood ratios were highest at 24 h postinjection at a value of 63 for both RP-593 and RP-582. At all time points, the kidney retention for RP-593 was significantly higher than that of RP-582, and was attributed to the presence of 2 arginine amino acid residues.

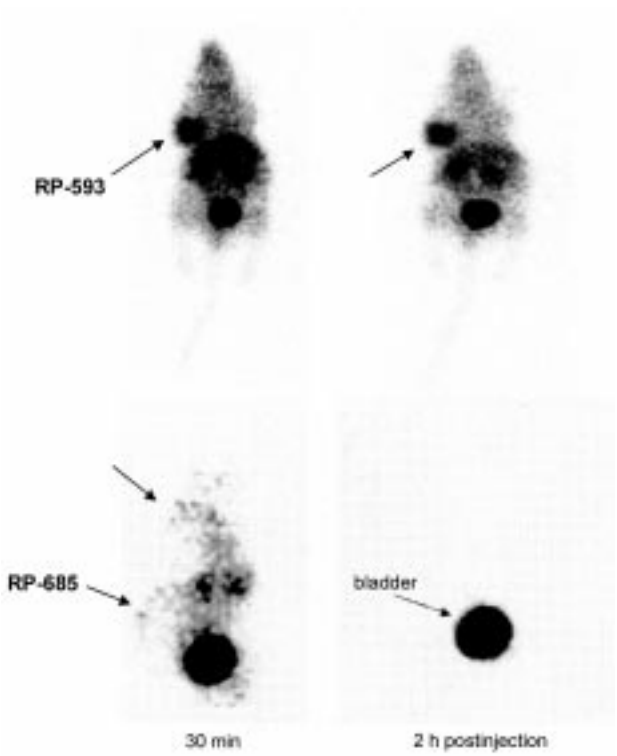

Fig. 5. Representative images of RP-593 (top) and RP-685 (bottom) at 30 min and 2 h postinjection in the c-neu oncomouse® model. Arrows indicate the presence of radioactivity in tumors or bladder. Images have not been filtered.

RP-593 was also tested in dogs with confirmed mammary adenocarcinomas. Figure 8 shows representative

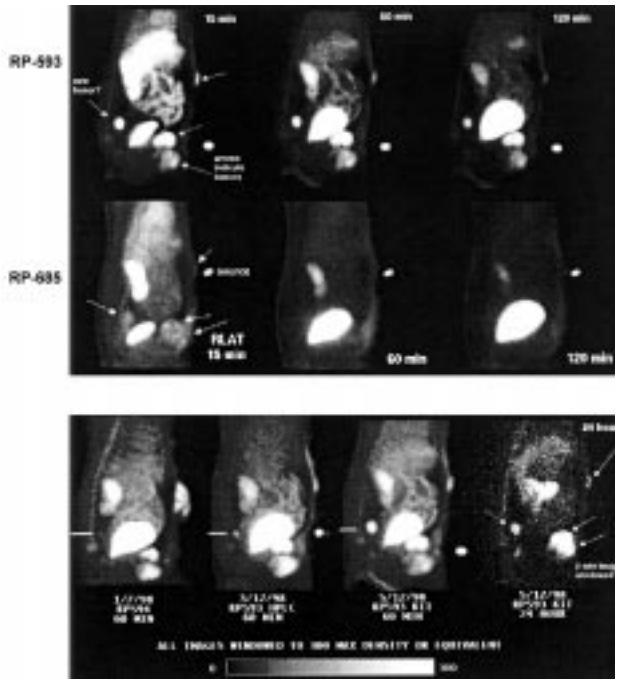

Fig. 6. Representative images of RP-593 (top) and RP-685 (bottom) at 30 min and 2 h postinjection in the canine with confirmed mammary adenocarcinoma. RP-685 was a negative control for imaging studies. When the dog was first administered RP-593 (1/7/98), scintigraphic images showed no indication of any metastatic tumor. Four months later, images of the dog administered RP-593 clearly showed the rapidly growing tumor (on the left side of each image).

images of the same dog separately administered RP-593 (top) or the negative control RP-685 (bottom) at 30 min and 2 h postinjection. Scintigraphic images of the dog administered with RP-593 clearly show the presence of tumors, including a rapidly growing tumor (on the left side of each image). In contrast, RP-685 shows no uptake in these tumors. 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 was clearly seen 60 min after injection of RP-593 (0.5 mCi/kg i.v.). As the tumor size increases, the images show increased localization of radioactivity in the tumor site. These studies clearly demonstrate that RP-593 has the potential as a new radiopharmaceutical for detection of solid tumors and for monitoring the tumor growth. However, the biodistribution data in the c-neu oncomouse® model shows that RP-593 is excreted via both renal and hepatobiliary routes. The radioactivity levels in blood, kidney and liver are relatively high, which makes RP-593 less than ideal as an imaging agent.

The fact that RP-593 shows superior tumor uptake and longer tumor retention time as compared to RP-582 is very intriguing. The receptor binding affinity of SQ-157 (peptide monomer: IC_{50} = 1.0 nM) and SQ-168 (peptide dimer: IC_{50} = 0.6 nM) in the ELISA assay is almost identical within the experimental error. Therefore, it is reasonable to believe that the difference between RP-593 and RP-582 is likely related to the presence of the 2 RGD-containing cyclic peptides in RP-593 and their receptor binding kinetics. It can be envisioned that the binding of one RGD-containing cyclic peptide to the integrin $\alpha_v\beta_3$ will significantly increase the "local concentration" of the second RGD-containing cyclic peptide in the vicinity of the receptor-binding site. The high "local RGD-peptide concentration" is expected to enhance the rate of receptor binding or/and reduce the rate of dissociation of the radiolabeled bioconjugate from the receptor site; thereby improving the localization and retention of radiolabeled bioconjugate in tumors.

Nonpeptide integrin $\alpha_v\beta_3$ receptor antagonists

A number of nonpeptide leads are useful as targeting biomolecules for development of integrin $\alpha_v\beta_3$-targeted radiopharmaceuticals. Figure 2 shows selected examples with the quinolone, indazole, isoxazoline and benzodiazepine scaffolds. A major challenge in using nonpeptide $\alpha_v\beta_3$ receptor antagonists as targeting biomolecules is their selectivity for integrin $\alpha_v\beta_3$. HYNIC conjugates derived from indazole and benzodiazepine scaffolds showed high receptor binding affinity, but a limited selectivity for integrin $\alpha_v\beta_3$. Although [^{99m}Tc] complexes of HYNIC-nonpeptide conjugates containing indazole and benzodiazepine scaffolds show excellent tumor uptake (66, 67), it is still difficult to develop them into commercial product due to lack of specificity and selectivity for integrin $\alpha_v\beta_3$.

Harris and coworkers at BMS recently reported a series of HYNIC conjugates of quinolone-based integrin $\alpha_v\beta_3$ receptor antagonists and the use of their radiometal complexes for tumor imaging and radiotherapy (30, 68). The quinolone-based nonpeptide integrin $\alpha_v\beta_3$ receptor antagonists are of particular interest due to their high binding affinity and selectivity for integrin $\alpha_v\beta_3$ over GPIIb/IIIa (68). Figure 7 shows selected examples of the synthesized HYNIC-nonpeptide conjugates. The receptor binding data for integrin $\alpha_v\beta_3$ and GPIIb/IIIa are listed in Table I. In all the cases, the HYNIC conjugates show very high receptor binding affinity for integrin $\alpha_v\beta_3$. The attachment of the technetium chelate has no significant impact on the receptor binding.

Ternary ligand [^{99m}Tc] complexes [^{99m}Tc(HYNIC-BM)(tricine)(TPPTS)] (HYNIC-BM = SG-386, SQ-102, SQ-103, SQ-104 and SG-391) were screened in the c-neu oncomouse® model for tumor imaging and biodistribution (69). The biodistribution data in selected organs for [^{99m}Tc] complexes are listed in Table II. Compared to those of HYNIC-peptide conjugates, ternary ligand [^{99m}Tc] complexes of HYNIC-nonpeptide conjugates show much higher tumor uptake (Table II) and better target-to-background ratios (Table III). For example, the tumor uptake of RP-678 is about 1.5-fold higher than that of RP-593 while the kidney and liver uptake of RP-678 is much lower than

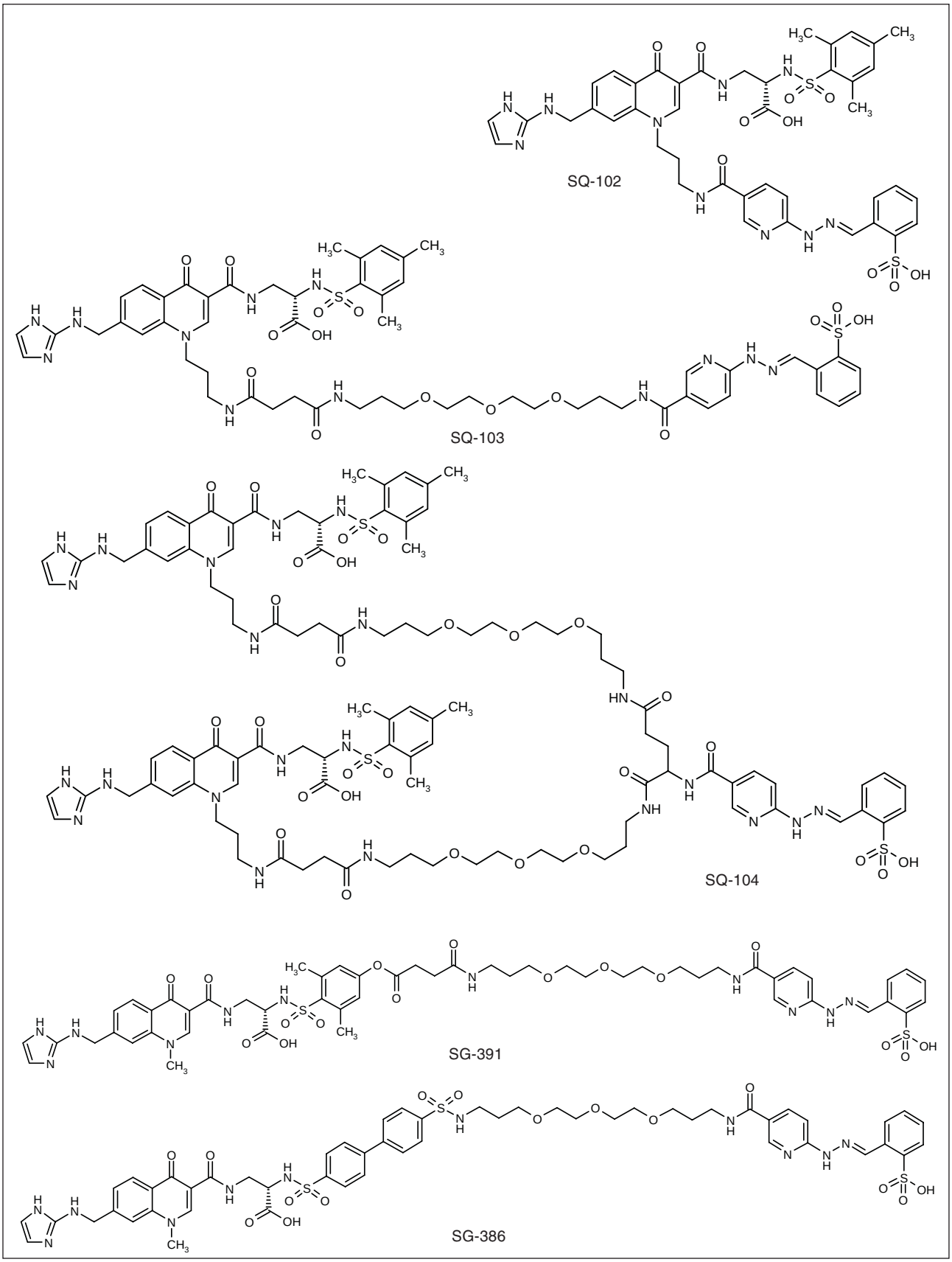

Fig. 7. Structures of selected HYNIC-nonpeptide conjugates.

Table II: Biodistribution data of selected ternary ligand [^{99m}Tc] complexes (administered i.v. at a dose of 2 mCi/kg) at 2 h postinjection.

[^{99m}Tc] Complex	Tumor Uptake (% ID/g)	Blood Activity (% ID(g)	Kidney Uptake (% ID/g)	Liver Uptake (% ID/g)
RP-593	4.65 (n=5)	1.1 (n=5)	12.3 (n=5)	5.18 (n=5)
RP-675	0.692 (n=2)	0.88 (n=2)	4.3 (n=2)	0.38 (n=2)
RP-685	0.61 (n=2)	0.91 (n=2)	3.4 (n=2)	0.51 (n=2)
RP-678	7.5 (n=3)	1.3 (n=3)	3.6 (n=3)	2.7 (n=3)
RP-692	9.6 (n=1)	1.3 (n=1)	4.4 (n=1)	7.1 (n=1)
RP-693	8.4 (n=2)	3.9 (n=2)	16.0 (n=2)	2.1 (n=2)
RP-696	8.5 (n=1)	1.9 (n=1)	42.6 (n=1)	1.8 (n=1)

Table III: Comparison of selected ternary ligand [^{99m}Tc] complexes with respect to tumor/nontarget ratios at 2 h postinjection in the c-neu oncomouse® model.

[^{99m}Tc] Complex	Tumor/Muscle Ratio	Tumor/Kidney Ratio	Tumor/Blood Ratio	Tumor/Liver Ratio
RP-593	4.64	0.38	4.23	0.90
RP-685	1.12	0.18	0.67	1.20
RP-678	7.45	2.08	5.77	2.78
RP-692	16.00	2.18	7.38	1.35
RP-693	8.64	0.53	2.15	4.00
RP-696	10.13	0.20	4.47	4.72

that of RP-593. In this respect, the [^{99m}Tc]-labeled quinolone-based integrin $\alpha_v\beta_3$ receptor antagonists offer advantages over the [^{99m}Tc]-labeled cyclic RGD peptides. However, [^{99m}Tc] complexes of HYNIC-nonpeptide conjugates still show a certain degree of hepatobiliary clearance. For example, RP-692 shows the highest uptake in tumors and liver. Both RP-693 and RP-696 show high tumor uptake; but the high kidney uptake and long retention time in kidneys make them less desirable as new imaging agents. Therefore, future research should be directed towards modification of ternary ligand [^{99m}Tc] complexes of HYNIC-nonpeptide conjugates.

Conclusions

Radiolabeled integrin $\alpha_v\beta_3$ receptor antagonists represent a new class of target-specific radiopharmaceuticals with the potential to be used not only for early detection of tumors but also for staging the extent of the tumor growth and monitoring the therapeutic response of cancer treatment. Since integrin $\alpha_v\beta_3$ is highly expressed in rapidly growing tumors and during tumor invasion and metastasis, radiopharmaceuticals targeting integrin $\alpha_v\beta_3$ are most like to be more specific for rapidly growing and metastatic solid tumors. Radiopharmaceuticals with an appropriate therapeutic radionuclide can be used for radiotherapy of solid tumors.

The use of peptide dimer in RP-593 as the targeting biomolecule is very intriguing. Giving the short distance between the 2 cyclic RGD peptides, it is unlikely that these 2 cyclic peptides would bind to the adjacent integrin $\alpha_v\beta_3$ receptors simultaneously. However, the binding of 1 cyclic RGD peptide to integrin $\alpha_v\beta_3$ will significantly increase the “local concentration” of the second cyclic RGD peptide in the vicinity of the receptor-binding site. The high “local RGD-peptide concentration” is expected to enhance the rate of receptor binding and reduce the rate of dissociation of the radiolabeled bioconjugate from the receptor site; thereby improving the localization and retention of radiolabeled bioconjugate in tumors. The same concept has been used for the design of SQ-103 and RP-696 , but the tumor uptake of RP-696 (quinolone dimer) is not significantly different from that of RP-693 (quinolone monomer). Therefore, the “dimer” or “polymer” concept needs to be further explored in the future.

There are many factors influencing the tumor uptake and tumor retention of a specific radiotracer. These include the receptor population on endothelial and tumor cells, receptor binding affinity, lipophilicity, protein binding, receptor binding and dissociation kinetics, and excretion route. The fact that the [^{99m}Tc]-labeled peptide αP2 has

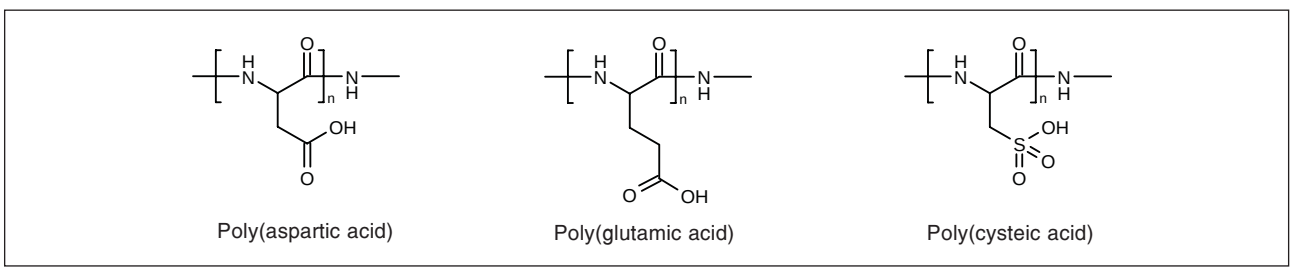

Fig. 8. Small peptides as pharmacokinetic modifiers for [^{99m}Tc]-labeled biomolecules.

been successfully used for imaging lymph node metastases in humans strongly suggests that there are sufficient integrin $\alpha_v\beta_3$ receptors on both tumor neovasculature and tumor cells for scintigraphic imaging.

Within the literature, there is an overwhelming emphasis on the impact of integrin $\alpha_v\beta_3$ binding affinity on the tumor uptake with very little attention paid to the improvement of receptor binding kinetics (association and dissociation rate) and the impact of receptor binding kinetics on the tumor uptake and retention time. There is also very limited information available to link the lipophilicity and protein binding characteristics of [^{99m}Tc]-labeled small biomolecules directly to their biodistribution and pharmacokinetic properties.

The main pharmacokinetic consideration for a new [^{99m}Tc] radiopharmaceutical is that it has a high tumor uptake with diagnostically useful target-to-background 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. The hepatobiliary excretion and high protein binding are detrimental for the improvement of target-to-background ratio. Therefore, an important aspect of the future research on new target-specific [^{99m}Tc] radiopharmaceuticals should be directed towards modification of lipophilicity and protein binding characteristics of the [^{99m}Tc]-labeled biomolecule to improve the target-to-background ratios.

Small biomolecules in the blood plasma are filtered through glomerular capillaries in the kidney and subsequently reabsorbed by the proximal tubular cells by carrier-mediated endocytosis. The membranes of the renal tubular cells contain negatively charged sites to which the positively charged groups (guanidine in the RGD sequence or imidazole in the quinolone scaffold) in small biomolecules are expected to bind. In this respect, negatively charged small peptide sequences (Fig. 8), such as poly(aspartic acid), poly(glutamic acid) and poly(cysteic acid), can be used as pharmacokinetic modifiers to reduce renal uptake of [^{99m}Tc]-labeled biomolecules. The carboxylic or sulfonic groups are expected to be deprotonated under physiological conditions, which results in formation of highly charged [^{99m}Tc] complexes. In this way, the interaction between the membranes of renal tubular cells and positively charged groups (guanidine in the RGD peptide sequence or imidazole in the quinolone scaffold) of small biomolecules can be minimized thereby reducing renal uptake of the [^{99m}Tc]-labeled small biomolecule. At the same time, the use of negatively charged pharmacokinetic modifiers is expected to increase the hydrophilicity of the [^{99m}Tc]-labeled biomolecule, which is expected to result in a faster renal excretion and improved target-to-background ratios.

It should be noted that radiopharmaceuticals targeting integrin $\alpha_v\beta_3$ are not just limited to diagnostic applications. Results from preclinical studies on [^{90}Y]-labeled integrin $\alpha_v\beta_3$ receptor antagonists (peptides and nonpeptides) are also very promising (29, 30, 68-70). One can always hope that one day these therapeutic radiopharmaceuticals can find the wide use for the treatment of cancer even though they may not become the elusive “magic bullet”. Even if it may not be a single agent, a cocktail of compounds (radiotherapy and chemotherapy) representing the Holy Grail of cancer research may be found in the foreseeable future.

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