
Single Photon Emission Computed Tomography Tracer

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Abstract

Single photon emission computed tomography (SPECT) is the state-of-the-art imaging modality in nuclear medicine despite the fact that only a few new SPECT tracers have become available in the past 20 years. Critical for the future success of SPECT is the design of new and specific tracers for the detection, localization, and staging of a disease and for monitoring therapy. The utility of SPECT imaging to address oncologic questions is dependent on radiotracers that ideally exhibit excellent tissue penetration, high affinity to the tumor-associated target structure, specific uptake and retention in the malignant lesions, and rapid clearance from non-targeted tissues and organs. In general, a target-specific SPECT radiopharmaceutical can be divided into two main parts: a targeting biomolecule (e.g. peptide, antibody fragment) and a γ -radiation emitting radionuclide (e.g. ^{99m}Tc , ^{123}I). If radiometals are used as the radiation source, a bifunctional chelator is needed to link the radioisotope to the targeting entity. In a rational SPECT tracer design these single components have to be critically evaluated in order to achieve a balance among the demands for adequate target binding, and a rapid clearance of the radiotracer. The focus of this chapter is to depict recent developments of tumor-targeted SPECT radiotracers for imaging of cancer diseases. Possibilities for optimization of tracer design and potential causes for design failure are discussed and highlighted with selected examples.

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Contents

1	Introduction.....	66
2	General Aspects for the Design of SPECT Tracers.....	69
3	Peptide-Receptor Radionuclide Imaging	73
3.1	Somatostatin Analogs.....	74
3.2	Bombesin Analogs.....	76
3.3	Neurotensin Analogs	79
3.4	Other Peptides-Based Radiotracers.....	81
4	Antibodies and Antibody Fragments	82
4.1	Targeting Fibronectin Extra-Domain B: Antiangiogenic Antibody Fragment L19...	84
5	Vitamin-Based Radiotracers.....	85
5.1	Folic Acid Conjugates.....	85
5.2	Vitamin B ₁₂ Conjugates.....	87
5.3	Other Vitamin Targeting Agents	89
6	Intracellular Targets	90
6.1	^{99m} Tc-Carbohydrate Complexes	90
6.2	Radiolabeled Nucleoside Analogs for Targeting Human Thymidine Kinase.....	91
6.3	Radioiodinated Meta-Iodobenzylguanidine	93
7	Optimization of SPECT Tracer Design and Potential Reasons for Failure.....	95
8	Summary and Conclusion	96
	References.....	97

1 Introduction

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are valuable molecular imaging modalities as both are capable of detecting minute amounts of radioactive tracer (Rowland and Cherry 2008; Spanoudaki and Ziegler 2008). Clinical PET is currently about 2–3 orders of magnitude more sensitive than SPECT, has a better spatial resolution, and offers superior quantification. Nowadays, many nuclear imaging centers possess PET or PET/CT scanners. However, the large infrastructure that is needed for the production of β^+ -emitting radioisotopes (e.g. ¹⁸F, ¹¹C, ⁶⁴Cu) make PET an expensive technology. Also, at the moment there are no approved clinical grade generators for PET radioisotopes (e.g. ⁶⁸Ga, ⁴⁴Sc). Hence, for routine application SPECT is still the state-of-the-art nuclear imaging modality because it is less expensive and can make use of a broader array of suitable and available radionuclides (Table 1). Importantly, SPECT imaging is a useful technology for monitoring targeted radionuclide therapy employing radioisotopes that emit—concomitantly with the therapeutic radiation— γ -rays of suitable energies for SPECT (e.g. ¹⁷⁷Lu, ^{188/186}Re, ⁶⁷Cu, ¹³¹I, ²¹³Bi) (Alford et al. 2009).

Generally, SPECT radiopharmaceuticals can be classified according to their biodistribution characteristics. There are those whose tissue distribution is determined exclusively by their chemical and physical properties and those whose distribution and accumulation are determined by their specific interaction with a biological target that is expressed at the site of interest (e.g. tumor-associated

Table 1 Selection of radioisotopes for SPECT imaging (and therapy)

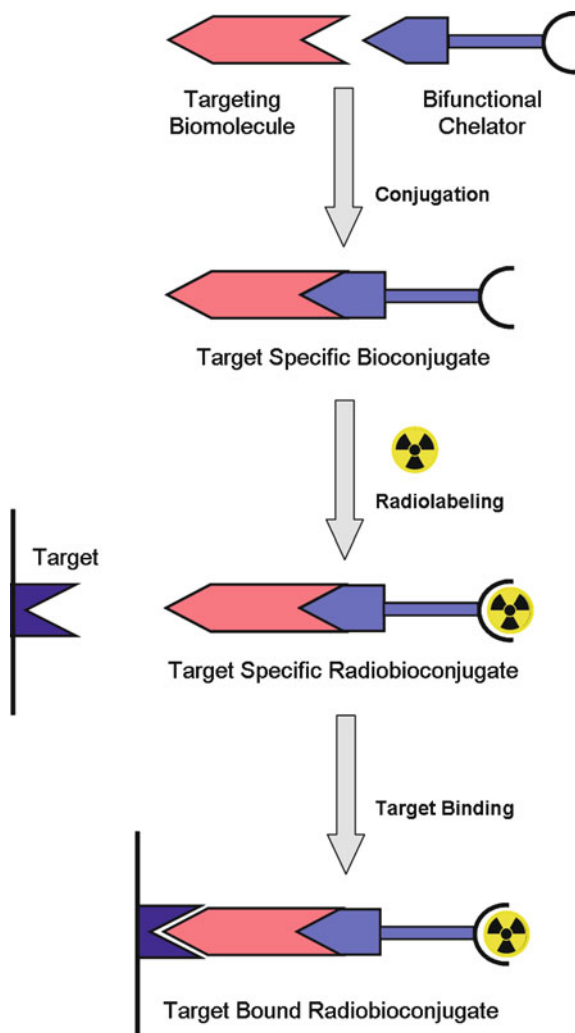
SPECT-isotopes	Half-life	γ -Energy	
^{99m}Tc	6.02 h	141 keV (89 %)	
^{111}In	2.80 d	171 keV (91 %), 245 (94 %)	
^{67}Ga	3.26 d	93 (39 %), 185 (21 %), 300 (17 %), 394 (5 %)	
^{123}I	13.22 h	159 (83 %)	
Therapy/SPECT isotopes	Half-life	β^- -Energy _{average} [keV]	γ -Energy [keV]
^{177}Lu	6.65 d	134 (100 %)	113 (10 %), 208 (10 %)
^{186}Re	3.72 d	347 (93 %)	137 (9.5 %)
^{188}Re	17.0 h	763 (100 %)	155 (16 %)
^{67}Cu	2.58 d	141 (100 %)	185 (49 %)
^{131}I	8.03 d	182 (100 %)	365 (82 %)

receptor) (Liu 2008; Bartholoma et al. 2010). Herein we focus on the development and (pre) clinical application of target-specific radiotracers. A target-specific SPECT radiopharmaceutical can be divided into two main parts: a targeting biomolecule and a γ -radiation emitting radionuclide (Liu 2008). In the case of using radiometals as the radiation source a bifunctional chelator is needed as an additional component of the radiopharmaceutical. Thus, a metallic radioisotope is coordinated by a suitable chelating agent that is conjugated to the targeting agent via a linker entity (Fig. 1). In a rational design of a SPECT tracer the single components have to be critically evaluated in order to achieve a balance among the demands of an adequate target binding and a rapid excretion.

The majority of diagnostic radiopharmaceuticals currently available in nuclear medicine make use of metallic radioisotopes. For SPECT imaging [^{99m}Tc]-technetium is the most widely applied radioisotope because of its ideal physical decay properties and easy availability by a generator system (Table 1). [^{111}In]-indium is another SPECT radioisotope frequently used in the clinics where it is often employed as a surrogate for [^{90}Y]-yttrium analogs since ^{90}Y that is used for therapeutic purposes is a pure β^- -emitter. In contrast, clinical application of [^{67}Ga]-gallium is relatively rare. Non-metallic radionuclides used for SPECT are basically the isotopes of iodine. [^{123}I]-Iodine has dosimetry and imaging characteristics that are superior to [^{131}I]-iodine and [^{125}I]-iodine and is therefore the preferred isotope for imaging purposes (Table 1).

A targeting biomolecule serves as a “carrier” for specific delivery of the radionuclide to the target expressing cells of interest. Such biomolecules could be specific antibodies (or antibody fragments) or small molecular weight molecules (e.g. peptides, vitamins, nucleosides). Each class of targeting agents has its pros and cons for its use in diagnostic nuclear medicine and for a potential translation to therapeutic applications. Peptide-based radiopharmaceuticals represent by far the largest group of tumor-targeted radioimaging agents currently in use.

Fig. 1 Schematic representation of a biomolecule that is conjugated to a radiometal. The bifunctional chelator that is conjugated to the target-specific biomolecule is necessary for coordination of the radiometal. The target-specific radiobioconjugate binds to the tumor cell surface associated target (e.g. receptor) and allows visualization of target-expressing malignant lesions via SPECT



During tracer development, the first steps are based on chemistry and molecular biology methods such as peptide syntheses, conventional or combinatorial chemistry, and phage display techniques for preparation, identification, and isolation of high-affinity binders to a particular receptor. Determination of the tumor-targeted radiotracer's stability *in vitro* and its ability to bind with high affinity to the target structure on cultured cancer cells are first requirements in this early development stage. The *in vitro* evaluation is followed by investigations *in vivo* using an adequate animal model, typically tumor-bearing small rodents. It is important to recognize that radiolabeled tumor imaging agents display different biodistribution and pharmacokinetics in animal models compared to humans due to a different metabolism, differences in the volume of distribution and potential

cross-reactivity of the targeting entity with normal tissues expressing the target receptor or antigen in humans (Buchsbaum 1997). Significant variability in the tissue distribution of radiotracers might occur among different animal models (e.g. mice vs. rats) or different animal strains (e.g. nude mice vs. normal mice). However, small rodents have emerged as generally the most useful and cost effective animal models for developing and evaluating radiotracers and to test new experimental approaches to increase their localization in tumors. Post-mortem biodistribution studies allow the detection and quantification of radioactive accumulation in targeted and non-targeted tissues, and thus the determination of the radiotracer's pharmacokinetic profile. Collection of blood and tissue samples for identification of metabolites at different time points after radiotracer application provides information about the radiotracer's circulation time and its in vivo stability. By increasing the availability of small-animal SPECT and SPECT/CT scanners in recent years, the process of radiotracer development has been significantly improved and accelerated while the number of test animals required has been reduced. Thus, a wide variety of targeted SPECT radiotracers are currently being developed and preclinically tested for in vivo imaging of various tumor types expressing one or more of the most relevant receptor types (Schottelius and Wester 2009).

The focus of this chapter is to present general aspects for the design of SPECT tracers followed by specific examples of recent SPECT imaging agents based on antibodies, antibody fragments, peptides, and other small-molecular weight biomolecules such as vitamins or nucleosides. The examples demonstrate possibilities for optimization of the tracer design by tuning single components of these imaging agents. Finally, potential causes for failures in SPECT tracer design are discussed.

2 General Aspects for the Design of SPECT Tracers

The ideal SPECT tracer exhibits excellent tissue penetration, high affinity to the target structure, specific uptake and retention in the target cells, and rapid clearance from non-targeted tissues and organs. In addition, it is highly stable in vivo, easy to prepare, and safe for human application. These aspects are crucial because injected radiotracers that are not stable, not bound to the target, or not rapidly excreted create high background signals resulting in low tumor-to-background contrast and unnecessary radiation dose burden to the patient (Alford et al. 2009).

In the case of metallic radioisotopes, a bifunctional chelator is needed that is covalently linked to a biomolecule (Liu 2008). Since the stability of the radiometal complex is a critical aspect for the success of a radiopharmaceutical, it is important to choose an ideal chelating system that allows the formation of radiometal complexes of high thermodynamic stability and kinetic inertness (Bartholoma et al. 2010). Among the SPECT isotopes currently in use, [^{99m}Tc]-technetium is still the workhorse of diagnostic nuclear medicine. It is used in the majority of diagnostic scans conducted each year in hospitals worldwide. The preferred use of ^{99m}Tc -radiopharmaceuticals reflects the ideal nuclear properties of the isotope and,

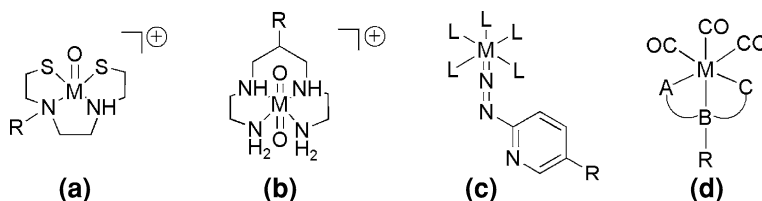


Fig. 2 The most frequently used $^{99\text{m}}\text{Tc}$ -complexes for radiobioconjugates. **a** $^{99\text{m}}\text{Tc}(\text{V})$ oxo-core, **b** $^{99\text{m}}\text{Tc}(\text{V})$ dioxo-core, **c** $^{99\text{m}}\text{Tc}(\text{V})$ organohydrazino-core (HYNIC) and **d** $^{99\text{m}}\text{Tc}(\text{I})$ -tricarbonyl-core. $\text{M} = ^{99\text{m}}\text{Tc}$, $\text{L} = \text{co-ligands}$

until recently, the convenient availability from commercial $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ -generators. Currently, a key challenge is the continuing global shortage of $^{99\text{m}}\text{Tc}$ because aging nuclear reactors that provide a large fraction of the world's supply require more frequent repair and/or routine maintenance.

Technetium is a transition metal that presents a major challenge with respect to designing radiopharmaceuticals with favorable *in vivo* properties. In order to link the radionuclide to a targeting molecule, $[\text{}^{99\text{m}}\text{Tc}(\text{VII})]$ -pertechnetate that is eluted from the $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ -generator must be reduced to build a complex with an appropriate bifunctional chelating system, most commonly in the oxidation state +I, +III or +V. The $^{99\text{m}}\text{Tc}(\text{V})$ -oxo and $^{99\text{m}}\text{Tc}(\text{V})$ -organohydrazino cores are most extensively studied (Fig. 2). The $^{99\text{m}}\text{Tc}(\text{V})$ -oxo-core generally adopts a square-pyramidal geometry with the π -bonding oxo-group in the apical position. The core is stabilized by σ - and π -donating groups where amino, amido, and thiolate ligands as well as tetradentate ligands of the N_xS_{4-x} -class have been investigated (Liu 2008; Bartholoma et al. 2010). A prominent example of a tetradentate chelator is the peptide-based chelator mercapto-acetylglucylglycylglycine (H_5MAG_3) (Lei et al. 1996).

An alternative approach is the use of the $^{99\text{m}}\text{Tc}(\text{V})$ -organohydrazino (HYNIC) core that was first introduced by Abrams et al. 20 years ago (Abrams et al. 1990; Schwartz et al. 1991). The advantages of this system are the facile functionalization of targeting entities via amide linkage. It has therefore been used for $^{99\text{m}}\text{Tc}$ -labeling of a variety of high, medium, and small molecular-weight biomolecules (Guo et al. 1999; Steffens et al. 1999; Decristoforo et al. 2000a, b; Tang et al. 2005; Ferro-Flores et al. 2006; von Guggenberg et al. 2007; Liu et al. 2008; Salouti et al. 2008). Since the HYNIC-chelator can only occupy one or two coordination sites on the metal, co-ligands such as tricine are needed to complete the coordination sphere of $^{99\text{m}}\text{Tc}$ (Edwards et al. 1997; Liu et al. 1998; Purohit et al. 2003). The possibility for selection of appropriate co-ligands is advantageous for an easy modulation of the hydrophilicity and pharmacokinetics of the $^{99\text{m}}\text{Tc}$ -HYNIC-derivatized biomolecules. However, the presence of multiple species in solution due to different bonding modalities of the HYNIC moiety and co-ligands might be problematic for a commercial development, because of the increasing regulatory hurdles and the requirements of fully characterized products. Another, less

frequently employed approach is the use of a $^{99m}\text{Tc(V)}$ -dioxo-core coordinated by nitrogen ligands that form octahedral complexes with the oxygens *trans* to each other (Kastner et al. 1982). The group of Nock and Maina made successful use of a tetramine chelator for ^{99m}Tc -radiolabeling of several peptide-based biomolecules forming monocationic polar complexes with the $^{99m}\text{Tc(V)}$ -dioxo-core (Maina et al. 2002; Nock et al. 2003, 2006). The advantages of this radiolabeling strategy include its easy formation at ambient temperature, its high stability in the biological milieu, and considerable hydrophilicity. A completely different ^{99m}Tc -radiolabeling strategy has been introduced by the development of the tricarbonyl-technique which offered new opportunities for the design of $^{99m}\text{Tc(I)}$ -radiotracers (Egli et al. 1997; Alberto et al. 1998; Alberto et al. 1999a, b; Egli et al. 1999; Schibli et al. 2000; Alberto et al. 2001; Schibli and Schubiger 2002). The water-soluble $^{99m}\text{Tc(I)}$ -tricarbonyl precursor's aqua-ligands are readily exchanged allowing the coordination of preferentially tridentate chelators that can be modified to provide complexes with cationic, neutral, or anionic overall charge (Schibli et al. 2000; Müller et al. 2006b; Garcia Garayoa et al. 2007b; Schweinsberg et al. 2008; Maresca et al. 2009). In addition, the tricarbonyl radiolabeling strategy is also accessible for the preparation of stable radiometal complexes using β -particle emitting rhenium isotopes ($^{186/188}\text{Re}$, Table 1). Hence, the production of iso-structural compounds with the “matched pair” $^{99m}\text{Tc}/^{188/186}\text{Re}$ for diagnostic and therapeutic purposes has become feasible thanks to the tricarbonyl strategy, a feature which is often not fulfilled with Re(V) -complexes (Müller et al. 2007).

Radiolanthanides (e.g. ^{177}Lu) and lanthanide-like isotopes as well as indium and gallium are used in an oxidation state +III. They can generally be coordinated by polyamino-polycarboxy chelating systems. Coordination numbers of lanthanides are typically between seven and ten whereas coordination numbers of eight or nine are most common in Ln(III) -complexes with polydentate chelators. The 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-chelator emerged as particularly useful for lanthanide coordination of therapeutic radiopharmaceuticals because of the formation of metal complexes of extremely high thermodynamic stability and kinetic inertness (Fig. 3). In addition, the hydrophilic acetate chelating arms of DOTA favor a fast clearance of radiotracers from the blood and non-targeted organs and tissues. Despite the similarities of the SPECT radioisotopes [$^{67}\text{Ga(III)}$]-gallium and [$^{111}\text{In(III)}$]-indium they are different in size and charge density. Ga(III) has a small ionic radius (0.65 Å) and the coordination number is six whereas the ionic radius of In(III) is larger (0.92 Å) and it is seven- or eight-coordinated in its complexes. The structural differences among Ga- and In-complexes might have an influence on the overall tissue distribution of one and the same bioconjugate as recently exemplified with a somatostatin-analog (Heppeler et al. 1999). A higher tumor uptake and lower kidney retention has been reported for ^{67}Ga -DOTATOC compared to that of ^{111}In -DOTATOC. Whereas DOTA appears to be an ideal chelator for coordination of lanthanide radioisotopes or $^{111}\text{In(III)}$, its coordination cavity is not ideal for Ga(III) as it is too large. On the other hand there is a perfect fit between the size of Ga(III) and the coordination cavity formed by the N_3O_3 donor atoms of the macrocyclic 1,4,7-triazacyclononane-1,4,7-triacetic acid

(NOTA)-chelator (Liu 2008). Consequently, a higher thermodynamic stability constant has been found for Ga-NOTA complexes compared to those of Ga-DOTA-complexes (Delgado and da Silva 1982). In some cases, open chelating systems are more favorable than macrocycles because they are capable to form radiometal complexes at ambient temperature which is particularly important for temperature-sensitive targeting agents. Examples are variable versions of diethylenetriamine pentaacetic acid (DTPA, CHX-DTPA, etc., Fig. 3). DTPA is one of the most commonly employed acyclic ligands in radiochemistry useful for coordination of ^{111}In , ^{67}Ga , and radiolanthanides. For ^{111}In -complexation DTPA emerged as the ideal chelating agent (Mardirossian et al. 1993).

In addition to the bifunctional chelator's function for stable coordination of the radiometal, the linker-entity is important for conjugation with the biomolecule and might influence the overall pharmacokinetics of the radiopharmaceutical. By affecting the biomolecule's lipophilic or hydrophilic characteristics the linker system can serve for controlling its *in vivo* behavior. Thus, the nature of a bifunctional chelator in terms of geometry, lipophilicity, and overall charge plays a crucial role in determining the biodistribution of (tumor-) targeted radiopharmaceuticals (Bartholoma et al. 2010).

Functionalization of amino acid side chains (e.g. lysine, cysteine) with chemically reactive probes of bifunctional chelators is a largely uncontrolled random process that results in a heterogeneous mixture of conjugates modified at variable sites. A considerable advantage of small molecular-weight biomolecules (e.g. peptides and vitamins) is the fact that derivatization with a bifunctional chelating agent can be governed by specific chemical reactions that yield a single, clearly defined species. In contrast, loss of binding affinity is of concern during the process of antibody derivatization because modification of the Fab region (antigen binding site) can possibly have deleterious effects on the target binding of the protein. Both loss of binding activity to the target and overlabeling effects are highly undesired processes because they result in unwanted background radiation and unspecific accumulation of the antibody radioconjugates in the liver. For this reason, recent endeavors were undertaken for the development of site-specific derivatization via enzymatic reactions that are selective for a particular amino acid (Josten et al. 2000; Mindt et al. 2008) or sugar residue (Boeggeman et al. 2009) at a specified site of the antibody.

Since small molecular-weight molecules are usually stable at a broad range of temperatures and pH values the radiolabeling procedure is mostly smooth and quantitative. In contrast, proteins are generally sensitive to elevated temperatures. Thus, commonly applied methods for radiometal-labeling of proteins are time-consuming due to the low reaction temperature applicable. To overcome this drawback, pre-labeling strategies have been proposed allowing the preparation of radioimmunoconjugates within a shorter period of time while preventing the risk of affecting the antibody's scaffold under possibly harsh conditions needed for direct radiolabeling strategies (Li et al. 1995; Wängler et al. 2009).

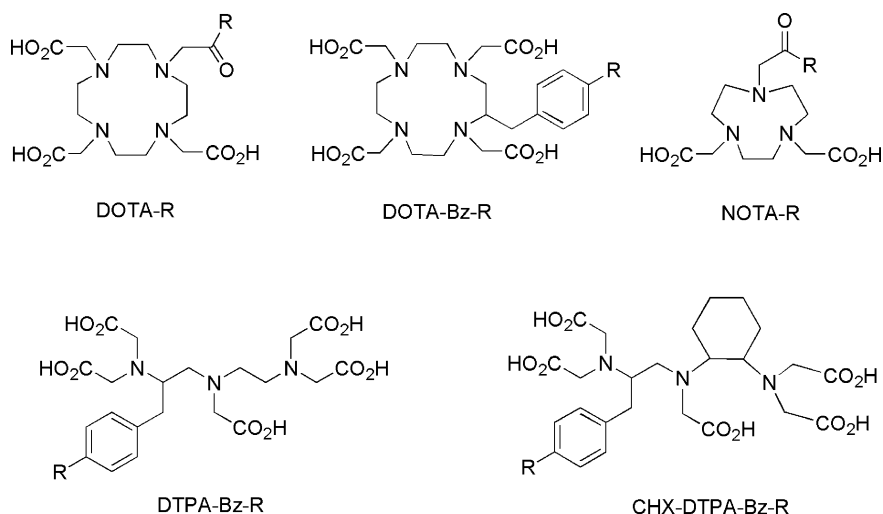


Fig. 3 The most frequently used macrocyclic (DOTA, NOTA) and acyclic (DTPA) chelators for complexation of radioisotopes for SPECT imaging (e.g. ^{111}In , ^{67}Ga) and combined therapy/SPECT imaging (e.g. ^{177}Lu)

3 Peptide-Receptor Radionuclide Imaging

Since receptors for regulatory peptides are overexpressed in a variety of human cancers, it is a prominent strategy to use radiolabeled analogs of these physiologically occurring peptides for tumor-targeted nuclear imaging. Advantages of using peptides are their good tissue penetration, a fast clearance, and minimal immunogenicity (Schottelius and Wester 2009). Small peptides of usually less than 40 amino acid residues are easily accessible through solid phase peptide synthesis. Their tolerance toward bulky modification and resistance toward harsh chemical conditions that are sometimes inevitable during radiolabeling procedures are further advantages of peptides. Importantly, a formulation of a radiolabeled peptide consists of identical molecules with a well-defined structure. Clearly, the most outstanding example of success in the field of peptide-based diagnostic and therapeutic nuclear medicine has been the use of somatostatin analogs for targeting the somatostatin receptor (Kwekkeboom et al. 2000). Somatostatin-derived tracers designed to image somatostatin receptor subtype 2 (sst2)-expressing tumors have enjoyed almost two decades of successful preclinical development and extensive clinical application. This example has paved the path for further exploration of radiolabeled peptides targeting other tumor-associated receptors such as gastrin releasing peptide receptors, neurotensin receptors, or cholecystokinin receptors (Behr et al. 2001).

3.1 Somatostatin Analogs

The prototypes of radiolabeled peptides for SPECT imaging are the somatostatin analogs commonly labeled with ^{111}In or $^{99\text{m}}\text{Tc}$. Somatostatin receptors are overexpressed on neuroendocrine tumors including pituitary adenomas, pheochromocytomas, paragangliomas, neuroblastomas, and medullary thyroid cancers. From the five subtypes of somatostatin receptors belonging to the G-protein coupled receptors, subtype 2 is the most widely overexpressed form in neuroendocrine tumors. In the beginning of their development, somatostatin analogs suffered from rapid degradation *in vivo*. Such limitations have been overcome by stabilization strategies through the development of synthetic peptides. Peptides of high chemical stability became accessible by introduction of D-amino acids or other unnatural amino acids at known cleavage sites, cyclization, or modification of C- and N-termini via amidation, reduction, alkylation, or acylation (Schottelius and Wester 2009). The clinically approved ^{111}In -labeled DTPA⁰-octreotide (OctreoScan) has proven to be a successful and versatile molecular imaging agent (Figs. 4 and 5). The most frequently used DOTA-coupled, somatostatin-based peptides are [DOTA⁰,Tyr³]-octreotide and [DOTA⁰,Tyr³,Thr⁸]-octreotate usually referred as DOTATOC and DOTATATE (Fig. 4). These analogs have also been successfully employed for therapeutic purposes when radiolabeled with particle-emitting radioisotopes (e.g. ^{177}Lu , ^{90}Y). Several sst2-binding somatostatin analogs are currently used in the clinic. Further research projects are focusing on the development of new and improved somatostatin analogs with a broader receptor subtype affinity profile. Such compounds would extend the range of targeted cancer candidates and increase the net tumor uptake when several receptor subtypes are expressed on the same tumor cell.

The generally high kidney uptake of radiometallated peptides due to their reabsorption in the renal proximal tubules is a drawback for peptide-based tumor targeting as it may lead to reduced contrast and quality of diagnostic imaging and damage radiosensitive kidneys if applied for therapeutic purposes (Gotthardt et al. 2007). Thus, several strategies to reduce tubular reabsorption of peptidic radiotracers have been investigated. One strategy relies on the chemical modification of the peptide with entities or overall charges that would potentially reduce renal uptake. A successful example of such modification is given by the work of Schwaiger and co-workers who developed ^{125}I -somatostatin analogs modified with carbohydrate-entities (Wester et al. 2002; Schottelius et al. 2002). Glycation modified the physicochemical behavior of the radiotracers in that pharmacokinetics were significantly improved as shown by reduced hepatic uptake and biliary excretion and a rapid clearance from the circulation via the kidneys without increasing renal accumulation of radioactivity. Another approach is based on the administration of additional substances for potential inhibition of peptide reabsorption. In this respect the co-infusion of the cationic amino acids lysine and arginine is the most prominent example since this combination successfully reduced renal accumulation of radiolabeled somatostatin analogs in preclinical studies (de Jong et al. 1996; Bernard et al. 1997; Verwijnen et al. 2005) and in patients (Hammond et al. 1993; Rolleman et al. 2003).

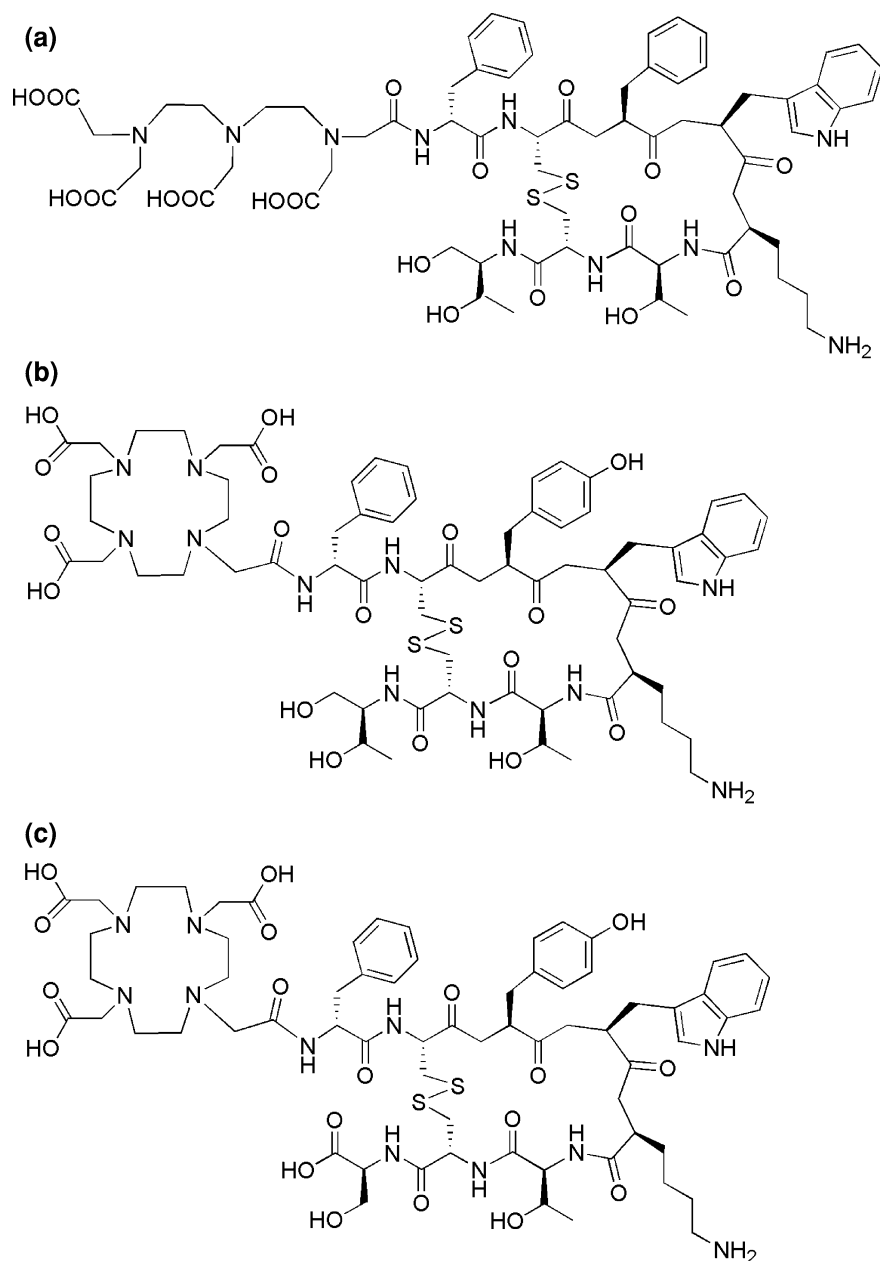
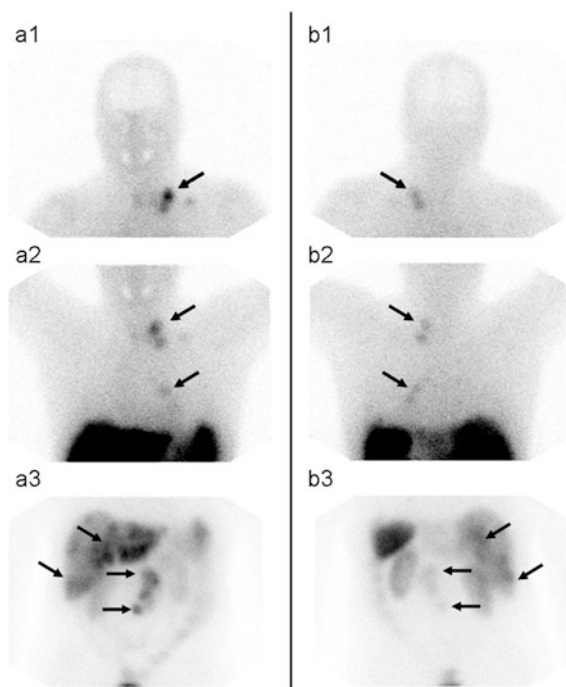


Fig. 4 Chemical structures of DTPA- and DOTA-modified somatostatin analogs for targeted diagnosis and therapy of somatostatin receptor-positive cancer diseases. **a** DTPA⁰-octreotide, **b** DOTA⁰-Tyr³-octreotide (DOTATOC), DOTA⁰-Tyr³-Thr⁸-octreotate (DOTATATE)

Fig. 5 Scintigraphy [multiple planar spot views, anterior (a) and posterior (b) of the head/neck (1), thorax (2) and abdomen (3)] performed 24 h after injection of ^{111}In -octreotide (OctreoScan; Covidien, Petten, the Netherlands) in a patient diagnosed with a well-differentiated endocrine carcinoma (carcinoid) with lymphogenic spread in the abdomen and supraclavicular and multiple metastatic lesions in the liver and lungs. The images have been kindly provided by J.J.M. Teunissen (MD, PhD), Erasmus Medical Center, Rotterdam, The Netherlands



Originally, it was proposed that peptide agonists that are efficiently internalized into receptor expressing cancer cells would be the best candidates for tumor imaging (Cescato et al. 2006). However, the two somatostatin analogs ^{111}In -DOTA-sst2-ANT and ^{111}In -DOTA-sst3-ODN-8 showed extremely high tumor accumulation despite being receptor antagonists (Ginj et al. 2006). It could be shown in vitro that a more than 15-fold increased number of binding sites per cell were accessible for antagonists compared to their agonist analogs and in addition slow ligand dissociation from the receptor was determined. These findings attracted the attention of many research groups and led to the development of further sst2-binding somatostatin-based antagonists. The studies confirmed that high-affinity somatostatin receptor antagonists that poorly internalize in tumor cells exhibit improved tumor targeting characteristics than corresponding agonists. The fact that this phenomenon was found not only for sst2-selective compounds but also for sst3-selective compounds suggests that this phenomenon is valid for more than just one particular receptor (Cescato et al. 2008).

3.2 Bombesin Analogs

The bombesin receptor family comprises four receptor subtypes whereof the gastrin releasing peptide (GRP) receptor or bombesin receptor subtype 2 (BB2) has been studied most thoroughly (Smith et al. 2003d, 2005). The impetus for

Table 2 Sequence of various bombesin analogs

Analogue	Chelator	Linker	1–6	7–13	14
Bombesin			pGlu-Gln-Arg-Leu-Gly-Asn-	-Gln-Trp-Ala-Val-Gly-His-Leu-	-Met-NH ₂
Demo-besin-1	N4	-BzDig-	-DPhe-	-Gln-Trp-Ala-Val-Gly-His-Leu- NH₂	
BBS-1	(N ² His)Ac-	-βAla-βAla-		-Gln-Trp-Ala-Val-Gly-His- Nle-	
BBS-2	(N ² His)Ac-	Lys(sha)-βAla-βAla-		-Gln-Trp-Ala-Val-Gly-His- Nle-	
BBS-3	(N ² His)Ac-	-Lys(Amd)-βAla-βAla-		-Gln-Trp-Ala-Val-Gly-His- Nle-	
BBS-4	(N ² His)Ac-	-Ala(^N TG)-βAla-βAla-		-Gln-Trp-Ala-Val-Gly-His- Nle-	
MP2653	DTPA		-aCMpip-Tha-	-Gln-Trp-Ala-Val-βAla-His- Tha-	-Nle-NH₂
MP2346	DOTA		-Pro-Gln-Arg-Tyr-Gly-Asn-	-Gln-Trp-Ala-Val-Gly-His-Leu-	-Met-NH ₂
Pesin	DOTA	-dPEG ₄ -		-Gln-Trp-Ala-Val-Gly-His-Leu-	-Met-NH ₂
AMBA	DOTA	-CH ₂ CO-Gly-4-aminobenzyl-		-Gln-Trp-Ala-Val-Gly-His-Leu-	-Met-NH ₂
RM 1	DOTA	-CH ₂ CO-Gly-4-aminobenzyl-	-Phe-	-Gln-Trp-Ala-Val-Gly-His- Sta-	-Leu-NH₂

Lys(sha) = lysine-coupled shikimic acid, Lys(Amd) = Amadori-Product; Ala(^NTG) = triazole-coupled glucose, Sta = statyl

targeting the GRP receptor is based on the fact that a variety of human tumors overexpress GRP receptors including prostate, breast, and small cell lung cancers (Moody et al. 1983; Gugger and Reubi 1999; Markwalder and Reubi 1999). The development of ^{99m}Tc-bombesin analogs has been the focus in recent years in many research laboratories (Table 2) (Baidoo et al. 1998; Van de Wiele et al. 2000; Nock et al. 2005). The tricarbonyl technique which was developed in view of the opportunity to use ^{99m}Tc and ¹⁸⁸Re as a “matched pair” for diagnosis and therapy (Alberto et al. 2001; Schibli et al. 2002) has been employed most extensively for radiolabeling of bombesin analogs (La Bella et al. 2002; Smith et al. 2003b, c; Garcia Garayoa et al. 2007a, b, 2008; Schweinsberg et al. 2008). A drawback of this strategy is, however, the fact that most ^{99m}Tc/¹⁸⁸Re-tricarbonyl-based bombesin derivatives are predominantly cleared via the hepatobiliary excretion pathway because of the tricarbonyl’s inherent lipophilicity (Decristoforo and Mather 2002). Increasing the hydrophilicity of radiolabeled GRP-targeting

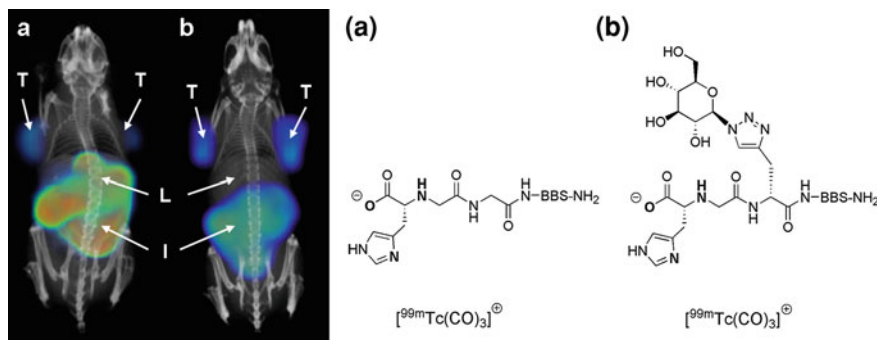


Fig. 6 SPECT/CT images of PC-3 tumor bearing mice 1.5 h after injection of **a** $^{99m}\text{Tc}(\text{CO})_3\text{-(N}^2\text{His)Ac-}\beta\text{Ala-}\beta\text{Ala-[Cha}^{13}\text{,Nle}^{14}\text{]BBS(7-14)-NH}_2$ (control compound) and **b** $^{99m}\text{Tc}(\text{CO})_3\text{-(N}^2\text{His)Ac-Ala}^{\text{NTG}}\text{-}\beta\text{Ala-}\beta\text{Ala-[Cha}^{13}\text{,Nle}^{14}\text{]BBS(7-14)-NH}_2$, ($^{\text{NTG}}$ = N-linked triazole-linked glucose). T = tumor, L = liver, I = intestines (Schweinsberg et al. 2008)

peptide conjugates is necessary because accumulation of radioactivity in the liver and intestinal tract would compromise their capacity to effectively image solid tumors and metastatic lesions in the abdomen. This has been accomplished for example by introduction of “innocent” peptide sequences such as polylysine, polyglycine, or polyaspartic acid residues (Liu and Edwards 1999). Also, it could be shown that the introduction of a polar serylserylserine spacer into ^{99m}Tc -tri-carbonyl pyrazolyl bombesin analogs resulted in a longer retention time of the radiotracer in the tumor tissue compared to analogs with more lipophilic linker entities consisting of β -alanine or glycylglycylglycine (Alves et al. 2006). Based on the promising results experienced with somatostatin analogs conjugated to carbohydrates (Wester et al. 2002; Schottelius et al. 2002), glycation of bombesin tracers was approached with the aim to increase their overall hydrophilicity (Schweinsberg et al. 2008). In this respect Garcia et al. tested three different bombesin analogs in vitro and in vivo. One of the derivatives was modified with a linker bearing a lysine that was coupled to the glycomimetic shikimic acid at the ε -amino group. Another bombesin derivative was glycosylated via an “Amadori rearrangement” and the third compound was a bombesin analog derivatized with an azido-glucose that was coupled to an alkyne-group of the linker entity via a “click”-chemistry reaction (Table 2). The introduction of polar carbohydrates had no negative effects on the in vitro stability and the internalization or efflux profile of the radiotracers in cultured tumor cells. In contrast, these modifications led to a significant reduction of abdominal radiotracer accumulation, a clearly higher tumor uptake and thus improved tumor-to-background ratios in vivo. The best results were obtained with the bombesin analog modified via a “click”-reaction that contained a triazole coupled glucose entity. The tissue distribution could be clearly ameliorated as demonstrated via SPECT/CT imaging studies where the tumor uptake was shown to be increased (Fig. 6).

On the other hand accumulation in the liver was significantly reduced. Despite the higher kidney uptake found for the carbohydrate bombesin analogs at early time points after injection, this decreased rapidly with time indicating that the radiotracers were not trapped in the renal tissues. By this example the strategy of radiotracer glycation has been demonstrated as a potent method to increase the overall hydrophilicity of a tracer and thus to improve the tissue distribution.

Based on the advantages of using trivalent radiometals for preparation of site-directed diagnostic/therapeutic radiopharmaceuticals (Smith et al. 2003d; Giblin et al. 2005), interest has been sparked into the synthesis and biological evaluation of trivalent radiometalated bombesin derivatives using radioisotopes such as ^{111}In or ^{177}Lu (Table 2) (Breeman et al. 2002; Hoffman et al. 2003; Smith et al. 2003a; Johnson et al. 2006; de Visser et al. 2007). One such example is the bombesin analog referred to as DOTA-AMBA useful for both diagnostic and therapeutic purposes (Lantry et al. 2006; Maddalena et al. 2009). Also, a so-called pan-bombesin analog has been designed with the special characteristic of displaying high affinity to all three bombesin receptor subtypes possibly allowing a broader field of application (Zhang et al. 2004).

The majority of research efforts into the design of bombesin-based radiotracers have been performed by using GRP receptor agonists. Such bombesin analogs undergo receptor-mediated endocytosis enabling residualization of the attached radiometal within the targeted tumor cell. However, $^{99\text{m}}\text{Tc}$ -demobesin-1 is a potent antagonist which clearly exhibited high affinity to the GRP receptor even though significant internalization into PC-3 prostate tumor cells was not observed. This radiotracer allowed imaging of PC-3 tumors in mice with better tumor-to-background contrast compared to the best available agonist analog (Nock et al. 2003). Thus, endeavors were directed also toward the development of bombesin antagonists. Recently, superior imaging properties of the ^{111}In -radiolabeled bombesin antagonist RM1 over the agonist ^{111}In -DOTA-AMBA have been demonstrated (Mansi et al. 2009). Whether or not bombesin antagonists are also favorable over agonists for therapeutic purposes remains to be investigated.

3.3 Neurotensin Analogs

Neurotensin (NT) is a linear tridecapeptide that can be found in the central nervous system and in peripheral tissues. Among the three NT receptors (NTR), NTR1 has been found in several neuroendocrine tumor types. Of special interest are exocrine pancreatic carcinomas that overexpress NTR1 with an incidence of 75–88 % (Reubi et al. 1998). Thus, several studies focused on the development of NT analogs for radiolabeling with SPECT radionuclides such as $^{99\text{m}}\text{Tc}$ (Garcia-Garayoa et al. 2006; Maina et al. 2007; Garcia-Garayoa et al. 2009) and ^{111}In (de Visser et al. 2003; Alshoukr et al. 2009). Similar to other small neuropeptides, neurotensin is rapidly metabolized in plasma by endogenous peptidases. Thus, neurotensin analogs which are stabilized at one or more of the three potential cleavage sites were developed. In this respect, the research group of Maina and

Table 3 Stability and affinity of different radiolabeled NT analogs (Garcia-Garayoa et al. 2009)

Analog	Amino acid sequence	In vitro stability		In vivo stability		Affinity Kd (nM)
		Plasma	HT-29	Blood		
^{99m} Tc-NT-II	(N ^z -His)Ac-Arg-Arg-Pro-Tyr-Ile-Leu	5.6 min	n.d.	< 1min		0.3±0.2
^{99m} Tc-NT-XII	(N ^z -His)Ac-Arg-(N-CH ₃)-Arg-Pro-Tyr-Tle-Leu	21 d	6.5 h	0.75 h		2.0±1.6
^{99m} Tc-NT-XIX	(N ^z -His)Ac-Arg-(N-CH ₃)-Arg-Pro-Dmt-Tle-Leu	28 d	2.4 d	1.40 h		15.0±9.2

The modifications in the binding sequence are marked in *bold*

(N^z His)Ac Retro[N^z -carboxymethyl-histidine], Tle tertiary-leucine, Dmt dimethyltyrosine, n.d. not determined

Nock developed several ^{99m}Tc(V)-neurotensin analogs, referred to as ^{99m}Tc-demotensin, employing amino acid substitutions and/or reduction of the amide bond Arg⁸/Lys⁸-Arg⁹ to the corresponding amine (Nock et al. 2006; Maina et al. 2007). Garcia and coworkers reported the biological evaluation of neurotensin analogs in which two of the three cleavage sites have been stabilized (Bruehlmeier et al. 2002; Garcia-Garayoa et al. 2006). These interventions allowed preparation of neurotensin analogs of high plasma stability, affinity to the NTR1 in the nanomolar range, and significant tumor uptake in preclinical and clinical studies. A promising candidate is the ^{99m}Tc(CO)₃-radiolabeled (N^z-His)Ac-Arg-(N-CH₃)-Arg-Pro-Tyr-Tle-Leu (^{99m}Tc-NT-XII), which has been stabilized at the cleavage sites 8–9 and 11–12. Other than in the case of bombesin derivatives (see Sect. 3.2), the introduction of a glycomimetic entity (shikimic acid) coupled to the side chain of an additional lysine residue did not result in an improved tissue distribution of the radiotracer. Although the expected lower kidney and liver uptake could be achieved, both the receptor affinity and the tumor uptake were unfavorably reduced. Recently, the group of Garcia reported the evaluation of a ^{99m}Tc(CO)₃-neurotensin analog, ^{99m}Tc-NT-XIX, modified at all three cleavage sites (Garcia-Garayoa et al. 2006, 2006; Garcia-Garayoa et al. 2009). Despite a slight decrease in receptor affinity and a lower rate of internalization, the in vitro and in vivo stability of this novel radiopeptide has been significantly increased (Table 3).

This example of a triple-stabilized neurotensin analog demonstrates the importance of the radiotracer's metabolic stability to increase its accumulation in the tumor tissue which was—in the case of ^{99m}Tc-NT-XIX—even able to compensate a slightly lower receptor binding affinity. The clearly improved tumor-to-background contrast of ^{99m}Tc-NT-XIX over ^{99m}Tc-NT-XII could be visualized by SPECT/CT-imaging (Fig. 7). Thus, the development of neurotensin ^{99m}Tc-radiotracers, where single amino acids have been substituted for peptide stabilization, is an example for optimization of a radiotracer's tissue distribution by increasing its in vivo stability.

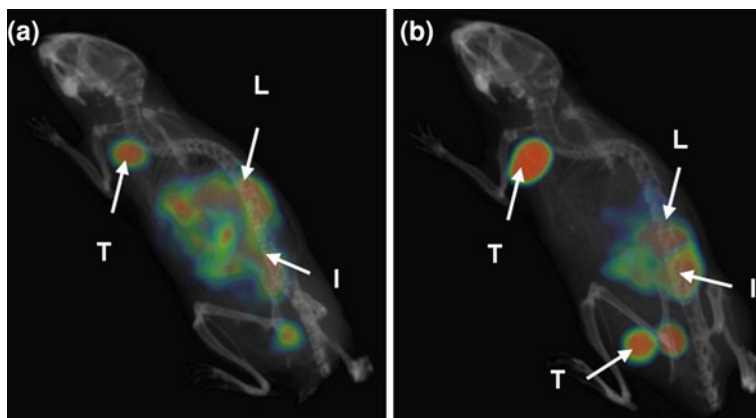


Fig. 7 SPECT/CT images of HT-29 tumor bearing mice 1.5 h after injection of **a** ^{99m}Tc -NT-XII and **b** ^{99m}Tc -NT-XIX. T = tumor, L = liver, I = intestines (Garcia-Garayoa et al. 2009)

3.4 Other Peptides-Based Radiotracers

Beyond somatostatin and GRP receptor targeting with bombesin and neurotensin analogs, many other regulatory peptide receptors are overexpressed on a variety of tumor types. Thus, peptide analogs in various stages of preclinical or clinical development include derivatives of cholecystokinin-2 (CCK-2) (de Jong et al. 1999), glucagon-like peptide-1 (GLP-1) (Körner et al. 2007), neuropeptide Y (NPY) (Zwanziger et al. 2008) and Arg-Gly-Asp (RGD) peptides (Schottelius et al. 2009) among others. CCK-2 receptors are expressed in medullary thyroid cancers. Initial gastrin-ligands for CCK-2 receptor targeting comprising a DTPA-DGlu-chelator showed unfavorable tumor-to-kidney ratios of radioactivity accumulation and were therefore not developed further. New gastrin derivatives lacking the glutamate-moiety showed excellent CCK-2 receptor affinity and lower renal retention in a rat AR42J tumor model (Good et al. 2008). Recently, it was found that GLP-1 receptors are highly overexpressed in virtually all insulinomas and gastrinomas (Körner et al. 2007). Metabolically more stable GLP-1 congeners referred to as exendin-3 and exendin-4 have been derivatized with a DTPA or DOTA chelating system for radiolabeling with ^{111}In or lanthanide radioisotopes. Remarkable tumor targeting was found in a human patient while employing ^{111}In -DOTA-exendin-4 (Christ et al. 2009). NPY analogs are of interest because of the frequent overexpression of NPY receptors in a variety of tumor types including breast cancer. A recent article reports on the synthesis and evaluation of a large number of NPY analogs where a DOTA-derivatized compound radiolabeled with ^{111}In performed as a potent radiotracer (Zwanziger et al. 2008). However, the in vivo studies with this tracer showed only low tumor uptake whereas radioactivity retention in the kidneys was extremely high. RGD-peptides that do not belong to the group of regulatory peptides are of particular interest for targeting integrin receptors such as the $\alpha_v\beta_3$ integrin. This integrin subtype is strongly expressed on

antibodies' targeting properties and for dosimetry. Common tumor associated targets for radioimmunoimaging (and -therapy) are epidermal growth factor receptors (EGFR) (Tolmachev et al. 2009; Xu et al. 2009), the carcinoembryonic antigen (CEA) (Hong et al. 2008), the prostate-specific membrane antigen (PSMA) (Leyton et al. 2008), cluster of differentiation antigens (e.g. CD20), the pancarcinoma antigen (TAG-72), and the HER2 receptor among others. In addition, a number of angiogenesis markers—protein antigens expressed either on blood vessels or in the adjacent matrix of vessels—have been characterized as targets for selective delivery of antibodies to the tumor neovasculature (Brack et al. 2004). Examples are the fibronectin extra-domain B (EDB) (Neri et al. 1997), the integrin $\alpha_v\beta_3$ (Posey et al. 2001), the vascular endothelial growth factor (VEGF) (Brekken et al. 1998), and annexin A1 (Oh et al. 2004).

Potential concerns for radioimmunodiagnosis and strategies for optimization have been summarized in several review articles (Buchsbaum 1995; Buchsbaum 1997; Verhaar-Langereis et al. 2000). The main disadvantage of antibodies, namely their immunogenicity, could be largely overcome by the application of humanized antibodies that evade the immune system and are resistant to degradation. However, the slow vascular clearance (days to weeks) of antibodies as a consequence of their high molecular weight (IgG antibodies: ~ 150 kDa) and the low tissue penetration are generally disadvantageous for radioimaging because of the resulting low target-to-non-target contrast at early time points after administration. Although it is generally accepted that antibodies are not the preferred biomolecules for nuclear imaging, the application of antibody fragments for SPECT have been successfully exemplified. Similar to peptides, antibody fragments are rapidly cleared from the blood and from non-targeted tissues. The results thereof are higher tumor-to-background ratios compared with intact antibodies and a lower radiation absorbed dose in non-targeted tissues and organs. A reduced percentage of injected doses of radioactivity in the tumor tissue and higher radiation doses in the kidneys are also consequences of the reduced size of antibody fragments (Buchsbaum 1997).

Efforts have been directed toward the development of antibody fragments such as $F(ab')_2$, $F(ab')$ and single chain Fv (scFv) fragments to achieve faster clearance from the blood and in addition a better tumor penetration (Yokota et al. 1992; Yokota et al. 1993). Application of high-affinity scFv resulted in a relatively high tumor uptake combined with a rapid blood clearance and hence favorable targeting ratios (Begent et al. 1996). Multimers of antibody fragments may result in improved tumor localization compared with monomeric species as a result of higher affinity and slower blood clearance (King et al. 1994). Another approach to achieve improved pharmacokinetics is the pre-targeting strategy. Pre-targeting involves an initial targeting agent, which itself can be bound by secondarily injected agents. Secondary agents are either quickly clearing radiotracers that bind the initial agent with high affinity (Goldenberg et al. 2008) or “chase” reagents that clear an unbound radiolabeled antibody in circulation (Kobayashi et al. 1994). The pre-targeting approach is, however, not commonly applied for SPECT. In contrast, this strategy is much more favorable for radioimmunotherapy in order to reduce the

radioactive dose burden to the bone marrow and thus to avoid potential hemotoxicity of long circulating antibodies labeled with particle-emitting radioisotopes.

Radioimmunoimaging is of particular interest to evaluate a potential application of antibodies for targeted radionuclide therapy by interchanging a diagnostic with a therapeutic radioisotope of similar chemical characteristics (e.g. ^{111}In and ^{90}Y) or using a therapeutic radionuclide that emits concomitantly with therapeutic radiation also diagnostic γ -rays of a suitable energy for SPECT (e.g. ^{177}Lu , Table 1). The most prominent example of an antibody employed for radioimmunotherapy is ibritumomab tiuxetan (Zevalin), a ^{90}Y -radiolabeled monoclonal anti-CD20 antibody for the treatment of non Hodgkins lymphoma. Its ^{111}In -radiolabeled counterpart is usually administered prior to therapy for detection of receptor-positive malignant tissue via SPECT imaging and for dosimetry.

4.1 Targeting Fibronectin Extra-Domain B: Antiangiogenic Antibody Fragment L19

Angiogenesis is an underlying process in many human diseases, including cancer. An established target in this respect is the extra-domain B of fibronectin (EDB), a domain of 91 amino acids, which is typically inserted in fibronectin molecules at sites of tissue remodeling but not in fibronectin molecules under normal conditions. Thus, the expression of EDB has been shown in malignant tumors but not in healthy tissues (Zardi et al. 1987). The Neri group has isolated a number of human monoclonal antibodies to EDB (Carnemolla et al. 1996; Neri et al. 1997; Pini et al. 1998). The human antibody fragment, scFv(L19) displayed subnanomolar affinity to EDB and has been shown to efficiently localize on tumoral neovasculature in animal models (Demartis et al. 2001). Importantly, the ^{123}I -labeled dimeric L19 antibody fragment L19(scFv)_2 has been evaluated for targeting primary tumors and metastatic lesions in cancer patients through immunoscintigraphy (Santimaria et al. 2003). This clinical study was performed with 20 patients whereof the majority had colorectal or lung cancer. It could be demonstrated that the antibody ^{123}I - L19(scFv)_2 selectively accumulated in malignancies and allowed distinguishing among actively growing and quiescent lesions. Another Phase I/II clinical immunoscintigraphy study used ^{123}I - L19(scFv)_2 in patients with head and neck squamous cell carcinoma (Birchler et al. 2007). It was observed that for head and neck scintigraphy, iodinated antibodies have severe disadvantages. Although the thyroid gland was protected by competitive application of cold iodide, there were substantial artifacts in this area in all cases as a result of the uptake of liberated free iodide that was always present to a certain degree. Since dehalogenases are present in the salivary glands, free iodide also gave a high background in the 4 h post injection scintigraphy in the parotid and submandibular glands as well as in the minor salivary glands of the oral and nasal mucosa. Although the ^{123}I - L19(scFv)_2 is probably less suited as a diagnostic imaging modality for head and neck cancer, L19(scFv)_2 offers a general potential to be used as a tumor targeting

agent for both diagnostic and therapeutic purposes. Because neovasculature and tissue remodeling are required for the growth of all aggressive solid tumors, imaging approaches that use angiogenesis markers can be used for different types of cancer. An advantage of this strategy might be the fact that noninvasive imaging of angiogenesis via EDB fibronectin targeting allows the discrimination between quiescent and actively growing lesions.

5 Vitamin-Based Radiotracers

The use of small molecular-weight targeting agents is favorable to surmount the drawbacks of long circulation times and thus poor tumor-to-background contrast as well as possible immunogenicity encountered with antibodies. In this respect the application of vitamins as targeting agents provides several advantages: vitamins are small in size, inexpensive, relatively easily amenable for chemical modification, and non-immunogenic. Rapidly dividing cancer cells have an increased demand for certain vitamins such as folates, vitamin B12 (cobalamin), biotin, and riboflavin. These B-group vitamins are required for cell survival and proliferation because they act as co-enzymes of biochemical reactions that are essential for the synthesis of amino acids and for nucleotide bases (Russell-Jones et al. 2004). The most thoroughly investigated vitamin to be used as tumor-targeting agent is folic acid. The utility of folic acid conjugates has been widely exemplified in a variety of (pre)clinical studies for targeting the folate receptor (FR) that is overexpressed on a wide variety of cancer types (Low et al. 2008). Also, it has been demonstrated that vitamin B₁₂ has the potential to be used as cancer targeting agent whereas only few studies have focused on the applicability of biotin for direct tumor targeting (Russell-Jones et al. 2004). Since vitamins are indispensable for sustaining life, it is unlikely that a mutational arrest of vitamin uptake would occur with concomitant failure of vitamin-mediated diagnosis or therapy. This is a distinct feature of vitamins and an advantage for their application as tumor targeting agents. Thus, using vitamin-based imaging agents is attractive and the strategy holds promise to also be used for therapeutic purposes.

5.1 Folic Acid Conjugates

Folic acid and folates (reduced forms) are water-soluble vitamins of the B-complex group. Humans cannot synthesize folates and hence must necessarily obtain them from food. Although only small quantities of folates are required, these vitamins are vital for various biochemical reactions including those for the synthesis of RNA and DNA, amino acid metabolism, and gene regulation. Cellular uptake of folates is accomplished by either carrier systems or the high-affinity folate receptor (FR). The FR is a glucosylphosphatidylinositol (GPI)-anchored protein that is frequently overexpressed in a variety of tumor types including cancers of the breast, ovaries, cervix, endometrium, lungs, kidneys, colon, and

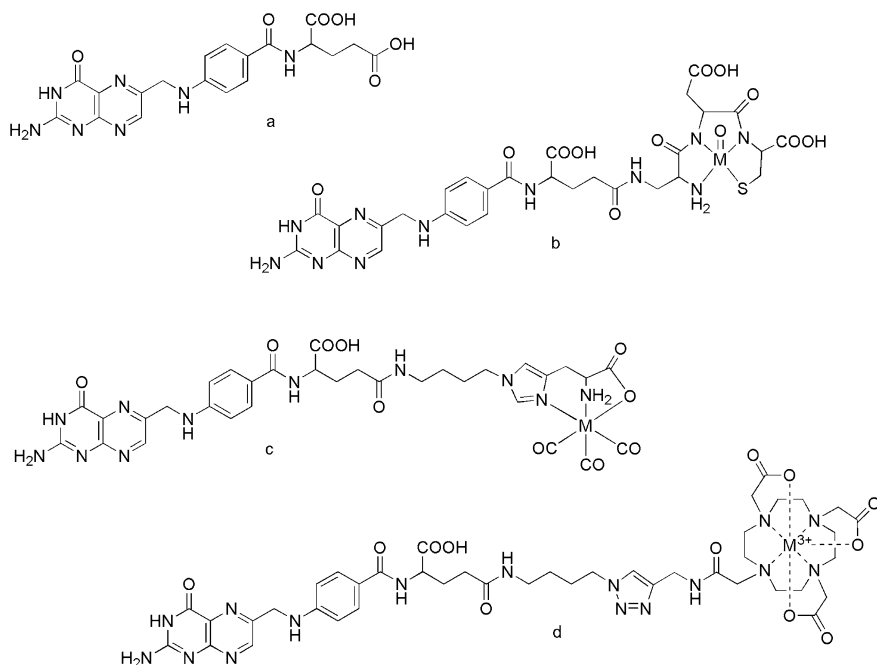
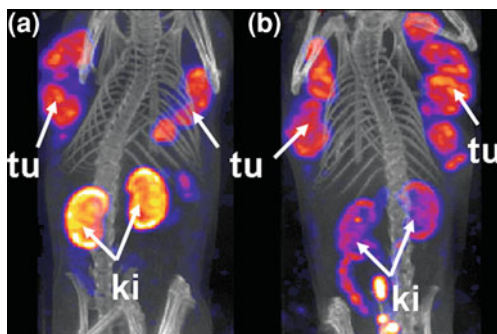


Fig. 9 Chemical Structures of **a** the vitamin folic acid, **b** EC20 ($M = {}^{99m}\text{Tc}$), **c** His-folate ($M = {}^{99m}\text{Tc}$, ${}^{188}\text{Re}$) and **d** DOTA-folate ($M = {}^{111}\text{In}$, ${}^{177}\text{Lu}$)

brain (Antony 1996; Parker et al. 2005). In normal organs and tissues, FR-expression is highly restricted to only a few sites where it is located on the apical side of polarized epithelia in the lung, the placenta, and the choroid plexus of the brain and in the proximal tubule cells of the kidneys (Weitman et al. 1992; Antony 1996; Parker et al. 2005). Thus, folic acid can be used as a molecular “Trojan horse” for selective delivery of attached probes to FR-positive cancer cells (Low et al. 2008). During the last two decades, a variety of folic acid conjugates of radioisotopes useful for SPECT imaging (${}^{99m}\text{Tc}$, ${}^{111}\text{In}$, ${}^{67}\text{Ga}$) has been developed and evaluated (Fig. 9) (Ke et al. 2003, 2004). Biodistribution studies of radiofolates in mice showed a specific uptake in FR-positive tumor (xeno)grafts, whereas unspecific radioactivity in background tissues was rapidly cleared in particular if the derivatives displayed hydrophilic properties. In the kidneys, however, high radioactivity retention was observed as a consequence of the specific binding of radiofolates to FRs expressed in the proximal tubule cells. This process results in unfavorably low tumor-to-kidney ratios of radiofolates in general. Clinical application of the two most promising candidates, ${}^{111}\text{In}$ -DTPA-folate (Wang et al. 1997; Mathias et al. 1998; Siegel et al. 2003) and ${}^{99m}\text{Tc}$ -EC20 (Leamon et al. 2002; Reddy et al. 2004; Fisher et al. 2008) revealed the same phenomenon in humans that was previously found in tumor bearing mice. While imaging of

Fig. 10 SPECT/CT of mice injected with **a** ^{111}In -DTPA-folate only and **b** in combination with pre-dosed pemetrexed



malignant tissue could be successfully achieved, high radioactivity uptake was found in the kidneys of patients where the FR is expressed to approximately the same level as in mouse kidneys (Parker et al. 2005).

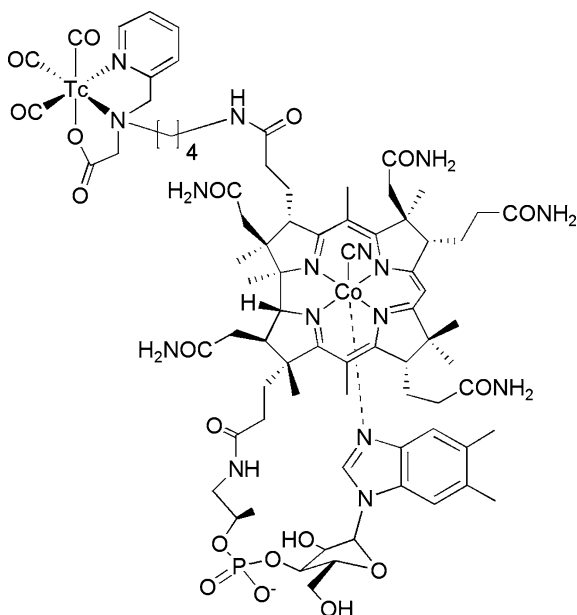
In an attempt to improve to low tumor-to-kidney ratio of radiofolates it was hypothesized that application of antifolates (e.g. pemetrexed) could increase the “appetite” of the tumor cells for folates and thus lead to an increased accumulation of folic acid conjugates. This hypothesis was confirmed *in vitro* (Müller et al. 2006a). However, in mice that were treated with antifolates, radiofolate uptake in tumor xenografts was not increased. While approaching this hypothesis, injection of pemetrexed was accomplished at different time points prior to the radiotracer. None of the experiments revealed an increased tumor accumulation of radioactivity, however, surprisingly administration of pemetrexed short before the radiofolate resulted in a significant reduction of kidney uptake (Müller et al. 2006a).

The result was a tremendous increase of the tumor-to-kidney ratio of radioactivity. This effect could be reproduced with a variety of folic acid conjugates radiolabeled with various radionuclides ($^{99\text{m}}\text{Tc}$, ^{188}Re , ^{111}In , ^{177}Lu) and in mouse models bearing different tumor (xeno)grafts (KB, IGROV-1, SKOV-3; M109) (Müller et al. 2007, 2008, 2009, 2010). The clearly superior SPECT imaging quality of mice that received pre-dosed pemetrexed could be impressively demonstrated while using ^{111}In -radiolabeled DTPA-folate (Fig. 10). This example demonstrates a pharmacological intervention by a non-radioactive substance that results in an improved tissue distribution of the radiotracer compared to the results obtained after radiotracer administration alone.

5.2 Vitamin B₁₂ Conjugates

The earliest studies of radiolabeled vitamin B₁₂ (cobalamin) using cobalt radioisotopes (^{57}Co , ^{58}Co , ^{60}Co) showed radioactivity accumulation in peripheral, actively growing tumors with highest accumulation in sarcomas (Flodh 1968; Flodh and Ullberg 1968; Blomquist et al. 1969). Other studies used radioiodinated arylstannylcobalamin conjugates showing enhanced uptake into renal carcinomas in nude mice when compared with other healthy tissues and organs (Wilbur et al.

Fig. 11 Chemical Structure of ^{99m}Tc -PAMA-C4-cobalamin, a TCII non-binder vitamin B₁₂ derivative



1996). Collins et al. developed ^{111}In -DTPA-analogs of cobalamin (DTPA cobalamin analogs = DACs) and tested them in preliminary biodistribution experiments in mice with CCL8 sarcomas and in swine (Collins and Hogenkamp 1997). The overall biodistribution of DACs showed tumor uptake and high radioactivity accumulation in healthy organs that was almost identical to previous studies performed with copper-radiolabeled vitamin B₁₂. The same group reported the first patient study performed with ^{111}In -DTPA-adenosylcobalamin for cancer imaging (Collins et al. 1999). ^{111}In -DTPA-adenosylcobalamin was found to be effective for detection of high-grade aggressive tumors in humans with the most successful results in patients with breast cancer and high-grade lung, colon, thyroid, and sarcomatous malignancies (Collins et al. 2000). However, the most significant uptake of these cobalamin derivatives was found in the liver, kidneys, and spleen followed by radioactivity accumulation in several glands. Vitamin B₁₂ is bound to soluble transport proteins in circulation, namely transcobalamin I (TCI), intrinsic factor (IF), and transcobalamin II (TCII) whereof the latter is the principle vitamin B₁₂ binding protein (Seetharam 1999; Seetharam and Li 2000; Seetharam and Yammani 2003). TCII-cobalamin binds to TCII-receptors that are ubiquitously expressed in cells for effective acquisition of this important vitamin. Originally, vitamin B₁₂-mediated tumor targeting was thought to be dependent on undisturbed interaction of cobalamin with these main transport systems and tumor uptake was believed to be mediated via up-regulated TCII receptors (Bauer et al. 2002; Russell-Jones et al. 2004). Later, it was hypothesized that selective TCII non-binders would lead to improved tissue distribution. Various cobalamin derivatives comprising a [pyridine-2-ylmethylamino]-acetic acid (PAMA)-chelator for coordination of the

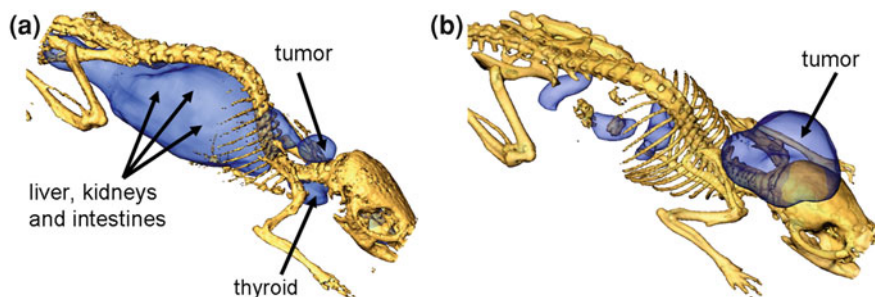


Fig. 12 Whole-body SPECT/CT scans of B16F10 tumor bearing mice, 24 h after injection of **a** $^{99m}\text{Tc}(\text{CO})_3\text{-PAMA-C6-cobalamin}$ (TCII binder) and **b** $^{99m}\text{Tc}(\text{CO})_3\text{-PAMA-C4-cobalamin}$ (TCII non-binder)

^{99m}Tc -tricarbonyl-core were developed with different spacer lengths [C-2 to C-6, i.e. $(-\text{CH}_2)_n$, $n = 2-6$]. $^{99m}\text{Tc}(\text{CO})_3\text{-PAMA-cobalamin}$ derivatives with a spacer length of C-5 or longer displayed TCII binding affinity whereas those with shorter spacer lengths (C-2 to C-4, Fig. 11) were identified as TCII-non-binders, but displayed retained interaction with IF and TCI (Waibel et al. 2008). The results of biodistribution studies in tumor-bearing mice performed with $^{99m}\text{Tc}(\text{CO})_3\text{-PAMA-C5-cobalamin}$ and $^{99m}\text{Tc}(\text{CO})_3\text{-PAMA-C6-cobalamin}$ were similar to previously evaluated $^{111}\text{In-DTPA-adenosylcobalamin}$ tracers (Waibel et al. 2008). In contrast, data of $^{99m}\text{Tc}(\text{CO})_3\text{-PAMA-cobalamin}$ derivatives with spacer lengths shorter than C-5 showed a significantly improved tumor-to-blood and tumor-to-kidney ratio of radioactivity. Thus, abolished interaction of the radiolabeled cobalamin tracer with TCII resulted in decreased accumulation of the radiotracer in the blood and in organs and tissues that would otherwise be predestined to have high cobalamin uptake such as kidneys and diverse glands (Fig. 12).

$^{99m}\text{Tc}(\text{CO})_3\text{-PAMA-C4-cobalamin}$ (Fig. 11) was selected as the most favorable candidate because it displayed the highest tumor-to-blood and tumor-to-kidney ratios in animal experiments. These findings suggest that the transport of cobalamin derivatives into malignant tissue is not dependent on the transport protein TCII but rather mediated via TCI. By this example it could be demonstrated that variation of the radiotracer's linker length could have a tremendous impact on the overall tissue distribution of a radiotracer and thus, on its successful application. Excellent results achieved in preclinical studies paved the path toward a clinical application of cobalamin-targeted radioimaging in patients using the TCI-selective organometallic ^{99m}Tc -vitamin B_{12} derivative.

5.3 Other Vitamin Targeting Agents

It is likely that carriers and receptors of vitamins other than folates and vitamin B_{12} could be used for tumor targeted radioimaging purposes. Among the vitamins of the B-group it was suggested that cancer cells also overexpress a biotin receptor that

could, however, not yet be identified (Minko et al. 2002; Russell-Jones et al. 2004; Yang et al. 2009). Also, a possible reason for the generally little interest in biotin as a direct tumor targeting agent could be the fact that renal filtration and reabsorption of biotin and its conjugates lead to high renal uptake of radioactivity in the kidneys. Recently, it was shown that vitamin C (ascorbate) conjugated nanoparticles could be delivered into the brain presumably via the sodium-dependent ascorbic acid transporter SVCT2 whose RNA was found in the choroid plexus epithelium (Salmaso et al. 2009). The SVCT2 carrier was found on rat glioma cells (C6 and F98) and on mouse fibroblasts (NIH/3T3). This study introduced the perspective of using the SVCT2 transporter for brain targeting through the choroid plexus where it is selectively expressed. There might also be a potential to use this vitamin C transporter for nuclear imaging purposes of cancer diseases in the future.

6 Intracellular Targets

6.1 ^{99m}Tc -Carbohydrate Complexes

The most frequently used radiotracer for nuclear imaging purposes is currently the glucose analog [^{18}F]-2-fluoro-desoxy glucose ([^{18}F]-FDG). This PET tracer is taken up by tumor cells mainly by facile diffusion through the glucose transport protein 1 (Glut1). In the cell interior [^{18}F]-FDG is phosphorylated by the enzyme hexokinase yielding [^{18}F]-FDG-6-phosphate which cannot escape the cell anymore. Thus, this trapping mechanism results in accumulation of radioactivity in metabolically active (cancer) cells. The clinical relevance of [^{18}F]-FDG promoted the development of inexpensive and readily available ^{99m}Tc -labeled glucose analogs. The first derivatives reported in the literature were $^{99m}\text{Tc}(\text{V})$ -glucose complexes (Risch et al. 1977; Torizuka et al. 1995; Ozker et al. 1999). However, these ^{99m}Tc -tracers did not match the criteria and features of [^{18}F]-FDG, such as active transport via Glut1 and phosphorylation via hexokinase. Endeavors have been undertaken by the group of Schibli and others to design organometallic glucose and glucosamine analogs using the matched pair $^{99m}\text{Tc}/^{188}\text{Re}$ (Petrig et al. 2001; Dumas et al. 2003; Bayly et al. 2004; Schibli et al. 2005). Biological characterization has been reported from a variety of organometallic $^{99m}\text{Tc}(\text{CO})_3$ -glucose complexes, derivatized at the C-1, C-2, C-3 and C-6 positions with various chelating systems. These compounds were tested for their ability to be internalized into Glut1 expressing cancer cells, HT29, and in addition it was investigated on whether or not they would be phosphorylated via the hexokinase reaction. Unfortunately, all of the complexes tested appeared not to be recognized and transported via Glut1. The authors stated the likeliness of $^{99m}\text{Tc}(\text{CO})_3$ -glucose complexes being sterically too demanding for recognition at the extracellular binding site and/or transportation via Glut1. Also, other than [^{18}F]-FDG, the organometallic glucose derivatives were not phosphorylated by hexokinase. Orvig and his collaborators reported several new approaches of organometallic

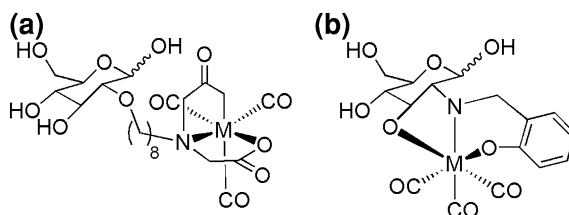


Fig. 13 C-2 functionalized glucose derivatives of an imino diacetic acid chelator (Schibli et al. 2005) and an N-(2'-hydroxybenzyl)-amino-chelating system (Bayly et al. 2004) radiolabeled with $^{99\text{m}}\text{Tc}$ -tricarbonyl ($\text{M} = ^{99\text{m}}\text{Tc}$)

carbohydrate complexes. Among others they synthesized N-hydroxybenzyl-amino-deoxy-glucose derivatives (Fig. 13) and carbohydrate-appended hydroxypyridinone derivatives (Bayly et al. 2004; Storr et al. 2005; Ferreira et al. 2006a, b; Ferreira et al. 2010). However, most of these compounds revealed neither to be hexokinase substrates nor inhibitors. Although basic cell data of these carbohydrate radiometal complexes is lacking, it is likely that they are not taken up via the Glut1 transporter or other specific transport mechanisms and thus would fail to accumulate in cancer cells *in vivo*.

6.2 Radiolabeled Nucleoside Analogs for Targeting Human Thymidine Kinase

In mammalian cells, salvage pathway phosphorylation of thymidine is catalyzed by two different thymidine kinases (TK): the cell-cycle regulated cytoplasmic TK1 and the constitutively expressed mitochondrial TK2. The human TK1 (hTK1) activity is known to fluctuate with cellular DNA synthesis, the activity being high in proliferating and malignant cells and low or absent in quiescent cells, whereas TK2 activity is low in both dividing and quiescent cells (Munch-Petersen et al. 1995). Since the activity of hTK1 is often dramatically increased in cancer cells, interest has been sparked in targeting this enzyme by radioactive thymidine analogs for selective imaging of proliferating cancer cells. In the cell interior nucleosides are rapidly phosphorylated to nucleotides, which renders them unable to penetrate biological membranes and thus they are “trapped” inside the cells. Thymidine and thymidine analogs labeled with PET radioisotopes such as ^{11}C -methyl-thymidine, ^{76}Br -fluoro-deoxyuridine, and ^{18}F -fluoro-deoxythymidine are either under development or already in use as proliferation marker (Gardelle et al. 2001; Buchmann et al. 2003). However, due to the high costs for the production of PET radioisotopes and the unfavorably short half-lives of PET isotopes, the use of SPECT radioisotopes $^{99\text{m}}\text{Tc}$ or ^{111}In would be more advantageous. Schmid et al. focused on the preparation of radiometal labeled thymidine complexes functionalized at position N3 with a DO3A-chelator suitable for radiolabeling with ^{111}In or lanthanide radioisotopes (Schmid et al. 2006). However, cellular uptake of the

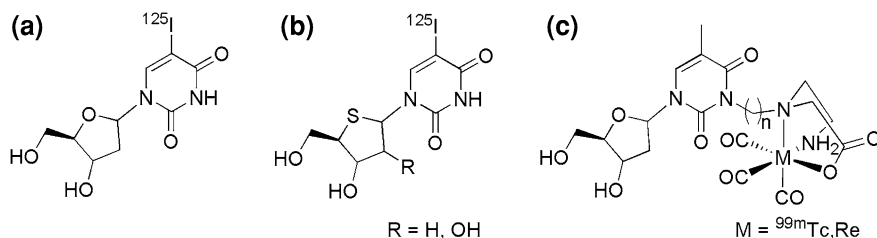


Fig. 14 Chemical structures of nucleoside-based SPECT tracers. **a** 5-[^{125}I]-Iodo-2'-deoxyuridine (IdUrd) (Dupertuis et al. 2001; Semnani et al. 2005), **b** 5-[^{125}I]-iodo-4'-thio-2'-deoxyuridine (ITdU, R = H) and 5-[^{125}I]-iodo-4'-thio- β -arabinofuranosyl-uracil (ITAU, R = OH) (Toyohara et al. 2002) and **c** $^{99\text{m}}\text{Tc}(\text{CO})_3$ -thymidine derivatives ($n = 2, 3, 5$ or 10) (Desbouis et al. 2008)

thymidine metal complexes in DoHH2 and HL60 cells failed. Clearly, there is an interest to develop thymidine derivatives suitable for radiolabeling with $^{99\text{m}}\text{Tc}$. Celen et al. reported the preparation and evaluation of a $^{99\text{m}}\text{Tc}(\text{V})$ -MAMA-propylthymidine complex as a potential probe for in vivo visualization of tumor cell proliferation via SPECT (Celen et al. 2007). However, this ligand could not be phosphorylated because it was too bulky. The group of Schibli focused on the development of thymidine analogs labeled with the organometallic $^{99\text{m}}\text{Tc}$ -tricarbonyl core (Fig. 14) (Desbouis et al. 2008; Struthers et al. 2008, 2009). The design of organometallic $^{99\text{m}}\text{Tc}$ -derivatives could be favorable as these complexes were sterically less demanding than previously prepared thymidine radiometal complexes. Those organometallic thymidine derivatives were systematically evaluated regarding the influence of the spacer length between the thymidine and the chelating system, the overall charge of the complex after radiometal coordination and the uptake in human neuroblastoma SKNMC cells. From these studies, it was concluded that neutral and anionic complexes are more readily accepted as substrates than cationic complexes.

Moreover, modeling experiments suggested that the flexibility of a longer spacer between the thymidine molecule and the organometallic core further improves the ability of the complex to be accommodated in the binding site of the enzyme. Cellular uptake was higher for complexes with log P values greater than one but still about 6-fold lower than for the ^3H -thymidine control compound. Although some of the organometallic thymidine complexes were identified as enzyme substrates, the low and often almost absent permeability of the thymidine metal complexes through the cellular membrane remains a major hurdle for these compounds.

Another strategy used iodinated thymidine analogs (e.g. 5-iodo-2'-deoxyuridine (IUdR)) as cell proliferation markers for nuclear imaging purposes and potential therapeutic application. However, the imaging quality was found to be impaired by the tracer's rapid in vivo degradation. Pre-application of 5-fluoro-2'-deoxyuridine (FdUrd) was tested with the aim to block thymidine synthesis and thus trigger the tumor uptake of ^{125}I -IUdR (Dupertuis et al. 2001). Indeed, as a result of FdUrd pre-dosing ^{125}I -IUdR incorporation into glioblastoma cells and tumors was increased and thus, the tumor-to-background contrast slightly improved. The same research

group reported a beneficial effect of combining the administration of ^{125}I -IdUrd with unlabeled IdUrd to increase the rate of DNA incorporation of ^{125}I -IdUrd in malignant gliomas (Dupertuis et al. 2002). Apparently, the C–N-glycosidic bond of IUdR is too labile in vivo which leads to metabolites that display reduced tumor affinity. In an attempt to increase the radiotracer's in vivo stability the tracer has been chemically modified by fluorination of the sugar moiety at different positions (3' or 2'-substitution). However, the preparation of fluorine-stabilized iodinated thymidine analogs with retained cellular uptake, cytosolic phosphorylation, and selectivity for hTK1, appears to be quite challenging (Gati et al. 1984; Mercer et al. 1989). A strategy for stabilizing the C–N-glycosidic bond without interfering with the cytosolic thymidine kinase has been carried out by the replacement of the furanose ring oxygen with sulfur for preparation of 5- ^{125}I -iodo-4'-thio-2'-deoxyuridine (ITdU) and 5- ^{125}I -iodo-1-(4-thio-d-arabinofurnosyl)uracil (ITAU) (Fig. 14) (Toyohara et al. 2002). ITdU exhibited a high resistance to the glycosidic bond cleavage reaction provoked by thymidine phosphorylase, while maintaining affinity to nucleoside kinases. Also, the increased in vivo radioiodination stability and rapid DNA incorporation of ITdU resulted in a preferential uptake of radioactivity in the proliferating organs making this tracer a promising tumor-imaging agent. A comparative study of six 5-iodonucleosides revealed that the in vivo proliferation-imaging potential of nucleosides might be estimated by their in vitro affinity for TK1 and their C–N-glycosidic bond stability (Toyohara et al. 2003). However, since these iodo-nucleosides have not been examined with regard to the important step of the nucleoside transport activity, further investigations would be necessary to allow a clear statement which radiotracer would be the most suitable for imaging of tumor cell proliferation.

By the examples of nucleoside derivatives and conjugates of carbohydrates it was demonstrated that the development of radiotracers for intracellular targets might be problematic if bulky metal chelates are employed since cellular uptake of these radiotracers via transmembrane-spanning carriers or passive diffusion could be hindered.

6.3 Radioiodinated Meta-Iodobenzylguanidine

Finally, we would like to highlight a long-serving but still frequently used tumor imaging agent with an intracellular target. Meta-iodobenzylguanidine (MIBG), a catecholamine analog, is suitable for radiolabeling with radioactive iodine (e.g. ^{123}I) for the purpose of SPECT imaging of neuroendocrine and carcinoid tumors, a subtype of neuroendocrine tumors (Khan et al. 2008). Radiolabeled MIBG was first synthesized at the University of Michigan as early as 1980 (Wieland et al. 1980). It localizes through the physiologic nor-epinephrine reuptake mechanisms with uptake into catecholamine storage vesicles of adrenergic nerve ending and the cells of the adrenal medulla. Carcinoid tumor cells share the common characteristic of a sodium-dependent ATP/Mg $^{2+}$ neuronal pump mechanism in their cell membranes that allows the accumulation of norepinephrine and MIBG where MIBG is not

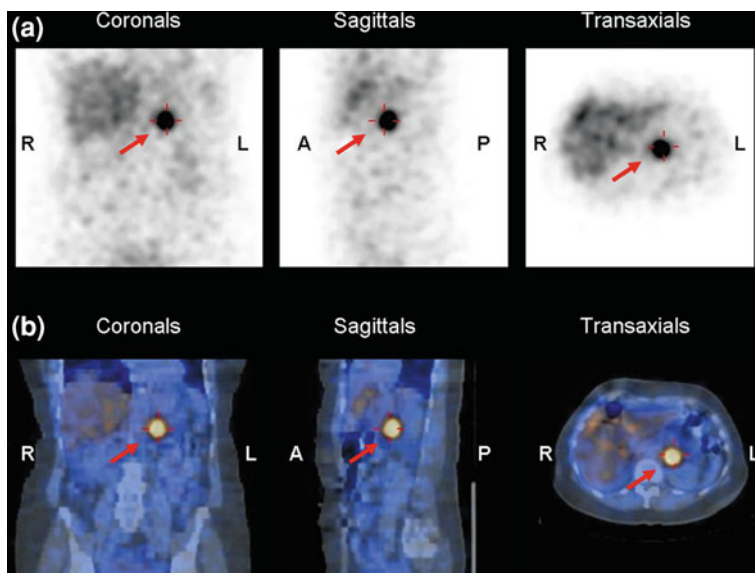


Fig. 15 SPECT images **a** and SPECT/CT overlay **b** of a patient with a neuroendocrine tumor (pheochromocytoma) in the upper thorax. Accumulation of ^{123}I -MIBG in the malignant tissue is indicated with *red arrows*. The images have been kindly provided by N. Schäfer, (MD, Ph.D.), University Hospital, Zurich, Switzerland

significantly metabolized. Initially, ^{131}I -radiolabeled MIBG was used for the detection of neuroendocrine tumors such as pheochromocytomas, but later its application has been extended also to scintigraphic visualization of neuroblastoma and carcinoid tumors. Although both ^{123}I - and ^{131}I -MIBG can be used for the purpose of radionuclide imaging, ^{123}I has dosimetry and imaging characteristics superior to ^{131}I and thus, it is the preferred radionuclide for SPECT imaging (Fig. 15). In contrast, ^{131}I is preferred for therapy due to the emission of β -particles and dosimetric considerations (Hoefnagel et al. 1987).

To develop an MIBG analog with improved uptake in tumors, no carrier added ^{131}I -MIBG has been developed. The methodology for producing high specific activity (no-carrier-added) ^{131}I -MIBG was originally described in 1993, but only recently it has been developed for clinical application. With this method every molecule of MIBG contains an ^{131}I -radiolabel, whereas prior methods provided a mixture of “cold” and “hot” tracer, wherein only 1 of 2,000 molecules of MIBG contained radioactive iodine. As a result of the high specific activity achieved by the no-carrier-added radiolabeling method, the mass of the MIBG administered can be reduced and thus undesired side-effects caused by the “cold” MIBG, such as hypertension during infusion could be minimized. The only concern of the no-carrier-added ^{131}I -MIBG has been that normal tissues and organs with relatively low levels of nor-epinephrine uptake might absorb more radioactivity because of the lack of competitive inhibition of radiotracer uptake by the “cold” MIBG.

Table 4 Potential reasons for failure of tumor-targeted nuclear imaging

Possibilities for Failure	Consequences	Examples
Expression of the target structure in normal tissues and organs	Radiotracer accumulation in normal tissues and organs	<ul style="list-style-type: none"> - Bombesin receptor (pancreas) - Somatostatin receptor (adrenals) - Folate receptor (kidneys)
Long circulation time	High background radiation in the blood—dose burden to healthy tissues (bone marrow)	- Monoclonal antibodies
Short circulation time	Low tumor accumulation	- Small molecular-weight targeting agents (e.g. folic acid)
Rapid enzymatic metabolism	Low tumor accumulation Accumulation of metabolites in kidneys and liver	- Non-stabilized neurotensin-analogs
Binding to physiological transport proteins	High background radiation in the blood	- Vitamin B ₁₂ /transcobalamin II
Intracellular targets	Cellular uptake via carrier systems or passive diffusion hindered by bulky radiometal complexes	<ul style="list-style-type: none"> - ^{99m}Tc-glucose analogs - ^{99m}Tc-thymidine analogs
Lipophilic character	Unspecific accumulation of the radiotracer in the bile, liver and intestinal tract	<ul style="list-style-type: none"> - ^{99m}Tc(CO)₃-moiety - Alkyl chain-spacers
Low specific activity	Low tumor uptake Undesired side-effects as a result of substantial amount of injected “cold” tracer	- [¹³¹ I]-MIBG

7 Optimization of SPECT Tracer Design and Potential Reasons for Failure

The design and development of a nuclear imaging probe comprises an appropriate biomolecule as targeting vector, a site for conjugation that does not interfere with the biomolecule's binding affinity to the tumor-associated target, a suitable linker length and a radioisotope that matches with an appropriate biomolecule. For stable coordination of metallic radioisotopes the choice of a suitable chelator is crucial. There are several possible strategies to optimize SPECT tracers with regard to their specificity to and selectivity for the targeted malignant tissue while minimizing their uptake in normal tissues and organs. Variation of the radionuclide, modification of the bifunctional chelator, introduction of linker-entities of variable spacer length for stabilization or modulation of the overall tracer characteristics, alteration of the radiolabeling technique and manipulation of the radiotracer's

blood, and normal tissue clearance by variation of the biomolecule's overall size (e.g. antibodies versus antibody fragments or peptides). Finally, optimization of the tissue distribution of radiotracers might also be accomplished by a combination with non-radioactive substances whereof the most prominent example is the application of positively charged amino acids (e.g. lysine) that block renal uptake of radiolabeled Fab fragments of antibodies (Behr et al. 1995, 1996) and peptides (de Jong et al. 1996; Rolleman et al. 2003; Verwijnen et al. 2005).

During the course of about two decades of (pre)clinical research with tumor-targeted SPECT tracers several reasons for potential failure of SPECT imaging agents could be identified (Table 4). Based on the data obtained with nuclear imaging agents that initially failed, new strategies to optimize the design and utility of SPECT tracers are currently being developed.

8 Summary and Conclusion

A variety of approaches for the design and improvement of SPECT tracers have been discussed herein. Each class of targeting agents, antibodies, peptides, and non-peptide-based small-molecules such as vitamins has its pros and cons for application in diagnostic nuclear medicine. In principle, it would be ideal to use SPECT tracers that accumulate specifically in malignancies and that are rapidly cleared via kidneys allowing high tumor-to-background contrast of radioactivity already short after administration. Such optimal characteristics are, however, not always easy to achieve.

The recent observation that somatostatin and bombesin analog antagonists provide superior characteristics over agonists with regard to their tumor accumulation is an unexpected finding that is not yet completely understood. Using oligomeric ligands to improve binding and targeting properties of radiolabeled peptides over their monomeric counterparts appears to be a more rational design that could be successfully proven for example with RGD-based analogs. Recently, vitamin-based radioimaging agents have been developed that are selectively accumulated in tumor cells. In the case of vitamin B₁₂, analogs with abolished binding to the ubiquitous protein transcobalamin II showed a reduced uptake in non-targeted tissues. In the case of FR-targeting it was the combined application with the antifolate pemetrexed that led to an improved tumor selectivity of folic acid based radioconjugates while undesired uptake in FR-positive kidneys could be reduced. Targeting of intracellular tumor markers such as the enzymes hexokinase or human thymidine kinase 1 turned out to be a more problematic strategy for SPECT tracers, particularly those that are based on radiometals, compared to the targeting of cell surface-exposed tumor markers. The necessity of the targeting agent to permeate cancer cell membranes via carrier systems or passive diffusion to reach intracellular targets could be a hindrance for a proper function of the targeting system in particular if the radioconjugate is composed of a bulky radiometal complex.

Finally, it has to be critically acknowledged that only a small selection of examples for tracer designs could be included in this chapter. The immense opportunities for the design of radiopharmaceuticals and the enormous potential it

provides for future development of new and improved SPECT tracers holds great promise for early clinical application of novel imaging agents in oncology.

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