Novel Single-Photon-Emitting Radiopharmaceuticals for Diagnostic Applications

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Abstract

The armamentarium of approved radiopharmaceuticals for either diagnosis or therapy is at the core of the clinical practice of today's nuclear medicine. Nevertheless, both because the currently approved agents do not meet all the clinical needs for radionuclide targeting and because advancing knowledge in the pathophysiology of tissues/organs open in turn new opportunities, investigations continue at the preclinical and clinical validation level for the development of new radiopharmaceuticals, most of which are not approved yet for commercial use. Concerning in particular the diagnostic applications of nuclear medicine to oncology, ongoing investigations in the search for tumor-targeting agents with better specificity and sensitivity are countless, possibly within the scenario of theranostics – that is, with the dual potential for imaging and for therapy, depending on the specific radionuclide employed for radiolabeling. We will focus this chapter on the most promising imaging agents labeled with single-photon-emitting radionuclides based on some of the mechanisms that are typical for tumor cells/tissues.

Keywords

Cancer biology • Molecular imaging in cancer • Single-photon emission imaging • Radiopharmaceuticals

Glossary

BTAP	Bis(thioacetamido)pentanoyl
DOTA	2-(4-Isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid (macrocyclic coupling agent to label compounds of biological interest with metal radionuclides)
DTPA	Diethylenetriaminepentaacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
FDA	United States Food and Drug Administration
GMP	Good manufacturing practice
HER	Human epidermal growth factor receptor
HPLC	High-performance liquid chromatography (formerly known as high-pressure liquid chromatography)
HYNIC	6-Hydrazinopyridine-3-carboxylic acid, also known as hydrazido-nicotinic acid/hydrazinonico-tinamide (a chelating agent)
MMP	Metalloproteinases, a family of matrix enzymes

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MRI	Magnetic resonance imaging
NIRF	Near-infrared fluorescence
PET	Positron emission tomography
RGD	Tripeptide composed of L-arginine, glycine, and L-aspartic acid (a sequence that is a common element in cellular recognition)
SPECT	Single-photon emission tomography
TGF	Transforming growth factor
TKI	Tyrosine kinase inhibitor
TPPTS	3,3,3”-Phosphanetriyltris (benzenesulfonic acid) trisodium salt, a ligand also known as sodium triphenylphosphine trisulfonate
VEGF	Vascular endothelial growth factor

Contents

Introduction and Background

The armamentarium of approved radiopharmaceuticals for either diagnosis or therapy is at the core of the clinical practice of today’s nuclear medicine. Nevertheless, advancing knowledge in the pathophysiology of tissues/organs opens new opportunities, for the development of new radiopharmaceuticals, most of which are not approved yet for commercial use. There is an ongoing search for tumor-targeting agents with better specificity and sensitivity. There are many new developments of agents with the dual potential for imaging and for therapy, depending on the specific radionuclide employed for radiolabeling [1–3].

Tumor cells and tissues exhibit different characteristics compared to normal cells and due to the altered physiology, tissue composition, and expression of intra- and extracellular molecules [4, 5]. These attributes can be the basis for developing new imaging targets for diagnostic applications as well as for developing new anticancer drugs and for assessing tumor response to treatment [6–9] (see also ▶ Chaps. 1, “Cancer Biology of Molecular Imaging,” and ▶ 2, “Principles of Molecular Targeting for Radionuclide Therapy” of this book).

Single-photon-emitting radiopharmaceuticals can be classified according to different properties, such as their biodistribution and targeting characteristics, their different chemical and physical properties, and their specific interaction with a target. Tumors are known to display an aberrant vascular network and microcirculation, which in turn influence an increase in interstitial pressure, as well as hypoxia and acidosis; all these features contribute to the expression of malignant phenotypes and to resistance to various treatments [10]. Within this environment, tumor cells can also display altered energy metabolism, as reflected, for example, in increased glucose uptake and shifted balances in metabolic products.

Thus, at the preclinical level, a variety of single-photon-emitting tracers are under evaluation for use as markers for (neo)angiogenesis [11–13], hypoxia [14, 15], acidosis [16], metabolic activity [17], and proteolytic activity [18, 19]. Besides metabolic tracers, efforts are being directed also toward the development and validation of single-photon probes specific for tumor target molecules such as cell surface antigens, receptors, or other molecules similarly overexpressed in tumor tissues [20]. The use of peptides interacting with receptors [21], of antibodies or antibody fragments targeting different epitopes of tumor-associated antigens [22], of vitamin-based radiopharmaceuticals [23], and of

nucleoside analogs [24–27] significantly increases the possibilities for tumor detection, localization, and staging. Specific issues of interest in translational preclinical imaging studies include efforts directed at improving specificity for tumor types [28], tumor uptake/retention [29], yet with minimal pharmacological effects of the imaging probes.

The successful choice of a target molecule potentially leads to the development not only of a molecular imaging probe but also of a therapeutic agent capable to inhibit the disease process – according to the approach of theranostics. Receptor targeting with small radiolabeled peptides for receptor-targeted tumor imaging (PET and SPECT) as well as for radionuclide therapy provides good examples of such theranostics potential.

Peptide Receptor Targeting

The best-known examples of this tumor-targeting mechanism are based on the use of somatostatin analogs. For imaging purposes either single-photon-emitting radionuclides (chiefly ^{111}In) or positron-emitting radionuclides (chiefly ^{68}Ga) are used in the clinical routine. For therapeutic purposes, different β^--emitting radionuclides (chiefly ^{90}Y and ^{177}Lu) are currently being used clinically or undergoing extensive validation; although the chief indications for the use of these radiopharmaceuticals regard neuroendocrine neoplasms (see ▶ Chaps. 29, "Diagnostic Applications of Nuclear Medicine: Neuroendocrine Tumors," and ▶ 43, "Neuroendocrine Tumors: Therapy with Radiolabeled Peptides" of this book), the indications for the use of these tracers, with either diagnostic or therapeutic purposes, are expanding to other tumors as well. Thus, the radiolabeled somatostatin analogs are prototypes for a whole spectrum of other peptide receptor systems that are overexpressed in a variety of tumors. The common feature shared by all these systems is that they are members of the so-called G-protein-coupled receptor superfamily and play a major role in the progression and angiogenesis of a number of malignancies.

Briefly, the peptide ligands that have yielded the most promising results so far are the analogs of bombesin, of cholecystokinin, of the vasoactive intestinal peptide, of gastrin, of glucagon, of substance P, of exendin, and of the α-melanocyte-stimulating hormone [30–36]. The use of the so-called RGD-based peptides for targeting the integrin receptor system (another member of the G-coupled family) is discussed further below in this chapter.

Apoptosis

Apoptosis is a process of regulated or programmed cell death, which in its classic form, does not cause inflammation. The mitochondria and ribosomes of the cells undergoing apoptosis remain intact, and surrounding cells internalize them along with the other components of the post-apoptotic cell. Necrotic cells, in contrast, lose membrane integrity, swell, and then release their contents to the surrounding tissue, causing inflammation and possibly initiating an immune response.

Lipid composition of the outer and inner leaflets of the plasma membrane is normally not symmetrical; for instance, some molecules such as phosphatidylserine and phosphatidylethanolamine are normally retained on the intracellular face of the cell membrane. When cells undergo apoptosis, this distribution is altered, so that these molecules are rapidly exposed to the outside of the cell membrane. The most promising single-photon-emitting radiopharmaceuticals targeting phosphatidylserine and phosphatidylethanolamine are represented by radiolabeled annexin and duramycin, respectively.

^{99m}Tc-Annexin V

The discovery of phosphatidylserine externalization as the event initiating apoptosis has opened the way to the search for compounds that have an affinity for phosphatidylserine and could therefore be used to localize in sites of apoptosis. To date, the compound that has received major interest in both the preclinical and clinical arena is annexin V, a non-glycosylated single-chain protein physiologically involved in the inhibition of hemostasis [37]. Annexin V, which is part of a protein family that binds to negatively charged

phospholipids in a Ca^{2+}-dependent manner, is distributed ubiquitously in the human body. Complete functional details of the annexin V pathway have yet to be clarified, but a large body of evidence suggests that annexin acts as a specific ligand for phosphatidylserine, and it is able to prevent activation of the immune response when cells undergo apoptosis [38].

In translational research, annexin V labeled with radionuclides has been used for studying, localizing, visualizing, and measuring apoptosis in vitro as well as in vivo, both in preclinical animal models and in patients [39, 40]. Radiolabeled annexin was first proposed to image blood clots in patients with atrial fibrillation; subsequently this compound gained attention for imaging of apoptosis in tumors [41]. Annexin V used for development of radiolabeled imaging agents is produced by the expression of annexin V complementary DNA in *Escherichia coli*. Several radiolabeled probes and other agents bearing different moieties have been explored preclinically to image annexin V uptake by means of SPECT, PET, MRI, and near-infrared fluorescence (NIRF).

As single-photon imaging probes, numerous methods for labeling annexin with ^{99m}Tc have been identified through the use of chelators such as the bifunctional N_2S_2 chelate to form ^{99m}Tc-4,5-bis(thioacetamido)pentanoyl-annexin V (or ^{99m}Tc-BTAP-annexin V, also known as ^{99m}Tc-Apomate) or hydrazinonicotinamide (HYNIC) to form ^{99m}Tc-HYNIC-annexin V. The former formulation is also available as a good manufacturing practice (GMP) product in a radiolabeling kit [42].

Moreover, animal experiments suggest an improved protocol for annexin labeling via the use of self-chelating annexin V mutants. In this case, the radiotracer exhibits reduced abdominal background and decreased renal radiation dose [43]. At the clinical level, various studies support the notion that ^{99m}Tc-annexin V SPECT allows for noninvasive, reproducible, quantitative apoptosis imaging and for assessing tumor response as early as 24 h after the start of treatment, with the goal of monitoring the effectiveness of therapy in cancer patients. With pioneering work, Belhocine et al. used ^{99m}Tc-BTAP-annexin V to monitor chemosensitivity in a variety of cancer types (e.g., lung cancer, lymphoma, and breast cancer), demonstrating that ^{99m}Tc-BTAP-annexin V uptake after chemotherapy was significantly related to survival and progression-free survival in cancer patients [44].

More recently ^{99m}Tc-annexin V has been probed in clinical studies to assess the efficacy of chemotherapy and radiotherapy [45, 46]. The promising results so far obtained offer the possibility also in this scenario to develop a personalized medicine approach, now primarily explored in genome-based medicine, applicable to all cancer patients.

However, the main problems in a wide clinical application remain the absence of ideal biological properties; experiences with radiolabeled annexin V have indicated that issues such as biodistribution and target-to-background ratio require further improvement [47]. In an attempt to reduce renal uptake, annexin V was also labeled with ^{123}I. ^{123}I-annexin V does indeed show good imaging features for imaging the abdominal region compared to ^{99m}Tc compounds (no liver nor renal radioactivity accumulation 12-h post-injection) but is subject to rapid in vivo dehalogenation and is more expensive, and the labeling procedure is more complex [48].

^{99m}Tc-Duramycin

Duramycin, a 19-amino acid peptide cross-linked by a disulfide bond, has been found to be capable of binding to phosphatidylethanolamine with high affinity and high selectivity; therefore, the radiolabeled ^{99m}Tc-duramycin represents now a novel molecular compound for apoptosis imaging [49].

Similar to phosphatidylserine (the target marker of the earliest apoptosis probe, annexin V), phosphatidylethanolamine is a major component of the inner leaflet of the cell membrane, and its expression on the surface of normal viable cells is extremely low [50–52]. When apoptosis occurs, phosphatidylethanolamine is exposed on the cell surface [53], because of redistribution of phospholipids across the bilayer. Phosphatidylethanolamine becomes accessible to the extracellular milieu during necrosis, because of the

compromised plasma membrane integrity. Thus, phosphatidylethanolamine constitutes a potential target molecule for cell death imaging in general.

Phosphatidylethanolamine expressed on the apoptotic cell surface appears to play a regulatory role in the so-called blebbing and formation of apoptotic bodies. In these constitutive processes of apoptosis, intracellular components are discretely packaged and earmarked for engulfment by scavenger cells without causing inflammation. As one of the morphologic hallmarks of apoptosis, blebbing includes profound membrane structural remodeling. The trans-bilayer movement of phosphatidylethanolamine is especially enhanced on the blebs of apoptotic cells. These morphologic changes are in part attributed to the phosphatidylethanolamine-mediated reorganization of actin filaments [54, 55].

As a novel molecular probe to target apoptosis imaging, duramycin is produced by *Streptoverticillium cinnamoneus* [56, 57]. Biologic activities of duramycin have been well characterized, and their phosphatidylethanolamine binding activity explored with in vitro biologic studies [58–62]. In particular, duramycin binds the head group of phosphatidylethanolamine with high affinity at a molar ratio of 1:1 [63–65]. Duramycin has a compact cyclic configuration, with a single binding pocket that specifically interacts with phosphatidylethanolamine. Stabilized by three internal thioether bonds, duramycin is the smallest known polypeptide that has a defined three-dimensional binding site [56, 57].

In 2008, Zhao et al. originally described the preparation of ^{99m}Tc-labeled duramycin, using a HYNIC ligand with tricine and phosphine as coligands [66]. The low molecular weight of duramycin (~2 kDa) confers favorable pharmacokinetics and biodistribution properties for in vivo imaging of apoptosis. However, in the original formulation, HPLC purification after radiolabeling was a prerequisite for intravenous injection in humans, a cumbersome procedure that limited clinical investigations. The goal to produce high-quality ^{99m}Tc-curamycin in a single-step kit formulation, without additional purification steps, was achieved in 2012 by Zhao and coworkers, with the development of a single-step kit formulation for ^{99m}Tc-labeling of HYNIC-duramycin [67]. An optimal formulation with tricine-to-TPPTS molar ratio of 10:1 was determined, which led to consistent production of high radiochemical purity ^{99m}Tc-duramycin without the need for further purification. The radiopharmaceutical so produced retained phosphatidylethanolamine binding affinity and specificity, while its clearance properties and in vivo biodistribution were consistent with those obtained in prior studies using radio-HPLC-purified preparation.

In a rat model of myocardial ischemia/reperfusion injury, ^{99m}Tc-duramycin showed specific higher uptake in apoptotic cells than in viable control cells, with favorable pharmacokinetic and biodistribution profiles. The tracer was rapidly cleared from the circulation via the renal system with a blood half-life of less than 4 min in rats and a very low liver and gastrointestinal uptake [67, 68]. Similar favorable targeting and biodistribution properties have been observed also in a porcine model of myocardial ischemia/reperfusion, showing high accumulation of ^{99m}Tc-duramycin in tissue sites of injury with high apoptotic activity [69]. In addition, ^{99m}Tc-duramycin has been evaluated for imaging ischemia/reperfusion injury in the brain using the rat model of middle cerebral artery occlusion [70], as well as in a model of oxidative lung injury [71–73], and finally in susceptible tissues after exposure to high-dose radiation [74].

Taken altogether, these investigations suggest the high potential of ^{99m}Tc-duramycin for use in oncology, an assumption that has recently been confirmed by preclinical studies demonstrating high specific uptake of ^{99m}Tc-duramycin in apoptotic cells of colon cancer or breast cancer xenografts responding to chemotherapy [75–77], thus supporting the hypothesis that ^{99m}Tc-duramycin is a potential candidate in cancer patients to assess response to therapy.

Angiogenesis

The formation of the new vessels ("angiogenesis") is an essential process in the growth of solid tumors. Once tumors have reached a size of >1 mm^3, diffusion alone from the capillary bed is no longer sufficient to supply the tumor cells with

adequate amounts of oxygen and nutrients. Further tumor growth is only possible when new blood vessels are formed. Nevertheless, while normal angiogenesis is orderly and highly regulated, tumor angiogenesis is chaotic and irregular. Angiogenesis represents an interesting molecular target not only for imaging but also for targeted forms of therapy. Examples for target anti-angiogenic therapies currently used in the clinical practice are cilengitide (that inhibits integrin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$) and bevacizumab, an antibody targeting the vascular endothelial growth factor (VEGF).

Different potential molecular targets to monitor angiogenesis are potentially available for imaging purposes. At the moment the most suitable candidates for tracer development are represented by integrin antagonists, expressed extracellular matrix protein inhibitors or matrix metalloproteinase, as well as by tracers binding to tyrosine kinases or growth factor receptors.

Integrins

Integrins are heterodimeric membrane receptors constituted by α and β subunits that mediate interactions between cells and the extracellular matrix and soluble molecules (such as growth factors). So far 18 different α and 8 different β subunits have been identified, corresponding to 24 different integrin receptors. Integrin $\alpha_v\beta_3$ is one of the most studied in oncology, because it is highly expressed on the cell surface of activated endothelial cells in newly formed blood vessels. In the preclinical setting, a large variety of imaging strategies have been successfully employed for imaging integrin expression.

All the tracers that are used for imaging of integrin expression are based on the tripeptide sequence arginine-glycine-aspartic acid (or RGD in the single letter code) [78]. RGD binds to the integrin containing the α_v subunit, which represents an abundant physiologic integrin-binding ligand in proteins of the extracellular matrix. Accordingly, a variety of radiolabeled RGD-based peptides have been developed for noninvasive imaging of integrin $\alpha_v\beta_3$ expression with either SPECT or, in most of the cases, PET. The ^{99m}Tc-labeled RGD peptides have been the subject of few investigations, and few peptides have been translated into clinical use, such as ^{99m}Tc-NC100692 and ^{99m}Tc-labeled RGD dimeric peptides with PEG_4 and Gly_3.

^{99m}Tc-αP2

This 10-amino acid ^{99m}Tc-peptide first described in 1988 contains two copies of the RGD motif for integrin-specific binding. It has been tested in a clinical study in a patient with malignant melanoma, resulting in a good detection rate of metastases in the neck, axilla, abdomen, and soft tissue 60–120 min after injection; however, sensitivity was somewhat lower for lesions located in the thorax, due to high blood pool activity in the heart and large vessels [79].

^{99m}Tc-NC100692 (or ^{99m}Tc-Maraciclatide)

^{99m}Tc-NC100692 is a new RGD-containing peptide labeled with ^{99m}Tc that binds to integrin $\alpha_v\beta_3$ with high affinity [80]. Clinical studies in patients with breast and lung cancer showed a good detection rate for malignant lesions greater than 1 cm, in the breast, brain, and lung, whereas the detection rate was considerably lower for bone and liver metastases [81–83].

On the other hand, in the scenario of theranostics, some investigators confirm that ^{99m}Tc-NC100692 does target the $\alpha_v\beta_3$ integrin in mice bearing glioma tumors and may, therefore, be useful for identifying patients prior to anti-$\alpha_v\beta_3$ therapy as well as for monitoring tumor response to antiangiogenetic therapy in these patients [84].

^{99m}Tc-3PRGD$_2$

^{99m}Tc-PEG_4-E[PEG_4-c(RGDfK)]$_2$ (or ^{99m}Tc-3PRGD$_2$) is a new single-photon tracer targeting

the integrin $\alpha_v\beta_3$-receptor and has been used preclinically for tumor imaging, for evaluating angiogenesis, and for monitoring antiangiogenic drug efficacy [85, 86].

The new types of RGD peptides show much higher in vitro integrin $\alpha_v\beta_3$-binding affinity than the single RGD tripeptide sequence and exhibit significantly increased tumor uptake and improved in vivo kinetics in animal models. ^{99m}Tc-3PRGD2 is excreted predominantly by the kidneys and has a rapid blood clearance, with less than 1% of the initial radioactivity remaining in the blood circulation at 60 min after injection. No adverse reactions have been observed in animal models or in humans to date. ^{99m}Tc-3PRGD$_2$ can easily be prepared in a kit formulation and has shown excellent in vivo patterns of biodistribution in nonhuman primates [87].

Recently, ^{99m}Tc-3PRGD$_2$ has been used in patients, in particular for characterizing solitary pulmonary nodules [88], as well as palpable and nonpalpable breast lesions [89]. It has also been used with satisfactory results in patients with lung cancer [90], in patients with iodine-refractory thyroid cancer [91], for imaging bone metastases in patients with lung cancer (versus the conventional ^{99m}Tc-MDP bone scan) [92], and for monitoring the response to treatment with antiangiogenetic therapy in a mouse model of glioma [93].

^{99m}Tc-RGD-BBN

A dual receptor-targeted probe, integrin $\alpha_v\beta_3$ and gastrin-releasing peptide receptor (GRPR)-targeted peptide, Glu-c(RGDyK)-bombesin (RGD-BBN) labeled with technetium-99m (^{99m}Tc-RGD-BBN), has been tested with promising results in an animal model [94] and then with pilot studies in humans. In particular, its biodistribution has been evaluated in healthy volunteers (exhibiting a safe profile) and in patients with breast cancer as a novel agent for scintimammography. In this clinical setting, ^{99m}Tc-RCD-BBN has shown excellent properties for tumor detection [95], with the potential of avoiding surgical biopsy in patients with equivocal breast lesions, thanks to its very high negative predictive value for malignancy [96].

Hybrid Radioactive/Fluorescent RGD

^{111}In-labeled RGD has been coupled with a fluorescent dye to produce a hybrid tracer, with the purpose of allowing visualization of tumor margins during surgery as well as the in vivo detection of the tumor and its distant metastases. Only very preliminary preclinical studies have been performed so far with this new hybrid tracer, which appears to exhibit optimal properties for targeted $\alpha_v\beta_3$ integrin detection, with very high tumor-non-tumor ratios [97].

Extracellular Matrix

Fibronectin is a polymorphic matrix protein belonging to the widely distributed family of universal cell-adhesion molecules. Fibronectin can exist in several isoforms implied in a variety of processes such as cell migration, wound healing, and oncogenic transformation. A particular isoform (the splicing variant ED-B) is important in vascular proliferation and is widely expressed in neoplastic tissues while showing a highly restricted distribution in normal tissues [98].

^{99m}Tc-AP39

The ^{99m}Tc-anti-ED-B fibronectin L19-(Gly)$_3$-Cys-Ala scFv antibody fragment (^{99m}Tc-AP39) is a radiolabeled molecular imaging agent developed for single-photon emission imaging of tumor angiogenesis and for guidance during antiangiogenic treatment for tumors.

The single-chain antibody fragment (scFv) derived from the L19 monoclonal antibody (with a high affinity to ED-B fibronectin) was developed by Pini et al. [99] and initially labeled with radioiodine for biodistribution in different animal models, where it exhibited excellent targeting to tumor vessels, without selective accumulation in the vessels of other organs [100].

In order to prepare a stable ^{99m}Tc-labeled L19 fragment, Berndorff et al. [101] inserted the amino

acid sequence $(Gly)_3$-Cys-Ala at the C terminus of L19 to produce the recombinant protein, AP39. These authors demonstrated that the ^{99m}Tc-labeled compound so formed (^{99m}Tc-AP39) has favorable biodistribution and imaging properties in mice bearing a murine embryonal teratocarcinoma. Since high levels of ED-B expression have been found in a variety of cancers including primary and metastatic breast, colorectal, and non-small cell lung cancers [102–104], this anti-fibronectin tracer has the potential of being useful for noninvasive imaging of tumor angiogenesis in all such cancers, virtually as a pancarcinoma-targeting agent.

Besides the potential for tumor targeting with imaging purposes as described above, the very high uptake of the anti-fibronectin ED-B monoclonal constructs in tumor tissues opens promising perspectives for developing new agents for radioimmunotherapy, as described in details in ► Chap. 8, "Novel Radiopharmaceuticals for Therapy" of this book.

Matrix Metalloproteinases

Due to their involvement in tumor metastasis and angiogenesis, the matrix metalloproteinases (MMP) are potential targets for molecular imaging. The MMP family consists of more than 18 different members, with levels in tissues that are controlled by a balance between synthesis of the proenzyme and expression of endogenous MMP inhibitors [105]. Increased proenzyme production causes degradation of the basement membrane and of the extracellular matrix, thus preparing the structural requirements for migration of endothelial cells and formation of vessels [106]. In particular, the MMP-2 and MMP-9 gelatinases are often detected in malignant tissue, and their overexpression correlates with tumor aggressiveness and metastatic potential. Ongoing investigations aim at developing synthetic compounds for targeting MMPs.

Although most studies are in a preliminary phase, some hydroxamate-based tracers with promising binding properties have been identified. In particular, among single-photon-emitting agents, the peptidomimetics [^{111}In]-DTPA-RP782 and [^{99m}Tc]-(HYNIC-RP805)(tricine)(TPPTS) and the picolyl-benzenesulfonamide [^{123}I]I-HO-CGS 27023A appear to specifically target the enzymatic action of MMPs in animal models of various diseases. Nevertheless, preclinical studies in animal models prove that these imaging agents might be more successful for investigating atherosclerosis than for tumor targeting [107].

Epidermal Growth Factor Receptor (EGFR)

The EGFR family consists of four transmembrane receptors, respectively, EGFR properly said (HER1/erbB-1), HER2 (erbB-2/neu), HER3 (erbB-3), and HER4 (erbB-4). EGFR is a glycosylated transmembrane protein composed of an extracellular ligand-binding region, a transmembrane region, and an intracellular tyrosine kinase domain. The extracellular domain binds endogenous growth factors, like epidermal growth factor (EGF) or transforming growth factor alpha (TGF-α). Binding of one of these endogenous ligands triggers erbB receptor aggregation, thus resulting in the formation of receptor homodimers and/or heterodimers, and internalization. Dimer formation leads to activation of the intrinsic receptor tyrosine kinase domain and to a cascade of intracellular signaling pathways.

This mechanism is involved in the regulation of cell growth, as well as in differentiation and survival of cells. These properties have attracted the interest of investigators especially in oncology, and EGFR has been investigated as a major target for the treatment of uncontrolled tumor growth [108]. In fact, EGFR is often overexpressed in human malignancies such as head and neck squamous cell carcinoma, gastrointestinal and abdominal carcinomas, lung carcinomas, carcinomas of the reproductive tract, melanomas, glioblastomas, and thyroid carcinomas [109]. Although data are heterogeneous in this regard, overexpression is often associated with an aggressive tumor phenotype and poor prognosis. To target tumor cell proliferation or growth via EGFR, monoclonal antibodies (mAbs) against this receptor have been developed for therapeutic purpose. One of these agents, cetuximab,

has been approved by the Food and Drug Administration (FDA) in 2004 for treatment of colorectal cancer.

Two predominant classes of EGFR inhibitors have been developed including mAbs that target the extracellular domain of EGFR, such as cetuximab (Erbitux) or trastuzumab (Herceptin), and small molecule tyrosine kinase inhibitors (TKIs) that target the receptor catalytic domain of EGFR, such as gefitinib (Iressa) and erlotinib (Tarceva) [110]. Several SPECT single-photon radiopharmaceuticals have been developed in preclinical trials, such as ^{99m}Tc-Cetuximab as stable complex with ethylenedicysteine [111,112]. Besides an unexpected high kidney uptake observed in rates bearing a human breast tumor, the 6-h physical half-life of ^{99m}Tc was too short for imaging, considering that mAb preparations like cetuximab the highest tracer accumulation in the tumor is expected 2–3 days post-injection. In a pilot clinical study, high uptake of this cetuximab conjugate in tumors was observed, however, without a sufficiently high target-to-background ratio and without a clear correlation with other clinical features in patients with head and neck cancer.

With its relative longer physical half-life (2.8 days), ^{111}In-labeled mAb conjugates obtained using the chelators DOTA or DTPA are in principle more suitable for tumor imaging than the ^{99m}T-labeled counterparts. Accordingly, these have been investigated in animal models [113, 114] and in humans [115]. Radiolabeled pertuzumab, a HER2 dimerization inhibitor that binds to an epitope different from that of trastuzumab, was also evaluated to image HER2 downregulation after 3 days of treatment with trastuzumab in human breast cancer xenografts. ^{111}In-diethylenetriaminepentaacetic acid-pertuzumab (^{111}In-DTPA-pertuzumab [116]) demonstrated a reduction in viable, HER2-positive tumor cells after 3 weeks of therapy [117].

Folate Receptor Overexpression

Due to their increased metabolic and structural requirements, tumor cells usually consume high amounts of folate, a compound involved in many biosynthetic processes including DNA synthesis. The folate transporter is usually overexpressed on the surface of tumors such as ovarian cancer and lung cancer, thus representing a possible target for molecular imaging, both for therapeutic [118] and for diagnostic purpose (^{99m}Tc-Etarfolatide or ^{111}In-DOTA-folate) [119]. Interference by important accumulation of this tracer in normal tissues, particularly in the kidneys, makes it difficult to analyze the results of clinical trials with ^{99m}Tc-etarfolatide. To prevent such problem, folic acid or pemetrexed combined with thymidine was administered in patients before tracer injection as an antidote to the potential toxicity [120]. The results of trials with this combination are promising, particularly in patients with ovarian cancer [121–123], and the compound is currently under review by the European Medicines Agency.

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