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Chapter 2

Radiopharmaceuticals: radiolabeled nanoparticles as multivalent systems for molecular imaging

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Radiofármacos: nanopartículas como sistemas multifuncionales para la obtención *in vivo* de imágenes moleculares

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Radiopharmaceuticals: radiolabeled nanoparticles as multivalent systems for molecular imaging

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Introduction

Molecular imaging comprises non-invasive monitoring of functional and spatiotemporal processes at molecular and cellular levels in humans and other living systems. Imaging techniques such as magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET) and optical fluorescence imaging (OI) have been used to monitor such processes.

Nanoparticles can be defined as a particle of any material having dimensions of 100 nm or less with novel properties that distinguish them from the bulk material due to size and surface effects and they can be prepared as stable, homogeneous and well characterized systems in size and shape. Nanoparticles produce multivalent effects due to multiple simultaneous interactions between the biomolecules conjugated on the nanoparticle surface and the cell surface, with specific receptors for those biomolecules. Nanoconjugates used in diagnosis or therapy must be nontoxic, biocompatible and stable in biological media with high selectivity for biological targets.

Regulatory peptide-receptors are proteins over-expressed in numerous human cancer cells. These receptors have been used as molecular targets for labeled peptides to localize tumors. The improvement of peptide analogues allows specific clinical imaging and therapy of different tumor types, including breast, prostate, lung, intestine, pancreas and brain tumors. Therefore, specific cancer targeting through selective peptides for diagnostic and therapeutic purposes is considered to be a promising strategy in oncology. Peptides for cancer imaging using the different medical diagnostic techniques such as those mentioned above, rely mostly on the use of radiopeptides and peptides conjugated to near-infrared fluorochromes, metallic nanoparticles or quantum dots (nanocrystals) (Figure 1).

1. Radiolabeled peptides as diagnostic agents

It is well known by the radiopharmaceutical community the use of peptides labeled with different radioactive nuclides to detect malignant tumors (^{111}In, ^{99m}Tc, ^{18}F, ^{68}Ga, ^{64}Cu) as well as for therapeutic schemes (^{90}Y, ^{177}Lu, ^{188}Re) applied in the practice of Nuclear Medicine and Molecular Imaging [1-5]. Therefore, it is possible to identify: gastrinomas, lung and pancreas cancer with somatostatin analogous; breast cancer with bombesin; *in vivo* angiogenesis with cycle-Arg-Gly-Asp (cRGD); infectious processes with the

antimicrobial peptide ubiquicidine (UBI) and thyroid differentiated cancer with cholecystokinin to mention some examples [6,7]. Penetrating peptides are emerging as attractive drug delivery tools. The HIV Tat(49-57) -derived peptide is a small basic peptide called “trojan horse” for successfully delivering a large variety of cargoes into cells such as nanoparticles, proteins, peptides and nucleic acids [8,9].

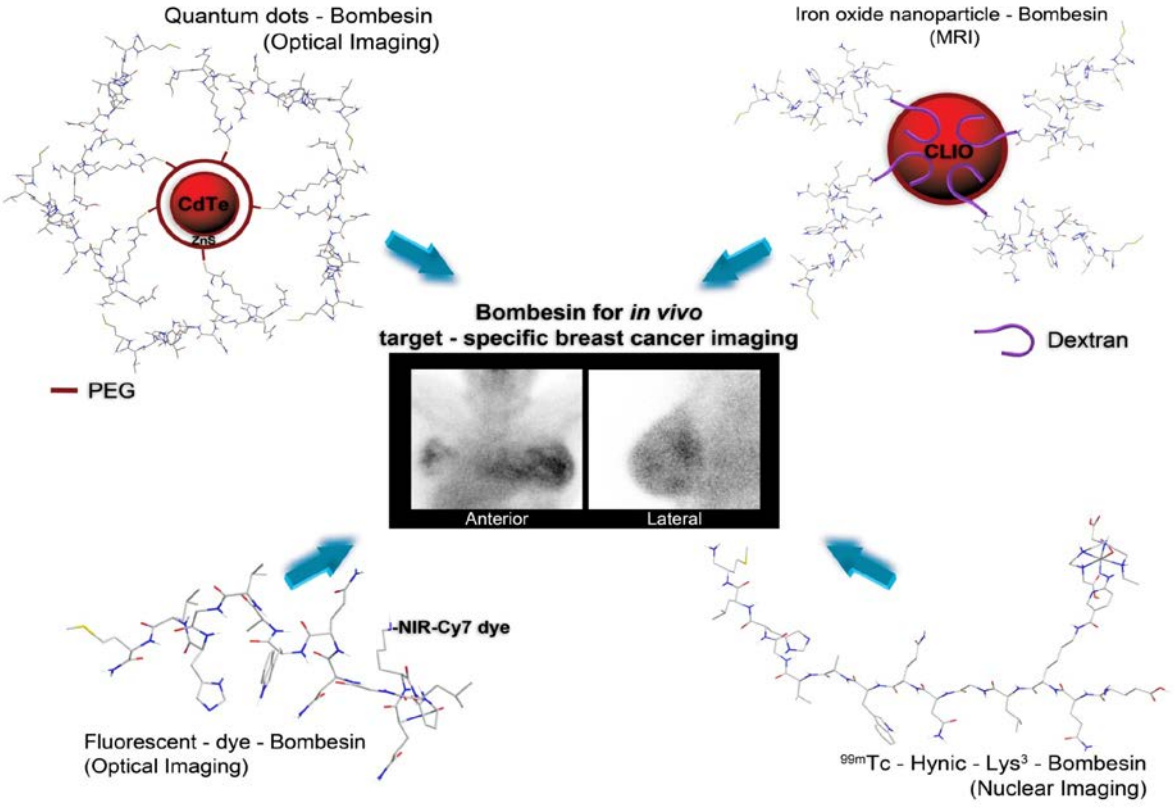

Figure 1. Schematic illustration of the multiple modalities for in vivo target-specific cancer imaging with peptides conjugated to quatum dots, metallic nanoparticles, near-infrared fluorochromes or radionuclides [2].

From the point of view of molecular recognition, peptides have excellent properties that allow them to participate in ligand-receptor molecular interactions. Nanoparticles coated with peptides increase their stability and biocompatibility allowing them to be directed to the desired target. Two approaches have been developed for targeting namely “passive” and “active”. Passive targeting depends on homing of the vectors in unhealthy tissues due to extravasation through leaky blood vessels (gaps ~100-800 nm).

An important aspect of carrier systems in the 5-20 nm scale is their ability to take advantage of the enhanced permeation and retention (EPR) effect [10,11]. On the other hand, active targeting presents on the carrier surface for specific recognition by cell surface

receptors. Combinations of both types of targeting will render an ideal carrier for *in vivo* delivery (Figure 2) [10,11].

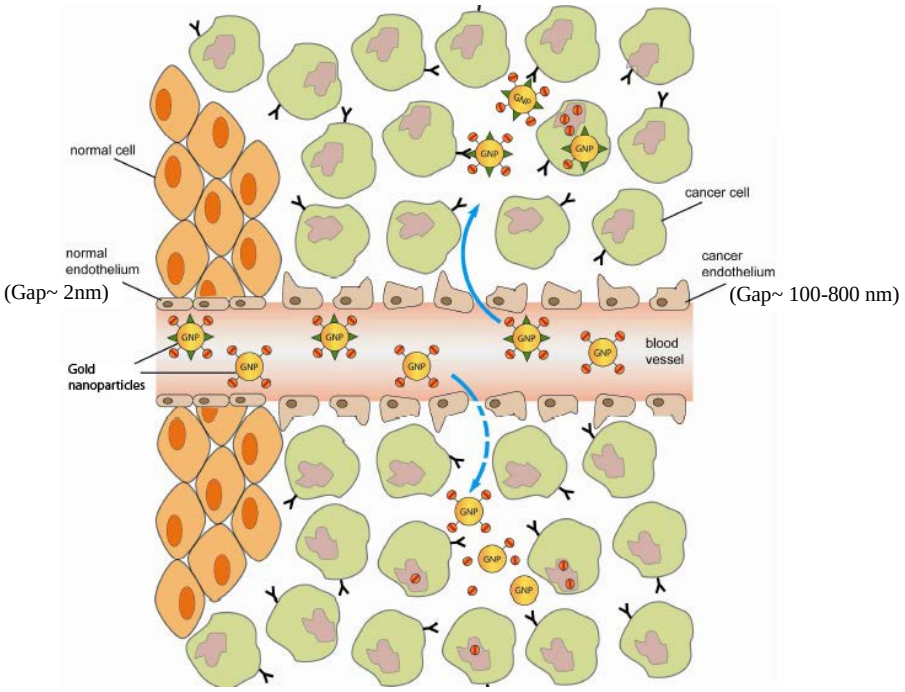

Figure 2. Passive targeting depends on homing of the nanoparticles in unhealthy tissues due to diffusion or convection across the leaky blood vessels (cancer endothelium: gaps 100–800 nm). It means that in the 20 nm scale, nanoparticles have the ability to take advantage of enhanced permeation and retention effect (EPR). Active targeting presents peptides on the nanoparticle surface for specific recognition by cell surface receptors [10,11].

2. Peptides in Optical Imaging

Optical imaging offers real-time, nonradioactive, and, depending on the technique, high-resolution imaging of fluorophores embedded in diseased tissues. Of the various optical imaging techniques investigated to date, near-infrared (NIR, 700-1000 nm wavelength) fluorescence-based imaging is of particular interest for noninvasive *in vivo* imaging because of the relatively low tissue absorption, scatter, and minimal autofluorescence of NIR light. This is because hemoglobin (the primary absorber of visible light), water and lipids (the primary absorbers of infrared light) have their lowest absorption coefficients in the NIR region. Deeper tissue areas are thus accessible for tomographic display of the optical signals.

Advanced fluorescence imaging techniques, such as fluorescence molecular tomography (FMT) and fluorescence reflectance imaging (FRI), commonly employ NIR wavelengths for *in vivo* molecular imaging applications. Fluorescence imaging methods are generally superior in terms of sensitivity and ease of use. However, NIR fluorescence imaging in

small animals cannot be directly scaled up to *in vivo* imaging in patients due to the limited optical signal penetration depth (≤ 7 mm by fluorescence resonance imaging and ≤ 20 cm by fluorescence molecular tomography). In clinical settings, fluorescence imaging is relevant for tissues close to the surface of the skin and tissues accessible by endoscopy and intraoperative visualization [12-14].

Quantum dots (QDs) or nanocrystals are fluorescent semiconductor nanoparticles (2-10 nm) with many unique optical properties including bright fluorescence, resistance to photobleaching, and a narrow emission bandwidth. Their fluorescence emission wavelength can be continuously tuned from 400 nm to 2000 nm by changing both the particle size and chemical composition, at room temperature. Recently, a detailed procedure for the preparation of QDs-RGD was reported for *in vivo* imaging of human glioblastoma tumors in mice [15]. Young and Rozengurt [16] demonstrated that QDs-bombesin conjugates can label the bombesin-preferring G protein-coupled receptors (GPCR) in living mice, suggesting that QDs technology can be adapted to monitor *in vivo* ligand binding to GPCRs. Ruan et al. have recently used QDs-Tat as a model system to examine the cellular uptake and intracellular transport of nanoparticles in living cells [17].

The fluorescence of gold nanoparticles (AuNP) rises from the surface plasmon resonance and can be enhanced, quenched or photobleached by the AuNP size, the nature of bounded cap to AuNP as well as the surrounding of the capped-AuNP. Fluorescence emission is very important in the study of AuNP conjugated to peptides since in general, peptides contain aromatic residues which transfer their energy to the AuNP following the pathway: fluorescence level (peptide) to phosphorescence level (peptide) to surface plasmon resonance level (AuNP) and the absorbed light re-emitted, usually in the NIR range or close to- if the NIR fluorescence emission is not quenched or masked by the luminescence background in particular, in tissues [10].

From a therapeutic point of view, there are two properties of gold that are most relevant: resistance to oxidation and plasmon resonance with light [18]. The plasmon resonance for ordinary gold nanospheres is at 520 nm, in the middle of the visible spectrum, but this can be red-shifted into the near infrared region (NIR) from 800 to 1200 nm. This is useful because body tissue is moderately transparent to NIR light [19], thereby providing an opportunity for therapeutic effects in deep tissues. Local application of heat is a known concept in therapeutic medicine that has been extensively explored for cancer treatment and other conditions. Excitation sources, such as infrared lamps, ultrasound or lasers, can be used in the process, but there is always the problem of limiting the heat generated to just the region of the target tissue. As mentioned earlier, this problem can be solved, in part, by using gold nanoparticles designed to absorb in the NIR spectrum so that the resulting localized heating causes irreversible thermal cellular destruction. NIR irradiation led to a rise in the temperature of the target regions from 40 to 50°C, which selectively destroyed the carcinomas [19].

3. Iron Oxide Nanoparticles Conjugated to Peptides for MRI

Recently, dextran coated superparamagnetic iron oxide nanoparticles (CLIO) were conjugated to bombesin to visualize tumors in a murine model of pancreatic ductal adenocarcinoma by MRI [20]. Montet et al. [21] prepared cRGD-CLIO labeled with the flourochrome Cy5.5. This magneto-fluorescent RGD nanoparticles were targeted to αvβ3-expressing tumor cells *in vivo* and were detectable by fluorescence reflectance imaging, fluorescence molecular tomography, and magnetic resonance imaging.

4. *Use of Gold Nanoparticles in the Design of New Radiopharmaceuticals at ININ.*

The aim of the development of cancer-receptor-specific AuNPs at ININ is to maximize the binding affinity via multimeric peptides based on the multivalency principle. Recent studies have demonstrated that conjugating gold nanoparticles (AuNP) to peptides produces biocompatible and stable multivalent systems with target-specific molecular recognition with unlimited future in biomedical applications [22-28]. Peptides can be conjugated to one AuNP by spontaneous reaction of the AuNP surface with a thiol (cysteine) or an N-terminal primary amine. The thiol group is considered to be the most important type of molecule to stabilise any size of AuNPs, by forming a 'staple motif' chemical model of two thiol groups interacting with three gold atoms in a bridge conformation [28]. Attaching multiple units of a receptor-specific peptide to one AuNP (1000 or more peptide molecules to one 20 nm AuNP) provide useful tools in improving imaging or therapy of tumors over-expressing peptide-receptors. Their combination of low inherent toxicity, ample surface area and biological stability provides them with unique attributes that should enable new cancer therapy strategies.

AuNP conjugated to [Tyr^3]octreotide (TOC) peptide was characterized by TEM, UV–Vis, infrared and fluorescence spectroscopy. AuNP and AuNP-TOC fluorescence emission spectra were obtained both in solution and in murine AR42J-tumour tissues. The fluorescence analyses in tissue revealed the recognition of the AuNP-TOC conjugate by the neuroendocrine tumor because of the lower energy position of the fluorescence resonance (692 nm) with respect to that of the AuNP in the same tumor tissue (684 nm). The emission band observed in the near infrared region (692 nm) opens the possibility for using AuNP-TOC in bioimaging. More than 500 TOC peptides can be bound to one 20 nm AuNP [23].

It has been shown that mannosylated macromolecules labeled with ^{99m}Tc can offer better uptake characteristics due to the well defined size, which is governed by the size of the macromolecule. Furthermore, these radiopharmaceuticals are considered target-specific because they exhibit specific binding to mannose receptors expressed on lymph node macrophages Hydrazinonicotinamide-Gly-Gly-Cys-NH_2 (HYNIC-GGC) peptide and a thiol-triazole-mannose derivative were synthesized, characterized and conjugated to gold nanoparticles (AuNP, 20 nm) to prepare a multifunctional system of HYNIC-GGC-AuNP-mannose by means of spontaneous reaction of the thiol (Cys) present in HYNIC-GGC sequence and in the thiol-mannose derivative (Figure 3). The nanoconjugate was characterized by transmission electron microscopy (TEM), IR, UV–Vis, Raman, Fluorescence and X-ray photoelectron spectroscopy (XPS). Technetium-99m labeling was

carried out using EDDA/tricine as coligands and $SnCl_2$ as reducing agent with further size exclusion chromatography purification. Radiochemical purity was determined by size exclusion HPLC and ITLC-SG analyses. *In vitro* binding studies were carried out in rat liver homogenized tissue (mannose-receptor positive tissue). Biodistribution studies were accomplished in Wistar rats and images obtained using a micro-SPECT/CT system. TEM and spectroscopy techniques demonstrated that AuNPs were functionalized with HYNIC-GGC and thiol-mannose through interactions with thiol groups and the N-terminal amine of cysteine. Radio-chromatograms showed radiochemical purity higher than 95%. ^{99m}Tc-EDDA/HYNIC-GGC-AuNP-mannose (^{99m}Tc-AuNP-mannose) showed specific recognition for mannose receptors in rat liver tissue. After subcutaneous administration of ^{99m}Tc-AuNP-mannose in rats (footpad), radioactivity levels in the popliteal and inguinal lymph nodes revealed that 99 % of the activity was extracted by the first lymph node (popliteal extraction). Biodistribution studies and *in vivo* micro-SPECT/CT images in Wistar rats showed an evident lymph node uptake (11.58 ± 1.98 % I.A. at 1 h) which was retained during 24 h with minimal kidney accumulation (0.87 ± 0.09 % I.A.) and negligible uptake in all other tissues (Figure 4). This study demonstrated that ^{99m}Tc-AuNP-mannose remains within the first lymph node during 24 h and therefore might be useful as a target-specific radionanoconjugate for sentinel lymph node detection. [25]

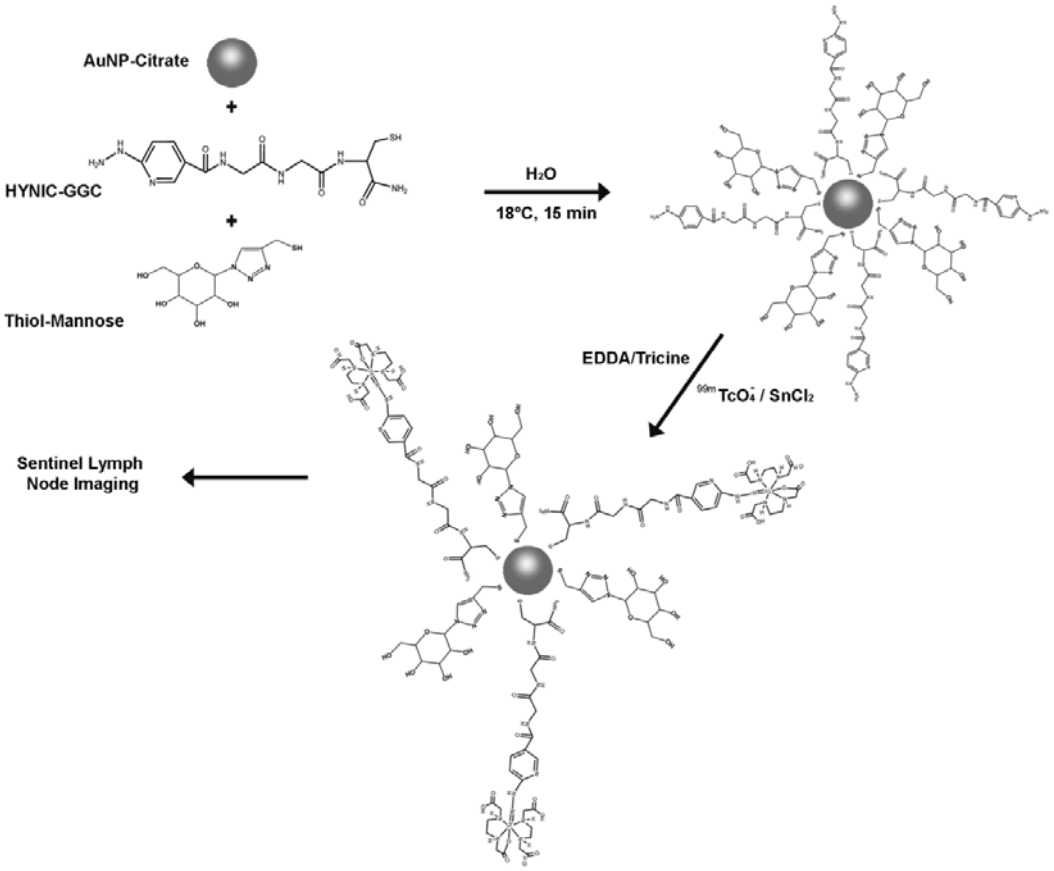

Figure 3. Overall scheme of ^{99m}Tc-EDDA/HYNIC-GGC-AuNP-mannose preparation for sentinel lymph node imaging.

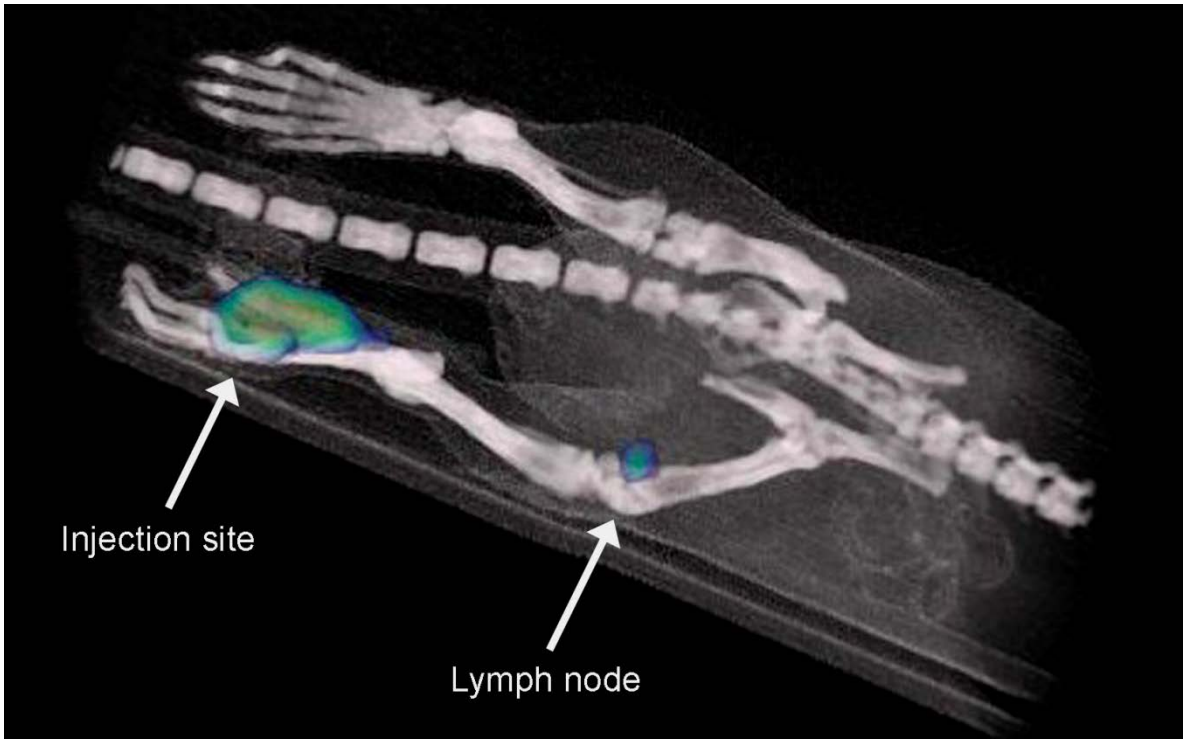

Figure 4. Micro-SPECT/CT image of ^{99m}Tc-AuNP-mannose in the first lymph node in Wistar rat 2 h after radiopharmaceutical administration

Angiogenesis is a physiological process involving the growth of new blood vessels and it is a requirement for tumor growth and metastasis. The angiogenic process is regulated by cell adhesion receptors, such as integrins. The αvβ3 integrin is expressed on the surface of normal endothelial cells at low levels, but is over-expressed in the tumor neovasculature and tumor cells of osteosarcoma, neuroblastoma, glioblastoma, melanoma, lung carcinoma and breast cancer. Radiolabeled peptides based on the Arg-Gly-Asp (RGD) sequence have been reported as radiopharmaceuticals with high affinity and selectivity for the αvβ3 integrin and are therefore useful in the non-invasive monitoring of tumor angiogenesis by molecular imaging techniques. Monomeric RGD radiopharmaceuticals tend to have fast blood clearance but relative good tumor uptake and rapid tumor washout, whereas dimeric, tetrameric and octameric cyclo-RGD peptides exhibit increased affinity and enhanced adhesion to target αvβ3 integrin due to an increase in multivalent sites. At ININ a multimeric system of ^{99m}Tc-labeled gold nanoparticles (20 nm) conjugated to 200 cyclo[Arg-Gly-Asp-Phe-Lys(Cys)] (c[RGDfK(C)]) peptides was successfully prepared with potential as a specific radiopharmaceutical for tumor angiogenesis imaging [26].

The gastrin-releasing peptide receptor (GRP-r) is a seven-transmembrane G-protein coupled receptor that is over-expressed in breast, prostate and small-cell lung carcinoma and lymph node metastases. Bombesin is a tetradecapeptide that binds with high affinity to GRP-r. Specifically, the Lys^3-bombesin analogue labeled with technetium-99m has been reported as a radiopeptide with high stability in human serum, specific cell receptor binding and rapid internalization [27]. Images of GRP-r expression in breast cancer patients using ^{99m}Tc-EDDA/HYNIC-Lys^3-bombesin have demonstrated distinct radioactivity accumulation in malignant tissue [5]. Recently we developed a multifunctional system of technetium-99m labeled gold nanoparticles conjugated to Lys^3-bombesin/HYNIC-GGC with potential as a new target-specific radiopharmaceutical for *in vivo* GRP-r imaging [28].

5. *Perspectives: Multimodal Techniques for Molecular Imaging*

Molecular imaging represents the future of diagnostic imaging: it evolves from both anatomic and functional imaging as well as advances in genomics, cell and molecular biology, chemistry, and physics. Different imaging techniques are, in general, complementary rather than competitive. Dual-labelled targeting imaging agents, allow cross validation and direct comparison for example between nuclear (the goldstandard) and fluorescence optical image, or MRI and fluorescence, or trimodal nuclear-MRI-fluorescence.

For example, SPECT and PET provide functional information but lack the anatomical information. Computer tomography (CT) is a tomographic imaging technique that uses external X-ray source to produce 3-dimensional anatomic image data. The SPECT/CT and PET/CT systems, currently used in the clinical practice, combine a gammacamara and an integrated X-ray transmission system mounted on the same gantry. The advantage of SPECT/CT or PET/CT is the ability to acquire an anatomic image with CT and a functional image with SPECT or PET sequentially.

In the same direction, a dual-modality PET/NIR fluorescent peptide has been recently reported by Cai et al. [29]. A QD with an amine-functionalized surface was modified with RGD (90 peptides per QD) and 1,4,7,10-tetraazacyclodocecane-N,N',N",N"'-tetraacetic acid (DOTA) chelators for integrin αvβ3-targeted PET/NIRF imaging. PET/NIRF imaging, tissue homogenate fluorescence measurement, and immunofluorescence staining were performed with U87MG human glioblastoma tumour-bearing mice to quantify the ^{64}Cu-DOTA-QD-RGD uptake in tumour and major organs. Excellent linear correlation was obtained between the results measured by *in vivo* PET imaging and those measured by *ex vivo* NIRF imaging and tissue homogenate fluorescence. Histologic examination revealed that ^{64}Cu-DOTA-QD-RGD targets primarily the tumour vasculature through a RGD-integrin interaction, with little extravasation. Authors concluded that this dual-function probe has significantly reduced potential toxicity and overcomes the tissue penetration limitation of optical imaging, requisite for quantitative targeted imaging in deep tissue [29].

The synthesis and *in vivo* characterization of an ^{18}F-CLIO was reported by Devaraj et al. [30]. This particle consists of cross-linked dextran molecules held together in core-shell formation by a superparamagnetic iron oxide core and functionalized with the radionuclide

^{18}F in high yield via "click" chemistry. Such nanoparticles could accurately detect lymph nodes (LNs), which are critical for assessing cancer metastasis. *In vivo* PET/MRI images could clearly identify small (~1 mm) LNs along with precise anatomical information.

NIR fluorescence has the potential to provide rapid, inexpensive, and nonradioactive population-based screening for breast cancer. Bhushan et al. [31] developed a system for detection of breast cancer microcalcifications using a dual-modality SPECT/NIR fluorescent probe.

In cancer therapy, one of the most important things to keep in mind is that "the magic bullet does not exist", and in order to increase therapeutic response, the application of combined modalities with different therapeutic agents is necessary. Radiolabeled gold nanoparticles conjugated to regulatory peptides, for example ^{177}Lu-DOTA-Gly-Gly-Cys-AuNP-Lys^3-bombesin (size = 10 nm), could represent a unique multifunctional target-specific pharmaceutical that administered as a single drug would be capable of acting as a combined therapy system: targeted radiotherapy, photothermal therapy, angiogenesis inhibition and apoptosis induction in prostate and breast cancer [24].

References

[1] Reubi JC, Maecke HR. Peptide-based probes for cancer imaging. *J. Nucl. Med.***41**,1735-1738, 2008.

[2] Ferro-Flores G, Ramírez F de M, Meléndez-Alafort L, Santos-Cuevas CL. Peptides for *In Vivo* Target-Specific Cancer Imaging. *Mini-Rev. Med. Chem.*, **10**, 87-97, 2010.

[3] Torres-García E, Ferro-Flores G, Murphy CA, Correa-González L, Pichardo-Romero P. Dosimetry and Biokinetics of ^{188}Re-anti-CD20 in Patients: Initial Experience *Arch. Med. Res.* **39**, 100-109, 2008.

[4] Ferro-Flores G, Murphy CA. Pharmacokinetics and Dosimetry of ^{188}Re-pharmaceuticals. *Adv. Drug Deliv. Rev.* **60**, 1389-1401, 2008.

[5] Santos-Cuevas CL, Ferro-Flores G, Murphy CA, Pichardo-Romero P. Targeted imaging of gastrin-releasing peptide receptors with ^{99m}Tc-EDDA/HYNIC-[Lys^3]-bombesin: biokinetics and dosimetry in women. *Nucl. Med. Commun.* **29**, 741-747, 2008.

[6] Vallejo E, Martínez I, Tejero A, Hernández S, Jiménez L, Bialostozky D, Sánchez G, Ilarraza H, Ferro-Flores G. Clinical Utility of ^{99m}Tc-Labeled Ubiquicidin 29-41 Antimicrobial Peptide for the Scintigraphic Detection of Mediastinitis after Cardiac Surgery. *Arch. Med. Res.* **39**, 768-774, 2008.

[7] Welling MM, Ferro-Flores G, Pirmettis I, Brouwer C. Current status of imaging infections with radiolabeled anti-infective agents. *Anti-invective Agents Med. Chem.* **8**, 272-287, 2009.

[8] Santos-Cuevas CL, Ferro-Flores G, Murphy CA, Ramírez F de M, Luna-Gutiérrez MA, Pedraza-López M, García-Becerra R, Ordaz-Rosado D. Design, preparation, *in vitro* and *in vivo* evaluation of ^{99m}Tc-N_2S_2-Tat(49–57)-bombesin: a target-specific hybrid radiopharmaceutical. *Int. J. Pharm.* **375**, 75-83, 2009.

[9] Santos-Cuevas CL, Ferro-Flores G, Rojas-Calderón EL, García-Becerra R, Ordaz-Rosado D, Murphy CA, Pedraza-López M. ^{99m}Tc-N_2S_2-Tat(49-57)-bombesin

internalized in nuclei of prostate and breast cancer cells: Kinetics, dosimetry and effect on celular proliferation. *Nucl. Med. Commun.* **32**, 303-713, 2011.

[10] Ghosh P, Han G, De M, Kyu-Kim C, Rotello V. Gold nanoparticles in delivery applications. *Adv. Drug Deliv. Rev.* **60**, 1307-1315, 2008.

[11] Maeda A, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Control. Release* **65**, 271-284, 2000.

[12] Tearney GJ, Brezinski, M. E., Bouma *et al*. In Vivo Endoscopic Optical Biopsy with Optical Coherence Tomography. *Science* **276**, 2037-2039, 1997.

[13] Ntziachristos V, Weissleder R. Charge-coupled-device based scanner for tomography of fluorescent near-infrared probes in turbid media. *Med. Phys.* **29**, 803-809, 2002.

[14] Ntziachristos V, Tung CH, Bremer C, Weissleder R. Fluorescence molecular tomography resolves protease activity *in vivo*. *Nat. Med.* **8**, 757-760, 2002.

[15] Cai W, Chen X. Preparation of peptide-conjugated quantum dots for tumor vasculature-targeted imaging. *Nat. Prot.* **3**, 89-96, 2008.

[16] Young SH, Rozengurt E. Qdot nanocrystal conjugated to bombesin or ANG II label the cognate G protein-coupled receptor in living cells. *Am. J. Physiol. Cell Physiol.* **290**, C728-732, 2006.

[17] Chen F, Gerion D. Fluorescent CdSe/ZnS nanocrystal−peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. *Nano Lett.* **4**, 827-32, 2004.

[18] Goodman CM, McCusker CD, Yilmaz T, Rotello VM. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjugate Chem.* **15**, 897-900, 2004.

[19] Hirsch LR, Stafford RJ, Bankson JA, Sershen SR, Rivera B, Price RE, Hazle JD, Hals NJ. Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 13549-13554, 2003.

[20] Montet X, Weissleder R, Josephson L. Imaging pancreatic cancer with a peptide-nanoparticle conjugate targeted to normal pancreas. *Bioconjugate Chem.* **17**, 905-911, 2006.

[21] Montet X, Montet-Abou K, Reynolds F, Weissleder R, Josephson, L. Nanoparticle imaging of integrins on tumor cells. *Neoplasia* **8**, 214-222, 2006.

[22] Bhattacharya R, Mukherjee P. Biological properties of "naked" metal nanoparticles. *Adv. Drug Deliv. Rev.* **60**, 1289-1306, 2008.

[23] Surujpaul PP, Gutierrez-Wing C, Ocampo-García B, Ramírez F de M, Murphy CA, Pedraza-López M, Camacho-López MA, Ferro-Flores G. Gold nanoparticles conjugated to [Tyr^3]octreotide peptide. *Biophys. Chem.* **138**, 83-90, 2008.

[24] Ferro-Flores G, Ocampo-García BE, Ramírez F de M, Gutiérrez-Wing C, Murphy CA, Santos-Cuevas CL. Chapter 11 "Gold Nanoparticles Conjugated to Peptides" in "Colloids in Biothechnology" Edited by Monzer Fanum. Ed. CRC Press/Taylor & Francis. Boca Raton, FL, 2010, p. 231-252.

[25] Ocampo-García BE, Ramírez F de M, Ferro-Flores G, De León-Rodríguez LM, Murphy CA, Pedraza-López M, Medina LA, Camacho-Lopez MA. Technetium-99m labeled gold nanoparticles capped with HYNIC-peptide/mannose for sentinel lymph node detection. *Nucl. Med. Biol.* **38**, 1-11, 2011.

[26] Morales-Avila E, Ferro-Flores G, León-Rodríguez LM, Ramírez F de M, Santos-Cuevas CL, Camacho-López MA, Medina LA. Multimeric system of ^{99m}Tc labeled gold

nanoparticles conjugated to c[RGDfK(C)] for molecular imaging of tumor αvβ3 expression. *Bioconjugate Chem.*, in press, 2011.

[27] Ferro-Flores G, Rivero IA, Santos-Cuevas CL, Sarmiento JI, Ocampo-García BE, García-Becerra R, Ordaz-Rosado D. Click Chemistry for [$^{99m}Tc(CO)_3$] labeling of Lys^3-bombesin. *Appl. Rad. Isot.* **68**, 2274-2278, 2010.

[28] Mendoza-Sánchez AN, Ferro-Flores G, Ocampo-García BE, Morales-Avila E, Ramírez F de M, León-Rodríguez LM, Santos-Cuevas CL, Medina LA, Rojas-Calderón EL, Camacho-López MA. Lys^3-bombesin conjugated to ^{99m}Tc-labeled gold nanoparticles for *in vivo* gastrin releasing peptide-receptor imaging *J. Biomed. Nanotech.* **6**, 375-384, 2010.

[29] Cai W, Chen K, Li ZB, Gambhir SS, Chen X. Dual-Function Probe for PET and Near-Infrared Fluorescence Imaging of Tumor Vasculature. *J. Nucl. Med.* **48**, 1862-1870, 2007.

[30] Devaraj NK, Keliher EJ, Thurber GM, Nahrendorf M, Weissleder R. Labeled nanoparticles for *in vivo* PET-CT imaging. *Bioconjugate Chem.* **20**, 397-402, 2009.

[31] Bhushan KR, Misra P, Liu F, Mathur S, Lenkinski RE, Frangioni JV. Detection of breast cancer microcalcifications using a dual-modality SPECT/NIR fluorescent probe. *J. Am. Chem. Soc.* **130**, 17648-17649, 2008.