Molecular Imaging Kits for Hexosamine Biosynthetic Pathway in Oncology

David J. Yang*[,1], Fan-Lin Kong[1], Takashi Oka[2] and Jerry L. Bryant[1]

[1]*Departments of Experimental Diagnostic Imaging and* [2]*Radiology; The University of Texas MD Anderson Cancer Center, Houston, Texas 77030 and Yokohama City University, Yokohama, Japan*

Abstract: Noninvasive imaging assessment of tumor cell proliferation could be helpful in the evaluation of tumor growth potential, the degree of malignancy, and could provide an early assessment of treatment response prior to changes in tumor size determined by computed tomography (CT), magnetic resonance imaging (MRI), Positron emission tomography (PET), Single-Photon emission computed tomography (SPECT) or ultrasonography, respectfully. Understanding of tumor proliferative activity, in turn, could aid in the selection of optimal therapy by estimating patient prognosis and selecting the proper management. PET/CT imaging of ^{18}F-fluoro-2-deoxy-glucose (FDG) is recognized as a technology for diagnosing the presence and extent of several cancer types. Recently, radiolabeled glucosamine analogues were introduced as a promising SPECT agent to complement FDG imaging to increase specificity and improve the accuracy of lesion size in oncology applications. Radiolabeled glucosamine analogues were developed to localize in the nuclear components of cells primarily via the hexosamine biosynthetic pathway whereas glucose localizes in the cytoplasm of cells through the glycolytic/TCA pathway. This paper reviews novel kit-based radiolabeled glucosamine analogues for metabolic imaging of tumor lesions. The novel radiolabeled glucosamine analogues may increase the specificity in oncology applications and can influence patient diagnosis, planning and monitoring of cancer treatment.

Keywords: Hexosamine pathway, glucosamine, chelation, theranostic, PET, SPECT.

IMPORTANCE OF NUCLEAR MOLECULAR IMAGING IN ONCOLOGY

Drug discovery is accelerating due to mapping of molecular targets and the rapid synthesis of high-throughput in vitro testing of compounds in their early stage of the drug development process. The development of radiolabeled biochemical compounds, understanding molecular pathways and imaging devices to detect the radioactivity by external imaging has expanded the use of nuclear molecular imaging studies in drug development. Nuclear molecular imaging modalities (Positron Emission Tomography, PET; Single Photon Emission Computed Tomography, SPECT) map the location and concentration of radionuclide-labeled compounds [1-3]. At present, PET and SPECT gamma cameras are hybrid with computed tomography (CT) to enhance their sensitivity to quantify drug properties *in vivo* in real time dynamic events. PET/CT and SPECT/CT are better than PET and SPECT alone because multiple slices by CT and serial images by PET and SPECT provide better delineation in tumor volumes. In addition, combining the anatomical and morphological location from CT, such as SPECT/CT and PET/CT provides the capability to accurately evaluate post-therapy anatomic alteration. PET/CT and SPECT/CT agents show high specific activities because they are made through a nuclear transformation and use carrier free forms of isotopes. Moreover, PET/CT and SPECT/CT chelator-based agents do not produce detectable pharmacologic effects but provide important information concerning the characterization of varieties diseases, such as vascular angiogenesis [4-6], hypoxia [7-9], apoptosis [10-11], cellular signaling and transcriptional activity [12-14]. PET/CT and SPECT/CT may assist in the determination of optimal therapeutic dosing, differential diagnosis between inflammation/infection and recurrence, sensitive or resistant to treatment response, grading of tumors, and the prediction of treatment response by selecting patient who may or may not respond to therapy. On the other hand, CT, magnetic resonance imaging (MRI), and ultrasound are prognostic tools because they do not provide cellular target information, thus, assessment of the effectiveness of cancer therapy is not optimal.

*Address correspondence to this author at the Department of Experimental Diagnostic Imaging, Unit 59, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA; Tel: (713) 794-1053; Fax: (713) 794-5456; E-mail: dyang@mdanderson.org

KITS CONTAINING RADIONUCLIDES AND CHELATION IN ONCOLOGY

Radionuclide generator systems that can be produced in a well controlled facility and have a long history of successful clinical application would provide an easy and accessible radioisotope for clinical radiopharmaceutical agents. A generator uses a parent-daughter nuclide pair wherein a relatively long-lived parent isotope decays to a short-lived daughter isotope which is used for imaging. The parent isotope, which is produced at a cyclotron facility, is shipped to clinical sites and from which the daughter isotope is eluted on site for clinical use. Nuclear Pharmacies at hospital frequently used generator-produced isotopes are shown in Table **1**. For example, PET radioisotpe ^{68}Ga- (68-minute half-life) agents are with significant commercial potential because the isotope can be produced from a ^{68}Ge generator (275-day half-life) on site and can be a convenient alternative to cyclotron-produced PET isotopes, such as ^{18}F or ^{124}I. Although the maximum positron energy of ^{68}Ga (max=1.90 MeV, mean=0.89 MeV) is higher than that of ^{18}F (max=0.63 MeV, mean=0.25 MeV), a study using Monte Carlo analysis on spatial resolution revealed that under the assumption of 3 mm spatial resolution of PET detectors, the conventional full width at half maximum (FWHM) of ^{18}F and ^{68}Ga is indistinguishable in soft tissue (3.01 mm vs 3.09 mm) [15]. It implies that with the spatial resolution at 5 to 7 mm of current clinical scanners, the imaging quality using ^{68}Ga-tracers can be as good as that of ^{18}F-agents. The results of this theoretical analysis are substantiated clinically by the images with satisfactory qualities presented in a recent study in which patients with carcinoid tumors were imaged with ^{68}Ga-DOTATOC (somatostatin receptor tracers) by PET [16].

Table 1. Clinical commonly used generator-produced radioiso-topes

Generator	Parent $T_{1/2}$	Daughter $T_{1/2}$	Frequency of Milking
^{99}Mo/^{99m}Tc	2.8 d	6 h	*
^{188}W/^{188}Re	70 d	17 h	24 h
^{62}Zn/^{62}Cu	9 h	10 min	30 min
^{68}Ge/^{68}Ga	271 d	68 min	6 h

1875-533X/12 $58.00+.00

Due to favorable physical characteristics (6 h half-life, 140keV), easy availability, and low price, SPECT radioisotope ^{99m}Tc is still an agent of choice for labeling radiopharmaceuticals. ^{99m}Tc can be produced from a ^{99}Mo generator (2.75-day half-life) on site. An imaging agent in a kit form (drug substance) labeled with ^{99m}Tc (drug product) could be easily achieved in high yield. This unique chelation technology has commercial interests and is an alternative to complex radiochemistry production. The longer half-life of ^{99m}Tc provides an opportunity for serial imaging studies that helps in differential diagnosis and responsiveness evaluation. The ineffective treatment could be stopped in early phases and possible switching to more effective treatments which may be beneficial to patients early on in the course of their treatment.

However, among the kits for theranostic radionuclides, ^{188}Re has good characteristics because of its ß energy (2.1 MeV), its short physical half-life (16.9 hr) and its 155keV γ-ray emission for dosimetric and imaging purposes. The short physical half-life of ^{188}Re allows for higher doses compared with long-lived radionuclides. Furthermore, the short half-life reduces the problems of radioactive waste handling and storage. In particular, ^{188}Re is available from in-house generator system similar to ^{99m}Tc generator. ^{188}Re can be obtained from a ^{188}W/^{188}Re generator, which makes it very convenient for clinical use. The generator-produced radionuclide, ^{188}Re, is carrier free and might offer higher specific activity (37,000–74,000 MBq/mg). ^{188}Re has been applied for a variety of therapeutic applications including radioimmuno-therapy [17,18], and use ^{188}Re-microspheres and colloids for potential treatment of rheumatoid arthritis of the synovial joints [19,20] and hepatoma [21,22]. An initial feasibility study with ^{188}Re-hydroxyethylidene diphosphonate (^{188}Re-HEDP) using different ^{188}Re sources demonstrated that application of ^{188}Re-HEDP is safe and successful in pain relief palliation [23]. In addition, dose escalation studied with ^{188}Re-HEDP in prostate cancer patients showed that 3.3 GBq (90 mCi) dose is the maximum tolerated [24].

Moreover, in order to develop the technology for "personalized medicine for right diagnosis and for early and right medication", a chelator-based technology is of essence. Because chelators could coordinate radiometals and metals for image-guided target assessment and patient selection, the approach maybe the cornerstone for theranostic applications. Several chelators have been reported such as N_4 (e.g. Cyclam-14, DOTA), N_3S (e.g. MAG-3), N_2S_2 (e.g. ECD), NS_3, S_4 (e.g. sulfur colloid), diethylenetriamine pentaacetate (DTPA), O_2S_2 (e.g. DMSA), and hydrazinenicotinamide (HYNIC) [25-29]. Among these chelators for example, DTPA has been shown to have a faster wash out and forms less stable complexes with ^{99m}Tc. ^{99m}Tc-HYNIC has been shown to be useful in imaging, but labeling HYNIC with ^{99m}Tc requires two chemicals, triphenylphosphine and tricine, which are inconvenient for the kit preparation. As for as the most stable formation, may be the nitrogen and sulfur combination which has been shown to be a very stable chelator for ^{99m}Tc-bis-aminoethanethiol tetradentate ligands, also called diaminodithiol compounds. These are also known to form very stable Tc(V)O-complexes on the basis of efficient binding of the oxotechnetium group to two thiosulfur and two amine nitrogen atoms. Therefore, L,L-ethylenedicysteine (EC) is the most successful example of N_2S_2 chelates [30-33] of this combination. In addition, EC can be labeled with radiometals efficiently with high radiochemical purity and the preparation remains stable for several hours [34-36]. The strong complexing property of such N_2S_2-tetraligand systems is also used for labeling of proteins and peptides with radiometals for theranostic applications after conjugation to a bisaminothiol derivative [37-39]. In this report, a series of EC, DTPA, and MAG-3 chelators in the imaging of hexosamine biosynthetic pathway in oncology are reviewed.

METABOLIC IMAGING OF HEXOSAMINE BIOSYNTHETIC PATHWAY IN ONCOLOGY

Molecular imaging in oncology has been focused on identification of specific markers and the application of these markers for evaluation of patient response to radiation therapy, chemotherapy or chemo/radiotherapy. The radiotracer could non-invasively assess diseases treatment endpoints which used to rely almost exclusively on biopsies, histopathological assays and now biomarkers. For example, one radiotracer which is considered a molecular imaging marker is ^{18}F-fluoro-2-deoxy-glucose (FDG), a gold standard for PET. This molecular imaging marker is complementary to the CT and MRI, which allows detection of unsuspected distant metastases disease. Though FDG-PET is concordant with the findings of CT and MRI in diagnosing various tumors, FDG also has its drawbacks. FDG utilizes two main glucose transporters and hexokinase phosphorylated processes but becomes trapped and gets cycled for tumor imaging [40-42]. This makes FDG exhibits poor differentiation between inflammation/ infection and tumor recurrence due to its high uptake in granulocytes, macrophages and in tissues which may take up high levels of glucose [43]. One example, FDG has poor contrast in brain tumors due to the high uptake of glucose in normal brain tissue [44]. Because of patterns like brain and white cells, FDG does not provide the selection of patients for therapeutic response. Therefore, it is amenable to develop an alternative radiotracer for better differential diagnosis and responsiveness in cancers.

Metabolic imaging glucose and glucosamine agents which enters into the cell via hexosamine biosynthetic pathway and its regulatory products of glucosamine-6-phosphate becomes that involves insulin activation, downstream signaling and translocation, which upregulate mRNA expression and tumor growth [45-52] may be of great interest in monitoring post therapy changes in patients treatment. The hexosamine biosynthetic pathway is shown in Fig. (**1**). In the hexosamine biosynthetic pathway, glutamine:fructose-6-phosphate amidotransferase (GFAT) uses the amide group of glutamine to convert fructose 6-phosphate to glucosamine 6-phosphate, thus formation of hexosamine products requires a supply of both glucose and glutamine [45-46]. Metabolic imaging glucosamine and N-acetylglucosamine interaction with uridine diphosphate (UDP) to UDP-N-acetylglucosamine to monitor its interaction with other cell factors is very important in nuclear molecular imaging. For example, *O*-linked *N*-acetylglucosamine transferase (OGT) catalyzes UDP-GlcNAc to a single GlcNAc in glycosylation to serine or threonine residues of transcription factors (i.e. NF-kB) for nuclear trafficking and transactivation. OGT protein may be a sensor to activate UDP-N-acetylglucosamine to interact with protein to form unique glycoprotein, which involves nuclear and cytosolic protein interactions. Being able to image and monitor this dynamic glycosylation of serine or threonine residues on nuclear and cytosolic protiens by OGT that is abundant in all multicellular eukaryotes may be the most important molcular imaging function to perform in nuclear medicine. In fact, having to correlate nuclear molecular imaging techniques with the activities of OGT which is a part of post-translational modification that appears to modify a large number of nucleocytoplasmic proteins changes is critcal in staging patients with cancer. This factor known as OGT, performs activities which exquisitely responsive to intracellular UDP-N-acetylglucosamine and UDP concentrations, which are in turn highly sensitive to glucose concentrations and other stimuli that may have an hugh impact on pateints that may be treated with chemodrugs. Since this phenomenom is so dynamic and is often in response to different stimuli, for example stress because so many cancer pateints are stressed, (stress, heat) and to monitor the true changes of the nucleus and its ability to become internalized by the cell nucleus and tract its interaction may provide a great value to nuclear medicince as a whole [47-50]. Therefore, this should be viewed as true molecular medicine which imaging

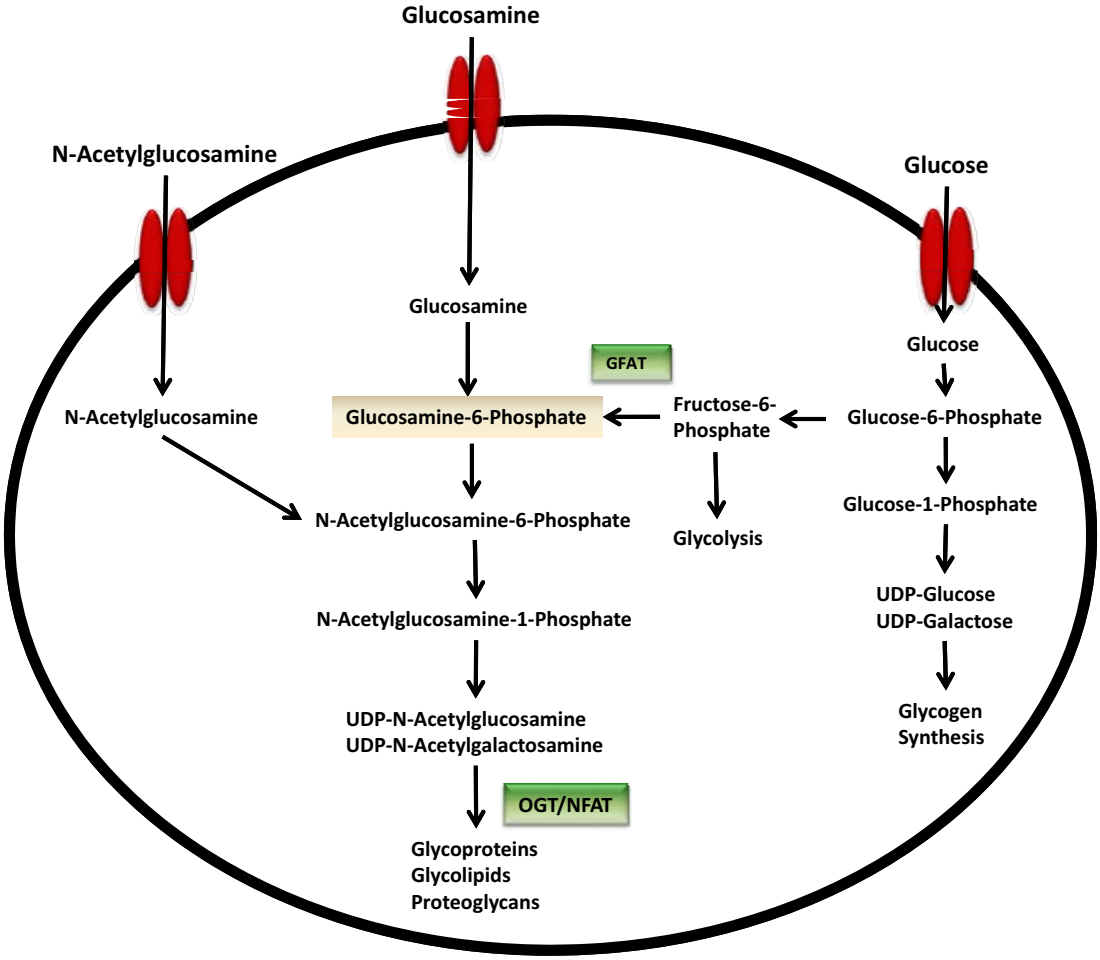

Fig. (1). Hexosamine biosynthetic pathway. (GFAT: glutamine:fructose-6-phosphate amidotransferase; OGT: *O*-linked *N*-acetylglucosamine transferase; UDP: uridine diphosphate).

can be intergrated for better understanding how the cell nucleus, can interact with ubiquitous transcription factors, such as Sp1 which can be extensively modified by OGT that's been tagged. Moreover, as one transcription factor like Sp1 becomes hyperglycosylated in response to hyperglycemia or elevated glucosamine [51-52], this process can be monitor and evaluated by nuclear imaging techniques.

RADIOLABELED GLUCOSAMINE ANALOGUES AS ALTERNATIVE CHELATOR-BASED RADIOTRACERS BEYOND FDG

The key to glucosamine analogue uniqueness as a target specific molecular compound is that it uses a chelator, radiolabeled with ^{99m}Tc which is commonly used in nuclear medicine and mimics the naive glucosamine in man. Three radiolabeled glucosamine analogues are reviewed. They are MAG-3-glucosamine [53], DTPA-glucosamine [54-58], and EC-glucosamine (EC-G) [59-61]. The structures are shown in Fig. (**2**). For example, EC-G is not radioactive, and thus, is a pharmaceutical compound. This compound only becomes a radiopharmaceutical when the ^{99m}Tc is added and reduced with a reducing agent. However, FDG is manufactured by a cyclotron, radiolabeled with ^{18}F and does not mimic navie glucose. In essence, when FDG is manufactured in a cyclotron, the radioisotope, fluorine-18 is an integral part of the compound. In other words, FDG is radioactive the moment it is manufactured. Although, EC-G and FDG bear certain common features, it's the dissimilarities that are the most important features to point out. For example, both compounds use sugar analogues for targeting when the compounds are taken up by the tumor cells that how they are utilized by the cell is what makes the difference so dramatic. FDG gets trapped in the cell's cytoplasm while EC-G is translocated into the cell's nuclei where the DNA resides. This explains, in part, why FDG gets taken up in inflamed or infected tissue and EC-G does not. Another feature is that because of FDG charge, it crosses the blood-brain-barrier and saturates a normal brain. However, EC-G does not cross a normal blood-brain-barrier unless there is a break down in the brain barrier due to an insult to the brain. ^{99m}Tc-EC-G has been shown to exhibit characteristics similar to FDG in terms of the glucose membrane transport process which involves the glycolytic and hexosamine pathways several past studies. Lastly, the mechanism of action and the negative charge of FDG are the primary reasons why FDG cannot be a platform for a therapeutic compound. Conversely, EC-G is an excellent platform for a therapeutic compound. However, several other chelators must be mention in this class but doesn't have the full capable of radiolabeled EC-G.

The difference in MAG-3-glucosamine, DTPA-glucosamine, and EC-G structures is that only EC-G has two glucosamine moieties in the molecule and others do not. Synthesis of DTPA-DG was achieved by reacting glucosamine with DTPA-dianhydride in the presence of triethylamine, followed by sephadex purification [54]. The chemical modification may change the characterics of a glycoprotein is transported in the cell. MAG-3 chelator was conjugated at the position 1 of glucosamine, which has similar chemistry that was performed in 1-thioglucose [62]. The authors

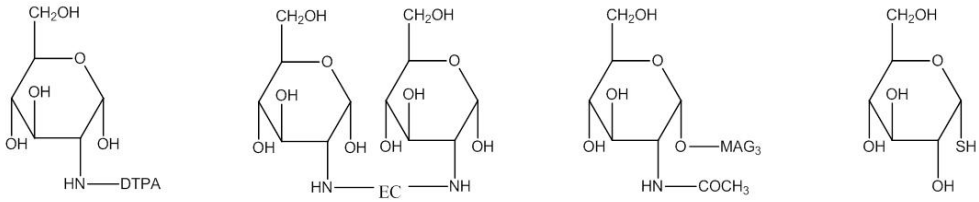

Fig. (2). Structures of chelator-based glucosamine analogues.

reported the similar biodistribution pattern in normal mice between MAG-3-glucosmaine and MAG-3-glucose. However, there were neither a tumor model nor human studies to affirm the effectivenss of MAG-3-glucosmaine. Although, DTPA-glucosamine was labeled with ^{99m}Tc and ^{188}Re, respectively [54-58], it exhibited a faster clearance in animal studies, thus, the tumor registration was low. Again, it has been discovered that radiolabeled EC-G behaves, in certain respects, quite differently than radiolabeded FDG. For instance, a hexokinase assay is based upon the reduction of NAD^+ through a coupled reaction with glucose-6-phosphate dehydrogenase to produce NADH. Both EC-G and FDG demonstrate that they can be phosphorylated and sequestered in glucose-energy-dependent cells similarly to glucose itself. However, the magnitude of EC-G to produce NADH is less than FDG suggesting that either the rate limiting step of phosphorylation in EC-G is slower than FDG or EC-G is more involved in downstream signature event than FDG [59]. Subsequently, our cell cycle analysis revealed that ^{99m}Tc-EC-G was able to transport across the nucleus membrane and involved in proliferation activity in all phases of cell cycles [60]. Moreover, the radiolabeded uptake studies of FDG, glucose and glucosamine correlated well with the thymidine incorporation assay studies with unlabeled EC-G and glucose suggesting both EC-G and glucose were involved in the proliferation/growth activity of cells, whereas FDG was not [59,60,63].

The lack of FDG involvement in DNA proliferation is attributable to the presence of the fluorine atom at position 2 of the molecule, which prevents its recognition by UDP and consequently its utilization in cell proliferation and growth. Two sets of glucose transporters are involved in this process which are transporters 1 and 3, and transporters 2 and 4 [47], respectfully. The clinical Phase I and II trials revealed that ^{99m}Tc-EC-G was safe and had favorable radiation dosimetry, and was able to differentiate tumor from infammation in lung cancer patients [61], respectfully. Consequently, ^{99m}Tc-EC-G offer the potential to be the standard SPECT imaging agent for screening, diagnosing and staging for all cancer types [61].

Cancer cells upregulate glycolysis, increasing glucose uptake to meet energy needs. A small fraction of a cell's glucose enters the hexosamine biosynthetic pathway which regulates levels of GFAT and OGT. Elevated expression of OGT, which is the enzyme catalyzing the addition of O-GlcNAc to proteins, enhances posttranslational modification of diverse nuclear and cytosolic proteins. Reduction of O-GlcNAcation through RNA interference of OGT in cancer cells may lead to inhibition of tumor growth both in vitro and *in vivo* and is associated with decreased cell-cycle progression. These findings identify O-GlcNAc as a novel mechanism through which alterations in glucose metabolism regulate cancer growth and invasion and suggest that OGT may represent novel therapeutic targets for cancers. Thus, the hexoamine biosynthetic pathway-based radiotracer may provide more accurate information on molecular image-guided therapy.

In summary, the cold kit precursor is considered as drug substance thus it is easier in chemistry, manufacturing and control (CMC) standardization. Kit-based radiotracers provide an opportunity for better differentiation between infection/ inflammation and tumor recurrence than FDG in human. The kit-based radiotracers may discontinue ineffective treatment in an earlier phase and switch to a more efficient treatment that would be beneficial to patients early on in the course of treatment.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENT

None declared.

REFERENCES

[1] Buerkle A, Weber WA. Imaging of tumor glucose utilization with positron emission tomography. Cancer Metastasis Rev, **2008**;27(4):545-54.

[2] Delbeke D., *et al*., Procedure guideline for tumor imaging with 18F-FDG PET/CT. J Nucl Med, **2006**;47(5):885-95.

[3] Strauss LG, Conti PS. The application of PET in clinical oncology. J Nucl Med. **1991**;32:623-48.

[4] Yang DJ, Kim KD, Schechter NR, *et al*. Assessment of antiangiogenic effect using ^{99m}Tc-EC-endostatin. Cancer Biother Radiopharm **2002**;17:233-245.

[5] Schechter NR, Wendt RE, Yang DJ, *et al*. Radiation Dosimetry of ^{99m}Tc-labeled C225 in Patients with Squamous Cell Carcinoma of the Head and Neck. J Nucl Med **2004**;45(10):1683-7

[6] Gong J, Yang DJ, Kohanim S, Humphreys R, *et al*. Novel *in vivo* imaging demonstrates upregulation of death receptors by paclitaxel and correlates with enhanced antitumor effects of receptor agonist antibodies. Molecular Cancer Therapeutics **2006**;5(12):2991-3000.

[7] Yang DJ, Ilgan S, Higuchi T, *et al*. Noninvasive assessment of tumor hypoxia with ^{99m}Tc-labeled metronidazole. Pharm Res **1999**;16(5):743-750.

[8] Song HC, Bom HS, Cho KH, *et al*. Prognostication of recovery in patients with acute ischemic stroke using brain spect with ^{99m}Tc-metronidazole. Stroke, **2003**;34:982-986.

[9] Ito M, Yang DJ, Mawlawi O, *et al*. PET and planar imaging of tumor hypoxia with labeled metronidazole. Academic Radiology **2006**;13(5):598-609.

[10] Kurihara H, Yang, DJ, Cristofanilli M, *et al*. Imaging and Dosimetry of ^{99m}TC EC Annexin V: Preliminary Clinical Study Targeting Apoptosis in Breast Tumors. Applied Radiation and Isotopes **2008**; 66:1175-1182.

[11] Vriens PW, Blankenberg FG, Stoot JH, *et al*. The use of technetium ^{99m}Tc annexin V for *in vivo* imaging of apoptosis during cardiac allograft rejection. J Thorac Cardiovasc Surg. **1998**; 116:844-853.

[12] Yang DJ, Ozaki K, Oh C-S, *et al*. ^{99m}Tc-EC-Guanine: Synthesis, Biodistribution and Tumor Imaging in Animals. Pharmaceutical Research **2005**;22 (9):1471-1479.

[13] Takahashi N, Yang DJ, Kohanim S, *et al*. Targeted functional imaging of estrogen receptors with ^{99m}Tc-GAP-EDL. Eur J Nucl Med Mol Imaging **2007**;34:354-362.

[14] Wu JY, Yang DJ, Angelo LS, *et al*. Molecular imaging of Bcr-Abl phosphokinase in a xenograft model. Molecular Cancer Therapeutics **2009**;8(3):703-10.

[15] Knoess C, Siegel S, Smith A, *et al*. Performance evaluation of the microPET R4 PET scanner for rodents. Eur. J. Nucl. Med. Mol. Imaging, **2003**; 30, 737-747.

[16] Hofmann M, Maecke H, Borner R, *et al*. Biokinetics and imaging with the somatostatin receptor PET radioligand ^{68}Ga-DOTATOC: preliminary data. Eur. J. Nucl. Med. **2001**; 28, 1751-7.

[17] Bernhardt P, Benjegard SA, Koelby L, *et al*. Dosimetric comparison of radionuclides for therapy of somatostain receptor-expressing tumors. Nucl. Med. Commun **2000**;21:566.

[18] Buchman I, Bunjes D, Seitz U, *et al*, Radioimmunotherapy for myeloablation prior to stem cell transplantation with Re-188 CD 66 a,b,c,d,e antibody in high risk leukemia patients. Eur. J. Nucl. Med**. 2000**;27**:**S81.

[19] Wang SJ, Lin WY, Chen MN, *et al*. Rhenium-188 microsphere: a new radiation synovectomy agent. Nucl. Med. Communication **1998**;19:427–433.

[20] Wang SJ, Lin WY, Hsieh BT, *et al*. Rhenium-188 sulfur colloid as a radiation synovectomy agent. European J. Nucl. Med. **1995**;22:505–507.

[21] Wang SJ, Lin WY, Chen MN, *et al*. Radiolabelling of Lipiodol with generator-produced ^{188}Re for hepatic tumor therapy. Appl. Radiat. Isot. **1996**;**47**:267–271.

[22] Wang SJ, Lin WY, Chen MN, *et al*. Intratumoral injection of rhenium-188 microspheres into an animal model of hepatoma. J. Nucl. Med. **1998**;39**:**1572–1577.

[23] Maxon HR, Schroder LE, Washburn LC, *et al*, Rhenium-188(Sn)HEDP for treatment of osseous metastases. J. Nucl. Med. **1998**;39:659–663.

[24] Palmedo H, Guhlke S, Bender H, *et al*, Dose escalation study with rhenium-188 hydroxyethlidene diphosphonate in prostate cancer patients with osseous metastases. European J. Nucl. Med. **2000**;27:123–130.

[25] Canet EP, Casali C, Desenfant A, *et al*. Kinetic characterization of CMD-A2-Gd-DOTA as an intravascular contrast agent for myocardial perfusion measurement with MRI. Magn Reson Med. **2000**;43(3):403-409.

[26] Mang'era K, VanbilloenH, Cleynhens B, *et al*. Synthesis and Evaluation of the 99mTc-Complexes of L-Cysteine Acetyldiglycine (a Hybrid of MAG3 and L,L-EC) and of L-β-Homocysteine Acetyldiglycine. Nuclear Medicine & Biology, **2000**;27: 781-789.

[27] Kao CH, ChangLai SP, Chieng PU, *et al*. ^{99m}Tc-Methoxyisobutylisonitrile chest imaging of small cell lung carcinoma: relation to patient prognosis and chemotherapy response--a preliminary report. Cancer. **1998**;83:64-68.

[28] Wu HC, Chang CH, Lai MM, *et al*. Using ^{99m}Tc-DMSA renal cortex scan to detect renal damage in women with type 2 diabetes. J Diabetes Complications. **2003**;17:297-300.

[29] Ohtsuki K, Akashi K, Aoka Y, *et al*. ^{99m}Tc-HYNIC-annexin V: a potential radiopharmaceutical for the in-vivo detection of apoptosis. Eur J Nucl Med. **1999**; 26(10):1251-1258.

[30] Van Nerom CG, Bormans GM, De Roo MJ, *et al*. First experience in healthy volunteers with ^{99m}Tc-L,L-ethylenedicysteine, a new renal imaging agent. Eur J Nucl Med. **1993**;20:738-46.

[31] Vanbilloe HP, Cleynhens BJ, Verbruggen AM. Synthesis and Biological Evaluation of the Four Isomers of ^{99m}Tc-Labeled Ethylenecysteamine Cysteine (^{99m}Tc-ECC), the Mono-acid Derivative of ^{99m}Tc-L,L-ethylenedicysteine. Nuclear Medicine Biology, **2000**;27:207-214.

[32] Ilgan S, Yang DJ, Higuchi T, *et al*. ^{99m}Tc-Ethylenedicysteine-Folate: A new tumor imaging agent. Synthesis, labeling and evaluation in animals. Cancer Biotherapy and Radiopharm. **1998**;13: 427-435.

[33] Yang DJ, Bryant J, Chang JY, *et al*. Assessment of COX-2 expression with ^{99m}Tc-labeled celebrex. Anti-Cancer Drugs **2004**;15:255-263.

[34] Kong FL, Zhang YH, Ali M, *et al*. Synthesis of ^{99m}Tc-EC-AMT as an imaging probe for amino acid transporter systems in breast cancer. Nuclear Medicine Communications. **2010**;31(8):699-707.

[35] Anderson CJ, John CS, Li YJ, *et al*. N,N'-Ethylene-di-L-cysteine (EC) complexes of Ga(III) and In(III): molecular modeling, thermodynamic stability and *in vivo* studies. Nucl Med Biol **1995**: 22:165-173.

[36] Sun Y, Anderson CJ, Pajeau TS, *et al*. Indium (III) and gallium (III) complexes of bis(aminoethanethiol) ligands with different denticities: stabilities, molecular modeling, and *in vivo* behavior. J Med Chem **1996**: 39:458-470.

[37] Gong J, Yang DJ, Kohanim S, Angelo LS, Kurzrock R. Molecular imaging of gefitinib activity in an epidermal growth factor receptor (EGFR)-bearing xenograft model. Cancer Biology Therapy. **2009**;8(23):1-9.

[38] Yang DJ, Azhdarinia A, Wu P, *et al*. *In vivo* and In Vitro Measurement of Apoptosis in Breast Cancer Cells Using ^{99m}Tc-EC- Annexin V. Cancer Biotherapy Radiopharm **2001**;16(1):73-84.

[39] Schechter NR, Yang DJ, Azhdarinia A, *et al*. Assessment of epidermal growth factor receptor with ^{99m}Tc-ethylenedicysteine-C225 monoclonal antibody. Anticancer Drugs **2003**;14:49-56.

[40] Delappe E and Dunphy M. ^{18}F-2-Deoxy-d-Glucose positron emission tomography-computed tomography in lung cancer. Semin Roentgenol. **2011**; \$6(3):208-23.

[41] Khan N, Islam MM, Mahmood S, *et al*. 18F-fluorodeoxyglucose uptake in tumor. Mymensingh Med J. **2011**; 20(2):332-42.

[42] Fang J, Luo XM, Yao HT, *et al*. Expression of glucose transporter-1, hypoxia-inducible factor-1α, phosphatidylinositol 3-kinase and protein kinase B (Akt) in relation to [(18)F]fluorodeoxyglucose uptake in nasopharyngeal diffuse large B-cell lymphoma: a case report and literature review. J Int Med Res. **2010**; 38(6): 2160-8.

[43] Chang JM, Lee HJ, Goo JM, *et al*., False positive and false negative FDG-PET scans in various thoracic diseases. Korean J Radiol, **2006**;7(1): 57-69.

[44] Rosenbaum SJ, Lind T, Antoch G, False-positive FDG PET uptake--the role of PET/CT. Eur Radiol, **2006**;16(5): 1054-65.

[45] Marshall S, Bacote V, Traxinger RR. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. J Biol Chem **1991**;266:4706-4712.

[46] Wells L, Gao Y, Mahoney JA, *et al*. Dynamic O-glycosylation of nuclear and cytosolic proteins: further characterization of the nucleocytoplasmic beta-N-acetylglucosaminidase, O-GlcNAcase. J Biol Chem **2002**;277:1755-1761.

[47] Scheepers A, Joost HG, Schürmann A. The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. JPEN J Parenter Enteral Nutr. **2004**;28(5):364-71.

[48] Wells, L, Vosseller, K, and Hart,GW. Glycosylation of Nucleocytoplasmic Proteins: Signal Transduction and O-GlcNAc. Science. **2001**;291, pp. 2376-2378.

[49] Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O. Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. Annu Rev Biochem. **2011**;80:825-58.

[50] Paterson AJ, Kudlow JE. Regulation of glutamine:fructose-6-phosphate amidotransferase gene transcription by epidermal growth factor and glucose. Endocrinology. **1995**;136(7):2809-16.

[51] Pal S, Claffey KP, Cohen HT, Mukhopadhyay D. Activation of Sp1-mediated vascular permeability factor/vascular endothelial growth factor transcription requires specific interaction with protein kinase C zeta. J Biol Chem **1998**;273:26277-26280.

[52] Black AR, Black JD, Azizkhan-Clifford J. Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol **2001**;188:143-160.

[53] Chopra A. ^{99m}Tc-Labeled *S*-benzoylmercaptoacetyl triglycine N-acetylglucosamine. Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US) **2010**; 2004-2010.

[54] Chen Y, Xiong QF, Yang XQ, *et al*. Evaluation of ^{188}Re-DTPA-deoxyglucose as a potential cancer radiopharmaceutical. AJR Am J Roentgenol. **2010**;194(3):761-5.

[55] Liang J, Chen Y, Huang Z, *et al*. Early chemotherapy response evaluation in tumors by ^{99m}Tc-DTPA-DG. Cancer Biother Radiopharm. **2008**;23(3):363-70.

[56] Zhang W, Chen Y, Guo da J, *et al*. The synthesis of a D-glucosamine contrast agent, Gd-DTPA-DG, and its application in cancer molecular imaging with MRI. Eur J Radiol. **2011**;79(3):369-74.

[57] Chen Y, Huang ZW, He L, *et al*. Synthesis and evaluation of a technetium-99m-labeled diethylenetriaminepentaacetate-deoxyglucose complex ([^{99m}Tc]-DTPA-DG) as a potential imaging modality for tumors. Appl Radiat Isot. **2006**;64(3):342-7.

[58] Xiong QF, Chen Y, He L, *et al*. Study of apoptosis induced by ^{188}Re-DTPA-DG in MCF-7 breast carcinoma and A549 pulmonary carcinoma cells. Cancer Biother Radiopharm. **2007**;22(4):543-50.

[59] Yang DJ, Kim CG, Schechter NR, *et al*. Imaging with ^{99m}Tc-EC-DG Targeted at the Multifunctional Glucose Transport System: Feasibility study with rodents. Radiology **2003**;226: 465-473.

[60] Yang DJ, Yukihiro M, Oh CS, *et al*. Assessment of therapeutic tumor response using ^{99m}Tc-Ethylenedicysteine-Glucosamine. Cancer Biotherapy Radiopharm. **2004**;19(4):444-458.

[61] Schechter NR, Erwin WD, Yang DJ, *et al*. Radiation dosimetry and biodistribution of ^{99m}Tc-ethylenedicysteine-deoxyglucose in patients with non-small cell lung cancer. Eur J Nucl Med Mol Imaging. **2009**;36 (10):1583-1591.

[62] Castelli R, Fernandez M, Porcal W, *et al*. Preparation and Primary Bioevaluation of ^{99m}Tc-labeled-1-thio-β-D-Glucose as Melanoma Targeting Agent. Curr Radiopharm. **2011**;4(4):355-60.

[63] Balkwill FR. The chemokine system and cancer. J Pathol. **2012**; 226(2): 148-57.

Received: February 07, 2012 **Revised: March 20, 2012** **Accepted: March 21, 2012**