Nuclear Engineering and Technology 48 (2016) 597–607

Available online at ScienceDirect

Nuclear Engineering and Technology

journal homepage: www.elsevier.com/locate/net

Invited Article

Development of Drugs and Technology for Radiation Theragnosis

Hwan-Jeong Jeong [a,1], *Byung Chul Lee* [b,c,1], *Byeong-Cheol Ahn* [d,*], *and Keon Wook Kang* [e,*]

[a] *Department of Nuclear Medicine, Biomedical Research Institute, Chonbuk National University Medical School and Hospital, 20 Geonjiro Deokjin-gu, Jeonju, Jeollabuk-do, Republic of Korea*
[b] *Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, 82 Gumiro 173 Beongil, Bundang-gu, Seongnam, Gyeonggi-do, Republic of Korea*
[c] *Center for Nanomolecular Imaging and Innovative Drug Development, Advanced Institutes of Convergence Technology, 864-1 Iui-dong, Yeongtong-gu, Suwon, Gyeonggi-do, Republic of Korea*
[d] *Department of Nuclear Medicine, Kyungpook National University School of Medicine and Hospital, 130 Dongdeok-ro, Jung-gu, Daegu, Republic of Korea*
[e] *Department of Nuclear Medicine and Cancer Research Institute, Seoul National University, 101 Daehakro, Jongno, Seoul, Republic of Korea*

ARTICLE INFO

Article history:
Received 10 April 2016
Accepted 13 April 2016
Available online 23 April 2016

Keywords:
Albumin
Cell Therapy
Molecular Imaging
Nanoparticles
Nuclear Medicine
Peptides
Radiation Theragnosis

ABSTRACT

Personalized medicine is tailored medical treatment that targets the individual characteristics of each patient. Theragnosis, combining diagnosis and therapy, plays an important role in selecting appropriate patients. Noninvasive *in vivo* imaging can trace small molecules, antibodies, peptides, nanoparticles, and cells in the body. Recently, imaging methods have been able to reveal molecular events in cells and tissues. Molecular imaging is useful not only for clinical studies but also for developing new drugs and new treatment modalities. Preclinical and early clinical molecular imaging shows biodistribution, pharmacokinetics, mechanisms of action, and efficacy. When therapeutic materials are labeled using radioisotopes, nuclear imaging with positron emission tomography or gamma camera can be used to treat diseases and monitor therapy simultaneously. Such nuclear medicine technology is defined as radiation theragnosis. We review the current development of drugs and technology for radiation theragnosis using peptides, albumin, nanoparticles, and cells.

* *Corresponding authors.*
E-mail addresses: abc2000@knu.ac.kr (B.-C. Ahn), kangkw@snu.ac.kr (K.W. Kang).
[1] H.-J. Jeong and B.C. Lee contributed equally to this work.
http://dx.doi.org/10.1016/j.net.2016.04.003

1. Introduction

Personalized therapy is a tailored medical treatment targeting the individual characteristics of each patient. Theragnosis, combining diagnosis and therapy, plays an important role in selecting appropriate patients. Pharmacogenomics or gene panel studies are actively getting into the clinical process to predict patients' the responses to drugs. However, biopsy-driven examinations are based on only a small sample of tissue that is removed from a part of the body. These cannot predict the response of each lesion, especially in cancer patients. After radionuclide therapy using ^{131}I for thyroid cancer, whole-body scans are generally performed to evaluate uptakes and distribution in the body. Lesions can now be more accurately localized and characterized using single photon emission computed tomography/computed tomography (SPECT/CT). SPECT/CT is a hybrid imaging system that colocalizes radionuclide accumulations on CT-based anatomical structures. We suggest defining a new term, theragnosis. Combining therapy and *in vivo* imaging, theragnosis simultaneously localizes therapeutic drugs, peptides, genes, or cells through labeling with radionuclides, fluorescent dye, contrast agents, etc. In this review, we will focus on current development drugs and technologies for radiation theragnosis in nuclear medicine.

2. Theragnosis using peptides

A variety of nuclear imaging probes led to the development of molecular imaging using SPECT and positron emission tomography (PET). Nuclear imaging techniques, which are based on the radiolabeling of specific molecular probes using radioisotopes, can provide insights into the phenotypic functional changes of the disease and the specific biochemical processes of probes. Using different isotopes labeling directly or by bifunctional chelators, recently peptide-based radiopharmaceuticals have been developed and used as biological tools for tumor receptor imaging as well as targeted radionuclide therapy. The typical peptide used as nuclear imaging probes consist of a relatively small number of amino acids (up to 30) with comparably favorable properties, including high receptor binding affinity, selective *in vivo* biological activity, and rapid pharmacokinetics, but usually do not have immunogenic features. The most widely used radioisotopes for peptide labeling for diagnostic and radiotherapy purposes are listed in Table 1.

The first clinical study of ^{123}I-labeled somatostatin derivative (^{123}I-labeled tyr-3-octreotide) in cancer patients was reported by Krenning *et al.* [1] in 1989. Subsequently, ^{111}In-labeled octreotide (^{111}In-Octreoscan, ^{111}In-pentetreotide) was developed and approved by the U.S. Food and Drug Administration (FDA) as the imaging agent for somatostatin receptor-positive cancer, such as neuroendocrine tumors, mammary cancer, and small cell lung cancer [2]. In addition, ^{99m}Tc-labeled somatostatin analogs were successfully developed by the preconjugation of the HYNIC ligand to the peptide and used in humans [3]. Owing to the development of macrocyclic chelators such as DOTA, DOTAOC, DOTATOC, DOTAVAP, DOTATATE, and lanreotide DOTALAN, somatostatin analogs are thus promising theragnosis for peptide receptor imaging (^{111}In or ^{68}Ga) and radionuclide therapy (^{90}Yo or ^{177}Lu for β emitters; ^{213}Bi or ^{225}Ac for α emitters) [4–10].

The above results also promote the development of other peptide radiopharmaceuticals, such as bombesin, neurotensin (NT), cholecystokinin (CCK)/gastrin, exendin, RGD, and substance P (Table 2).

Bombesin and gastrin-releasing peptide (GRP) share a highly conserved seven-amino acid C-terminal sequence (Trp-Ala-Val-Gly-His-Leu-Met-NH_2) and also play an important role in the growth of different type of cancers [11]. Therefore, ^{68}Ga-labeled Pan-bombesin analog (^{68}Ga-BZH_3) was developed and used to evaluate the impact of peptide receptors scintigraphy on the diagnosis and the potential therapy [12]. Consistently, new radiolabeled bombesin analogs have been developed, and their encouraging preclinical results are applied to clinical oncology [13–17].

NT is a tridecapeptide found in several human cancers including Ewing sarcoma, meningioma, astrocytoma, and pancreatic carcinomas [11]. An example for radiolabeled NT, reported by Franz Buchegger, is the synthesis of ^{99m}Tc-NT-XI containing tricarbonyl ^{99m}Tc moiety [18–20]. Despite the favorable preclinical animal studies of ^{99m}Tc-NT-XI, the initial clinical findings are not promising because of the high nonspecific uptake of radioactivity in the kidneys.

Based on receptor autoradiographic studies in humans, CCK and gastrin, which are highly expressed in the intestines and brain, are highly expressed in 90% of medullary thyroid carcinomas and in a high percentage of other tumors such as stromal ovarian cancers, small cell lung cancer, astrocytoma, gastroenteropancreatic neuroendocrine tumors, and gastrointestinal stromal tumors [11,21].

In a comparison of three promising CCK-2 receptor-binding peptides [^{99m}Tc-N_4-Gly-D-Glu-$(Glu)_5$-Ala-Tyr-Gly-Asp Trp-Met-Asp-Phe-NH_2 (^{99m}Tc-demogastrin 2), ^{111}In-DOTA-D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (^{111}In-DOTA-CCK), and ^{111}In-DOTA-D-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (^{111}In-DOTA-MG11)], ^{99m}Tc-demogastrin 2 showed better detectability and was able to visualize tumor regions in human medullary thyroid cancer patients better than ^{111}In-DOTA-CCK and ^{111}In-DOTA-MG11 [22].

The glucagon-like peptide receptor (GLP-1R) is a member of the G-protein-coupled receptor family, and was found to be overexpressed in insulinomas, gastrinomas, and medullary thyroid carcinomas. Exendin-4 consists of 39 amino acids with metabolic resistance and shares an approximately 50% homology with the human GLP-1. For diagnosis and internal radiotherapy, ^{111}In labeled exendin-4 was developed and evaluated for its therapeutic efficiency. In particular, ^{111}In labeled [Lys^{40}(Ahx-DTPA)-NH_2]-exendin-4 was able to distinguish between benign and malignant insulinomas. Moreover, ^{111}In labeled [Lys^{40}(Ahx-DOTA)-NH_2]-exendin-4 is a very promising peptide radiopharmaceutical for visualizing insulinomas and clinically detects pancreatic and ectopic insulinomas [23–25].

One of the most frequently studied peptides are cyclic RGD (Arg–Gly–Asp) peptides, which have been evaluated for imaging integrin $\alpha_v\beta_3$ expressed tumors. The vitronectin receptor, integrin $\alpha_v\beta_3$ receptor, is known to play an important role in tumor-induced angiogenesis and tumor metastasis. A

Table 1 – Different radioisotopes and methods for labeling peptides.

Radioisotopes	Half-life	Labeling methods	Application
Fluorine-18	1.83 hr	Indirect labeling (prosthetic groups)	Diagnosis
Technetium-99m	6.02 hr	Direct labeling (S–S bonds) Chelators (MAG_3, DADT, HYNIC)	Diagnosis
Rhenium-186/rhenium-188	3.7 d/16.9 hr	Same as for technetium-99m	Therapy
Iodine-123/iodine-131	13.2 hr/8 d	Direct labeling (tyrosine) Indirect labeling (Bolton–Hunter reagent)	Diagnosis/therapy
Gallium-68	1.13 hr	Chelators (NOTA, DOTA)	Diagnosis
Copper-64/copper-67	12.7 hr/2.6 d	Chelators (TETA, DOTA, NOTA)	Diagnosis/therapy
Indium-111	67.2 hr	Chelators (DTPA, DOTA)	Diagnosis/therapy
Lutetium-177	160.8 hr	Chelator (DOTA)	Therapy
Yttrium-90	64.1 hr	Chelator (DOTA)	Therapy
Bismuth-213	45.6 min	Chelator (DOTA)	Therapy
Actinium-225	10 d	Chelator (DOTA)	Therapy

DADT, diaminedithiol; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; HYNIC, 2-hydrazinonicotinic acid; MAG_3, mercaptoacetyltriglycine; NOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid; TETA, 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid.

Table 2 – Radiolabeled peptides for cancer theragnosis.

Peptide	Target receptor	Tumor type
Somatostatin	sst_2	Neuroendocrine tumors (gastroenteropancreatic tumors), lymphoma, paraganglioma, carcinoids, breast, brain, renal, small cell lung cancer
Bombesin	BB_2, GRP-R	Breast, prostate, pancreas, gastric, colorectal, small cell lung cancer
Neurotensin	NTR_1	Ewing sarcoma, astrocytoma, breast, prostate cancer, colon, small cell lung cancer
Exendin	GLP-1	Insulinomas, gastrinomas, pheochromocytomas, paragangliomas and medullary thyroid carcinomas
RGD	Integrin $\alpha_v\beta_3$	Glioma, breast cancer, melanomas
Substance P	NK_1	Glioblastoma, medullary thyroid cancer, small cell lung cancer

BB2, bombesin receptor 2; GLP-1, glucagon-like peptide receptor 1; GRP, gastrin-releasing peptide; NK_1, neurokinin type 1; NTR_1, neurotensin receptor 1; sst_2, somatostatin receptor 2.

number of RGD peptides have been radiolabeled with ^{99m}Tc, ^{111}In, ^{123}I, ^{68}Ga, ^{64}Cu, ^{18}F, and ^{90}Y. One of the first RGD peptides to be clinically tested was [^{18}F]Galacto-RGD, which demonstrated noninvasive quantitative assessment of integrin expression pattern in patients with malignant tumors [26]. For diagnostic imaging or radiotherapy of tumors, [^{111}In/^{90}Y]-DOTA-E-[c(RGDfK)]$_2$ and [^{99m}Tc/^{188}Re]-IDA-D-[c(RGDfK)]$_2$ were synthesized and showed favorable biodistribution and *in vivo* tissue clearance in mice bearing $\alpha_v\beta_3$-positive OVCAR-3 and U87-MG cells [27,28].

This ^{99m}Tc-IDA-D-[c(RGDfK)]$_2$ also offered the strong possibility of the noninvasive imaging of atherosclerotic plaque in preclinical studies [29]. In recent studies, RGD-based radiolabeled hybrid peptides, such as RGD-somatostatin and RGD-BBN, were developed and showed efficient tumor targeting with high affinity as compared to the corresponding monometic peptides [30,31].

In order to make up the weak points of inconsistent expression of somatostatin type 2 receptor limited application in glioblastoma, radiolabeled substance P derivative was prepared by conjugating the macrocyclic chelators (DTPA or DOTAGA) to Arg1 of substance P [32,33]. The thymus expressed enough substance P receptors to allow *in vivo* visualization by [^{111}In-DTPA-Argl]–substance P. α-Emitted radioisotope labeled substance P, [^{90}Y]-DOTAGA–substance P, in particular, was used as the local intratumoral treatment in malignant glioma patients. [^{90}Y]-DOTAGA–substance P substantially inhibited further growth and led to radiation-induced tumor necrosis [34].

3. Theragnosis using albumin

Albumin is the most abundant plasma protein that is synthesized in the liver. Its distribution is primarily intravascular, and its plasma half-life is 19 days [35]. Its molecular weight is 66.5 kDa and small enough to filter out through glomerulus in the kidney. Like antibodies, albumin binds to the neonatal Fc receptor (FcRn), which is expressed in proximal tubular cells, and is reabsorbed [36]. Albumin is a carrier protein for steroids, thyroid hormone, retinoids, other lipophilic hormones, and lipophilic drugs [37]. When Evans blue–albumin complex was injected into tumor-bearing mice via a tail vein, it gradually accumulated in the tumor [38]. When Evans blue–albumin complex was injected into the solid tumor, it was retained in the tumor for a long period. This phenomenon is now called the enhanced permeability and retention (EPR) effect on the tumor. Many nanodrug delivery systems, including liposome, use this strategy for the passive targeting of tumors.

Radiolabeled human serum albumin (HSA) was used for the detection of protein losing enteropathy [39]. A ^{99m}Tc-HSA

scan showed blood pool imaging reflecting its intravascular distribution [40]. In patients with protein losing enteropathy, radioactivity accumulated in the abdomen and migrated distally. ^{99m}Tc-HSA was injected in a tumor-bearing mouse, and radioactivity accumulated slowly in the tumor (Fig. 1). It has not yet been elucidated why albumin accumulates in the tumor. Some mechanism other than the EPR effect is suggested. Abraxane is a nanoparticle albumin-bound paclitaxel and became the first albumin-based antineoplastic agent approved by FDA. GP60, a 60-kDa glycoprotein expressed on the plasma membrane of endothelial cells, binds albumin and enhances tumor penetration of Abraxane by endothelial transcytosis [41]. A secreted protein, acidic and rich in cysteine (SPARC) is highly expressed in malignant cells and secreted into interstitial space. Because albumin has an affinity to SPARC, SPARC may play a role in albumin accumulation in the tumor [42].

For the targeted drug delivery of albumin to the liver or a hepatocellular carcinoma, galactose residues were introduced on the surface of albumin [43]. These neoglycoprotein conjugates selectively bind to the asialoglycoprotein receptor of hepatocytes or well-differentiated hepatocellular carcinoma cells. The (6-maleimidocaproyl) hydrazine derivative of doxorubicin (DOXO-EMCH) was coupled to a thiolated form of lactosaminated human albumin (L-HSA). We radiolabeled L-HSA using ^{64}Cu, a positron emitter with a 12.7-hour half-life, via click chemistry. We made a xenografted mouse model, inoculating HepG2 (well differentiated hepatocellular carcinoma), Hep3B (poorly differentiated hepatocellular carcinoma), and HT29 (colon cancer) cells in a mouse. Then, we injected ^{64}Cu-L-HSA and ^{64}Cu-HSA into tumor-bearing mice via a tail vein. When we took PET scans, lactosaminated HSA accumulated specifically in HepG2 tissues, whereas ^{64}Cu-HSA accumulated in HepG2, Hep3B, and HT29 similarly (Fig. 2). Molecular imaging by PET scan proved targeted delivery of albumin based drug to tumor over-expressing asialoglycoprotein receptor. Radiolabeled albumin drugs have a potential for theragnosis agents performing imaging and targeted therapy simultaneously.

4. Theragnosis using nanoparticles

Nanotechnology is the engineering field of handling of materials and devices with nanometer sizes. Colloidal gold, iron oxide nanoparticles, quantum dots (QDs), and nano-sized liposome are examples of nanoparticles whose size generally ranges from 1 to 200 nm. This nanotechnology led to a new area—"nanomedicine," the application of nanotechnology in human healthcare for the diagnosis, monitoring, treatment, prediction, and prevention of disease. Since the mid-1990s, when Doxil was approved by FDA, various nanoconstructs have entered the market and have been used in clinical trials. However, many obstacles exist in applying nanomaterials to humans. The application of nanotechnology to medicine offers advanced technology including the early detection, imaging, treatment of cancers, medical analysis, drug manipulation, and multifunctionality. Eventually, advances in nanomedicine could promise greatly improved life of patients with respect to several diseases. To translate to clinical use, a deep understanding is needed regarding the chemical and physical properties of particles and their pharmacokinetic behaviors in the body, such as biodistribution, toxicity, and biocompatibility. To understand nanoparticle-based theragnosis, we first have to learn more about nanoparticles. This review is intended to introduce several nanoparticles—superparamagnetic iron oxide nanoparticle (SPION), gold nanoparticle (AuNP), QDs, and liposome—which are widely investigated in nanomedicine in terms of molecular imaging and theragnosis. Besides these nanoparticles, there are numerous nanosubstances or combinatory nanomaterials using two or three materials, such as chitosan nanoparticles, and block copolymer nanoparticles, etc. The medical doctors and researchers who work in the field of medicine should pay attention to nanotechnology oriented from engineering to cooperate with engineers and develop new medical technology.

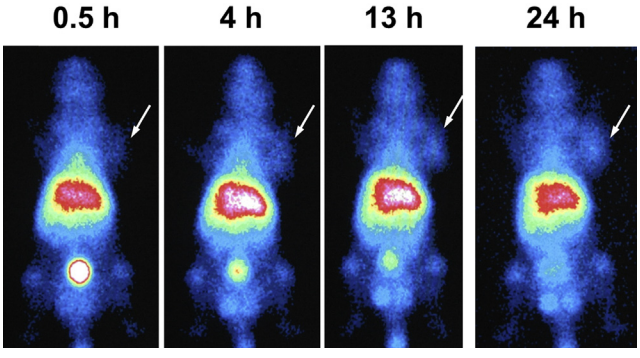

Fig. 1 – ^{99m}Tc-albumin scan in a tumor bearing mouse. Radioactivity slowly accumulated in the tumor.

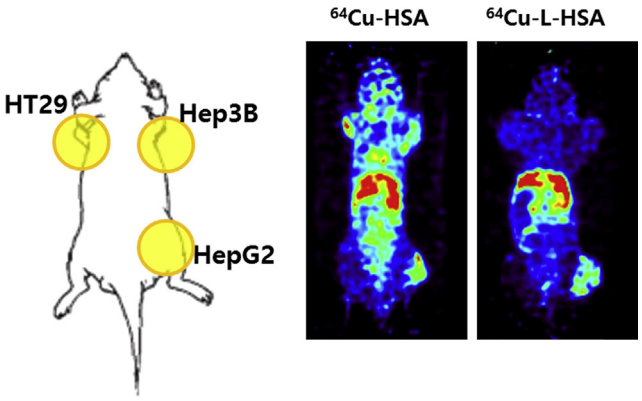

Fig. 2 – PET scan image in a tumor bearing mouse after 46 hours of injection with ^{64}Cu-HSA and ^{64}Cu-lactosaminated-HSA (^{64}Cu-L-HSA) each. ^{64}Cu-L-HSA accumulated specifically in HepG2 tissues, whereas ^{64}Cu-HSA accumulated in HepG2, Hep3B, and HT29 similarly. HSA, human serum albumin; L, lactosamine; PET, positron emission tomography.

4.1. *Superparamagnetic iron oxide nanoparticles*

SPIONs contain one or more superparamagnetic iron oxide cores composed of a mixture of γ-Fe_2O_3 (maghemite) and Fe_3O_4 (magnetite) and a biocompatible coating [44]. Two main

classes of SPIONs based materials are currently used for medical applications: (1) SPIO with a mean particle diameter of 50–100 nm (coating included); and (2) ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles with a size below 50 nm (hydrodynamic size coating included) [45]. Basically, SPIO and USPIO are known as negative contrast agents because they can significantly enhance T_2^* relaxation, resulting in signal void in T_2^*-weighted images, unlike gadolinium-based contrast agents, which create a bright signal in magnetic resonance (MR) images [46]. Interest on SPIONs has dramatically increased in recent decades. Because of their low toxicity, large magnetic moments, and superparamagnetic properties, they have been investigated for many medical applications such as (1) drug or gene delivery, (2) magnetic separation (e.g., in rapid DNA sequencing), (3) antitumor treatment with magnetic hyperthermia therapy, (4) magneto-transfection, and (5) stem cell tracking, etc. The large surface area of SPIONs has a particular advantage in its conjugation with targeting moieties, drug molecules, and imaging probes. Widder *et al.* [47] used the first magnetic drug delivery systems. They found that doxorubicin and magnetite were encapsulated in albumin microspheres. This led researchers to perform targeted drug delivery and imaging using magnetic nanoparticles. The decisive point for high-yield drug delivery using magnetic nanoparticles is their targeting capabilities. In order to increase the tumor targeting capability, it is essential to attach targeting moieties (i.e., antibodies, nucleotides, hormones, and receptor ligands) to the nanoparticle surface. Many target strategies have been tried to deliver the drug to the target site. SPIONs have been investigated as site-specific drug release from SPIONs, targeted prodrug delivery, and imaging-guided drug delivery (IGDD), etc. For example, Yang *et al.* [48] reported the pH-dependent release of doxorubicin from SPIONs-loaded amphiphilic triblock copolymer nanoparticles. Hwu *et al.* [49] reported paclitaxol conjugated to the SPIONs through a phosphodiester bond and then paclitaxol molecules released from particles by the intracellular phosphodiesterase. In the case of IGDD, nanomaterials are being developed more and more for theragnosis. Theragnosis can provide noninvasive biodistribution imaging and monitoring of drug release, so we can predict therapeutic responses and facilitate therapeutic intervention [50]. Fan *et al.* [51] synthesized SPIO-conjugated, doxorubicin-loaded microbubbles for brain tumor drug delivery. In this study, drug release was controlled by focused ultrasound, and then released SPIO particles, after destruction, were deposited within brain tumors by a magnetic transducer. The overall process was monitored by magnetic resonance imaging (MRI). Here, MRI instruments have a lower sensitivity than fluorescent and radionuclide imaging. The concept of "platform" can be introduced to use SPIONs with pros and cons. Coated SPIONs can be used as a platform conjugated with targeting moiety and the other imaging probes, such as fluorescent dyes for optical imaging, and radioisotopes for gamma imaging that have more sensitivity than an MRI probe [52,53]. The other method for molecular imaging by using SPIONs is to make nanoparticles including SPIONs or to make layers containing iron oxide [54]. These particles can be used in dual or triple imaging platforms.

4.2. *Gold nanoparticle*

AuNPs have been used in various biomedical applications such as diagnosis, treatment of disease, and pharmaceutical drug delivery. AuNPs have certain characteristics, such as their facile synthesis, easy surface functionalization, unique optical properties, and biocompatibility. High-quality, high-yield, and size controllable colloidal gold can be prepared using the citrate reduction method [55]. AuNPs are detected by X-ray examination, so these can be used as a theragnosis substance.

The properties of superior light absorption, scattering (1,000 times higher than organic dyes), and converting into thermal energy by surface plasmon resonance of AuNPs quickly found a use in photothermal cancer therapies [56]. When AuNPs absorbed a heating source such as near-infrared light, the excited electrons (collective coherent oscillation) return to the ground state, and this rapid relaxation makes energy in the form of heat, and as a result the surrounding temperature is simultaneously raised and cancer cells are destroyed. As mentioned above, this phenomenon can be used to provide contrast for photoacoustic imaging or photothermal therapy (PTT). Photothermal heating only occurs surrounding AuNPs, and high temperatures can easily be localised to reduce the negative side effects of cancer therapies [57]. The temperature rise is primarily related to shape and concentration, incubation time, laser fluency (power per unit area), and laser exposure time. Near-infrared light is more optimal for *in vivo* therapy of tumors within deep tissues because of its deep penetration, and minimal absorption of the hemoglobin and water molecules in tissues. PTT in recent years have been used with gold nanorods (AuNRs). Recently, a new nanoparticle platform for PTT was reported. Li *et al.* [58] reported a new delivery and photothermal ablation system based on AuNRs-laden-macrophage (macrophages are Trojan horses carrying 7-nm-diameter AuNRs). After intratumoral injection, the bovine serum albumin (BSA)-coated sAuNRs-laden-macrophages show greatly improved photothermal conversion almost everywhere in the tumor, resulting in minimized tumor recurrence rates compared to free BSA-coated sAuNRs. Piao *et al.* [59] reported that porous and hollow-structure AuNPs were coated with red blood cell (RBC) membrane. RBC-AuNPs exhibit significantly enhanced *in vivo* blood retention and circulation and drastically enhanced tumor uptake when administered systematically, and mice that received PTT cancer treatment showed 100% survival over a span of 45 days. Rengan *et al.* [60] reported on biodegradable gold-coated liposome nanoparticles (lipid–gold hybrid material). The combination treatment of this hybrid with a laser results in a 4.63-fold reduction of the tumor bioluminescence signal compared with controls.

To increase the sensitivity of detection and the therapeutic effects of AuNPs, many researchers had been trying to label with radionuclides such as ^{111}In, ^{18}F, and ^{177}Lu on gold nanostructures [61–63].

4.3. *Quantum dots*

QDs are light-emitting colloidal semiconductor nanocrystals with a core–shell structure and a diameter typically ranging from 2 to 10 nm and contains a small finite number of

conduction band electrons, valence band holes, or excitons. In 1993, Murray *et al*. [64] developed a simple route to produce high-quality CdSe QDs using a high-temperature organometallic procedure. After that, tremendous research efforts have been devoted to fabricating high-quality QDs for applications in biology and medicine. QDs have been investigated a little as a theragnosis agent because of the composite's toxicity. Recent advances on cadmium-free synthesis have pushed QDs to become important fluorescent probes for bioimaging research. In comparison with organic dyes and fluorescent proteins, QDs offer several unique advantages, such as tunable emission from visible to near-infrared wavelengths by size and composition. In addition, a large surface area/volume ratio permits the attachment of a large quantity of target moieties or drugs, and their excellent stability for long investigation times are the main advantages of QDs compared to other fluorescent agents [65]. Targeting moieties on QDs have been studied with aptamers and antibodies [66,67]. QDs exert their therapeutic effect as conjugation, inclusion, or mix-up with drugs containing therapeutic genes [68–70]. New synthetic techniques such as alloying, core/shell, and doping, developed in recent years, will likely play key roles in the future development of tuning the band gap.

4.4. *Liposome*

Liposomes have been studied as potential drug carriers, but recently, there has been a focus on their use as an imaging agent. Liposome has many attractive features as imaging agents for clinical applications. The surface of a liposome can be modified easily by adding special ligands for selective targeting and uptake such as transferrin receptors and pancreatic adenocarcinoma [68,71]. One field of application for imaging agents is the lymphatic system. They are ideal for transport through the lymphatic system because of their size and biocompatibility. Liposome can also contain imaging and/or therapeutic substances inside. For example, using these characteristics of liposome, my colleagues reported a mannosylated liposome containing encapsulated indocyanine green for sentinel lymph nodal optical imaging agent [72]. We also suggested liposomal nanoparticles as a theragnosis agent to treat ischemic lesions by delivering angiogenic peptides into ischemic lesions, as well as by imaging the place where nanoparticles are accumulated in the body [73,74].

5. Theragnosis using cells

Cell-based therapy, the therapy using microbial or human cellular materials, is a new pharmaceutical frontier [75]. Cells have more distinct therapeutic capabilities compared to small molecule drugs and biologics (e.g., antibodies, hormones, growth factors), especially in the arena of regenerative medicine. However, cell-based therapy can also be applied to treat infections, autoimmunity disorders, cancers, metabolic diseases, and tissue degeneration [75,76].

Cells can actively move toward organs that have disorders or tissues that have injuries by recognizing specific signals for the lesions, and they have abilities to sense their microenvironments and repair disordered biology or heal injured tissue [75]. A comprehensive understanding of the biology of therapeutic cells is essential to harness cell-based therapy as a strong armor against various intractable diseases.

Theragnosis with cells is drastically improving through the advancement of molecular imaging technologies, which allow the visual representation, characterization, and quantification of biological processes at the cellular and subcellular levels within intact living organisms [76]. *In vivo* tracking of cells is an indispensable technology for the development and optimization of cell-based therapy by monitoring transplanted stem cells or immune cells in preclinical animal and clinical human models [76,77].

Stem cell therapy has become a promising therapeutic modality of tissue regeneration for diseases that are irreversible (e.g., myocardial infarction, stroke, alopecia) by conventional therapies. The therapeutic cells are believed to be responsible for growth, wound healing, and replacing cells that are lost through pathological conditions or daily wear and tear. The therapeutic effect of the stem cell therapy may be related to the *in vivo* survival of therapeutic cells and generation of target tissue by differentiation of the cells [76].

Direct labeling of therapeutic cells with signal producing elements (radionuclides, fluorophore, nanoparticles, etc.) can feasibly visualize the cells in *in vivo* animal models, which have a great advantage for basic pathophysiologic and therapeutic studies of diseases that are difficult, or impossible, to perform in humans. However, the direct labeling technique has critical pitfalls for the purpose of *in vivo* monitoring of the labeled cells. Therapeutic cells are able to proliferate after introduction into the subject; however, the average intensity of imaging signals from the directly labeled cell is degraded by the division of the cell—the so-called dilution effect (Fig. 3). The death of the labeled cell cannot turn off the signals via the presence of the signal-producing element in the deceased cells, even after being engulfed by scavenger cells (Fig. 4). Elution of the signal-producing elements from the cells is another issue. The limitations permit the use of direct labeling mainly for short-term and early-phase *in vivo* monitoring studies. Indirect labeling of the therapeutic cells using transduction of imaging reporter genes into the cells can resolve the limitations of direct labeling. Daughter cells from the division of the transduced therapeutic cells have exactly the same reporter genes in their nucleus; therefore, signal intensity from the daughter cells should be the same as the parent therapeutic cells. In addition, the death of parent or daughter cells will turn off the signals.

Indirect labeling with reporter gene driven by a tissue-specific promoter can visualize differentiation of stem cells noninvasively in both *in vitro* and *in vivo* models [77,78]. The reporter gene strategy using a specific promoter system can be used in high-throughput *in vitro* screening platforms for differentiating agents and in assessing *in vivo* differentiation of the administered therapeutic stem cells (Fig. 5).

Some therapeutic immune cells, such as natural killer cells or cytotoxic T cells, can seek and destroy intractable cancers, which are not responsive to conventional radiation or chemotherapy because of their innate characteristics. Migration of the cells to the target cancer is an essential part of the therapeutic success, and molecular imaging for the

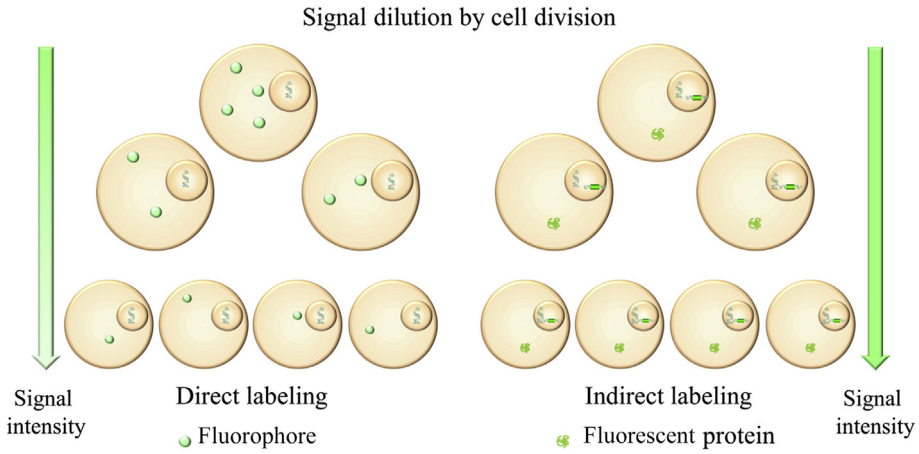

Fig. 3 – Signal dilution effect by cell division in cells directly labeled with signaling elements (fluorophores), but not in indirectly labeled cells using reporter genes (fluorescent protein).

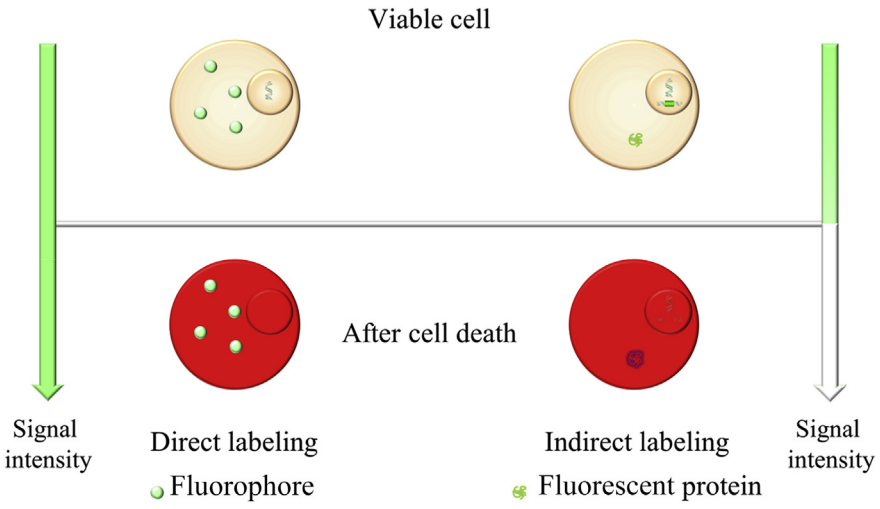

Fig. 4 – Signals from cells directly labeled with signaling elements (fluorophores) persist after death of the cells; however, signals from cells indirectly labeled cells using reporter genes (fluorescent protein) are turned off after death of the cells.

therapeutic cells can visualize the targeting ability of the cells toward cancers; therefore, the therapeutic effects of the cell for the cancer can be forecast with the imaging.

Other immune cells, such as dendritic cells, have the ability to present antigens to cells of the adaptive immune system (T and B cells) in lymphatic tissues, and the therapeutic effect of the dendritic cell therapy requires the successful migration of the cells to the lymphatic organs; therefore, the therapeutic effect of the cell can be predicted by molecular imaging demonstrating *in vivo* migration of the cells to the lymphoid organs [79,80].

Therapeutic cells can be engineered to manage disorders more efficiently and to broaden their applications [75]. The disease-targeting ability of therapeutic cells can be enhanced by the modification of the extracellular targeting domain of the cell, and the effect of the modification can be feasibly assessed by *in vivo* molecular imaging by direct or indirect labeling methods [81].

New nanoparticles or other synthetic delivering agents are under development to deal with some diseases that are intractable, mainly because of the lack of efficient drug delivery strategies that can target diseased areas. However, a large portion of the new drug-delivering vehicles can still be sequestered in the reticuloendothelial system. As an alternative, certain types of cells that are capable of homing to diseased or injured tissues can be used as unique carriers for theragnosis agents. For liver injury, mesenchymal stem cells have a natural tendency to home in on injured areas of the liver; therefore, the cells can be used as drug delivering beacon, and in addition, the mesenchymal stem cells can also help in the regeneration of the injured liver tissue [82].

Cell-based therapy is emerging as a hope for medicine as a new pillar of therapeutics to the conventional small molecule drugs and biologics, and application of the theragnosis strategy using molecular imaging techniques to the therapy speeds up the realization of its clinical applications.

Radiation theragnosis for thyroid cancer using radioiodine is an old practice but still practical to use. Whole-body scans following radioiodine therapy enables lesion-by-lesion monitoring of treatment. If we radiolabel therapeutic materials or

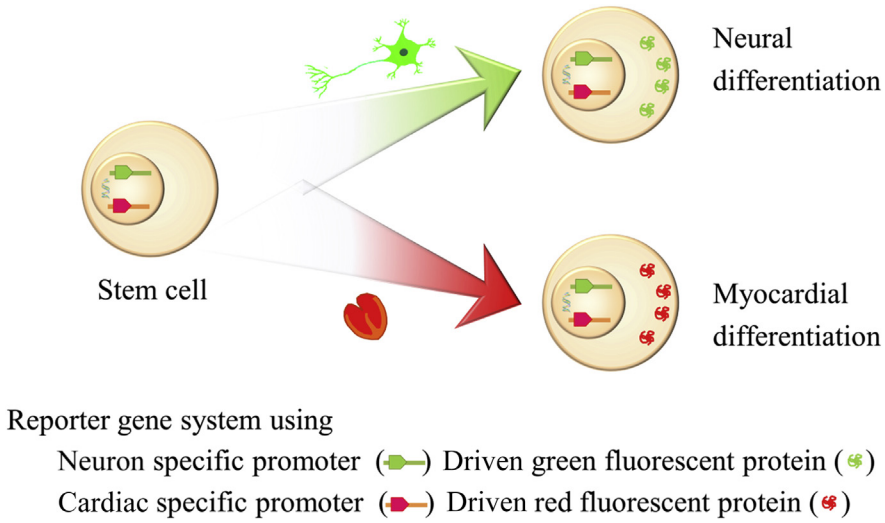

Fig. 5 – Dual reporter gene system using both neuron specific promoter driven green fluorescent protein (GFP) and cardiac specific promoter driven red fluorescent protein (RFP) can noninvasively visualize stem cells differentiation in both *in vitro* and *in vivo* environments. Neuronal differentiation of the stem cell makes production of GFP and cardiac differentiation of the stem cell makes production of RFP instead.

drug delivery systems such as peptides, albumin, nanoparticles, and cells, they are applicable as theragnosis agents for various diseases. Radionuclide imaging can also act as a tool for the development of drugs and therapeutics.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

This research was supported by the National Nuclear R&D Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant number: NRF-2012M2A2A7014020) and by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI14C1072).

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