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Molecular Imaging: New Applications for Biochemistry

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Abstract Molecular imaging can reveal in vivo analysis and quantification of biochemical reactions. To enable cell-surface imaging of receptors, novel ligands have been developed which can be radiolabeled or imaged by bioluminescence. Specific examples include somatostatin receptors, estrogen and progesterone receptors, receptors involved in adhesion and externalization of phosphatidyl serine as an indicator of apoptosis. Central nervous system imaging can be carried out using ligands for receptors including dopamine, serotonin and Gamma amino butyric acid (GABA). In addition, tumor and metabolic imaging can be carried out with the Na-K ATPase pump using the tracer thallium-201 for SPECT or F-18 FDG for PET imaging. Finally, novel receptors or endogenous metabolic pathways can be analyzed combining cell-gene therapy to create specific tracer targets in cells that can be studied by molecular imaging. The challenge of molecular imaging is to first identify key pathways that are unique for a specific disease processes, such as atherosclerosis, cancer, CNS disorders, immunologic and arthritis disorders and next to devise a high-affinity specific small molecular ligand that can be adapted to be a radiolabeled tracer to study this pathway. Advances in genomics and proteomics combine with new peptide-chemistry approaches should provide a large number of targets and tracers in the near future to achieve these imaging objectives. J. Cell. Biochem. Suppl. 39: 162–171, 2002.

Key words: PET; SPECT; in vivo imaging; receptor; metabolism; apoptosis; gene expression

"Molecular imaging" has the potential to enable in vivo analysis and quantification of biochemical reactions. Presently, techniques developed in nuclear medicine imaging allow non-invasive measurement of receptor number, receptor binding affinity, metabolic rates of physiological pathways, and concentrations of molecular end-products, in addition to other normal and disease-specific signatures of molecular activity in humans. In vivo imaging can, for example, determine the metabolic rate of oxygen utilization in the brain ($CMRO_2$) and biochemical reaction rates such as rates of enzymatic processes and second messenger activity. This imaging is made possible by understanding the biochemistry of a specific pathway and developing a radiopharmaceutical that can be used to interrogate the activity (molecular concentrations and rate) of this pathway.

The use of I-131 for measuring thyroid functional parameters and imaging the gland represented one of the first in vivo "molecular imaging" procedures about 40 years ago since iodine was naturally taken up by the gland and bound to tyrosyl moieties to form MIT and DIT, which were coupled to form tri-iodothyronine (T3) and thyroxine (T4). I-131 can be used to assess the binding and provide a molecular image of the activity of the thyroid gland. The similar principal has been conceived to measure uptake, molecular pathway activity, and molecular concentrations in numerous other molecular reaction determinations in most organ systems throughout the human body.

It is important to distinguish between the anatomical and functional aspects of in vivo

Abbreviations used: MRP-1, multi-drug resistant (MDR)-associated protein; GABA, gamma amino butyric acid; CCD, charged coupled device; BLI, bioluminescence imaging; PET, positron emission tomography; CAT, computerized axial tomography.

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imaging. Anatomic analysis includes planar radiographs, computed axial tomography (CAT) scans, and magnetic resonance imaging (MRI) scans, which, like a routine chest radiograph, presents a representation of the internal structure of an organ (Fig. 1). Using different contrast agents, these anatomic scans can bestow some physiological information. For example, in the case of MRI, in the presence of receptor or metabolic-pathway specific contrast reagent, measurement of T-cell migration and activity can be monitored. Nuclear medicine imaging using computerized tomographic techniques such as single photon emission computerized tomography (SPECT) or positron emission tomography (PET) provides functional information with greater contrast and organ-specific localization (Fig. 1). These two complementary methods of disease detection are currently used together in routine hospital diagnosis of patient disease. Advances have recently been made to develop sophisticated imaging modalities that combine these complementary imaging procedures into a single imaging device and imaging session, allowing fusion of anatomy and physiology (Fig. 1).

For example, combined PET–CT scanning utilizes F-18 labeled fluorodeoxyglucose (F-18 FDG) which exhibits increased uptake due to an increase in glycolysis associated with anaerobic tissues (due to hypoxia in tumors) [Warburg, 1931] combined with a CT scan, to provide the precise anatomic location necessary to distinguish "areas of increased uptake" seen on PET as being associated with a malignant tumor mass. For PET and SPECT scans, a typical study dose is about 10–20 milliCuries (mCi) or 370–740 megaBecquerels (MBq). Although correlation of molar concentrations of anatomic contrast agents and molecules of radioactivity cannot be strictly compared, it can be estimated by taking the molecular concentration necessary to produce a molecular image of radioactivity. Nuclear imaging requires a thousand

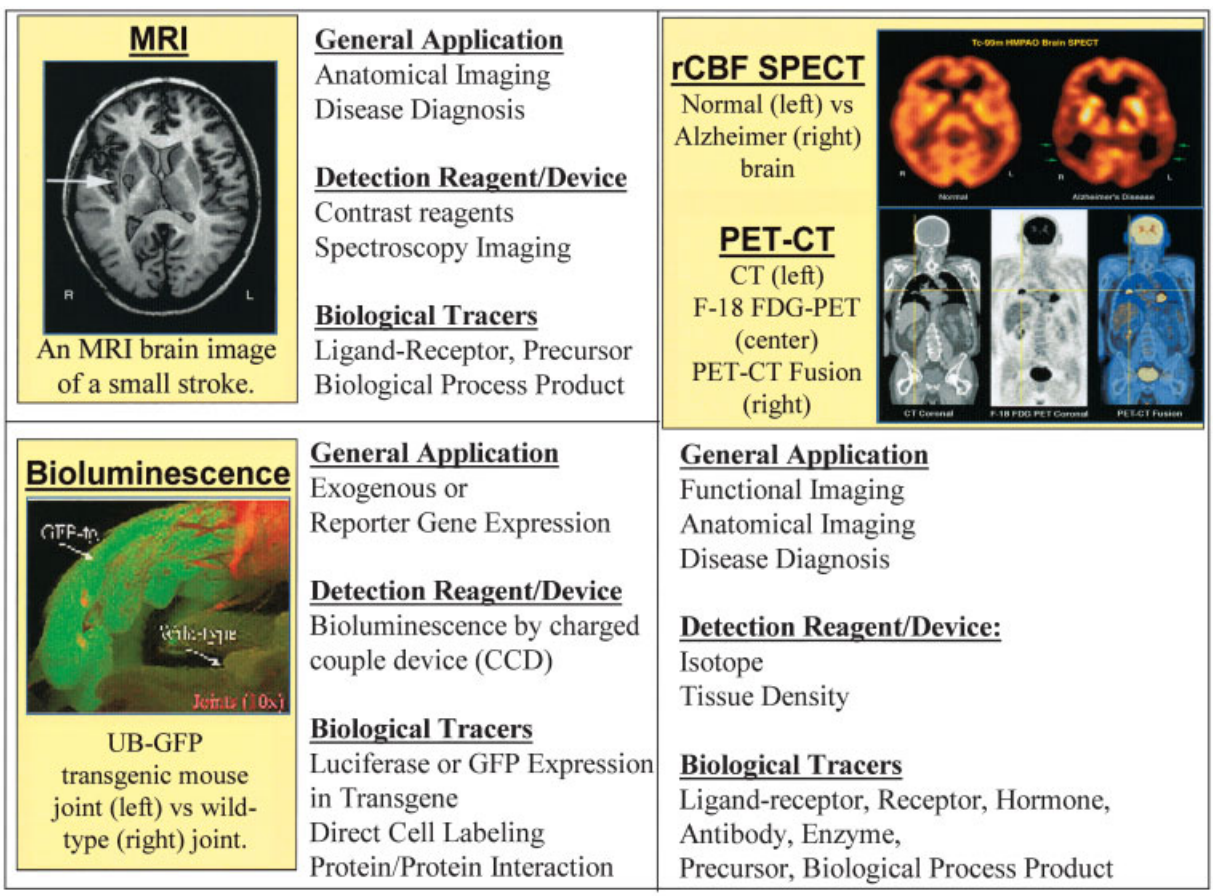

Fig. 1. Summary of the general methods used for molecular imaging.

to a million times lower molecular concentrations than those necessary to produce a physiological image of anatomy through methods of magnetic resonance spectroscopic imaging (MRSI, approximately 1 mM). Much higher concentrations of contrast agents to change proton susceptibility for detection of different vascular changes and tissue types are necessary. Therefore, PET and SPECT scanning is many-fold times more sensitive for a given molecular tracer concentration to detect physiological change compared to MRI or CT. This requirement for low concentration of tracer uptake to detect physiological change is an important advantage when developing tracers for molecular imaging of biological processes since they do not alter the metabolic rates of the natural substrates by competitive inhibition.

Another attractive modality for molecular imaging is the use of in vivo bioluminescence imaging (BLI) [Leaback and Haggart, 1989; Contag and Bachmann, 2002]. This uses luciferase that exhibits cell and tissue-specific detection due to either specific promoters that drive luciferase expression, labeled cell population or protein/protein interactions (Fig. 1). This imaging technique is based on light emission in the red to infrared range (greater than 600 nM) which is selectively transmitted to tissue due to the relatively low absorption of these wavelengths. Hemoglobin is the primary absorber of light in vivo, which decreases above 600 nM whereas the absorption of water begins at 900 nM and, therefore, emission between these two wavelengths is optimal.

Detection of bioluminescence is carried out with charged coupled device (CCD) cameras that are ultra-cooled to have a high efficiency at wavelengths greater than 600 nM [Leaback and Haggart, 1989; Contag and Bachmann, 2002]. Light collection systems enable the use of lenses to alter the depth and to focus images. A limitation of BLI is caused by the significant light absorption with distance transversed in vivo and light imaging at present is not practical beyond 2–3 mm of tissue.

IMAGING OF RECEPTOR EXPRESSION

Meta-iodobenzylguanidine (MIBG) binds to the norepinephrine receptor and is the classic example for synthesis of a peptide modeled after a receptor [Beierwaltes, 1987]. For successful imaging of receptors, the absolute number of receptors expressed in quiescent cells or following a specific stimulus needs to be moderately high. The half-life of the receptor needs to be considered, and internalization of tracer can be beneficial in receptor imaging. Finally, the number of different receptors on different lesions, and their specificity needs to be considered. This will be discussed under somatostatin receptor imaging below.

SOMATOSTATIN RECEPTORS

There are six somatostatin receptors (SSTR), SSTR-1, SSTR-2A and 2B, SSTR-3, SSTR-4, and SSTR-5 [Hofsli, 2002; Menda and Kahn, 2002]. SSTR-2 is expressed to varying degrees and heterogeneity on certain human cancers whereas SSTR-1 and SSTR-4 is found in prostate carcinoma as well as in normal and hyperplastic endothelium. Somatostatin plays a role in tumor growth inhibition. Tc-99m depreotide (Neotect) or somatostatin analogs are approved for clinical use in the US and Europe [Virgolini et al., 2001]. SSTR-2 receptor imaging also has been successfully used by incorporation of the SSTR-2 gene into different delivery vectors to enable expression in other cell types, such as immune cells. In addition to somatostatin, other peptide-based receptor binding radiopharmaceuticals including gastrin/cholecystokinin (CCK), substance P, the vasoactive intestinal peptide (VIP) are being developed.

ESTROGEN AND PROGESTERONE

A second ligand–receptor imaging pair has been the application of estrogen and progesterone imaging associated with breast cancer. Both Fluorine-18 labeled estrogen analogs and I-131 and I-123 labeled compounds have been synthesized [Yang et al., 1994]. The radioiodinated estrogen analog has been successfully used to determine the estrogen receptor status in the study of women with breast carcinoma using both planar and SPECT radio-nuclide imaging. Progesterone receptor imaging with a progesterone analog Z-[I-123]-IPG2 is under development [Rijks et al., 1998].

RECEPTOR–LIGAND FOR ANGIOGENESIS

Tumor imaging employs the fact that tumor growth involves endothelial cells lined with neovascularization that supply microcirculation to most solid tumors and are also needed

for tumor growth and metastases. This neovascularization results in over-expression of specific cell markers including Factor VIII, acidic and basic heparin-binding fibroblast growth factor (BFGF), insulin-like growth factor (IGF), platelet derived growth factor (PDGF), and also CD32 (one of the human IgG Fc receptors) and CD34 (a monocyte-lineage stem cell marker). Vascular cells also express integrins, the one being most studied is the αVβ3 integrin which has an active receptor binding component of repeats of three amino acids, arginine, glycine, and aspartate, also referred to as RGD. This is the most abundant integrin expressed on the surface of proliferating endothelial cells. Blocking of αVβ3 with RGD-containing peptides or antibodies causes apoptosis of angiogenic endothelium cells and involution of tumor capillaries in vitro and in vivo. Peptides with high affinity for the αVβ3 integrin have been identified by the in vivo screening of phage display libraries. A cyclic pentapeptide cyclo (–Arg–Gly–Asp–D-Phe–Val–) has been identified as having a high affinity (Kd < 10 nmole/L) binding to the αVβ3 integrin. This has been modified to enable labeling with iodine or Fluorine-18 enabling in vivo imaging [Haubner et al., 2001a,b; Su et al., 2002]. Other androgenesis factors have been imaged which include the insulin growth factor (IGF) receptors IGF-1 and IGF-2 [Sun et al., 1997]. These receptors, also referred to as somatomedans are elevated in a number of tumors including Wilms neoblastoma, hepatoma, neuroblastoma, gleomas, leiomyosarcoma lipomas, colon carcinoma, and pheochromcytoma, in tumors bound by IGF1 with a Kd of 0.1–1.1 nM. Ligands to these receptors include IGF-1, bombesin (BN)–gastrin releasing protein (GRP), VIP, and transferrin.

IMAGING OF APOPTOSIS USING TC-99M LABELED ANNEXIN V

Apoptosis is a cell death process, usually mediated by signaling through a death domain receptor, and results in the early upregulation of service molecules that enable the apoptotic cell to enter into the monocyte/phagocytic system [Zhou et al., 2002]. Entry into phagocytes prevents the apoptotic cells from undergoing cell lysis and release of intracellular components, which can result in an inflammatory or autoimmune response. One of the molecules associated with early apoptosis and phagocytosis is externalization of phosphatidyl serine (PS), which is normally expressed on the inner membrane leaflet, but is redistributed to the outer membrane leaflet during apoptosis. Annexin-V has been labeled with Tc-99m and used with SPECT in humans and in animals to image apoptosis. In the near future, Annexin-V can also be labeled with F-18 to obtain higher resolution PET imaging [Bennett et al., 1995; Post et al., 2002; Russell et al., 2002]. Applications for apoptosis imaging include not only tumor imaging but also detection of cardiac allograft rejection, myocardial ischemia associated with apoptosis and inflammation and autoimmune disorders as well as screening for vulnerable atherosclerotic plaques indicated by the presence of apoptotic cells.

ANTIBODY IMAGING

In the past, antibody imaging of a particular cell receptor has been performed using SPECT tracers labeled with Tc-99m or I-131 [Mariani et al., 1997]. An antibody can be developed with a very high affinity to any surface receptor. One limitation of antibody imaging is that this is a foreign protein that can invoke an immune response. The second limitation is that the Fc receptor can be cleared through the reticuloendothelial system. This second limitation may be abrogated at lease in part by either blocking this receptor with "cold" Fc or by using radiolabeled antigen-binding portion (Fab) of the antibody. Because of these limitations, antibody imaging is being replaced by the availability of specific ligands that can be labeled.

IMAGING TRACERS FOR THE CENTRAL NERVOUS SYSTEM (CNS)

A new tracer which will have a significant impact on molecular imaging is I-123 labeled *N*-methyl-2 beta-carbomethoxy-3 beta-(4-iodophenyl) tropane (I-123-β-CIT), which is used for imaging of the dopamine and serotonin transporters by SPECT [Emond et al., 1997; Tatsch et al., 1997]. It has currently been used for evaluations of patients with Parkinson's disease. In most patients with Parkinson's disease there is a marked asymmetric uptake in the putamen and caudate head nuclei. The degree of diminution can be used to estimate the extent of nigrostriatal degeneration in Parkinson's disease patients. Several studies have now established that the posterior-inferior aspect of the

globus pallidus shows diminution of this tracer. Use of this tracer in conjunction with anatomic–functional fusion imaging can be used for precision stereotactic ablation or dopamine supplementary implants.

Gamma amino butyric acid (GABA) is the most abundant inhibitory transmitter in the CNS that is distributed throughout the brain. When GABA inhibitory activity exceeds that of excitatory inputs, symptoms of sedation, ataxia, and amnesia occur. Benzodiazepines have widespread clinical use and act by potentiating the effects of GABA on the chloride ion channel of the $GABA_A$–benzodiazepine receptor complex. A central benzodiazepine receptor ligand Ro 16-0154 (Iomazenil) has been identified to bind the human benzodiazepine receptors with high affinity. Benzodiazepine receptor binding analogs for radio-nuclide imaging include ^{11}C flumazenil for PET, and I-123 iomazenil for SPECT. These have shown promise in understanding of anxiety disorders, schizophrenia, and temporal lobe epilepsy.

MOLECULAR IMAGING OF METABOLISM

Thallium-201 has been shown to have affinity for brain tumors as early as the 1970s. Thallium-201 has high sensitivity for detection of new, recurrent, or residual viable tumors, which are difficult to differentiate from post-radiation necrosis and edema on CT or MRI. The distribution and retention of thallium-201 in the normal brain and tumors is an active process related to blood flow, loss of integrity of the blood–brain-barrier, tumor cell viability, tumor type, tumor cell membrane function, and the Na–K ATPase pump activity. Brain SPECT imaging employs the tracer thallium-201 to detect new, residual, or recurrent viable tumor due to the fact that there is transport of thallium-201 across the breakdown in the blood–brain barrier, and uptake of thallium-201 into regions of cerebral hypermetabolism. Thallium accumulates in the residual or recurrent viable tumor cells in proportion to malignant grade and total viable tumor bulk.

MULTI-DRUG RESISTANT (MDR) P-GLYCOPROTEIN (PGP)

A major obstacle to chemotherapy of cancer is the expression by cancer cells of the MDR transporter, which transports chemotherapeutic drugs. The MDR is a 170,000 transmembrane glycoprotein PGP that is a product of the MDR-1 gene as well as a related 190,000 membrane glycoprotein, the MDR-associated protein (MRP-1). PGP and MRP-1 are members of an ATP-binding cassette (ABC) super family of membrane transport proteins that control resistance to a broad spectrum of xenobiotics including natural drug products, conjugated compounds, chemotherapeutic drugs, and other cytotoxic drugs. To image the activity of MDR-1 PGP, several compounds have been developed [Kim et al., 1999]. The most studied is Tc-99m-sestamibi. Tc-99m sestamibi accumulates within cells due to physiologically negative mitochondrial and plasma membrane potential. Tc-99m sestamibi (Tc-99m MIBI) is a monovalent cation complex formed by a central technetium atom surrounded by six 2-methoxy-2-isobutyl isonitrile groups. In brain tumors, the mechanism of tumor uptake is also thought to be dependent on mitochondrial activity and the presence of PGP. Tc-99m sestamibi imaging scan shows intense uptake in residual or recurrent viable tumor cells with better anatomic delineation of the tumor boundary compared to thallium-201 due to the more favorable imaging qualities of the 140 keV Tc-99m photon.

GLUCOSE TRANSPORTERS AND HEXOKINASE

Hexokinase catalyzes the initial rate-limiting step in glycolysis, which is phosphorylation of glucose to glucose-6 phosphate. FDG is an analog of glucose that enters the cell through the same pathways of the glucose transporters Glut-1 and Glut-4 and is phosphorylated by hexokinase. However, FDG-6 phosphate is not further metabolized and remains trapped within the cells. Several investigators have shown that the Glut-1 protein is increased on cancer cells. In hypoxic conditions, Warburg [1931] showed that the accumulation of the FDG-6 phosphate increases as glucose transporter levels increase and probably preserves glucose metabolism at levels needed for survival of cells, and this occurs where highest levels of Glut-1 expression are located in hypoxic areas of tumors, and near necrotic areas. FDG uptake is not unique to cancer since FDG traces glucose metabolism rather than tumor-specific glucose metabolism. After FDG uptake occurs in other glucose-utilizing cells such as macrophages and leukocyte [Mochizuki et al., 2001]. This

non-specificity of FDG incorporation can be overcome in part by combining PET imaging with CT imaging to identify both anatomic and physiologic high uptake areas of FDG.

PET imaging using 2-[Fluorine-18] fluoro-2-deoxy-D-glucose (F-18 FDG) has been shown to have advantages compared to SPECT radiopharmaceutical imaging in detection of recurrent or residual brain tumor due to it's higher image resolution. However, F-18 FDG is a less sensitive but more specific tracer for the detection of recurrent or residual viable tumor as compared to thallium-201 or Tc-99m sestamibi which is a more sensitive but a less specific tracer. The lack of sensitivity of F-18 FDG is due to the fact that it has relatively high uptake in normal brain. The lack of specificity of thallium-201 is due to the fact that it accumulates at the site of blood–brain barrier breakdown prior to its uptake through the Na–K ATPase pump.

One of the major clinical applications for F-18 FDG imaging is in the detection and staging of cancer. Recent medicare approval for detection, staging and restaging in lung, colorectal, lymphoma, melanoma, head and neck, and breast carcinoma has greatly increased the number of PET scans performed in the United States for these indications.

IMAGING OF CELL PROLIFERATION USING EXOGENOUS THYMIDINE KINASE

Since FDG PET is not selective for tumor cells, radiolabeled thymidine has been used as a more selective marker of cell proliferation. Since thymidine is rapidly incorporated into newly synthesized DNA, thymidine analogs radiolabel with PET isotopes can provide imaging of tumor cell proliferation. The first experiments were carried out with ^{11}C-thymidine labeled in the methyl position. This tracer is limited since the short life half-life (20 min for C-11) made this isotope difficult to use. Longer-lived isotopes were sought that utilized F-18 (half-life 110 min), I-124 (half-life 4.2 days), Br-76 (half-life 110 min). Therefore, I-124-dU and Br-76-dU have been used, but these may not directly reflect thymidine precursor incorporation into DNA since these are only analogs [Boni et al., 1999]. BrdU labeling is commonly used in analysis of proliferation for immunohistochemical study and flow cytometry. Other F-18 analogs including 2′-F-ara-deoxyuridine (FAU) and 3′-deoxy-3′-[F-18] fluorothymidine (FLT). FLT has been shown to correlate with independent studies using Ki-67 immunostaining of lung cell proliferation [Shields et al., 1998].

CHOLINE KINASE

Choline is actively transported into cells, incorporated into phosphoryl choline, a product of choline kinase, and is the first intermediate in the incorporation and trapping of choline into the phospholipid pool. Choline has, therefore, been developed as a PET tracer for tumor imaging. Analogs include C-11 choline, F-18 fluoroethylcholine, and F-18 fluoromethylcholine (FCH) [Hara et al., 2002]. These have been used to detect cancers including brain, lung, head, neck, colon, and bladder, as well as prostate and prostate cancer metastases.

SODIUM/IODINE SYMPORTER (NIS)

NIS is a membrane glycoprotein that uses the trans-membrane sodium gradient maintained by the sodium/potassium ATPase to co-transport iodine and sodium into cells. Secondary active transport of iodine by NIS occurs in the thyroid gland, lactating breast epithelium, salivary gland, and gastric mucosal. Such tissues, therefore, concentrate radioactive isotopes of iodine including I-123 and I-131 and also Tc-99m pertechnetate, which can be used for diagnosis of these cancers as well as thyroid gland pathology [Riedel et al., 2001]. The NIS transporter has also been used for specific and significant accumulation of I-123 to enable imaging as well as use of ^{131}I to induce death of certain thyroid and other tumor cells. Recent studies indicate that imaging of NIS in breast cancer in mouse models may result in specific killing of the NIS positive breast parenchyma but not normal cells. Therefore, certain transporters such as NIS can be used for both diagnosis and radiolabeled-incorporation to treat certain tumors. Such imaging combined with treatment has also been used with other specific tracers.

EX VIVO AND IN VIVO IMAGING OF EXOGENOUS GENES

Imaging of Reporter Genes

There are several considerations when constructing a transgenic mouse or producing cell lines that can be transfected to express a reporter gene to be used for molecular imaging.

First, the reporter itself can be any of the receptors previously mentioned such as the SSTR-2 or dopamine-2 receptor (D2R) or a receptor that facilitate metabolic function such as herpes simplex virus-1 thymidine kinase (HSV-1 TK). A choice of reporter system requires that this reporter is expressed at low levels or is absent in the desired tissue or cell to be imaged, and thereby reducing background signal. Second, it just depends upon the image modality that one is considering. For PET imaging, D2R would enable detection with fluorodeoxyglucose dopamine [Kugaya et al., 2000]. For SPECT imaging, the expression of the somatostatin type II receptor would enable imaging with Tc-99m Neotect [Virgolini et al., 2001]. For BLI expression of luciferase would enable detection with a super-cooled CCD or microscope. A second consideration is the method used to express the reporter gene. The reporter gene could be placed under the control of a cell-specific receptor or a receptor for a biologic process of interest. For example, for T-cell imaging, the T-cell specific CD2 promoter, and enhancer results in long-term expression and does not require stimulation of the T cell. For biologic process, the BLI has been placed under the regulation of the prostate-specific antigen promoter [Contag and Bachmann, 2002]. For MRI, the HIV tat sequence can be coupled to super paramagnetic cross-linked iron oxide (CLIO) to create a CLIO-tat nanoparticle that can be transferred with high efficiency into T cells [Dodd et al., 2001].

A third consideration when transfecting a reporter gene is that the transfection system should not significantly interfere with the cell to be imaged. For T-cell imaging, the CD2 promoter and reporter is preferable a CMV promoter-reporter since the CMV promoter is not strong in the absence of T-cell stimulation. In addition, the transfection system is a consideration. For some cells, such as fibroblasts, it is easy to transfect DNA or use a gene therapy transfer technique. For other cells, such as T cells, gene therapy transfer is possible only after expression of the receptor such as the human coxsackie-adenovirus receptor (hCAR) for adenovirus transfection.

DOPAMINE-2 RECEPTOR

The D2R can be targeted using replicative-incompetent adenovirus, which will target the liver or can target an implanted tumor to stably express D2R [MacLaren et al., 1999]. Once expression of the D2 receptor is induced in vivo, this can be imaged by PET using (F-18-fluorethyl) Spiperone (F-18-FESP) and in SPECT imaging using I-123-iodobenzamine. However, one limitation using the D2R as a reporter protein is that transfection of adenovirus may induce cell changes or antigenicity. Also, the life span of expression of an adenovirus gene therapy in the liver is limited to 1 month. If longer imaging is required, it may be necessary to place the D2R into an adeno-associated virus vector or for even longer-term expression, into a Lenti virus vector. For longest expression, one must turn to a transgenic mouse to have integrated and high level expression of an exogenous reporter or receptor. Overcoming the problem of targeting expression of exogenous receptors to cells or tissue in humans for long-term imaging is a major hurdle at the present time.

CYTOSINE DEAMINASE (CD) AND HSV-1 TK EXPRESSION

Escherichia coli expression of CD, which converts cytosine to uracil. The CD gene from *E. coli* can be used to image CD with radio tracers but this is limited by the slow uptake of 5-fluorocytosine and rapid diffusion of the 5-fluorourasil out of cells [Haberkorn et al., 1996].

HSV-1 TK has advantages for therapeutic as well as imaging applications due to the broad substrate specificity of HSV-1 TK activity relative to the endogenous eukaryotic thymidine kinase. HSV-1 TK enables high phosphorylation of nucleoside analog such as ganciclovir compared to endogenous thymidine kinase following active transport of a nucleoside analog through a cell membrane, the agent is selectively phosphorylated only by viral TK. This mono-phosphorylated nucleoside is trapped within cells and subsequently converted to nucleoside triphosphate, which terminates DNA synthesis. This results in trapping of the nucleoside analog like ganciclovir selectively within cells which express HSV-1 TK. Cells that do not express the viral TK do not accumulate ganciclovir. This accumulation causes cell death at normal concentrations. However, tracer amounts of nucleoside analogs can be trapped inside cells without causing toxicity, thus enabling repeated imaging of HSV-1 TK activity

with SPECT or PET [Iyer et al., 2001]. For SPECT imaging uracil nucleoside derivatives such as 5-ioda-2′-fluoro-2′-dyoxie-1-β-D-arabiono-furanosyl-5-iodouracil (FIAU) labeled with I-124 or I-131 can be used. For PET imaging, a similar analog, which is 8-F-18-fluoro-9-2 hydroxyl-1-hydroxymethyl ethoxymethyl guananine (8-F-18-fluoroganciclovir) (F-18-FGCV) and other analogs can be used for PET imaging.

Using HSV-1 TK in imaging, one can also place this under different promoters. The feasibility of imaging constitutive human promoters such as the elongation factor 1A or nuclear factor of activated T cell (NF-AT) [Ponomarev et al., 2001]. Therefore, exogenous genes can be used to image gene transcription.

EXOGENOUS GENE EXPRESSION, BIOLUMINESCENCE

A novel method for in vivo molecular imaging is the application of bioluminescence using luciferase as the reporter gene. This reporter gene can be genetically programmed to non-invasively interpret the presence of activation of specific biologic events. This is most efficient when carried out using a luciferase that emits at approximately 600 nM, which penetrates well through tissue and is detected well using a super-cooled CCD. Unlike SPECT, PET, or MRI, only cells that express the luciferase emit light and there is no background signal. Therefore, in principle, BLI is predicted to be 100- to 1,000-fold more sensitive than PET detection of reporter genes in muscle and 10-fold more sensitive than PET in the liver. The luciferase is either expressed in a luciferase transgenic mouse with a tissue-specific promoter of interest or luciferase can be constitutively after stable integration into a tumor cell line. The tumor cell line can then be transferred into a mouse and imaged by administration of luciferine at any time after tumor transfer. Both the primary tumor as well as metastases can be imaged with high accuracy using BLI. BLI has also been used to image T cells that localize to collagen-II arthritis in mice. A collagen-specific T-cell clone was transduced with a retrovirus vector containing a dual-function reporter gene comprised between a coding sequence for GFP and luciferase. The GFP component enabled selection of cells that were transduced with the reporter genes. After transfer of the transduced CII-specific T cells to animals that exhibited clinical signs of collagen-induced (CII) autoimmune arthritis, bioluminescence of the T cells could be correlated with the joint lesion. In addition, treatment of the mice with anti-inflammatory therapy reduced arthritis and BLI from the reporter gene. Therefore, this BLI can be used to detect inflammatory cells.

SUMMARY AND CONCLUSION

Molecular imaging is now possible since radiolabeling with either SPECT or PET tracers that are specific for receptor or a cell process is possible. BLI is another method, this requires introduction of a light emitting gene such as luciferase into the desired cell, and is also limited to an imaging depth of approximately 3–4 cm. MRI or MRSI imaging can be used to achieve molecular imaging. MRI imaging requires loading, for example, T cells with the super paramagnetic iron oxide such as CLIO-tat [Dodd et al., 2001] or for detection of apoptosis using CLIO-Annexin V [Schellenberger et al., 2002]. MRSI can detect the presence of chemical using magnetic spectroscopy. The combination of these functional imaging modalities with the anatomical imaging such as computerized axial tomography (CAT) scan or MRI scanning can provide a comprehensive picture of anatomy and physiology. Future challenges will be to find more specific receptors for a given cell type or process, such as specific receptors for cancer cells. Secondly, specific ligands for these receptors that have high affinity binding for these receptors, can be labeled in vivo with a SPECT or PET tracer or MRI ligand without interrupting the binding activity, and third development of tracers that do not have high background, and are cleared quickly except from the area where specific binding occurs. Significant advances in these three areas will enable even greater applications of molecular imaging to understanding biologic processes.

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