Journal of Cellular Biochemistry 90:454–463 (2003)

Real Time Non-Invasive Imaging of Receptor–Ligand Interactions In Vivo

Paul Winnard, Jr. and Venu Raman*

Department of Radiology, MRI Division, Johns Hopkins University, Traylor 340, 720 Rutland Avenue, Baltimore, Maryland 21205-2196

Abstract Non-invasive longitudinal detection and evaluation of gene expression in living animals can provide investigators with an understanding of the ontogeny of a gene's biological function(s). Currently, mouse model systems are used to optimize magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and optical imaging modalities to detect gene expression and protein function. These molecular imaging strategies are being developed to assess tumor growth and the tumor microenvironment. In addition, pre-labeling of progenitor cells can provide invaluable information about the developmental lineage of stem cells both in organogenesis and tumorigenesis. The feasibility of this approach has been extensively tested by targeting of endogenous tumor cell receptors with labeled ligand (or ligand analog) reporters and targeting enzymes with labeled substrate (or substrate analog). We will primarily discuss MRI, PET, and SPECT imaging of cell surface receptors and the feasibility of non-invasive imaging of gene expression using the tumor microenvironment (e.g., hypoxia) as a conditional regulator of gene expression. J. Cell. Biochem. 90: 454–463, 2003. © 2003 Wiley-Liss, Inc.

Key words: cell-surface receptor; targeted imaging; MRI; PET; SPECT

The study of gene expression or protein function is usually carried out ex vivo. Generally, these experiments require that tissue samples or cultured cells be subjected to fixation or consumed prior to analyses resulting in the loss of native structure and function. The resulting preparations are then probed with the desired reagents (e.g., antibodies, ligands, oligonucleotides, or enzymatic substrates) which have been labeled with a reporter moiety or are reacted with a second labeled compound. Labels include fluorochomes, radionuclides, spin labels, or precursors whose metabolites are; for example, precipitated dyes or reactive oxygen species, all of which provide a means for detection. These techniques attempt to provide information about a macromolecule's expression pattern (cellular or tissue), structural integrity, interactions, or function. However, information about the native state of the system can only be obtained by probing living organisms over a period of time. A goal of non-invasive imaging modalities is to detect gene expression and harness information regarding the spatial-temporal expression pattern as well as its effect on the cellular environment in real-time.

Currently, technologies are being developed to facilitate continuous detection of gene expression. Emerging techniques are demonstrating that evidence of biochemical events such as gene expression, protein–protein interactions, enzymatic activity, and apoptosis can be obtained in real-time in vivo with little or no artificial manipulations of cultured cells or living animals [Jacques and Desreux, 2002]. An advantage of these non-invasive approaches is the longitudinal evaluation of gene expression in living cells and animals, which can provide investigators with a better understanding of the ontogeny of a gene's biological function(s). In addition, this repeated imaging capability can be applied to in situ tracking of cell migration including stem cells and metastatic progression. Continued advances in imaging

Grant sponsor: The Johns Hopkins University (ICMIC Program); Grant number: P20-CA86346.

*Correspondence to: Venu Raman, Department of Radiology, MRI Division, Johns Hopkins University, Traylor 340, 720 Rutland Avenue, Baltimore, MD 21205-2196.
E-mail: vraman2@jhmi.edu

Received 11 June 2003; Accepted 13 June 2003
DOI 10.1002/jcb.10616

© 2003 Wiley-Liss, Inc.

technologies are at the heart of this revolution and include magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), as well as various optical imaging methods that are based on fluorescence, fluorescence resonance energy transfer (FRET), near infrared fluorescence (NIRF), multiphoton fluorescence, and luminescence. A detailed discussion of each of these fields is beyond the scope of this review and the reader can find specifics in several recent articles and references there in [Centonze and White, 1998; Weissleder et al., 1999; Dodeigne et al., 2000; Chatziioannou, 2002; Jacques and Desreux, 2002; Yamagoe et al., 2003]. We have limited our discussion to the use of MRI, PET, and SPECT imaging with appropriately labeled ligands for targeting of cell-surface receptors. During the course of reviewing this material, a very brief introduction to the use of labeled substrates for detection of enzymatic activity also emerges. Finally, we will briefly discuss the future use of imaging modalities, which we believe well be based on the detection of the controlled expression of genes.

CELL-SURFACE RECEPTOR IMAGING

In vivo applications of targeted imaging began with the production of antibodies to cell surface antigens [Hazra and Sharma, 1982]. The rationale behind this approach was that antibodies, particularly monoclonal antibodies, could be produced with very high specificities to a cell surface antigen of choice. Here, high specificity refers to the interaction of ligand or antibody with a single unique epitope as well as a dissociation constant (K_d) at or below the nanomolar (nM) range. Taking advantage of the fact that certain tumors or disease tissues have abnormal expression of specific antigens, detection of these antigens by labeled antibodies continues to be pragmatic and a feasible approach to image diseased lesions. The bulk of this work has centered on imaging tumors or delivering therapeutic agents tethered to the antibodies to tumors [Baxter and Jain, 1996]. Recent advances in this area include the production of chimeric humanized monoclonal antibodies. To facilitate diffusion, other antibody subtypes are being designed that have much lower molecular weights, referred to as minibodies (80 kDa) and the 55 kDa diabodies [Wu et al., 1999; Wu et al., 2000; see Fig. 1]. These antibodies have less antigenicity in humans compared to murine monoclonal antibodies that elicit an immune response precluding their use in longitudinal radiotherapeutic regimes, which require several doses of radiolabeled antibody over the course of weeks or months. The development of humanized antibodies was undertaken to address this problem. In addition, the smaller chimeric antibodies have pharmacokinetic properties [Thomas et al., 1989; Williams et al., 2001] that favor their use in PET studies where the half-lives of the radionucleotides are on the order of hours (^{18}F ~1.9 h and ^{64}Cu ~12 h). Diabodies labeled with ^{64}Cu, are showing very promising results as imaging agents [Wu et al., 1999]. These studies indicate that the previous difficulties of delivering antibodies to the site of interest and antibody antigenicity are being significantly reduced.

A major hurdle in imaging specific antigens is the diffusion of large labeled molecules to the target site. Large molecules like whole IgG (150 kDa) have low diffusion coefficients and exhibit decreased extravasation from the capillaries to the interstitial spaces [Thomas et al., 1989]. In addition, antibodies can be internalized following their interaction with the cognate antigen, making longitudinal studies dependent upon the antigen turnover rate. Given these limitations, investigators have been seeking other cell surface targets that bind much smaller molecules and several laboratories are exploring the use of normal human cell-surface receptors as imaging targets (Table I). Investigators have attempted to target both endogenously expressed receptors and those on engineered tumor cells that overexpress a receptor of choice (Fig. 2). Tumor cells have been engineered to express particular receptors in situ through gene therapy-like techniques by injection of viral vectors carrying the gene of choice to subcutaneous xenografts (SCXs) or ex vivo by standard transfection methodologies. The receptors that are being studied can be targeted with low molecular weight, i.e., $\leq$2 kDa, ligands (Fig. 1), which should possess traits of high blood pool clearance through the kidneys and rapid uptake at the targeted site. Generally, the rationale remains the same as that for antibody use, i.e., the delivery of a highly specific reporter molecule (see Table I for K_ds) to a cell surface molecule

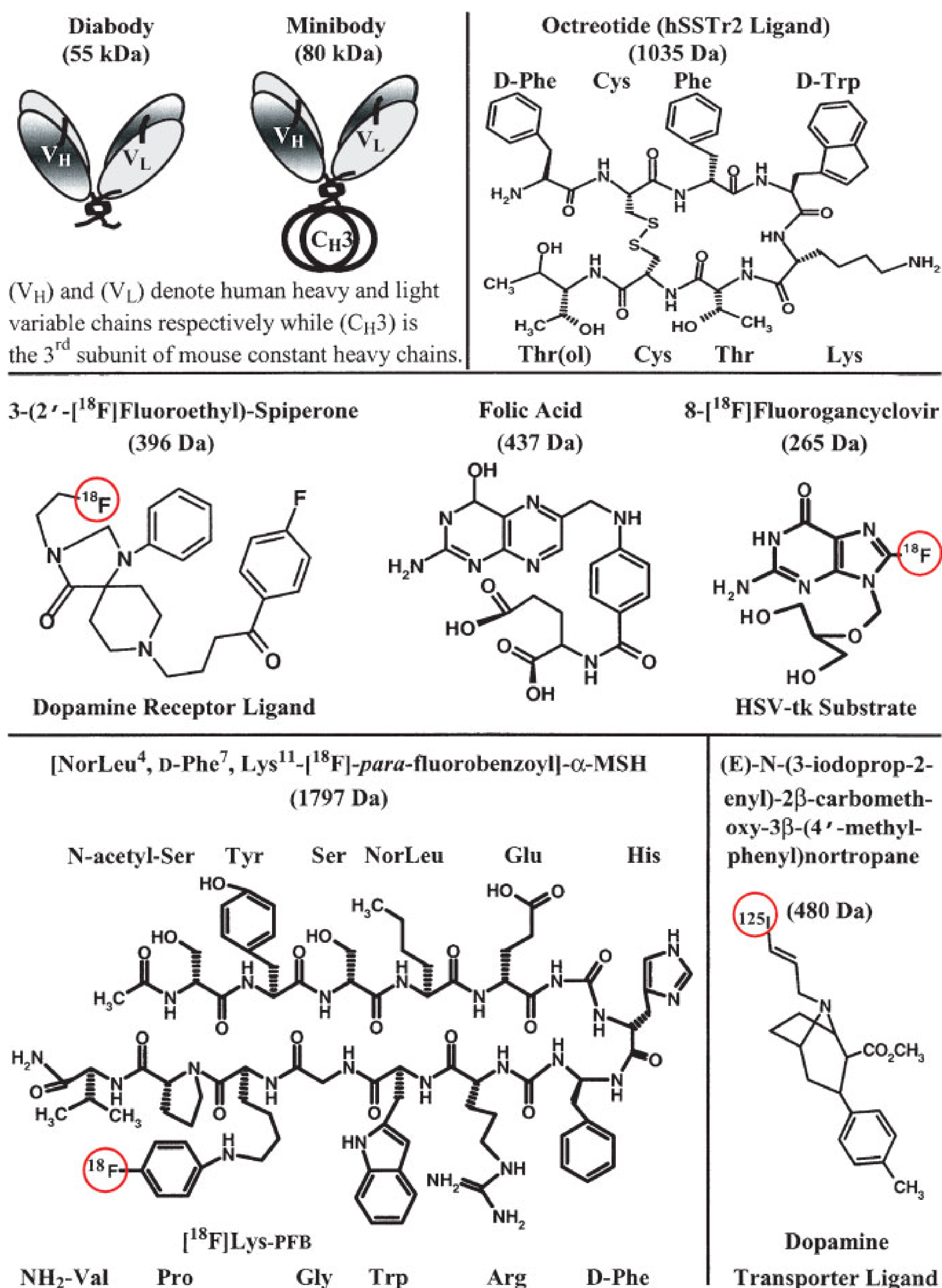

Fig. 1. Examples of antibodies and ligands being tested in cell-surface antigen or cell-surface receptor directed molecular imaging. In the case of the antibodies metal chelators are usually conjugated to amino groups while in the case of Octreotide chelators have been coupled to the amino-terminus' NH_2 group and for folic acid attachment has been done through the carboxyls. Such chelators are necessary if metal labels such as ^{64}Cu, ^{99m}Tc, or Gd are to be attached to these peptides. ^{18}F and ^{125}I labels are circled. hSSTr2 denotes human somatostatin type-2 receptor. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Cell-Surface Receptor/Ligand Pairs Currently Being Developed for Non-Invasive Imaging

Receptor	Ligand	K_d (nM)	Targeted tissue	Non-targeted tissue
hSSTr2	Somatostatin analogs	6.0–7.0	SCXs, lung, neuroendocrine, and ovarian tumors	Liver, spleen, thyroid, breast, and renal cysts
Transferrin	Transferrin	1.0–3.6	Brain tumors, stem cells, and liver	Liver and spleen
D2R	Dopamine analogs	0.04–1.7	Striatum	Brain, pituitary, adrenal gland, and kidney
Folate	Folate	0.07–1.6	Ovarian tumors and SCXs	Liver, kidney, ovary, and intestines
DopTrans	Dopamine analogs	1.5–2.8	Striatum	Substantia nigra, olfactory tubercles, and accumbens nuclei[a]
$5\text{-}HT_{1A}$	Serotonin analogs	3.4	Hippocampus	Frontal cortex, liver, lung, spleen, kidney, and intestine
Sigma-2	Sigma-2 analogs	1.5–45	Mouse mammary adenocarcinoma (as SCX)	Thyroid, skin, liver, lung spleen, kidney, and heart
α-MSH	α-MSH analogs	1.3	Melanoma (as SCX)	Kidney and intestine
GRP	GRP analogs	2	Ovarian tumors (as SCX), breast cancer cells, and breast tumor sections	Small intestine, spleen, lining of abdominal cavity, and uterus
NeTen	Neurotensin analogs	1.5	Colon adenocarcinoma (as SCX)	Liver and kidney
VIP	VIP analogs	1.2–3.0	Carciniods and breast tumors (as SCX)	Liver and intestine
Insulin	Insulin	2	Distribution of normal insulin receptors	—

K_d is the dissociation constant.
hSSTr2, human somatostatin receptor type 2; D2R, dopamine type-2 receptor; DopTrans, dopamine transporter; $5\text{-}HT_{1A}$, serotonin 1A receptor; α-MSH, α-melanocyte stimulating hormone receptor; GRP, gastrin-releasing peptide receptor (GRPr); NeTen, neurotensin receptor; VIP, vasoactive intestinal peptide receptor; SCX, subcutaneous xenograft.
[a]In this case only uptake in the brain was reported and the uptake at other organs was not given. References are Zinn and Chaudhuri [2002], Weissleder et al. [1999], Satyamurthy et al. [1986], Leamon et al. [2002], Guilloteau et al. [1998], Choi et al. [2001], Vaidyanathan and Zalutsky [1997], Rogers et al. [1997], Garcia-Garayoa et al. [2002], Raderer et al. [2000], and Iozzo et al. [2002].

that exhibits preferential/specific expression in diseased tissue.

SOMATOSTATIN TYPE-2 RECEPTOR

Human somatostatin type-2 receptor (hSTTr2) is a member of the G-protein coupled family of receptors [Zinn and Chaudhuri, 2002] that are characterized by seven transmembrane domains and a cytosolic domain that interacts with G-proteins. Its naturally occurring ligand, somatostatin, is a 14 amino acid cyclic peptide (AGCKNFFWKTFTSC). Interest in hSSTr2 as an imaging target came about when it was recognized that many types of tumors overexpress it and that ligand binding initiates an anti-proliferative signal cascade. Thus, targeting hSTTr2 has been considered as a therapeutic strategy for modulating tumor growth as well for imaging. It was discovered early on that somatostatin could not be used as an imaging ligand because of its short circulating half-life of <3 min, resulting from its enzymatic degradation in the blood stream. To overcome this problem several somatostatin analogs, such as Octreotide (Fig. 1), have been prepared that are not readily degraded in the blood stream and still exhibit a high affinity for hSSTr2 (Table I). These analogs have been labeled with various radionuclides for PET (^{18}F, ^{66}Ga, and ^{64}Cu) and SPECT (^{99m}Tc) imaging studies, as well as with therapeutic radionuclides such as ^{188}Re [Zinn et al., 2000; Ugur et al., 2002]. Imaging of hSSTr2 is being extended to the indirect detection of gene expression during gene therapy [Zinn and Chaudhuri, 2002]. In this case, the hSSTr2 is the reporter gene in a dual expression vector that allows a second gene of choice to be co-expressed with hSSTr2 (Fig. 3). Thus, detection of hSSTr2 indicates that the same cells harbor the gene of choice, which can encode a therapeutic gene [Iyer et al., 2001] or tumor suppressor gene such as *p53* or *p16* [Steiner et al., 2000; Lane and Lain, 2002].

DOPAMINE D_2 RECEPTOR (D2R)

The human D2R is another G-protein coupled receptor [Senogles, 1994]. Labeled dopamine and dopamine analogs such as 3-(2′-[^{18}F]fluoroethyl-spiperone (Fig. 1) are being used to detect and monitor D2R expression [Satyamurthy et al., 1986]. Generally, D2R imaging provides a non-invasive means to assess dopamine uptake in the striatum. These are functional studies as

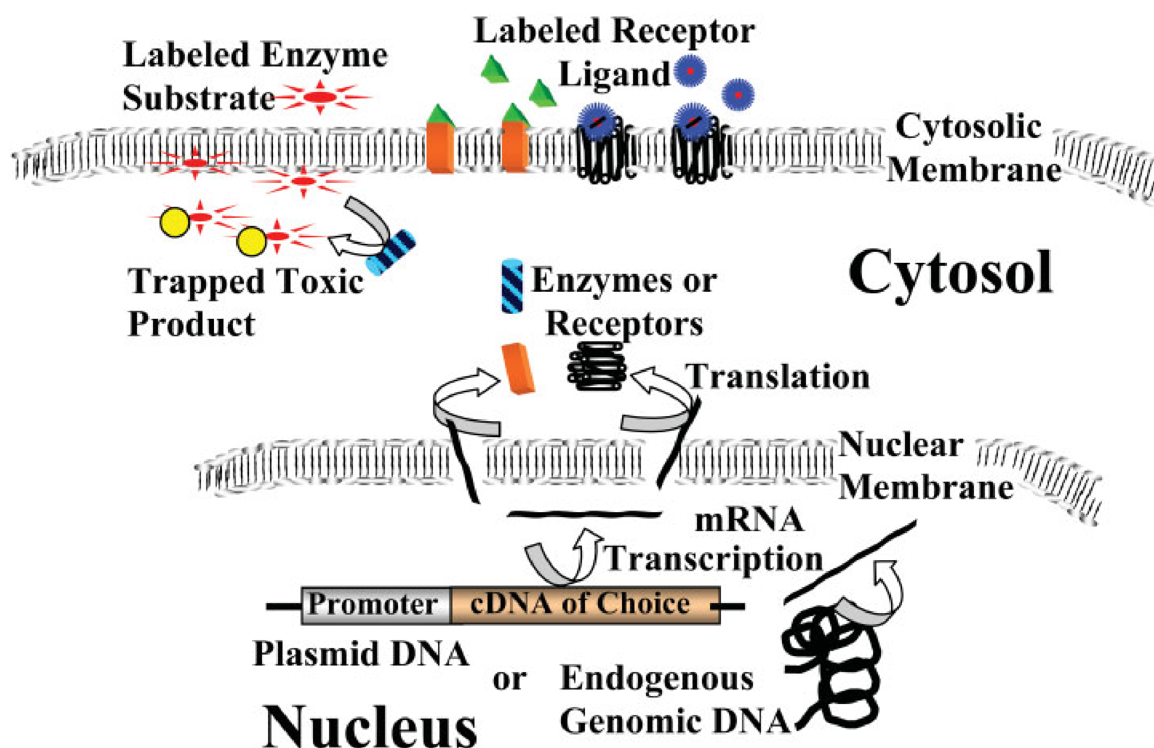

Fig. 2. Schematic representation of cellular pathways that generate molecular imaging targets. Targets can provide functional information about gene expression during gene therapy or gene expression in engineered cells or about gene expression that is controlled by microenvironmental conditions (see Fig. 4). Nuclear transcription from an exogenously introduced plasmid or from endogenous genomic DNA and cytosolic translation of the resulting mRNAs is depicted. This provides the protein targets, i.e., cell-surface receptors or cytosolic enzymes. Cell-surface receptors; for example, somatostatin type-2 or folate or enzymes, such as, Herpes simplex virus thymidine kinase (HSV*tk*), are then targeted with appropriately labeled receptor ligand or enzyme substrate. Labeled enzyme substrates; for example, 8-[^{18}F]Fluorogancyclovir (see Fig. 1), are designed in a manner that allows them to readily transverse the cytosolic membrane. However, upon metabolism the then labeled products, which are usually cytotoxic; for example, triphosphorylated 8-[^{18}F]Fluorogancyclovir, remain trapped within the cell. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

low amounts of reporter binding should reflect loss of dopamine receptor and the cellular brain functions that it regulates. These studies indicate the feasibility of obtaining clinically relevant information on brain status an area that may otherwise be inaccessible. In addition, it has now been shown that D2Rs are expressed throughout the gastrointestinal tract and thus, targeted imaging of gastrointestinal tumors via D2R is being explored [Lemmer et al., 2002]. High affinity D2R ligands have been developed (Table I) and labeled with a variety of

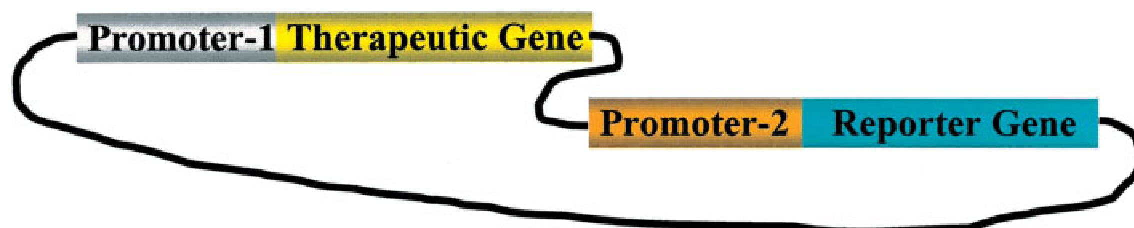

Fig. 3. Illustration of a dual expression plasmid or vector construct showing in tandem a promoter-therapeutic gene linked with a promoter-reporter gene. Examples of therapeutic genes that are being used in these types of constructs include HSV*tk*, *p53*, and *p16*. Examples of reporter genes include β-galactosidase, green fluorescence protein, luciferase, hSTTr2, and dopamine type-2 receptor. Promoter-1 and promoter-2 can be identical or different. In addition these promoters can be constitutive such as the human cytomegalovirus promoter or tissue specific such as the whey acidic protein promoter or conditional such as the hypoxia response element (HRE) promoter. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

radionuclides for PET (^{11}C and ^{18}F) and SPECT (^{123}I) imaging. D2R is also being tested as a potential reporter gene (Fig. 3) as described for hSSTr2 [MacLaren et al., 1999].

FOLATE RECEPTOR

Human folate receptor (hFR) sometimes referred to as folate-binding-protein, is a glycosylphosphatidylinositol membrane anchored protein that possesses high affinity for folate (Table I) and transports it into cells [Holm et al., 1999]. hFR is overexpressed on a variety of tumors and may be a marker for breast, cervical, ovarian, colorectal, renal, and nasopharyngeal cancers. Thus, similar to hSSTr2, targeted imaging of the hFR is an important strategy for the diagnostician because it may allow for the non-invasive detection of cancerous lesions as well as the monitoring of tumor growth/regression following cancer therapy. Folate (Fig. 1) has been labeled with ^{99m}Tc for scintigraphy (although SPECT could be applied) [Leamon et al., 2002] and Gd for MRI [Konda et al., 2002].

TRANSFERRIN RECEPTOR

Transferrin receptors shuttle iron into cells via the iron binding protein transferrin, through endocytosis. Normally, this is not a continuous process and transferrin receptor expression is regulated by the iron concentration inside the cell. Once sufficient iron concentration is attained, transferrin receptor expression is down-regulated. Weissleder et al. [1999] have engineered a variant human transferrin receptor to circumvent this regulatory circuit. They have shown that cancer cells that overexpress these mutant receptors can continuously load large amounts of iron into the cell. They have conjugated transferrin to monocrystalline iron oxide nanocompounds (MIONs) and used this reagent to target tumor cells that overexpress the mutant transferrin receptor. This strategy allows them to image tumors that overexpress the mutant transferrin receptor using MRI. The principle advantage of MRI imaging is its high spatial resolution however, sensitivity is low and the ability to acquire specific MRI images requires the presence of high local concentrations of contrast agent, such as iron oxide. Weissledder's experiments suggest that receptor-mediated MRI of tumors is possible.

OTHER RECEPTORS UNDER DEVELOPMENT FOR TARGETED IMAGING

As indicated in Table I, several other receptors are under development for targeted imaging. Targeted SPECT imaging has been used to quantify and evaluate the functional status of dopamine transporter in the brain [Guilloteau et al., 1998; see Fig. 1 for an example of a dopamine transporter ligand]. As with targeted D2R imaging, obtaining information on the functional status of brain biochemistry can only be realized through non-invasive imaging techniques and targeting the dopamine transporter is one option (e.g., monitoring Parkinson's disease by measuring dopamine transporter content in the striatum). Along these lines, serotonin-1A receptors ($5\text{-}HT_{1A}$) which regulate mood, sleep, as well as learning, are being explored as targets of PET imaging [Plenevaux et al., 2000]. This would allow the monitoring of physiologic changes at this receptor in patients with sleep disorders, for example.

In understanding cancer biogenesis, the sigma-2 receptor is thought to be overexpressed in many tumors and such expression may be correlated to the proliferative state of some tumor cells. Thus, sigma-2 based scintigraphy studies have been initiated to test the ability to monitor tumor proliferation in breast cancer [Choi et al., 2001]. Also, melanoma is being targeted via the α-melanocyte stimulating hormone receptor (α-MSHr) using appropriately labeled α-MSH analogs (Fig. 1), which are metabolically stable versions of α-MSH and thus, are not readily degraded in the blood stream [Vaidyanathan and Zalutsky, 1997]. It is thought that pulmonary and gastrointestinal neuroendocrine tumors might be specifically targeted with labeled analogs of gastrin-releasing peptide after induction of gastrin-releasing peptide receptor (GRPr). This approach is being tested [Rogers et al., 1997]. Neurotensin receptor is also being studied as a possible target for gastrointestinal neuroendocrine tumors using labeled stabilized neurotensin [Garcia-Garayoa et al., 2002]. Carcinoids constitute the highest incidence of neuroendocrine cancers and predominately occur as gastrointestinal tumors. Vasoactive intestinal peptide (VIP) is a 28 amino acid peptide with high affinity for VIP receptor (VIPR). Overexpression of VIPR on carcinoids may be correlated to the proliferative state of these tumors. Thus, VIPR has been targeted for

scintigraphy studies by ^{123}I-labeled VIP as a mode of detecting and monitoring carcinoids [Raderer et al., 2000].

Finally, targeting the insulin receptor with ^{124}I-labeled insulin for PET imaging is being studied from the perspective that in vivo evaluation of insulin receptor expression in inaccessible areas such a brain and myocardium may provide a better understanding of the pathogenesis of obesity and degenerative disease [Iozzo et al., 2002].

CAVEATES OF RECEPTOR BASED IMAGING

The targeting of endogenous receptor expression suffers from a number of potential confounding drawbacks. As indicated in Table I, absolute specificity is usually lost due to uptake of labeled ligand outside the targeted site. This occurs because receptor expression occurs in several types of tissue. Some of the consequences of this are false positives during data interpretation, the possible masking of the signal or obstruction of lesion detection inside of an area of high non-specific uptake, as well as subjecting normal tissue to toxic labeled ligand. Furthermore, although the amount of non-specific uptake in any one tissue may be relatively low, the cumulative effect can be substantial. This removes the amount of labeled ligand that is available for interactions at the site of interest and requires higher than otherwise needed doses of labeled ligand. There is also the problem of competition of administered ligand with endogenous ligand [Cumming et al., 2002]. This can cause an attenuation of signal at the targeted site. Moreover, many of the receptors under evaluation are the first components of potent signal transducing pathways [Liang et al., 2001]. Thus, ligand binding by these receptors triggers intracellular signaling cascades that can bring about changes in gene expression. Such changes disrupt normal cellular physiology and if coincident with paracrine or endocrine events might bring about physiological changes throughout a tissue as well as systemically. These changes conformed the measurement of native physiological processes. Finally, while most receptor studies are currently geared towards tumor targeting, one needs to recognize that many tumors have altered receptor expression that can vary during the course of tumorigenesis [Edwards, 1985]. Often a receptor's expression is diminished or lost at some point in the progression of the cancer. Such losses can give rise to false negatives when evaluating targeted ligand binding studies.

Some of these difficulties have been partially addressed. For instance, a mutant D2R, which upon ligand binding does not initiate its signal cascade, has been studied [Liang et al., 2001]. Also, enhanced continuous receptor expression can be obtained by linking the gene of interest to a tissue specific promoter.

FUTURE IMAGING OF CELL SURFACE RECEPTORS

Emerging imaging strategies continue to focus on optimizing the target to non-target ratio. One approach is the use of gene therapy techniques, which are based on viral vector delivery systems such as adenoviral packaged vectors. The genetic constructs carried by these vectors are designed for controlled expression of the gene of choice. This is achieved by placing the gene under the control of tissue specific promoters or conditional promoters (Fig. 4). In the latter case, enhancement or repression of gene expression is regulated by transcription factors that are repressed, induced, or stabilized by alterations in the tumor microenvironment; for example, oxygen concentration or pH.

Currently GRPr placed under the control of tumor specific promoters, either erbB-2 or DF3 (MUC1), has been shown to be expressed solely on breast and pancreatic tumor cells [Stackhouse et al., 1999]. In another example, the Herpes simplex virus type-1 thymidine kinase (HSV1*tk*) gene placed under the control of α-lactalbumin promoter showed expression of HSV-tk was limited to breast tumor cells, which were subsequently susceptible to gancyclovir (Fig. 1) treatment (as exemplified in Fig. 2) [Anderson et al., 1999]. Similarly, retroviral delivery of reporter β-galactosidase (β-gal) under whey acidic protein has provided a means of limiting β-gal expression to mammary tumor cells [Ozturk-Winder et al., 2002]. Also, luciferase-reproter gene has been placed under the control of chimeric prostate specific enhancers for prostrate specific expression [Wu et al., 2001]. All of these tissue specific promoters could be tailored for use with receptor expression as has been reported for the GRPr.

For conditional receptor expression the hypoxia response element (HRE), grp78 stress-inducible promoter, or pH response element

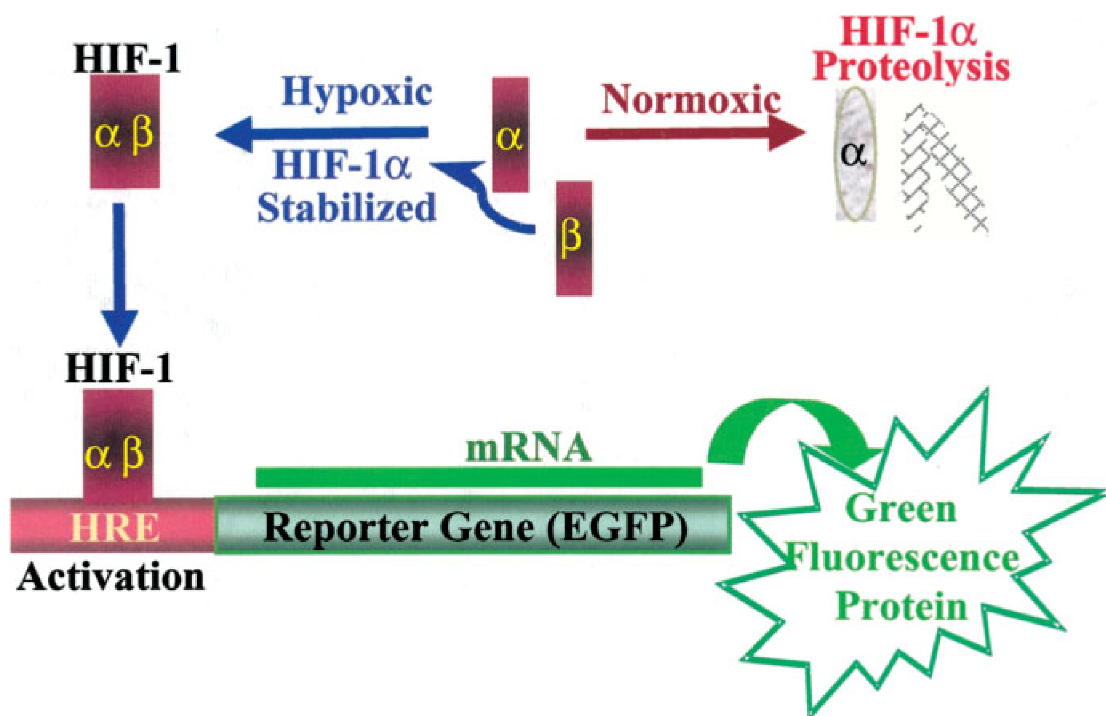

Fig. 4. Representative model of use of conditional promoter to control gene activity. Here the use of the HRE is being illustrated. Under normoxic conditions hypoxia inducible factor-1α (HIF-1α) is targeted for degradation via the ubiquitination/proteosome pathway. Under hypoxic conditions the ubiquitination of HIF-1α is disabled and HIF-1α dimerizes with HIF-1β forming HIF-1, which activates a variety of genes by interacting with the HRE(s) of their promoters. In this figure, enhanced green fluorescence protein (EGFP) gene is shown to be activated during hypoxia. However, any desired reporter gene under the control of a variety of promoters or enhancers (see text for examples) can be constructed. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

can be used to control gene expression. Genes under HRE control exhibited controlled expression of the reporter gene to those areas of a solid tumor that were under low oxygen tension [Dachs and Tozer, 2000; Wouters et al., 2002]. The grp78 stress-inducible promoter has been used for the conditional control of suicide therapy [Chen et al., 2000]. Finally, the pH response element ought to be capable of limiting gene expression to those regions of a tissue or tumor that undergo changes in pH [Laterza and Curthoys, 2000].

In summary, a variety of non-invasive imaging modalities have been under development for some time now [Wrenn et al., 1951; Hinshaw et al., 1978; Hoffman and Phelps, 1986]. A general goal continues to be focused on devising methods that resolve, in real time, the functional status of individual tissues and organ systems in vivo at the molecular level. As we learn more about the differential expression of cell specific receptors and thus, tissue specific receptors, our ability to view the functional status or diseased state of these living systems with highly specific probes will continue to increase. Moreover, the use of conditional expression systems will heighten our abilities to achieve specificity and will provide us with probing systems that will allow us to better understand the tumor microenvironment as well as the cellular alterations that occur during tumor biogenesis.

ACKNOWLEDGMENTS

We thank Dr. Zaver Bhujwalla, Dr. Dmitri Atremov, Dr. Richard Roden, Dr. Saraswati Sukumar, Dr. Chris Umbricht, and Dr. Scott Kominsky for critically reading the manuscript and helpful discussions.

REFERENCES

Anderson LM, Swaminathan S, Zackon I, Tajuddin A-K, Thimmapaya B, Weitzman SA. 1999. Adenovirus-mediated tissue-targeted expression of the HSV*tk* gene for the treatment of breast cancer. Gene Therapy 6:854–864.

Baxter LT, Jain RK. 1996. Pharmacokinetic analysis of the microscopic distribution of enzyme-conjugated antibodies

and prodrugs: Comparison with experimental data. Br J Cancer 73:447–456.

Centonze VE, White JG. 1998. Multiphoton excitation provides optical sections from deeper within scattering specimens than confocal imaging. Biophys J 75:2015–2024.

Chatziioannou AF. 2002. Molecular imaging of small animals with dedicated PET tomographs. Eur J Nucl Med 29:98–114.

Chen X, Zhang D, Dennert G, Hung G, Lee AS. 2000. Eradication of murine mammary adenocarcinoma through HSVtk expression directed by the glucose-starvation inducible grp78 promoter. Breast Cancer Res Treat 59:81–90.

Choi S-R, Yang B, Ploss LK, Chumpradit S, Wey S-P, Acton PD, Wheeler K, Mach RH, Kung HF. 2001. Development of a Tc-99m labeled sigma-2 receptor-specific ligand as a potential breast tumor imaging agent. Nucl Med Biol 28:657–666.

Cumming P, Wong DF, Dannals RF, Gillings N, Hilton J, Scheffel U, Gjedde A. 2002. The competition between endogenous dopamine and radioligands for specific binding to dopamine receptors. Ann NY Acad Sci 965: 440–450.

Dachs GU, Tozer GM. 2000. Hypoxia modulated gene expression: Angiogenesis, metastasis and therapeutic exploitation. Eur J Cancer 36:1649–1660.

Dodeigne C, Thunus L, Lejeune R. 2000. Chemiluminescence as diagnostic tool. A review. Talanta 51:415–439.

Edwards PAW. 1985. Heterogenous expression of cell-surface antigens in normal epithelia and their tumours, revealed by monoclonal antibodies. Br J Cancer 51:149–160.

Garcia-Garayoa E, Blauenstein P, Bruchlmeier M, Blanc A, Iterbeke K, Conrath P, Tourwe D, Schubiger A. 2002. Preclinical evaluation of a new, stabilized neurotensin (8–13) pseudopeptide radiolabeled with ^{99m}Tc. J Nucl Med 43:374–383.

Guilloteau D, Emond P, Baulieu J-L, Garreau L, Frangin Y, Pourcelot L, Mauclaire L, Besnard J-C, Chalon S. 1998. Exploration of the dopamine transporter: In vivo and in vitro characterization of a high-affinity and high-specificity iodinated tropane derivative (E)-*N*-(3-iodoprop-2-eny)-2β-carbomethoxy-3β-(4′-methylphenyl)-nortropane (PE2I). Nucl Med Biol 25:331–337.

Hazra DK, Sharma RC. 1982. Specific tumour targeting in imaging and therapy with special reference to monoclonal radionuclidic agents. Nucl Med Commun 3:210–223.

Hinshaw WS, Andrew ER, Bottomley PA, Holland GN, Moore WS. 1978. Display of cross sectional anatomy by nuclear magnetic resonance imaging. Br J Radiol 51: 273–280.

Hoffman EJ, Phelps ME. 1986. In: Positron emission tomography and autoradiography: Principles and applications for the brain and heart. Phelphs ME, Mazziotta J, Schelbert H, editors. New York: Raven Press.

Holm J, Hansen SI, Hoier-Madsen M, Birn H, Helkjaer P-E. 1999. High-affinity folate receptor in human ovary, serous ovarian adenocarinoma, and ascites: Radioligand binding mechanism, molecular size, ionic proerties, hydrophobic domain, and immunoractivity. Arch Biochem Biophys 366:183–191.

Iozzo P, Osman S, Glaser M, Knickmeier M, Ferrannini E, Pike VW, Camici PG, Law MP. 2002. In vivo imaging of insulin receptors by PET: Preclinical evaluation of iodine-125 and iodine-124 labeled human insulin. Nucl Med Biol 29:73–82.

Iyer M, Barrio JR, Namavari M, Bauer E, Satyamurthy N, Nguyen K, Toyokuni T, Phelps ME, Herschman HR, Gambhir SS. 2001. 8-[^{18}F]fluoropenciclovir: An improved reporter probe for imaging HSV1-*tk* reporter gene expression in vivo using PET. J Nucl Med 42:96–105.

Jacques V, Desreux JF. 2002. New classes of MRI contrast agents. Topics Curr Chem 221:123–164.

Konda SD, Wang S, Brechbiel M, Wiener EC. 2002. Biodistribution of a ^{153}Gd-folate dendrimer, generation = 4, in mice with folate-receptor positive and negative ovarian tumor xenografts. Invest Radiol 37: 199–204.

Lane DP, Lain S. 2002. Therapeutic exploitation of the p53 pathway. Trends Mol Med 8(Suppl):S38–S42.

Laterza OF, Curthoys NP. 2000. Effect of acidosis on the properties of glutaminase mRNA pH-response element binding protein. J Am Soc Nephrol 11:1583–1588.

Leamon CP, Parker MA, Vlahov IR, Xu L-C, Reddy JA, Vetzel M, Douglas N. 2002. Synthesis and biological evaluation of EC20: A new folate-derived, ^{99m}Tc-based radiopharmaceutical. Bioconjug Chem 13:1200–1210.

Lemmer K, Ahnert-Hilger G, Hopjner M, Hoegerle S, Faiss S, Grabowski P, Jockers-Scherubl M, Riecken EO, Zeitz M, Scherubl H. 2002. Expression of dopamine receptors and transporter in neuroendocrine gastrointestinal tumor cells. Life Sci 71:667–678.

Liang Q, Satyamurthy N, Barrio JR, Toyokuni T, Phelps MP, Gambhir SS, Herschman HR. 2001. Noninvasive, quantitative imaging in living animals of a mutant dopamine D_2 receptor reporter gene in which ligand binding is uncoupled from signal transduction. Gene Ther 8:1490–1498.

MacLaren DC, Gambhir SS, Satyamurthy N, Barrio JR, Sharfstein S, Toyokuni T, Wu L, Berk AJ, Cherry SR, Phelps ME, Herschman HR. 1999. Repetitive, non-invasive imaging of the dopamine D_2 receptor as a reporter gene in living animals. Gene Ther 6:785–791.

Ozturk-Winder F, Renner M, Klein D, Muller M, Salmons B, Gunzburg WH. 2002. The murine whey acidic protein promoter directs expression to human mammary tumors after retroviral transduction. Cancer Gene Ther 9:421–431.

Plenevaux A, Weissmann D, Aerts J, Lemaire C, Brihaye C, Degueldre C, Le Bars D, Comar J-F, Luxen A. 2000. Tissue distribution, autoradiography, and metabolism of 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2′-pyridinyl)-*p*-[^{18}F]fluorobenzamido]ethyl]piperazine (*p*-[^{18}F] MPPF), a new serotonin 5-HT_{1A} antagonist for positron emission tomography: An in vivo study in rats. J Neurohem 75:803–811.

Raderer M, Kurtaran A, Leimer M, Angelberger P, Niederle V, Virhapper H, Vorbeck F, Hejna MHL, Scheithauer W, Pidlich J, Virgolini I. 2000. Value of peptide receptor scintigraphy using ^{123}I-vasoactive intestinal peptide and ^{111}In-DTPA-D-Phe^1-octreotide in 194 carcinoid patients: Vienna University experience, 1993 to 1998. J Clin Oncol 18:1331–1336.

Rogers BE, Rosenfeld ME, Khazaeli MB, Mikheeva G, Stackhouse MA, Liu T, Curiel DT, Buchsbaum DJ. 1997.

Localization of iodine-125-mIP-Des-Met[14]-bombesin (7–13)NH_2 in ovarian carcinoma induced to express the gastrin releasing peptide receptor by adenoviral vector-mediated gene transfer. J Nucl Med 38:1221–1229.

Satyamurthy N, Bida GT, Barrio JR, Luxen A, Mazziotta JC, Huang S-C, Phelps ME. 1986. No-carrier-added 3-(2′-[^{18}F]fluoroethylspiperone, a new dopamine receptor-binding tracer for positron emission tomography. Nucl Med Biol 13:617–624.

Senogles SE. 1994. The D_2 dopamine receptor isoforms signal through distinct $G_{i\alpha}$ proteins to inhibit adenylyl cyclase. J Biol Chem 269:23120–23127.

Stackhouse MA, Buchsbaum DJ, Kancharla SR, Grizzle WE, Grimes C, Laffoon K, Pedersen LC, Curiel DT. 1999. Specific membrane receptor gene expression targeted with radiolabeled peptide employing the erb-2 and DF3 promoter elements in adenoviral vectors. Cancer Gene Ther 6:209–219.

Steiner MS, Zhang Y, Farooq F, Lerner J, Wang Y, Lu Y. 2000. Adenoviral vector containing wild-type *p16* suppresses prostate cancer growth and prolongs survival by inducing cell senescence. Cancer Gene Ther 7:360–372.

Thomas GD, Chappell MJ, Dykes PW, Ramsden DB, Godfrey KR, Ellis JRM, Bradwell AR. 1989. Effect of dose, molecular size, affinity, and protein binding on tumor uptake of antibody or ligand: A biomathematical model. Cancer Res 49:3290–3296.

Ugur O, Kothari PJ, Finn RD, Zanzonico P, Ruan S, Guenther I, Maecki HR, Larson SM. 2002. Ga-66 labeled somatostatin analogue DOTA-DPhe^1-Tyr^3-octreotide as a potential agent for positron emission tomography imaging and receptor mediated internal radiotherapy of somatostatin receptor positive tumors. Nucl Med Biol 29:147–157.

Vaidyanathan G, Zalutsky MR. 1997. Fluorine-18-labeled [Nle^4, D-Phe^7]-α-MSH, an α-melanocyte stimulating hormone analogue. Nucl Med Biol 24:171–178.

Weissleder R, Tung C-H, Mahmood U, Bogdanov A, Jr. 1999. In vivo imaging of tumors with protease-activated near-infrared fluorescent probes. Nat Biotech 17:375–378.

Williams LE, Wu AM, Yazaki PJ, Andrew AL, Raubitschek AA, Shively JE, Wong JYC. 2001. Numerical selection of optimal tumor imaging agents with application to engineered antibodies. Cancer Biother Radiopharm 16: 25–35.

Wouters BG, Weppler SA, Koritzinsky M, Landuyt W, Nuyts S, Theys J, Chiu RK, Lambin P. 2002. Hypoxia as a target for combined modality treatments. Eur J Cancer 38:240–257.

Wrenn FR, Jr., Good ML, Handler P. 1951. The use of positron-emitting radioisotopes for the localization of brain tumors. Science 113:525–527.

Wu AM, Williams LE, Zieran L, Padma A, Sherman M, Bebb GG, Odom-Maryon T, Wong JYC, Shively JE, Raubitschek AA. 1999. Anti-carcinoembryonic antigen (CEA) diabody for rapid tumor targeting and imaging. Tumor Target 4:47–58.

Wu AM, Yazaki PJ, Tsai S-w, Nguyen K, Anderson AL, McCarthy DW, Welch MJ, Shively JE, Williams LE, Raubitschek AA, Wong JYC, Toyokuni T, Phelps ME, Gambhir SS. 2000. High-resolution microPET imaging of carcinoembryonic antigen-positive xenografts by using a copper-64-labeled engineered antibody fragment. Proc Natl Acad Sci USA 97:8495–8500.

Wu L, Matherly J, Smallwood A, Adams JY, Bellderun A, Carey M. 2001. Chimeric PSA enhancers exhibit augmented activity in prostate cancer gene therapy vectors. Gene Therapy 8:1416–1426.

Yamagoe S, Kanno T, Kanno Y, Sasaki S, Siegel RM, Lenardo MJ, Humphrey G, Wang Y, Nadatani Y, Howard BH, Ozato K. 2003. Interaction of histone acetylases and deacetylases in vivo. Mol Cell Biol 23: 1025–1033.

Zinn KR, Chaudhuri TR. 2002. The type 2 human somatostatin receptor as a platform for reporter gene imaging. Eur J Nucl Med 29:388–399.

Zinn KR, Buchsbaum DJ, Chaudhuri TR, Mountz JM, Grizzle WE, Rogers BE. 2000. Noninvasive monitoring of gene transfer using a reporter receptor imaged with a high-affinity peptide radiolabeled with ^{99m}Tc or ^{188}Re. J Nucl Med 41:887–895.