REVIEW

In vivo imaging of molecular targets and their function in endocrinology

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Abstract

Imaging is one of the fastest growing fields of study. New technologies and multimodal approaches are increasing the application of imaging to determine molecular targets and functional processes *in vivo*. The identification of a specific target, transporter, or biological process using imaging has introduced major breakthroughs to the field of endocrinology primarily utilizing computed tomography, magnetic resonance imaging, ultrasonography, positron emission tomography, single-photon emission computed tomography, and optical imaging. This review provides a general background to the specific developments in imaging that pertains to *in vivo* function and target identification in endocrine-based diseases.

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Introduction

The field of *in vivo* imaging offers the opportunity to define the morphological size and location of organs and diseased tissue in three dimensions. Accumulating studies indicate that the field of imaging can work toward providing patient-specific information, for example, in cancer the tumor localization, staging, and treatment follow-up response. The ability to detect abnormal masses of cells in the body allows the diagnosis of benign and malignant tumors as well as other changes in endocrinology. Advances in the imaging field provide the chance to couple these morphological datasets with functional biological pathways in an attempt to better understand the properties of specific organs in normal and diseased tissue.

Functional imaging is necessary because macroscopic alterations in tissues from disease are often the end result of changes that occurred in a molecular and signaling profile prior to the increase in growth. Locating and determining the size of an abnormal growth in one tissue might only reflect benign disease and not the need for medical intervention. In addition, knowing the size and location of a particular abnormal tissue would provide little or no information regarding the proper treatment strategy. Therefore, developing *in vivo* imaging modalities that depict changes in target expression and function are necessary for the field of endocrinology.

Imaging modalities

Imaging technologies that report on the regulation of specific pathways involve positron emission tomography (PET), single-photon emission tomography (SPECT), magnetic resonance imaging (MRI), and optical imaging using fluorescence and bioluminescence. PET and SPECT utilize radiolabeled molecules to image the distribution of tracers within the body and report on the expression level of a target or the activity of cells. The advantage of PET and SPECT is the high-sensitivity level for detecting subtle biological changes using limited quantities of the imaging agent. SPECT gathers imaging information based on the amount of gamma-emitting radionucleotides that emit a single photon, but it is generally not as sensitive and less quantifiable than PET. PET detects biochemical processes in cells by measuring the positrons emitted by the probe as it decays and collides with electrons *in vivo* (Doubrovin *et al.* 2004, Herschman 2004). The PET emissions are two photons released 180° from one another and detected in an array, which measure the volume and concentration of the probe (Blasberg 2002). MRI generally requires the use of contrast agents that increase the signal intensity of tissues containing the agent as compared with surrounding tissues (Allen & Meade 2004). Most contrast agents use gadolinium (III) because of its high magnetic moment and the abundance of unpaired electrons. The ability of gadolinium to increase signal intensity relies on the interaction between unpaired

electrons and water protons resulting in an increase in T1 and a longer signal. In general, gadolinium (III) is stably chelated due to toxicity of free gadolinium in the body. Superparamagnetic species are utilized in MRI as T2 agents. The large magnetic field of a superparamagnetic species decreases the signal intensity in tissues resulting in a contrast enhancement (Allen & Meade 2003, Allen *et al.* 2004). Optical imaging often takes advantage of light transmission through a biological tissue. However, as many samples are hidden from light sources due to the interior location of the body, only small animals that are relatively transparent can be readily imaged. The targeted imaging approaches highlighted in this review are summarized in Fig. 1. Some of the advantages and disadvantages are also summarized in Table 1.

Glucose metabolism

Integral to the field of endocrinology is the process of glucose metabolism. The most commonly used PET agent

for functional imaging clinically is fluorine-18-labeled deoxyglucose (^{18}F FDG or FDG). FDG is a glucose analog that is transported via glucose transporters (GLUTs) such as GLUT-1 and GLUT-3 (facilitated glucose transporter member 1) and type II hexokinase (Tohma *et al.* 2005, Zhao *et al.* 2005). Its metabolite, 2-fluorodeoxyglucose-6-phosphate, is effectively trapped intracellularly and cannot undergo further metabolism. The rapid metabolism and uptake of glucose by cancer cells makes accumulation of the PET agent an excellent marker for malignant disease, dependent on the genetic expression of the various GLUTs (Mankoff *et al.* 2007). The efficiency of the tumor to accumulate FDG provides prognostic value for how well the patient will respond to chemotherapy, as the high metabolically activated state correlates with tumor cell division, the biological target of many chemotherapeutic drugs (Eriksson *et al.* 2005). Despite its widespread use and strengths, FDG-PET does not provide useful information for tumors that are relatively slow growing (Lewington & Clarke 2001). Also, the spatial resolution of PET imaging makes the detection of small tumors difficult, especially if they are located near the

Figure 1 Major classifications of *in vivo* imaging agents for evaluating function. The basic concept behind several methods of functional imaging is shown. The PET agent FDG is transported through GLUT channels and trapped inside the cell to demonstrate glucose metabolism. In neuroendocrine tumors, accumulation of metabolites of several amino acids allows for functional imaging. For diseases with receptor overexpression, imaging agents that bind to either extracellular or intracellular targets offer insight into the expression level of the receptor and possible downstream pathways. Enzyme-activated reporters indicate the function of the protein, for example, matrix metalloproteinases activate reporter agent while degrading extracellular proteins. Finally, reporter genes containing a regulatory element in the promoter linked to an easily assayed protein, such as green fluorescent protein, luciferase, or β -galactosidase. When the gene of interest is transcribed, the reporter protein is activated and can be easily quantified *in vivo*.

Table 1 Advantages and disadvantages of imaging modalities used for functional imaging

	Advantages	Disadvantages
Imaging modality		
Bioluminescence	Spatial and temporal information Low non-specific luminescence Monitors cell viability	Enzyme most commonly used not naturally in humans: engineered Requires injectable substrates and cofactors
Fluorescence	Low cost No tissue damage Multiple fluorophores	Diffraction and penetration through organs difficult Non-specific fluorescence
MRI	High spatial resolution Well tolerated Excellent delineation of anatomical structures	Low (milli or micro molar) sensitivity Mostly restricted to extracellular domains
PET	High sensitivity Readily reaches target	Requires radioactivity Low spatial resolution
SPECT	High sensitivity	Requires radioactivity Less quantifiable than PET

bladder where excretion of the agent can obscure imaging (Israel & Kuten 2007). Finally, FDG more specifically marks cells that express the GLUT-1 transporter as opposed to having a higher metabolism and therefore macrophages and sites of inflammation are sometimes confused for tumors. Often neoplastic cells have altered biochemical pathways that result in the upregulation of the GLUT, altered glucose metabolism and even alterations in glycolytic enzymes associated with the hypoxic environment that may activate metabolic steps (Pauwels *et al.* 1998). Despite these drawbacks, FDG remains the most highly utilized functional imaging agent and demonstrates that a known endocrine process can be exploited to develop *in vivo* imaging tools.

Amino acid metabolism

One of the hallmarks of neuroendocrine tumors is the uptake and metabolism of amino acids into their decarboxylated forms also known as amine precursor uptake and decarboxylation (APUD). The term APUD defines a series of cells with endocrine functions based on the secretion of amine or polypeptide hormones. The neuroendocrine tumors include carcinoid tumors, paragangliomas, medullary thyroid carcinomas, and pancreatic islet cell tumors (Tamm *et al.* 2007). Neuroendocrine tumors show a high uptake and metabolism of two well-characterized agents, ^{11}C -L-dopa and ^{18}F -L-DOPA (Becherer *et al.* 2004). PET imaging can successfully image the neuroendocrine tumor tissue and some of the metastases including those in the liver, lymph, and bone clinically (Nanni *et al.* 2006). Another amino acid utilized in the study of neuroendocrine tumors is the ^{11}C -5-hydroxytryptophan (5-HTP) serotonin derivative (Sundin *et al.* 2007). In comparative studies, PET imaging with ^{11}C -5-HTP had a higher sensitivity than computed tomography (CT) and better determined liver metastases

(Orlefors *et al.* 1998). The ability of ^{11}C -5-HTP to image both primary tumors and metastasis was superior to that of the L-dopa compound; however, excretion of the tracers in the urine obscured detection of kidney satellite populations of tumor cells (Sundin *et al.* 2004, Krausz & Israel 2006). Studies utilizing a compound called carbidopa, an inhibitor of the urinary metabolite of ^{11}C -5-HTP, significantly reduced renal signal obstruction and increased the concentration of the PET agent in the tumor (Orlefors *et al.* 2006). Amino acid and glucose metabolism are both examples of how a known function or genetic alteration in endocrine disorders can be exploited by *in vivo* imaging agents such that they accumulate and are targeted to specific cell types for functional imaging.

Cell surface receptors

Cell surface receptors represent one type of target for imaging agents that provide information about signal pathway expression (Groves *et al.* 2007). While the expression of the receptor alone is often an important parameter for staging and disease diagnosis, it does not directly indicate pathway activation. However, internalization of receptors in response to ligand binding is one mechanism for sequestering imaging agents. In addition, overexpression of a receptor often represents the upregulation of the gene encoding for that receptor, which frequently corresponds with the aggressive state of a tumor.

Somatostatin receptors have been identified on the surface of many neuroendocrine tumors and represent a valid target for imaging. The most common analog produced to bind to somatostatin receptors is octreotate (^{111}In -pentetreotide), which preferentially binds type 2 somatostatin receptors (Rufini *et al.* 2006). New variations on the ^{111}In -labeled drug utilizing

beta-emitting radiolabels like ^{90}Y or ^{177}Lu improve the therapeutic profile such that decreases in tumor size after treatment can be measured using ^{111}In -labeled existing PET tracers in the clinic (Van Essen *et al.* 2007). Whole-body imaging can be performed after administration of the agent to visualize tumors and metastasis expressing high levels of the receptor (de Herder *et al.* 2005). Identifying small metastatic populations of cells based on overexpression of a cell surface receptor is a major breakthrough for *in vivo* imaging agents.

Tyrosine kinase receptors are another popular cell surface target for the treatment of both breast and prostate cancers. Many generations of preclinical epidermal growth factor (EGF) imaging agents targeting the receptor family have been engineered as well as combinations of imaging agents including PET, SPECT, MRI, and CT (Cai *et al.* 2007). Both endogenous ligands and drugs have been coupled to various imaging modalities to measure EGF receptors. The monoclonal antibody herceptin (trastuzumab) has also been used to create an imaging agent specific for the Her-2/neu (ErbB2) receptor (Artemov *et al.* 2003a,b, Funovics *et al.* 2004). Due to the incredibly complicated signaling network regulated by the EGF receptor family and the cross-talk with other signaling pathways, expression of these receptors may not translate into valuable information for treatment. Therefore, the tyrosine kinase family of imaging agents offers one key example of how imaging a receptor may provide little information on specific downstream pathways, because the receptor is present in many isoforms that each activate unique and complicated signaling cascades. However, expression of the receptor and its subsequent inactivation does have therapeutic potential in mitigating cancers regardless of the downstream pathway information thus illustrating the goal of imaging receptors in the absence of specific downstream signaling information.

Intracellular receptors

Intracellular receptors are a separate category of imaging agents that must both target a relevant binding partner and cross the cell membrane. The estrogen receptor is an intracellular receptor that is used to diagnose and stage breast cancers. Because the current methodologies for screening estrogen and progesterone receptor require invasive biopsies, identification of an *in vivo* imaging agent could improve the diagnosis of the disease. Several PET imaging agents have been developed preclinically to evaluate the expression of these nuclear intracellular receptors such as ^{18}F -fluoroestradiol, $^{99\text{m}}\text{Tc}$, and ^{68}Ga -flutamate peptide-estradiol, and ^{18}F -16 α -ethyl-19-norprogesterone (Jonson & Welch 1998, Hostetler *et al.* 1999, Jonson *et al.* 1999, Skaddan *et al.* 2000). While many

of these molecules have been plagued with metabolic inactivation, binding to serum hormone binding globulin, and poor chemical synthetic yield, positive results have been reported for 18-fluoroestradiol (FES). A study performed on two patients utilizing ^{18}F -FES and ^{18}F -FDG detected metabolically inactive, estrogen receptor (ER)-positive tumor populations (Kumar *et al.* 2007). Two MRI agents have been developed for targeting progesterone receptor, but both need validation that they can detect subtle changes in receptor levels before their ability to image tumors can be substantiated (Lee *et al.* 2005, 2007). MRI generally is considered not sensitive enough to detect small changes in receptor concentration but immobilization and concentration inside of cells in the low micromolar dose might still generate viable images (Hanaoka *et al.* 2008). Radiolabeled tamoxifen agents have also been derived for PET imaging and may improve treatment decisions for patients responsive to the therapy (Van de Wiele *et al.* 2001). Aromatase imaging agents are under investigation preclinically and current options take advantage of binding inhibition to demonstrate where the aromatase protein is expressed as opposed to monitoring the enzyme activity (Takahashi *et al.* 2006, Wang *et al.* 2007). Androgen receptor PET agents have also been created but indicate whether a therapy is reducing androgen levels as opposed to staging prostate cancers, because androgen receptors are usually present even in androgen-independent cancers (Van Den Bossche & Van de Wiele 2004, Parent *et al.* 2007). Prostate cancer differs from some other hormonally regulated cancers in that it retains expression of the androgen receptor even in the context of low circulating androgens (Kaarbo *et al.* 2007). Importantly, while steroid receptors are often important in the staging and responsiveness of endocrine-related cancers, they may also be utilized to study developmental processes and other diseases due to their broad activity in endocrinology (Takahashi *et al.* 2007).

Enzyme catalysis

One specific methodology for imaging function involves capturing the activity of enzymes important in endocrinology (Torigian *et al.* 2007). Most of the enzyme-activated agents are designed to demonstrate the activity of proteins important in cancer biology. However, the general concept of applying functional groups onto imaging agents for activation by an enzyme has broad-ranging implications for the field of endocrinology. For example, the matrix metalloproteinases are critical molecules in development and cancer, which allow cells to degrade extracellular matrix proteins and invade surrounding areas. Enzyme-activated matrix metalloproteinase (MMP) tracers are now in the development

of both the MR and PET fields as diagnostic tools for human disease (Breyholz *et al.* 2007). Another enzyme activity imaging strategy involves caspases, enzymes responsible for DNA degradation during apoptosis (Faust *et al.* 2007). The imaging probe selectively binds to the activated enzyme thereby demonstrating where *in vivo* apoptosis is occurring as part of disease or therapy. A final example uses an enzyme-activated form of the substrate for β -galactosidase. When a sugar moiety is cleaved by the β -galactosidase enzyme, a gadolinium ion in the imaging agent is now capable of interacting with water molecules increasing T1 signal enhancement using MR (Louie *et al.* 2000). Generating enzyme-activated substrates offers one of the most promising methods for measuring function as it focuses on the protein activity rather than the amount of a receptor or a transporter (Louie 2006). However, the field is still developing and reagents directly applicable for endocrine diseases are still sparse.

Gene promoters via marker proteins

The imaging field is most likely to generate functional data in the area of reporter gene constructs. The system usually combines a reporter protein, which is an easily assayed gene, controlled by a regulatory element of a promoter of interest (Fig. 2; Rome *et al.* 2007). Expression of the gene is measured and correlates with the transcriptional activity of the gene under investigation. The most common reporter proteins are green fluorescent protein (GFP), luciferase, thymidine kinase, and β -galactosidase. The choice of the reporter and the specific agent or biological process used to activate the expression will influence whether the system is imaged with PET, MRI, or optical techniques. Because expression relies on the genetic engineering of the reporter and the promoter, most applications are relevant to animal models and cell lines. First, the technology can be applied at both the cellular and whole-body level. Neurons

Figure 2 Basic strategy for utilizing reporter gene as *in vivo* imaging agent. The reporter gene contains the promoter of a gene of interest linked to an easily assayed protein. The reporter gene is then transfected into the target cell through transgene expression in a mouse, introduction by a virus, or transiently into cell populations. Promoters can be constantly activated or inducible so that function could be imaged continuously or during a specific biological process. Reporters are then detected usually in the presence of the substrate for the reporter protein, i.e., luciferin is injected into mice expressing luciferase. The functional activation of the reporter is then imaged accordingly based on the selected protein.

responsible for responding to glucocorticoid in the hippocampal portion of the brain have been imaged using GFP driven by a specific promoter to detect neuroendocrine function *in vivo* (Nishi *et al.* 2007). The *in vivo* differentiation of sexually dimorphic gonads was similarly visualized using the GATA-4 promoter linked to GFP in mice (Mazaud Guittot *et al.* 2007). When the promoter is expressed in many tissues then whole-body imaging can be developed. The vascular endothelial growth factor (VEGF) promoter was linked to GFP and illustrated the importance of this gene during vascularization of the human embryo and again during mammary carcinogenesis (Faley *et al.* 2007). The luciferase and β -galactosidase genes have been introduced into mice under the control of the estrogen response element creating animals with bioluminescence or colorimetric changes in tissues that are activated by estrogens, genistein, and bisphenol A (Ciana *et al.* 2001, 2003, Nagel *et al.* 2001, Lemmen *et al.* 2004b). The ERE-reporter mice have been instrumental in elucidating where and when estrogens act in both males and females during development and after exposure to environmental toxins (Ciana *et al.* 2003, Lemmen *et al.* 2004a). Whole-body imaging can be time dependent when the reporter construct is not visualized unless its substrate has been injected and then the promoter of interest is depicted in a tissue- and time-specific manner (Zhang *et al.* 2001). Chemical modifications of luciferase are now available such that the protein is secreted allowing for its quantification in the serum of transgenic animals (Wurdinger *et al.* 2008). The generation of reporter technology for animals and cell lines is almost boundless, however, the requirement to have transgenic promoters driving expression of a reporter protein limits its direct application in humans. A major advantage of utilizing reporter genes is that the signal demonstrates that the protein required for luminescence is functional *in vivo* and that the promoter driving its expression is activated in the physiological context.

Cell tracking

Stem cells, cultured primary cells, or any therapeutic cells engineered with reporter genes can be utilized to image populations reintroduced into the human, so they may non-invasively be trafficked and identified. When individuals have cells transplanted into diseased tissue for regrowth, such as β -islet transplantation, the cells can be labeled with different reporter proteins to indicate whether the transplant is in the correct location (Acton & Zhou 2005). Pancreatic cells labeled with GFP and luciferase have been utilized both to study development and tissue survival (Puri & Hebrok 2007). In the field of reproductive biology, fertility studies indicating the viability of cryopreserved and vitrified green fluorescent

eggs have been reported after transplantation into wild-type animals (Hani *et al.* 2006). Utilizing reporter genes to display whether a transplanted tissue or cell type remains viable or trafficked to the correct location offers a promising clinical application for *in vivo* imaging of function in endocrinology.

Multimodal approaches

With the advent of new technologies, the opportunity to combine approaches and generate sophisticated targeted imaging agents is possible. Probably the most exciting use of *in vivo* imaging will combine multiple types of technologies and imaging approaches to generate information with the highest possible specificity, resolution, and targeted biological function. Each imaging modality has positive and negative attributes in terms of spatial resolution, image intensity, sensitivity, and the clearance rate from the body (Frullano & Meade 2007). In addition, the technique used might favor one imaging modality over another (Seevinck *et al.* 2007). Multimodal agents might conjugate two imaging tracers together such that one molecule contains both a PET and MR ion. Alternatively, the imaging agents could combine nuclear tracers (PET) and optical imaging molecules like tetramethylrhodamine. Surgical removal of a tumor with a specific signature of receptors and transporters could be identified through PET or MR and then during the procedure further demarcated under fluorescent light with optical imaging to fine tune the resection (Josephson *et al.* 2002, Kircher *et al.* 2003). Combining more than one type of scan with CT and PET, or MRI might integrate superior imaging modalities for localization and functional properties of the diseased tissue (Acton & Zhou 2005). For example, X-ray tomography and MR imaging might provide the best contrast of both the bone and soft tissue. If multiple functional imaging modalities were utilized, a combination of attributes about a diseased organ could be explored. For example, a breast tumor could be imaged for estrogen and progesterone receptors with two agents, or a tumor could be quantified for metabolic activity and expression of the Her2/neu receptor. Multimodal agents might also integrate aspects of therapy along with imaging properties, so that the efficacy of a drug to shrink a tumor might be imaged *in vivo* (Frullano *et al.* 2006). Multimodal applications could also link one imaging strategy with an alternative method of measurement such as the creation of a secreted form of luciferase that can be measured quantitatively in the blood upon transgene activation (Wurdinger *et al.* 2008). The application could be extended even further in the presence of transgenic cell lines and animal models to study the development of one organ, the action of a drug on that organ, and the

effect of the drug on the physiology of the tissue *in vivo* (Dickinson 2006).

Conclusions

Endocrinology is a fast-growing field. The technologies are now being developed to determine the gene expression and function of important biomarkers for endocrine disorders. As more technologies develop in the preclinical stage, the opportunity will arise for imaging to increase individualized medicine with clinicians utilizing specific functional imaging agents to identify the location and exact alteration in a person's health. In some cases, the imaging breakthroughs will improve upon quantitation, while others will elucidate a function gone awry as in cancer or glucose metabolism. Because more types of imaging will be utilized together in a cogent manner to describe human health, the integration of technology and researchers at all levels will be critical. The field will rely heavily in the future on the integration of many areas of science including biology, chemistry, imaging, pharmacokinetic clearance, and medicine. Another challenge will be developing technologies that have succeeded in small animal models to the phase where they are applicable to humans. The process of bringing each new imaging agent through Food and Drug Administration approval for use in the clinic could be hastened by combining existing agents using multimodal approaches. Endocrinology will benefit greatly from enhanced early detection, functional analysis, and localization of signaling changes that the field of imaging brings to this diverse discipline.

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