A Database of Imaging Reporters and Multimodal Molecular Bio-Imaging

R.Sharma[1], A.Sharma[2], C.J.Chen[3]

[1]Engineering and Technology division, TCC/Center of Nanomagnetics and Biotechnology, Florida State University, Tallahassee, FL 32304 USA
[2]Nanotechnology Lab, Maharana Pratap A&T University, Udaipur, Rajasthan, India
[3]Department of Mechanical Engineering, FAMU-FSU College of Engineering, Tallahassee, FL 32304 USA

ABSTRACT

Oligonucleotides are biomarkers in gene expression imaging and proteins for imaging proteomics based on these end product molecules as visible by MRI and PET to give structural and metabolic information in diseased tissue. We compiled the database based on oligonucleotides and proteins, their biochemical electrophoretic properties and matched measurable imaging signal properties (relaxation constants for MRI; SUV for PET; and OD for bioluminescence) generated by different proteins and oligonucleotides available for bioimaging of tumors. The novelty of using database is the unique selection of molecule(s) as biosensor or reporter with its specific sensitivity to the imaging modality to translate the mechanistic chemical details on digitized maps (image) from tissue in non-invasive manner. The database has utility in developing new modalities of tumor diagnostic imaging. *Key words: proteins, oligonucleotides, MRI-PET, multimodal imaging, gene expression*

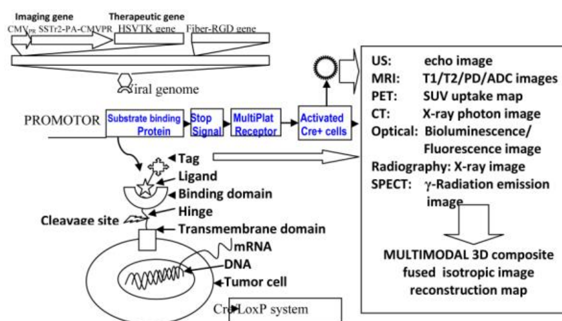
1 INTRODUCTION

The molecular imaging exploits the use of specific molecular probes to generate the image contrast. The molecular probes utilize the imaging technology specific microscopic physical, physiological, metabolic events and changes to differentiate pathology form normal tissue. This approach has enhanced the utility of imaging technique from nonspecific macroscopic information to specific micro- and nanoscopic scale with details of specific molecular event. Molecular imaging is based on imaging transgene expression. The present paper highlights the need of a database with measurable resource of oligonucleotide and protein molecules with utility in bioimaging. With this aim, molecular imaging techniques and their applications are identified and defined for: 1. noninvasive in vivo imaging methods for specific molecular gene expression and protein-protein interactions; 2. to monitor time-based several molecular events in the tissues; 3.to visualize and follow-up trafficking and targeting of cells; 4. to optimize conditions of gene therapy and monitoring drug therapeutics; 5. to develop rapid drug chemosensitivity assay to image the drug effect at cellular and molecular level. In this direction, an imaging technique is reported to visualize a cell in animal or human body including oral, intraperitoneal, intravenous, intrathecal administration of a binding domain ligand into the animal. The binding domain ligand interacts with the binding domain. The ligand on binding domain is visualized based on the fact that ligand acts as secretory or plasma membrane trafficking signal domain. Today, the main challenge in developing molecular imaging techniques is to achieve goal of imaging in noninvasive, rapid, reproducible and quantitative manner that can give information in time-dependent experimental, developmental, environmental and therapeutic influence on gene products and proteins in tumors of animals or patients. The database of molecules as potential reporters by MRI, PET, bioluminescence will be useful resource of selecting molecules to develop techniques of molecular imaging the cells or molecular events during influence of drugs, metabolism, physiology, experimental, environmental and genetics in the body.

What is needed in imaging transgene expression reporter? -<u>An extracellular, membrane bound protein for docking with a tagged particle</u>

A detailed description of membrane bound proteins, docking mechanism and tagged particles is available to introduce with the imaging reporter system and its components. Transgene expression reporter molecule can be imaged by using multimodal imaging techniques to evaluate the cell function as shown in Fig 1.

How transgene expression synthesizes reporter molecule in the cell and reportor works?



What molecular imaging database technology offers?

It offers the ability to perform real-time and steady state imaging reporting in at least five different modalities while at the same time offering the ability to control the pharmacokinetics of the substrates utilized.

1. Targeted Modification of Cells: The chimeric receptors were used for targeted modification of cells

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marked by the receptor. The specificity of the ligand-receptor interaction can be used to bring liposomes and other transmembrane delivery vehicles including transmembrane targeting peptides, such as HIV Tat protein, close to the cell membrane of specific cells, thereby accelerating the cellular uptake. Main applications are:

1. Targeted Ablation or Gene Expression Activation in Specific Cells: By labeling the ligand with a molecule which is excited by a specific radiofrequency (e.g. ultrasound) or a specific frequency of electromagnetic radiation (e.g. microwaves or x-rays) it is possible to ablate cells. Cells bearing the chimeric receptor are transplanted into patients and subsequently become harmful (e.g. cancerous) or studies in which cell or tissue specific targeted ablation may be desired (e.g. tumors, diseased tissues etc.). This technology can also be used to activate engineered, heat-shock genes by using a lower dose of radiofrequency/radiation. By labeling the ligand with a molecule which is excited by a specific radiofrequency or specific frequency of electromagnetic radiation and using a lower dose of radiation (shorter time and/or intensity), can increase the temperature to activate heat shock genes[1]. Receptors can target cells for one or two step destruction. The single step ligand-mediated delivery of any toxin or anticellular agent, such as diphtheria toxin, DNAase or barnase and two step delivery of an iron oxide particle to the cell surface destroy cells with exposure to radiofrequency or microwave radiation. It leads to iron oxide particle vibration and heat-mediated destruction of the tagged cells, for example delivery of thymidine kinase, which upon internalization leads to cell death in the presence of administered gancyclovir.

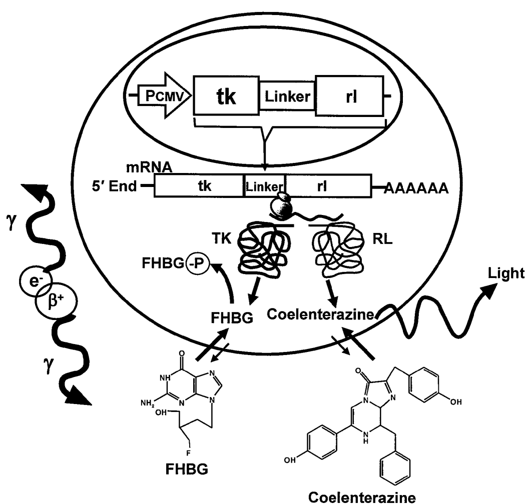

Figure 2: A sketch of multimodal imaging reporter system is shown measuring gene expression by 2 imaging techniques when two reporter genes are expressed from fusion of vector, tk (for PET) with rl luciferase (for optical) with help of polynucleotide coding for spacer and CMV driven expression. After transcription, mRNA translation makes polypeptide having partial fused proteins[2].

2. Molecular Imaging and Cancer: Molecular mechanisms involved in different types of cancer is the key in identifying and targeting various steps in cancer progression for therapeutic intervention. Therapeutic treatments can be monitored for efficacy as related to these specific mechanisms and signal transduction pathways. Mouse disease models can be raised as research tool for monitoring microinvasion and micrometastasis. The in-vivo, multi-modality reporter allows early detection of cancer progression in mouse cancer models, and consequent monitoring of therapeutic treatment efficacy in these models. Mice shows expression of chimeric receptor on cancer cells (FIG. 1) and facilitates imaging by multiple modalities (e.g. CT/MRI/PET/Optical). Chimeric receptor is single pass transmembrane domain and an extracellular maltose-binding domain. Activation of this reporter in pre-malignant cells detects proliferation, micro-invasion and micrometastasis. The chimeric receptor's ligand, maltose can be labeled with gadolidium, iodine, radiolabeled substrates, a red fluorescent protein or cy5.5 (probe). Labeled maltose probes are then intravenously injected into the genetically engineered mice. The labeled maltose binds with tumor cell-specific chimeric receptors. Consequently, this binding activity allows for real-time multi-modality in vivo imaging using MRI, CT, PET, fluorescent or optical imaging (depending on the label bound to the maltose injected). The use of steady-state tumor cell reporting (visualization of specific gene expression) in vivo using anatomically high resolution imaging (CT/MRI) and molecularly sensitive (PET/Optical) imaging emerged as technical advance. Currently, the best available technologies are limited to three different non-high resolution modalities (PET/Luminescent/Fluorescent) using a tri-fusion protein. Through the use of a labeled ligand, temporal control of contrast-enhanced multi-modal imaging offers greater flexibility to visualize cell populations in living transgenic animals through a minimally invasive means to study the steady-state of tissues, the health of specific tissues in animal models of diseases. Multimodal imaging offers in-depth study of the molecular mechanisms involved in tumorigenesis and cancer progression (e.g. metastasis). Knowledge gained through the application of this technology to monitor drug efficacy leads to better treatment, resulting in the inhibition of cancer progression and ultimately cancer regression. This technology can also be applied in a variety of other applications including real-time in vivo gene expression analysis for physiologic and disease models.

3. **Database of Imaging Reporters:** Imaging reporters can visualize a tissue or cell in vivo. Imaging reporter itself can be identified by fluorescence or radiolabeling

[1] http://wwwmgs.bionet.nsc.ru/mgs/papers/stepanenko/hs-trrd/

[2] US patent 2009/7524674:Gambhir SS, Ray P. http://www.patentstorm.us/patents/pdfs/patent_id/7524674.html

inside the cell or tissue. Imaging reporters are made up of a transmembrane domain with hinge domain, a binding domain, reporter binding sites, antibody recognition sites, specific cellular trafficking signals, or domains that can be used for purification. Following description is devoted on database of imaging reporter proteins with detail of maltose binding protein.

a) Transmembrane Domains: Available transmembrane sequence include tyrosine kinase receptors, single-pass domains such as the tyrosin family (epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR)), g-protein receptor; multi-pass domains, such as G-proteins, or other such transmembrane domains[3].

b) Binding Domains: Binding domain bind with maltose (with maltose binding protein), biotin (with avidin), glutathione (with GST), or hyaluran polymers (with cd-44 or RHAMM protein). The substrate is linked with imaging tags such as fluorescence or radiography, cy5.5, iodine for animal computed tomography, a stable isotope for SPECT or PET imaging, gadolidium for magnetic resonance imaging. In following section, binding proteins are described as maltose binding-, periplasmic-, streptavidin, cd-44, antibody proteins as examples.

Binding proteins may be present in animal or may not be present in the animal binding domains and they bind with nontoxic substrate to the animal. Maltose Binding Protein (SEQ ID NO:15) binds with its ligand maltose, a nontoxic metabolite in mammals. Maltose binding protein is a periplasmic bacterial protein encoded by a 1.2 kb gene. It is known to bind several substrates including linear maltodextrins of two to at least seven alpha 1,4 linked glucosyl units, for example, maltose, maltotriose, and maltohexose, as well as cyclic maltodextrins such as cyclomaltohexose and cyclomaltoheptaose with high affinities (K_d=1.6-40×10^{-7} M) [1]. Mutant maltose binding proteins can bind substrates with higher affinities [2]. The mutations of the last two altered base pairs converting Met (M) (position 347 of SEQ ID NO:1) and Gln (Q) position 351 of SEQ ID NO:1) to Ala (A) results in an increased affinity for maltose of K_D=70 nm (from 1200 nm). Deletion of the first four amino acids (Glu (E) (position 198 of SEQ ID NO: 1), Asn (N) (position 199 of SEQ ID NO: 1), Lys (K) (position 201 of SEQ ID NO: 1) and Tyr (Y) (position 202 of SEQ ID NO: 1)) results in an increased affinity for maltose of K_D = 110 nm. Combination of both these mutations results in an increased affinity for maltodextrins and especially an increase in affinity for maltotriose of K_D = 6 nm. Three dimensional structure of maltose binding protein (critical bonds and molecular interactions between the substrates maltose, maltotriose and maltodextrose and maltose binding protein) speculate structural and functional relationship at substrate linkage sites for multimodal imaging.

Linear maltodextrins and cyclodextrins are broken down into monomers by enzymes in the intestines of mammals so labeled maltose is an excellent candidate for infusion into the blood. Maltose is also further broken down in the kidney by maltase. Maltose has been shown to be safe for intravenous infusion and distributed in the extracellular space and rapidly cleared from the body by the kidney. Labeled maltose is distributed to cells and subsequently it binds to cells expressing the chimeric protein, while remaining non-bound labeled maltose is rapidly cleared. Maltose is labeled with radioactive isotopes (^{14}C, ^{13}C, ^{15}O), ANDS (Flux Instruments), Spin and amino aromatic compounds. Pharmacokinetics can be expressed by maltase in the bile pathway of the liver causing the catabolism of maltose in the bile ducts of the liver.

2 3D-MOLECULAR IMAGING OF GENE EXPRESSION BIOMARKERS BY NANOPARTICLES

- $\alpha_V\beta_3$ integrin and collagen III molecules in carotid artery wall predict the status of restenosis and mural injury in carotid artery disease. Molecular probes offer the potential to characterize biochemical features by targeting of biochemical epitopes such as perfluorocarbon nanoparticles are echogenic for US, usable for MRI, CTI, SPECT imaging of thrombosis and angiogenesis[3,4].
- New approaches of multi-labeling techniques are emerging for multimodal imaging techniques. Nanotechnology is recently applicable mode of multimodal imaging platform by fusing two or three image types. The superoxide myoglobin based SPIOM particles were prepared with co-precipitation method as previously described in detail [5,6]. Other significant nanoparticles in atherosclerosis are transferrin, meso-2,3-dimercaptosuccinic acid bound anti-human Troponin with linker ethyl-3-[3-dimethylaminopropyl] carboiimide hydrochloride [6,7]. The internalized transferrin receptors are promising tools of transgene expression in imaging[4]. The general structures schemes of nanoparticles are shown as micelles (see Figure 3).
- The iron oxide based nanoparticle MION (monocrystalline iron oxide nanoparticle), were reported for MR imaging with other multimodal imaging techniques. The dextran coating around the nanoparticle was reported crosslinked with epichlorin hydrin aminated as shown in Figure 12, and labeled with near-infrared fluorochrome Vivotag-680 with following characteristics [87].
- Derivatization with the chelator DTPA to attach with radiotracer ^{64}Cu.

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[3] US patent 2008/0260646. Keller C, Hawkes PJ. http://www.freepatentsonline.com/20080260646.pdf

[4] USP 6511967 Weissleder R, Basilion JP, Chiocca EA. http://www.patentstorm.us/patents/pdfs/patent_id/6511967.html

- Iron oxide core provided contrast in MRI (T2, T2*, or steady-state free-precession sequences).
- Fluorochrome for fluorescence imaging, fluorescence microscopy, flow cytometry, and fluorescence-mediated tomography.
- Crosslinked aminated polysaccharide coating for biocompatibility, determined blood half-life, and provided linker for attachment of tracers and potentially affinity ligands.
- ^{18}FDG PET for hybrid PET-CT Imaging on X-PET PET-CT system (Mercury Computer Systems, Carlsbad, Calif).
- MRI Microimaging Studies on 7-T horizontal-bore scanner (Bruker Pharmascan, Billerica, Mass).
- In Vivo Fluorescence Reflectance Imaging, Fluorescence Microscopy, Phosphorimaging. Autoradiography, Flow Cytometry using triple fluorescent labeled imaged with an upright epifluorescence microscope (Eclipse 80i, Nikon, Melville, NY)
- Histopathology to compare the detectable regions and morphology.

3 BOTTLENECKS OF GENE EXPRESSION IN CLINICAL DIAGNOSIS AND HUMAN GENE THERAPY: JOURNEY FROM YEAR 2000 TILL TODAY

- With little success, gene therapy focused primarily on cardiovascular disorders, genetic diseases (hemophilia, cystic fibrosis) and cancer, with particular emphasis on clinical trials and advanced preclinical studies. Phase III clinical trials using retroviral-based suicide gene therapy for cancer revealed no significant therapeutic benefit in patients suffering from glioblastoma multiforme. Nevertheless, new and promising approaches for cancer gene therapy are being developed that rely on the use of targetable vectors and conditionally replicating vectors that replicate specifically in cancer cells [90]. Vector technology, gene expression profiling, sodium symporters, preclinical tumor animal models, injectable nanoparticles, engineered antibody fragments, non-invasive monitored gene delivery systems have emerged as possible clinical gene therapy monitoring tools in future with greater hopes [91-107]. In current decade, major emphasis was focused on development of real-time, 3D-isotropic fusion multimodal PET-MRI-US, CT-PET-MRI imaging, thymidine kinase, robust image processing segmentation and registration algorithms with high diagnostic accuracy with high precision and imaging signal sensitivity to the disease at micron level [108-116].

FUTURISTIC GOALS

Today, much cannot be said about the success of clinical gene therapy and its medical option. Transgene expression mechanism and its components will be better understood and role of different gene expression proteins at metabolic level will speculate minute user-friendly gene manipulation events at regulatory level. Better understanding of gene delivery and its implications will provide opportunity of safe and affordable clinical trials in near future.

4 CONCLUSION

The gene expression reporters have emerged as specific molecular imaging markers for in vivo monitoring of cell function and metabolism and act as contrast agent in imaging techniques. However, gene transcript and translation during gene expression is a complex and less specific process that limits the specificity of gene expression and success of gene delivery based clinical trials. It needs extensive research on safety and specificity of nucleic acid delivery systems with more sensitive imaging reporters to visualize in vivo events inside the cell.

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