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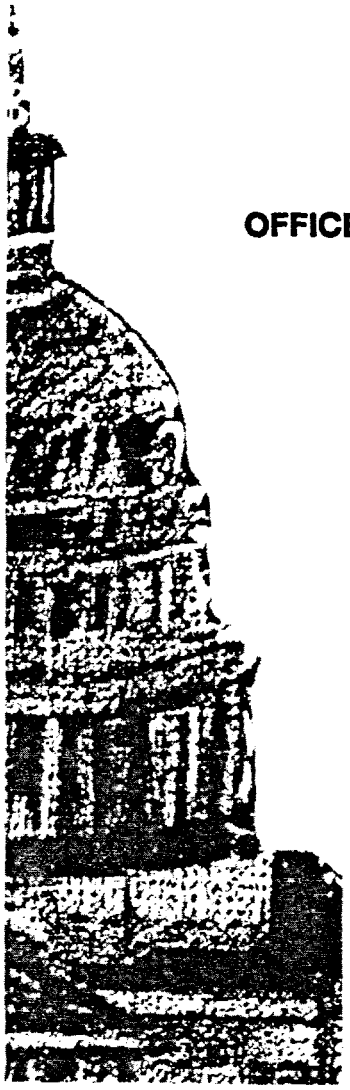
ABSTRACTS



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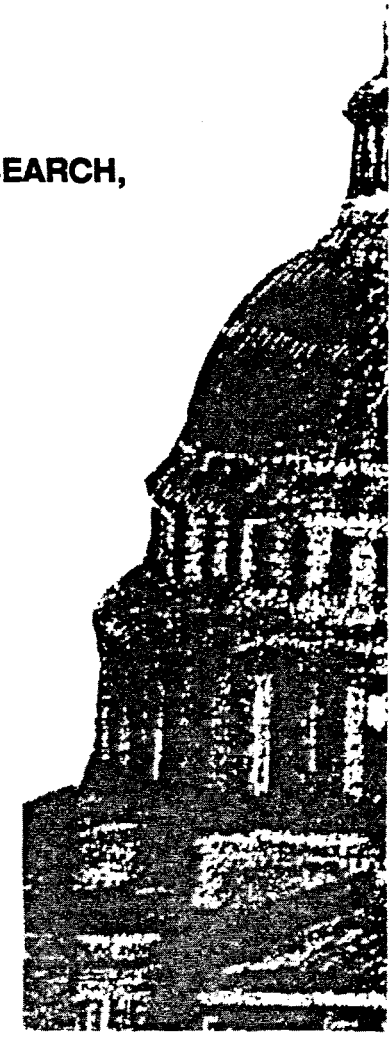

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PRESENTS

FRONTIERS IN NUCLEAR MEDICINE SYMPOSIUM: NUCLEAR MEDICINE & MOLECULAR BIOLOGY

RICHARD C. REBA, M.D., FACNP
PROGRAM CHAIRMAN

PRESENTED AT

STOUFFER MAYFLOWER HOTEL
WASHINGTON, DC
SEPTEMBER 9, 1993

FRONTIERS IN NUCLEAR MEDICINE SYMPOSIUM: NUCLEAR MEDICINE & MOLECULAR BIOLOGY

Contents

Program Chairman - RICHARD C. REBA, M.D., FACNP

Growth Factor Mitogenic Pathway and Oncogenesis

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The growth and differentiation of normal cells are under the control of growth factors. Tumor cells, however, have escaped the growth factor dependence and grow out of control or abnormally. A central issue in understanding how growth factors regulate cell growth and differentiation and how a normal cell can be transformed to become a cancer cell is the mechanism of signal transduction. The signals are initiated at the cell surface and transmitted, through the cytoplasm, to the nucleus, leading to a cascade of events taking place inside the cell and ultimately resulting in cell growth and differentiation. The goal of our research is to delineate the signal transduction pathway by growth factor receptors.

A large group of serum growth factors stimulate the cellular responses by binding to and activating a family of cell surface receptors which possess an intrinsic protein tyrosine kinase activity. These receptors are composed of an extracellular domain for ligand binding, a single transmembrane segment which anchors the receptor through the membrane and a cytoplasmic tyrosine kinase domain, essential for signal transduction. We have studied the early events of receptor tyrosine kinase signal transduction including 1) the mechanism of transmembrane signaling; 2) interaction of the receptor tyrosine kinase and down-stream signaling molecules; 3) mechanism of communication between the receptor and "mitogenic switch" $p21^{ras}$.

It is clear now that ligand-induced receptor dimerization is responsible for activation of the receptor tyrosine kinase and transmitting the signal across the membrane. The direct evidence supporting the dimerization model comes from the demonstration that a functionally defective receptor can block the signal transduction of its wild-type counterpart. The receptors for colony-stimulating factor-1 (CSF-1) and platelet-derived growth factor (PDGF) will be used as examples to elucidate this mechanism.

The immediate event upon activation of the receptor tyrosine kinase is receptor autophosphorylation in tyrosine residues. The phosphorylated tyrosine residues in the receptors then provide binding sites for the cytoplasmic signaling molecules containing src-homology 2 (SH2) and src-homology 3 (SH3) domains, initially described as non-catalytic sequences found in $pp60^{c\text{-}src}$. The SH2 domains of the enzymatically otherwise diverse signaling molecules bind to phosphorylated tyrosine residues in the receptor and the SH3 domains interact with downstream effectors, thereby connecting the single activated receptor to the multiple downstream signaling pathways. The ras family of low molecular weight guanine nucleotide-binding proteins are proto-oncogene products that play a pivotal role in the receptor tyrosine kinase stimulated cell growth and differentiation. Mutated alleles of the cellular ras gene were found in many human cancer cells. However,

the mechanism by which the tyrosine kinase receptors communicate with the ras protein in the cell had been a mystery until the recent discovery of the adapter molecule called GRB2/Sem-5. We cloned GRB2 (Growth factor Receptor Binding protein 2) cDNA by screening cDNA expression libraries using autophosphorylated (^{32}P) EGF receptor carboxy terminus as the probe. GRB2, like ras gene, is expressed in all tissues and cell lines studied. GRB2, composed exclusively of one SH2 and two SH3 domains, binds to the activated tyrosine kinase receptor, via its SH2 domain, and to the guanine nucleotide releasing factor Sos, through its SH3 domains. Sos then activates ras protein by converting it from inactive GDP-bound form to active GTP-bound form. The function of GRB2 is evolutionarily conserved since a similar mechanism has been indicated in Drosophila and C. elegans.

Nck, another GRB2-like cellular oncogene, is composed entirely of one SH2 and three SH3 domains and is a common target for variety of both tyrosine kinase and non-kinase cell surface receptors. In order to understand the biological function of Nck, we undertook the following three approaches: 1) oncogenesity test by overexpressing Nck in NIH 3T3 cells; 2) assessment of its role in the PDGF receptor signaling by identifying its binding site in the PDGF receptor and then study the effect of mutations at the binding site; 3) identification of downstream effectors of Nck by expression cloning using radiolabeled GST-SH3 domain fusion protein as a probe. We showed that overexpression of Nck causes transformation of NIH 3T3 cells, indicating its involvement in growth control. Nck protein binds to the tyrosine 751 in the PDGF receptor, which is important for mitogenic function of the receptor. We have recently cloned two downstream effectors of Nck using radiolabeled Nck SH3 domains as the probe. Functional characterization of these effectors may reveal the mechanism of Nck oncogenesis.

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DEVELOPMENT AND CLINICAL APPLICATIONS OF A SMALL PEPTIDE AS RADIOLABELED IN VIVO DIAGNOSTIC PROBE

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Several dozens of small peptides, widely distributed in the human body, highly potent and important regulators of biological processes in numerous tissues, have been identified in the past several years. One of those, somatostatin (SS), the first of such peptides used in the nuclear medicine field, has been developed as in vivo labeled diagnostic probe for a variety of pathologies. Basic knowledge on SS, SS receptors and SS target tissues, as well as on the clinical implications of this diagnostic tool are reviewed.

SS has a wide range of actions in the human body: inhibition of pituitary hormone secretion, inhibition of pancreatic and gastrointestinal hormone secretion, inhibition of gut function, regulation of the immune and kidney function and neurotransmittory properties (Reichlin, 1983). In recent years a number of observations have suggested that SS plays also a role in cancer. In numerous animal tumor models and cultured tumor cell lines, SS and SS analogs inhibit tumor growth. Octreotide, the first clinically used stable SS analog, successfully controls hormonal hypersecretion in patients with pituitary tumors, carcinoids and islet cell tumors, and tumor-growth inhibitory effects have been observed in part of these patients (Lamberts et al., 1991).

All these SS and octreotide actions are mediated by specific, high affinity SS receptors (SS-R) present in the brain, pituitary, pancreas, gastrointestinal tract, kidney and lymphoid tissue, as shown with in vitro SS receptor binding and autoradiography. Not only healthy tissues but also several human pathologies, in particular cancers, are expressing SS-R as well. SS-R are found in most neuroendocrine tumors, i.e. GH and TSH producing pituitary tumors, endocrine gastroenteropancreatic (GEP) tumors, paragangliomas, pheochromocytomas, MTC and SCLC. SS-R are also expressed in a majority of malignant lymphomas, in several brain tumors (all meningiomas, most astrocytomas), in renal cell carcinomas and in breast tumors (Reubi et al., 1992). The majority of tumors expressing SS-R are rather differentiated (i.e. astrocytomas vs. glioblastomas), but exception exists (lymphomas). Certain categories of tumors (ovarial and GEP tumors, MTC) express SS-R subtypes. In pituitary and GEP tumors, SS-R are functional, mediating hormone secretion inhibition and therefore

playing a predictive role for assessing the therapeutical efficacy of SS analogs (Reubi et al., 1992).

The high density of SS-R in many of these tumors and the availability of potent and stable SS analogs as radioligands made it possible to develop an in vivo technique of scanning SS-R positive tumors and their metastases in the patients. After injection of isotope-coupled octreotide (123-I-[Tyr-3]-octreotide or 111-In-[DTPA]-octreotide) primary SS-R positive tumors, as well as their metastases, are visualized in most patients with neuroendocrine tumors (i.e. carcinoids, islet cell tumors, paragangliomas, pheochromocytomas, small cell lung cancer) (Krenning et al., 1989; Lamberts et al., 1990). Subsequently, also meningiomas, well-differentiated astrocytomas, neuroblastomas, malignant lymphomas and part of the primary breast cancers could be visualized in vivo by SS-R scintigraphy. In several cases, tumor sites unrecognized previously with other diagnostic methods could be identified. The results of imaging in vivo correlated with the SS-R status of the tumors in vitro.

SS-R scintigraphy has the advantage to be a simple and very sensitive technique for demonstrating tumor localization in the majority of patients with neuroendocrine tumors and also in patients with lymphomas and breast tumors. Apart from its merit in tumor and metastases localization in vivo, SS-R imaging, in consequence of its ability to demonstrate SS-R positive tumors, could be used to select those patients with neuroendocrine tumors who are likely to respond favorably to octreotide treatment. Furthermore, it may be possible to take advantage of the expression of these membrane peptide hormone receptors in cancers by carrying out radiotherapy with isotope-labeled SS analogs, as well as to perform radio-isotope guided surgery of SS-R positive tumors after administration of a low-energy gamma photon-emitting radionuclide.

Our more recent studies showed that not only cancers but also non-neoplastic pathologies had often a high expression of SS-R. This is the case for granulomas in sarcoidosis and in tuberculosis, for rheumatoid arthritis and for a variety of reactive lymph node inflammations (Reubi et al., 1993). SS-R can be identified in vitro in receptor autoradiographic studies, as well as in vivo in the patients in these inflammatory pathologies. Interestingly, the expression of SS-R seems to be related to the activity of the disease; octreotide scintigraphy may therefore help to monitore and to evaluate the course of the illness. However, these results also mean that octreotide scintigraphy is not specific for tumor diagnosis.

SS-R imaging represents the first example of the clinical use of a small peptide as efficient in vivo diagnostic tool. Such peptides may be considered a powerful alternative to (fragments of) monoclonal antibodies. SS-R may therefore, be

considered as a paradigm for further research on the role and the potential diagnostic use of other peptide receptors in pathological states.

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RADIOLABELING OF PROTEINS PEPTIDES AND OTHER MOLECULES OF ONCOLOGICAL INTEREST

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With the increasing interest in "molecular nuclear medicine" there are major chemical challenges in the labeling of proteins, peptides, oligonucleotides and other molecules. In this presentation, examples of techniques for labeling these classes of compounds will be discussed.

One example of peptides in the labeling of somatostatin analogs, somatostatin-14, in its naturally occurring form, is unsuitable for in vivo use due to its very short biological half-life. An eight-amino acid peptide analogue of somatostatin, Sandostatin (or octreotide) has been synthesized that has a longer half-life as well as inhibitory properties more potent than the native somatostatin.[1] The ability to visualize somatostatin receptors in vivo for a large number of different tumor types using nuclear medicine will have a great impact on the biological characterization of cancers, and will also be of value in making therapeutic decisions. The presence of somatostatin receptors in a tumor predicts a good therapeutic response. Octreotide has been labeled with ^{123}I and imaging studies in humans showed visualization of endocrine pancreatic tumors, metastatic carcinoids, pituitary tumors, as well as well-differentiated brain tumors and meningiomas. Within 2 minutes after injection of ^{123}I-labeled Tyr^3-octreotide, approximately 50% of the activity cleared from the blood pool and localization of a variety of tumors and their metastases was possible.[2] Analysis of the ^{123}I activity in the plasma indicates mainly peptide-bound radioiodine in the first 3 hours, with the concentration of free iodine increasing after 3 hours.[3] For routine use, ^{123}I-labeled Tyr^3-octreotide has drawbacks which include: (1) the cost and limited availability of high specific activity ^{123}I; (2) the time-consuming labeling and purification procedures; (3) the profuse hepato-biliary excretion which hinders the visualization of tumors in the abdomen.[4]

To overcome the disadvantage of ^{123}I-labeled Tyr^3-octreotide, octreotide has been conjugated with the chelate DTPA and labeled with ^{111}In.[5] The binding affinity to somatostatin receptors of ^{111}In-labeled DTPA-D-Phe^1-octreotide was somewhat lower than that of ^{123}I-Tyr^3-octreotide;[5] however, in rats the radioactivity was cleared predominantly through the kidneys as the intact labeled peptide.[6] Imaging studies involving ^{111}In-DTPA-D-Phe^1 octreotide in humans also showed the activity to clear rapidly through the kidney, although the blood clearance of the ^{111}In-labeled peptide was considerably slower than that of ^{123}I-Tyr^3-octreotide.[7] Because ^{111}In-labeled DTPA-D-Phe^1-octreotide remains intact in vivo, the background radioactivity in the body is much lower than that of the iodinated peptide, making ^{111}In-labeled DTPA-D-Phe^1-octreotide more sensitive

for imaging certain types of tumors.

We have also labeled octreotide with copper-64, an isotope with potential both with positron emission tomography and for therapy. The details of this technique will be described.

Methods for labeling proteins with carbon-11 and fluorine-18 will be discussed. These techniques will have applications when small fragments of antibodies are prepared that clear more rapidly from the circulation than currently available fragments. We have developed a series of radiolabeled polypeptides which are concentrated in the lysosomes by receptor mediated endocytosis. Receptor mediated endocytosis utilizes specific high affinity cell surface receptors to capture dilute ligands. This receptor ligand is then internalized and resulting intracellular vesicles, acidify either through a proton ATPase or cation exchange. The receptor recycles to the plasma membrane while the ligand is delivered to the lysosome. In this way more than 250 ligand molecules can be delivered by a single receptor.[8] Techniques for labeling these receptor ligands and their applications will be discussed.

The estrogen, progestin and androgen receptors are all target for radiopharmaceutical delivery. With the knowledge of the structure of receptor sites, ligands can be designed in a more specific manner than has been applicable in the past. Approaches to the designs ligands for these receptors labeled with halogens and technetium-99m will be discussed.

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ADVANCES IN THE MOLECULAR BIOLOGY OF HUMAN TUMORS: THE ROLE FOR NUCLEAR MEDICINE IN IMPROVED DIAGNOSIS AND THERAPY

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Nuclear medicine uses radiotracers to evaluate molecular alterations in normal and abnormal tissues. Recent advances in our understanding of the molecular biology of cancer suggest an important role for nuclear medicine in noninvasive study of human tumors for the purpose of improving diagnosis and therapy.

It is now known that specific genetic alterations in human cells can predispose to the development of tumors. One such alteration involves the tumor suppressor genes. For example $_p$53 is a protein which is important to normal growth regulation during cell division. $_p$53 gene resides on chromosome 17p. The deletion of this tumor suppressor gene has been associated with the development of a malignant phenotype in human cells. Frequently, the negative feedback loop which controls the cellular concentration of normal $_p$53 is disrupted in malignant cells so that the mutated $_p$53 is overexpressed in the cancer.

In addition to tumor suppressor genes, proto-oncogenes are important to the development of a proliferative state that may lead to cancer. An example is the growth control system, epidermal growth factor receptor, as well as the production of certain molecules by the tumor cells such as TGF alfa which are important to autocrine stimulation of growth of human cells. EGF receptors are expressed on lung cancers. Antibodies which recognize these receptors can be radiolabeled with In-111 or I-131 and used as imaging agents to detect the presence of EGF receptor in carcinoma of the lung. At Memorial Sloan-Kettering Cancer Center, EGF receptor antibody targeting has been shown to be feasible (Divgi, et al).

It is likely that in the future, specific radioantibodies or radioligands will be developed that recognize a variety of products of oncogenes or tumor suppressor genes. Tumors will then be characterized *in vivo* and noninvasively with radioactive probes that bind to tumor cells with specific functions. In this way more effective treatments may be planned, based on better characterization of the tumor. For example, if EGF receptor expression is present, a combination of EGF receptor antibody and anthracycline therapy may be effective in antitumor activity (Baselga, et al). Also, somatostatin receptors are expressed on many human tumors, including APUD tumors and lymphomas. ^{111}In pentetreotide, as a diagnostic agent to define tumors which will respond to specific therapies which block somatostatin receptors shows

considerable promise (Kwekkeboom, et al).

Molecular biology will also serve nuclear medicine for the development of specific antibody and antibody forms that are more suitable for targeting to tumors. Dr. Schlom and colleagues have shown the feasibility of creating fragments of antibodies which consist essentially of active binding sites of the hypervariable regions of what were originally murine antibodies. These humanized Fv fragments have more favorable characteristics for targeting human tumors than the whole IgG. In principle this will lead to better penetration of the tumor and a better therapeutic index of the tumor (Milenic, et al).

Through genetic engineering, we are currently working on the development of more suitable forms of the antibody 3F8, which targets GD-2 antigen on tumors of neuroectodermal origin.

The anti-tumor effectiveness of systemic chemotherapy is limited by the development of human cancer cells which are resistant. The "multi-drug resistant phenotype", which affects chemically diverse drugs derived from biologic sources is due to the expression of a membrane bound glycoprotein (p-glycoprotein) which pumps drugs such as vincristine and adriamycin out of the tumor cells. The use of radiolabeled colchicine (Mehta, et al) and radiolabeled antibody (Scott, et al) may make it possible to monitor the emergence of tumor chemo-resistance *in vivo*. Also these same methods may help determine the effectiveness of attempts to reduce tumor cell resistance.

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TARGETING THE MULTIDRUG RESISTANT P-GLYCOPROTEIN WITH CATIONIC ORGANOTECHNETIUM COMPLEXES AND TECHNETIUM LABELED ANTI-SENSE OLIGODEOXYRIBONUCLEOTIDES

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The multidrug resistant P-glycoprotein, a 170 kDa plasma membrane protein encoded by the mammalian multidrug resistance gene (*MDR1*), functions as an energy-dependent efflux pump of many of the most potent chemotherapeutic drugs in cancer treatment (1, 2). Increased levels of P-glycoprotein or P-glycoprotein messenger RNA (mRNA) have been detected in all forms of human cancers, including leukemias, lymphomas, sarcomas, and carcinomas (3), and transfection of cloned P-glycoprotein is sufficient to cause multidrug resistance in experimental systems. Overexpression of the human *MDR1* gene is associated with resistance to a broad spectrum of diverse chemotherapeutic agents which include anthracyclines, Vinca alkaloids, taxol, and actinomycin D. P-glycoprotein expression in human tumors has been characterized by immunohistochemical techniques with monoclonal antibodies, RNA expression analysis, and flow cytometry of fluorescent substrates. However, these approaches require serial biopsies, are limited by the invasiveness and labor intensity of current protocols, and suffer from deficiencies attributable to sampling errors of tissue specimens. An *in vivo* scintigraphic probe of the *MDR1* gene, its transcripts, or the P-glycoprotein gene product would be a significant advance for cancer chemotherapy.

Functional Imaging of MDR P-glycoprotein. Most cytotoxic as well as MDR reversing drugs tend to be hydrophobic and positively charged at neutral pH (4). This general feature and the demonstrated ligand binding properties of P-glycoprotein raise the possibility that hexakis(2-methoxyisobutyl isonitrile) technetium(I) (^{99m}Tc-SESTAMIBI), a lipophilic cationic radiopharmaceutical useful in perfusion imaging, could be a suitable substrate for the *MDR1* gene product. We tested the hypothesis that ^{99m}Tc-SESTAMIBI is recognized by P-glycoprotein as a novel organometallic transport substrate by characterizing the transport and inhibition profile of ^{99m}Tc-SESTAMIBI in mammalian multidrug-resistant cell lines *in vitro*. In chinese hamster drug-sensitive V79, and progressively multidrug-resistant 77A and LZ derivative cell lines, steady-state contents of ^{99m}Tc-SESTAMIBI were 10.0 ± 0.5 (n=9), 3.6 ± 0.5 (n=8) and 0.4 ± 0.02 (n=9) fmol (mg protein)$^{-1}$ (nM_o)$^{-1}$, respectively, consistent with enhanced extrusion of the imaging agent by P-glycoprotein-

enriched cells. Maximal doses (>100 μM) of the MDR reversing agents verapamil and cyclosporin A enhanced ^{99m}Tc-SESTAMIBI accumulation in V79, 77A, and LZ cells by approximately 10-fold, 25-fold and 200-fold, respectively. ^{99}Tc-SESTAMIBI inhibited photolabeling of P-glycoprotein by [^{125}I]iodoaryl azidoprazosin in a concentration-dependent manner. In athymic *nu/nu* mice, ^{99m}Tc-SESTAMIBI accumulation was 35% ± 4% (n=4) lower in P-glycoprotein-enriched KB-8-5 human tumors compared to parental KB tumors implanted in the opposite flank. These experiments demonstrate the feasibility of scintigraphically imaging P-glycoprotein function *in vivo* with ^{99m}Tc-SESTAMIBI (5).

Baculoviral Expression System. Insertion and overexpression of the target gene into an expression system can aid direct investigation into effects of the protein product on transport and binding of novel radiopharmaceuticals. We have taken advantage of the helper-independent baculoviral expression system (6) to directly demonstrate P-glycoprotein-mediated transport of ^{99m}Tc-SESTAMIBI. Host *Spodoptera frugiperda* (Sf9) insect cells were infected with a recombinant *Autographa californica* nuclear polyhedrosis baculovirus, containing the human *MDR1* gene under the control of the strong polyhedron promoter, to achieve high levels of expression of the multidrug transporter. Several properties of the host Sf9 cells could be exploited, including: 1) cells could be grown in monolayer culture, thereby facilitating transport assays, 2) parental Sf9 cells have little or no natural expression of P-glycoprotein, providing a convenient baseline for ^{99m}Tc-SESTAMIBI accumulation, and 3) baculovirus-infected Sf9 cells are able to perform many higher eukaryotic postranslational modifications, such as glycosylation and phosphorylation, and therefore, have been extensively characterized for the successful overexpression of a variety of cytoplasmic and integral membrane proteins in active functional states. In our system, ^{99m}Tc-SESTAMIBI accumulation asymptotically approached a plateau of 650 fmol (mg protein)$^{-1}$ (nM_O)$^{-1}$ in parental Sf9 cells and 337 fmol (mg protein)$^{-1}$ (nM_O)$^{-1}$ in wild-type baculoviral infected (control) cells. Overexpression of recombinant human *MDR1* P-glycoprotein in the baculoviral expression system as determined with the monoclonal antibody C219 was maximal at 72 hr post-infection; this reduced tracer accumulation to <3% of control (12 fmol (mg protein)$^{-1}$ (nM_O)$^{-1}$). Verapamil (500 μM), the classical MDR modulator, produced a 4-fold enhancement of ^{99m}Tc-SESTAMIBI accumulation in Sf9 cells expressing *MDR1* P-glycoprotein, but less than a 50% enhancement in parental and wild-type infected Sf9 cells, consistent with verapamil-induced inhibition of P-glycoprotein-mediated ^{99m}Tc-SESTAMIBI efflux. We conclude that: 1) the recombinant transporter is expressed in a functional state in Sf9 cell membranes, and 2) ^{99m}Tc-SESTAMIBI is a transport substrate recognized by the human *MDR1* P-glycoprotein.

Tc-99m Labeled Anti-Sense Oligodeoxyribonucleotides. Anti-sense oligonucleotides (ASO) bind to and inhibit translation of mRNA *in vitro* and hence, block production of a variety of proteins. Efforts are currently well underway to exploit oligonucleotides as pharmacological agents to block translation of target mRNA for therapeutic applications in disease. We are testing the feasibility of using ASOs as radiopharmaceutical imaging agents to detect cells within tissues that harbor high levels of *MDR1* mRNA. We have synthesized a candidate ASO that flanks the initiation codon in the hamster mdr1 mRNA as the phosphorothioate with a six carbon primary amine linker attached at the 5' end. The ASO was reacted with diethylenetriamine pentaacetic acid (DTPA) anhydride, and the resulting product mixed with a preparation of Tc-99m-gluconate for labeling. Successful labeling of the ASO-DTPA complex was confirmed by the observation of co-elution of UV absorbing material and Tc-99m activity in fractions collected off a G50 column. Pilot studies with hamster multidrug-resistant LZ cells show accumulation of the labeled ASO to a plateau of ~23 pmol ASO (mg protein)$^{-1}$(μM ASO in the incubation buffer)$^{-1}$. This indicates nearly a 3-fold concentration (or association) of the ASO within the cells with respect to the extracellular spaces, and forms the basis for targeting *MDR1* transcripts with these radioprobes.

Summary. Functional SPECT imaging with Tc-99m-isonitrile complexes such as ^{99m}Tc-SESTAMIBI or assessment of mRNA levels with targeted ASO probes may provide novel mechanisms to rapidly characterize *MDR1* P-glycoprotein expression in human tumors *in vivo*, in particular, in breast, lung, lymphoma, and pediatric neuroblastoma, aid the targeting of new P-glycoprotein reversing agents, and ultimately provide a means to direct patients to specific cancer therapies.

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RADIOLABELED ANTISENSE PROBES FOR DIAGNOSIS OF CANCER

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Antisense drugs, phosphodiester, nuclease-resistant derivatives, pharmacokinetics:
Recombinant DNA technology helped us in identifying the sequences of oncogenes (1). Antisense or anticodon oligonucleotides (2) had been found to inhibit a variety of oncogenes (c-ras, c-myc, c-myb, bcr/abl). Phosphorothioates and methylphosphonates were found to be nuclease-resistant and more effective in inducing phenotypic changes and inhibition of gene expression at high concentration (10-100 μM). This inhibition may result from antisense effect and nonspecific effect of a polyanion (7). The sequences working on the antisense mechanism were chosen in our studies for generation of antisense radiopharmaceuticals, labeled with a variety of gamma-emitting metallic and nonmetallic radionuclides of ^{125}Iodine, ^{123}Iodine, ^{111}Indium, ^{99m}Technetium, ^{67}Gallium and ^{153}Samarium (2-6,8). Some of these probes label neutrophils spontaneously after intravenous administration. This binding is mediated by a 80,000 D receptor on a variety of cell-membranes. These probes could be used for the diagnosis of infection and inflammation. For tumor detection, these sequences should be avoided in favor for enhanced blood clearance and tumor-delivery of radionuclides mediated by other screened specific oncogene sequences.

Antisense Radiopharmaceuticals, Oncogenes and Diagnosis of Proliferative Diseases: Myc-protein, a product of c-myc gene, functions in cellular differentiation, proliferation and tumor formation. The c-myc oncogene is amplified in cancer making the c-myc mRNA a suitable target for noninvasive imaging of oncogene activation. Knowing the nucleotide sequence, we could select a specific sequence; this radiolabeled sequence may work as a "molecular velcro" for the localization of primary tumor and metastatic sites. The kinetics of uptake of ^{111}In-labeled antisense (AS), sense (SN, control) and oxo and thio derivatives by P388 cells was evaluated. The 15-mer oligonucleotide sequence for the initiation-codon domain was synthesized with a DNA-synthesizer (Applied Biosystems Inc.), aminolinked [sense (SN) and antisense (AS) phosphodiester (O) and monothioester (S)] and coupled to isothiocyanate of DTPA and aliquots (10μg) were lyophilized. In-111 chloride (100-500 μCi) was chelated to probe; aliquots of 1-2 μCi (0.1 μg) was added to P388 cells (1X10^6 cells/ml in logarithmically growing [L] and nutritionally deprived plateau [P] phase) and incubated at 37°C for 10, 20, 40 and 60 minutes, washed and cell-uptake CU(%) was calculated and tabulated below:

	10 min,L	60 min,L	10 min,P	60 min,P
AS(S)	18.7±2.8	48.3±9.6	12.7±0.7	30.4±1.9
SN(S)	15.4±2.1	22.2±4.7	14.1±3.1	19.8±4.7
AS(O)	18.9±2.5	50.1±8.8	12.8±0.7	30.5±1.7
SN(O)	16.8±2.1	22.1±3.2	13.1±2.7	23.4±5.2

The [L] phase cells with more mRNA copies hybridized more INASON probes than [P] phase cells; CU(%) of sense (O) and (S) probes was significantly lower ($p < 0.001$). The thio and oxo probes showed similar high CU(%) suggesting efficient probe transport through membrane, hybridization with c-myc mRNA suitable for noninvasive imaging of proliferation

in a tumor model.

Five-six microcuries (1 μg) were injected intravenously in mammary tumor-bearing Balb/c mice ($1X10^6$ cells subcutaneously, 8 days post-inoculation). They were imaged and sacrificed at 2, 6, 12, 24, 48 and 72 hours; biodistribution (percent of injected dose:ID%), tumor/blood (T/B) and tumor/muscle (T/M) at 2 hours were tabulated:

	T/B	T/M	ID%
Antisense(S)	3.4±0.2	34.6±2.6	13.9±2.5
Antisense(O)	2.8±1.1	33.5±2.5	12.4±3.2
Sense(S)	0.6±0.2	5.8±1.9	2.9±0.7

Highest uptake was observed at 2 hours with stable RAS-thio probes and small tumor could be imaged noninvasively; this level declined post-injection. Uptake was least with the sense probe. The mechanism of in vivo hybridization of INASON probe with mRNA of c-myc oncogene is under investigation. The antisense radiopharmaceuticals may provide a new sensitive and specific avenue for noninvasive imaging of a variety of malignant tumors at an earlier stage.

SUMMARY: Radiopharmaceuticals labeled with nonmetallic (^{123}I, ^{18}F) and metallic radionuclides (^{99m}Tc, ^{67}Ga, ^{111}In, ^{90}Y, ^{153}Sm) are used routinely for diagnosis and therapy. We developed a new type of radioligands based on antisense oligodeoxynucleotide analogs labeled with ^{123}I, ^{99m}Tc and ^{111}In and named them "antisense radiopharmaceuticals". The specific oligodeoxynucleotides provided us with a new avenue of intracellular delivery of radionuclides, drugs and toxins post-internalization. We have developed and tested these radiolabeled probes in both in vitro (cultured cells and tissues) and in vivo systems (mice, dogs and pigs). A DTPA-conjugated amino-hexyloligonucleotide kit (freeze-dried) was developed for direct labeling with metallic radionuclides. Quality assurance of RASON probe is carried out by thin-layer, gel-electrophoresis and size-exclusion (HPLC) techniques for checking chemical and radiochemical impurities in these probes. Size-exclusion techniques were used for the quantification of hybridization kinetics of RASON probe with specific mRNA of interest. In vitro uptake was dependent on nucleotide-sequence/length, time and temperature. For therapeutic RASON probes labeled with ^{153}Sm, ^{131}I (ß,γ), ^{186}Re (ß,γ), ^{188}Re (ß), ^{90}Y (ß) etc., could be used. In vitro studies of RASON probes with extracted mRNAs, cultured cells and fixed tissues identified the parameters of stability, specificity of cellular uptake and hybridization-kinetics. The pharmacokinetics of several ^{111}In-labeled deoxynucleotides (oxo and thio derivatives, sense and antisense) of different nucleotide length and sequence, specific for binding mRNAs of ß-actin, histone2/4, c-myc, c-myb, c-fos and c-erbB2 were studied in Balb/c and nude mice, Beagle dogs and Yorkshire pigs. The paradigm of maximal probe uptake was tested in mammary-tumor bearing Balb/c and human tumor xenograft (MCF-7) in nude mice. For neutrophil labeling, we have targeted the histone4 mRNA in the neutrophils for direct labeling in whole blood. Using the P388 cell-line (murine monocytic leukemia) and neutrophils from canine, porcine and human blood as model cells, we had screened several labeled deoxyoligonucleotides for optimum nucleotide length (15, 20, 25-mer). Highest uptake (12-15)% of injected dose of ^{111}In- and ^{99m}Tc-labeled c-myc and c-erbB2 probes was found at 2 hours post-injection in a small transplanted breast-tumor in Balb/c mice and human tumor (MCF-7)-xenograft in nude mice. The phosphorothioate derivative cleared faster from blood than phosphodiester. In vivo studies

characterized the parameters of specific activity on biodistribution, in vivo metabolism and biodistribution, radiation dosimetry and optimum imaging time with these new probes. Since probes are internalized efficiently in target cancer cells of interest, after intravenous injection, high amount of radiation could be delivered for early imaging of small tumor by gamma-emitters and and cell-killing by beta-emitters, using this Trojan horse strategy. Supported by DOE/OHER, NCI and NHLBI-Shannon Award.

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NEW HORIZONS IN ONCOLOGY

Henry N. Wagner, Jr. M.D.

No field of medicine is better equipped to transfer the advances in molecular biology to the care of patients, the most immediate applications being in cancer. For example, cancer can arise in tissues that are target tissues for hormones. Molecular biology provides an approach to the design of new *in vivo* tracers, including those for detecting biochemical recognition sites. In the past, drugs were developed by screening synthetic organic compounds for an observed biological effect. Recognition sites can now be identified and quantified *in vivo* by radiolabeling selective agonists or antagonists. In situ hybridization is one way to develop specific probes for the mRNA for a specific subtype of receptor.

The demonstration of stereospecific uptake helps prove that the molecule is binding to a receptor. Displacement is another form of evidence. Drugs based on hormone agonists or antagonists can be useful in regulating the growth of the cancer. Octreoscan-111, an eight-peptide analog of somatostatin, is now widely used in many types of neuroendocrine and other tumors. It is likely that other ligands for imaging peptide hormones will soon be available.

Detecting estrogen receptors or progesterone receptors help determine whether the lesions will respond to hormonal therapy. Of those that contain estrogen receptors, about half will respond to therapy. Other examples include dopamine and somatostatin receptors in pituitary and other tumors.

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