Journal of Cellular Biochemistry Supplement 39:184–193 (2002)

Grappling With Metastatic Risk: Bringing Molecular Imaging of Met Expression Toward Clinical Use

Rick Hay,* Brian Cao, Ilan Tsarfaty, Galia Tsarfaty, James Resau, and George Vande Woude

Van Andel Research Institute, 333 Bostwick Avenue N.E., Grand Rapids, Michigan 49503

Abstract The availability of a test to assess the likelihood that a given tumor will invade or metastasize would be a useful development in clinical oncology. We propose that multimodality imaging of tumor expression of Met could serve as a prototype for metastatic risk stratification (MRS). Met, a receptor protein tyrosine kinase, is expressed by most solid tumors, and aberrant expression of Met is associated with poor clinical prognosis. We summarize the current status and predict the future direction of research in four areas of molecular imaging and cancer therapy that exploit Met. J. Cell. Biochem. Suppl. 39: 184–193, 2002. © 2002 Wiley-Liss, Inc.

Key words: receptor tyrosine kinase; immunohistochemistry; confocal laser scanning microscopy (CLSM); nuclear imaging; ultrasound; magnetic resonance imaging (MRI); immunotherapy

"The very rich are different from you and me."—F. Scott Fitzgerald.

"Yes, they have more money."—Ernest Hemingway, in reply.

The American Cancer Society estimates that 1.3 million people in the United States will learn they have a new cancer in 2002 [American Cancer Society, 2002]. At the time of diagnosis, about 40% of them will be told that the cancer has already spread beyond its site of origin; the rest will be told it has not. But *no person* with a newly discovered cancer thought to be anatomically confined will be told how likely it is to spread in the future, because nobody knows for sure.

Wouldn't it be great if we did? Wouldn't it be convenient if every "very malignant" cancer had more of something—like money—that we could see and count, so that we could give people who just learned they have cancer some accurate odds to consider, and to help them decide what to do next?

The biomedical imaging community has already succeeded on this front in the field of cardiovascular disease. We routinely assess myocardial perfusion by noninvasive imaging methods in conjunction with physical or pharmacological stress testing, a process known as "cardiac risk stratification" (CRS). Both ultrasonic and nuclear imaging techniques are widely used for CRS. Both are highly accurate in the hands (and eyes) of experienced imaging specialists. Myocardial perfusion imaging accounts for a large share of the total number of nuclear imaging and sonographic studies performed in the United States annually. Every patient after her or his first cardiac event, most patients undergoing post-event or post-intervention periodic surveillance, many patients planning to undergo elective surgery, and nearly everyone with a complaint of chest discomfort—of a typically cardiac pattern or not—can expect to undergo between one and several noninvasive imaging studies for CRS.

The critical value of assessing myocardial perfusion as an adjunct to physiologic stress testing protocols (i.e., exercise on a treadmill or cycle, or infusion of cardiotropic drugs, in conjunction with monitoring of vital signs, electrocardiographic patterns, and signs and symptoms) is that it permits one to expand a crude few-point CRS scale (e.g., the Duke score) based on physiologic test results alone into a 30-fold "gray scale" of CRS. For instance, a person with a low Duke score and a normal stress nuclear myocardial perfusion imaging study is only at 0.3% likelihood of experiencing a

*Correspondence to: Rick Hay, Van Andel Research Institute, 333 Bostwick Avenue N.E., Grand Rapids, MI 49503.

Received 4 November 2002; Accepted 4 November 2002

DOI 10.1002/jcb.10441

Published online in Wiley InterScience (www.interscience.wiley.com).

© 2002 Wiley-Liss, Inc.

myocardial infarction or cardiac death within 2 years, compared to a 10% likelihood for a person with a high Duke score and a highly abnormal imaging study. Having this noninvasive imaging information to supplement clinical evaluation, a physician can recommend a suitable protocol for further diagnostic testing, treatment, and clinical surveillance that is objectively based on an individual patient's needs. Parker has recently reviewed this topic [Parker, 2001] in an article that readers unfamiliar with the intricacies of clinical imaging will find enlightening.

Is a parallel achievement possible in the field of oncology? Can we develop a straightforward, objective, inexpensive, widely embraced, and well-reimbursed test for most or all patients with newly diagnosed, clinically confined cancers—a test that would amount to (or contribute to) "metastatic risk stratification" (MRS)? A person with a low MRS score would be considered to have a tumor at low risk of metastatic or invasive behavior, and could be monitored and treated conservatively; one with an intermediate MRS score could be treated conservatively but monitored frequently; and one at high risk by MRS would have an objective basis for agreeing to and enduring a correspondingly more aggressive therapy and intensive monitoring protocol. Through MRS, we could objectively individualize the treatment and monitoring of patients with cancer in a way that has not heretofore been thinkable.

Every dividing cell has the potential to become neoplastic, and every neoplasm has the potential to become frankly malignant, manifested as the ability to invade and metastasize. Molecular oncologists have sought and scrutinized molecules involved in carcinogenesis and cancer progression for the last 20 years or so, and now, armed with the technical capacity to perform high-throughout gene expression microarray analysis and proteomic analysis on thousands of molecules at a time, the process is accelerating [Huang et al., 2001; Takahashi et al., 2001; Miller et al., 2003]. We can only hope that governmental regulators, approval processes, and evaluation boards accept and understand the new paradigms that will develop. There is an ever-growing list of potential candidate molecules that might play a role in conferring "very malignant" status, or that serve as extratumoral indicators of that status, for every type of cancer that has been interrogated with this technology. We now face the task of categorizing and making sense of this mountain of data, confidently hopeful that at least a few molecules will emerge as useful markers of and targets for treating very malignant cancers. This is an admirable and important endeavor, and we are committed to pursuing it. On the other hand, certain molecules whose presence and form of expression give information about metastatic risk were known before the recent explosion in gene expression analysis technology. We might already be able to exploit these molecules for MRS, or at least as prototypes for the MRS algorithms of the future. One such example is the molecule known as Met.

Met, the protein product of the c-met-protooncogene, was discovered in the Vande Woude laboratory at the National Cancer Institute in 1984 [Cooper et al., 1984; Dean et al., 1985; Iyer et al., 1990]. Met is a receptor protein tyrosine kinase of the same family as epidermal growth factor (EGF) receptors. It acts as the cell surface membrane receptor for hepatocyte growth factor/scatter factor (HGF/SF), dimerizing after binding ligand to form the active kinase. Under normal conditions, Met is a keystone molecule, acting on the molecular signaling pathways responsible for cellular differentiation, motility, proliferation, organogenesis, angiogenesis, and apoptosis [Haddad et al., 2001]. In neoplastic cells, the aberrant expression of Met and HGF/SF leads to emergence of an invasive/metastatic phenotype. This conclusion is supported both by transfection experiments and by retrospective analysis of many types of human solid tumors, including cancers originating in the head and neck, thyroid, lung, breast, stomach, liver, pancreas, colon and rectum, kidney, urinary bladder, prostate, ovary, uterus, skin, bone, muscle, and other connective tissues [Stuart et al., 2000; van der Voort et al., 2000; Haddad et al., 2001]. Both paracrine and autocrine mechanisms of Met activation by HGF/SF occur in human neoplasms (Fig. 1). Moreover, activating mutations in Met—either inherited in the germ line or found in sporadic cancers—have been shown to contribute to a variety of human cancers [Schmidt et al., 1997].

Across the spectrum of tumors that have been investigated, the level of Met-HGF/SF expression in general correlates inversely with clinical outcome. This correlation has been examined in greatest detail for human breast and prostate

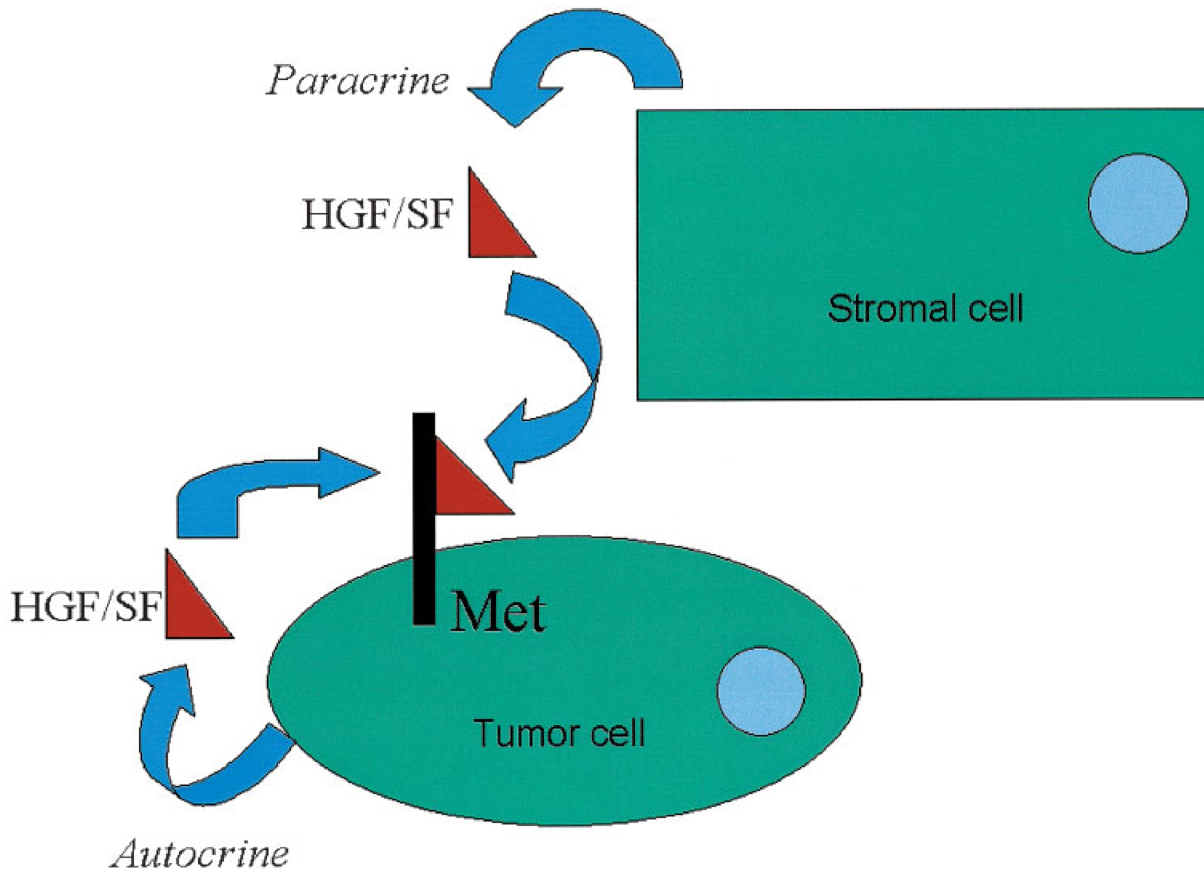

Fig. 1. Paracrine and autocrine activation of Met by hepatocyte growth factor/scatter factor (HGF/SF). Paracrine activation occurs when Met receptor molecules associated with neoplastic cells bind HGF/SF ligand produced by nearby stromal mesenchymal cells. Autocrine activation occurs when the same cells as those expressing the Met receptor also produce ligand.

carcinomas. Met overexpression in breast tumors is associated with breast cancer progression [Niemann et al., 1998; Tsarfaty et al., 1999; Firon et al., 2000], and high HGF/SF expression also correlates with poor survival in ductal breast carcinomas [Yamashita et al., 1994; Ghoussoub et al., 1998]. Tsarfaty et al. [1999] quantified Met expression in uninvolved (N) relative to tumor (T) tissue in the same primary breast carcinoma sections. The overall Met distribution in this patient group was ~40% with $T < N$, ~40% with $N = T$, and 20% with $T > N$. Higher Met expression in tumors than in normal tissue was associated with poor patient outcome. Three groups of investigators [Tuck et al., 1996; Jin et al., 1997; Edakuni et al., 2001] have examined Met and HGF/SF expression in benign and malignant breast tissue. They found that frequently both receptor and ligand are expressed, and that expression is higher in breast cancer and carcinomas in situ than in benign breast tissue. While Met is mainly detected in epithelial breast cancer cells, HGF/SF is detected in tumor cells as well as in stromal cell types, implying that HGF/SF contributes to growth and invasiveness of breast cancer cells by autocrine and paracrine mechanisms. This conclusion is also supported by recent experiments showing increased tumorigenic and metastatic activity accompanied by reduced tubule formation of breast cancer cells after transfection with Met and HGF/SF [Firon et al., 2000]. There is a growing body of clinical and experimental evidence that Met also plays a critical role in the behavior of human prostate carcinoma. Four independent laboratories have reported aberrant expression of Met by about one-half to two-thirds of localized prostate cancers, but evidently by *all* osseous metastases. This phenomenon suggests that Met provides a strong mechanism of selection for metastatic growth in prostate cancer [Humphrey et al., 1995; Pisters et al., 1995; Watanabe et al., 1999; Knudsen et al., 2002].

In lay terms, until or unless something better comes along, Met might be considered the poster child of very malignant cancers, a veritable Beacon of Bad Biological Behavior. Put another way, it is conceptually useful to consider these points:

- "Very malignant" cancers express molecules in common, such as Met, that can be independent of their tissues of origin;
- Met is a process-specific rather than tissue-specific marker for cancer, an indicator of tumor destiny rather than of tumor origin.

With these notions in mind, the goal of molecular imaging that exploits Met is to determine the status of Met expression in a particular solid tumor in vivo, and armed with that information, to design Met-directed therapy that will alter tumor destiny toward a more favorable clinical outcome.

We have been developing molecular imaging tools and approaches to clarify the behavior of Met at the cellular level, and applying these approaches to in vivo animal models of human cancer and to naturally occurring human cancers. Our recent and ongoing efforts to exploit Met as a molecular imaging and therapeutic target fall into four general areas, with the status of each summarized below.

Microscopic Molecular Imaging: Immunohistochemistry, Immunofluorescence, and Confocal Laser Scanning Microscopy (CLSM)

Our collaborators and we employ conventional immunohistochemical techniques with commercially available anti-Met polyclonal antibodies and our repertoire of murine anti-Met and anti-HGF/SF monoclonal antibodies (mAbs) to examine the cellular distribution of Met and its ligand within samples of human tissues, including samples of surgically resected human neoplasms [Knudsen et al., 2002; Qian et al., 2002] (Fig. 2). With the availability of CLSM, we can analyze immunofluorescent

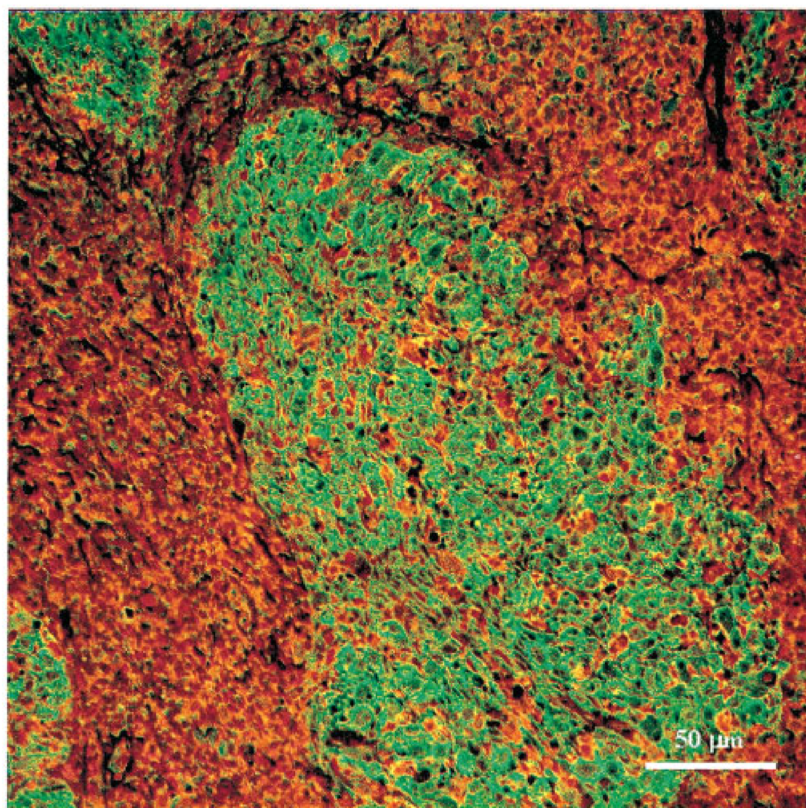

Fig. 2. Immunofluorescent image of Met and hepatocyte growth factor/scatter factor (HGF/SF) expression in human nasopharyngeal carcinoma. Met (green) in the neoplastic cells is identified with C-28 polyclonal rabbit anti-Met antibody followed by FITC-conjugated anti-rabbit antibody. HGF/SF (red) in the surrounding stroma is identified with a murine anti-HGF/SF mAb followed by rhodamine-conjugated anti-mouse antibody. (Courtesy of Chao-Nan Qian and Bin Teh.)

images generated with polyclonal or monoclonal antibodies of known epitopic specificity to determine to what extent antigen species colocalize within a given cell. For example, it is now possible to visualize simultaneously the distributions of ligand (HGF/SF) and receptor (Met), as depicted by respective suitably fluorophore-conjugated antibodies, within the same autocrine cell. In this way, we can determine either whether different antibodies react with identical or separate antigens within a cell, or to what extent different antigens interact at the subcellular level with each other. We are refining this technique, drawing on advances in instrumentation and in conjugation methods, to examine the interactions between individual molecules of HGF/SF with individual molecules of Met within a single cell. This molecular imaging approach is currently invasive, in that living tissue or cells must be harvested and preserved before analysis, but we expect in the near future to extend this technology to examine the interactions of fluorophore-conjugated Met and HGF/SF molecules in intact living organisms, including human tumor xenograft-bearing mice.

A powerful characteristic of immunofluorescence combined with CLSM is that the fluorescence in the images can be quantified at the microscopic level. With proper controls and statistical analysis, the relative abundance of different antigens can be determined within a single cell, across different histologic regions (e.g., epithelium vs. stroma), and indeed, when coupled with tissue array methodology [Rimm et al., 2001], across multiple different tissue or tumor samples on a single microscope slide. The reader is referred to recent references for examples of how we have applied this technology to analyze Met expression in human tissue samples [Tsarfaty et al., 1999; Qian et al., 2002].

Microscopic imaging may seem a non-flashy way to perform what is considered "molecular imaging" in todays scientific news environment, but we believe it constitutes a sound foundation for the development of an in vivo molecular imaging program. We use the results of microscopic molecular imaging to direct new experiments involving in vivo molecular imaging, and conversely, we validate our in vivo imaging results with microscopic analysis. We further believe that wider awareness, acceptance, and availability of microscopic molecular imaging analysis of Met within the diagnostic pathology and clinical oncology communities must be realized before in vivo imaging of Met expression can succeed at the clinical level.

Nuclear Molecular Imaging: Radioimmunoscintigraphy

Radioimmunoscintigraphy is an important and attractive modality for experimental and clinical molecular imaging of cancer. One can raise, characterize, and propagate mAbs reactive against virtually any given protein antigen, even those present as minor components of complex protein mixtures or as minor surface components of whole cells. Established methods for radiolabeling mAbs in suitable quantity and of appropriate quality for scintigraphy are widely available, technically feasible, relatively inexpensive, and adaptable to virtually any mAb regardless of its epitopic specificity. New radiolabeling methods are continually emerging, and many laboratories are evaluating a wide range of antibody derivatives—from full-length chimeric and humanized molecules, to monomeric and multimeric antibody fragments, to immunoconjugates—as potentially superior imaging and therapeutic agents, with improved targeting selectivity and more favorable biological turnover kinetics [Program and Abstracts, Ninth Conference on Cancer Therapy with Antibodies and Immunoconjugates, 2002].

Moreover, the reagents, supplies, and equipment required to perform radioimmunoscintigraphy in experimental animals and in humans are commonplace. For decades decommissioned or refurbished clinical gamma cameras have proven satisfactory for animal imaging applications, and they continue to do so. Modified or custom-built gamma cameras adapted for small animal imaging are becoming more widely available, but whether they provide sufficient improvements in image quality to offset their higher cost remains to be established.

The major advantage of scintigraphy as a molecular imaging modality (and not limited to imaging with antibodies) is that the acquired images are inherently quantitative. The physics of gamma radiation and the mathematical analysis of nuclear images, including corrections for photon attenuation and other artifacts, are well understood. In animal models as well as in human studies, we can noninvasively and accurately measure net accumulation and some kinetic parameters of radiopharmaceutical

interactions with target lesions, and the concurrent collection of even a small set of biological samples (e.g., blood and excreta) for direct counting combined with quantitative analysis of diagnostic images enables us to make useful dosimetry estimates for therapeutic purposes.

Having established a collection of mAbs reactive against many epitopes of the Met-HGF/SF complex, we are now developing animal models to examine the interactions of radiolabeled mAbs with human tumor xenografts. We are evaluating individual clones of anti-Met mAbs and neutralizing mixtures of anti-HGF/SF mAbs [Cao et al., 2001] alone and in combination for this purpose, surveying xenografts of different tissue origins and exhibiting varying patterns of Met expression (Fig. 3). We previously published our findings from nuclear imaging experiments evaluating a radioiodinated mixture of anti-Met and anti-HGF/SF mAbs in four types of xenografts in mice [Hay et al., 2002a], and we recently completed a series of imaging experiments evaluating Met3—anti-Met mAbs from a single hybridoma clone (2F6)—for its ability to discriminate among xenografts exhibiting differing patterns or levels of Met expression [Hay et al., 2002b]. Key findings from our studies so far can be summarized as follows.

- Met-expressing tumor xenografts in nude mice can be visualized as early as 1 h following injection of radiolabeled anti-Met or/and anti-HGF/SF mAbs, with peak image contrast (activity in tumor vs. whole body) occurring at about 3 days postinjection;
- Met-expressing tumor xenografts exhibit a wide range of radiolabeled mAb initial uptake, from ~5 to 20% of the estimated injected activity (%IA), with tumor-associated radioactivity constituting from ~ 10 to 40% of total body activity at peak image contrast;
- The turnover of radiolabeled mAbs appears to be substantially more rapid in tumor xenografts exhibiting higher initial uptake values.

While continuing to conduct nuclear imaging experiments, we are using microscopic imaging techniques combined with immunochemical and biochemical analyses to understand the molecular bases for these phenomena, e.g., to determine the relative contributions of such parameters as total cellular Met levels, surface access of Met to mAbs, the state of Met activation, and rates of receptor turnover, to the imaging characteristics of Met-expressing tumors in vivo.

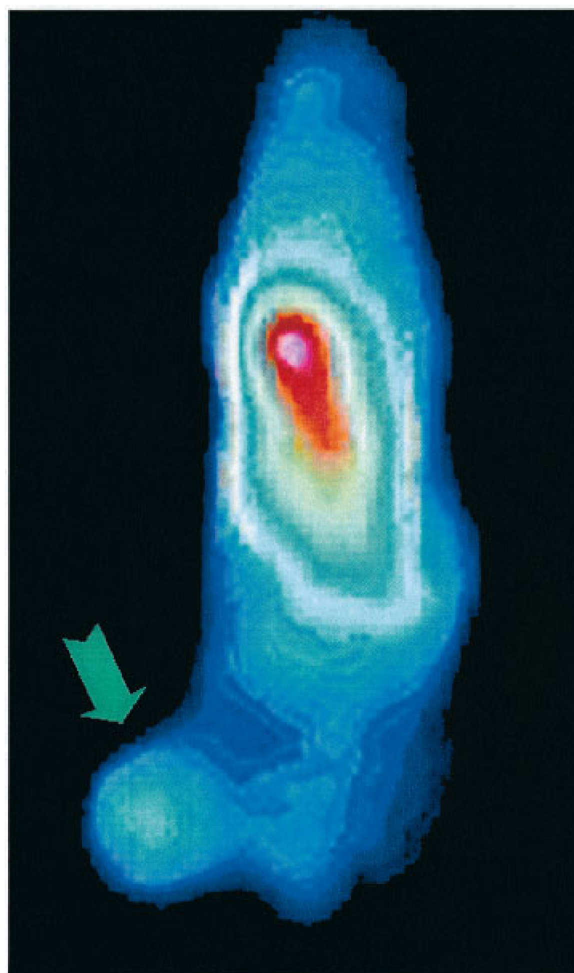

Fig. 3. Radioimmunoscintigraphy of human leiomyosarcoma xenograft in a nude mouse. A tumor xenograft was induced in a nude mouse by subcutaneous injection of SK-LMS-1 cells (autocrine for Met-hepatocyte growth factor/scatter factor (HGF/SF)) into the posterior aspect of the right thigh. The animal was injected intravenously with I-125-Met3, an anti-Met mAb, and imaged with a Biospace γ Imager 1 h later. The arrow indicates the position of the xenograft. (Courtesy of Jon McKown, IN/US Systems.)

"Provocative" Functional Molecular Imaging: Assessing Tumor Physiology by Magnetic Resonance Imaging (MRI) and Ultrasonography

Both MRI and ultrasonography—traditionally considered "anatomic" or "structural" imaging modalities—may also be used effectively as functional molecular imaging tools. The most

powerful advantage of these modalities as applied to small animal imaging is their excellent spatial and temporal resolution. When combined with "provocative" testing (analogous to CRS by stress echocardiography), both MRI and ultrasonography offer rapid and accurate noninvasive measurements of short-term changes in tumor physiology following activation of Met. Using MRI, for example, one can evaluate changes in the pattern of blood flow and in regional blood oxygen levels that occur near and within a tumor or normal tissue following deliberate physiologic provocation, e.g., an intravenous bolus injection of HGF/SF. In parallel, one can use Doppler ultrasonography to quantify changes in blood flow velocity near and within a Met-expressing tumor under stimulated and non-stimulated conditions. Once these parameters are understood, biochemical or pharmacologic agents that either mimic or block the interaction of HGF/SF with Met or that interfere with the intracellular sequelae of Met activation could be administered in vivo, and their effects monitored accurately and noninvasively. Moreover, with these tools, one can monitor changes in tumor physiologic responsiveness longitudinally in the same animal, e.g., changes that are dependent on tumor growth or angiogenesis or that occur in response to tumor therapy. Both imaging modalities offer the additional advantage of precise tumor size determination, and may be used serially to monitor rates of tumor growth and rates of change in tumor dimensions in response to therapy.

As an example of the utility of this approach, we recently published the results of a collaborative study using MRI and ultrasonography as functional molecular imaging tools to study the physiological consequences of Met activation in mice bearing paracrine Met-expressing mammary adenocarcinomas [Shaharabany et al., 2001] (Fig. 4). The activation of Met in vivo by bolus intravenous injections of human HGF/SF alters the hemodynamics of both malignant and normal Met-expressing tissues. Tumors and organs expressing high levels of Met show the greatest changes in blood oxygenation levels as measured by blood oxygenation level–dependent MRI (BOLD-MRI). Following HGF/SF injection, Met-expressing tumors show up to a 30% change in signal by BOLD-MRI in dose-dependent fashion, while no significant difference is observed in tumors or organs that do not express Met. As assessed by color Doppler ultrasonic imaging, dramatic changes in the pattern of regional blood flow are visualized in

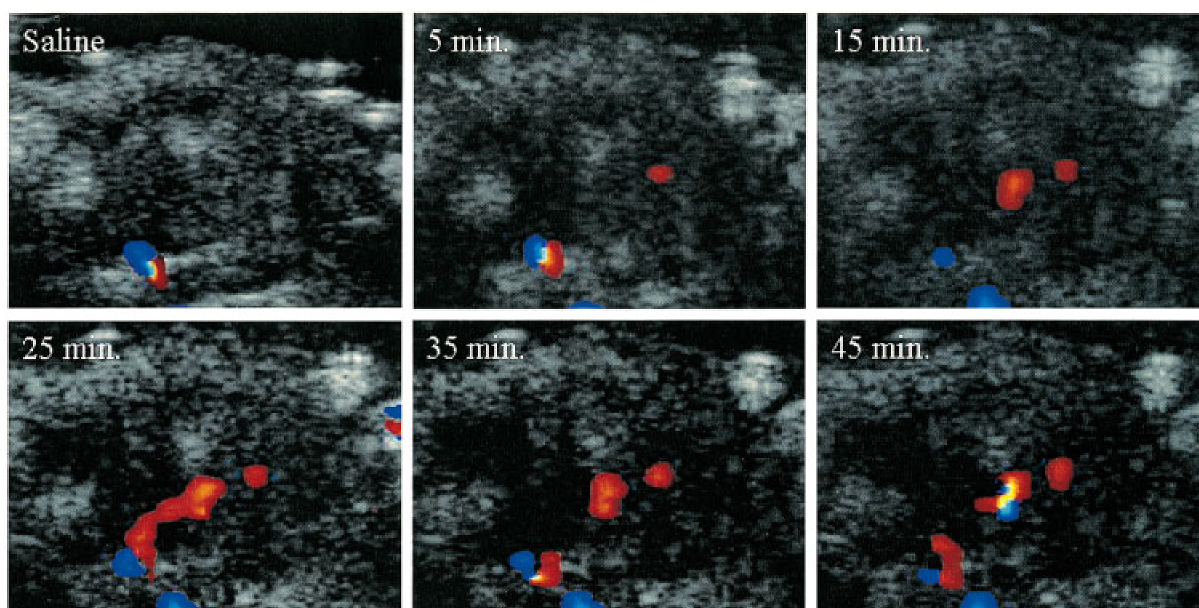

Fig. 4. Noninvasive imaging of increased tumor blood flow following activation of Met. A mouse bearing a DA3 breast tumor (exhibiting paracrine activation of Met by hepatocyte growth factor/scatter factor (HGF/SF)) was injected intravenously with saline, followed by human HGF/SF. Color Doppler ultrasound measurements (Accuson, Sequoia) were performed before HGF/SF injection and at 5, 15, 25, 35, and 45 min afterward. Blood vessels are depicted as *blue* and *red spots*; *yellow* and *green* colors represent regions of higher blood flow velocity.

and near tumors following HGF/SF administration, with a marked reduction in peripheral blood flow and a concurrent enhancement of central blood flow within the tumors. These hemodynamic changes are evident within a few minutes after HGF/SF infusion.

Met-Directed Forms of Cancer Therapy

The ultimate purpose of the diagnostic molecular imaging tests outlined above, whether noninvasive or invasive, is to determine appropriate therapy. Once a tumor has been found to express high levels of Met, an obvious form of therapy is to use biological or chemical agents that inhibit formation or activity of the Met-HGF/SF receptor-ligand complex. For example, we have recently reported that mixtures of monoclonal antibodies directed against HGF/SF are able to inhibit or arrest the growth of Met-expressing tumor cells in vitro and of homologous xenografts in vivo [Cao et al., 2001]. This dramatic finding opens the door for us also to consider radioimmunotherapy with suitably radiolabeled anti-Met mAbs, as well as Met-directed therapy with geldanamycin [Webb et al., 2000] or with selective inhibitors of downstream responders to Met activation [Haddad et al., 2001; Koo et al., 2002].

For the near future, we are committed to exploring different approaches and optimizing protocols for imaging Met abundance and physiologic responsiveness in animal models of human cancer, including murine and canine models of spontaneously occurring Met-expressing cancers. Concurrently, we are validating our noninvasive molecular imaging findings by quantitative biochemical methods and by analytical microscopy. Finally, we will continue to analyze human cancer tissue specimens by state-of-the-art analytical microscopic methods to develop a more complete understanding of the relationships between Met expression and clinical factors.

Before these molecular imaging approaches will become clinically useful on a broad scale, there are three major hurdles to surmount:

- We need to increase the awareness and acceptance of Met as a molecule deserving of scrutiny among practicing oncologists and pathologists. In addition to our continued efforts to validate this notion experimentally and by retrospective and prospective analysis of human tissue specimens, this will require a concerted public relations drive at the national and international levels on behalf of scientists working in the area.
- We must bring our preclinical noninvasive imaging studies to a level of completeness that will satisfy local and federal regulatory bodies to approve these combinations of reagents and modalities for early clinical testing.
- In close collaboration with our clinical colleagues, we must develop rational and practical algorithms for utilizing molecular imaging of Met in the clinical setting.

In principle, we propose that every solid human tumor that is biopsied or excised should be interrogated routinely by immunohistochemistry to characterize its Met-expression status. All patients with Met-positive tumors could then undergo a Met-directed nuclear imaging study to disclose residual or clinically occult lesions and assess their abundance of Met, or to document that none are evident. Any patient with residual or newly disclosed lesions could be evaluated by provocative diagnostic MRI and/or ultrasonography to determine the physiologic responsiveness of their tumors, and an appropriate therapy regimen (chemotherapy, immunotherapy, radioimmunotherapy) could then be devised. Finally, either provocative functional imaging or Met-directed nuclear imaging could be used to monitor changes in Met abundance and activity in response to therapy.

We are perhaps 3–5 years away from performing noninvasive Met-directed imaging in humans with cancer, yet our progress so far in animal models is most encouraging. If we can confirm, as all our preclinical studies suggest, that the "very malignant" indeed have more of Met in terms of abundance or provocable activity, the concept of using Met as a reliable clinical indicator of metastatic risk could become reality.

ACKNOWLEDGMENTS

We are grateful to Chao-Nan Qian and Bin Teh and to Jon McKown for providing images, and to the Jay and Betty Van Andel Foundation, the Michigan Life Sciences Corridor, the Michigan Universities Commercialization Initiative, CapCURE, the U.S. Army, and the National Cancer Institute for supporting research in the

authors' laboratories. We also thank Beatrice Knudsen, Milton Gross and Brain Ross for their collaborative imaging support.

REFERENCES

American Cancer Society. 2002. Cancer Facts and Figures 2002. New York.

Cao B, Su Y, Oskarsson M, Zhao P, Kort EJ, Fisher RJ, Wang L-M, Vande Woude GF. 2001. Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models. Proc Natl Acad Sci USA 98:7443–7448.

Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, Vande Woude GF. 1984. Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature 311:29–33.

Dean M, Park M, Le Beau MM, Robins TS, Diaz MO, Rowley JD, Blair DG, Vande Woude GF. 1985. The human met oncogene is related to the tyrosine kinase oncogenes. Nature 318:385–388.

Edakuni G, Sasatomi E, Satoh T, Tokanuga O, Miyazaki K. 2001. Expression of the hepatocyte growth factor/c-Met pathway is increased at the cancer front in breast carcinoma. Pathol Int 51:172–178.

Firon M, Shaharabany M, Altstock RT, Horev J, Abramovic A, Reseau JH, Vande Woude GF, Tsarfaty I. 2000. Dominant negative Met reduces tumorigenicity-metastasis and increases tubule formation in mammary cells. Oncogene 19:2386–2397.

Ghoussoub RAD, Dillon DA, D'Aquila T, Rimm EB, Fearon ER, Rimm DL. 1998. Expression of c-Met is a strong independent prognostic factor in breast carcinoma. Cancer 82:1513–1520.

Haddad R, Lipson KE, Webb CP. 2001. Hepatocyte growth factor expression in human cancer and therapy with specific inhibitors. Anticancer Res 21:4243–4252.

Hay RV, Cao B, Skinner RS, Wang L-M, Su YL, Resau JH, Vande Woude GF, Gross MD. 2002a. Radioimmunoscintigraphy of tumors autocrine for human Met and hepatocyte growth factor/scatter factor. Mol Imaging 1:56–62.

Hay R, Cao B, Skinner RS, Wang L-M, Su Y, Resau J, Knudsen B, Vande Woude G, Gross M. 2002b. Radioimmunoscintigraphy of human Met-expressing tumor xenografts with Met3, an anti-Met monoclonal antibody. Cancer Biother Radiopharm 17:482 (Abstract).

Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS, de la Chapelle A. 2001. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proc Natl Acad Sci USA 98:15044–15049.

Humphrey PA, Zhu X, Zarnegar R, Swanson PE, Ratliff TL, Vollmer RT, Day ML. 1995. Hepatocyte growth factor and its receptor (c-MET) in prostatic carcinoma. Am J Pathol 147:386–396.

Iyer A, Kmiecik TE, Park M, Daar I, Blair D, Dunn KJ, Sutrave P, Ihle JN, Bodescot M, Vande Woude GF. 1990. Structure, tissue-specific expression, and transforming activity of the mouse met protooncogene. Cell Growth Differ 1:87–95.

Jin L, Fuchs A, Schnitt SJ, Yao Y, Joseph A, Lamszus K, Park M, Goldberg ID, Rosen EM. 1997. Expression of scatter factor and c-Met receptor in benign and malignant breast tissue. Cancer 79:749–760.

Knudsen BS, Gmyrek GA, Inra J, Scherr DS, Vaughan ED, Nanus DM, Kattan MW, Gerald WL, Vande Woude GF. 2002. High expression of the Met receptor in prostate cancer metastasis to bone. Urology (in press).

Koo H-M, VanBrocklin M, McWilliams MJ, Leppla SH, Duesbery NS, Vande Woude GF. 2002. Apoptosis and melanogenesis in human melanoma cells induced by anthrax lethal factor inactivation of mitogen-activated protein kinase kinase. Proc Natl Acad Sci USA 99:3052–3057.

Miller JC, Zhou H, Kwekel J, Cavallo R, Burke J, Butler EB, Teh BS, Haab BB. 2003. Antibody microarray profiling of human prostate cancer sera: Antibody screening and identification of potential biomarkers. Proteomics (in press).

Niemann C, Brinkmann V, Spitzer E, Hartmann G, Sachs M, Naundorf H, Birchmeier W. 1998. Reconstitution of mammary gland development in vitro: Requirement of c-Met and c-erbB2 signaling for branching and alveolar morphogenesis. J Cell Biol 143:533–545.

Parker JA. 2001. Cardiac nuclear medicine in monitoring patients with coronary heart disease. Sem Nucl Med XXXI:223–237.

Pisters LL, Troncoso P, Zhau HE, Li W, von Eschenbach AC, Chung LWK. 1995. C-Met proto-oncogene expression in benign and malignant human prostate tissues. J Urol 154:293–298.

Program and Abstracts, Ninth Conference on Cancer Therapy with Antibodies and Immunoconjugates. 2002. Cancer Biother Radiopharm 17:465–494.

Qian C-N, Guo X, Cao B, Kort EJ, Lee C-C, Chen J, Wang L-M, Mai W-Y, Min H-Q, Hong M-H, Vande Woude GF, Resau JH, Teh BT. 2002. Met protein expression level correlates with survival in patients with late-stage nasopharyngeal carcinoma. Cancer Res 62:589–596.

Rimm DL, Camp RL, Charette LA, Costa J, Olsen DA, Reiss M. 2001. Tissue microarray: A new technology for amplification of tissue resources. Cancer J 1:24–31.

Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, et al. 1997. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 16:68–73.

Shaharabany M, Abramovitch R, Kushnir T, Tsarfaty G, Ravid-Megido M, Horev J, Ron R, Itzchak Y, Tsarfaty I. 2001. In vivo molecular imaging of Met tyrosine kinase growth factor receptor activity in normal organs and breast tumors. Cancer Res 61:4873–4878.

Stuart KA, Riordan SM, Lidder S, Crostella L, Williams R, Skouteris GG. 2000. Hepatocyte growth factor/scatter factor-induced intracellular signalling. Int J Exp Pathol 81:17–30.

Takahashi M, Rhodes DR, Furge KA, Kanayama H, Kagawa S, Haab BB, Teh BT. 2001. Gene expression profiling of clear cell renal cell carcinoma: Gene identification and prognostic classification. Proc Natl Acad Sci USA 98:9754–9759.

Tsarfaty I, Alvord WG, Resau JH, Altstock RT, Lidereau R, Bieche I, Bertrand F, Horev J, Klabansky RL, Keydar I, Vande Woude GF. 1999. Alteration of Met protooncogene product expression and prognosis in breast carcinomas. Anal Quant Cytol Histol 21:397–408.

Tuck A, Park M, Sterns EE, Boag A, Elliott B. 1996. Coexpression of hepatocyte growth factor and receptor (Met) in human breast carcinoma. Am J Pathol 148:225–232.

van der Voort R, Taher TEI, Derksen PWB, Spaargaren M, van der Neut R, Pals ST. 2000. The hepatocyte growth factor/Met pathway in development, tumorigenesis, and B-cell differentiation. Adv Cancer Res 79:39–90.

Watanabe M, Fukutome K, Kato H, Murata M, Kawamura J, Shiraishi T, Yatani R. 1999. Progression-linked overexpression of c-Met in prostatic intraepithelial neoplasia and latent as well as clinical prostate cancers. Cancer Lett 141:173–178.

Webb CP, Hose CD, Koochekpour S, Jeffers M, Oskarsson M, Sausville E, Monks A, Vande Woude GF. 2000. The geldanamycins are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasminogen activator-plasmin proteolytic network. Cancer Res 60: 342–349.

Yamashita JI, Ogawa M, Yamashita SI, Nomura K, Kuramoto M, Saishoji T, Shin S. 1994. Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. Cancer Res 54:1630–1633.