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Molecular Imaging of Tumor Angiogenesis

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Abstract The emergence of angiogenesis as an important target for cancer therapy has led to increased research aimed at understanding the mechanisms underlying the development, maintenance, and destruction of tumor vasculature. Concurrently, molecular imaging technologies have been developed and are being incorporated as integral components of biomedical research due to their ability to noninvasively monitor in vivo molecular events. With the evaluation of numerous anti-angiogenic agents in clinical trials, the adaptation and validation of molecular imaging modalities for monitoring angiogenesis is actively being pursued. The importance of selecting appropriate molecular targets in the study of angiogenesis has become increasingly complex due to the pleiotropy of vascular phenotypes. Furthermore, due to both the relatively low abundance of endothelial cells in tumor tissue and the inherent difficulties of detecting molecular events, molecular imaging of vasculature necessitates continued improvements in achieving higher sensitivity. While several studies have been published that set the groundwork for imaging angiogenesis, much has yet to be accomplished. Various tumor models and transgenic mice provide an excellent resource for developing molecular imaging technologies for the understanding of angiogenesis. This research may play a particularly crucial role in evaluating mechanism and efficacy during pre-clinical testing of anti-angiogenic drugs. Due to practical limitations, however, the implementation of angiogenesis-directed molecular imaging may not extend beyond highly specialized clinical trials. That is, imaging modalities that evaluate angiogenesis at a functional level may prove more appropriate. Despite future technical challenges, molecular imaging will become an important research and clinical tool in evaluating tumor angiogenesis. J. Cell. Biochem. Suppl. 39: 72–78, 2002. Published 2002 Wiley-Liss, Inc.†

Key words: magnetic resonance imaging; optical imaging; intravital videomicroscopy; positron emission tomography; ultrasound

Angiogenesis, or the recruitment of new vasculature from existing blood vessels, is a vital component of many normal physiological processes. Under inappropriate conditions, however, angiogenesis may serve as a crucial factor in disease development and progression [reviewed by Carmeliet and Jain, 2000]. Pathological angiogenesis has been demonstrated in several diseases including cancer, hypertension, rheumatoid arthritis, and diabetic retinopathy.

Briefly, angiogenesis is a complex process regulated through a fine balance of pro-angiogenic and anti-angiogenic molecules. Thus, a relative increase in pro-angiogenic stimuli will result in blood vessel recruitment. While endothelial cells serve as the building blocks of blood vessels, the immediate microenvironment, hormonal influences, and multiple cell types all influence the angiogenic process. Tumors may switch to an angiogenic phenotype under various types of metabolic, mechanical, or immunological stressors. This switch results in progression from microscopic disease to expansive tumor growth and metastases. Thus, the initiation and maintenance of pathological angiogenesis has been the focus of active research as a putative target of cancer therapy.

Abbreviations used: bFGF, basic fibroblast growth factor; BOLD, blood oxygenation level dependent; CT, computed tomography; FDG, 18F-fluorodeoxyglucose; GFP, green fluorescent protein; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MMPs, matrix metalloproteinases; NIR, near-infrared; PET, positron emission tomography; SNR, signal to noise ratio; VEGF, vascular endothelial growth factor.

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Molecular imaging has emerged as an exciting tool for serially evaluating in vivo molecular events noninvasively. More precisely, molecular imaging technology adapts current imaging technologies, such as magnetic resonance imaging (MRI), positron emission tomography (PET), and optical imaging, to monitor molecular events. While active efforts by several groups to develop molecular imaging technologies for evaluating angiogenesis are in progress, few if any molecular imaging methods have been validated as versatile methods of monitoring angiogenesis. This is a result of the molecular complexity of angiogenesis and the relative infancy of molecular imaging as a field. This review addresses multiple topics in the development and application of molecular imaging to tumor angiogenesis, from the selection of a molecular target to the role of molecular imaging in angiogenesis-related clinical trials.

SELECTING MOLECULAR TARGETS IN ANGIOGENESIS

Since angiogenesis is a complex process involving multiple cell types and molecular mediators, the selection of molecular imaging targets is extremely important. For instance, prior to the elucidation of separate angiogenic pathways mediated by integrins related to either vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF) [Friedlander et al., 1995], an investigator targeting one specific integrin could miss important changes in angiogenesis modulation. Thus, a specific target should be explored and thoroughly validated before extensive research efforts are directed towards developing a specific molecular imaging application.

The discovery of distinct vascular markers in different tissues through phage display technology provides an important avenue for directed molecular imaging of angiogenesis [Trepel et al., 2002]. This has increased importance in molecular imaging applications that use intravenous delivery of contrast or substrate agents. Such agents could be targeted to specific tissues through their endothelial "zip code," thus improving localization and overall efficiency. This is particularly important for the concurrent delivery of therapeutic and imaging agents in the treatment and monitoring of cancer. Also, tissue-specific angiogenesis may be targeted by exploiting pathways unique to a particular tissue [LeCouter et al., 2002]. For the general evaluation of angiogenesis, a universal target for the evaluation of tumor vasculature would be of great benefit and would likely have increased applicability to general clinical practice. However, a putative universal target has yet to be robustly validated. We have developed a versatile system using murine MRI and microarray technology for querying differences within imaged tumors [Costouros et al., 2002]. Briefly, tumors are imaged, excised with concordant orientation, sectioned, and co-registered. Tissue can then be examined histologically with appropriate stains and immunohistochemical methods, or RNA can be extracted for microarray analysis from specific tumor regions using laser capture microdissection (Figure 1). This methodology can be applied using various imaging modalities and targeted molecular reporters or contrast agents for the discovery of future molecular targets. Also, imaging observations may be correlated with molecular events in validating assumptions made through imaging. Some promising targets for reporters of tumor angiogenesis include the $\alpha_v\beta_3$ integrin adhesion receptor, the neovasculature specific Tie2 promotor, hypoxia inducible factor-1α, and various matrix metalloproteinases (MMPs) involved in angiogenesis progression and metastasis.

SENSITIVITY, SPECIFICITY, AND RESOLUTION

A major technical hurdle for most current molecular imaging applications is poor sensitivity for low-level molecular activity. In the imaging of angiogenesis, this difficulty is compounded by the relatively small proportion of endothelial cells within tumors. Thus, methods for improved sensitivity and signal to noise ratio (SNR) are crucial to the feasibility of molecular imaging in angiogenesis. While anatomic localization of molecular activity may not be essential for specific experiments, pre-clinical and clinical applications of molecular imaging will require either concurrent anatomic resolution or the capability for accurate co-registration across imaging modalities.

The recent development of CT-PET imaging may address both of these issues. CT-PET combines the advantages of its component modalities—the excellent sensitivity and functional imaging of PET and the anatomic detail of CT (sub-millimeter resolution). However, despite

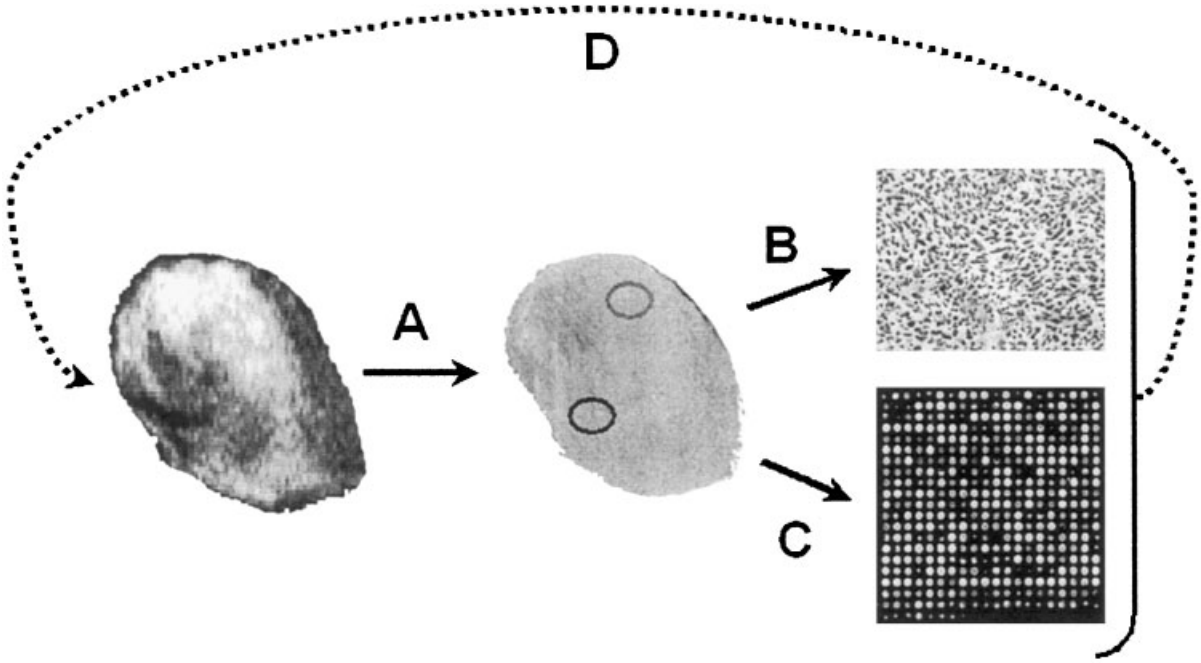

Fig. 1. Correlating imaging with biology. Image of tumor (left) shows distinct regions based on contrast or reporter delivered intravenously. Tissue can then be removed, sectioned, and co-registered with image (**A**). From the sectioned tissue, biological correlation with imaging can be performed, for instance, through immunohistochemistry for describing protein (or protein-expressing cell type) distribution (**B**) or microarray gene expression analysis (**C**) from distinct enhancing regions (light and dark elipses). Compiled information can then be used to make biological inferences based on the contrast agent or reporter used for imaging, as well as to define new molecular targets for imaging (**D**).

general anatomic co-registration and localization with CT-PET, finer details are not appreciated with PET due to the intrinsic limitations in resolution (several millimeters). This can be overcome in small animal imaging through the use of micro-PET (sub-millimeter resolution) [reviewed by Chatziioannou, 2002]. As tumors tend to be heterogeneous in their vascular distribution, more robust information may be achieved with molecular imaging modalities that concomitantly provide anatomic detail, such as MRI.

In contrast to PET, MRI offers excellent anatomic resolution (millimeter to sub-millimeter resolution with increasing magnetic field strength). However, current contrast agents lack the necessary specificity and SNR for molecular imaging applications. Molecular MRI is based on the premise that magnetic reporters are specifically targeted through a receptor-ligand or antibody fragment. Also, specificity may be achieved through magnetic agents that are trapped or activated in the appropriate molecular environment, e.g. through enzymatic modification [Louie et al., 2000]. Despite the localized accumulation and specificity of these agents, current MRI is unable to image molecular contrast agents with adequate sensitivity. Thus, several schemes for MRI contrast agent signal amplification are being investigated, including directed enzyme conjugates [Bogdanov et al., 2002] and targeted contrast polymer agents [Curtet et al., 1998].

In addition to PET, CT, and MRI, optical imaging methods are becoming increasingly powerful techniques for studying angiogenesis in vivo. Currently, the most commonly employed optical makers are fluorescent proteins (e.g. green fluorescent protein (GFP)), bioluminescent enzymes (e.g. luciferase), and near-infrared (NIR) probes (e.g. Cy5.5 labeled proteins). Because these techniques rely on optical phenomena such as fluorescence and bioluminescence, which are significantly impacted by factors such as tissue absorption and autofluorescence, they are to date limited in application to small animal imaging. An exception is NIR imaging; because longer wavelengths transmit through tissues more efficiently than shorter ones, NIR imaging is more suited for in vivo

applications than fluorescent (e.g. GFP) imaging. The greatest inherent SNR of the optical methods is provided by bioluminescent imaging, because in light-tight imaging chambers, signal is only derived from tissues expressing luciferase. While tissue penetration is an obvious difficulty for all optical methods, the power of these techniques as research tools should not be underestimated. Advantages of optical imaging methods include their low-cost, the relatively simple technology allowing for high throughput experiments, and the simultaneous use of multiple markers with different optical characteristics to study several biological processes in the same experiment. Encouraging evidence is provided by results such as the noninvasive visualization of relatively small numbers of luciferase-expressing cells in the abdominal organs of a mouse [Edinger et al., 1999]. This level of sensitivity may be more appropriate for visualizing angiogenesis. Currently, transgenic mice have been developed expressing GFP (FVB/N-TgN(TIE2GFP)287Sato) or beta-galactosidase (FVB/N-TgN(TIE2-lacZ) 182Sato) under control of the Tie2 promotor (The Jackson Laboratory, Bar Harbor, ME). For in vivo imaging applications, however, the development of a transgenic mouse with the luciferase gene under control of the Tie2 promotor is needed for an adequate SNR.

THE STUDY OF ANGIOGENESIS

The application of molecular imaging to angiogenesis can be divided into direct and indirect (surrogate) detection of molecular activity. Direct detection involves detecting primary molecular events, such as receptor expression on endothelium or enzymatic activation of a reporter. As previously mentioned, this type of imaging typically suffers from poor sensitivity. In contrast, indirect detection monitors sequelae of primary molecular events for which inference is made based on indirect imaging findings, such as changes in pH due to altered angiogenesis. While limitations exist in making inferences based on indirect imaging methods, this approach can help to overcome problems associated with direct imaging. For instance, indirect imaging could provide information of the downstream effects from multiple angiogenesis pathways, thus avoiding missing the correct molecular event. Also, indirect imaging methods could be directed towards molecular activity that results in biological amplification, such as through a signal cascade, thus providing improved sensitivity.

Direct Molecular Imaging

Several methods of direct molecular imaging of angiogenesis are currently being evaluated. A powerful approach to studying angiogenesis in vivo at a microvascular, cellular, and now molecular level is provided by intravital fluorescence videomicroscopy. The advantage of this technique is that it provides a high spatial resolution, noninvasive, direct, and continuous visualization of the tumor microvasculature. Single cells labeled with optical markers can be detected and followed as they migrate through blood vessels, and tumor biology can be studied in dorsal skin chambers created in living animals. Coupled with the advances in fluorescent and bioluminescent reporter genes, this technology is now shedding light on molecular events. For instance, using a transgenic mouse expressing GFP under the control of the promoter for VEGF, the importance of host stromal cells in the angiogenic tumor environment has been demonstrated with intravital videomicroscopy [Fukumura et al., 1998]. As previously mentioned, a transgenic line expressing GFP under control of the Tie2 promoter has also been created [Motoike et al., 2000]. Further recent advances include the use of the multiphoton laser-scanning microscope. Its advantages include high three-dimensional resolution of processes such as gene expression in tumors, including both tumor surface and deep regions [Brown et al., 2001].

Lower spatial resolution optical techniques are afforded by whole-body fluorescent and bioluminescent imaging of small animals. Due to poor sensitivity resulting from the low abundance of endothelium and angiogenesis-associated molecular events relative to tissue volume, these techniques are limited in detecting primary events. However, the technology has been used to study tumor angiogenesis in a variety of ways. For instance, fluorophore coupling has been used to image a fibronectin isoform that is present in tumor vessels during angiogenesis [Neri et al., 1997]. Using the contrast between bright GFP-expressing tumor tissue and darker intratumoral blood vessels, in vivo fluorescent imaging has been utilized to quantify tumor angiogenesis in real time at relatively transparent, orthotopic sites, such as

the mouse breast fat pad [Yang et al., 2001]. The enzymatic activity of matrix metalloproteinase-2 (MMP-2), which had previously been shown in a tumor model to be necessary for the switch to the angiogenic phenotype [Fang et al., 2000], has been visualized noninvasively in vivo using MMP substrates linked to quenched NIR fluorochromes [Bremer et al., 2001]. The action of MMP-2 on these substrates caused activation of the fluorochromes and enabled the imaging of enzymatic activity (and the activity by clinically relevant inhibitors) in the live mouse.

The utility of PET has been demonstrated in the targeted imaging of $\alpha_v\beta_3$ integrins, a cell adhesion receptor enriched on tumor endothelium, via an RGD peptide labeled tracer in mice [Haubner et al., 2001]. The $\alpha_v\beta_3$ integrin receptor has also been exploited using MRI with targeted paramagnetic liposomes [Sipkins et al., 1998] or nanoparticle contrast agents [Anderson et al., 2000]. Ultrasound has been used to target activated endothelium using microbubble contrast agent against ICAM-1 cell adhesion receptors [Villanueva et al., 1998]. However, the utility of ultrasound in molecular imaging may suffer due to substantial variability in acquiring images and interpreting results.

Indirect Molecular Imaging

Current indirect imaging applications related to angiogenesis exploit changes in tumor microenvironment, such as oxygenation, pH, and metabolism, that are related to vascular status. While molecular imaging using PET is typically targeted for direct imaging, the widespread radiotracer 18F-fluorodeoxyglucose (FDG) can be categorized as an indirect imaging reporter that measures increased glycolysis from well oxygenated, actively proliferating tumor cells. However, conflicting literature correlating angiogenesis and FDG uptake supports the observation over the last decade that tumor cells may selectively switch their metabolic profile independent of the angiogenic status of the tissue [Aronen et al., 2000; Veronesi et al., 2002].

MRI has great potential for indirect molecular imaging applications using advanced imaging techniques such as magnetic resonance spectroscopy (MRS) and blood oxygenation level dependent (BOLD) imaging. MRS is emerging as a useful tool for measuring metabolite levels in tissue. Current MRS applications are limited by the number of observable metabolites and typically use proton signal due to its higher sensitivity compared to other detectable isotopes. Feasibly imaged on most current clinical MRI scanners, the anaerobic production of lactate could be considered a marker for hypoxic tissue with inadequate angiogenesis. Like FDG, however, the use of lactate as a marker of hypoxia is confounded by variable tumor metabolism independent of tissue oxygenation. Another approach could measure adenosine phosphate nucleotide energy dynamics using phosphorus MRS, correlating ATP production and consumption associated with aerobic metabolism. However, phosphorus imaging still suffers from decreased sensitivity in comparison to proton MRS. Through technical improvements and the implementation of higher field strength MR scanners, this technology should provide an effective means for indirectly measuring angiogenesis modulation. BOLD imaging exploits the paramagnetic nature of deoxyhemoglobin, thus using an intrinsic contrast agent for detecting tissue oxygenation levels. This has been used for assessing changes in angiogenesis modulation in response to changes in VEGF [Abramovitch et al., 1999] or Met activation by hepatocyte growth factor scatter factor [Shaharabany et al., 2001]. Furthermore, pH sensitive contrast agents, such as liposomes that undergo a conformation change in acidic pH and consequently deposit a contrast agent or reporter, may also prove useful for monitoring the pH in the tumor microenvironment [Lokling et al., 2001].

FUTURE ROLE OF MOLECULAR IMAGING IN ANGIOGENESIS

While molecular imaging has demonstrated impressive potential for understanding angiogenesis in the research setting and will play an important role in pre-clinical assessment of novel anti-angiogenisis drugs, the true hope for any new noninvasive diagnostic technology is its application to patient care. From assessing the extent of disease and planning appropriate therapy to evaluating treatment responses or relapses, molecular imaging will play an important role in anti-angiogenic therapy. This role is intimately linked to current trends in pharmaceutical design and the general cytostatic nature of anti-angiogenesis therapy. For instance, with drugs designed using molecular-

based rationale such as the protein-tyrosine kinase inhibitor Gleevec™ (Novartis Pharmaceuticals, East Hanover, NJ) and the push for developing clinically appropriate gene-therapy vectors, future therapy will require the ability to monitor associated molecular events. Since effective anti-angiogenic therapy will often show changes on the scale of hours to days, far before appreciable changes in tumor size, standard imaging methods are insufficient for assessing therapeutic response. In addition, intravenous targeting of imaging agents to tumor bears similar problems as chemotherapeutic delivery. That is, vascular heterogeneity and variable interstitial pressures may inhibit universal distribution of the targeted substance within the tumor, giving an inaccurate assessment of tumor angiogenesis. Thus, other imaging modalities, such as techniques describing the functional status of tumor vasculature [Libutti et al., 1999], may be more indicative of the angiogenic state of a tumor. As with any new imaging technology, molecular imaging of angiogenesis will serve as an adjunct modality to well-established techniques, and will likely be implemented to describe particular angiogenic phenotypes pertinent to cancer therapy.

REFERENCES

Abramovitch R, Dafni H, Smouha E, Benjamin LE, Neeman M. 1999. In vivo prediction of vascular susceptibility to vascular endothelial growth factor withdrawal: Magnetic resonance imaging of C6 rat glioma in nude mice. Cancer Res 59:5012–5016.

Anderson SA, Rader RK, Westlin WF, Null C, Jackson D, Lanza GM, Wickline SA, Kotyk JJ. 2000. Magnetic resonance contrast enhancement of neovasculature with alpha(v)beta(3)-targeted nanoparticles. Magn Reson Med 44:433–439.

Aronen HJ, Pardo FS, Kennedy DN, Belliveau JW, Packard SD, Hsu DW, Hochberg FH, Fischman AJ, Rosen BR. 2000. High microvascular blood volume is associated with high glucose uptake and tumor angiogenesis in human gliomas. Clin Canc Res 6:2189–2200.

Bogdanov AA, Matuszewski L, Bremer C, Petrovsky AV, Weissleder R. 2002. Oligomerization of paramagnetic substrates result in signal amplification and can be used for MR imaging of molecular targets. Molecular Imaging 1:16–23.

Bremer C, Tung CH, Weissleder R. 2001. In vivo molecular target assessment of matrix metalloproteinase inhibition. Nat Med 7:743–748.

Brown EB, Campbel RB, Tsuzuki Lei, Xu P, Carmeliet D, Fukumura RK. 2001. In Vivo measurement of gene expression, angiogenesis and physiological function in tumors using muliphoton laser scanning microscopy. Nat Med 7:864–868.

Carmeliet P, Jain RK. 2000. Angiogenesis in cancer and other diseases. Nature 407:249–257.

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

Costouros NG, Lorang D, Zhang Y, Miller MS, Diehn FE, Hewitt SM, Knopp MV, Li KCP, Choyke PL, Alexander HR, Libutti SK. 2002. Microarray gene expression analysis of murine tumor heterogeneity defined by dynamic contrast-enhanced MRI. Molecular Imaging 1:301–308.

Curtet C, Maton F, Havet T, Slinkin M, Mishra A, Chatal JF, Muller RN. 1998. Polylysine-Gd-DTPAn and polylysine-Gd-DOTAn coupled to anti-CEA F(ab')2 fragments as potential immunocontrast agents. Relaxometry, biodistribution, and magnetic resonance imaging in nude mice grafted with human colorectal carcinoma. Invest Radiol 33:752–761.

Edinger M, Sweeney TJ, Tucker AA, Olomu AB, Negrin RS, Contag CH. 1999. Noninvasive assessment of tumor cell proliferation in animal models. Neoplasia 1:303–310.

Fang J, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, Harper J, Tamvakopoulos G, Moses MA. 2000. Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. Proc Natl Acad Sci 97:3884–3889.

Friedlander M, Brooks PCs, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. 1995. Definition of two angiogenic pathways by distinct alpha v integrins. Science 270: 1500–1502.

Fukumura D, Xavier R, Sugiura T, Chen Y, Park E, Lu N, Selig M, Nielsen G, Taksir T, Jain RK, Seed B. 1998. Tumor induction of VEGF promoter activity in stromal cells. Cell 94:715–725.

Haubner R, Wester HJ, Weber WA, Mang C, Ziegler SI, Goodman SL, Senekowitsch-Schmidtke R, Kessler H, Schwaiger M. 2001. Noninvasive imaging of alpha(v)-beta3 integrin expression using 18F-labeled RGD-containing glycopeptide and positron emission tomography. Cancer Res 61:1781–1785.

LeCouter J, Lin R, Ferrara N. 2002. Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. Nat Med 8:913–917.

Libutti SK, Choyke P, Carrasquillo JA, Bacharach S, Neumann RD. 1999. Monitoring responses to antiangiogenic agents using noninvasive imaging tests. Cancer J Sci Am 5:252–256.

Lokling KE, Fossheim SL, Skurtveit R, Bjornerud A, Klaveness J. 2001. pH-sensitive paramagnetic liposomes as MRI contrast agents: In vitro feasibility studies. Magn Reson Imaging 19:731–738.

Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. 2000. In vivo visualization of gene expression using magnetic resonance imaging. Nat Biotechnol 18:321–325.

Motoike T, Loughna S, Perens E, Roman BL, Liao W, Chau TC, Richardson CD, Kawate T, Kuno J, Weinstein BM, Stainier DY, Sato TN. 2000. Universal GFP reporter for the study of vascular development. Genesis 28:75–81.

Neri D, Carnemolla B, Nissim A, Leprini A, Querze G, Balza E, Pini A, Tarli L, Halin C, Neri P, Zardi L, Winter G. 1997. Targeting by affinity-matured recombinant antibody fragments of an angiogenesis associated fibronectin isoform. Nat Biotechnol 15:1271–1275.

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.

Sipkins DA, Cheresh DA, Kazemi MR, Nevin LM, Bednarski MD, Li KC. 1998. Detection of tumor angiogenesis in vivo by alphaVbeta3-targeted magnetic resonance imaging. Nat Med 4:623–626.

Trepel M, Arap W, Pasqualini R. 2002. In vivo phage display and vascular heterogeneity: Implications for targeted medicine. Curr Opin Chem Biol 6:399–404.

Veronesi G, Landoni C, Pelosi G, Picchio M, Sonzogni A, Leon ME, Solli PG, Leo F, Spaggiari L, Bellomi M, Fazio F, Pastorino U. 2002. Fluoro-deoxi-glucose uptake and angiogenesis are independent biological features in lung metastases. Br J Cancer 86:1391–1395.

Villanueva FS, Jankowski RJ, Klibanov S, Pina ML, Alber SM, Watkins SC, Brandenburger GH, Wagner WR. 1998. Microbubbles targeted to intercellular adhesion molecule-1 bind to activated coronary artery endothelial cells. Circulation 98:1–5.

Yang M, Baranov E, Li XM, Wang JW, Jiang P, Li L, Moossa AR, Penman S, Hoffman RM. 2001. Whole-body and intravital optical imaging of angiogenesis in orthotopically implanted tumors. Proc Natl Acad Sci 98: 2616–2621.