Journal of Cellular Biochemistry Supplement 39:11–17 (2002)

Functional and Molecular MR Imaging of Angiogenesis: Seeing the Target, Seeing it Work

Michal Neeman*

Department of Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel

Abstract Intensive research over the last years led to the discovery of multiple molecular pathways and intricate regulatory network controlling the growth and regression of blood vessels in general and angiogenesis in particular. The difficulties in elucidation of the regulation of angiogenesis, stems from the inherent complexity due to participation of many cell types, under a dominant impact of physiological and environmental effects of flow, perfusion, and oxygenation. Major advances were achieved with the use of sophisticated transgenic mice models engineered so as to provide spatially and temporally controlled expression of specific factors alone or in combination. In vivo analysis of these models frequently requires the use of non-invasive imaging modalities for measurement of functional parameters of the vasculature along with dynamic molecular information. Optical methods are extensively applied for the study of angiogenesis [Brown et al., 2001] but provide very limited tissue penetration. MRI offers the advantage of being non-invasive with uniform and relatively high spatial resolution for deep tissues. Multiple MRI approaches for monitoring angiogenesis were developed over the last years, each looking at a particular step in the process. The aim of this paper is to analyze the clinical, pharmaceutical, and biological needs for imaging of angiogenesis, and to critically evaluate the strengths and weaknesses of functional and molecular imaging for monitoring angiogenesis. The inherent problem of validation of different measures of angiogenesis, and the advantages and limitations associated with application of MRI based methods, as surrogates for other measurements of angiogenesis will be discussed. The terms molecular imaging and functional imaging are frequently loosely defined with a significant overlap between the two. For the sake of this paper we will apply a narrower definition of both terms, where molecular imaging will apply to methods directed towards detection of specific biological molecules that participate directly in (regulation of) a physiological process; while functional imaging will be used to describe those methods that aim to detect the physiological response to a defined (molecular) stimulus. J. Cell. Biochem. Suppl. 39: 11–17, 2002.

Key words: MRI; angiogenesis; functional imaging; molecular imaging; VEGF

MRI MAPPING OF ANGIOGENESIS

MRI methods for imaging angiogenesis cover an array of parameters including functional measurements of blood volume, perfusion, permeability, and vasoreactivity, along with development of novel contrast materials for selective targeting of angiogenesis associated molecular markers. These approaches were utilized in a wide range of applications including the study of physiological and tumor angiogenesis, analysis of the effects of altered expression of genes involved in angiogenesis, and for monitoring the response to antiangiogenic therapy.

Measurement of blood volume and especially, monitoring changes in blood volume over time, are reflective of de novo growth of blood vessels and, therefore, measure the final product of the process of angiogenesis. A number of studies attempted to apply blood volume measurements for quantitative determination of angiogenesis. Many of these studies attempted to correlate blood volume measurements from dynamic contrast enhanced MRI with histological determination of micro-vessel density (number of vessels per unit area; MVD) in angiogenic hotspots. A number of studies reported significant correlation [Hawighorst et al., 1998a,b; Brasch and Turetschek, 2000; Pathak et al., 2001];

Grant sponsor: Israel Ministry of Health; Grant sponsor: The Israel Science Foundation; Grant sponsor: USA NIH; Grant number: RO1 CA75334.

*Correspondence to: Michal Neeman, Department of Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel.
E-mail: michal.neeman@weizmann.ac.il

Received 3 October 2002; Accepted 3 October 2002
DOI 10.1002/jcb.10399
Published online in Wiley InterScience (www.interscience.wiley.com).

while other studies reported weak correlation suggesting that other factors contribute to the MRI data [Buckley et al., 1997]. An inherent flaw in this correlation stems from the well known increased vessel diameter in tumors, resulting in increased blood volume fraction that does not necessarily reflect increased number of vessels as determined by MVD. Moreover, steady state or R_1 based MRI measurements of blood volume using blood pool agents are particularly sensitive to overestimation in regions of angiogenesis due to vascular hyperpermeability resulting in significant extravasation of even high molecular weight contrast materials.

We applied intrinsic MR contrast originating from blood deoxyhemoglobin for determination of the apparent vessel density (AVD; [Abramovitch et al., 1998a]). Significant correlation was found with independent optical imaging analysis of vessel density in the same samples. While this approach provides only qualitative determination of vessel density, it provides the advantage of relying on contrast material which is intravascular, and because no contrast agents are administered, measurements can be repeated easily allowing for detailed kinetics measurements of vascular development [Abramovitch et al., 1995; Schiffenbauer et al., 1997; Abramovitch et al., 1998b; Gilead and Neeman, 1999].

The link between angiogenesis and hyperpermeability has been established through the seminal work of Harold Dvorak on vascular endothelial growth factor (VEGF), which is also an extremely potent vascular permeability factor (VPF) [Dvorak et al., 1999]. Upon activation of VEGF receptors by VEGF, microvessels become hyperpermeable to plasma proteins and other circulating macromolecules. Such hyperpermeability was found to accompany angiogenesis in tumors, healing wounds, retinopathies, inflammatory conditions, and physiological ovarian angiogenesis. Hyperpermeability to plasma proteins results in the formation of a fibrin-rich extracellular matrix that supports the ingrowth of fibroblasts and endothelial cells. Hyperpermeability is provided by a number of VEGF-induced mechanisms including vesiculo-vacuolar organelles (VVOs) and trans-endothelial openings that have been variously interpreted as inter-endothelial cell gaps or trans-endothelial cell pores [Dvorak et al., 1999; Feng et al., 2000]. Beyond inducing vascular permeability and angiogenesis, VEGF is also a key survival factor for endothelial cells in immature neovasculature [Benjamin and Keshet, 1997].

However, recent studies undermined the tight link between permeability, angiogenic, and endothelial survival activities of VEGF by demonstrating specific molecular pathways that dissociate the different activities. Thus angiogenesis without hyperpermeability could be induced by VEGF either in the presence of elevated levels of angiopoietin-1 [Thurston et al., 2000] or upon inactivation of src [Eliceiri et al., 1999; Ferrara, 2001]. Consequently, while hyper permeability is frequently indicative of VEGF-induced angiogenesis, it cannot be used as an absolute surrogate for either VEGF or angiogenesis.

Despite the need for caution in associating VEGF and permeability, a large number of studies confirmed the link between the two, suggesting that in many cases VEGF activity can be mapped using hyperpermeability, which is easily detectable by contrast enhanced MRI. Thus spatial registration was observed between regions of high expression of VEGF mapped by immuno-histochemistry and MRI mapping of permeability to albumin–GdDTPA [Bhujwalla et al., 2001b]; Suppression of VEGF using anti-VEGF antibodies [Brasch et al., 1997], or using inhibitors for the VEGF receptor [Drevs et al., 2002] significantly reduced vascular permeability. Similarly, reduced permeability was detected in prostate tumors after androgen ablation therapy, which suppresses hormonally induced expression of VEGF [Padhani et al., 2001].

Acute administration of VEGF in normal tissue resulted in immediate increased extravasation of macromolecular albumin–GdDTPA [Dafni et al., 2002b]. Detailed analysis of the kinetics of contrast extravasation revealed that the hyperpermeability induced by VEGF under these conditions is transient and is diminished within 1–2 h through inactivation of the administered growth factor, rather than by desensitization of the receptor [Dafni et al., 2002b]. This experimental approach can be used for in vivo functional mapping of the dynamics of receptor mediated growth-factor internalization and recycling as well as for mapping of receptor desensitization [Dafni et al., 2002b].

Prolonged exposure to elevated levels of VEGF was studied using tumors engineered

to express VEGF under switchable promoter [Benjamin and Keshet, 1997]. Albumin triple-labeled with GdDTPA, fluorescein, and biotin was used for multi-modality imaging by MRI, confocal microscopy, and histology, respectively. High levels of VEGF were associated with hyperpermeability, interstitial convection, and lymphatic drain, all of which could be abrogated by switching off over-expression of VEGF [Dafni et al., 2002a].

Mismatch between the spatial pattern of vascular permeability and blood volume in tumors demonstrated the complexity of using either of these parameters as an independent measure of angiogenesis [Bhujwalla et al., 2001b, 2002]. Thus, regions of high vascular volume frequently showed relatively low permeability whereas high permeability was typically observed in regions of low blood volume. The relation between metabolic heterogeneity and tumor vascularity were studied by elegant correlations of NMR spectroscopic data, MRI mapping of pH, and dynamic contrast enhanced MRI measurements of blood volume and permeability using albumin–GdDTPA [Bhujwalla et al., 2001a, 2002]. Specific patterns linking the different parameters may help define common regulatory pathways that dictate the final tumor micro-environment.

Intrinsic contrast MRI can be sensitized to blood oxygenation and deoxyhemoglobin content by application of experimental protocols sensitive to R2* relaxation (Blood oxygenation level dependent (BOLD) contrast; [Ogawa et al., 1990]). Using this type of contrast, vascular function, namely, the capacity to respond to changes in inhaled oxygen by changes in hemoglobin saturation and oxygen delivery can be qualitatively mapped from signal changes in response to hyperoxia. Vasoreactivity, imaged from signal changes in response to hypercapnia (5% CO_2), is conferred to new blood vessels by recruitment of pericytes and smooth muscle cells, and thus signal changes in response to hypercapnia could be used for mapping vascular maturation [Abramovitch et al., 1999]. This approach was validated by comparing spatial patterns of maturation in tumors as detected by MRI and by histological staining with endothelial markers and with alpha-smooth muscle actin for staining the contractile perivascular cells. In addition, the protective role of maturation in maintaining vascular survival upon VEGF withdrawal could be reproduced by MRI [Abramovitch et al., 1999]. The physiological basis for this method was revealed by intravital microscopy, showing the role of the perivascular cells in controlling capillary blood flow and hematocrit [Neeman et al., 2001]. A similar approach was used for the study of angiogenesis induced by organ specific over expression of VEGF in the liver of transgenic mice [Dor et al., 2002]. Those studies showed the critical period of exposure to VEGF necessary for vascular stabilization, and resistance to VEGF withdrawal.

Developments in molecular imaging of angiogenesis focused on the generation of contrast materials that target specific cell surface receptors, adhesion molecules, and components of the extracellular matrix. Thus novel contrast agents were developed for labeling of the integrin alpha-v beta-3 [Sipkins et al., 1998; Anderson et al., 2000], and its binding-site recognition peptide RGD [Johansson et al., 2001]. Additional studies reported the development of contrast agents targeting endothelial cell receptors including E-selectin [Kang et al., 2002] and ICAM-1 [Sipkins et al., 2000]; and the provisional matrix fibrin [Lanza et al., 1998; Yu et al., 2000]. Demonstration for specificity of binding were obtained from in vivo models, but none of these studies tested the ability to follow temporal and spatial changes in expression in the same animal. It is in that capacity where MRI could potentially provide unique information unattainable by conventional molecular biology approaches. Similarly, the direct biological effects of these contrast materials was not determined so far with respect to potential activation or suppression of signaling of the imaged target molecule.

PROMISE OF MOLECULAR AND FUNCTIONAL IMAGING

Advanced functional and molecular MRI of angiogenesis will be valuable for clinical, pharmaceutical, and basic research applications. Clinical imaging methods hold promise for enabling non-invasive tailoring of therapy by following individual response using molecular and functional surrogate markers based on the specific mode of action of treatment. Imaging methods could help monitor drug delivery, drug activation, and expression of reporters during gene therapy.

Drug development efforts may be assisted by novel imaging methods that would aid in monitoring the efficacy of molecular targeting in vivo. Additional methods may allow assessment of the available target for therapy by molecular imaging (mapping receptor density), followed by functional imaging for mapping physiological response to treatment.

One of the striking recent events in molecular biology is the rapid development of new experimental models that allow precise and sophisticated alterations of gene expression in a tissue and time specific manner. The challenge for these models (cells and animals) is to characterize the phenotype generated by the genetic alterations alone, in combination and under an array of physiological conditions. Functional and molecular imaging methods are an integral part of this effort, and are frequently the rate-limiting step in progress due to the inherent low throughput of these experiments and the very small number of laboratories engaged in this effort. In order to increase accessibility, newly developed methods should become robust and automated enough to be performed by researchers without extensive background in imaging.

Developments in imaging of gene expression [Louie et al., 2000; Moore et al., 2001] provide the prospect that it may become possible to derive spatial and temporal expression patterns in the live animal, and combine these with functional imaging of outcome. For those systems that follow a precise well-orchestrated program (such as embryonic development), MRI provides in most cases little advantage over conventional methods. However, these methods will be extremely valuable for the study of chaotic processes where high variance complicates comparison between different animals. Examples include wound repair and tumor growth, both of which include an active angiogenic component. Similarly, when imaging a single time point with no follow-up, the information obtained by MRI will be at best comparable to that obtained by histology. The major advantage and promise of in vivo methods lies in the ability to monitor processes over time. To achieve that aim, it is critical that the imaging method will not alter the process studied. Thus, the contrast material used for either functional or molecular imaging must be provided at infinite dilution, so as not to alter the biology or exert independent agonist or antagonist biological activity.

FEASIBILITY OF NON-INVASIVE FUNCTIONAL AND MOLECULAR IMAGING BY MRI

MRI is exquisitely sensitive to changes in soft tissues, and particularly to changes in molecular dynamics of water including molecular diffusion and flow. Through the paramagnetic property of deoxyhemoglobin, MRI is sensitive also to changes in blood oxygenation. Low and high molecular weight contrast materials allow mapping of blood flow, perfusion, and permeability (permeability surface area product). Blood flow and blood volume can be mapped over a few orders of magnitude in scale by application of methods ranging from steady state intrinsic contrast to the use of slowly clearing blood pool agents. Thus, MRI is highly sensitive to the functional aspects of microcirculation. Moreover, repeated measurements of the same mice were demonstrated so as to monitor developmental changes over the course of days. It is at this time scale that MRI can probe directly angiogenesis itself, by following the generation of new blood vessels where non existed before.

For functional measurements of angiogenesis, microvasculature, and microcirculation, the parameters measured such as perfusion, and blood volume scale on the order of 1% of the total MR signal. For water this implies about 500 mM. Thus any contrast material dissolved in water in the sub millimolar concentration can be regarded as a dilute tracer. Magnetic resonance spectroscopy (MRS) provides a bridge between functional and molecular imaging. Here metabolites that are in the millimolar concentration range are detected directly using their own NMR signal.

The concentration of molecules such as lipids and ECM components, is much lower and contrast material when applied, is used at relatively high concentration so as to achieve sufficient signal enhancement. However, these molecules are abundant enough so that biological effects can be minimized. This is not so for growth factors and their receptors, the popular targets for molecular imaging. The optimal activity for these molecules is typically in the pico to micro molar range. To achieve sufficient sensitivity, the MR contrast material must be applied at close to saturating concentration. In addition, a very bulky contrast material is frequently used so as to provide sufficient sensitivity.

So far no detailed studies tested the biological effects of molecular imaging contrast agents. For application of these tools it is critical to determine the direct effects of these materials on biological signaling through the imaged receptor–ligand system. It is important to evaluate whether the ligand can displace the molecular contrast material thereby biasing the result to underestimate receptor levels. Alternatively, the contrast material might displace the natural ligand, and interfere with receptor internalization leading to sequestration of receptor-contrast material complexes on the cell surface leading to over-estimation of the contrast material.

MOLECULAR MARKERS AS SURROGATES FOR FUNCTION AND VICE VERSA

Insight for possible applications for molecular and functional imaging can be derived from previous experience in using functional and molecular markers by non-imaging modalities. Traditionally molecular markers often served as surrogates for detection of changes in function. In fact the entire field of pathology of fixed histological samples rests on the assumption that specific static molecular and structural features of the tissue can predict the progression of a disease, namely, provide functional information. Examples for this type of rational can be found in the use of markers such as PCNA to predict cell proliferation; tumor specific markers as predictors of tumor growth, and staining for DNA breaks as a molecular marker for apoptotic cell death. Similarly, histological and biochemical analysis of the expression of angiogenic growth factors were used as a marker for a dynamic process such as angiogenesis. Diagnosis based on disease symptoms, is a classical example for the exact opposite philosophy, namely, analysis of function as means for detecting molecular events. An important example is the use of glucose levels in diabetic patients as a surrogate for monitoring insulin.

PITFALLS OF SURROGATE MARKERS

Surrogate markers fail when the coupling between the measured parameter and the parameter of interest breaks. Disruption of coupling tends to occur specifically for biologically critical checkpoints and can be directly linked with pathology. For this reason it is often important to measure both the molecular signal and the functional response. Since both occur in that particular sequence, namely, a molecular signal is subsequently followed by a functional response, typically with a 5–50 h delay necessary for expression, translation, cell migration, and differentiation, it is frequently impossible to combine invasive molecular biology methods for detection of the molecular signal. For example, without development of new non-invasive tools for molecular imaging, it is not possible to measure expression of VEGF, and monitor the subsequent hyperpermeability, and angiogenesis, in the same system. Thus, molecular imaging might prove to be particularly useful when coupled with functional MRI measurements.

It is elegant but not truly essential that both functional and molecular measurements will be done using the same methodology. Possible promising combinations include the use of positron emission tomography (PET) [Gambhir, 2002] or optical imaging as molecular imaging methods (high sensitivity but poor resolution in the former and either poor tissue penetration or poor resolution for the later); combined with MRI for high resolution functional imaging.

CONCLUSIONS

Functional MRI methods of angiogenesis provide at their current state of development important information on vascular development, permeability, and maturation. Molecular MRI of angiogenesis provides at this point promising prospects for future applications. The targeted contrast agents reported so far have not been evaluated with respect to their ability to provide spatial and temporal resolution that would enable mapping of changes in expression pattern. Moreover, these materials were not evaluated with respect to their ability to probe the angiogenic process non-invasively, without altering its course. The inherent low sensitivity of MRI predicts that it may be difficult to achieve truly non-invasive molecular imaging for the key regulatory biological molecules that control angiogenesis. However, predictions of this sort tend to stimulate work aimed to disprove them, and thus it is plausible that despite the above reservations, non-invasive molecular MRI of angiogenesis will become feasible and will complement functional imaging in providing non-invasive methods for simultaneously seeing the target and seeing it work.

ACKNOWLEDGMENTS

This work was supported in part by research grants from the Israel Ministry of Health, The Israel Science Foundation, and by the USA National Institute of Health RO1 CA75334.

REFERENCES

Abramovitch R, Meir G, Neeman M. 1995. Neovascularization induced growth of implanted C6 glioma multicellular spheroids: Magnetic resonance microimaging. Cancer Res 55:1956–1962.

Abramovitch R, Frenkiel D, Neeman M. 1998a. Analysis of subcutaneous angiogenesis by gradient echo magnetic resonance imaging. Magn Reson Med 39:813–824.

Abramovitch R, Marikovsky M, Meir G, Neeman M. 1998b. Stimulation of tumour angiogenesis by proximal wounds: Spatial and temporal analysis by MRI. Br J Cancer 77: 440–447.

Abramovitch R, Dafni H, Smouha E, Benjamin LE, Neeman M. 1999. In vivo prediction of vascular susceptibility to vascular susceptibility 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.

Benjamin LE, Keshet E. 1997. Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: Induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. Proc Natl Acad Sci USA 94:8761–8766.

Bhujwalla ZM, Artemov D, Aboagye E, Ackerstaff E, Gillies RJ, Natarajan K, Solaiyappan M. 2001a. The physiological environment in cancer vascularization, invasion, and metastasis. Novartis Found Symp 240:23–38.

Bhujwalla ZM, Artemov D, Natarajan K, Ackerstaff E, Solaiyappan M. 2001b. Vascular differences detected by MRI for metastatic versus nonmetastatic breast and prostate cancer xenografts. Neoplasia 3:143–153.

Bhujwalla ZM, Artemov D, Ballesteros P, Cerdan S, Gillies RJ, Solaiyappan M. 2002. Combined vascular and extracellular pH imaging of solid tumors. NMR Biomed 15: 114–119.

Brasch R, Turetschek K. 2000. MRI characterization of tumors and grading angiogenesis using macromolecular contrast media: Status report. Eur J Radiol 34:148–155.

Brasch R, Pham C, Shames D, Roberts T, van Dijke K, van Bruggen N, Mann J, Ostrowitzki S, Melnyk O. 1997. Assessing tumor angiogenesis using macromolecular MR imaging contrast media. J Magn Reson Imaging 7:68–74.

Brown EB, Campbell RB, Tsuzuki Y, Xu L, Carmeliet P, Fukumura D, Jain RK. 2001. In vivo measurement of gene expression, angiogenesis, and physiological function in tumors using multiphoton laser scanning microscopy. Nat Med 7:864–868.

Buckley DL, Drew PJ, Mussurakis S, Monson JR, Horsman A. 1997. Microvessel density of invasive breast cancer assessed by dynamic Gd-DTPA enhanced MRI. J Magn Reson Imaging 7:461–464.

Dafni H, Israely T, Bhujwalla ZM, Benjamin LE, Neeman M. 2002a. Over-expression of vascular endothelial growth factor 165 drives Peri-tumor interstitial convection and induces lymphatic drain: Magnetic resonance imaging, confocal microscopy, and histological tracking of triple labeled albumin. Cancer Res Nov 15, 2002.

Dafni H, Landsman L, Schechter B, Kohen F, Neeman M. 2002b. MRI and fluorescence microscopy of the acute vascular response to VEGF165: Vasodilation, hyperpermeability, and lymphatic uptake, followed by rapid inactivation of the growth factor. NMR Biomed 15:120–131.

Dor Y, Djonov V, Abramovitch R, Itin A, Fishman GI, Carmeliet P, Goelman G, Keshet E. 2002. Conditional switching of VEGF provides new insights into adult neovascularization and pro-angiogenic therapy. EMBO J 21:1939–1947.

Drevs J, Muller-Driver R, Wittig C, Fuxius S, Esser N, Hugenschmidt H, Konerding MA, Allegrini PR, Wood J, Hennig J, Unger C, Marme D. 2002. PTK787/ZK 222584, a specific vascular endothelial growth factor-receptor tyrosine kinase inhibitor, affects the anatomy of the tumor vascular bed and the functional vascular properties as detected by dynamic enhanced magnetic resonance imaging. Cancer Res 62:4015–4022.

Dvorak HF, Nagy JA, Feng D, Brown LF, Dvorak AM. 1999. Vascular permeability factor/vascular endothelial growth factor and the significance of microvascular hyperpermeability in angiogenesis. Curr Top Microbiol Immunol 237:97–132.

Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, Cheresh DA. 1999. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. Mol Cell 4:915–924.

Feng D, Nagy JA, Dvorak AM, Dvorak HF. 2000. Different pathways of macromolecule extravasation from hyperpermeable tumor vessels. Microvasc Res 59:24–37.

Ferrara N. 2001. Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol Cell Physiol 280:C1358–C1366.

Gambhir SS. 2002. Molecular imaging of cancer with positron emission tomography. Nat Rev Cancer 2:683–693.

Gilead A, Neeman M. 1999. Dynamic remodeling of the vascular bed precedes tumor growth: MLS ovarian carcinoma spheroids implanted in nude mice. Neoplasia 1: 226–230.

Hawighorst H, Knapstein PG, Knopp MV, Weikel W, Brix G, Zuna I, Schonberg SO, Essig M, Vaupel P, van Kaick G. 1998a. Uterine cervical carcinoma: Comparison of standard and pharmacokinetic analysis of time–intensity curves for assessment of tumor angiogenesis and patient survival. Cancer Res 58:3598–3602.

Hawighorst H, Weikel W, Knapstein PG, Knopp MV, Zuna I, Schonberg SO, Vaupel P, van Kaick G. 1998b. Angiogenic activity of cervical carcinoma: Assessment by functional magnetic resonance imaging-based parameters and a histomorphological approach in correlation with disease outcome. Clin Cancer Res 4:2305–2312.

Johansson LO, Bjornerud A, Ahlstrom HK, Ladd DL, Fujii DK. 2001. A targeted contrast agent for magnetic resonance imaging of thrombus: Implications of spatial resolution. J Magn Reson Imaging 13:615–618.

Kang HW, Josephson L, Petrovsky A, Weissleder R, Bogdanov A, Jr. 2002. Magnetic resonance imaging of

inducible E-selectin expression in human endothelial cell culture. Bioconjug Chem 13:122–127.

Lanza GM, Lorenz CH, Fischer SE, Scott MJ, Cacheris WP, Kaufmann RJ, Gaffney PJ, Wickline SA. 1998. Enhanced detection of thrombi with a novel fibrin-targeted magnetic resonance imaging agent. Acad Radiol 5(Suppl 1):S173–S176; discussion S183-S184.

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.

Moore A, Josephson L, Bhorade RM, Basilion JP, Weissleder R. 2001. Human transferrin receptor gene as a marker gene for MR imaging. Radiology 221:244–250.

Neeman M, Dafni H, Bukhari O, Braun RD, Dewhirst MW. 2001. In vivo BOLD contrast MRI mapping of subcutaneous vascular function and maturation: Validation by intravital microscopy. Magn Reson Med 45:887–898.

Ogawa S, Lee TM, Nayak AS, Glynn P. 1990. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. Magn Reson Med 14:68–78.

Padhani AR, MacVicar AD, Gapinski CJ, Dearnaley DP, Parker GJ, Suckling J, Leach MO, Husband JE. 2001. Effects of androgen deprivation on prostatic morphology and vascular permeability evaluated with MR imaging. Radiology 218:365–374.

Pathak AP, Schmainda KM, Ward BD, Linderman JR, Rebro KJ, Greene AS. 2001. MR-derived cerebral blood volume maps: Issues regarding histological validation and assessment of tumor angiogenesis. Magn Reson Med 46:735–747.

Schiffenbauer YS, Abramovitch R, Meir G, Nevo N, Holzinger M, Itin A, Keshet E, Neeman M. 1997. Loss of ovarian function promotes angiogenesis in human ovarian carcinoma. Proc Natl Acad Sci USA 94:13203–13208.

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.

Sipkins DA, Gijbels K, Tropper FD, Bednarski M, Li KC, Steinman L. 2000. ICAM-1 expression in autoimmune encephalitis visualized using magnetic resonance imaging. J Neuroimmunol 104:1–9.

Thurston G, Rudge JS, Ioffe E, Zhou H, Ross L, Croll SD, Glazer N, Holash J, McDonald DM, Yancopoulos GD. 2000. Angiopoietin-1 protects the adult vasculature against plasma leakage. Nat Med 6:460–463.

Yu X, Song SK, Chen J, Scott MJ, Fuhrhop RJ, Hall CS, Gaffney PJ, Wickline SA, Lanza GM. 2000. High-resolution MRI characterization of human thrombus using a novel fibrin-targeted paramagnetic nanoparticle contrast agent. Magn Reson Med 44:867–872.