Advances in Anatomic, Functional, and Molecular Imaging of Angiogenesis

Angiogenesis is a fundamental process in various physiologic and pathologic processes. The ability to visualize and quantify angiogenesis will allow early diagnosis and monitoring for clinical determination of angiogenesis states before, during, and after adjuvant antiangiogenic and therapeutic angiogenesis treatments.

**Key Words:** angiogenesis; volumetric computed tomography (VCT); dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI); integrin; vascular endothelial growth factor receptor (VEGFR)

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In recent years, angiogenesis has become one of the most important and intensely studied areas of cancer research, with the future holding great promise. Angiogenesis is a fundamental process in various physiologic and pathologic processes. The ability to visualize and quantify angiogenesis will allow early diagnosis and monitoring for clinical determination of angiogenesis states before, during, and after adjuvant antiangiogenic and therapeutic angiogenesis treatments. Investigators have identified more than 20 angiogenic growth factors, their receptors, and the details of their signal transduction pathways. Achievements with the vascular endothelial growth factor (VEGF) antibody bevacizumab (Avastin; Genentech) in combination with standard cytotoxic chemotherapy (VEGFR) (Fig. 1). Advances with the vascular and vascular volumes, and blood flow. Dynamic contrast-enhanced MRI has been used for evaluation of drug efficacy in several trials of antiangiogenic drugs. Overall, dynamic contrast-enhanced MRI is promising as an imaging biomarker in clinical trials. However, data on its performance, especially for response assessment, are not uniform and seem to depend strongly on the therapy protocol and tumor type.

**FUNCTIONAL IMAGING OF ANGIOGENESIS**

Dynamic contrast-enhanced MRI is a noninvasive method of imaging microvascular structure by tracking the pharmacokinetics of an injected contrast agent as it passes through tumor vasculature. This technique measures changes in vascular permeability, extravascular and vascular volumes, and blood flow. Dynamic contrast-enhanced MRI has been used for evaluation of drug efficacy in several trials of antiangiogenic drugs. Overall, dynamic contrast-enhanced MRI is promising as an imaging biomarker in clinical trials. However, data on its performance, especially for response assessment, are not uniform and seem to depend strongly on the therapy protocol and tumor type.

The simplicity, ease of use, speed, and safety of ultrasound for angiogenesis imaging has led to its increased use in disease diagnosis, treatment assessment, follow-up, and therapy guidance. Ultrasound can be applied to image the microcirculation using both Doppler and microbubble techniques. Power Doppler can be quantified to give an estimate of relative fractional vascular volume and blood velocity. Innovations in the field of ultrasound imaging...
physiologic meaning of results from these studies often remains
enhanced MRI are widely used to assess hemodynamic parameters, the
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tissues.
affect both image resolution and the depth of light penetration in
optical imaging is tissue light scattering and absorption, which
in situ scanning force microscopy (scanning microscopy, multiphoton laser scanning microscopy, and
vivo such as intravital fluorescence microscopy, confocal laser
multimodality imaging of integrin expression (PET, SPECT, MRI,
ultrasound, and optical) using appropriate cyclic RGD peptides and
monoclonal antibodies against integrin αβ3 have been published
(11–16). Among these, PET of integrin αvβ3 using the radiotracer 18F-
galacto-RGD has been tested in humans with good contrast and with a
low radiation dose, comparable to that of 18F-FDG (Fig. 2) (17–20). It
has also been shown that the intensity of 18F-galacto-RGD uptake
 correlates with αvβ3 expression in murine tumor xenografts and in
patients with cancer. The same tracer is now being evaluated for
clinical use in different tumor entities.

Integrins are a family of cell adhesion molecules playing key roles
during tumor angiogenesis and metastasis (8,9). Integrins expressed
on endothelial cells modulate cell migration and survival during
tumor angiogenesis, whereas integrins expressed on carcinoma cells
potentiate metastasis by facilitating invasion and movement across
blood vessels. The αvβ3 integrin, which binds to arginine-glycine-
aspartic acid (RGD)–containing components of the extracellular
matrix, is significantly upregulated on tumor vasculature but not on
quiescent endothelium (9,10). Several comprehensive reviews on
multimodality imaging of integrin expression (PET, SPECT, MRI,
ultrasound, and optical) using appropriate cyclic RGD peptides and
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Most monomeric RGD peptide–based tracers tend to have fast
blood clearance accompanied by relatively low tumor uptake and
rapid tumor washout, presumably because of the suboptimal
receptor-binding affinity/selectivity and inadequate contact with
the binding pocket in the extracellular segment of integrin \(\alpha_v\beta_3\). Multimerization of cyclic RGD peptides has been repeatedly demonstrated to increase integrin affinity and enhance antiadhesive ability against integrin \(\alpha_v\beta_3\) and thus significantly improve tumor targeting over the monomeric RGD analogs (11,12,21–26). The polyvalency effect has been most beneficial in tumors with low to medium integrin expression (26). Several dimeric and multimeric RGD peptide–based imaging probes are in the process of clinical translation for the first studies on humans. Whether such new probes will have advantages over \(^{18}\)F-galacto-RGD remains to be elucidated.

Integrin \(\alpha_v\beta_3\) is not an angiogenesis marker per se because of its nonexclusive expression on the neovasculature. Small molecules, even antibody-based probes for integrin expression, do not provide an accurate readout of tumor angiogenesis because these probes bind to integrin \(\alpha_v\beta_3\) expressed on both the tumor vasculature and tumor cells. Because of their relatively large sizes and rigid structure, integrin-targeted nanoparticles that do not extravasate are probably true vascular integrin–specific probes (27,28). A few such examples include RGD peptide–coupled quantum dots for near-infrared fluorescence imaging, single-walled nanotubes for Raman and photoacoustic imaging, liposomal and perfluorocarbon nanoparticles for \(T_1\)-weighted MRI, ultrasmall superparamagnetic iron oxide particles for \(T_2\)-weighted MRI, and microbubbles for contrast-enhanced ultrasound. Several of these nanoparticle conjugates have also been suitably labeled for radionuclide imaging to assess the tumor-targeting efficacy and in vivo kinetics of the particles in a more quantitative manner.

The angiogenic actions of VEGF are mediated mainly via 2 endothelium-specific receptor tyrosine kinase receptors, Flt-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2). Both VEGFRs are restricted largely to vascular endothelial cells, and all VEGF-A isoforms bind to both VEGFR-1 and VEGFR-2. It is now generally accepted that VEGFR-2 is the major mediator of the mitogenic, angiogenic, and permeability-enhancing effects of VEGF. The critical role of VEGF-A in cancer progression has been highlighted by the approval of the humanized anti-VEGF monoclonal antibody bevacizumab (Avastin; Genentech) for first-line treatment (29). The flourishing investigations of both radionuclide- and non–radionuclide-based VEGFR imaging studies stem from the successful initial clinical evaluation of \(^{123}\)I-VEGF\(_{165}\) in patients with gastrointestinal cancer (30,31). Development of VEGF- or VEGFR-targeted molecular imaging probes is a potential new paradigm for assessing antiangiogenic therapies and clarifying the role and expression profile of VEGF/VEGFR in angiogenesis-related diseases (32).

Imaging probes based on wild-type VEGF-A isoforms bind to both VEGFR-1 and VEGFR-2. The kidneys have high VEGFR-1 expression that can take up VEGF-A–based tracer and thus usually makes the kidney the dose-limiting organ (33,34). Because VEGFR-2 is generally accepted to be more functionally important than VEGFR-1 in cancer progression, the ability to image VEGFR-2 expression can be a valuable tool for evaluating patients with a variety of malignancies, particularly those undergoing anti-VEGFR-2 therapies. Alanine-scanning mutagenesis has been used to identify a positively charged surface in VEGF\(_{165}\) that mediates binding to VEGFR-2 (35). Arg\(_{82}\), Lys\(_{84}\), and His\(_{86}\), located in a hairpin loop, were found to be critical for binding VEGFR-2, whereas negatively charged residues, Asp\(_{63}\), Glu\(_{64}\), and Glu\(_{67}\), were associated with VEGFR-1 binding. A VEGFR-2–specific PET tracer has thus been developed using the D63AE64AE67A mutant of VEGF\(_{121}\) (VEGF\(_{DEE}\)) generated by recombinant DNA technology. Renal uptake of \(^{64}\)Cu-tetraazacyclododecanetetaacetic acid (DOTA)-VEGF\(_{DEE}\) was significantly lower than that of \(^{64}\)Cu-DOTA-VEGF\(_{121}\) (Fig. 3) (36). Further improvement in VEGFR-2 binding affinity/specificity, pharmacokinetics, and tumor-targeting efficacy by generation of other VEGF\(_{121}\) mutants is considered a good direction for clinical translation of VEGF protein-based imaging probes.

**FUTURE PERSPECTIVES**

Numerous imaging techniques are now available to visualize tissue vasculature on a structural, functional, and molecular level. All these techniques have undergone significant preclinical development in
recent years and should aid in future assessment of angiogenesis, monitoring of antiangiogenic treatment, and antiangiogenic drug development. Only vascular imaging of functional hemodynamic parameters such as $K_{trans}$, blood flow, and blood volume are currently being used clinically to assess antiangiogenic and cytotoxic chemotherapies. However, the physiologic meaning of these results is often difficult to interpret.

With molecular imaging of angiogenesis, only a few radiotracers have been used in humans thus far, and the role of these tracers in assessing response to antiangiogenic therapies is still undetermined. Although integrin $\alpha_\beta_3$ is by far the most extensively studied angiogenic factor for imaging, future trials still need to elucidate which target structure is optimal for assessing angiogenesis. In regard to which imaging technique is optimal, the radiotracer approach will likely be the first to be used on a wider scale in patients in the intermediate term because of its high sensitivity and the low amounts of tracer required. However, over the long term, MRI might be a better imaging alternative because of its lack of ionizing radiation and high spatial resolution. Overall, it is likely that angiogenesis will eventually be assessed not using a single parameter, target structure, or imaging technique but rather a combination of parameters that allow for a multimodal imaging evaluation of the intricacies of the angiogenic cascade. Assessing the different aspects of angiogenesis at the structural, functional, and molecular levels for clinical determination of angiogenesis states before, during, and after antiangiogenic therapy or therapeutic angiogenesis will likely become a reality and help further steps toward personalized medicine.

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