

Imaging of angiogenesis

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Imaging of angiogenesis is an important tool in the diagnosis of disease. Small molecule or nanoparticulate probes are currently developed to identify angiogenic endothelium with non-invasive imaging techniques such as magnetic resonance and ultrasound imaging. These techniques are very valuable since they would not only contribute to improved diagnosis, but will also allow monitoring of angiogenesis intervention therapies, early detection of disease, as well therapy development. Such techniques will ultimately also contribute to the introduction of personalized medicine. The current special issue of *Angiogenesis* is put together to highlight several interesting and new developments in this field.

Intervention in angiogenesis has currently made its way into daily clinical practice. Several angiogenesis inhibitors have been approved by the Food and Drug Administration (FDA) for therapeutic use in the therapy of disease, most commonly in oncological pathologies. Since angiogenesis is a multidisciplinary theme, application of angiogenesis inhibition is also expected for other angiogenic diseases such as rheumatoid arthritis, atherosclerosis, endometriosis and psoriasis [1]. Clinical application has been approved for the therapy of many cancer types and eye diseases, such as age-related macular degeneration. In the field of cancer, neutralization of the key angiogenic growth factor VEGF is

daily clinical practice, mostly in combination with chemotherapeutic agents. While the first approved agent, Avastin/bevacizumab, is an anti-VEGF antibody based therapeutic, later generation drugs are collectively based on inhibition of VEGF (and other growth factor) signaling by inhibition of tyrosine kinase inhibitors. This type of inhibitors is now widely tested and clinical studies have led to FDA approval for several of them. Application is possible now for many tumor types and is especially successful in renal cell cancer, where monotherapy with Sutent/sunitinib is nowadays first line therapy.

While these examples picture the exploitation of angiogenesis for development of therapy, there is also an active field of science exploiting angiogenesis or angiogenesis inhibitors for diagnostic purposes making use of imaging technologies. Imaging of diseased tissue by exploiting vascular permeability or vascular targeting is a promising tool in modern medicine. The latter approach depends on identification of targets that are upregulated at angiogenic blood vessels as compared to quiescent normal blood vessels and on the availability of probes that bind selectively and with high affinity to such targets. Different genomic screening approaches, performed worldwide, identified markers of angiogenic endothelial cells. Among these markers are molecules such as $\alpha_v\beta_3$ integrin, CD13, vimentin, VEGF receptor, and galectin-1, which have shown promise in imaging of angiogenesis [2–4].

The field of angiogenesis research has been initiated by the hypothesis that the growth of tumors is dependent on the formation of new blood vessels, put forward by Folkman [5] in the early 1970s. This idea indicated that angiogenesis inhibitors might be discovered and employed as therapy against angiogenic diseases. The process of angiogenesis is an intricately regulated cascade of processes that occurs in growing tissues where, for example,

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conditions of hypoxia have turned on the production of angiogenic growth factors such as the families of vascular endothelial cell growth factors (VEGFs) and fibroblast growth factors (FGFs). Preexisting endothelial cells in capillaries can sense that and subsequently produce proteases to dissolve the basement membrane and extracellular matrix. Thereby endothelial cells migrate into the direction of the stimulus. Endothelial cells subsequently proliferate and form new vascular sprouts that become functional blood vessels after the attraction of accessory cells such as pericytes and the formation of a new rigid extracellular matrix [6]. This angiogenesis cascade provides opportunities for intervention in every single step separately, and inhibitors for each of these steps have been discovered and are being developed in clinical studies. Anti-angiogenesis compounds can specifically and directly inhibit the proliferation of endothelial cells (TNP-470/caplostatin, platelet factor-4) or interfere directly with the migratory activity of these cells (endostatin, integrin antagonists). Alternatively, they can inhibit the production or activity of metalloproteinases (MMPs), inducing a hampered mobility of endothelial cells. However, the best developed angiogenesis inhibitors are the ones that act indirectly, either by clearing angiogenic growth factors from the circulation, blocking the corresponding growth factor receptors, or by intervention in the intracellular signaling pathways activated by these growth factors.

A variety of imaging modalities is available to visualize and characterize the angiogenic vasculature [7]. In a clinical setting these include magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), ultrasound imaging and computed tomography (CT) [8]. Preclinically, all the aforementioned imaging modalities are available, but usually as dedicated small animal scanners with a smaller field of view and higher spatial resolution [9]. In addition, optical *in vivo* imaging techniques such as intravital microscopy, near infrared fluorescence (NIRF) and bioluminescence imaging are frequently employed to evaluate angiogenesis in small laboratory animals [10].

Dynamic contrast enhanced (DCE) MRI is one of the most widely and commonly used imaging methods to visualize tumor angiogenesis in cancer patients and to evaluate angiostatic therapies [11]. The application of contrast enhanced CT imaging is also being explored for perfusion imaging of cancers in patients. Both DCE MRI and CT provide information about the vascular permeability of tumors, but do not directly visualize the newly formed vasculature. Fluorodeoxyglucose (FDG) PET imaging allows quantitative imaging of glucose metabolism. Since tumor glucose metabolism is expected to decrease when nutrient vessels regress after the onset of angiostatic therapy, as an indirect marker for angiogenesis,

a decrease in glucose metabolism may be visualized by FDG-PET. Unfortunately, changes in vascular permeability and FDG uptake may not necessarily be the result of changes in ongoing angiogenesis, but can also be the result of a variety of different processes, including hypoxia or the collapse of the tumor microvasculature. More recently, contrast enhanced MRI and CT as well as FDG-PET have been explored for the study of neovascularization in atherosclerosis in a number of studies [12, 13]. Although in its infancy, these studies have shown a clear correlation between atherosclerotic plaque permeability, microvessel density and vulnerability. Interestingly, Calcagno et al. [12], who also contributed to this special issue, have shown parameters obtained *in vivo* with DCE-MRI and FDG-PET to correlate with the plaque microvessel density determined *ex vivo*, in a rabbit model of atherosclerosis.

To enable a more specific evaluation of angiogenesis, target-specific imaging methods have been developed for PET and SPECT imaging, MRI, ultrasound as well as optical imaging [14]. Such molecular imaging methods exploit probes that, after intravenous administration, specifically target molecular epitopes upregulated at the angiogenic vasculature. These probes are labeled with tracers and/or materials that allow their visualization with one (or more) of the aforementioned imaging techniques. The nuclear imaging techniques (PET and SPECT) inherently rely on target-specific probes. To that end, targeting ligands such as antibodies, proteins and peptides are labeled with radioactive isotopes. Most notably, the $\alpha_v\beta_3$ integrin specific RGD peptide has been studied and applied in both preclinical and clinical studies, which has resulted in the development of a wide variety of radiolabeled RGD analogs [15]. In addition, different other endothelial cell markers have been exploited to image angiogenesis by PET and SPECT, including the VEGF receptor or adhesion molecules [16].

The development of nanoparticulate molecular imaging probes has shown great progress in the past decade [17]. Such probes, carrying a high payload of contrast generating materials, have shown to be especially useful for MR molecular imaging of angiogenesis, since MRI is a relatively insensitive technique that requires μM concentrations of contrast agents to accumulate to allow their visualization [18]. For optical techniques, quantum dots have shown great potential because these semiconductor nanoparticles exhibit some unique properties that make them resistant to photobleaching and provide a very narrow and tunable excitation wavelength [19]. Interestingly, nanoparticles allow the integration of multiple materials and agents for multimodal imaging purposes and/or the combination of target-specific therapy [20].

For the current special issue on “*Imaging of Angiogenesis*” we have invited a series of renowned expert

investigators to contribute a state-of-the-art review. We attempted to put together an issue that covers the above-mentioned wide range of applications in this field. The issue starts with a contribution, which also provided the cover art, about vascular permeability and lymphatic drainage imaging in experimental cancer by Vandoorne, Addadi and Neeman [21]. Calcagno et al. [12] [22] show the potential of contrast enhanced MR imaging to identify the vulnerability of atherosclerotic plaques'. MRI of angiogenesis in the brain as a result of stroke has been reviewed by investigators of the NRM group of Dijkhuizen in Utrecht, The Netherlands [23]. A contribution from a Norwegian group by Hak et al. [24] exemplifies the use of window chamber models to visualize nanoparticle targeting in tumors by intravital microscopy. We have contributed a mini review about paramagnetic quantum dots for multimodal of tumor angiogenesis [25], while Snoeks, Löwik and Kaijzel from the Leiden University Medical Center in The Netherlands highlight the latest developments in optical approaches to image and characterize angiogenesis [26]. Investigators Jansen, Koutcher and Shukla-Dave at Memorial Sloan-Kettering Cancer Center [27] reviewed the field of head and neck squamous cell carcinoma imaging. The Biomedical NMR group of the Eindhoven University of Technology (Nicolay, Strijkers et al.) summarized their work on multimodal liposomes [28], while Willmann et al. from Stanford University School of Medicine wrote an overview of their ultrasound based tumor angiogenesis imaging work [29]. The special issue is wrapped up with a great contribution by Lanza et al. [20] from Washington University of Medicine. They give a summary of their work with angiogenesis specific perfluorocarbon nanoparticles for molecular MRI and therapy of cancer and atherosclerosis [30].

We are confident that this special issue of Angiogenesis is a valuable documentation of the current technologies available to image neovasculation formation.

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