

# THE BASICS: TUMOR VASCULATURE

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Angiogenesis or the *de novo* formation of blood vessels is one of the ‘hallmarks’ of cancer and a key prerequisite for tumor progression and metastasis (Hanahan et al. 2011). The healthy body controls angiogenesis through a balance of ‘on’ and ‘off’ switches known as angiogenesis stimulating growth factors (e.g. vascular endothelial growth factor or VEGF) and angiogenesis inhibitors (e.g. thrombospondin-1), respectively (Bergers et al. 2003). However in cancer, the body loses control of this homeostatic balance, resulting in the excessive growth of tumor blood vessels that are structurally and functionally distinct from the vasculature of healthy tissue (Carmeliet et al. 2000; Jain et al. 2001).

Tumor-induced blood vessels are typically sinusoidal, exhibit discontinuous basement membranes, and lack tight endothelial cell junctions making them highly permeable to macromolecules (Konerding et al. 1995; Nagy et al. 2010). Other characteristics include spatial heterogeneity, loss of branching hierarchy, arterio-venous shunts, acutely and transiently collapsing vessels, a dearth of smooth muscle cell lining, and an inability to match the elevated metabolic demand of cancer cells, resulting in areas of hypoxia and necrosis (Semenza 2003). Pioneering work by Jain, Vaupel and others has demonstrated that these morphological anomalies profoundly alter tumor hemodynamics, blood rheology, and perfusion (Jain 1988; Vaupel et al. 2007).

These observations led Folkman to hypothesize that inhibiting tumor growth with specialized ‘antiangiogenesis’ drugs could profoundly affect the quality of life of cancer patients (Folkman 1971). Over the past several years, a large number of angiogenesis inhibitors have been identified and are currently in clinical trials (Folkman 2007). Most of these inhibitors achieve their effects by inhibiting VEGF (Ferrara 2002). Recently, it was shown that antiangiogenic therapies can transiently ‘normalize’ the chaotic tumor vasculature (Jain 2001; Jain 2005), improve tumor blood flow and enhance delivery and efficacy of anti-cancer drugs (Batchelor et al. 2007; Batchelor et al. 2013). While VEGF inhibition has been shown to be effective in multiple cancers, response in metastatic cancer has been limited, with the disease eventually progressing (Ebos et al. 2011). This has been attributed to the rapid development of resistance to VEGF targeting drugs (Sennino et al. 2012). Preclinical studies have demonstrated that tumors respond to VEGF inhibition with a loss of blood vessels (i.e. vascular pruning), which decreases perfusion and elevates hypoxia, inducing mechanisms of evasive resistance to antiangiogenic therapy that include revascularization via alternative proangiogenic pathways (Bergers et al. 2008), increased invasiveness and enhanced metastasis (Ebos et al. 2011).

With these developments in the field of angiogenesis has come a crucial need for reliable biomarkers to: (i) test novel antiangiogenic drugs; (ii) assess their therapeutic efficacy in vivo; (iii)

provide early measurable signs of tumor relapse/recurrence; (iv) stratify patients according to their angiogenic/tumor phenotype; (v) identify the best combinatory drug regime (i.e. antiangiogenic drug plus chemo- or radio-therapy); and (vi) repeatedly monitor patients with maximal safety (Pathak et al. 2008).

A wide array of non-invasive imaging modalities have been used to image the tumor vasculature. These include x-ray computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), ultrasound, and different types of optical imaging, each with its own distinct advantages as a tool in the non-invasive, in vivo assessment of tumor angiogenesis (McDonald et al. 2003). However, the assortment of available 'functional' contrast mechanisms in conjunction with the development of novel imaging probes, has made MRI invaluable for the functional and molecular imaging of tumor vasculature (Pathak et al. 2010). This includes probing tumor vasculature using the endogenous contrast produced by paramagnetic deoxyhemoglobin in tumor microvessels or the blood oxygenation level dependent (BOLD) contrast mechanism (Ben Bashat et al. 2012; Boult et al. 2013), and imaging tumor perfusion with arterial spin labeling (ASL), in which the intrinsic magnetization of arterial blood water serves as the endogenous tracer (Silva et al. 2000). Exogenous low and high molecular weight gadolinium (Gd)-based complexes, as well as para- and superparamagnetic contrast agents have been used in pre-clinical and clinical studies of tumor angiogenesis (Pathak et al. 2004). The MR pulse sequences used to characterize tumor vascularization depend on the physical properties and pharmacokinetics of the contrast agent used, following which a range of tumor vascular parameters (e.g. blood volume, blood flow, vascular permeability, microvessel density and radius etc.) can be calculated from tracer kinetic principles and MR biophysical principles (Zaharchuk 2007; Pathak 2009).

The recent development of novel contrast agents directed to cell-surface receptors expressed on tumor endothelial cells using peptides, ligands or antibodies has made feasible 'molecular' imaging of tumor angiogenesis with MRI (Pathak et al. 2010). Additionally, multi-modal probes that can be used in combination with two or more imaging modalities are also being developed. For example, high-resolution SPECT-CT/MRI of angiogenesis in a tumor model was recently reported using probes that consisted of  $\alpha_v\beta_3$ -targeted  $^{99m}\text{Tc}$  nanoparticles (Lijowski et al. 2009). It is worth mentioning that new preclinical MR imaging methods in conjunction with computational models of angiogenesis are helping us gain a deeper understanding of the cellular and molecular regulation of tumor angiogenesis (Kim et al. 2012). For example, 3D mapping of murine neurovasculature using high-resolution magnetic resonance microscopy ( $\mu\text{MRI}$ ) has been combined with diffusion tensor imaging (DTI) to examine the interplay between the brain tumor vasculature and white matter reorganization as well as phenotype the brain tumor microenvironment (Kim et al. 2011; Pathak et al. 2011). Incorporation of high-resolution 3D tumor vascular imaging data facilitates development and validation of predictive, multiscale computational models of tumor angiogenesis (Stamatelos et al. 2013) and more realistic biophysical models for validating MRI-based angiogenesis biomarkers (Pathak et al. 2008).

MRI is continuing to evolve as an important modality for the molecular-functional characterization of tumor vasculature. As new contrast mechanisms and imaging methods are developed, these will facilitate and usher in the era of 'personalized' cancer therapy.

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