

Non-invasive imaging of angiogenesis in head and neck squamous cell carcinoma

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Abstract Squamous cell carcinoma of the head and neck etiologically associated with tobacco smoking and alcohol (HNSCC) is the seventh most common cancer in the United States. Angiogenesis, the process by which new blood vessels are formed, is an essential element at the basis of both tumor growth and metastases. This review discusses pertinent aspects of the role of imaging modalities in assessing angiogenesis and anti-angiogenic therapy in advanced HNSCC.

Keywords Head and neck squamous cell carcinoma
Angiogenesis Anti-angiogenic treatment
Imaging techniques Magnetic resonance imaging
Computed tomography Positron emission tomography
Ultrasound Molecular imaging

Advanced head and neck cancers

In 2009, it was estimated that 48,010 new cases of head and neck (HN) cancer would be diagnosed in the United States and 11,260 deaths would occur due to the disease. HN cancers generally stem from the mucosal epithelia of the oral cavity, pharynx, and larynx and have been

etiologicaly associated with tobacco smoking and alcohol consumption [2]. Due to the wide variation in tissues of origin, HN cancer is a heterogeneous disease, representing a variety of histologies and differentiation patterns. The most common histology is squamous cell carcinoma (SCC), which accounts for greater than 90% of HN cancers. Early HN cancers are treated with radiation or surgery and have encouraging locoregional control [3]. After standard therapy (induction or concurrent chemotherapy administered with radiation or prior to surgery) for advanced HN disease, only 60% of patients survive 5 years [1]. Even with aggressive intervention, HN cancers often metastasize to the cervical lymph nodes before invading distant organs including the lungs. Despite advances in surgical techniques and oncologic treatment, the survival rate has remained the same over the past decade. The two main reasons for poor outcome in advanced HN cancer are a delayed diagnosis leading to locoregional failure and late intervention with salvage treatment when disease recurs [5, 6]. Therefore, even if the initial carcinoma is successfully removed, the risk of a patient with HN cancer dying from this or a second malignancy remains high. This review will focus on advanced HNSCC.

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Angiogenesis and therapy

Angiogenesis is a critical element of tumor growth and metastasis. It is a complex process, consisting of multiple steps concerning endothelial cell cessation, proliferation and migration of endothelial cells, and formation of new blood vessels [7]. During angiogenesis, vessels form to provide the expanding tumor with cells of endothelial origin, which divide much faster than normal endothelial cells. Once tumors grow beyond a diameter of a few

millimeters, passive diffusion cannot sustain the viability of malignant cells anymore, and hence the formation of new vascular network is necessary. Tumors that grow beyond this stage, therefore, are capable of activating the “angiogenic switch.” Induction of this angiogenic switch depends on how heavily the balance tips in favor of angiogenesis. The induction can occur at different stages of tumor formation, depending on factors such as tumor type and the microenvironment. Blood vessel formation will persist as long as the tumor is growing and is aimed at feeding essential nutrients and oxygen to hypoxic and necrotic areas of the tumor. Angiogenesis plays an important role in head and neck squamous cell carcinoma (HNSCC) as in any other solid tumor. The ability of the tumor to start angiogenesis is a prerequisite for metastatic spread. Traditionally, microvessel density (MVD) has been the parameter most often used for assessing angiogenesis. Another approach involves the assessment of circulating angiogenic serum markers (e.g., vascular endothelial growth factor (VEGF)) [9].

Several studies [10–12] have shown that monitoring tumor angiogenesis has prognostic value. Angiogenesis is linked to an increased risk of local regional recurrence, distant metastases, and reduced survival in patients with cancers at various organs, including breast, lung, and ovary. Hence, angiogenesis is an interesting area of oncologic research. However, accurate means are needed for detecting tumor angiogenesis and monitoring treatment-induced changes in angiogenesis that precede changes in tumor size [7]. Promising developments in HNSCC angiogenesis research have engendered a need for a reliable pre-clinical platform to develop new therapies and validate surrogate markers of their efficacy in vivo [8].

The “gold-standard” MVD measure of angiogenesis is the histological estimate of the average number of microvessels within a selected region. Several studies have found that intratumoral MVD is predictive of outcome in a number of HNSCC disease sites and could aid the identification of patients who may need aggressive or adjuvant therapy [13–15]. However, the histological MVD assessment is inherently invasive and cannot clarify whether there are functional vessels with blood flow and whether vessels are of a hyperpermeable nature. Also, the assessment of serum marker VEGF lacks sufficient sensitivity and specificity [9].

Tumors are heterogeneous. Angiogenesis usually peaks in the tumor rim, and therefore MVD measures will vary at different locations where the biopsy is obtained and may underestimate the degree of angiogenesis. Hence, the MVD parameter is more similar to a static “snap-shot” that does not lend itself to either dynamic situ assessment of the tumor vasculature or of the molecular factors that regulate its growth [15]. Intratumoral

and MTT values reduced relative to the values of these (interstitial) space. The contrast agent decreases the T1 parameters in normal adjacent structures. (longitudinal relaxation time) value of blood and hence ally, Hermans et al. [23] demonstrated that perfusion enhances the signal intensity on T1-weighted imaging. The assessed on CTP in HNSCC is an independent predictor of outcome after definitive radiation therapy with or without imaging covering the tissue or organ of interest using chemotherapy. Specifically, they reported that a lower pre-intervals in the seconds range, for periods of up to several treatment (radiation) perfusion rate had a significantly higher local failure rate.

Interestingly, Gandhi et al. [24] and Zima et al. [25] demonstrated that CT-determined BV be converted into parameters reflecting tumor biology. The before treatment or after induction chemotherapy contrast enhancement on T1-weighted images can be modulated significantly with response in advanced HNSCC. High pre-treatment (induction chemotherapy) BV was linked to beneficial outcome; and a reduced BV post-enhancement, maximum slope, and washout slope. Quantitative methods can provide parameters like peak transfer constant in min^{-1} , v_e (volume fraction of the parameters BF and BV correlated positively with MVD as determined through histology, indicating the potential of CTP for assessing angiogenesis in vivo (Fig. 2).

DCE-MRI

In DCE-MRI, injection of the contrast agent gadopentetate dimeglumine (Gd-DTPA) intravenously is followed by the agent passing from the intravascular space to the interstitial space at a rate that depends on perfusion, vascularity, and tissue permeability. So far, few studies have described the characteristic DCE-MRI enhancement patterns of tumors in the HN region. Konouchi et al. [29] have studied 30 patients with

Fig. 1 Contrast-enhanced neck CT scans (a, c) and CT perfusion maps (b, d) of a 45-year-old man with stage IV (a–b) and a 57-year-old woman with stage III (c–d) HNSCC in the base of the tongue. Solid tumor masses are indicated with T in (a, c). CT perfusion map (b) shows elevated blood flow (190.15 ml/100 g/min), which was confirmed by relative increased intratumoral MVD (47.2 vessels per square millimeter), whereas CT perfusion map (d) shows decreased blood flow (39.13 ml/100 g/min), which was confirmed by relative decreased intratumoral MVD (19.2 vessels per square millimeter). Reprinted with permission from [26]

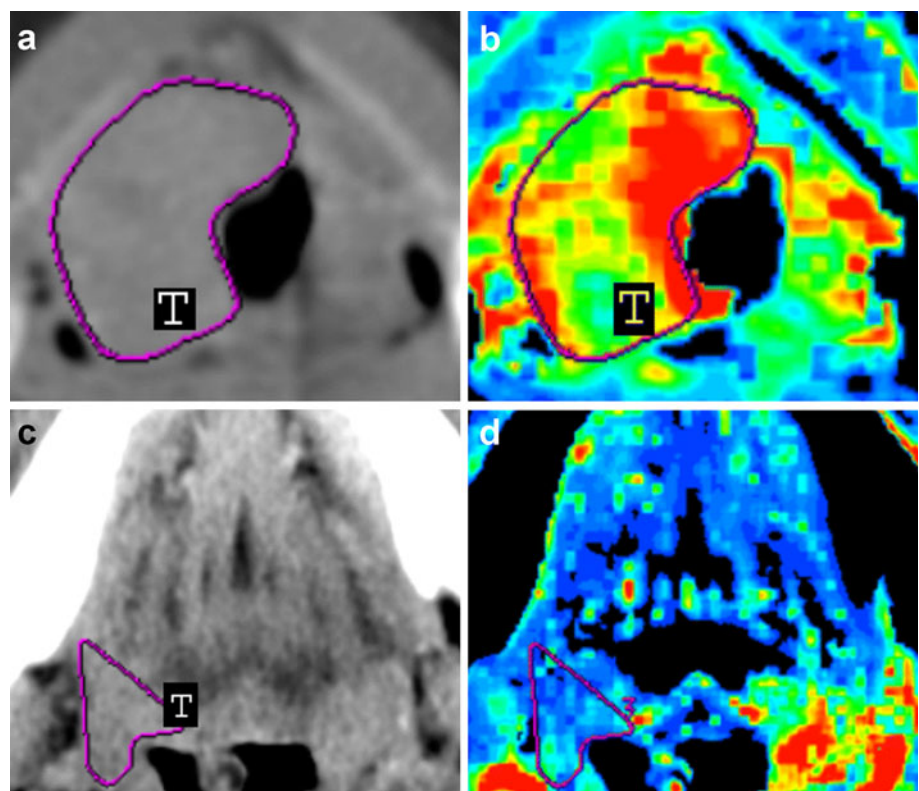
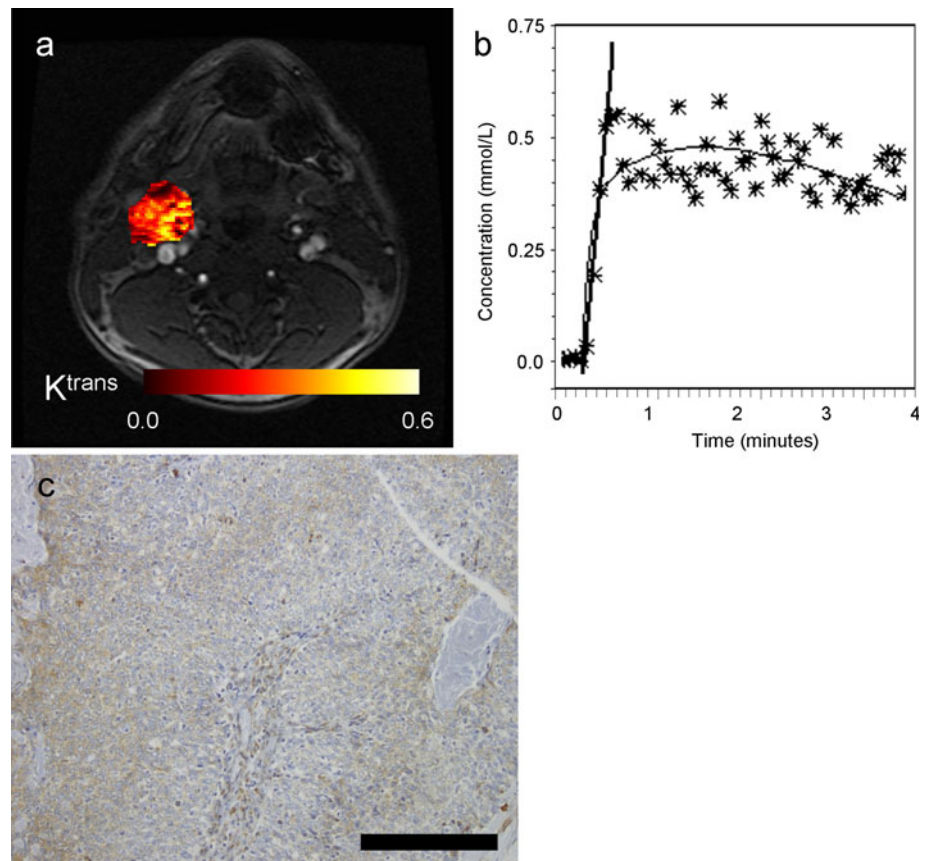


Fig. 2 Images illustrating the left neck lymph node of a patient with HNSCC (male, 49 years old, primary cancer unknown). **a** Post-contrast axial MR image extracted from the DCE-MRI scan with the calculated parametric K^{trans} map of the node overlaid on the DCE-MR image. **b** DCE-MRI signal, converted into Gd-DTPA concentrations, as a function of acquisition time. The *stars* indicate the individual data points (averaged over the ROI), the *thin black line* is the t_1 , and the *thick black line* indicates the slope. **c** VEGF immunostaining of corresponding tumor surgical specimen, indicating high expression. *Black bar* indicates 0.2 mm



primary oral cavity and oropharyngeal SCC with DCE-favorable treatment outcomes [23–25, 32] and that a large MRI and correlated the findings with PCNA (proliferating cell nuclear antigen). The PCNA labeling index showed a better response [24, 30]. Conflicting results, where a significant correlation with maximum contrast index (CI) increase in BV after treatment is linked to favorable outcome and maximum CI gain. Hoskin et al. [30] found in 13 patients with advanced HN cancer that tumors with diminished perfusion at the end of radiotherapy were those endpoints for local control, and data analyses methods [30] most sensitive to treatment and that tumors which showed greater enhancement after accelerated radiotherapy were likely to fail locally. Cao et al. [31] performed quantification of blood volume (BV) and blood flow (BF) from

DCE-MRI data pre-therapy and 2 weeks after initiation of chemoradiation in 14 HNSCC patients with advanced disease; they showed that an increase in available primary

tumor blood (i.e., BV) for oxygen extraction during RT Diffusion-weighted Imaging (DWI) is a technique that was associated with increased local control. Finally, in a cohort of 33 patients with HNSCC with neck nodal metastases, Kim et al. [32] have shown that the average microscopic level and may be used for in vivo tumor pre-treatment DCE-MRI parameter K^{trans} value was pre-characterization [33]. Since freedom of translational motion of water molecules is hindered by interactions with other molecules and cellular barriers, such as membranes or different organelles, DWI abnormalities exhibit changes of tissue organization at the cellular level. These micro-

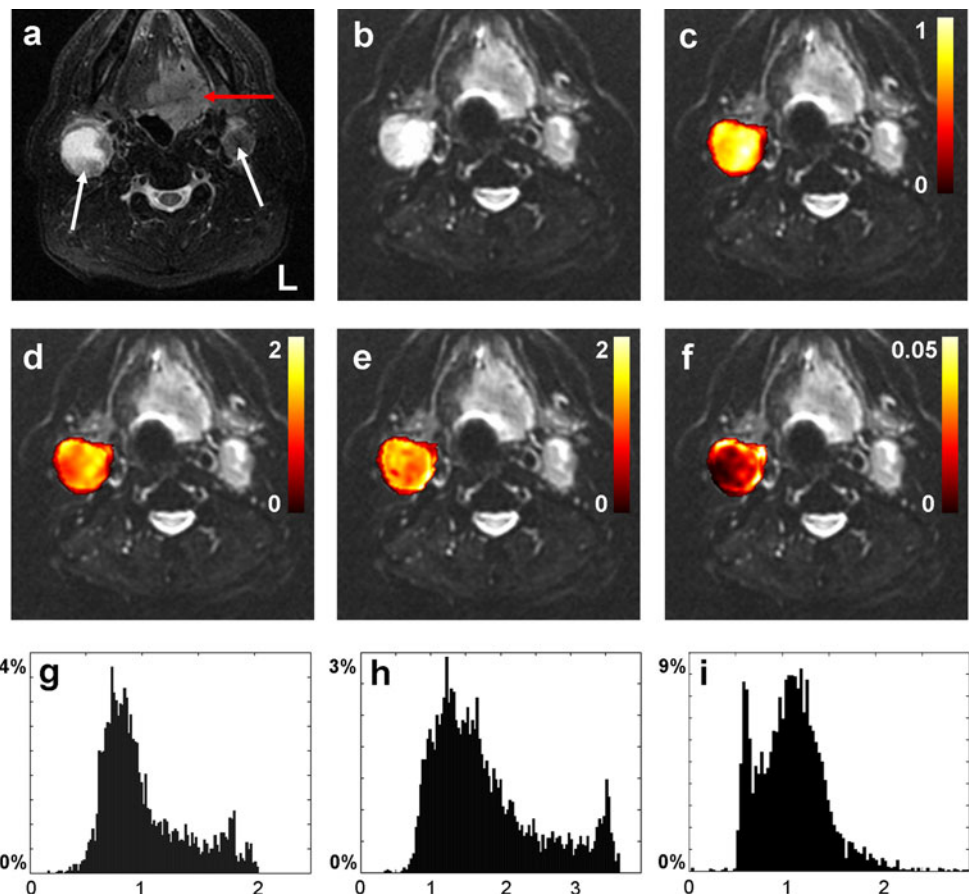
structural changes affect the (hindered) motion of water molecules and consequently alter the water diffusion

properties and therefore the MRI signal. The signal loss in tumor vessels which predominate at the edge of necrotic DWI can be quantified using the apparent diffusion coefficient (ADC), which can be interpreted as a measure of the anti-angiogenic agent bevacizumab. Another tentative link between angiogenesis and ADC was given by Liu et al. [43] in a preliminary report, who performed ADC, MVD, and VEGF measurements in 38 patients with prostate cancer. They observed a negative correlation between ADC values and MVD (and VEGF). This can be interpreted as the signal intensity decay is of monoexponential origin. Higher cell density will result in restricted water motion (diffusion). However, these results need to be validated.

In most DWI studies in patients with HN cancer, a combination of 2 or 3 b-factors was typically acquired, and the ADC values were calculated using the assumption that the signal intensity decay curves obtained from performing DWI with an extended range of b-values in patients with benign and cancerous tissue, and to assess residual or recurrent tumors [34–40]. Wang et al. [35] studied 97 patients with HN at 1.5T and reported mean ADC values for lesions with different underlying pathologies. The method also provided an additional parameter, K_{app} , which may provide added value. (Fig. 3).

The link between DWI findings and angiogenesis is not direct, but it has been hypothesized previously that tumors with a higher pre-treatment ADC are more susceptible to the effects of therapy with vascular disrupting or anti-tumor angiogenesis agents [4]. In particular, Koh et al. [42] suggested, based on results of DWI in 15 patients with solid tumors (mostly colorectal and ovarian), that immature ADC values of regions of interest could yield important

Fig. 3 Axial MR images from the oral cavity of 60-year-old man diagnosed with HNSCC in the base of the tongue. **a** T2 short tau inversion recovery (STIR) image. **b** realigned mean $b = 0$ image. The red and white arrows in (a) indicate the primary tumor and metastatic nodes, respectively. Figures (c–f) display voxel-by-voxel calculation outcomes for the right node presented as mask overlays on the realigned mean $b = 0$ image. **Graphs (g–i)** display the corresponding histogram distribution plots of the measures from (e). **c, g** Apparent diffusion coefficient obtained from Gaussian monoexponential fitting ($10^{-3} \text{ mm}^2/\text{s}$). **d, h** Apparent diffusion coefficient ($10^{-3} \text{ mm}^2/\text{s}$). **e, i** apparent kurtosis coefficient (dimensionless), and **reduced χ^2 error estimate**, all obtained from the non-Gaussian diffusional kurtosis analysis. Reprinted with permission from [41]



information about salivary gland function before and after phosphomonoesters (PMEs, phospholipid precursors); therapy and holds promise for investigating radiation- and glycerol esters of phosphocholine and phosphoethanolamine-induced xerostomia. In HNSCC, there is some evidence that DWI is useful for predicting and detecting early sphoethanolamine, collectively referred to as phosphodiester (PDEs) (phospholipid catabolites). PME and use of DWI for the prediction and detection of early PDE metabolites are elevated in tumors with high cell membrane turnover and are therefore markers of proliferation. In HN cancers, ^1H -MRS has been used mainly to correspond to better outcome and that an increase of ADC's after treatment was also favorable for response. Additionally, Galban et al. [46] showed that DWI could provide both prognostic and spatial information during study cell membrane metabolism, pH, and tumor energy non-surgical organ preservation therapy in HNSCC. The status that may be reported in this study were in agreement with Kim et al. that increase in ADC values after treatment (chemoradiation) was linked to favorable outcome.

PET

MRS

Magnetic resonance spectroscopy (MRS) is a technique which allows quantification of in vivo metabolite concentrations of tissue, thus offering a window onto cell metabolism [47]. MRS has been performed in HN cancers for both primary tumors and neck nodal metastases. The metabolites most commonly studied with MRS are total choline (tCho, a product of phospholipid metabolism) and lactate (a product of glycolysis). There is not a direct one-to-one link between angiogenesis and glucose consumption which can be quantified by PET metabolism seen on MRS. However, elevated levels of lactate may be observed during the process of aggressive tumor development accompanied by angiogenesis (the evolution of the microenvironment) [49]. Also, the rate of tumor cell proliferation has been linked to choline metabolites seen with phosphorus (^31P) MRS [50, 51].

Numerous investigators have found elevated total choline in tumors and it has been suggested that the elevated tCho peak reflects an increased cell proliferation rate [48]. The accumulation of lactate in tumors may be caused by both aerobic glycolysis (a common process in tumors) and by anaerobic glycolysis due to hypoxic conditions. Detection of lactate (a potential biomarker) in tumor tissues using non-invasive MR is difficult because of the presence of lipids, which typically have high co-resonating peaks. Hence, data acquisitions need to be tailored to observe these peaks in vivo. In HN cancer, spectral editing techniques have been used to observe the lactate peak [54]. ^31P MRS can show a number of metabolites that may offer critical information about the tumor: nucleoside triphosphates (NTPs); phosphocreatine (PCr) (energy metabolism); inorganic phosphate (Pi, intracellular pHi); phosphocholine and phosphoethanolamine, jointly denoted as

Positron emission tomography (PET) is a highly sensitive non-invasive technology that is well suited for the visualization of changes in metabolism in cancer. Before PET imaging, radiolabeled tracers are injected in non-pharmaceutical doses. Three-dimensional PET data can then be obtained and reconstructed to show the concentrations and locations of the radionuclide. PET imaging, like MRS, offers a window into the tumor microenvironment. ^{18}F -urodeoxyglucose (^{18}F -FDG) is the radiotracer most commonly used to image HN cancer [55]. ^{18}F -FDG uptake is proportional to tissue glucose consumption. Most tumors have an elevated ^{18}F -FDG uptake. This tracer has applications ranging from diagnosis to prediction of outcome in HN cancer [56]. Many other PET tracers have been used to better understand the tumor microenvironment, especially angiogenesis, and related processes such as hypoxia and proliferation. The radiotracer ^{18}F -Galacto RGD has been developed for PET imaging of $\alpha v \beta 3$ expression of a receptor involved in angiogenesis and metastases [58]. Beer et al. [57] tested the feasibility of using this tracer in eleven patients with HNSCC. They performed immunohistochemistry (IHC) using $\alpha v \beta 3$ -specific antibody and expression of the antibody was seen in all cases. Galacto RGD PET identified 10 of the 12 tumors correctly, and two tumors of size < 0.5 mm were missed. They established the feasibility of using this tracer to open up new possibilities for studying angiogenesis with PET. An important application of this new radiotracer would be in monitoring treatment response of anti-angiogenic therapy. ^{18}F -uromisonidazole (FMISO) PET has been used to study hypoxia [58, 59]. Hypoxia is a pathological condition in which tissue is deprived of adequate oxygen supply. Numerous investigators have stated that tumor oxygenation (using invasive Eppendorf pO_2 measurements) in HN

US

Ultrasound offers an inexpensive and readily accessible means to evaluate cervical adenopathy, and it is the standard tool used for this purpose in the clinic [66]. Typically, clinical examinations are performed with a high-frequency, linear transducer, usually with Doppler capability.

Substitution of neoplastic tissue for normal lymph node tissue makes lymph nodes unusually clear on ultrasonographic examination. The complex, multi-step process of angiogenesis is critically associated with blood flow and blood flow regulation. It has been hypothesized that laser Doppler ultrasound could provide a non-invasive and reliable method of quantifying blood flow within tumors [67]. Jacob et al. [67] showed that laser Doppler ultrasound is a potentially useful tool for studying blood flow in the HN region.

Molecular imaging

Molecular imaging (MI) has been defined as the in vivo visual representation, characterization, and quantification of biologic processes and molecular pathways at a cellular and molecular level [68]. As long as a suitable target, probe, and detection method are available, the MI concept can be applied to various imaging modalities, including MR, CT, PET, US, and optical imaging. MI is rapidly gaining acceptance in oncology, as it detects differences of molecular properties in vivo between cancer and normal tissue that arise as a result of malignant transformation [69].

The above mentioned ^{18}F -Galacto-RGD PET approach using the $\alpha v\beta 3$ -specific antibody is an excellent example of MI use in angiogenesis [56]. Receptors play a key role in signal transduction and proliferation of cancer cells [70]. Another example of such a receptor is the transferrin receptor (TfR), which is a cell membrane-internalizing receptor that is responsible for iron sequestration in mammalian cells [71]. Expression of TfR is elevated in malignant cells due to a need for iron as a cofactor for the ribonucleotide reductase enzyme [76].

TfR is overexpressed in the majority of cases of HNSCC but is minimally expressed in normal oral squamous epithelium and benign lesions [72]. A high expression level of TfR indicates a high grade of tumor malignancy and a poor prognosis. TfR expression can be targeted in vivo by conjugating a transferrin (Tf) probe to a nanoparticle suited for MI, as shown elegantly by Shan et al. [73] in an animal model of HNSCC.

In addition to the two aforementioned MI approaches in HNSCC (TfR and $\alpha v\beta 3$), many other angiogenesis-related receptors can be targeted using MI. As new molecular therapies are by definition directed at specific targets, MI directed at the same targets can be useful in predicting the response to anti-angiogenic drug treatment. Such a targeted

approach depends on the binding of labeled molecules to highly expressed markers. Interestingly, nanoparticles used for MI of angiogenesis may be designed such to have both diagnostic and anti-tumor features. This allows investigators to precisely monitor drug delivery and efficacy in tumors (theranostics) [74].

Molecules that are possible targets for angiogenesis include VEGF and its receptors, integrins, and matrix metalloproteinases (MMPs) [7]. The application of MI of angiogenesis in HNSCC has been very limited so far, therefore we would like to refer to articles by Turkbey et al. [17] and Cai et al. [75] who provide excellent, more extensive reviews of potential targets for MI of angiogenesis.

Current HNSCC treatment protocols for advanced disease include aggressive induction chemotherapy and concurrent chemoradiation, with an increasing role for novel, rationally designed, molecularly targeted agents [76]. There is a growing need for additional clinical diagnostic tests to help select treatments with maximal efficacy and minimal toxicity [8]. Unlike cytotoxic chemotherapy, which interrupts normal mitotic activity in dividing cells, targeted therapies are designed to interact primarily with cancer-specific attributes and signaling pathways [8]. One of these pathways is angiogenesis, a characteristic of all advanced cancers.

Pre-clinical and early clinical trials provide evidence of the promise of anti-angiogenic therapy for HNSCC [8]. [8] recently provided an overview of the currently available data and future directions for treatment, including ongoing pivotal clinical trials. Contrary to earlier fears that anti-angiogenic therapies could augment hypoxia and thereby might make treatment failure more likely, anti-angiogenic therapies in pre-clinical HNSCC models so far do not appear to have a propensity for resistance and go well with traditional therapies (such as radiation).

Clinical use of anti-angiogenic agents for HNSCC often focus on inhibitors of tyrosine kinase receptors, including bevacizumab, sorafenib, sunitinib, semaxanib, and others. Their use is currently limited to clinical trials, and therefore, literature on the use of imaging to monitor angiogenesis in HNSCC patients treated with anti-angiogenic drugs is scarce. It is pertinent to point out that most of the aforementioned studies of the use of imaging techniques to monitor treatment in HNSCC did not pertain to anti-angiogenic drugs, but to radiation or cytotoxic chemotherapy [8]. In a recent Phase II study by Machiels et al. [77], 38 patients with HNSCC were treated with sunitinib and a small subset of patients underwent DCE-MRI monitoring pre- and post-treatment. The DCE-MRI results

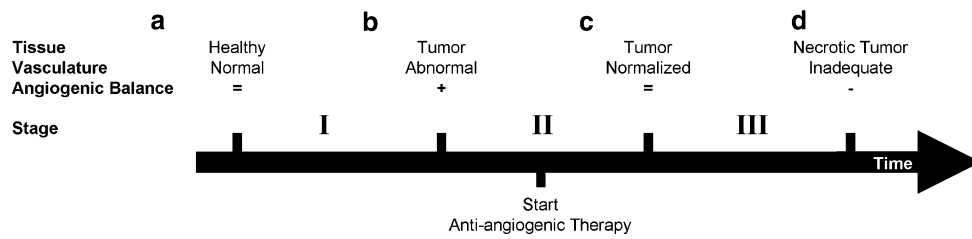


Fig. 5 Schematic of changes in tumor vasculature during tumornormalized tumor vasculature (as proposed by Jain) [The formation and the course of anti-angiogenic therapy]. The resulting network is more effective for the transport of therapeutics has normal vasculature, which is composed of mature vessels and had nutrients. Rapid pruning of tumor vasculature reduces the an exact balance of pro- and anti-angiogenic molecules. The integrity of vasculature network. The network is unable to facilitate activation of the angiogenic switch enables the tumor to engage tumor growth and could result in tumor dormancy, which is the surrounding blood vessels and promote formation of new vessels. The ultimate goal of anti-angiogenic therapy. Figure conceptualized from abnormal tumor vasculature provides the tumor with oxygen and nutrients, which facilitate more growth. Administration of anti-angiogenic therapies prune immature vessels, which results in more suited imaging modalities for their assessment

showed that in all but one of the patients studied, a significant decrease was observed in K^{trans} after treatment, suggesting that sunitinib indeed decreased the amount of angiogenesis. In another Phase II study, Fury et al. [78] used power Doppler ultrasound and serum VEGF levels to monitor anti-angiogenic activity of semaxanib in 35 anti-angiogenic patients with HNSCC. They found that tumor vascularity decreased during semaxanib treatment even in patients with progression of disease. They concluded that semaxanib restores vascular integrity [79]. Clinically, imaging can be achieved suboptimal inhibition of angiogenesis and changes observed with ultrasound were probably not related to anti-tumor activity of semaxanib. These Phase I presented imaging techniques and their relation to angiogenesis is provided in Fig. 5 and Table 1.

Table 1 Application of imaging modalities for monitoring angiogenesis-related processes in tumor formation and anti-angiogenic therapy

Process	Imaging modality	Stage		
		I Malignant transformation	II Start anti-angiogenic therapy	III Starvation of tumor
Angiogenesis	F-Galacto RGD-PET	+	-	-
Proliferation	FLT-PET, 1H-MRS (choline), 31P MRS (PME and PDE)	+	-	-
Vascularity	CT perfusion, DCE-MRI	+	-	-
Hypoxia	FMISO PET, 1H-MRS (lactate)	+	-	-
Blood flow	US	-	+	-
Perfusion	CT perfusion, DCE-MRI	-	+	-
Necrosis	DWI (ADC), MRI/CT	NA	NA	+
Growth in volume	MRI/CT	+	-	-
Biomarker upregulation	Molecular imaging	±	±	±

The processes represent the overall characteristics of the tumor and do not take into account the intratumor heterogeneity

+, positive effect; -, negative effect; NA, not applicable

^a Due to the diversity of possible biomarkers for MI, the process can either be up- or down-regulated

Do we really image angiogenesis?

As indicated above, most imaging modalities, with the exception of ^{18}F -Galacto RGD PET, do not provide direct information about angiogenesis itself, but merely about a related process. Therefore, the exact relevance of the changes observed in tissue contrast (as measured using one of the imaging modalities) to angiogenesis remains speculative in nature. However, sufficient evidence is available showing that techniques as CTPE [76], DCE-MRI [80], and US [81] closely correlate with MVD measurements, which can be regarded as a gold-standard. Unfortunately, for other techniques, a similar validation with MVD is not conclusive (DWI [43], FDG PET [82], and FMISO PET [83]) or lacks completely (MRS and FLT PET). Another relevant issue for all techniques used for imaging angiogenesis or angiogenesis-related processes is the proper analysis (e.g. modeling) of the data and the reproducibility of the imaging measurements. These are prerequisites in order to objectively infer whether measured changes in imaging parameters truly reflect the biological process.

Conclusion

Considering the diversity and demonstrated potential of available imaging methods, the importance of imaging in assessing angiogenesis in HNSCC is expected to grow.

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