

Review

Molecular Imaging of Tumor Blood Vessels in Prostate Cancer

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Abstract. *In the past three decades many efforts have been undertaken to understand the mechanisms of tumor angiogenesis. The introduction of anti-angiogenic drugs in tumor therapy during the last few years necessitates the establishment of new techniques enabling molecular imaging of tumor vascular remodelling. The determination of tumor size as commonly used is not appropriate since the extended necrosis under anti-angiogenic therapy does not necessarily result in the reduction of tumor diameter. The basis for the molecular imaging of tumor blood vessels is the remodelling of the tumor vessels under anti-angiogenic therapy which obviously occurs at an early stage and seems to be a convincing parameter. Beside the enormous progress in this field during the last few years the resolution is still not high enough to evaluate the remodelling of the micro tumor vessels. New imaging approaches combining specific molecular markers for tumor vessels with the different imaging techniques are needed to overcome this issue as exemplarily discussed for prostate cancer in this review. Molecular contrast agents targeting the vasculature will allow clinicians the visualization of vascular remodelling processes taking place under anti-angiogenic therapy and improve tumor diagnosis and follow-up.*

Prostate cancer is the most common malignancy of men and one of the leading causes of cancer-related mortality (1). Imaging of the prostate plays a central role for diagnosis, localization and staging of prostate cancer and is needed to

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guide treatment selection and treatment planning. Resolution of modern imaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET)/CT reaches up to micrometers. Transrectal ultrasonography (TRUS) has also advanced, but is still restricted to biopsy guidance and brachytherapy seed placement. However, these imaging techniques have limited value in the detection of cancer and are still far from providing an exact analysis of prostate cancer. Molecular imaging is based on the combination of conventional imaging procedures with the tumor specific molecular markers. The aim of this review is to provide a particular insight into the impact of molecular imaging of the tumor blood vessels in this regard.

Morphological and Molecular Characteristics of Tumor Blood Vessels

Initiation of tumor angiogenesis is marked by structural destabilization of existing blood vessels with endothelial fenestration, opening of inter-endothelial contacts and formation of transendothelial gaps (2, 3). This leads to detachment of endothelial cells from the basal lamina and peri-endothelial cells, which results in migration and proliferation of formerly quiescent and mature endothelial cells. Chemotactic impulses of pro-angiogenic factors such as VEGF and FGF-2 secreted from tumor cells lead to migration of new endothelial cells towards the tumor cells and finally, to formation of new blood vessels (Figure 1). These processes of vascular destabilization and new blood vessel formation result in abnormal leakiness of these vessels and in loss of hierarchic arrangement of large, middle sized and small blood vessels as found in the normal vascular system. Therefore it is generally accepted that the tumor vascular bed is chaotically organized (4). For a long time it has been believed that vascular remodelling of newly formed blood vessels in form of a restabilization does not exist in

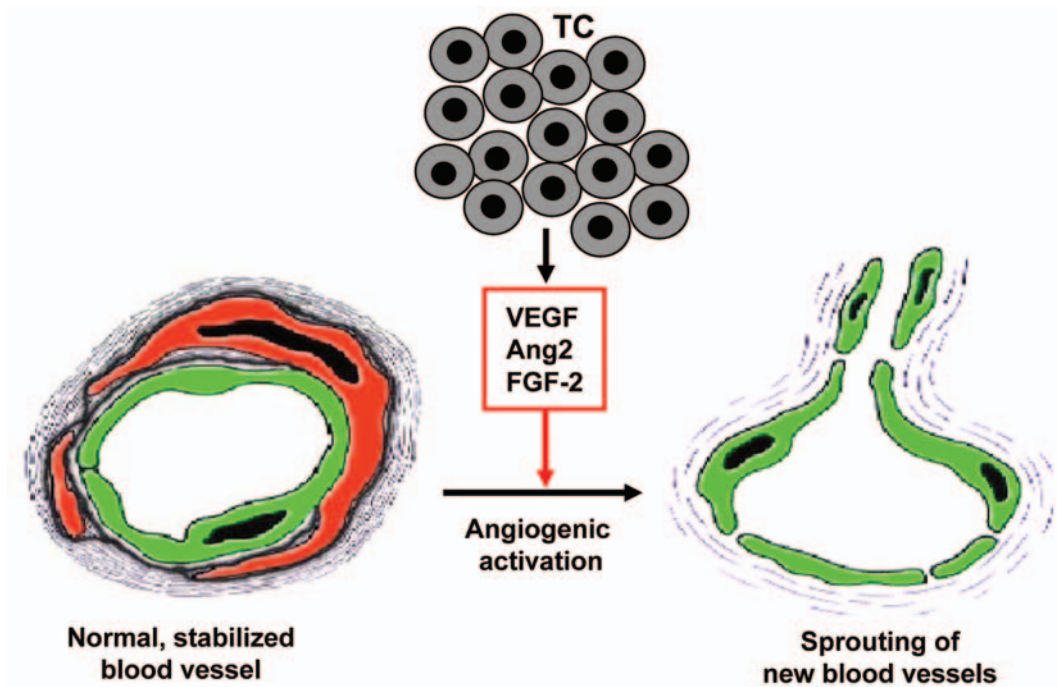


Figure 1. The scheme shows a stabilized capillary with closed endothelium (green), pericytes (red) tightly attached to the endothelium and a basement membrane (dark grey) clasping both cell types. Under the effect of pro-angiogenic factors, such as VEGF and FGF-2, secreted by tumor cells (TC) the vessel wall is nearly completely disintegrated. Detached endothelial cells (green) begin to migrate and proliferate. This leads to sprouting of new blood vessels from pre-existing blood vessels, namely angiogenesis.

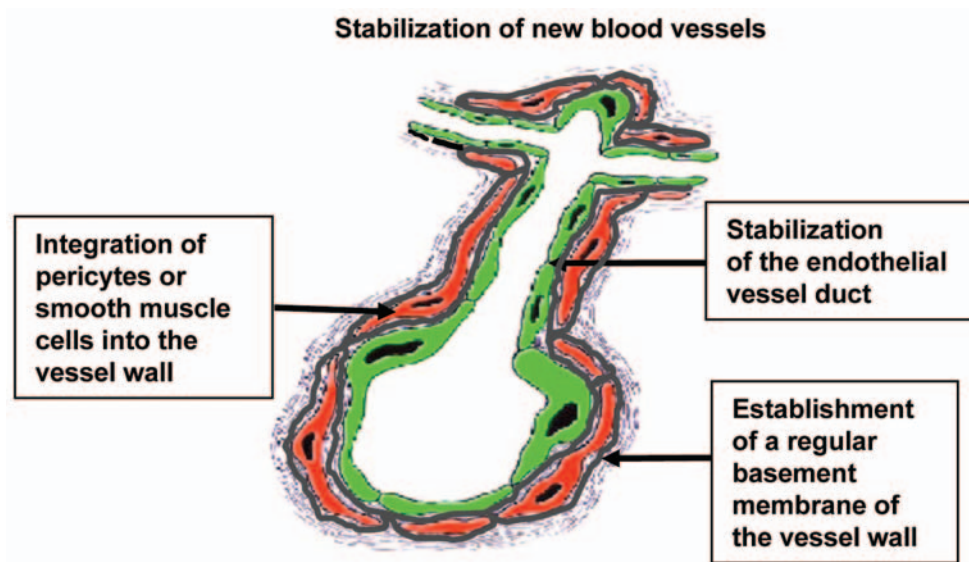


Figure 2. This graphic schematically shows the re-stabilization of the endothelial cell layer (green), re-integration of pericytes (red) into the vessel wall and re-establishment of the basement membrane.

the tumor. The heterogeneity of tumor vessels and the process behind it were neglected assuming that all tumor vessels are immature and instable (5-7). Results of studies performed in the last years including microscopic analyses

as well as modern imaging techniques have shown that tumor blood vessels also undergo a stabilization process (Figure 2) (8). Studies of anti-angiogenic therapy have shown that the process of vascular remodelling in tumors is influenced by

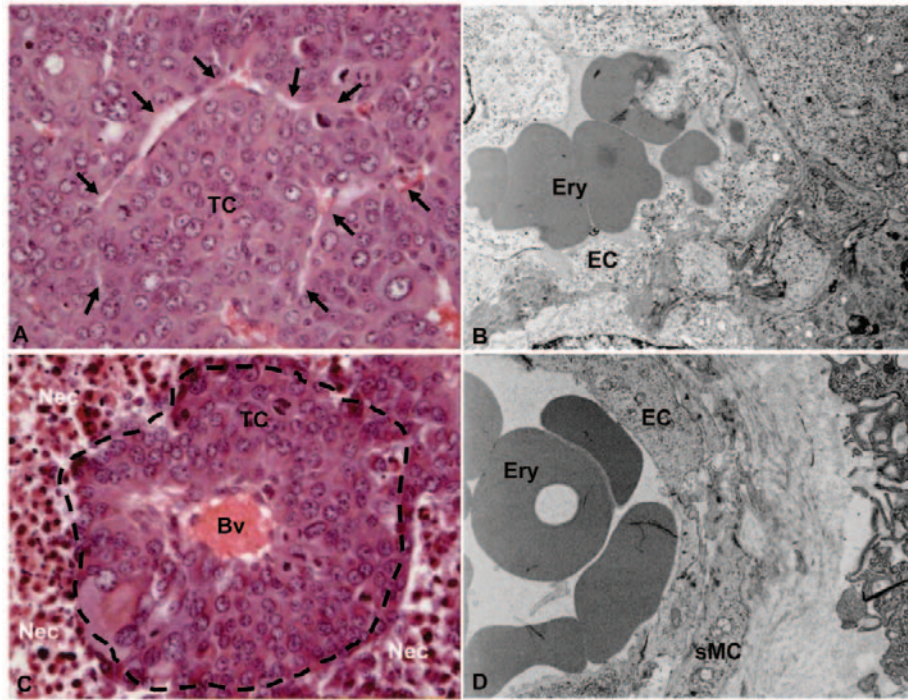


Figure 3. Histological section of an experimental tumor. A) Dense network of immature capillaries (arrows) encloses tumor cell groups (TC) in the tumor margin. B) Electron microscopy analyses of those areas in the tumor margin show non-stabilized endothelium (EC) and no basement membrane. C) In contrast blood vessels (Bv) with higher diameter dominate the tumor center. Tumor cells coronary arranged around those stabilized blood vessels survive while more distant tumor cells undergo necrosis (Nec). D) Electron microscopy analyses of those areas of the tumor center show at least 1-2 smooth muscle cell layers (sMC) around the endothelium (EC; Ery erythrocytes).

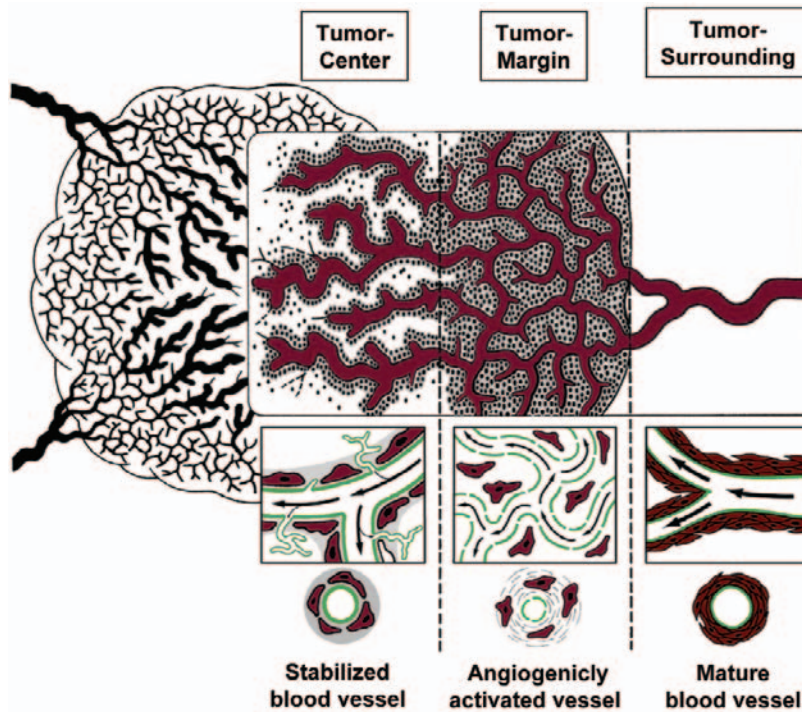


Figure 4. This graphic summarizes the blood vessel stabilization in the tumor center, which is associated with extended necrosis of tumor tissue, while a dense network of partly chaotically organized and immature blood vessels in the tumor margin show a tight relation to the tumor cell clusters. This blood vessel remodelling is to be captured with the new methods of molecular imaging.

angiogenesis inhibitors (9-11) and anti-angiogenic drugs in clinical studies (4, 12, 13). The tumor vascular bed is composed of blood vessels with differing morphological state. Electron microscopy studies reveal a heterogenous pattern of blood vessels with structurally stabilized among only partly stabilized blood vessels. Tumor blood vessels also differ regarding the expression pattern of signalling molecules, receptors and cell adhesion molecules in their wall, especially in their endothelial cells (14-19). This may enable the visualization of different states of the tumor vascular bed by coupling molecular markers and imaging techniques.

Tumor Blood Vessel Remodelling and its Impact on Tumor Tissue

Remodelling of blood vessels describes the morphogenetic processes involved in structural stabilization of newly formed blood vessels (Figure 3). Critical parameters include the formation of cell-cell contacts between endothelial cells and the basal lamina of the vessel wall as well as the integration of peri-endothelial cells, pericytes in the case of capillaries and smooth muscle cells in the case of larger blood vessels (5). In particular, studies by Suri *et al.* and Maisonpierre *et al.* regarding angiopoietins (Ang) 1 and 2 have shown that the first immature blood vessels composed of only endothelial cells undergo regression in case of missing integration of peri-endothelial cells into the vessel wall (20, 21). Ang1 promotes the integration of peri-endothelial cells into the vessel wall, while Ang2, especially in conjunction with VEGF, leads to detachment of peri-endothelial cells from the vascular wall and thus, antagonistically to the effect of Ang1, to destabilization of the vessel wall. However, Ang2 in combination with a low VEGF expression leads to regression of new blood vessels. It was later shown that Ang1-mediated vascular stabilization is associated with blockage of VEGF-induced vascular leakage (22). Whether similar processes also take place in the tumor vascular bed remains to be answered. Recent data support that some tumor blood vessels undergo immense structural changes of their wall during the process of tumor growth (7, 8). Especially blood vessels of the central areas of tumors are partially stabilized which is associated with a clear reduction of vascular density and extended tumor necrosis (Figure 3). These studies in experimental tumor models show that only those tumor cells that are in the tumor areas circularly arranged in 4-7 tumor cell rows around stabilized blood vessels, and thus are adequately supplied, survive. Tumor cells far from these blood vessels undergo necrosis (7) because of regression of instable and immature blood vessels supplying these areas. This process is apparently accelerated by tumor therapy with anti-angiogenic agents such as endostatin in experimental tumor models (8). Based on similar observations it has been

postulated that anti-angiogenic therapy would lead to a "normalization" of tumor blood vessels and open a "therapeutic" window enabling a more effective use of established therapies as chemo- or radiation therapy (4, 10). The inhibitory effect of vascular stabilization towards tumor growth also becomes clear by the experimental overexpression of the vessel-stabilizing factor Ang1 which leads to a significant slow-down of tumor growth (23). Corresponding to the experimental data, clinical studies with anti-angiogenic substances revealed a reduction of blood vessel permeability, an increase of tumor blood vessel diameter and necrosis of tumor tissue without a significant change of conventional parameters such as tumor size and diameter (24, 25).

The results described above indicate that a remodelling of the tumor blood vessel bed takes place without therapy, but is extensively increased under anti-angiogenic therapy. This process is accompanied by remarkable necrosis of tumor tissue (Figure 4). The changes in the vascular bed, however, cannot yet be sufficiently assessed and visualized with conventional imaging methods. This however is indispensable for an effective combination of anti-angiogenic therapy and conventional therapy regimes and, furthermore, for Micro-Imaging in the diagnosis and follow-up of tumors.

Advances in Prostate Cancer Imaging

Prostate biopsy is still considered the gold standard for diagnosis of prostate cancer. Conventional procedures, such as computed tomography (CT) and magnetic resonance imaging (MRI) as well as transrectal ultrasound (TRUS), have not yet been proven reliable in the diagnosis of prostate cancer (26). Despite restrictions in the informational value of these techniques, which can be overcome, all possible advances regarding prostate cancer imaging have been reached in the last decade (27).

Positron emission tomography (PET) has been shown to be particularly suitable for the detection of malignant processes (28). Several tracers appear to be promising for the diagnosis of prostate cancer. Although ^{18}F -Fluorodeoxyglucose-Uptake (^{18}F -FDG-PET) seems to correlate with Gleason-score, PSA and PSA-velocity, this tracer only exhibits a limited value for prostate cancer diagnosis (27, 29-31). The majority of prostate tumors show an only low increase in glucose metabolism compared to other, more aggressive tumor entities, which may be explained by the slow proliferation rate of prostate cancer (29, 30). Therefore other radiotracers need to be used for prostate cancer. Available PET tracers for assessment include ^{11}C - or ^{18}F -choline and acetate as well as ^{11}C -methionine (32, 33). ^{11}C -choline PET is sensitive and accurate in the preoperative staging of pelvic lymph nodes in prostate cancer (34). A few studies are available but there are no PET or

PET/CT studies with a large number of patients for tissue confirmation of prostate cancer (35). Further investigations are required.

The improved performance of MRI and combined MRI/MRS (magnetic resonance spectroscopy) in prostate cancer detection and staging over the last decade has been due to the maturation of magnetic resonance technology, refinement of the morphological criteria for identifying extra-prostatic disease and increased reader experience (36, 37). MRI is able to demonstrate zonal anatomy with excellent contrast resolution and is helpful in the decision-making process regarding whether to preserve or to resect neurovascular bundles during surgical management of prostate cancer. MRI can be used for targeted prostate biopsies (38, 39). However, the clinical practicality is questionable. MRI has no impact in detecting positive lymph nodes.

Transrectal ultrasonography (TRUS) remains the gold standard for biopsy guidance and brachytherapy seed placement (26). Introduction of microbubble contrast agents (“echo signal enhancers”) for ultrasound significantly increased the diagnostic potential of ultrasound, making it possible to visualize increased vascular flow. Contrast enhanced transrectal ultrasound can be used to target a biopsy procedure into areas of increased vascular flow (40). Among subjects with carcinoma, targeted cores were twice as likely to return a positive biopsy as compared with sextant cores (41). Future randomized clinical trials will have to confirm the value of contrast-enhanced targeted TRUS-guided biopsy.

Molecular Imaging of Tumor Blood Vessels in Prostate Cancer

The demonstration of blood vessels and their permeability as well as the visualization of blood flow directly or indirectly provides a glance into the inner life of a tumor. However, none of the frequently used imaging techniques such as MRI, PET/CT and transrectal ultrasound is able to visualize the microvascular bed of the tumor reliably and reproducibly and nor can be used repeatedly without harm to the patient.

As described in the beginning of this review, the tumor vascular bed is heterogenous regarding structure and function. The endothelium of tumor vessels differs from endothelium of blood vessels with regard to surface markers such as receptors VEGFR-1 and -2 (42, 43), E- (44) and P-Selectin (45), avb3-integrin (46) and CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule-1) (17). Tumor vessel endothelium expresses VEGF-receptors in much higher amounts than normal blood vessel endothelium. Furthermore, some cell adhesion molecules such as E-Selectin (47), avb3-integrin (48) and CEACAM1 (17) are nearly exclusively expressed by endothelial cells of small tumor blood vessels. P-Selectin is transferred to the surface from intra-cytoplasmic

reservoirs after activation of endothelial cells, as exemplarily shown in inflammatory cases, and provides adhesion of leukocytes to the endothelium. Antibodies and synthetically produced ligands, which serve as target molecules, could possibly be used to mark tumor blood vessels and thus make them accessible for imaging procedures. Currently, several research groups are working intensively on the development of targeted microbubbles for use in ultrasound. The goal of these efforts is to immobilize specific antibodies or ligands on the surface of microbubbles, which can be injected into the vascular system and bind on the endothelium of tumor vessels. They can accumulate in the vascular bed of tumor tissue, but not in the normal vascular bed. Microbubbles can be visualized by ultrasound imaging. Experimental studies with P- and E-Selectin (45) or avb3-integrin have already shown promising results (49, 50). Further analyses are necessary to improve the properties of microbubbles and their binding molecules as well as to evaluate tumor specific differences. Furthermore it is necessary to test the behaviour of targeted contrast agents during the remodelling process of the tumor vascular bed. This will make it necessary to immobilize a combination of molecules on a carrier or to use a combination of carriers.

Conclusion

Improvement of conventional imaging procedures is an essential aspect in clinical treatment of tumors. The aim is to reach a “vitreous” tumor, which can be looked into without the use of invasive methods. This can apparently be reached *via* the combination of molecular tools with available imaging techniques. The use of new targeted contrast agents will allow an improvement of resolution of the tumor vascular bed and observation of its remodelling process under anti-angiogenic therapy. There is still a long way to go, especially due to a difficult visualization of microvessels. The molecular imaging procedures will improve diagnosis and enable the clinician to work out a more effective, tumor specific therapy scheme and to observe therapy success or failure on time.

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