What About the Clinic?

12

The list of pathologies in which an excess of angiogenesis is observed is relatively long (see Table 12.1). However, the importance of angiogenesis in the pathophysiology of these diseases varies. It certainly plays a preponderant role in cancer and neovascular eye diseases. However, even in cancer, the importance of angiogenesis seems to vary from one cancer to another and from one stage of the disease to another.

The pharmaceutical industry has long been undecided about the development of molecules interfering with angiogenesis. This is because endogenous inhibitory molecules such as angiostatin and endostatin have not lived up to their promise in preclinical and clinical trials.

However, Genentech, under the impetus of Napoleone Ferrara, developed a humanized monoclonal antibody that had excellent inhibitory effects in preclinical models [251]. Clinical trials were then conducted, and in 2004, in the renowned *New England Journal of Medicine*, a very important article was published that definitely proved that inhibition of angiogenesis was a promising strategy for the treatment of cancer [139]. Hurwitz and colleagues showed clearly that patients with colon cancer with metastasis treated with a combination chemotherapy-VEGF inhibitor had significantly increased progression-free survival. This inhibitor is called bavacizumab (Avastin^{TM}) and has been used clinically for several years in different cancers.

Another strategy was developed at the same time. This involved small chemical molecules that bind to the tyrosine kinase domain found in the intracellular domain of angiogenic factor receptors such as the VEGF receptor. These molecules often have a more or less narrow specificity, that is, they can bind to several receptors with varying affinities. Molecules include sunitinib (Sutent^{TM}), sorafenib (Nexavar^{TM}), or PTK 787 or temsirolimus. Sutent^{TM} and sorafenib preferentially, but not exclusively, bind the VEGF receptor. Sutent^{TM}, for example, can also bind FGF and PDGF receptors and bind to other molecules within a cell. The affinities are nevertheless lower for the latter substrates.

A. Bikfalvi, *A Brief History of Blood and Lymphatic Vessels*,
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Table 12.1 Angiogenesis in human pathology

Pathology	Excess of angiogenesis	Defect in angiogenesis
Cancer	+	
Retinopathy	+	
Age related macular dystrophy (AMD)	+	
Alzheimer's disease	+	
Lateral amyotrophic sclerosis (SLA, Lou Gehring disease)		+
Atherosclerosis	+	
Hypertension	+	
Crohn's disease	+	
Lupus	+	
Nephropathy		+
Chronic wounds		+
Coronary artery disease		+
Diabetic ulcer		+
Multiple sclerosis	+	
Vascular malformations	+	
Obesity	+	
Psoriasis	+	
Allergic dermatitis	+	
Kaposi's sarcoma	+	
Hypertension of the pulmonary artery	+	
Asthma		
Mucoviscidosis	+	
Intestinal inflammatory disease	+	
Parodontal disease	+	
Liver cirrhosis	+	
Diabetic nephropathy	+	
Arthritis	+	
Ovarian kysts	+	
Endometriosis	+	
Uterine bleeding	+	
Osteomyelitis	+	

How does anti-angiogenesis therapy work at the tumor level? It is surprising to find that most anti-angiogenic molecules are effective when they are administered together with chemotherapy. How then can we explain this effect? Two opposing concepts exist. In the first, defended by Robert Kerbel, professor at the University of Toronto, anti-angiogenesis acts as an additive to chemotherapy [252]. Anti-angiogenesis increases intra-tumor hypoxia, leading to tumor regression. In contrast, Rakesh Jain, a professor at the Massachusetts General Hospital in Boston, developed the concept of normalization of tumor vessels, which we have already briefly discussed [253] (Fig. 12.1). We know that, in tumors, vessels have an abnormal

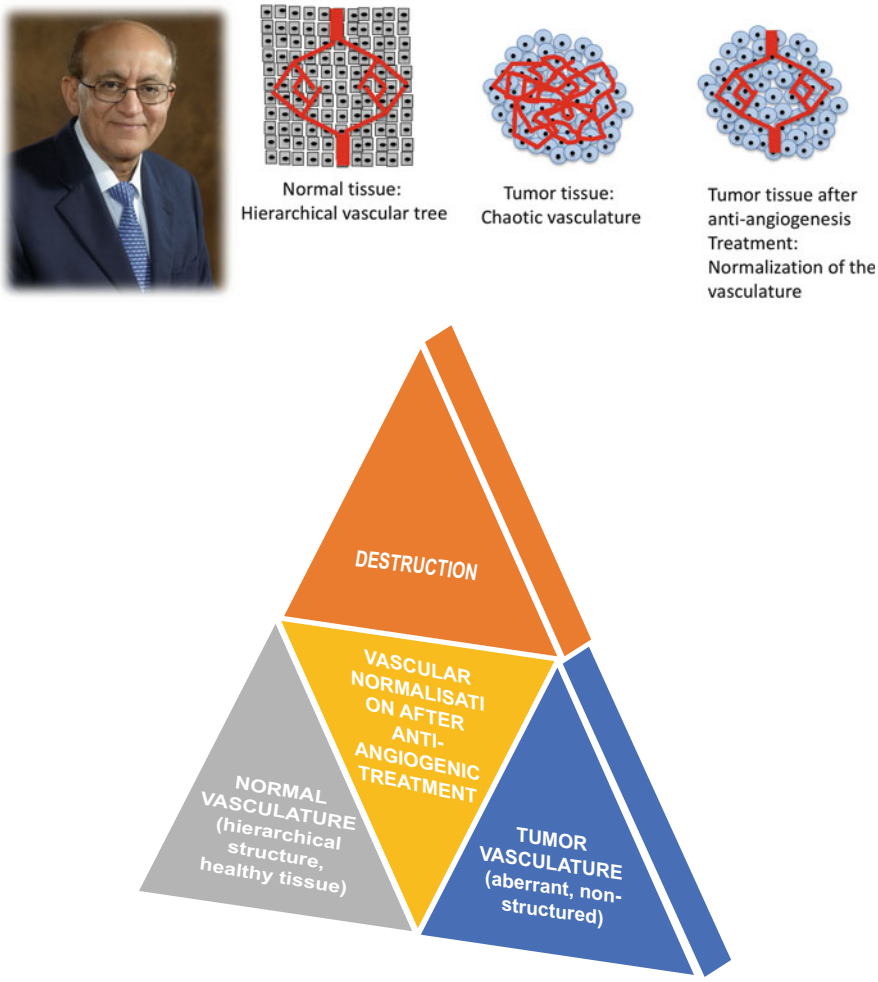

Fig. 12.1 Concept of vascular normalization. The tumor vasculature is abnormal in structure and functions. Anti-angiogenic therapy initially improves the structure and function of tumor vessels. However, aggressive or prolonged anti-angiogenic treatment may eventually destroy the vessels, resulting in a non-functional vascular system resistant to further processing. The dynamics of vascular normalization induced by VEGFR2 blockade (*top right*). Normal blood vessels in skeletal muscle (*left*); the following images show the vascularization of human colon carcinoma in mice (*middle*) at days 0 and 3 after the administration of specific VEGR2 antibodies (*right*). The figure is modified from Jain [255]. Schematic representation (*bottom*). Figures of the author. The photo was generously provided by Rakesh Jain

structure with a disturbed hierarchy and a faulty maturation. In this concept, anti-angiogenic therapy results in a return to a normal vessel structure as seen in healthy tissues. This concept is called "normalization." The vascular normalization concept is still widely debated and, as already mentioned, additional explanations exist. For

example, some have thought that chemotherapy mobilizes hematopoietic cells from the bone marrow (which have a detrimental effect on the therapy) and this could be blocked by anti-angiogenic therapy [254]. Another explanation is the presence of VEGF receptors at the level of the tumor cells themselves and, thus, the anti-angiogenic treatment can also act directly on the tumor cells [228].

Another aspect is the escape of the tumor from the anti-angiogenic treatment. Greene already observed that some tumors develop, under certain circumstances, without a vascularization [64]. He wrote in 1938: "*Despite these conditions, the human tumor has grown in seven of the twelve rabbits used. Growth first became apparent toward the end of the third week. The extension of blood vessels from the iris into the transplant occurred in four of the animals between the thirty-fifth and fortieth days. Vascularization has not occurred to date in three of the animals in which primary growth was observed, but notwithstanding the fragments have continued to increase in size*."

Different mechanisms are responsible for tumor escape (Fig. 12.2). The first mechanism is the reactivation of angiogenesis by the induction of new factors and their receptors. The second mechanism is the activation of invasive properties directly at the level of the tumor cell. This involves the induction of molecules promoting invasion of tumor cells. The most important molecules are constituted by the cMET receptor and its ligand (factor which binds this receptor) HGF we have already encountered. Other additional mechanisms have been described and are discussed in this book.

Indeed, very interesting work has recently been done on this subject in the context of glioblastoma. Glioblastoma is a brain tumor with a poor prognosis. The current treatment consists of radiotherapy and chemotherapy, most often associating a molecule called temozolamide. The problem with glioblastoma is that it can modulate its dependence on vascularization. As indicated in a previous chapter, glioblastoma may have an "angiogenic" phenotype and an "infiltrative" phenotype. Given here are a few additional explanations as this point is important. In the "angiogenic" phenotype, many new vessels are present in the tumor. As for the "infiltrative" phenotype, there are no new vessels formed, but the tumor cells use the pre-existing vessels (normal vessels) to attach and to migrate as a train would use rails to advance. In patients, the angiogenic and infiltrative phenotypes are often mixed and this is variable. Glioblastoma can be experimentally manipulated to acquire an angiogenic or invasive phenotype and this can be used to reveal how these processes proceed on a molecular level. It has been shown that human glioblastomas implanted in animals initially have an invasive phenotype and may acquire an angiogenic phenotype after several passages [256]. Additional mechanisms, which involved the cellular stress response or CXCR3/LRP1 cross-talk as well as the role of the EGF receptor in the invasive process, have already been discussed in this book. Furthermore, Rolf Bjerkvig's team has described that metabolic adaptation occurs after anti-angiogenic treatment with Bevacizumab [257]. Indeed, an increase in glucose incorporation is observed in treated tumors with an increase in glycolytic activity and reduction in oxidative metabolism. Is this adaptation linked to the infiltrative phenotype and does this adaptation involve glycolysis when its migration is stimulated? This is in

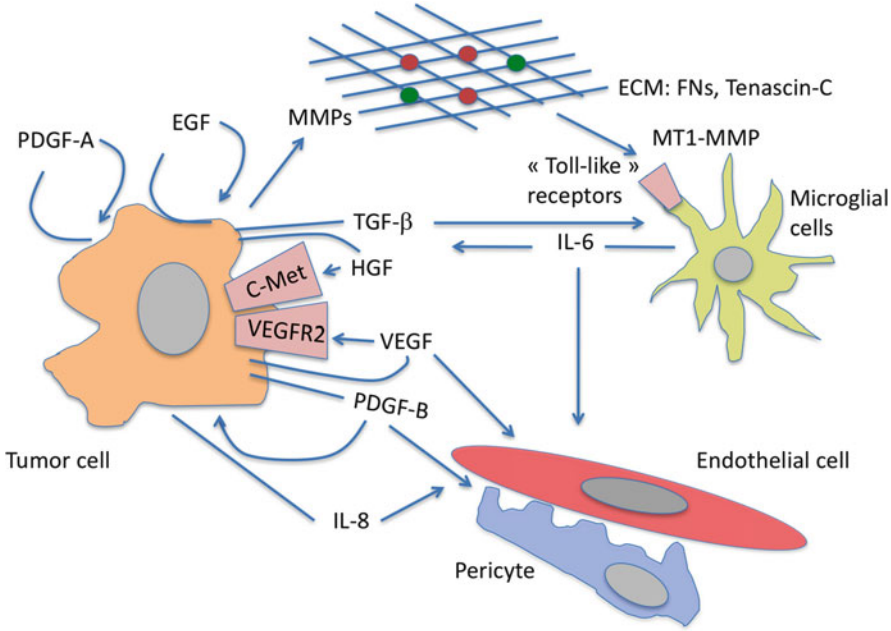

Fig. 12.2 Mechanisms of tumor invasion in brain tumors. Tumor cells interact with vascular cells, microglial cells, and components of the extracellular matrix. These interactions involve a variety of soluble regulatory factors and membrane receptors. On tumor cells, five growth factor systems are involved – PDGF/PDGFR, EGF/EGFR, VEGF/VEGFR2, TGFβ/TGFβR, and HGF/c-Met. At the level of vessels, various factors (VEGF, PDGF, IL-8, IL-6) are acting. In addition, microglial cells are activated by Toll-like receptors, membrane proteases (MT1-MMP), and matrix components (Fibronectin, tenascin). This leads to the migration of tumor cells into the cerebral parenchyma. The nature of pro-migration factors in the different structures of the cerebral parenchyma (vessel, nerve fibers, interstitial brain tissue) is not well-understood. Bradykinin is one of the only factors identified to date to attract tumor cells to blood vessels and allow migration using vessels as rails for invasion. Figure by the author reproduced with permission (Javerzat S, Godard V, Bikfalvi A. Future Neurology (2013) 8(2), 159–174. ISSN 1479-6708) [239]

apparent contradiction to the observation that oxidative metabolism is stimulated during invasion [258]. It is true that the latter observation was made in breast cancer, but one can wonder about these divergent results. Adaptation may be specific to the cellular or tissue context, but it would be better to have a more general explanation. In any case, no general conclusion can be drawn at the present time.

What is absolutely remarkable is that angiogenesis is completely blocked when tumor cells acquire an invasive phenotype. The vessels are tetanized by the assault of tumor cells.

Another target for clinical development was PLGF [172, 173] but this was abandoned because of conflicting results and failure in clinical studies. Indeed, based on the work of Peter Carmeliet, of which we have spoken previously, an anti-PLGF antibody was developed which had excellent effects concerning the

inhibition of angiogenesis in some angiogenic tumors in an experimental setting. Nevertheless, conflicting results have been published which show either the absence of an effect or, in contrast, an acceleration of the tumor growth when the PLGF is inhibited and an increase of resistance to anti-VEGF treatment [174, 175].

Nevertheless, in eye disease, the results with regard to the inhibition of angiogenesis were spectacular [259]. Indeed, the use of anti-VEGF had very convincing results in the treatment of age-related macular degeneration (AMD). For ophthalmological use, Lucentis™ was granted marketing authorization (MA) [260]. Nevertheless, the cost of this treatment is very high and much higher than that of Avastin™. However, more recent studies have shown that Avastin™ has exactly the same efficacy as Lucentis™ [261]. It is therefore possible to treat these patients with Avastin™ rather than with Lucentis™ to reduce the cost. The disadvantage of these treatments is that these drugs must be administered by intraocular injection. Alternative strategies should be developed that use a more manageable mode of administration.

As mentioned above, various clinical trials have been conducted with the aim of stimulating angiogenesis in cardiovascular diseases and only mixed results were observed. In this case, cell therapy seems to be the more effective (see Chap. 9).

Another way of exerting a therapeutic effect on vascularization is vascular targeting. The idea of this approach is that it is more important to target a pathological vascularization than to target a function. Anti-angiogenic approaches aim to interrupt the function of an angiogenic factor or receptor, which is the case for all the anti-angiogenic treatments to date. Avastin™, for example, blocks the function of VEGF, as do VEGF receptor inhibitors (sunitinib, sorafenib, etc.). The "targeting" approach is quite different. The function is not important. What is important is that the marker, which can be a molecule that has no proven function, is expressed in a pathological vessel. This marker serves just as a recognition molecule, which is targeted by a suitable chemical (peptides, antibodies, etc.) coupled to cytotoxic drugs, vascular destruction agents, etc.) bearing a label that can be attached.

Philippe Thorpe carried out pioneering studies by coupling an antibody directed against activated endothelial cells to tissue factor, a molecule that induces blood coagulation [72]. Tissue factor is an activator of the extrinsic blood coagulation pathway leading to the thrombus formation made of fibrin and platelets. As we have seen, Paul Broca had proposed galvanopuncture, which aims to obliterate vessels and no longer allows the passage of fluids and nutrients. This is similar to what Philippe Thorpe did a century later using molecular tools that specifically attacked the tumor vasculature.

There are two distinct approaches to targeting (Fig. 12.3). One is using small peptides, the other using antibodies. Using the phage display strategy, Erkki Ruhoslahti and Renata Pasqualini (Burnham Institute, San Diego, USA) were able to identify labels specifically recognizing different types of endothelium [262, 263]. Thus, they were able to identify small peptide sequences that recognize only tumor vessels but not normal vessels. In addition, Erkki Ruhoslahti was able to identify peptide sequences that bind specifically to lymphatic vessels. The respective receptors of these peptides have been partially identified. It is evident that these

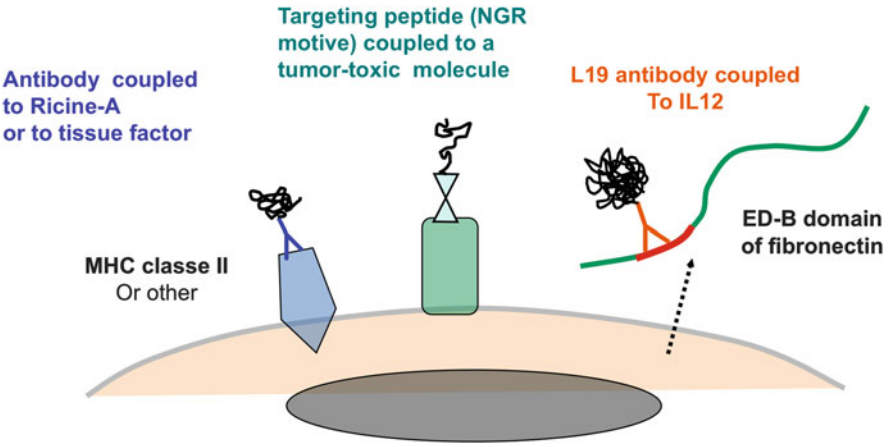

Fig. 12.3 Principles of vascular targeting. Figure by the author

peptides can be used for therapeutic and even diagnostic purposes by coupling them to an anti-tumor agent (for therapy) or to a tracer (for diagnosis).

Another approach is to use antibodies whose specificity for tumor or pathological (inflammatory) vascularization is proven. These antibodies can be obtained using the antibody phage display method. This was done by Dario Neri (ETH, Zurich) [264], who was able to identify antibodies specifically recognizing the EDB domain of fibronectin. This antibody has been coupled to various cytotoxic agents, TNF-α, or radioisotopes. The antibody is currently in clinical development and has shown promising results.