Tumor Angiogenesis

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KEY POINTS

- To grow over a certain size of a few millimeters in diameter, solid tumors need a blood supply from surrounding vessels.
- Small tumors can stay dormant for a very long time period until the so-called angiogenic switch occurs.
- Tumor-induced angiogenesis is mainly sustained by the production and secretion of angiogenic factors originating from tumor and stroma cells.
- The VEGF family of growth factors and the receptor tyrosine kinases play a key role in tumor angiogenesis and targeted therapy strategies.
- High VEGF expression promotes vascular permeability, leading to high interstitial and intratumoral pressure.
- The chaotic layout of tumor vasculature leads to inconsistent oxygen delivery within the tumor and creates regions of hypoxia.
- It is assumed that antiangiogenic drugs 'normalize' the tumor vasculature.
- Inhibiting tumor angiogenesis is a rational and potentially valuable therapeutic strategy.
- The available preclinical and clinical data strongly support the introduction of antiangiogenic drugs into combined modality treatment regimens that include radiation therapy.

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Abstract

Since the first description of angiogenesis and the discovery of its crucial role in tumor growth, extensive efforts have been made to develop antiangiogenic drugs. Some targeted therapies have been established as the first-line therapy in certain tumor types. However, the

pathophysiological principles are not fully understood, and little is known about the interaction of antiangiogenic drugs in combination with other classical antitumoral therapies like chemotherapy or radiation. A combination of all three strategies represents a very powerful tool to treat cancer aggressively, but also increases the risk of side effects. To understand the rationale of these combinational therapies, it is critically important to understand the angionesis and pathophysiology of antiangiogenic drugs on the one hand and the effects of radiation and chemotherapy on the other.

3.1 Introduction

A correlation between malignant tumors and surrounding blood vessels was first described at the annual meeting of Internal Medicine in 1908 by Elia Metschnikoff, a Russian clinician and noble prize winner. In 1971, the hypothesis that tumor growth was angiogenesis-dependent was raised by Judah Folkman (1971): To grow over a certain size of a few millimeters in diameter, solid tumors need a blood supply from surrounding vessels.

Solid tumors of up to 2–3 mm^3 can grow without a blood vessel supply. Nutrition and oxygen are provided via diffusion from the surrounding tissue. Above this size, diffusion becomes insufficient due to the negative surface/volume ratio. Based on a good balance between angiogenic and anti-angiogenic growth factors, a tumor of this size can stay dormant for a very long time period until the so-called angiogenic switch occurs. Based on several possible stimuli, a misbalance between angiogenic and anti-angiogenic factors in favor of pro-angiogenic factors leads to the proliferation of new blood vessels that originate from the existing vascular system. These blood vessels grow into the tumor and thus provide the necessary nutrients and growth factors for tumor progression. At the same time, the newly formed blood vessels allow tumor cells to disseminate and form metastases in distant organs (Fig. 3.1). Normally, vascular homeostasis is regulated by a balance of angiogenic and antiangiogenic mechanisms. Tumor-induced angiogenesis is mainly sustained by the production and secretion of angiogenic factors originating from tumor and stroma cells.

3.2 VEGF and Tumor Growth

The VEGF family of growth factors and the receptor tyrosine kinases play a key role in tumor angiogenesis and targeted therapy strategies. The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF), and they bind with different affinity and signaling response to VEGF receptors 1 (VEGFR-1), VEGF receptor 2 (VEGFR-2), and VEGF-receptor 3 (VEGFR-3) (Fig. 3.2).

VEGF promotes the growth of tumor vasculature to allow oxygen and nutrients to reach the rapidly dividing cancer cells. However, this tumor vasculature is abnor-

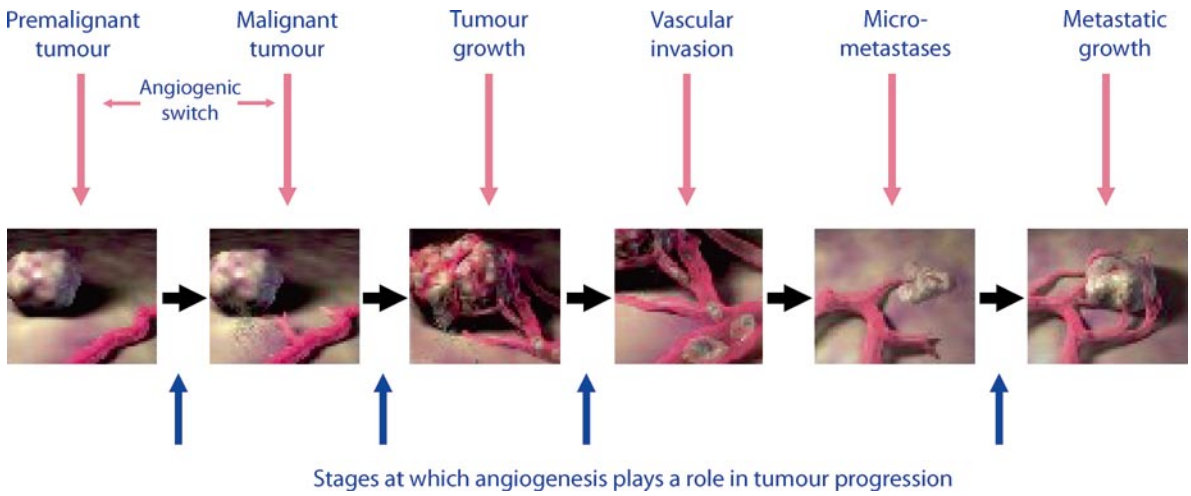

Fig. 3.1. Principles of tumor angiogenesis

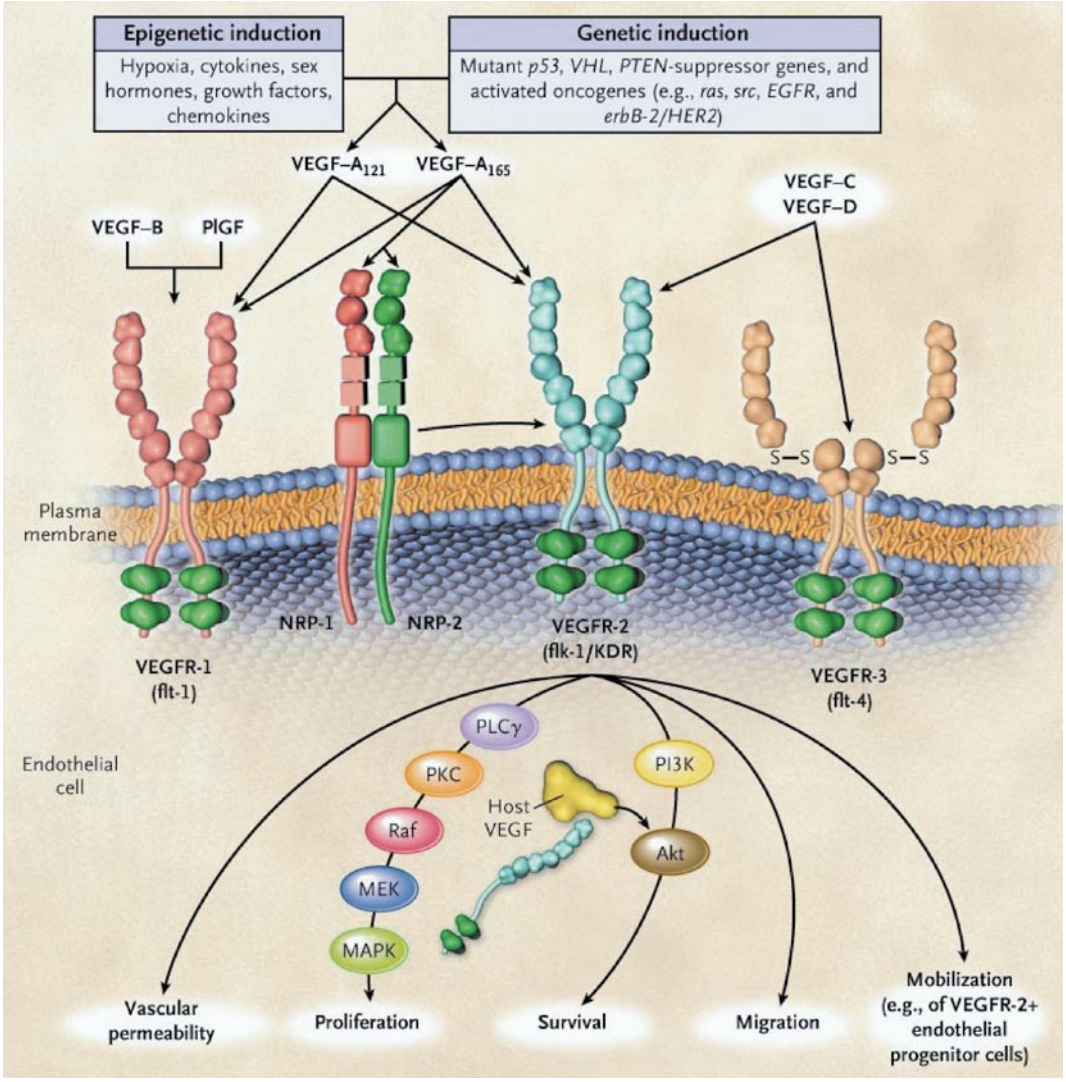

Fig. 3.2. The VEGF-receptor family. Binding and activation of VEGF-receptors and induction of intracellular signaling pathways. *$VEGF_{121}$* and *$VEGF_{165}$*: isoforms of VEGF; *VEGFR-2* VEGF receptor 2; *KDR* kinase-insert domain–containing receptor; *Flk-1* fetal liver kinase 1; *PLC* phospholipase C; *PKC* protein kinase; *MAPK* mitogen-activated protein kinase; *PI3K* phosphatidylinositol 3'–kinase; *EGFR* epidermal growth factor receptor; *flt-1* fms-like tyrosine kinase 1; *PlGF* placental growth factor; *PTEN* phosphatase and tensin homologue; *S–S* disulfide bond; *VHL* von Hippel–Lindau. Adapted from: Kerbel 2008 (N Engl J Med 2008;358:2039–49)

mal both in structure and function, with the vessels being immature, leaky, and tortuous, with a reduction or absence of supporting cells. The effect of VEGF on endothelial cells is important in the development of these abnormal vessels. High VEGF expression also promotes vascular permeability, leading to high interstitial and intratumoral pressure, which may allow tumor cells to enter the bloodstream and metastasizes, and which impairs the delivery of chemotherapy to the tumor (Jain 2001, 2003). The chaotic layout of tumor vasculature leads to inconsistent oxygen delivery within the tumor; this creates regions of hypoxia, which are resistant to radiotherapy (Brown 2002). Tumor blood vessels also have a reduction or absence of supporting pericyte and smooth muscle cells, which are essential to the functioning of the vasculature by stabilizing vessel walls and helping to regulate microcirculatory blood flow, as well as influencing endothelial permeability, proliferation, survival, migration, and maturation. An absence of pericytes sensitizes tumor vessels to VEGF inhibi-

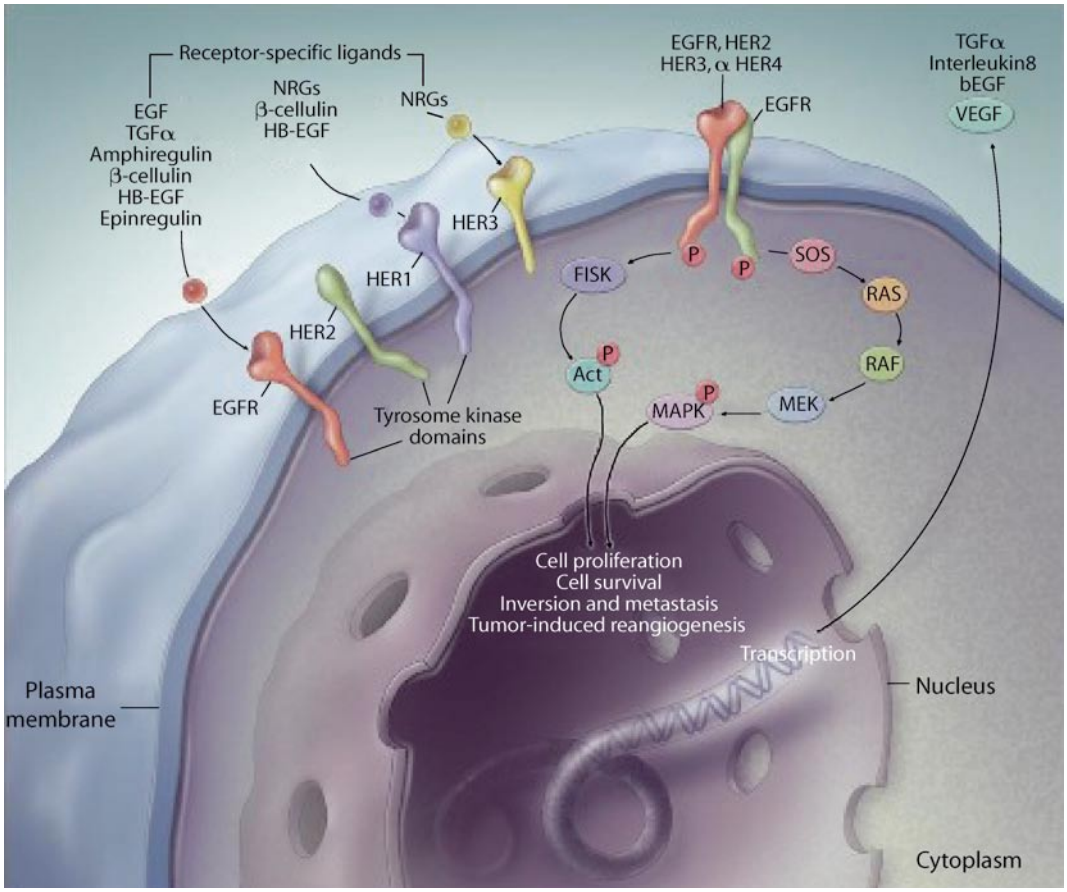

Fig. 3.3. The EGFR signal transduction pathway. After binding of a receptor-specific ligand to the extracellular portion of the EGFR or of one of the EGFR-related receptors (HER2, HER3, or HER4), the receptors build functionally active homodimers or heterodimers and cause the ATP-dependent phosphorylation of specific tyrosine residues in the EGFR intracellular domain. The two major intracellular pathways activated by EGFR are the RAS–RAF–MEK–MAPK pathway, which controls gene transcription, cell-cycle progression from the G1 phase to the S phase, and cell proliferation, and the PI3K–Akt pathway, which activates a cascade of anti-apoptotic and prosurvival signals. *bFGF* basic fibroblast growth factor, *HB-EGF* heparin-binding EGF, *MAPK* mitogen-activated protein kinase, *P phosphate* PI3K phosphatidylinositol 3,4,5-kinase, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor. Adapted from (Ciardiello and Tortora 2008)

tors, as shown in a number of mouse xenograft models (Abramsson et al. 2002; Morikawa et al. 2002; Baluk et al. 2005).

Further, endothelial cells and circulating bone-marrow-derived endothelial progenitor cells mainly express VEGFR-2 and, activated by VEGF-A, play a key role in tumor angiogenesis. In contrast, the role of VEGFR-1 remains uncertain in respect to VEGF-induced angiogenesis. In breast cancer cells, an intracellular intracrine mechanism of receptor and ligand interaction was postulated, giving VEGF and VEGFR-1 an autocrine function. VEGFR-1 is also associated with vascular development, and it may have a function in quiescent endothelium of mature vessels not related to cell growth. Some tumor cells produce VEGF, but due to a lack of VEGF receptors on their own surface, they do not respond to VEGF directly. Recent findings also suggest that the amount of VEGF produced by platelets and muscle cells are sufficient to induce tumor angiogenesis.

3.3 EGFR and Intracellular Signaling

Epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). In tumor cells overexpression of EGFR is associated with more aggressive disease, increased resistance to radiation therapy and chemotherapy, and finally with more aggressive spread of metastases and overall with a poor prognosis. After binding of receptor-specific ligands like epidermal growth factor (EGF), transforming growth factor α (TGFα), or further ligands, a functionally active dimer of EGFR with EGFR, HER2 HER4, or HER 3 occurs, and intracellular signaling cascades are initiated. EGFR mainly induces two pathways: the RAS–RAF–MAP–MAPK-pathway, which controls gene transcription and cell proliferation, and the PI3K-Akt-pathway, which activates a cascade of antiapoptotic and prosurvival signals (Fig. 3.3).

In patients with metastatic colorectal cancer, the success of anti-EGFR therapy with cetuximab depends on the nonmutated KRAS status. In patients with mutant KRAS, the intracellular signaling continues despite EGFR therapy. Mutated KRAS genes have been detected in about 40% of metastatic colorectal cancer patients. A retrospective analysis of tumor types revealed that patients with wild-type KRAS respond to cetuximab in combination with leucovorin, fluoruracil, and irinotecan (FOLFIRI) with an increase in progression-free survival from 25% without cetuximab to 43% (Van Cutsem et al. 2008). These results also describe a further step to individualized and customized treatment of cancer with targeted therapies.

Inhibiting tumor angiogenesis by targeting VEGF and also EGFR signaling is therefore a rational and potentially valuable therapeutic strategy. Approaches include the development of anti-VEGF-antibodies, anti-VEGF-receptor antibodies, antibodies to EGFR, small molecule inhibitors of receptor tyrosine kinases, and soluble VEGF-receptors.

3.4 Pathophysiology of Angiogenesis and Radiation

Blood vessels play a crucial role in the reaction to radiation exposure. Endothelial cells that line capillary blood vessels are situated very close to normal tissue cells, for example, such as epithelial cells in the gut mucosa. This close apposition enables endothelial cells and epithelial cells to communicate with each other by release of growth factors and hormones. Epithelial cells are also able to derive oxygen and nutrients from blood vessels. In contrast, tumor cells form multiple layers around a capillary blood vessel such that the most remote tumor cells are oxygen-deprived (hypoxic or anoxic) (Folkman and Camphausen 2001). The acute vascular reaction is mediated in a dose-dependent manner by the release of inflammatory substances, which in the literature are described as functional radiation effects. Already after the first or a few fractions of a conventional fractionated radiotherapy scheme, inflammatory cytokines such as interleukin-1 and TNF-alpha are expressed. Further the synthesis of prostaglandins and the activity of the nitric oxide (NO)-synthase are found to be increased in endothelial cells (Dörr and Trott 2000).

Sonveaux and colleagues (2003) specifically examined the effects of irradiation on endothelial cells to identify signaling cascades induced by ionizing radiation that could lead to alterations in endothelial cell phenotype and changes in angiogenesis. Earlier studies of several investigators had confirmed that treatment with growth factor antibodies or tyrosine kinase inhibitors can indeed increase the antitumoral effect of ionizing radiation and that such a combination could have super-additive effects, allowing a gain in efficacy by acting on two different targets, namely, tumor cells and endothelial cells (Gorski et al. 1999; Lee et al. 2000; Geng et al. 2001; Hess et al. 2001; Kozin et al. 2001; Camphausen and Menard 2002; Griffin et al. 2002; Huang et al. 2002).

Addressing the impact of irradiation on endothelial cells and the tumor vasculature, they demonstrated that the potentiation of the nitric oxide (NO) signaling pathway after irradiation induces profound alterations in the endothelial phenotype leading to tumor angiogenesis and that the inhibition of NO production suppresses these provascular effects of irradiation.

It has also been shown that NO modulates VEGF-induced angiogenesis and vascular permeability in vivo (Fukumura et al. 2001). There are three differently distributed and regulated isoforms of NO synthase (NOS): neuronal NOS (nNOS, also referred to as type I NOS), inducible NOS (iNOS, type II NOS), and endothelial NOS (eNOS, type III NOS). Endothelial NOS predominantly mediates this process, and iNOS appears to have a small, but additive effect. Thus, selective modulation of eNOS activity by targeting the VEGF pathway alters angiogenesis and vascular permeability in vivo. Other

physiologic vascular changes mediated by NO are blood flow and vessel diameter, respectively, vasorelaxation (Fukumura et al. 2001).

Endothelial cells react differently to radiation, depending on the inflammation. In inflammation endothelial cells are in a special physiologic condition, rapidly proliferating, actively synthesizing many pro-inflammatory and other peptides and proteins and responding differently to radiation than resting endothelial cells. The functional consequences of radiation exposure of these activated endothelial cells might be different from those induced in endothelial cells in healthy normal tissues studied (Trott and Kamprad 1999).

NO is also known as an important mediator in the status of inflammation in addition to its wide range of physiological and pathophysiological activities, including the regulation of vessel tone and angiogenesis in wound healing, inflammation, ischemic cardiovascular diseases, and malignant diseases. Depending on the dose and fractionation schedule, in the status of inflammation low-dose radiation attenuates the acitivity of iNOS and therefore mediates the acute inflammation in vivo. This appears to be one of the possible pathways explaining the well-known anti-inflammatory effect of low-dose radiotherapy (Review by Rischke et al. 2007).

Other effects of irradiation on endothelial cells are cytotoxic effects that participate in the antitumor treatment. As a chronic effect of radiation exposure to blood vessels, histopathologic investigation reveals a capillary rarefication, which means a markedly reduced density of capillaries in irradiated tissues, which can occur, depending on the tissue type, even after many years. The depletion of capillaries and mircrovessels is supposed to be the consequence of an impaired cellular function leading to destruction of capillaries. The exact mechanisms are still not known (Trott 2002).

An interesting approach postulates that angiogenic growth factors such as platelet-derived growth factor, insulin-like growth factor-1, and vascular endothelial growth factor lead to reduced long-term toxicity in the spinal cord in pre-clinical studies in a spinal-cord irradiation rat model (Andratschke et al. 2005).

3.5 Antiangiogenic Substances

Anti-VEGF-therapies can lead to regression of already existing tumor vascularization. VEGF is essential for tumor vessel cells to survive; it protects them from apoptosis and promotes tumor growth. Without a continuing supply of VEGF, endothelial cell apoptosis occurs, and newly developed tumor microvessels decay. VEGF inhibition also can lead to both structural and functional changes on surviving vessels, a phenomenon described as vessel normalization (Jain 2005).

3.5.1 Bevacizumab (Avastin™)

Bevacizumab (Avastin™) is a recombinant humanized monoclonal antibody directed against VEGF. Bevacizumab binds to VEGF and inhibits VEGF receptor binding. A precursor antibody to Bevacizumab was A4.6.1, a murine antibody cloned by Ferrara (Leung et al. 1989) and bound with high affinity to different isoforms of VEGF. It inhibited cell growth in immortalized tumor cell lines by a significant reduction of vascular density. As a murine protein, it provoked anaphylactic reactions and needed to be humanized.

In preclinical studies, the combination of Bevacizumab with chemotherapy led to synergistic activity. In xenotransplants, the combination of Bevacizumab with capecitabine inhibited tumor growth more effectively and longer than any other tested substance (Sachsenmaier 2001). It also showed synergistic effects in combination with paclitaxel and Trastuzumab (Herceptin™), a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor. In further in-vivo studies, the application of Bevacizumab to animals previously treated with capecitabine, topotecan, or cisplatin showed more successful tumor suppression. Also, repeated application of Bevacizumab proved to be safe and well tolerated.

3.5.2 Cetuximab (Erbitux ™)

Cetuximab (Erbitux ™), a monoclonal antibody, binds to the extracellular domain of EGFR, competing with its specific ligands and inhibiting intracellular signaling. Further, as an IgG1 immunoglobulin, it could elicit host antitumor immune responses such as cell-mediated antibody-dependent cytotoxicity and also EGFR internalization, down-regulation, and finally receptor degradation.

Among the EGFR targeting substances, Cetuximab has been approved for combination with radiotherapy for the treatment of locally advanced squamous-cell carcinoma of the head and neck (SCCHN) or as a single agent in patients who have had prior platinum-based therapy. Side effects of Cetuximab treatments include

acne-like skin affections, fever, and chills, asthenia, and nausea.

3.5.3 Small Molecule Tyrosine Kinase Inhibitors

Small molecule tyrosine kinase inhibitors (TKI), such as sorafenib (Nexavar™) and sunitinib (Sutent™), also represent antiangiogenic agents. Sorafenib is a potent orally available protein kinase inhibitor. Originally identified as a Raf kinase inhibitor, Sorafenib also inhibits VEGFR-1 and 2, platelet-derived-growth factor receptor (PDGFR-β), and c-Kit-Protein. Sorafenib has a dual antitumoral target affecting the tumor cell and its blood vessels. In human endothelial cells and in smooth muscle cells, VEGFR-2 signaling and activation of extracellular signal-regulated kinase (ERK) are induced.

Sunitinib (Sutent™) is also an orally available multitargeted TKI. Especially in renal cell carcinoma and in gastrointestinal stroma tumors (GIST), sunitinib proved to be superior to earlier therapy strategies and is now established as first-line treatment.

Sorafenib and sunitinib are both approved for the treatment of renal cell carcinoma. A clinical phase III trial studying sunitinib compared to sorafenib or placebo in treating patients with kidney cancer that has been removed by surgery (ClinTrails.gov NCT00326898) is currently recruiting patients.

3.5.4 Cediranib (Recentin™)

Cediranib (Recentin™), known as AZD2171, is an oral, highly potent, inhibitor of VEGF signaling that selectively inhibits all known VEGFR tyrosine kinase activity (VEGFR-1, -2 and -3; Fig. 3.4). Encouraging results obtained to date with Cediranib in a range of clinical studies show its potential as a new antiangiogenic drug in combination with radiotherapy.

The ability of Cediranib to inhibit growth factor-stimulated receptor phosphorylation was determined in a range of cell lines (Wedge et al. 2005). Furthermore, this effect was also associated with inhibition of MAP kinase phosphorylation, a downstream marker of VEGF signaling. These data suggest that Cediranib can selectively inhibit VEGFR-dependent proliferation, but appreciable functional selectivity is evident versus other targets, including EGFR, FGFR, and PDGFR-α.

The in vivo activity of Cediranib was also investigated in a model of vascular sprouting. In nude mice implanted with a VEGF-containing Matrigel plug, Cediraninb completely abolished VEGF-induced vessel formation (Wedge et al. 2005). Furthermore, Cediranib

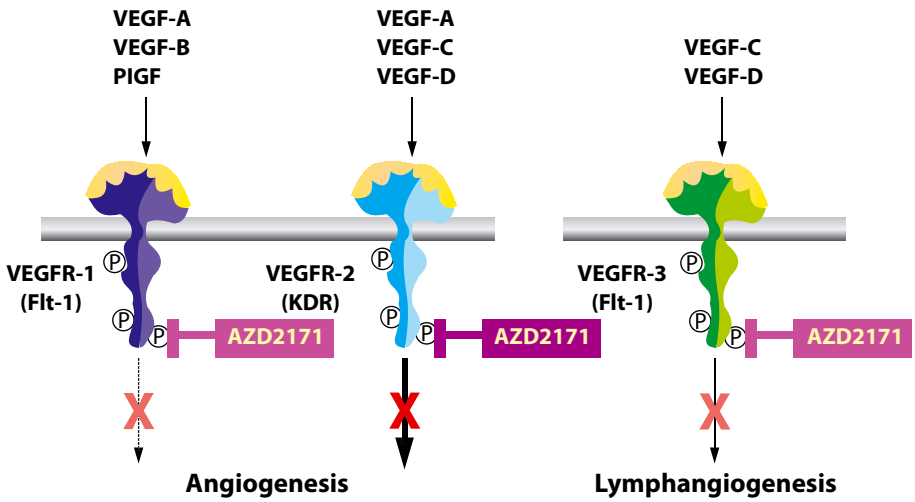

Fig. 3.4. Intracellular signaling inhibition by Cediranib

has demonstrated antitumor efficacy in a number of in vivo preclinical studies, including xenograft, orthotopic, metastatic, and spontaneous models of human cancer (Wedge et al. 2005).

Administration of Cediranib produced dose-dependent inhibition of tumor growth in a range of histologically distinct human tumor xenografts (lung, colon, breast, prostate, and ovarian) and also decreased primary tumor growth, metastasis, and microvessel density in an orthotopic model of murine renal cell carcinoma (Drevs et al. 2004).

Taken together, Cediranib has shown anti-tumor activity in a range of preclinical in vivo models consistent with inhibition of VEGF signaling and an antiangiogenic mode of action rather than a direct antiproliferative effect on tumor cells. In an extensive phase I program, Cediranib was tested as monotherapy in prostate cancer, with carboplatin and paclitaxel in non-small cell lung cancer (NSCLC), with selected chemotherapy regimens in advanced cancer, and with gefitinib in advanced cancer.

Cediranib is one of the most potent inhibitors of VEGFR-2 tyrosine kinase activity in development. Preclinical studies have demonstrated that Cediranib inhibits VEGF-dependent signaling, angiogenesis, and neovascular survival. Cediranib is also a potent inhibitor of VEGFR-1 and -3 tyrosine kinases, and shows selectivity for VEGFRs versus a range of other kinases. Consistent with an antiangiogenic effect, once-daily treatment with Cediranib produced dose-dependent inhibition of tumor growth in a broad range of established human tumor xenografts.

A series of phase I studies have been conducted to investigate Cediranib in patients with cancer, both as monotherapy and in combination with certain other anticancer strategies. These investigations have shown Cediranib to be generally well tolerated, with a side effect profile that is tolerable and manageable. Currently available pharmacokinetic data are supportive of a once-daily oral dosing schedule for Cediranib. Furthermore, preliminary efficacy data demonstrate that Cediranib has potential antitumor activity in multiple tumor types. Recruitment to a number of clinical trials has been initiated to further determine the activity of Cediranib in a wide range of tumors. Currently ongoing trials address the effect of Cediranib on metastatic colorectal cancer in combination with different chemotherapies. Encouraging preliminary results were reported for Cediranib in patients with glioblastoma suggesting an increase in overall survival (Batchelor 2008).

3.6 Chemotherapy and Antiangiogenic Therapy

Bevacizumab in combination with certain chemotherapy regimens has demonstrated clinically relevant improvements in survival or in progression-free survival in patients with colorectal, lung, and breast cancer.

Bevacizumab in combination with IFL (Irinotecan, 5-FU, and leukovorin) was studied as first-line therapy for patients with metastatic colorectal cancer. Eight hundred thirteen patients were randomly assigned, and 402 patients received IFL with bevacizumab. The addition of bevacizumab to fluorouracil-based combination chemotherapy results in statistically significant and clinically meaningful improvement in survival (Hurwitz et al. 2004).

Bevacizumab was also evaluated in patients with non-squamous NSCLC also chemotherapy naive. Four hundred thirty-four patients received bevacizumab in combination with carboplatin and paclitaxel versus 444 patients with carboplatin and paclitaxel alone. Overall survival was also significantly improved by addition of bevacizumab (Cohen et al. 2007).

A trial focusing on patients with HER2-negative and chemotherapy naive metastatic breast cancer comparing treatment with bevacizumab in combination with paclitaxel versus paclitaxel alone showed significant improvement of progression-free survival (median of 11.8 versus 5.9 months). In contrast, overall survival was not improved. As the reason for this discrepancy, the authors discuss possible rebound effects on subsequent treatments after ending the therapy or a correlation between bevacizumab resistance and resistance to other therapeutic attempts. Interestingly, progression-free survival and high response rates were seen early in metastatic disease, suggesting that development of metastases as a VEGF-dependent event is more vulnerable to bevacizumab treatment, and the question of bevacizumab effects in an adjuvant setting needs to be answered (Miller et al. 2007). Based on this trial the FDA granted an accelerated approval for bevacizumab in combination with paclitaxel.

However, the observation that antiangiogenic drugs combined with chemotherapeutic agents improve antitumoral effects is surprising, because one would expect the intratumoral delivery of drugs to be suppressed. Different models have been discussed to explain the chemosensitizing activity of antiangiogenic drugs. It is assumed that antiangiogenic drugs 'normalize' the tumor vasculature, enhancing the efficacy of chemotherapeutic drugs. Tumor vessels in general are structurally

abnormal, showing absence of hierarchically structured patterns such as reduced basement membranes and dilated vessels, making them leaky and resulting in altered perfusion or blood flow. Even highly vascularized tumors can be hypoxic, which again is known as being an angiogenesis-inducing factor. Antiangiogenic therapy can reverse these alterations, a phenomenon known as vessel normalization, and thus enhance antitumor effects of chemotherapeutic agents. After vessel normalization, a synergistic effect of bevacizumab with chemotherapy is assumed regarding the tumor cell recovery and repopulation. The rate of tumor cell repopulation after MTD of conventional chemotherapy or radiation does not necessarily decline in proportion to the number of treatment cycles. In fact, the observed trend suggests the opposite effect. Consequently, exposing the tumor to an antiangiogenic drug during the break periods between courses of chemotherapy is sought to reduce oxygenization and delivery of nutrients to repopulating cells. Taking those hypotheses into consideration, the timing of combinational therapies needs to be optimized. This also supports the suggestion to apply bevacizumab between chemotherapy cycles when tumor cell repopulation after cytotoxic chemotherapy is increased and repopulating cells demand for oxygen is high.

Further, for cytotoxic chemotherapy itself, antiangiogenic effects enhancing antitumoral activity have been described. It has been hypothesized that these drugs could damage endothelial cells that proliferate during the formation of new blood vessels, and also destruction of circulating bone marrow cells leads to impaired tumor angiogenesis. These endothelial cells include circulating endothelial progenitor cells (EPCs) that can incorporate into the lumen of nascent vessels and differentiate into mature endothelial cells (Asahara et al. 1997; Shaked et al. 2005). Given the well-established myelosuppressive effects of cytotoxic chemotherapy, one might predict that at least some of these proangiogenic bone marrow cell types would be sensitive to chemotherapy. Many of these cell populations can be mobilized into the peripheral blood by growth factors such as VEGF; thus, the combination of a VEGF-targeting agent with chemotherapy would be expected to have an additive, if not synergistic, suppressive effect on these cells.

Because of its low toxicity, metronomic chemotherapy, continuously administered low doses of chemotherapeutic drugs below toxicity levels, may be well suited for long-term combination with antiangiogenic drugs; such combinations have had marked antitumor effects in preclinical models (Klement et al. 2000; Kerbel and Kamen 2004; Pietras and Hanahan 2005). Both antibody-based and small-molecule antiangiogenic drugs enhance the effects of metronomic chemotherapy in preclinical models. Phase II trials of metronomic chemotherapy (Colleoni et al. 2002; Kieran et al. 2005), sometimes used in combination with antiangiogenic drugs, have yielded encouraging results in patients with advanced cancer (Canady 2005), but larger randomized trials are needed to validate the concept. There is also a need for surrogate markers to help determine the optimal biologic dose of this therapy. Circulating EPCs have been used successfully as a marker in preclinical studies (Shaked et al. 2005), but are not yet validated clinically.

3.7 Radiation and Antiangiogenic Therapy

Because of the encouraging results of antiangiogenic therapy combined with chemotherapy, consequently combinational therapies including antiangiogenesis and radiation with or without chemotherapy are becoming the focus of clinical interest. Besides developing new therapeutic strategies to improve curative cancer treatment, also the safety of combinational therapies needs to be addressed since tumor patients receiving antiangiogenic drugs might also receive radiation therapy for palliation.

The rationale for combining radiation with antiangiogenic drugs is based on several pathophysiological considerations. Tumor response to radiation therapy is caused by DNA damage to tumor cells and also depends on intracellular pathways controlling apoptosis, autophagy, and cell death induced by radiation. Oxygene is a potent radiosensitizer, and its interaction with radicals formed by radiation induces DNA damage. Hypoxia leads to radiation resistance. Radiation induces the secretion of cytokines that inhibit apoptosis in endothelial cells. Hypoxia inducible factor (HIF)-1α is activated when cells are hypoxic, it dimerizes with HIF-1β, and this leads to an increase in VEGF transcription. This is associated with a lower radiation response and tumor progression, mainly experienced in head and neck tumors, uterine cervix tumors, and sarcomas. Radiation itself also induces hypoxia and thus increases VEGF production and VEGFR expression. Hypoxia can be measured directly by determination of oxygen pressure or more recently by PET using misonidazol or 2nitroimidazole as tracers (Koch and Evans 2003; Rischin et al. 2006; Thorwarth et al. 2007). Again, vessel normalization enhances oxygenation and thus radio-

sensitivity in tumor cells. In preclinical studies antiangiogenic therapy has been shown to enhance radiation-induced cell death (Lee et al. 2000; Hess et al. 2001).

Factors known to control angiogenesis, such as fibroblast growth factor 2 (FGF2), EGF, VEGF, the alpha-vβ3 and alpha-vβ5 integrins, and some GTPase proteins, have been clearly demonstrated to be involved in controlling intrinsic radiation resistance.

The induction of this radiation resistance is also mediated by a small G protein, RhoB, known to be activated by various stresses, such as UV, but also ionizing radiation or hypoxia as well as by growth factors, such as EGF or FGF2 (Moyal 2008). Normalization of tumor vasculature by anti-VEGFR-2 antibody has also led to enhanced radiation-induced tumor response (Winkler et al. 2004). This study demonstrated the necessity to optimize timing of radiation and chemotherapy and supports the advantage of this therapy during the normalization phase.

There are also several trials ongoing, studying antiangiogenic drugs in combination with radiotherapy in patients with rectal cancer, pancreatic cancer, head and neck tumors, and brain tumors, e.g., with bevacizumab, imatinib (Gleevec™), and sunitinib.

The use of cetuximab in combination with radiotherapy is approved by the FDA for squamous cell carcinoma of the head and neck (SCCHN). Four hundred twenty-four patients with untreated SCCHN entered a phase III trial (Bonner et al. 2006) and were randomly assigned to radiotherapy alone or in combination with cetuximab. Overall survival and progression-free survival were significantly increased in the experimental arm (24.4 vs. 14.9 months). Interestingly, this trial also showed that radiation side effects were not increased. It has been noted critically that this trial did not compare chemoradiation as standard therapy with radiotherapy and cetuximab. Cetuximab has been studied in combination with chemotherapy, including 5-FU, paclitaxel, and cisplatin (Burtness et al. 2005; Bourhis et al. 2006) in patients with SCCHN. In a small number of patients, cetuximab has reverted cisplatin resistance, and it was suggested that cetuximab is the only second-line treatment with significant response rates available.

The widespread use of bevacizumab in multimodal attempts to treat different tumor entities logically demands an extension to address the advantages of bevacizumab in combination with radiation with or without chemotherapy. To date, no phase III studies have been completed, so that the extent of the benefit that bevacizumab seems to have remains to be determined. In patients with rectal carcinoma, Willet and colleagues (2007) demonstrated in a continuation of a dose-escalation phase I trial in addition to the dose limiting toxicity of bevacizumab that the combination of bevacizumab with chemoradiation may have high response rates. In this study, two consecutive cohorts of three patients with locally advanced rectal carcinoma were treated with bevacizumab (10 mg/kg), and concurrent administration of bevacizumab with 5-FU chemotherapy and pelvic radiation therapy. Surgery was scheduled 7 to 9 weeks after completion of therapy. Functional, cellular, and molecular studies were performed before and after initial bevacizumab monotherapy. Following the National Cancer Institute trial guidelines, they terminated the dose-escalation component of their study when two consecutive patients developed dose-limiting toxicities (DLT) of diarrhea and colitis during the combined treatment. Following recovery from toxicity, these patients were able to resume and complete radiation therapy and 5-FU. Because of these DLT, only five patients were enrolled at the 10 mg/kg dose. All the patients underwent surgery. Of considerable interest in respect of combining antiangiogenic substances with radiation or chemoradiation has been the fact that the patients receiving 10 mg/kg bevacizumab showed two complete pathologic responses, as compared to no complete pathologic response in the 5 mg/kg bevacizumab group (Willett et al. 2004, 2007). These tumor responses were also detected on computed tomography (CT) and positron emission tomography (PET) scans after completion of chemoradiation therapy, stressing that PET/CT-scans may be a valuable tool acting as an appropriate surrogate marker, while it is a non-invasive, sensitive, semi-quantitative, and reproducible method.

The few phase I publications studying toxicity of antiangiogenics in association with radiotherapy mainly investigated the acute effects. One study combined radiotherapy and 15 mg/kg bevacizumab with oxaliplatin and capecitabine in escalating doses in patients with rectal adenocarcinoma and showed that the antiangiogenics increased the toxicity of the combination of capecitabine, oxaliplatin, and radiotherapy, the DLT being grade 4 diarrhea (Czito et al. 2007). The combination of bevacizumab with radiotherapy and capecitabine in patients with pancreatic carcinoma showed grade 3 ulcerations with bleeding or perforation in four patients. These events occurred up to 20 weeks after the end of the combination of radiation with chemotherapy, particularly in patients whose tumor invaded the duodenum (Crane et al. 2006).

The available recent preclinical and clinical data strongly support the introduction of antiangiogenics into combined modality treatment schemes that include radiotherapy. The ultimate benefit of these therapeutic combinations needs to be determined with longer follow-up of the effect of these antiangiogenic agents

and by studying surrogate markers by metabolic and functional imaging (perfusion MRI, PET/CT–FDG, PET/CT-misonidazole) in early clinical studies, notably concerning the effect on tumor oxygenation and vascularization, in order to choose the optimal sequence and administration time of these drugs compared to radiotherapy. Elucidating the mechanisms by which radiosensitization is obtained and the molecular interplay between radiation toxicity to normal organs and antiangiogenics is also important to facilitate the design and testing of clinical strategies aimed at minimizing toxicity.

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