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REVIEW

Oncogenes and angiogenesis: a way to personalize anti-angiogenic therapy?

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Abstract The acquisition of oncogenic mutations and promotion of angiogenesis are key hallmarks of cancer. These features are often thought of as separate events in tumor progression and the two fields of research have frequently been considered as independent. However, as we highlight in this review, activated oncogenes and deregulated angiogenesis are tightly associated, as mutations in cancer cells can lead to perturbation of the pro- and anti-angiogenic balance thereby causing aberrant angiogenesis. We propose that normalization of the vascular network by targeting oncogenes in the tumor cells might lead to more efficient and sustained therapeutic effects compared to therapies targeting tumor vessels. We discuss how pharmacological inhibition of oncogenes in tumor cells restores a functional vasculature by bystander anti-angiogenic effect. As genetic alterations are tumor-specific, targeted therapy, which potentially blocks the angiogenic program activated by individual oncogenes may lead to personalized anti-angiogenic therapy.

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Anti-angiogenic therapy: where are we?

Angiogenesis is the biological process that drives the formation of new blood vessels from a pre-existing vasculature. Throughout embryonic development, physiological angiogenesis allows for expansion of the primitive vascular network formed by vasculogenesis, thanks to branching, remodeling, and maturation of the vascular bed [1]. During adulthood, angiogenesis normally occurs in only a few processes, such as in the female reproductive apparatus, and in pathological situations including wound healing, diabetic retinopathy, rheumatoid arthritis, and cancer [2].

More than 50 years ago, angiogenesis was described as a hallmark of tumor biology, and for the first time anti-angiogenic therapy was proposed as a cancer cure. Folkman and colleagues were pioneers in demonstrating that growing tumors need neovascularization when reaching a critical volume (around 1–2 mm^3) in order to continue their expansion [3, 4]. The induction of tumor vasculature, also known as "angiogenic switch", represents a complex and time-regulated process in cancer progression during which both the cancer cells and the tumor microenvironment secrete signals that recruit and expand the vascular network [5].

Tumor angiogenesis is central for tumor progression since blood vessels provide essential nutrients as well as oxygen to the proliferating malignant cells. Beyond the importance of angiogenesis for primary tumor expansion, blood vessels are an important route for cancer cell dissemination to distant organs. Indeed, the vascular system provides the motorway through which cancer cells disseminate, a process facilitated by the fact that the integrity of

the endothelial layer is significantly impaired in the tumor vasculature [6, 7]. Tumor blood vessels were initially spotted as attractive targets in cancer at two levels: to inhibit tumor growth by cutting nutrient delivery to proliferating tumors, and to prevent metastases formation by blocking the main route for cancer cell dissemination. As angiogenesis is a peculiar feature of a growing tumor mass, its blockade was considered highly tumor selective, with limited potential for side effects. Moreover, most solid cancers are dependent on angiogenesis for their expansion and for this reason anti-angiogenic therapy was envisioned as broadly applicable in clinical oncology.

Among the angiogenic factors, vascular endothelial growth factor, or vascular permeability factor (VEGF/VPF) has historically been considered the main target for the development of anti-angiogenic drugs [8–10]. Indeed, the most widely used anti-angiogenic inhibitor, bevacizumab, is a humanized monoclonal antibody that binds and selectively neutralizes the biological function of VEGF [11]. Over the years, other kinase inhibitors, such as sorafenib and sunitinib, have been developed to target the VEGFR pathway and interfere with VEGF-driven angiogenesis [12–14]. Multiple preclinical studies showed that anti-VEGF therapy that blocks tumor angiogenesis could delay tumor growth in animal models [15, 16].

In 2004, bevacizumab became the first anti-angiogenic FDA-approved drug for the treatment of metastatic colon cancer. This decision followed a phase III clinical trial where patients with metastatic colorectal cancer had an improvement in progression-free survival from 6.2 to 10.6 months when treated with bevacizumab in combination with chemotherapy, compared to chemotherapy alone [17]. Afterwards, bevacizumab therapy was extended to other malignancies such as non-small cell lung cancer, HER2-negative breast cancer, renal cell cancer, and glioblastoma [18–21]. In parallel, small-molecule tyrosine kinase inhibitors blocking the VEGFR pathway, like sunitinib, sorafenib, and pazopanib showed efficacy in the treatment of renal cell cancer and hepatocellular carcinoma [22–25].

While the initial results were regarded as highly promising, clinical evidence indicated that anti-VEGF therapy also had limitations. Bevacizumab was rarely successful as a single agent and the clinical benefits, reached only in combination with chemotherapy, were shown to be only transitory. Notably, multiple clinical studies quickly established that even within the same tumor subtype (i.e., colorectal and lung cancer) not all patients display the same rate of response to anti-angiogenic drugs and importantly, demonstrated that anti-angiogenic therapy can be overcome [17, 21, 26].

Several reasons can contribute to primary or secondary resistance to anti-VEGF clinical approaches [27]. An important consideration is that, in contrast to predictions, not all tumors are addicted to angiogenesis for their expansion and treatments that merely target blood vessels cannot provide benefit in these types of cancer [28, 29]. A relevant observation was revealed by the transcriptional analyses of angiogenic factors in primary breast tumors compared to adjacent normal tissue. In this study, the majority of pro-angiogenic molecules appeared to be down-regulated in the tumor tissue and thus leading the authors to suggest that breast cancer primary tumor is not a site of active angiogenesis [28]. We now know that tumors can use alternative ways to become vascularized and in this context a therapy directed to sprouting angiogenesis would exert a limited effect. Tumor vascularization can occur by multiple mechanisms including: co-option of pre-existing vessels, tumor cells can surround pre-existing tissues vasculature, vascular mimicry, when dedifferentiated tumor cells contribute to the formation of blood vessels, and postnatal vasculogenesis through the recruitment of bone marrow-derived endothelial precursors [30–34]. Of note, the different processes are often mixed within the tumor mass and can provide an alternative route of vascularization exploited by tumors to escape anti-angiogenic treatment [35, 36]. A second mechanism that can lead to refractoriness to anti-VEGF therapy is that tumors are biologically diverse and angiogenesis can be stimulated by alternative molecules. A clear example came from the analyses of biopsies from different grades of primary breast cancer. As previously shown, VEGF expression was correlated with poor prognosis, but its expression was higher in the early stage of the disease, while in high-grade breast cancer a wide range of other angiogenic factors, like FGF2, were more prominent [37]. This suggests not only that different tumors use diverse stimuli but also that within the same malignancy the tumor stage influences angiogenic pathway activation and thereby the response to therapy.

The most common mechanism of resistance to anti-angiogenic drugs appears to be tumor adaptation to the loss of vessels. The process involves the onset of compensatory stimuli that drive neovascularization, thereby evading anti-angiogenic approach [27]. Several angiogenic molecules secreted in the tumor microenvironment are thought to be involved in the angiogenesis rebound, such as FGF2, ephrins, angiopoietins, PDGF, SDF1, and G-CSF. These factors can directly stimulate angiogenesis or act by recruiting inflammatory cells, as it has been described for SDF1 and G-CSF, like tumor-associated macrophages (TAM) and bone marrow-derived cells (BMBC) that in turn provide angiogenic stimuli [27, 38–40]. In the RIP-Tag pancreatic mouse tumor model, treatment with a VEGFR-blocking antibody causes an initial response followed by tumor regrowth and rebound angiogenesis. A broad analysis revealed that several hypoxia-mediated genes are up-regulated in the environment

and, among them, FGF2 was shown to have an essential role in driving tumor revascularization [41]. A similar phenomenon has been observed in the clinic; glioblastoma patients treated with VEGFR2 small-molecule inhibitors display elevated FGF2 and SDF1 levels at the time when tumors became refractory to treatment [42].

Several reports have pointed to the tumor stroma as an important mediator of secondary resistance to anti-angiogenic therapy. For instance, tumor-associated fibroblasts release growth factors like PDGFC, Angptl2, and SDF1 that are involved in resistance to anti-VEGF treatment [43, 44]. BMDCs are recruited to the tumor microenvironment and promote cancer growth and angiogenesis rebound by providing alternative growth factors like Bv8 (or prokineticin-2) and HGF in anti-VEGF therapy refractory tumors [45–47].

Recent observations obtained in mouse model systems indicate that anti-angiogenic drugs might have concurrent deleterious side effects due to the generation of hypoxic stress. In preclinical models, preconditioning of the so-called metastatic niche or short-term treatment with anti-VEGF targeted therapy have been shown to enhance tumor invasiveness and metastatic burden in response to hypoxic stress [48–50]. These unexpected outcomes suggest that the generation of hypoxia resulting from blood vessels pruning may increase tumor aggressiveness, raising concerns about the clinical application of classic anti-angiogenic therapy. Importantly, the clinical relevance of these findings is still under investigation as evidence that patients treated with bevacizumab have a shorter time of progression-free survival are lacking [51]. However, glioblastoma tumors that relapse after bevacizumab treatment can have a more infiltrative phenotype [52, 53]. Similar observations have been made in renal cell cancer patients, where, after interruption of VEGFR tyrosine kinase inhibitor treatment, tumor growth rebounded with a concomitant increase in metastases [54].

The negative impact of the hypoxic stress can be extended to other aspects of tumor maintenance. The generation of a hypoxic niche is pivotal to support cancer stem cell population and to increase the expression of stem cell markers, at least in glioblastoma [55, 56]. Anti-angiogenic therapy is associated with this phenomenon since, as demonstrated in breast cancer preclinical models, the onset of hypoxia as consequence of anti-VEGF therapy increases the population of cancer stem cell within the tumor [57]. This is a new challenging aspect to be considered as cancer stem cells have been proposed as key actors in resistance to therapy and tumor recurrence [58–60].

Emerging concepts of tumor angiogenesis

In recent years, the process of tumor angiogenesis has been further detailed. We have learned, for example, that tumor neovascularization does not merely reflect an increase in capillary number but also a general modification in the physiology of the vasculature [6, 61]. Secretion of pro-angiogenic signals by both tumor cells and the microenvironment causes the formation of an abnormal vasculature, with chaotic and tortuous blood vessels and aberrant function, due to vasodilatation and increased vascular permeability [6, 62]. An aberrant vasculature results in hypoxia and necrosis that negatively impacts tumor progression, and vessel leakiness causes suboptimal blood flow and tumor perfusion. Impaired tumor perfusion affects the response to standard therapies due to reduced cytotoxic drug delivery within the tumor mass and defective production of oxygen radical species required for successful radiotherapy [63].

This additional knowledge must now be incorporated in the definition of new anti-angiogenic therapies. The latter should be implemented also considering the goal of reverting abnormal vasculature and to restore normal blood flow. In principle, this strategy should improve tumor perfusion while decreasing interstitial pressure and hypoxia-driven tumor aggressiveness [63, 64].

As mentioned above, another important consideration is that the tumor mass is a complex environment composed not only of cancerous cells but also of many other cell types such as fibroblasts, endothelial, and BMDCs. Considering the continuous and dynamic cross-talk among these cell lineages, current treatments are unlikely selective for only one cell type. Indeed, targeting blood vessels affects tumor cell proliferation and migration, and similarly targeting cancer cells deeply impinges on the tumor environment. This phenomenon, which has been referred to as “accidental anti-angiogenic therapy”, can be a consequence of chemotherapy or small molecules inhibitors [65]. In the case of small-molecule inhibitors, possible off-target effects are an important consideration. For example, it has been reported that targeting oncogenic BRAF with the tyrosine kinase inhibitor sorafenib not only inhibits cell proliferation in mutated tumor cells but directly affects angiogenesis because it blocks VEGFR2 and PDGFR activity [12]. A different mechanism acts in bystander anti-angiogenic therapy where targeting oncogenic events in the epithelial cell compartment indirectly impinges on the tumor environment [66].

Oncogenes in tumor angiogenesis

Genetic modifications occurring in the genome initiate the transformation process that leads to cancer. Two types of genetic aberration can drive tumorigenesis: mutation/amplification of oncogenes, and inactivation/deletion of oncosuppressor genes. The discovery that cancer cells rely on specific genetic alterations for their survival has driven

the development of targeted therapy with the aim of inhibiting the proliferation of tumor cells [67–70]. Activation of oncogenic pathways triggers profound modifications in the expression profile of tumor cells. Among others, the expression of several cytokines and growth factors is directly affected by the activity of individual oncogenes, thereby influencing the tumor environment [66, 71, 72].

Angiogenesis, just like most biological processes, relies on an appropriate balance of factors to maintain an optimal physiological condition. In cancer, the delicate equilibrium between pro- and anti-angiogenic factors is lost, with the abundance of pro-angiogenic cytokines being the main driver of tumor angiogenesis. A therapeutic approach that depletes or inactivates an angiogenic pathway, such as anti-VEGF therapy, causes vessel pruning and an inadequate vasculature with necrosis and hypoxic stress that negatively effects the tumor environment [63]. We suggest that an alternative strategy to target tumor angiogenesis could be to rescue the equilibrium of angiogenic signals by targeting the mutated oncogenes, which play a central role in this process.

Several examples of oncogene-driven angiogenesis have been described [65, 66]. Indeed, activation of MAPK and PI3K-AKT pathways, which are usually deregulated in cancer, enhances the expression of pro-angiogenic factors by acting at both the transcriptional and translational levels [73–75]. These findings may explain why targeted therapy, which usually has a cytostatic effect on tumor cells, also affects the tumor environment and normalize tumor vasculature (Table 1).

We recently described how targeting oncogenic BRAF, a serine threonine kinase, affects angiogenesis [71]. BRAF is frequently mutated in human cancer and the BRAFV600E mutation can influence the tumor environment by increasing expression of HIF1α, VEGFA, IL1β, and IL8, and by lowering levels of the angiogenic blocker thrombospondin 1 [71, 76–80]. We found that the most common BRAF variant (the BRAFV600E mutation) modulates the production of angiogenic molecules by cancer cells. Furthermore, we evaluated the effect of the specific BRAFV600E inhibitor PLX4720 on tumor angiogenesis and demonstrated that targeting BRAF stabilizes the tumor vasculature and abrogates hypoxia in tumor xenografts. Intriguingly, we found that PLX4720 acts by specifically switching-off the MAPK pathway in BRAF-mutated cells, thereby decreasing the expression of angiogenic molecules. These data led us to suggest that pharmacological inhibition of oncogenes in tumor cells can restore a functional vasculature and potentially blocks the specific angiogenic program activated by individual tumors. This mechanism of action provides a clear example of bystander anti-angiogenic therapy.

Similarly to BRAF, the RAS oncogene is a master regulator of the MAPK pathway that has been directly linked to induction of tumor angiogenesis [81]. Activated RAS increases the expression of VEGF and other angiogenic chemokines like CXCL1, CXCL5, and IL8 while suppressing expression of the angiogenesis inhibitor thrombospondin 1 [82–86]. A concomitant mechanism by which oncogenic RAS stimulates the angiogenic program is by up-regulating proteases important for matrix remodeling, such as uPA, MMP2, and MMP9 [87, 88]. The role of KRAS in driving angiogenesis is supported by clinical data in non-small cell lung cancer and in pancreatic tumor showing that KRAS activating mutations correlate with high VEGF expression [89, 90]. Inhibition of RAS activity by gene silencing suppresses VEGF expression. Moreover, decreased VEGF expression in KRAS-mutated colon cancer cells reduces the tumorigenic potential in vivo, highlighting the importance of VEGF expression in KRAS-driven tumors [91].

The PI3K-AKT-mTOR axis, which is often deregulated in human cancer, is another important pathway that controls the angiogenic program in tumor cells. The activation of this signaling pathway up-regulates the expression of HIF1α and VEGF and consequently promotes tumor angiogenesis [92–94]. Small-molecule inhibitors blocking different signaling nodes of this pathway have shown important effects on vascular normalization with consequent improvement in vascular blood flow and tumor oxygenation [95, 96].

Myc and p53 also act as master regulators of angiogenic factors. C-Myc triggers the expression of VEGF while it down-regulates thrombospondin 1 [97, 98]. p53 is an oncosuppressor gene involved in the down-regulation of pro-angiogenic factors like VEGF and FGF as well as in the up-regulation of thrombospondin 1 [99–101]. For example the expression of p53 is required to reverse the angiogenic program in hematopoietic malignancy. In this type of tumor, Myc inactivation is sufficient to induce tumor regression, but its effect is less prominent when p53 is lost. Expression of p53 reverses tumor angiogenesis by controlling the up-regulation of thrombospondin 1 and allows a sustained

Table 1 Summary of the main oncogene-targeted therapies that are reported to normalize tumor vasculature

Oncogenes targeted therapies with anti-angiogenic properties	Oncogene	References
Vemurafenib-PLX4720	BRAF	[71]
NVP-BEZ235	PI3K-mTOR	[96]
PI-103	PI3K	[95]
Nelfinavir	AKT	[95]
Erlotinib	EGFR	[104]
Iressa	EGFR	[104, 105]
Herceptin	HER2	[107]

tumor regression after Myc inactivation [102]. A recent study shows that p53 can also repress the thrombospondin 1 promoter in prostate cancer cell lines, suggesting a context-dependent regulation of thrombospondin 1 by p53 [103].

The inhibition of EGFR or HER2, tyrosine kinase receptors that activate MAPK and PI3K, is another example of vessel normalization by bystander oncogene targeting. EGFR can be amplified and mutated in several tumors including lung, colon, and glioblastoma. Pharmacological inhibition of EGFR decreases the expression of HIF1α and VEGF by tumor cells and treatment of tumor xenografts with Erlotinib or Iressa, two different tyrosine kinase inhibitors, also lead to vessel normalization [95, 104, 105]. As mentioned above, this effect on the tumor microenvironment can improve the success of both cytotoxic chemotherapy and radiotherapy. A preclinical study has shown that pretreatment of tumor xenografts with Erlotinib increases the delivery of chemotherapeutic agents within the tumor and results in higher inhibition of tumor growth compared to the single treatment [106]. Moreover, pretreatment with Erlotinib enhances the effect of radiation therapy in vivo but not in vitro, demonstrating that EGFR targeting may positively affect the tumor microenvironment [106]. HER2 is an important oncogene overexpressed in aggressive breast cancer. It has been found that targeting HER2-positive tumors with Herceptin strongly influences vascular structure and function and cause vessel normalization. Herceptin treatment slows down the secretion of VEGF, PAI-1, TGF-α, and Angiopoietin1, all important mediators of angiogenesis and in parallel up-regulates the expression of the anti-angiogenic factor thrombospondin 1 [107].

In conclusion, multiple evidences show that oncogene-targeted drugs might also impact tumor angiogenesis, suggesting an innovative strategy to revert aberrant vasculature and positively impact tumor environment. The limitation of this approach relies in the ability of tumor cells to develop secondary resistance towards targeted therapy. It is known that a tumor can overcome the dependency on a specific oncogene through various mechanisms: by involving compensatory genes through the activation of alternative molecular pathways or by the acquisition of new genetic alterations due to the intrinsic genomic instability of cancer cells [108]. The finding that targeting oncogene addiction in tumor cells results in abrogation of pro-angiogenic signals suggests that once acquired resistance is established reactivation of oncogenic pathways may trigger an angiogenic rebound. Therefore, any anticancer therapy (be it directed to the tumor cells or to the surrounding stroma) will always be limited by secondary resistance. Overcoming the latter is key in providing long-lasting clinical benefits.

Conclusions and perspectives

Both tumor angiogenesis and oncogenic addiction are considered hallmarks of cancer [62, 109]. In this review, we highlight the connection between these two events and discuss the hypothesis that targeting oncogenes can positively affect the tumor environment. We summarized examples of therapies aimed at blocking oncogenes that concomitantly were shown to have a clear effect on vascular normalization and tumor perfusion. At the same we note that targeting oncogenes can improve blood vessel structure and tumor oxygenation without having any obvious effect on tumor cell proliferation [95]. This suggests that oncogenic pathways can be involved in the activation and maintenance of the angiogenic program even in cancer cells that are not addicted to the targeted oncogenic mutations for proliferation.

By comparing the anti-angiogenic effect of oncogene-targeted therapy with an anti-VEGF approach important considerations can be made (see Fig. 1). An anti-VEGF approach causes blood vessel pruning and hypoxic stress that can result in an adaptive response with associated rebound in neoangiogenesis and in some case enhanced tumor aggressiveness. This is a feasible explanation for the short clinical benefit observed in patients treated with anti-angiogenic drugs [110, 111]. In contrast, oncogene-targeted therapy causes blood vessel normalization by restoring the equilibrium of angiogenic molecules and it is predicted that this effect is more sustainable and should allow a prolonged response [64].

A second relevant consideration is that anti-VEGF therapy is directed towards a single angiogenic stimulus and acts by blocking the VEGFR signaling pathway. The first limitation of this strategy is that to obtain a functional vasculature, the maintenance of a correct amount of angiogenic cytokines and not a complete depletion is important. Moreover, tumors develop resistance to anti-VEGF treatment and drive revascularization by alternative angiogenic programs. Despite the fact that the main read-out described for oncogene-driven angiogenesis is VEGF, other angiogenic stimuli are modulated by oncogenic mutations and blocking oncogenes with targeted inhibitors has the advantage of affecting a wide range of pro- and anti-angiogenic molecules [27, 64].

All these factors suggest that standard anti-angiogenic therapy is unlikely to succeed in all tumors, but treatment strategies need to be adapted to individual cancers. Selective biomarkers are needed to predict which patients will benefit from anti-angiogenic therapy and, considering that a specific angiogenic profile can be activated in different cancers, to select the appropriate therapy. Moreover, several studies have aimed at identifying molecular changes that correlate with the response to anti-angiogenic therapy,

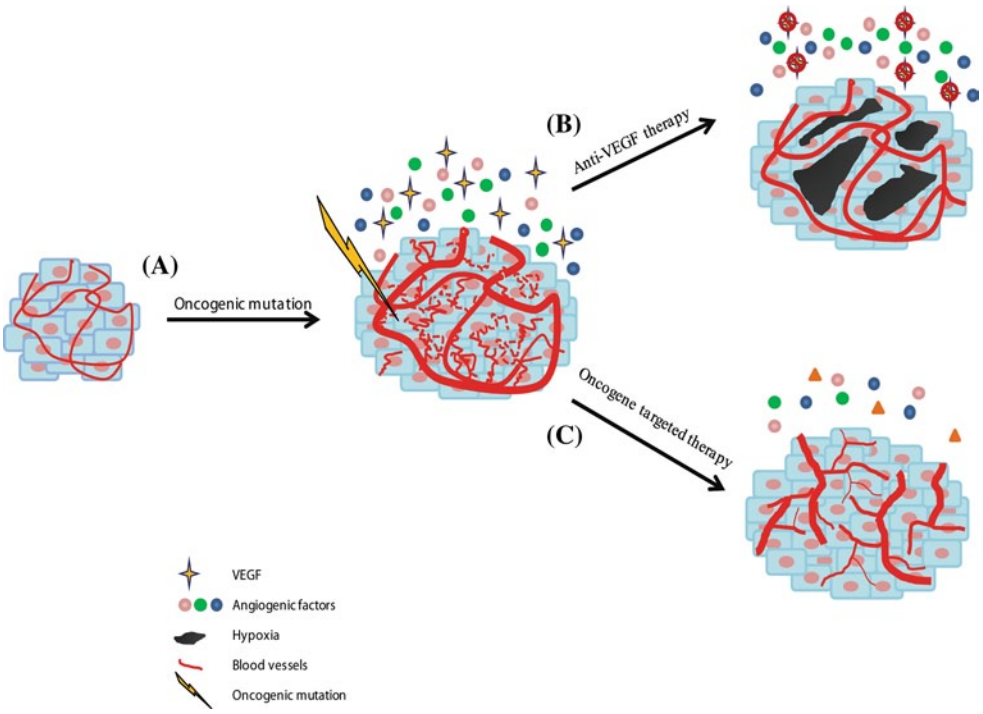

Fig. 1 Anti-VEGF and anti-oncogene-targeted therapy in tumor angiogenesis. **a** Oncogenic mutations drive tumor angiogenesis by increasing the expression of pro-angiogenic factors. The unbalance between pro- and anti-angiogenic factors causes an aberrant and missfunctional vasculature. **b** Anti-VEGF therapy causes blood vessel pruning and hypoxia that negatively impacts tumor progression. **c** Oncogene-targeted therapy potentially slows down the angiogenic program in tumor cells and restores the balance between pro- and anti-angiogenic factors, thereby leading to blood vessel normalization

which is an important parameter for the early identification of response or resistance to the therapy [112–114].

To date, reliable markers to predict response to anti-angiogenic treatment are not available; for example in metastatic colorectal cancer, neither VEGF nor microvessel density was predictive for response to bevacizumab [115]. Oncogenic mutations are already considered an important clinical parameter to stratify patients and identify suitable therapies. However, oncogenes have failed to be predictive for response to classical anti-angiogenic therapy [116], but it is possible that they will become useful for choosing alternative strategies. The knowledge about how the tumor microenvironment is influenced by targeted therapy will allow a better understanding of the clinical outcome and hopefully a clearer prediction of patient response.

We propose that blocking oncogenic pathways may result in inhibition of cancer cell proliferation, while concomitantly normalizing tumor vasculature. This approach opens possibilities for combinatorial treatments with chemotherapeutic agents or radiation therapy that would rely on the positive effects of vascular normalization on blood flow and tissue perfusion. Furthermore, by selectively blocking oncogenes, it should be possible to stall, at least temporarily, the angiogenic program. As oncogenes are activated in a tumor-specific fashion, we envision a personalized anti-angiogenic therapy that normalizes tumor vasculature even in cancers that are intrinsically refractory to anti-VEGF treatment thereby overcoming some of the limits of current anti-angiogenic drugs.

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