Tumor Neoangiogenesis and Flow Congestion
A Parallel to the Braess Paradox?

Stefan Kippenberger, Markus Meissner, Roland Kaufmann, Igor Hrgovic, Nadja Zöller, Johannes Kleemann

Although tumor angiogenesis is considered as a critical parameter in tumor growth, antiangiogenic monotherapy lags far behind expectations. Applying a mathematical model from route planning, known as the Braess paradox, provides an explanation by showing that in some constellations, more roads can lead to more traffic jams.

Tumor Angiogenesis
It is one of the most widely accepted paradigms in tumor biology that growth of solid tumors is restricted to the ample supply of nutrients. Accordingly, it is assumed that passive diffusion is sufficient for tumor growth only to a size of ≈2 mm in diameter; thus, the tumor connects to capillaries to satisfy its hunger. It was Folkman1 who first established in 1971 the concept of a tumor that actively attracts capillaries sprouting into the tumor tissue. In this model, the hypoxia-inducible factor 1, a heterodimeric transcription factor consisting of the constitutively hypoxia-inducible factor 1α and the oxygen-sensitive hypoxia-inducible factor 1α subunit, plays a key role. Under normoxic conditions, hypoxia-inducible factor 1α is proteasomally degraded, whereas hypoxia stabilized the dimer allowing transcription of a set of genes. Among those, members of the vascular endothelial growth factor (VEGF) family are of particular importance as they direct endothelial cell migration along the hypoxic gradient.2 Together with the proliferation-inducing properties of VEGF, new blood vessels are formed connecting the tumor to the blood circulation. These findings inspired the development of antiangiogenic tumor therapy as an effective tool to combat the growth of solid tumors.

Antiangiogenic Tumor Therapy—Unfulfilled Expectations
As a result, >60 antiangiogenic compounds of different origin have been identified and tested in patients to date.3 In particular, antibodies directed against VEGF-A (bevacizumab) and VEGF receptor 2 (ramucirumab), as well as endogenous inhibitors of angiogenesis, such as angiostatin and endostatin, are viewed as highly potential drugs to halt vessel growth.4,5 Furthermore, regimens utilizing metronomic low-dose chemotherapy have been suggested to offer antiangiogenic effects.6 Unfortunately, antiangiogenic monotherapies have not yet provided the expected quantum leap in tumor therapy. A monotherapy with antiangiogenic compounds is practically of no relevance in today’s tumor therapy. Instead, many tumors were treated with a combination of antiangiogenic and other antitumor agents, such as standard chemotherapeutics and small molecules blocking cellular signaling cascades (see below). At first glance, this does not seem convincing as the antiangiogenic regime rather protects the tumor from chemotherapy, making adverse systemic effects more likely.

Braess Paradox
In the present study, we suggest an explanation for this observation by referring to a phenomenon from traffic networks known as the Braess paradox.7,8 In Figure 1, a constellation in traffic is shown where the addition of an extra road augments the network’s capacity but reduces its efficiency. It is based on the concept that the addition of a self-referential link to non-interfering parallel connections increases congestions. This counterintuitive result is proven in real life and is nowadays considered in route planning. Moreover, the Braess paradox is shown to be relevant in a variety of networks, including electric circuits (Wheatstone bridge),9 distributed computer systems,10 water-supply pipe networks,11 natural-gas transportation,12 and human crowds.13 It is most likely that in many cases, tumor vasculature shares characteristics described by the Braess paradox. In particular, self-referential connections of the type shown in the model lead to a significant deterioration in network efficacy, similar to what is observed in many river deltas. Different from regular wound healing, the emerging network of capillaries in tumors is not well ordered and coordinated as under regular conditions but offers a chaotic architecture featuring dilated and contorted vessels with shunts and excessive branching.14 Moreover, vessel permeability, vessel diameter, tortuosity, tumor interstitial fluid pressure, and abnormalities in lymph vessels are characteristics of tumor vasculature with effect on tumor supply. Already in 2001, Jain15 suggested that antiangiogenic therapy may lead to a normalization of the vasculature before the vessel network finally collapses. The inefficacy of antiangiogenic monotherapies shows that the expected breakdown of tumor vasculature is at least insufficient. To study the effect of antiangiogenic therapy, an in vitro angiogenesis assay was utilized (Figure 2A). A coculture of endothelial cells and fibroblasts was seeded into a gel matrix. By the addition of 10 ng/mL VEGF, a complex maze of tubes is formed within 11 days. To examine the effect of

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an antiangiogenic treatment, VEGF was combined with increasing concentrations of bevacizumab (0.01, 0.1, and 1.0 mg/mL). Representative images show a concentration-dependent breakdown of the maze. Image analysis confirms this impression by showing a decrease of the cell-covered area from 24% to 2.6% under treatment with 1 mg/mL bevacizumab (Figure 2B), most likely due to the antiproliferative properties of bevacizumab. Corroboratively, a decrease of total tube length (from 6106, measured in pixel, to 618), tube number (from 223 to 8 pixel), and branching points (from 125 to 0 pixel) was measured. Interestingly, an inverse correlation was observed for the mean tube length. Here, an increase from 27 to 77 pixel was measured under treatment with 1 mg/mL bevacizumab. These data support the assumption that increasing concentrations of bevacizumab lead to a more ordered maze with less branching points but increased mean tube lengths.

Combination Therapies

Assuming that tumor vasculature displays vessel connections similar to what is described by the Braess paradox, treatment with an antiangiogenic drug normalizes tumor vasculature, by decreasing self-referential connections and increasing vessel stability and therefore improving tumor nourishment. In this model, an antiangiogenic therapy used as monotherapy is not only useless but detrimental. In view of this background, it becomes evident that the antiangiogenic approach paves the way for an improved delivery of other antitumor drugs, such as chemotherapeutics. This concept is backed by large clinical trials showing a significant benefit of combination therapies in various tumor entities, including ovarian cancer, metastatic colon–rectal cancer, renal cell carcinoma, non–small cell lung cancer, metastasized breast cancer, and advanced cervical cancer.

Future of Antiangiogenic Therapy

The counterintuitive finding of Dietrich Braess, who gave his name to the paradox, revolutionized the general

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**Figure 1.** Braess paradox—new roads increase traffic congestion. A, Road users traveling from city A to city D have 2 options: (1) drive directly to city C via the motorway and then take a country road to city D or (2) use the country road to city B and from there take the motorway. A direct linear connection between cities A, B, C, and D is not possible because of an obstacle (eg, a mountain). In this example, the 6 drivers starting at city A will split up equally between both options (3–3). B, Installation of a tunnel through the mountain offers a third option utilizing exclusively country roads to reach city D. The country road sections (A–B and C–D) become bottlenecks carrying those who solely take country roads and those who use both motorways and country roads. This leads to a congestion inhibiting traffic flow on all roads. As shown for (A), the distribution of the 6 drivers starting at city A is shown.

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**Figure 2.** Two-dimensional in vitro angioassay. A, To study the effect of bevacizumab, a vascular endothelial growth factor (VEGF) antagonist, on vessel architecture, the AngioKit assay (TCS Cellworks, Buckinghamshire, United Kingdom) was used according to the manufacturer’s instructions. Briefly, endothelial cells seeded in a fibroblast-containing matrix were treated with either VEGF (10 ng/mL) or a combination of VEGF and increasing concentrations of bevacizumab (0.01, 0.1, and 1.0 mg/mL) for a total period of 11 days. The medium was replaced 3 times. The experiment was terminated by staining the endothelial cells using an anti-CD31 (platelet/endothelial cell adhesion molecule-1) primary antibody in combination with an anti-IgG alkaline phosphatase-conjugated second antibody together with an insoluble substrate (BCIP/NBT [5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium]). Finally, CD31-positive cells were examined using a microscope (Zeiss, Stemi 2000-C; magnification, ×50). Representative images were taken and are shown here. B, Image analysis of the images shown in (A) was performed with Wimasis image analysis.
understanding of transportation in networks. As aforementioned, this model is applied to many mechanical and electric networks but yet not to tumor vasculature. The clinical success of combination therapies supports the hypothesis that Braess-like vessel constellation is also present in at least some tumors. Nonetheless, as long as we have no detailed information of the momentary vasculature of a specific tumor, it is difficult to forecast the outcome of an antiangiogenic therapy. This is aggravated by the fact that although tumor vasculature shares characteristics of transport networks, there are also some differences. Vessels are not leak proof tubes, and VEGF-A, the target of bevacizumab, is a pleiotropic effector not only affecting angiogenesis but also known to disrupt endothelial barrier function and inducing matrix metalloproteinases. These characteristics increase the complexity and therefore the assessment of tumor vasculature. However, if the function of the >6 members of the VEGF family, their numerous splicing variants, and counterparts on the receptor level were better understood, therapeutic interventions would be more effective. It could be speculated that in some scenarios, the paradigm of antiangiogenic therapy will experience a complete shift. The addition of a finely tuned cocktail of VEGF molecules may promote the generation of a highly recursive vessel network, eventually so congested that the tumor will perish. Necrotic zones that are frequent in large solid tumors reflect such processes. Then, analogous to the Braess paradox the flow collapses—an unwanted event in car transportation but a desirable event in tumor therapy.

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References

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