

Significance of Blood Vessel Leakiness in Cancer¹

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Abstract

Despite major advances in the field of tumor angiogenesis, relatively little attention has been paid to the permeability of blood vessels in tumors. The leakiness of tumor vessels is well documented in experimental tumor models and in human cancer, but the mechanism is poorly understood, as are the implications to the rate of cancer growth, predisposition to metastasis, and delivery of macromolecular therapeutics to tumor cells. Sixteen experts in the fields of cancer biology and vascular biology gathered at the William Guy Forbeck “Focus on the Future” Conference to discuss this topic. The meeting was the first of its kind focused on the significance of blood vessel leakiness in tumors. The participants discussed the cellular basis of tumor vessel leakiness, endothelial barrier function of blood vessels, monitoring tumor vessel leakiness, mediators of endothelial leakiness, consequences of tumor vessel leakiness, genomic analysis of vascular targets, targeting drugs to tumor vessels, and therapeutic manipulation of tumor vessels. The group concluded that a more complete understanding of the basic biology of tumor vessels will be necessary to fully appreciate the consequences of vessel leakiness in cancer. New research tools such as intravital measurements of tumor blood flow and vessel leakiness, *in vivo* phage display, magnetic resonance imaging, and use of selective angiogenesis inhibitors will contribute to this understanding.

Introduction

There is general agreement that blood vessels in tumors are abnormal. One manifestation of this abnormality is a defective and leaky endothelium. Blood vessel leakiness not only influences the internal environment of tumors and perhaps the rate of angiogenesis, but it also governs access of therapeutic antibodies, gene therapy vectors, and liposomal drug delivery systems to tumor cells. The purpose of the William Guy Forbeck “Focus on the Future” Conference was to provide an opportunity for scientists who study blood vessel barrier function to join forces with scientists working on tumor angiogenesis and other areas of cancer biology to define the state of the art and significance of blood vessel leakiness in cancer and to set out directions for future research in the field.

At the outset of the conference, Dr. Donald M. McDonald (University of California, San Francisco, CA) invited the participants to consider five questions: (a) Why are the blood vessels in tumors leaky, and what are the consequences of the leakiness? (b) How can blood vessel leakiness be assessed in experimental tumors and in

human cancer? (c) What strategies are available to target therapeutics to tumor vessels with the goal of normalizing or destroying the vessels? (d) Would it be therapeutically beneficial to decrease the leak, and if so, how could this be achieved? (e) Would “normalization” of tumor vessel structure and function be beneficial in cancer therapy, and if so, how could this be achieved?

Cellular Basis of Tumor Vessel Leakiness

Most tumor vessels have an irregular diameter and an abnormal branching pattern and do not fit well into the usual classification of arterioles, capillaries, or venules. Even large-caliber vessels have thin, leaky walls. Dr. Peter Baluk (University of California, San Francisco, CA), who studies the morphological abnormalities of tumor vessels, portrayed the structural irregularity, heterogeneity, and leakiness of tumor vessels as bizarre hallmarks of a propensity to break all of the rules of normal blood vessel construction. Although some reports suggest that tumor vessels lack endothelial cells, pericytes (mural cells), or basement membrane, recent work indicates that all of these components are present but abnormal (1, 2). Endothelial cells, although present on most if not all tumor vessels, do not form a normal monolayer and thus do not have a normal barrier function (1, 3). The cells are disorganized and irregularly shaped. Endothelial cells of some tumor vessels overlap one another, have luminal projections, and give rise to abluminal sprouts. The cells also have loose interconnections and focal intercellular openings, which are likely to be responsible for much of the vessel leakiness. The size of the openings (<2 μm in diameter) between endothelial cells examined by scanning electron microscopy fits the functional measurements of the pore cutoff size of tumor vessels. Pericytes with α -smooth muscle actin and/or desmin immunoreactivity are present on most tumor vessels, but they have an abnormally loose association with endothelial cells, and many have cytoplasmic processes that extend deep into the tumor tissue (2). The vascular basement membrane, whether visualized by type IV collagen, laminin, fibronectin, or nidogen immunoreactivity, covers most of the surface of tumor vessels. Nonetheless, the basement membrane has an abnormally loose association with endothelial cells and pericytes and consists of multiple layers. The redundant layers of basement membrane represent a historical record of continuous vascular remodeling.

Dr. Raghu Kalluri (Harvard Medical School, Beth Israel Hospital, Harvard Medical School, Boston, MA) elaborated on the abnormal structure and composition of the basement membrane of tumor vessels. The vascular basement membrane, which is formed by and envelops endothelial cells, pericytes, and smooth muscle cells of blood vessels, is distinct from other components of the extracellular matrix (4, 5). Vascular basement membrane is 50% type IV collagen and 30% laminin, plus significant amounts of nidogen (entactin), fibronectin, and other proteins and heparan sulfate proteoglycans. Analytical methods have revealed differences between vascular basement membrane and other basement membranes. Furthermore, the composition of the vascular basement membrane is not identical in all blood vessels. Vascular basement membrane is now recognized as undergoing important changes in disease and may be distinctive on

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tumor vessels. For example, deletion of the laminin $\alpha 4$ chain gene results in hemorrhage in knockout mice as well as bleeding and impaired vessel growth in angiogenesis assays. Some $\alpha 4$ chain-containing laminins (e.g., laminin-8) are associated with tumor vessels.

Basement membrane is also a potential source of pro- and antiangiogenic molecules. Some structural proteins of basement membrane can be broken down by enzymes to yield smaller molecules with potent actions. One example is endostatin, which is a COOH-terminal fragment of collagen XVIII. Another, tumstatin, is the noncollagenous-1 domain of the $\alpha 3$ chain of type IV collagen. A third example, arresten, is the noncollagenous-1 domain of the $\alpha 1$ chain of type IV collagen (4, 5). These molecules show great potential in slowing cancer growth in preclinical models and are already or will soon be in clinical trials. Dr. Kalluri concluded that (a) abnormalities in tumor vessels include changes in the composition, assembly, and architecture of the vascular basement membrane and (b) changes in the vascular basement membrane are likely to be important in the selective permeability of tumor vessels and, along with abnormalities in the endothelium, may influence the extravasation of plasma and limit the influx of immune cells into tumors.

What about the cause of these abnormalities in tumor vessels? Here, there are more questions than answers. Soluble and matrix-associated factors produced by tumor cells and the hypoxic environment are assumed to be important. Stromal cells are probably also involved, perhaps by participating in the imbalance of growth factors in tumors. VEGF,³ which can trigger the formation of very bizarre vessels in experimental models, is a likely contributor to the vascular abnormalities. Ang1, which may be made by pericytes, Ang2, which may be overexpressed by endothelial cells in tumors, and PDGF, which is necessary for normal pericyte development, are other contributors, but less is known about their role in tumor vessel leakiness.

Endothelial Barrier Function of Blood Vessels

Multiple factors influence the leakage of substances from blood vessels. Dr. Fitz-Roy Curry (University of California, Davis, CA) described the physiological mechanisms regulating the movement of substances out of normal blood vessels. Major factors are the luminal surface area and permeability of the vessel wall, concentration and driving forces (hydrostatic and osmotic gradients) across the endothelium, and blood flow (6, 7). An important goal will be to understand the relative contribution of each of these factors in the extravasation of macromolecules such as plasma proteins, therapeutic antibodies, or viral vectors from tumor vessels, but there is not a solid understanding at present. One reason is that the variables governing leakage, which can readily be measured in experimental models such as frog or rat mesenteric blood vessels, are difficult to assess in tumors. Dr. Curry described how, under normal conditions, the permeability of a single microvascular bed to water-soluble solutes can vary by a factor of at least 10,000, with electrolytes on one end of the spectrum and macromolecules on the other (6, 7). Permeabilities of different vascular beds range from the lowest values in the brain, through those in skeletal muscle and cardiac muscle, to those in mesentery, intestine, salivary glands, and renal glomeruli. Water and small water-soluble solutes have the largest variation. Differences among vascular beds are based on several factors, including the number of tight junctional strands and pores in the endothelium as well as the permeability of the glycocalyx lining the luminal surface of the endothelium.

Small solutes move out of vessels mainly by diffusion, but large solutes can move by diffusion and by convection, where extravasation

is coupled with water flow, particularly where vessels are very leaky. Dr. Curry highlighted the importance of distinguishing the permeability or leakiness of the vessel wall from the flux and accumulation of extravasated fluid and solutes outside vessels, which depend on multiple factors, including permeability, surface area, and forces that drive water and solutes across vessel walls.

Based on principles learned from normal blood vessels, where flux across vessel walls is the product of conductance and driving force, Dr. Curry estimates that a single hole $>0.5 \mu\text{m}$ in diameter in the endothelium of a tumor vessel would make the permeability of that vessel large enough for solute extravasation to be limited by blood flow. Thus, blood flow would limit the leakage of macromolecules, and increased blood flow should improve delivery of macromolecular therapeutics to perivascular cancer cells. Delivery to cancer cells located some distance from blood vessels would be limited by interstitial transport as well as by blood flow.

The dependency of the delivery of macromolecular drugs on blood flow raises the issue of tumor vessel "normalization" in response to certain treatments. According to Dr. Rakesh Jain (Massachusetts General Hospital, Harvard Medical School, Boston, MA), VEGF inhibitors, angiogenesis inhibitors, and even Herceptin may normalize tumor vessels (8, 9). This would result from decreased vessel tortuosity; reduced variability in vessel size, shape, and branching; correction of vessel wall abnormalities; and perhaps even diminished leakiness. Dr. Jain made the suggestion, which at first seems counterintuitive, that normalization of tumor vessels may improve drug delivery to tumor cells. In theory, the process of normalization would increase blood flow by decreasing resistance due to vessel tortuosity and irregular diameter. Increased blood flow could improve drug delivery, even if vessel leakiness diminished to some extent during normalization. However, increased blood flow could also stimulate tumor growth. To test these possibilities, Dr. Jain emphasized the need for more research in several areas, including: (a) the molecular, cellular, and physiological mechanisms of vascular normalization in tumors during therapy; (b) imaging methods, including the use of surrogate markers, to determine the conditions of tumor vessel normalization and to improve combination therapies; and (c) dynamic studies of endothelial cell/pericyte/basement membrane/stromal cell interaction to better understand the consequences of manipulating the function of tumor vessels. Dr. Curry added the suggestion that if technically feasible, the effect of increased blood flow should be tested on single tumor vessels before and after normalization under conditions where blood flow and permeability are measured.

Tumor vessels are an order of magnitude leakier than normal vessels. The pore cutoff size (indicating the largest particles that can cross vessel walls) is measured in hundreds of nanometers rather than in tens of nanometers (8, 10). However, the extravasation of proteins from tumor vessels is slower than would be predicted from the pore size if the vessels were otherwise normal. An abnormally small hydrostatic gradient from the inside to the outside of vessels, resulting from the high interstitial hydrostatic pressure within tumors, reduces the driving force for convective movement. In the absence of convection, the movement of macromolecules out of tumor vessels is driven mainly by diffusion, a slow process for large molecules. The high interstitial pressure in tumors is a consequence of leakage from blood vessels coupled with impaired fluid clearance through lymphatics and perhaps rigid or contractile stromal cells that decrease tissue compliance. Additional experiments are needed to determine the fate of substances that extravasate from tumor vessels and why functional lymphatics fail to develop in tumors, even in the presence of the lymphangiogenic growth factor VEGF-C. This is recognized as a very fertile area for experimentation because of the recent availability of

³ The abbreviations used are: VEGF, vascular endothelial growth factor; Ang, angiopoietin; PDGF, platelet-derived growth factor; VEGFR, VEGF receptor; MRI, magnetic resonance imaging.

reagents that can localize and stimulate or block the formation of lymphatics.

Little is known about the fate of solutes that extravasate from leaky tumor vessels. Because of the absence of functional lymphatics, extravasated solutes may leave tumors slowly by diffusion, return to the blood, be retained by binding to tumor or stromal cells, or be metabolized. The link between the poor penetrance of macromolecules into tumors and absence of lymphatics raises the possibility that drug delivery could be improved by normalizing the lymphatic drainage from tumors. However, the growth of functional lymphatics into tumors could predispose tumors to metastasis.

Monitoring Tumor Vessel Leakiness

Methods are being developed to monitor vascular permeability in tumors. This has been achieved in experimental models where tumor vessels are visible *in vivo* through a transparent window. Noninvasive approaches for use in human cancer are more challenging. Macromolecular tracers injected into the bloodstream can be detected in tumors using MRI, and mathematical models are being used to quantify extravasation.

Dr. Robert Brasch (University of California, San Francisco, CA) has developed MRI methods to measure the leakiness of tumor vessels in rodents and humans and to test drugs that may change the leakage. He has pioneered efforts using albumin-gadolinium complexes and dextran-coated iron oxide particles as macromolecular tracers to image tumor vessels and assess their leakiness by MRI (11, 12). One reason for imaging the leakiness of tumor vessels is to estimate the severity or grade of tumors, which correlates with amount of leakage. Imaging also provides a means for identifying drug-responsive tumors and monitoring the response of tumors to treatment. Dr. Brasch showed that irradiation increases the leakage of macromolecular MRI contrast agents from tumor vessels and that an anti-VEGF antibody decreases the leakage. Dr. Brasch concludes that leakiness of tumor vessels is a consistent and useful physiological property that can be measured in animals and humans and can be manipulated to clinical advantage and reflects the biological status of tumors. He recommended additional studies with the goal of identifying the morphological determinants and ways of manipulating the leakage.

Although there are still questions about the interpretation and quantification of the signal in MRI studies of vascular permeability, this approach is important because it offers a potential means of assessing tumor vessel leakiness noninvasively in humans and may be useful in evaluating the efficacy of angiogenesis inhibitors.

Mediators of Endothelial Leakiness

Dr. Neil Granger (Louisiana State University, Shreveport, LA) considered how inflammatory mediators increase blood vessel leakiness and how, under some conditions, leakage may be related to the migration of leukocytes through vessel walls (13). Many mediators, including histamine, bradykinin, serotonin, substance P, and certain cytokines, increase endothelial permeability, but some (nitric oxide, for example) can either increase or decrease vascular permeability under different circumstances. Leukocytes are key to blood vessel leakiness in some inflammatory responses and under conditions of ischemia followed by reperfusion but are not involved in many other types of leakage. The functional pore size in endothelial cells of the intestine can increase simply by exposure to certain dietary constituents. In terms of the mechanisms of leakiness of tumor vessels, Dr. Granger recommended additional work on (a) the structural basis for the leakiness of tumor vessels, (b) the role of dividing and growing or migrating endothelial cells in tumor vessel leakiness, and (c) the relative contributions of altered vascular permeability and impaired

lymphatic drainage to the accumulation of extravasated macromolecules in tumors.

Consequences of Tumor Vessel Leakiness

What is the relationship between blood vessel leakage and tumor growth? Dr. Harold Dvorak (Beth Israel Hospital, Harvard Medical School) presented a model system using focal transfection of skin with an adenoviral vector to study the effects of vascular growth factors in mice (14, 15). High expression of VEGF in this model stimulates the proliferation of large "mother vessels" and tortuous "glomeruloid bodies" that have structural and functional features in common with some tumor vessels. This model system presents an opportunity to create a "virtual tumor" consisting of blood vessels without tumor cells that is simpler to study than real tumors. Dr. Dvorak also considered the role of fibrinogen extravasation from leaky tumor vessels and subsequent fibrin deposition in establishing the environment within tumors that promotes angiogenesis and tumor growth. Relevant questions include: (a) Is fibrin essential for angiogenesis in tumors? Do tumors grow more slowly in fibrinogen knockout mice? (b) What are the abnormalities in the endothelial glycocalyx in tumor vessels? (c) Why do more erythrocytes extravasate from tumor vessels than platelets or leukocytes? (d) Why does VEGF-C induce the formation of new lymphatics in normal tissues but not in tumors?

Genomic Analysis of Vascular Targets

Remarkable progress has been made in profiling gene expression in endothelial cells and tumor cells. Dr. Richard Murray (Eos Biotechnology, Inc., South San Francisco, CA) described high-throughput gene expression studies as tools to discover novel targets for anticancer drugs. Eos Biotechnology, Inc. has amassed a database of expression values for 40,000 human genes in hundreds of tumors and normal tissues. Data from these specimens, analyzed using powerful bioinformatics tools, have enabled Eos Biotechnology, Inc. scientists to identify unique genetic differences between quiescent and growing endothelial cells and between tumors and normal tissues, with a special focus on identifying potential drug targets on the cell surface. Antibodies made to these molecules, in some cases coupled to toxic drugs, could target tumors. As to the future, Dr. Murray focused on the speedy translation of basic science discoveries into drug candidates as key to getting new drugs into the clinic rapidly. Proof of principle studies are most useful when they provide data relevant to subsequent clinical studies. In this regard, experiments on tumor vessel leakiness should take into account the practical aspects of delivering drugs preferentially to tumor vessels.

Targeting of Drugs to Tumor Vessels

There is general agreement on the utility of finding molecules on tumor vessels and cancer cells that can be used as targets for diagnostics and therapeutics. The identification of molecules that are preferentially expressed or uniquely accessible on tumor vessels is moving the drug targeting field from theory to reality, but the best targets and the best methods of finding them are still not settled. Molecules identified by gene expression studies, *in vivo* phage display, and other approaches offer great promise in concentrating systemically administered therapeutics in tumors while reducing side effects.

Dr. Philip Thorpe (University of Texas Southwestern Medical Center, Dallas, TX) described several potential targets on tumor vessels. Among these were $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin, VEGF-VEGFR-2 complex, endoglin (CD105), prostate-specific membrane antigen,

ED-B domain of fibronectin, endosialin, matrix metalloproteinase-9, and phosphatidylserine (16, 17). According to Dr. Thorpe, phosphatidylserine, which is normally expressed on the cytoplasmic surface of cell membranes, flips to the external surface in tumor vessels and is exposed to the bloodstream. Flipping may be triggered by endothelial cell activation or injury produced by hypoxia, inflammatory cytokines, or reactive oxygen species generated in the tumor. Antibodies to phosphatidylserine are nontoxic and have an antitumor effect in mice. Another strategy targets clotting factors to tumor vessels with the goal of triggering intravascular thrombosis within tumors (16, 17). These approaches can destroy tumor vessels and elicit regression of solid tumors in experimental models. Studies show that vascular targeting agents can rapidly shut down the vasculature of tumors, are effective at low dosage, and shrink small and large solid tumors in mice. Their attributes are distinct from and complementary to those of angiogenesis inhibitors that block tumor growth by suppressing blood vessel formation.

Drs. Renata Pasqualini and Wadih Arap (both from University of Texas M. D. Anderson Cancer Center, Houston, TX) are using *in vivo* phage display to identify proteins that are preferentially expressed on tumor vessels and can serve as targets for drug delivery (18–21). Experiments in mice show that targeted chemotherapeutics are effective at much lower doses than untargeted drugs. Using libraries of bacteriophage that express random peptides, the laboratory of Drs. Pasqualini and Arap has identified specific molecular targets (Zip codes) on vessels in tumors as well as in normal brain, lung, pancreas, adipose tissue, and prostate (18, 19). Most of the targets are on endothelial cells, but some in tumors may be on pericytes that are accessible via defects in the endothelium (20). Effective targets take advantage of the molecular heterogeneity of the vasculature and site-specific abnormalities in endothelial barrier function. Because of the nature of the technology, targets identified by *in vivo* phage display would also be accessible to macromolecular therapeutics, such as antibodies, liposomes, or viral vectors. Although most studies using *in vivo* phage display have been performed on mice, ground-breaking studies are under way in which *in vivo* phage display is performed on cancer patients (21). Ongoing studies are identifying molecular targets on blood vessels in tumors and in healthy tissues of humans. The interleukin 11 receptor is one such target identified in the prostate (21). To exploit the full potential of *in vivo* phage display for drug targeting research, additional work is needed to develop a more complete understanding of the cellular localization and accessibility of vascular Zip codes in tumors and healthy tissues.

Therapeutic Manipulation of Tumor Vessels

Transgenic mice that develop spontaneous, multifocal, multistage tumors are being used to compare the efficacy of drugs in preventing tumor growth and causing tumor regression. Dr. Douglas Hanahan (University of California, San Francisco, CA) described recent advances made in dissecting the steps involved in tumor angiogenesis and the effects of drugs on the process (22, 23). His group found that inhibition of VEGF signaling in endothelial cells prevents the growth of pancreatic islet cell tumors in RIP-Tag transgenic mice but seems less effective in causing tumor regression. Inhibition of VEGF-R2 signaling in tumor vessels would be expected to affect mainly endothelial cells. The presence of pericytes on tumor vessels as well as the supportive function of pericytes on endothelial cells and the dependency of pericytes on PDGF raises the possibility that a combination of VEGF and PDGF inhibitors would be more effective in destroying tumor vessels and causing tumor regression. However, the interpretation of such experiments depends on the selectivity and access of the inhibitors to their targets. Also, it remains to be determined whether

such inhibitors will destroy some tumor vessels, normalize others, and leave some unchanged. In relation to blood vessel leakiness, Dr. Hanahan recommended continued efforts to manipulate vascular permeability in tumors, with the goal of understanding the relevance of increased permeability to small molecules, proteins including antibodies, and leukocytes. Whereas some therapeutic strategies may benefit from normalizing tumor vasculature and decreased leakiness, others may be more effective when permeability is increased, as in immunotherapies where increased leukocyte flux into tumors would be beneficial.

Tumor vessel leakiness may result from an imbalance of growth factors that produces defects in endothelial cells, pericytes, and basement membrane. Dr. Gavin Thurston (Regeneron Pharmaceuticals, Inc., Tarrytown, NY) suggested that correction of this imbalance could reverse the abnormalities but cautioned that systemically administered growth factors or their inhibitors may not mimic the normally focal actions of their endogenous counterparts. Some growth factors (VEGF, for example) may be produced in amounts that trigger angiogenesis and the abnormal phenotype of tumor vessels, whereas other factors, such as Ang1 and VEGF inhibitors, drive tumor vessels toward a more normal phenotype. Alternatively, Ang2 and VEGF inhibitors may cause tumor vessels to regress and disappear. To evaluate these possibilities, it is necessary to understand the players. VEGF is a potent endothelial cell mitogen. Newly formed blood vessels tend to be VEGF dependent. VEGF-Trap, which is an inhibitor of VEGF consisting of soluble VEGFR-1 and VEGFR-2 coupled to an immunoglobulin Fc tail, blocks the early growth of tumors but tends to normalize blood vessels in established tumors. Ang1, which is not an endothelial cell mitogen, acts selectively on endothelial cells via Tie2 receptors and seems to have antileakage and anti-inflammatory roles (24, 25). Ang2 also acts on Tie2 receptors and mimics the effects of Ang1 under some conditions but blocks Ang1 and favors vessel regression under others. Ang2 may be a marker of endothelial cells in tumors and may promote lymphangiogenesis. In the absence of Ang2, vascular remodeling does not progress normally during development. Important questions that need to be addressed to evaluate these issues include: (a) Is VEGF overexpression the key imbalance with respect to the abnormalities of tumor vessels? How does VEGF inhibition change cellular morphology, permeability, and receptor expression of tumor vessels? Do the vessels normalize or regress? (b) What is the role of Ang2 in tumors? Does the ratio of Ang1 to Ang2 influence blood vessel morphology? (c) Would it be clinically more beneficial in cancer therapy to normalize tumor vessels or to make them even more abnormal to the point they regress?

Dr. David Cheresh (The Scripps Research Institute, La Jolla, CA) described his recent experiments on intracellular signaling pathways involved in VEGF-induced plasma leakage. His experiments identified the tyrosine kinase Src as a key player in intracellular signaling leading to leakage produced by VEGF but not by other mediators (26). Src is downstream to VEGF receptors and is linked via β -catenin to vascular endothelial-cadherin at intercellular junctions. VEGF does not cause leakage in mice in which *Src* – or its relative *Yes* – is knocked out. VEGF-induced leakage can also be blocked by the Src inhibitor PP1. Dr. Cheresh reported that tumors grown in *Src*-null mice are larger and have less fibrin at their surface and speculated that fibrin formed from extravasated fibrinogen may be involved in the containment of tumors. Src inhibition also reduces plasma leakage in the brain and increases survival in a mouse model of stroke, illustrating how research in the field of angiogenesis and vascular permeability may unexpectedly benefit other clinical situations (27). In this model, brain swelling associated with stroke leads to ischemia, tissue hypoxia, increased VEGF production, and more plasma leakage that exacerbates brain swelling. Src inhibitors ameliorate this problem. Dr.

Cheresh concluded that greater knowledge of the control of vascular permeability is likely to have a profound impact on cancer as well as other disease processes including inflammation and ischemia. For that to happen, more attention needs to be paid to the molecular mechanisms and cellular basis of changes in vascular permeability and blood flow in tumors and other diseases. Although the role of Src in VEGF-induced leakage in tumors is just beginning to be studied, *Src* knockout mice may be useful in assessing the functional significance of tumor vessel leakiness.

Conclusion

Leakiness is one of multiple abnormalities of tumor vessels that not only influences angiogenesis, tumor growth, and metastasis but also affects drug delivery and present novel targets for therapeutic intervention in cancer. Plasma leakage in tumors is a manifestation of structural defects in vessel walls resulting from the abnormal environment within tumors. An imbalance of VEGF, PDGF, Ang1, Ang2, and other growth factors is likely to play an important role. Vessel leakiness contributes to the abnormal environment and supports vessel sprouting and growth. Despite a severely defective barrier function, tumor vessels do not leak as much as expected because the high interstitial pressure in tumors limits the extravasation of fluid and macromolecules. The absence of functional lymphatics contributes to the high interstitial pressure. Therapeutic interventions that affect tumor vessels may destroy some vessels and leave others with a more normal phenotype. Normalization of tumor vessels may increase blood flow and improve the delivery of macromolecular therapeutics. However, the consequences of increased flow and decreased leakiness on tumor growth and the delivery of cell-based therapies are still unclear. Multiple new approaches are being used to identify molecules on tumor vessels that can serve as targets for preferential drug delivery. This strategy should have the benefit of reducing doses and side effects of anticancer drugs. It may also improve the efficacy of antiangiogenic therapy. With optimism over such new therapeutic approaches, the time is ripe to obtain further information about the nature and functional abnormalities of tumor vessels and how their leakiness contributes to growth and metastasis of tumors.

References

- McDonald, D. M., and Foss, A. J. Endothelial cells of tumor vessels: abnormal but not absent. *Cancer Metastasis Rev.*, 19: 109–120, 2000.
- Morikawa, S., Baluk, P., Kaidoh, T., Haskell, A., Jain, R. K., and McDonald, D. M. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am. J. Pathol.*, 160: 985–1000, 2002.
- Hashizume, H., Baluk, P., Morikawa, S., McLean, J. W., Thurston, G., Roberge, S., Jain, R. K., and McDonald, D. M. Openings between defective endothelial cells explain tumor vessel leakiness. *Am. J. Pathol.*, 156: 1363–1380, 2000.
- Colorado, P. C., Torre, A., Kamphaus, G., Maeshima, Y., Hopfer, H., Takahashi, K., Volk, R., Zamborsky, E. D., Herman, S., Sarkar, P. K., Ericksen, M. B., Dhanabal, M., Simons, M., Post, M., Kufe, D. W., Weichselbaum, R. R., Sukhatme, V. P., and Kalluri, R. Anti-angiogenic cues from vascular basement membrane collagen. *Cancer Res.*, 60: 2520–2526, 2000.
- Kamphaus, G. D., Colorado, P. C., Panka, D. J., Hopfer, H., Ramchandran, R., Torre, A., Maeshima, Y., Mier, J. W., Sukhatme, V. P., and Kalluri, R. Canstatin, a novel matrix-derived inhibitor of angiogenesis and tumor growth. *J. Biol. Chem.*, 275: 1209–1215, 2000.
- Michel, C. C., and Curry, F. E. Microvascular permeability. *Physiol. Rev.*, 79: 703–761, 1999.
- Bates, D. O., Heald, R. I., Curry, F. E., and Williams, B. Vascular endothelial growth factor increases Rana vascular permeability and compliance by different signalling pathways. *J. Physiol.*, 533: 263–272, 2001.
- Jain, R. K. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat. Med.*, 7: 987–989, 2001.
- Izumi, Y., Xu, L., di Tomaso, E., Fukumura, D., and Jain, R. K. Tumour biology: Herceptin acts as an anti-angiogenic cocktail. *Nature (Lond.)*, 416: 279–280, 2002.
- Carmeliet, P., and Jain, R. K. Angiogenesis in cancer and other diseases. *Nature (Lond.)*, 407: 249–257, 2000.
- Pham, C. D., Roberts, T. P. L., van Bruggen, N., Melnyk, O., Mann, J., Ferrara, N., Cohen, R. L., and Brasch, R. C. Magnetic resonance imaging detects suppression of tumor vascular permeability after administration of antibody to vascular endothelial growth factor. *Cancer Investig.*, 16: 225–230, 1998.
- Turetschek, K., Huber, S., Floyd, E., Helbich, T. H., Roberts, T. P. L., Shames, D. M., Tarlo, K. S., Wendland, M. F., and Brasch, R. C. MR imaging characterization of microvessels in experimental breast tumors by using a particulate contrast agent with histopathologic correlation. *Radiology*, 218: 562–569, 2001.
- Granger, D. N., Grisham, M. B., and Kvietys, P. R. Mechanisms of microvascular injury. In: L. R. Johnson (ed.), *Physiology of the Gastrointestinal Tract*, pp. 1693–1722. New York, NY: Raven Press, 1994.
- Pettersson, A., Nagy, J. A., Brown, L. F., Sundberg, C., Morgan, E., Jungles, S., Carter, R., Krieger, J. E., Manseau, E. J., Harvey, V. S., Eckelhoefer, I. A., Feng, D., Dvorak, A. M., Mulligan, R. C., and Dvorak, H. F. Heterogeneity of the angiogenic response induced in different normal adult tissues by vascular permeability factor/vascular endothelial growth factor. *Lab. Investig.*, 80: 99–115, 2000.
- Sundberg, C., Nagy, J. A., Brown, L. F., Feng, D., Eckelhoefer, I. A., Manseau, E. J., Dvorak, A. M., and Dvorak, H. F. Glomeruloid microvascular proliferation follows adenoviral vascular permeability factor/vascular endothelial growth factor-164 gene delivery. *Am. J. Pathol.*, 158: 1145–1160, 2001.
- Huang, X., Molema, G., King, S., Watkins, L., Edgington, T. S., and Thorpe, P. E. Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. *Science (Wash. DC)*, 275: 547–550, 1997.
- Thorpe, P. E., and Ran, S. Tumor infarction by targeting tissue factor to tumor vasculature. *Cancer J. Sci. Am.*, 6 (Suppl. 3): S237–S244, 2000.
- Trepel, M., Arap, W., and Pasqualini, R. Modulation of humoral immune response by targeting antigens to lymph nodes. *Cancer Res.*, 61: 8110–8112, 2001.
- Lahdenranta, J., Pasqualini, R., Schlingemann, R., Hagedorn, M., Stallcup, W. M., Bucana, C. D., Sidman, R., and Arap, W. An anti-angiogenic state in mice and humans with retinal photoreceptor cell degeneration. *Proc. Natl. Acad. Sci. USA*, 98: 10368–10373, 2001.
- Curnis, F., Arrighini, G., Sacchi, A., Fischetti, L., Arap, W., Pasqualini, R., and Corti, A. Differential expression of CD13 isoforms in tumor vessels, epithelia and myeloid cells: a mechanism for the tumor homing properties of drugs containing the NGR motif. *Cancer Res.*, 62: 867–874, 2002.
- Arap, W., Kolonin, M. G., Trepel, M., Lahdenranta, J., Cardo-Vila, M., Giordano, R. J., Mintz, P. J., Ardelt, P. U., Yao, V. J., Vidal, V. I., Chen, L., Flamm, A., Valtanen, H., Weavind, L. M., Hicks, M. E., Pollock, R. E., Botz, G. H., Bucana, C. D., Koivunen, E., Cahill, D., Troncoso, P., Baggerly, K. A., Pentz, R. D., Do, K. A., Logothetis, C. J., and Pasqualini, R. Steps toward mapping the human vasculature by phage display. *Nat. Med.*, 8: 121–127, 2002.
- Bergers, G., Javaherian, K., Lo, K. M., Folkman, J., and Hanahan, D. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science (Wash. DC)*, 284: 808–812, 1999.
- Bergers, G., Brekken, R., McMahon, G., Vu, T. H., Itoh, T., Tamaki, K., Tanzawa, K., Thorpe, P., Itoharu, S., Werb, Z., and Hanahan, D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat. Cell. Biol.*, 2: 737–744, 2000.
- Thurston, G., Suri, C., Smith, K., McClain, J., Sato, T. N., Yancopoulos, G. D., and McDonald, D. M. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science (Wash. DC)*, 286: 2511–2514, 1999.
- Thurston, G., Rudge, J. S., Ioffe, E., Zhou, H., Ross, L., Croll, S. D., Glazer, N., Holash, J., McDonald, D. M., and Yancopoulos, G. D. Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nat. Med.*, 6: 460–463, 2000.
- Eliceiri, B. P., Paul, R., Schwartzberg, P. L., Hood, J. D., Leng, J., and Cheresh, D. A. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. *Mol. Cell*, 4: 915–924, 1999.
- Paul, R., Zhang, Z. G., Eliceiri, B. P., Jiang, Q., Boccia, A. D., Zhang, R. L., Chopp, M., and Cheresh, D. A. Src deficiency or blockade of Src activity in mice provides cerebral protection following stroke. *Nat. Med.*, 7: 222–227, 2001.

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