Vascular endothelial growth factor (VEGF)-A holds an exceptional position among the many molecules implicated in the regulation of blood vessel formation. During embryonic development, it controls a large number of processes, spanning from the expansion of the earliest cell progenitors of the vasculature to the control of proliferation and migration of endothelial cells, vessel remodeling, and arteriovenous specification (3). A correct level of VEGF-A protein is absolutely critical for vessel development, because a reduction of expression by half or an increase by twofold are both fatal conditions for a mouse embryo.

Given the array of critical roles for this multitargeted cytokine, it would appear dangerous to expose humans to potent VEGF-pathway inhibitors. However, the ability of such inhibitors to block formation of tumor vessels and cause regression of tumors in experimental animals has provided a strong rationale for also testing VEGF-pathway blocking agents in human cancer therapy (4). Some of these agents have already shown promise in clinical trials, and one of them, the Genentech anti-VEGF-A antibody bevacizumab (Avastin), was approved by the Food and Drug Administration in 2004 for treatment of metastatic colorectal carcinoma. The fact that relatively mild and few side effects were reported from the use of Avastin in these patients came as a pleasant surprise but at the same time appeared to confirm a generally held assumption that the mature and quiescent blood vessels of normal tissues are independent of VEGF-A. There are reasons for caution, however, because regional capillary regression has been noticed in adult experimental animals as a result of inhibition of VEGF signaling. The side effects of Avastin may also have been underestimated in some of the trials conducted thus far, because treated patients are both preselected and seriously ill. In fact, Genentech recently (September 23, 2005) announced the discontinuation of a phase II clinical trial of Avastin in ovarian cancer patients because of gastrointestinal perforations in 11% of the patients (see www.medicalnewstoday.com, 29 Sep 2005).

The observed side effects, e.g., hemorrhage, proteinuria, hypertension, congestive heart failure, arterial thromboembolism, and gastrointestinal perforation (www.avastin.com), in fact, underscore the suspicion that VEGF-A could have important homeostatic vascular functions also in adult humans. VEGFs may have extracellular functions as well. VEGF receptors are expressed by several other cell types than endothelial cells, including neuronal cells, and neurotrophic and neuroprotective effects of VEGF-A have been demonstrated in animals (9). Clearly, more studies are required to provide a better understanding of the roles of VEGF in the adult organism. This issue of American Journal of Physiology—Heart and Circulatory Physiology publishes two very timely reports from McDonald’s laboratory (1, 5) that carefully analyze in adult mice the vascular effects of inhibition of VEGF signaling. These studies provide a series of observations that significantly forward our understanding of the homeostatic functions of VEGF in the adult (Fig. 1). They also add important pieces of information to the puzzle of vascular stability and phenotypic plasticity of blood vessels.

The studies used, for most if not for all of the analyses, four different VEGF-pathway inhibitors with different modes of administration, mechanism of action, potency, and pharmacokinetics. The combination of the four inhibitors provides a degree of control for specificity that is unusual for this type of study. The kinase inhibitor AG-013736, which was used, potently blocks other receptor kinases than the VEGF receptors (VEGFRs), including platelet-derived growth factor receptor-β that has in itself a role in vascular development, whereas the three soluble VEGFRs are differentially specific for each VEGF. The agents also have different routes of administration and pharmacokinetic properties. In general, the four substances had similar effects, allowing for the important conclusion that the observed effects were caused by inhibition of VEGF-A.

Kamba et al. (5) report the effect of VEGF inhibition for 2–3 wk on vascular densities in 17 normal organs in adult mice. This survey confirmed and extended data previously reported from the McDonald’s laboratory. Vascular densities were reduced by ~20% to 70% in the thyroid, pancreatic islets, pituitary, small-intestinal villi, adrenal cortex, epididymal fat pads, and the choroid plexus. The extent of capillary bed regression was different among organs, and, depending on inhibitor, however, consistently strong effects were noticed in thyroid and pancreatic islets (with reduction up to 70%).

Interestingly, the VEGF-dependent capillary beds share common features; they are fenestrated and have high expression of VEGFR-2 and VEGFR-3. These features were also prominently downregulated in the capillaries that remained after VEGF inhibition. Previous studies (2a) have indicated that VEGF stimulation may induce fenestrations, but the effects have been weak and difficult to interpret, due, in part, to limitations of the in vitro systems studied. Ultrastructural studies have also revealed that fenestrated areas occur as differently sized patches of extreme thinning of the endothelial cell, in which the density of fenestrae is relatively constant. Kamba et al. used both scanning and transmission electron microscopy to show that the size of the fenestrated patches...
clearly the very surprising and provocative findings on blood glucose handling by other organs, e.g., liver or adipose tissue. How can these data be reconciled? The expected decrease in glucose tolerance, despite similarly low-density of caveolae as that of the fenestrations in the areas of cell thinning. These observations lend support to the idea that fenestrations may represent caveolae that fuse to both sides of the endothelial cell when cell thickness falls below the caveolar diameter. The way by which VEGF regulates fenestration may therefore involve regulation of endothelial cell shape. It was previously described that VEGFR-3 is highly expressed by fenestrated capillaries in endocrine organs (7). The observations by Kamba et al., that virtual extinction of VEGFR-3 expression accompanies loss of fenestration as a result of VEGF inhibition, may suggest a specific role for VEGFR-3 signaling in mediating VEGF-A effects on fenestration in adult microvessels.

Reduction of vessel density and area of fenestration in endocrine organs would be assumed to have substantial effects on endocrine functions. Although the exact role for the capillary fenestrations in most organs is unclear (with the exception of the kidney glomeruli, where the fenestrations are needed for filtration), one would assume that the fenestrations facilitate both the rapid sensing of changes in blood composition and the response to it (i.e., rapid hormone distribution), which are characteristic features of many endocrine glands. To address the physiological consequences of VEGF inhibition, Kamba et al. made a preliminary record of certain basic physiological parameters in the treated mice. The clearest result, induced by all VEGF inhibitors, was lowered steady-state blood glucose levels and increased glucose tolerance. This is exactly the opposite of what one would expect. The result also opposes that of genetic VEGF-A ablation in islets, which produced an expected decrease in glucose tolerance, despite similarly lowered vessel density and reduced fenestration as observed after VEGF inhibition (6). How can these data be reconciled? The answer probably lies in the effect of VEGF inhibition on glucose handling by other organs, e.g., liver or adipose tissue. Clearly, the very surprising and provocative findings on blood glucose level after VEGF inhibition deserves further study, which may lead to the discovery of new and perhaps beneficiary side effects of anti-VEGF therapy.

Proteinuria was an important parameter to score in the VEGF-inhibited mice, given the profound VEGF dependence of developing glomerular capillaries (2) and the fact that proteinuria is one of the reported side effects of Avastin (13; see also Avastin Safety Sheet at www.avastin.com). Although 2 wk of VEGF inhibition did not cause consistent proteinuria in all treated animals, it should be remembered that proteinuria likely requires changes of the glomerular podocyte foot processes and their joining size-selective slit diaphragms (11). Such changes may occur successively to primary changes in other cell types in the glomerulus (endothelial or mesangial cells) and develop with increased time of VEGF inhibition. It will therefore be important to carefully monitor proteinuria and kidney function also after prolonged exposure to VEGF inhibitors.

In the study by Baffert et al. (1), McDonald’s laboratory addresses the nature and sequence of events leading to capillary regression on VEGF inhibition. To this end they focused their attention on a single organ, the trachea, where the capillary pattern is regular and simple. Within 1 day, they observed that individual branches lost patency, as demonstrated by disrupted marker perfusion. This was accompanied by the formation of fibrin clots and endothelial apoptosis. Loss of endothelial cells led to the formation of empty basement membrane sleeves, to which the pericytes initially remained attached. Subsequently, pericytes were lost from the sleeves, probably by retreat to the nearest intact vessel segment. Finally, the empty sleeves disintegrated. This sequence of events appears most logical. The trigger of vascular regression seems to be a local detachment or death of endothelial cells, which is in agreement with the established function of VEGF as an endothelial survival factor. It is obviously less clear why the role of VEGF as a fenestration and survival factor should go hand-in-hand in the adult microcirculation.

At first glance, this sequence of events differs from that reported to occur in tumor vessels after inhibition of VEGF signaling. A recent study (12) from Jain’s laboratory demonstrated by live recordings of tumor vasculature that VEGFR-2 inhibition by a blocking antibody initially led to vessel normalization (as reflected by both anatomical and functional parameters), accompanied by a substantial increase in pericyte coverage. Subsequently, the pericyte-covered vessels regressed. A number of factors could explain the seemingly opposing results, including different inhibitor specificities and diverging interpretations based on the different methodologies used (e.g., static vs. dynamic imaging) and the different biology of tumor and normal vessels. However, the results of the two studies may be reconciled if one considers that tumor vessels are initially abnormal and if these abnormalities partly result from too high VEGF levels. Several lines of evidence suggest that superphysiological VEGF levels lead to morphological and functional vessel abnormalities, including the loss of pericytes (8). If tumor vessel abnormalities result from too much of VEGF signaling, one would also expect them to be more sensitive to VEGF inhibition than the normal vessels, which is, in fact, observed by Kamba et al. It is therefore important to ask whether the further regression of the normalized tumor vessels might be similar to the regression of the normal tracheal vessels in its course of events and sensitivity to VEGF-pathway inhibition. If so, one may further ask whether the most beneficial effect of VEGF inhibition on tumor vessels might be a functionally normalized vasculature, providing facilitated drug delivery to the tumor and improved radiation
response (10). Is it at all warranted to aim at reducing tumor vessel density beyond the initial normalization window, creating an over-regressed tumor vasculature? Could potent VEGF inhibition, with the idea of regressing tumor vasculature maximally, cause the combined unwanted effects of hypoxia-induced increase in tumor aggressiveness, radiation resistance, obstructed drug delivery, and, additionally, serious side effects from regression of normal vessels? The fact that these questions currently lack answers points to the importance of careful studies that monitor the nature, effects, and sequence of events in vessel regression after VEGF inhibition, as exemplified by the two current papers from McDonald’s laboratory (1, 5).

REFERENCES


