

# Incipient Angiogenesis

Judah Folkman

The hypothesis that tumor growth is angiogenesis dependent (1,2) was first proposed in 1971 based on observations that expansion of a tumor mass was limited in the absence of angiogenesis. Since then, considerable experimental supporting evidence for this concept has been assembled from inhibition of angiogenesis by the following observations: 1) mechanical separation of tumor cells from their nearest vascular bed (3), 2) blockade of tumor-derived angiogenic factors (4), 3) administration of angiogenesis inhibitors (5), 4) blockade of endothelial receptors for angiogenic factors (6), 5) endogenous production of angiogenesis inhibitors from tumor cells (7,8), and 6) demonstration of the preangiogenic phenotype in spontaneous tumors (9). The size limits of experimental tumors when angiogenesis is blocked or absent are between approximately 0.2 mm in diameter [for lung metastases in mice (10)] and 2 mm, i.e., a tumor population of  $10^5$ – $10^6$  cells (for avascular chondrosarcomas in rats [Fang J, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, et al.; unpublished results]). The differences in size of avascular preangiogenic tumors may be due in part to the capacity of tumor cells to survive under differing degrees of hypoxia (11).

In an elegant report in this issue of the Journal, Li et al. (12) analyzed the earliest events that take place before and during the onset of tumor neovascularization. The authors implanted mammary cancer cells with the angiogenic phenotype into a transparent skin chamber in mice and rats. The cancer cells were visible because they expressed green fluorescence protein. This report shows that, if cancer cells are already angiogenic at the time of implantation, they can initiate angiogenesis long before the tumor population would have reached the limiting size of a non-neovascularized tumor, i.e., 0.2–2 mm.

In the study by Li et al., after only 20–50 tumor cells were injected into the chamber, the cells elongated by day 2, migrated toward the nearest microvessels in a parallel orientation by day 4, divided unidirectionally and induced vascular dilation and tortuosity by day 6, and stimulated new vascular sprouts with intermittent blood flow by day 8. By that time, a microscopic colony of up to 300–400 cells existed. Tumor cells preferentially grew contiguous to the microvessel sprouts, and by day 20 the tumor was filled with a newly formed vasculature.

A provocative finding of the study by Li et al. is that injection of a truncated soluble receptor for vascular endothelial growth factor (VEGF) led to tumor cell apoptosis, tumor regression, or suppression of tumor growth within 5 days before the appearance of neovascular sprouts. This receptor (ex-flk1), however,

had no effect on tumor cells *in vitro*, while it had a potent antiproliferative effect on endothelial cells. This result provides compelling evidence for the operation of a two-way paracrine exchange of growth factors and survival factors between tumor cells and neighboring vascular endothelial cells. In this model, tumor cells secrete angiogenic proteins that activate endothelial cells to elaborate chemoattractants for tumor cells. Simultaneously, the activated endothelium is preparing to send new sprouts toward the tumor. Vascular endothelial cells can produce at least 20 mitogens and antiapoptotic factors (13). Many of these proteins, such as basic fibroblast growth factor and heparin-binding epithelial growth factor, are stored in the extracellular matrix and could be mobilized by VEGF stimulation of endothelial cells to secrete proteases (14).

It is also possible that VEGF elaborated from the tumor cells increases the permeability of local microvessels so that the tumor microcolony is bathed in nutrients even before neovascularization begins. The diffusion of nutrients (and oxygen) into the tumor bed would also be facilitated by loss of pericytes from microvessels due to endothelial elaboration of angiopoietin-2 (15). Endothelial cells express angiopoietin-2 in the presence of tumor cells by an unknown mechanism. Furthermore, plasma and fibrin leakage from the microvessels could facilitate chemotaxis and alignment of tumor cells (16), as well as subsequent migration of endothelial cells (17).

The findings reported by Li et al. in this issue also provide an explanation for why long-term antiangiogenic therapy of tumor-bearing animals prevents the growth of some tumors and appears to eradicate others (5,18). It remains to be seen if long-term antiangiogenic therapy in humans will have a similar effect.

The vasodilation and tortuosity of microvessels that precede sprout formation may result from increased synthesis of nitric oxide, which is induced by VEGF [(19,20); reviewed in (21)]. By allowing endothelial cell elongation and spreading, vasodilation of microvessels may permit mitosis and migration of endothelial cells in response to mitogens (e.g., VEGF from the tumor cells) (21). Even highly transformed neoplastic cells respond more efficiently to mitogens when the cells are elongated or spread than when they are rounded (22).

---

Correspondence to: Judah Folkman, M.D., Children's Hospital, Hunnewell 103, 300 Longwood Ave., Boston, MA 02115 (e-mail: foss@hub.tch.harvard.edu).

© Oxford University Press

The most important contribution of the report by Li et al. is that it enlarges our understanding of the mechanism of antiangiogenic therapy. Until now, the conventional wisdom among angiogenesis researchers has been that angiogenesis inhibitors operate only after neovascularization has occurred. The authors clearly show that at least one angiogenesis inhibitor can function before a tumor has become neovascularized and lead to inhibition or eradication of a nascent tumor. If this finding holds up for other angiogenesis inhibitors, it provides a rational basis for a new use of antiangiogenic therapy: prevention of cancer in patients at high risk for the disease (23). It also provides a novel conceptual framework for the antiangiogenic activity of epigallocatechin-3-gallate in green tea (24), as well as for the antiangiogenic activity of aspirin in the prevention of human colon cancer (25).

The take-home message from the report by Li et al. is that close association of tumor cells with vascular endothelial cells, in the absence of any restraint on the paracrine chemotactic and mitogenic interactions between these two cell populations, is potentially dangerous to the host.

## REFERENCES

- (1) Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285:1182-6.
- (2) Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg* 1972;175:409-16.
- (3) Gimbrone MA Jr, Leapman S, Cotran R, Folkman J. Tumor dormancy *in vivo* by prevention of neovascularization. *J Exp Med* 1972;136:261-76.
- (4) Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature* 1993;362:841-4.
- (5) Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* 1997;390:404-7.
- (6) Millauer B, Shawver LK, Plate KH, Risau W, Ullrich A. Glioblastoma growth inhibited *in vivo* by a dominant-negative Flk-1 mutant. *Nature* 1994;367:576-9.
- (7) Bouck N. Tumor angiogenesis: the role of oncogenes and tumor suppressor genes. *Cancer Cells* 1990;2:179-85.
- (8) Cao Y, O'Reilly MS, Marshall B, Flynn E, Ji RW, Folkman J. Expression of angiostatin cDNA in a murine fibrosarcoma suppresses primary tumor growth and produces long-term dormancy of metastases. *J Clin Invest* 1998;101:1055-63.
- (9) Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;86:353-64.
- (10) Holmgren L, O'Reilly MS, Folkman J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat Med* 1995;1:149-53.
- (11) Helmlinger G, Yuan F, Dellian M, Jain RK. Interstitial pH and pO<sub>2</sub> gradients in solid tumors *in vivo*: high-resolution measurements reveal a lack of correlation. *Nat Med* 1997;3:177-82.
- (12) Li CY, Shan S, Huang Q, Braun RD, Lanzan J, Hu K, et al. Initial stages of tumor cell-induced angiogenesis: evaluation via skin window chambers in rodent models. *J Natl Cancer Inst* 1999;92:143-7.
- (13) Rak J, Filmus J, Finkenzeller G, Grugel S, Marme D, Kerbel RS. Oncogenes as inducers of tumor angiogenesis. *Cancer Metastasis Rev* 1995;14:263-77.
- (14) Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat Med* 1999;5:623-8.
- (15) Holash J, Wiegand SJ, Yancopoulos GD. New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. *Oncogene* 1999;18:5356-62.
- (16) Senger DR, Brown LF, Claffey KP, Dvorak HF. Vascular permeability factor, tumor angiogenesis and stroma generation. *Invasion Metastasis* 1994-92;14:385-94.
- (17) Kadish JL, Butterfield CE, Folkman J. The effect of fibrin on cultured vascular endothelial cells. *Tissue Cell* 1979;11:99-108.
- (18) O'Reilly MS, Holmgren L, Chen C, Folkman J. Angiostatin induces and sustains dormancy of human tumors in mice. *Nat Med* 1996;2:689-92.
- (19) Ziche M, Morbidelli L, Choudhuri R, Zhang HT, Donnini S, Granger HJ, et al. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J Clin Invest* 1997;99:2625-34.
- (20) Papapetropoulos A, Garcia-Cardena G, Madri JA, Sessa WC. Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. *J Clin Invest* 1997;100:3131-9.
- (21) Garcia-Cardena G, Folkman J. Is there a role for nitric oxide in tumor angiogenesis? *J Natl Cancer Inst* 1998;90:560-1.
- (22) Wittelsberger SC, Kleene K, Penman S. Progressive loss of shape-responsive metabolic controls in cells with increasingly transformed phenotype. *Cell* 1981;24:859-66.
- (23) Bergers G, Javaherian K, Folkman J, Hanahan D. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science* 1999;284:808-12.
- (24) Cao Y, Cao R. Angiogenesis inhibited by drinking tea. *Nature* 1999;398:381.
- (25) Shiff SJ, Rigas B. Aspirin for cancer. *Nat Med* 1999;5:1348-9.