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Tumor angiogenesis: A physiological process or genetically determined?

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Abstract

Continued tumor growth is dependent upon the growth of new blood vessels. This commentary reviews the mechanisms whereby tumors become vascularized and examines whether tumor angiogenesis is solely an example of a normal physiologic process or is part of the genetic program of the tumor. The likelihood that neovascularization of tumors combines both of these components, that is, utilizing tumor-specific elements as well as capacities common to all cells, is discussed.

It is well-established that continued tumor expansion is dependent upon the growth of new vessels for the delivery of oxygen and nutrients and for the removal of waste products. Early studies by Folkman and his coworkers revealed static tumor size in the absence of vessel ingrowth and an exponential increase in tumor volume upon vascularization [1]. More recently, studies in which the activities of known angiogenic factors were manipulated have provided direct evidence for the dependency of tumors on neovascularization. Kim et al. have shown that the administration of vascular endothelial cell growth factor (VEGF) neutralizing antibodies inhibits the growth of subcutaneous human xenografts in nude mice [2]. Millauer and her colleagues, using a retrovirus expressing a dominant negative flk-1 protein, a VEGF receptor, demonstrated the suppression of C6 glioma in nude mice [3]. Finally, Warren et al. reported that the administration of VEGF neutralizing monoclonal antibodies to nude mice with subcutaneous implants of human colon carcinomas led to smaller, less vascularized tumors [4]. In addition, metastases in these animals were dramatically reduced in number, and were all smaller than a cubic millimeter and avascular.

The goal of this commentary is to raise the question of whether tumor angiogenesis is an inherent physiologic process, that is, an example of a tissue signaling its environment to provide increased vascularization, or whether the capacity to induce vessels is itself a part of the tumorigenic process. For the sake of argument, these two hypotheses are stated below at their most extreme (Figure 1).

Hypothesis 1: Tumor angiogenesis, like angiogenesis of non-neoplastic tissues, is physiologically regulated by the tissue's metabolic needs

Angiogenesis will occur when there is a sustained and local need for increased blood flow. The two conditions that lead to neovascularization include: (1) increased metabolic load, as in chronic exercise, formation of the placenta, fetal development or increased tissue mass (including tumors) and (2) insufficient blood flow, usually the result of a vaso-occlusive process, as in proliferative diabetic retinopathy and ischemic heart disease. When these conditions occur, normal physiologic responses will

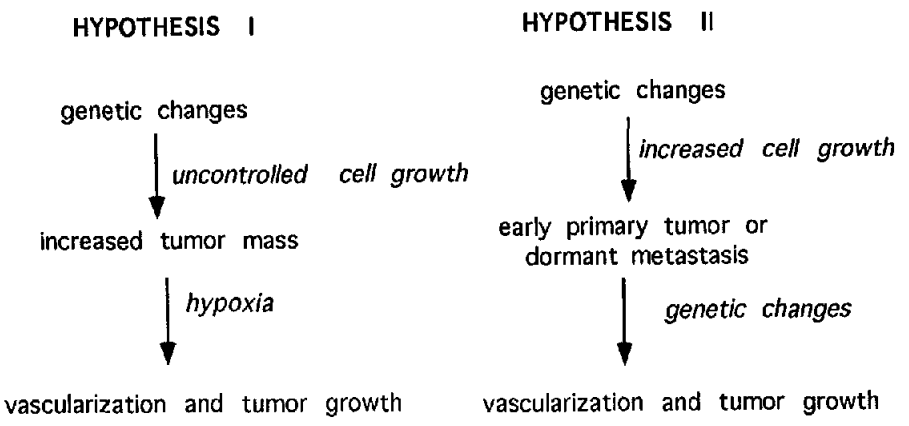

Figure 1. Two hypotheses on the mechanism of tumor vascularization. Hypothesis 1 states 'Tumor angiogenesis, like angiogenesis of non-neoplastic tissues, is physiologically regulated by the tissue's metabolic needs'. Hypothesis 2 states 'Neovascularization of tumors is a part of the genetically-based tumor progression'.

be invoked, including the expression of angiogenic factors which in turn increase vascularity.

Hypothesis 2: Neovascularization of tumors is a part of the genetically-based tumor progression

In the transition from a normal to a tumorigenic cell, there are changes that lead not only to unregulated cell growth, but also to the ability of those cells to invoke new blood vessels.

The first hypothesis states that 'Tumor angiogenesis, like angiogenesis of non-neoplastic tissues, is physiologically regulated by the tissue's metabolic needs.' Increased tissue mass leads to reduced local oxygen concentrations, reflecting the 'need' for vessels, and inducing angiogenesis in an hypoxic/ischemia driven-fashion. A local deficit in oxygen can be caused by traumatic injury to tissues, such as occurs in wounding, by vaso-occlusion that characterizes proliferative diabetic retinopathy and sickle cell retinopathy or by an increased metabolic load, which is observed in chronic exercise, tissue hyperplasia (e.g. atherosclerotic plaques) and tumor growth. Each of these conditions leads to new vessel growth.

Clear examples of this cause-and-effect relationship between oxygen requirements and vasoproliferation have been provided for vascularization during development and in the adult. Stone & Keshet and their coworkers have shown that the developmental vascularization of the mouse retina (which occurs postnatally) is driven by hypoxia that results from the increased oxygen consumption of the differentiated neural tissue [5]. Supplying the needed oxygen exogenously (by rearing the animals in hyperoxia) blunted the hypoxic signal and suppressed vessel growth [5]. The studies of Olga Hudlicka address new vessel growth in the adult and quantitatively document an increase in capillary density in skeletal muscle of rodents subjected to a long-term stimulation [6, 7].

As this concept applies to tumor growth, it suggests that tumor angiogenesis is mediated by built-in, normal physiological process(es). In tumorigenesis, a series of genetic aberrations leads to the enhanced capacity of cells to divide and survive outside of the normal rules of differentiation and tissue architecture. After accumulating enough advantageous alterations, a net shift in tumor cell survival is initiated (clonally) with the resulting increased metabolic load triggering an angiogenic response to support the increased tissue mass. Thus, once tumor cells escape the confines of normal growth control processes and begin to grow, they behave as the cells of any other tissue, exercising the ability to elicit new blood vessels.

What molecule(s) do tumor cells use to induce neovascularization? Convincing evidence has been generated to suggest that neovascularization is, un-

der many circumstances, mediated by VEGF in an hypoxia-driven process. Many cells have the capacity to synthesize VEGF and may normally do so constitutively at a low level. VEGF expression can be increased by hypoxia, which in a tissue culture system is an artificial means of simulating an increased metabolic load [8, 9]. With the recognition that ischemia/hypoxia is common to virtually all conditions which are characterized by neovascularization, the concept arises that local hypoxia leads to the induction of VEGF, which acts, in turn, in a paracrine manner to induce neovascularization.

Two groups independently reported that tumor cells of glioblastoma multiforma express VEGF mRNA in a pattern consistent with expression that is driven by relatively low oxygen concentrations [8, 10]. Keshet and his coworkers showed by *in situ* hybridization that VEGF mRNA is localized to a population of cells bordering necrotic foci (palisade cells), whereas mRNA for the VEGF receptor flk is in neighboring endothelial cells. In an elegant *in vitro* demonstration of this phenomenon, Keshet and colleagues, using a tumor spheroid system, demonstrated upregulation of VEGF in the more centrally located, hypoxic cells. Transplantation of the spheroids into nude mice led to their neovascularization and to the down-regulation of VEGF [11]. Coupled with a demonstrated role for VEGF in experimental tumor angiogenesis (and growth) [2–4], the observation of VEGF expression in a wide variety of tumors [12] suggests that the growth of many tumors is VEGF-dependent.

Two other situations in which VEGF has been directly demonstrated to mediate neovascularization relate to the retina. Retinal ischemia has been demonstrated to lead to the upregulation of VEGF, which causes an induction of new blood vessels on the retina [13] and iris [14, 15]. Similarly, the normal vascularization of the retina, which has been postulated to be an hypoxia-driven event (described above), has been shown to be mediated by VEGF [5].

Thus, it seems likely that at least some proportion of angiogenesis is hypoxia driven and mediated by VEGF. The question remaining is whether all angiogenesis is controlled in this fashion. A plethora of angiogenic factors have been described in the past decade and it is unclear where these polypeptide growth factors fit into the picture. There is little convincing data that directly demonstrate a causal role for *any* of the other angiogenic factors in *any* physiologic angiogenesis, be it normal or pathologic.

The second hypothesis states that 'Neovascularization in tumors is a part of the unique tumorigenic process.' The assumption which underlies this hypothesis is that tumor cells acquire an advantage, via a genetic alteration, that promotes angiogenesis and *allows* tumor growth. Evidence consistent with this hypothesis comes from several experimental systems.

Hanahan and his coworkers (reviewed in [18]) have developed several transgenic mouse lines that provide models to examine the progression of tumor cells from a hyperplastic stage to an invasive tumor. Mice expressing the bovine papilloma virus develop fibrosarcomas and this model has been used to study the cellular export of endothelial cell mitogens as a basis for the angiogenic 'switch'. Kandel and her coworkers correlated the ability of fibrosarcoma cells to release bFGF with their vascularity and tumorigenicity [19]. Whereas cells isolated from tumors early in development (mild fibromatoses) synthesized bFGF that remained cell-associated, more advanced tumors (fibrosarcomas) released more that 75% of their bFGF. Though cell death is often cited as a mechanism for bFGF release, lactate dehydrogenase levels indicate that cell lysis is not the mechanism for growth factor release in this model. The authors, therefore, hypothesize that the tumor cells 'switch' on a novel mechanism for the export of bFGF. A critical piece of evidence to prove this hypothesis would be the direct demonstration that the tumors in question are, in fact, bFGF-dependent for their continued growth. It will also be of interest to learn more about the mechanisms by which bFGF is released in this system. Though there are numerous anecdotal reports of bFGF release, there are no convincing data on the means by which this protein, which lacks a signal peptide, is exported.

In another transgenic mouse model in which the large 'T' antigen of SV40 is under the control of the insulin promoter, tumors arise from pancreatic is-

lets in a reproducible manner, with 4–10% of the islets becoming vascularized tumors within 2 months. The molecular basis the progression of this islet subpopulation to tumors is not fully understood. It may be an event that leads directly to the expression of angiogenic factors. However, though these cells have been shown to express several known angiogenic factors, including aFGF and VEGF, their levels do not change concomitant with vascularization [17]. Alternatively, as the authors postulate, the progression to tumors may result from a final genetic change that leads to loss of growth control, with vascularization resulting from the increased tissue load. In support of the latter concept, Hanahan and his colleagues reported that the transition of these cells to a tumor phenotype is correlated with loss of heterozygosity on chromosomes 9 and 16, possibly representing the loss of a tumor suppressor [20].

Normal physiologic angiogenesis is strongly suspected to be locally controlled by a fine balance between positive and negative regulators (for review see [16, 17]). Tumor angiogenesis appears to be influenced by these same regulators. The concept of an angiogenic switch was expanded by the work of Polverini, Bouck and their coworkers to include the elimination of angiogenesis inhibitory signals. Initial studies reported an activity capable of suppressing neovascularization in the conditioned media of hamster cells [21]. Using transformants and revertants, the investigators showed that the presence of the inhibitor, later shown to be thrombospondin (TSP) [22], was linked to a tumor suppressor gene. In studies of late passage fibroblasts from Li-Fraumeni patients, Bouck and her colleagues found that the switch to an angiogenic phenotype, measured by the effect of conditioned media on corneal neovascularization, involved the loss of the wild-type allele of the tumor suppressor p53 [23]. In support of a direct relationship between the loss of p53 and TSP-1 down-regulation, reintroduction of p53 into the fibroblasts led to increased TSP-1 mRNA and restoration of the anti-angiogenic phenotype [23].

Unanswered questions regarding these data include issues of the universality of the phenomenon (i.e. does this apply to cells other than fibroblasts). Consistent with this concept, a line of EC immortalized by polyoma middle T was reported to lack TSP-1 [24]. Reintroduction of TSP-1 into these cells restored their normal phenotype and suppressed tumorigenesis [25]. In another report, p53 null glioblastoma cells were shown to express angiogenic activity that was blocked upon the introduction of an inducible form of wild type p53. Though this inhibitor was not identified as TSP-1, these observations are consistent with the earlier correlation noted between the loss of p53 and reduction in anti-angiogenic activity. A second critical question is whether the loss of TSP-1 directly facilitates the ability of tumors to induce vascularization *in vivo*.

An alternative mechanism for the disruption of local vascular control is a tip in the balance in favor of growth stimulation (reviewed [26]). Oncogenic transformation of NIH 3T3 cells with either v-Ha-ras or v-raf was shown to lead to enhanced expression of VEGF mRNA [27]. Furthermore, expression of mutant ras resulted in the upregulation of VEGF mRNA in transformed epithelial cells; genetic disruption of mutant k-ras lead to a reduction of VEGF activity [28].

Although VEGF can induce new vessel growth, the key question with respect to the relevance of these observations is whether overexpression of VEGF can drive tumor growth. Data to suggest that this is so comes from recent work of Claffey et al. [29]. A human melanoma line, SK-MEL-2, which was shown to express low levels of VEGF *in vitro*, formed small and poorly vascularized tumors in nude mice. Following stable transfection with VEGF cDNA the cells formed large, well-vascularized tumors and were more metastatic. Cells stably transfected with an antisense VEGF construct expressed negligible levels of VEGF and led to tumors that were vascularized even less than those arising from the wild type counterpart. These data support the concept that low VEGF levels can be rate limiting for tumor growth. However, it does not directly address the question of whether overexpression (of the kind resulting from oncogenic transformation) is a prerequisite for successful tumorigenesis or whether it confers any growth advantage to the tumors. All of the oncogenes used to transform cells results in multiple phenotypic changes, making it difficult to assess the relative contribution of a single variable. In addition, stud-

ies are necessary to definitively demonstrate that growth factor overexpression (e.g. in the absence of hypoxia) is essential to the growth of non-experimental tumors *in situ*.

Tumor angiogenesis is controlled by both physiological and genetic events

Angiogenesis is a complex process that is critical to the growth and survival of most tissues. The possibility that tissue vascularity is regulated by oxygen tension is an appealing concept because of its self-regulating nature and economic efficiency. When oxygen deficit is acute and confined (such as in a burst of exercise), the tissue's response is equally acute and local, resulting in vasodilation and local increases in blood flow. When the hypoxia is systemic (as occurs at high altitudes), the response is systemic. The hormone erythropoietin is induced, leading to elevated red cell production and increased oxygen carrying and delivery capacity. If the local oxygen deficit is chronic, as occurs with vaso-occlusion or increased tissue mass, the response is increased vascularity. According to this 'rule', the neovascularization of a tumor may not be qualitatively different from the new blood vessels that form in response to the increased muscle mass in body builders.

If uncontrolled cell growth is itself sufficient to elicit neovascularization, how does one explain dormant metastases? Since metastases arise from the same cells that 'successfully' initiated the primary tumor, why should there be a stage of dormancy? If the cells of the primary tumor have undergone genetic changes that have rendered them exempt from normal growth control mechanisms and/or rendered them angiogenic, why are these same cells not able to induce angiogenesis and grow at the new site?

Firstly, it is known from the recent studies of Holmgren, O'Reilly and Folkman that dormancy is not equivalent to quiescence [30]. They showed that the proliferation of tumor cells in dormant lung metastases in mice is not significantly different from that in growing metastases. Dormant tumors, however, have a three-fold higher incidence of apoptosis compared to the growing metastases. Secondly, it is not known what occurs in the early stages of a primary tumor. Though it is well-accepted that a combination of genetic aberrations leads to the loss of normal growth control, there is no direct evidence that the final genetic change leads to immediate uncontrolled cell growth and tumor formation. It is reasonable to believe that the normal mechanisms, which regulate vascularization in normal tissues, are sufficient to control and/or suppress vascularization in the early stages of tumorigenesis. In fact, this is what appears to occur in carcinoma *in situ*, where there is hyperplasia, but no neovascularization. Thus, it is possible that the dormancy observed in metastases is not different than that occurring in the development of primary tumors. In other words, angiogenic signals, following metabolic demands, might be balanced by counteracting signals.

What then allows the dormant metastasis to be released from its 'dormancy'? One possibility (discussed above) is that the cells begin to make and/or export increased levels of angiogenic factors. However, since even normal cells appear to have the capacity to elicit new vessel growth, why should the ability to produce angiogenic factors be rate-limiting? Alternatively, consider the possibility (reviewed recently by Folkman [17, 31])) that the control lies at the level of the microenvironment.

What are the mechanisms that tissues use to negatively control vascularization? Numerous candidates for angiogenesis inhibitors have been postulated, including interaction between endothelial cells and pericytes, leading to the local activation of TGF-β [32], the local expression of TSP-1 [22], and the production of circulating angiostatic factors (e.g. angiostatin) by the primary tumor [33], to name a few. Normal mechanisms of growth regulation may therefore successfully control the micrometastases, until the balance is disrupted. A disturbance in the microenvironment may take many forms, including alterations in the basement membrane mediated by the tumor cells (i.e. their proteolytic enzymes), loss of local inhibitors (e.g. TGF-β) due to altered vascular cell interactions, loss of circulating inhibitors (angiostatin) due to removal of the primary tumor or an introduction of cytokines secondary to an inflammatory response.

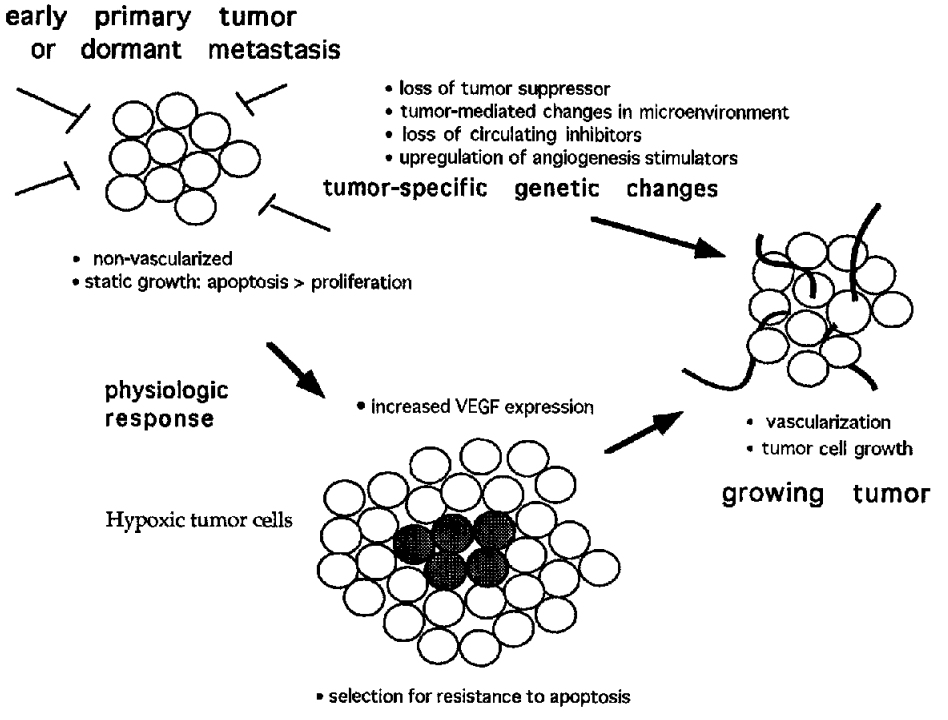

Figure 2. Tumor angiogenesis is controlled by both physiological and genetic events.

These, again, can be sorted into physiological mechanisms (cell-cell interactions or inflammatory response) and tumor-specific genetic changes (e.g. loss of a tumor suppressor that impacts on vascular control) (reviewed by [34]). A recent study provides clear evidence of the convergence of physiological mechanisms with tumor-specific processes. Graeber et al. report that although hypoxia can induce apoptosis in oncogenically transformed cells, further genetic alterations, such as the loss of p53, reduce hypoxia-induced cell death [35]. Thus, in addition to acting as a stimulus for the production of angiogenic factors, hypoxia appears 'to provide a selective pressure in tumours for the expansion of variants that have lost their apoptotic potential'. Further, cells lacking p53 are not only resistant to hypoxia [35], but they have lost their ability to make a negative regulator of angiogenesis [23]. Thus, the growth promoting effects of hypoxia and p53 loss provide both a direct growth advantage to tumor cells and facilitate the tumor neovascularization (Figure 2) [36].

In conclusion, the contributions of (i) vessel recruitment by angiogenic stimuli, mediated by normal physiologic mechanism (largely hypoxia-driven); (ii) overproduction of angiogenic factor by tumors; and, (iii) loss of local negative regulators due to genetic or epigenetic tumor-specific alterations, should all be considered in understanding the regulation of tumor angiogenesis.

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References

1. Folkman J: Anti-angiogenesis: new concept for therapy of solid tumors. Ann Surg 175: 409–416, 1972
2. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N: Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth *in vivo*. Nature 362: 841–844, 1993
3. Millauer B, Shawver LK, Risau W, Ullrich A: Glioblastoma growth inhibited *in vivo* by a dominant-negative Flk-1 mutant. Nature 367: 576–579, 1994
4. Warren RS, Yuan H, Matli MR, Gillett N, Ferrara N: Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. J Clin Invest 95: 1789–1797, 1995
5. Stone J, Itin A, Alon T, Pe'er J, Gnessin H, Chan-Ling T, Keshet E: Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. J Neurosci 15: 4738–4747, 1995
6. Hudlicka O: Development and adaptability of microvasculature in skeletal muscle. J Exp Biol 115: 215–228, 1985
7. Hudlicka O, Brown M, Egginton S: Angiogenesis in skeletal and cardiac muscle. Physiol Rev 72: 369–417, 1992
8. Shweiki D, Itin A, Soffer D, Keshet E: Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359: 843–845, 1992
9. Shima DT, Adamis AP, Yeo K-T, Yeo T-K, Allende R, Folkman J, D'Amore PA: Hypoxic induction of endothelial cell growth factors in retinal cells: identification and characterization of vascular endothelial growth factor (VEGF) as the mitogen. Mol Med 1: 182–193, 1995
10. Plate KH, Breier G, Weich HA, Risau W: Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo*. Nature 359: 845–848, 1992
11. Shweiki D, Neeman M, Itin A, Keshet E: Induction of vascular endothelial growth factor expression by hypoxia and by glucose deficiency in multicell spheroids: implication for tumor angiogenesis. Proc Natl Acad Sci USA 92: 768–772, 1995
12. Dvorak H, Brown L, Detmar M, Dvorak A: Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Path 146: 1029–1038, 1995
13. Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LEH: Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc Natl Acad Sci USA 92: 905–909, 1995
14. Adamis AP, Shima DT, Tolentino MJ, Gragoudas ES, Ferrara N, Folkman J, D'Amore PA, Miller JW: Inhibition of VEGF prevents ocular neovascularization in a primate. Arch Ophthalmol 114: 66–71, 1996
15. Miller JW, Adamis AP, Shima DT, D'Amore PA, Moulton RS, O'Reilly MS, *et al.*: Vascular permeability factor/vascular endothelial cell growth factor is temporally and spatially correlated with ocular angiogenesis in a primate model. Am J Pathol 145: 574–584, 1994
16. D'Amore PA: Mechanisms of endothelial growth control. Am J Respir Cell Mol Biol 6: 1–8, 1992
17. Folkman J: Angiogenesis inhibitors generated by tumors. Mol Med 1: 120–122, 1996
18. Christofori G, Hanahan D: Molecular dissection of multistage tumorigenesis in transgenic mice. Semin Cancer Biol 5: 3–21, 1994
19. Kandel J, Bossy-Wetzel E, Radvanyi F, Klagsbrun M, Folkman J, Hanahan D: Neovascularization is associated with a switch to the export of bFGF in the multistep development of fibrosarcoma. Cell 66: 1095–1104, 1991
20. Dietrich WF, Radany EH, Smith JS, Bishop JM, Hanahan D, Lander ES: Genome-wide search for loss of heterozygosity in transgenic mouse tumors reveals candidate tumor suppressor genes on chromosomes 9 and 16. Proc Natl Acad Sci USA 91: 9451–9455, 1994
21. Rastinejad F, Polverini PJ, Bouck NP: Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. Cell 56: 345–355, 1989
22. Good DJ, Polverini PJ, Rastinejad F, Le Beau MM, Lemons RS, Frazier WA, Bouck NP: A tumor suppressor dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. Proc Natl Acad Sci USA 87: 6624–6628, 1990
23. Dameron KM, Volpert OV, Tainsky MA, Bouck N: Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 265: 1582–1584, 1994
24. RayChaudhury A, Frazier WA, D'Amore PA: Comparison of normal and tumorigenic endothelial cells: differences in thrombospondin production and responses to transforming growth factor-beta. J Cell Sci 107: 39–46, 1994
25. Sheibani N, Frazier WA: Thrombospondin 1 expression in transformed endothelial cells restores a normal phenotype and suppresses their tumorigenesis. Proc Natl Acad Sci USA 92: 6788–6792, 1995
26. Rak J, Filmus J, Finkenzeller G, Grugel S, Marme D, Kerbel RS: Oncogenes as inducers of tumor angiogenesis. Cancer Met Rev 14: 263–277, 1995
27. Grugel S, Finkenzeller G, Weindel K, Barleon B, Marme D: Both v-Ha-ras and v-raf stimulate expression of the vascular endothelial growth factor in NIH 3T3 cell. J Biol Chem 270: 25915–25919, 1995
28. Rak J, Bayko L, Filmus J, Shirasawa S, Kerber RS: Mutant ras oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. Cancer Res 55: 4575–4580, 1995
29. Claffey KP, Brown LF, del Aguila LF, Tognazzi K, Yeo K-T, Manseau EL, Dvorak HF: Expression of vascular perme-

ability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. Cancer Res 56: 172–196, 1996
30. Holmgren L, O'Reilly MS, Folkman J: Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nature Med 1: 149–153, 1995
31. Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nature Med 1: 27–31, 1995
32. Antonelli-Orlidge A, Saunders KB, Smith SR, D'Amore PA: An activated form of TGF-β is produced by cocultures of endothelial cells and pericytes. Proc Natl Acad Sci USA 86: 4544–4548, 1989
33. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, *et al.*: Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 315–328, 1994
34. Lu C, Kerbel RS: Cytokines, growth factors and the loss of negative growth control in the progression of human cutaneous malignant melanoma. Curr Opin Oncol 6: 212–220, 1994
35. Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW, Giaccia AJ: Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors. Nature 379: 88–90, 1996
36. Kinzler KW, Vogelstein B: Life (and death) in a malignant tumor. Nature 73: 19–20, 1996

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