Inhibition of Tumor Angiogenesis[a]

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The dependence of solid tumor growth upon neovascularization provides a therapeutic opportunity to modulate the growth of neoplasms by inhibiting tumor-induced angiogenesis.[1-3] Although the experimental and clinical evidence that solid tumor growth is angiogenesis-dependent has been summarized in detail elsewhere,[4-7] a brief review of this topic will introduce potential applications for the treatment of cancer.

Angiogenesis and Solid Tumor Growth

Solid tumor growth occurs in two phases: an avascular phase and a vascular phase. During the initial avascular phase of growth, tumors exist as small aggregates of neoplastic cells supported by simple diffusion of oxygen and nutrients. Tumor volume increases at a slow linear rate to a maximum of less than 3 cubic millimeters. This volume is determined by the limits of diffusion;[8] no further three-dimensional growth occurs until the tumor becomes vascularized. When deprived of a vascular supply, as in the anterior chamber of the eye, tumors remain viable for prolonged periods, but they are dormant from a growth perspective.[9] A balance exists between cell proliferation at the periphery and cell death at the center of the tumor.

Tumors produce diffusable angiogenic factors that induce host capillary endothelial cells to proliferate, migrate, and form new vessels which supply the neoplastic cells. Once vascularized, tumors are maintained by perfusion and their growth becomes rapid. During this phase, tumor volume increases exponentially.[9-11]

INHIBITION OF TUMOR ANGIOGENESIS

Within the last two decades, a variety of seemingly unrelated classes of drugs have been found to inhibit angiogenesis *in vivo* and *in vitro*. These have been summarized elsewhere[6,7] and are presented in TABLE 1.[12-59] A few examples are reviewed in greater detail below.

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Cartilage

Cartilage, a normally avascular tissue resistant to invasion by most tumors,[60] develops from a vascularized embryonic form that subsequently loses its blood vessels in the early neonatal period.[61,62] These observations suggested to Brem and Folkman the possibility that neonatal cartilage may contain a factor that inhibits vessels, a hypothesis that was confirmed in 1975. With use of the rabbit cornea angiogenesis assay, it was shown that cartilage produces a potent diffusible inhibitor of tumor-induced angiogenesis.[12] Once isolated and purified,[13] the cartilage-derived factor delivered by selective intra-arterial infusions was found to modulate angiogenesis and tumor growth in rabbits and mice.[63] Further purification and characteriza-

TABLE 1. Reported Inhibitors of Angiogenesis

Inhibitor	Refs.	Inhibitor	Refs.
Extracts from avascular tissues		**Agents that bind heparin**	
• Cartilage-derived factor	12–15	• Protamine	45
• Vitreous extract	16, 17	• Platelet factor-4	46
Steroid hormones		**Other modulators of collagen biosynthesis**	
• Cortisone, hydrocortisone, tetrahydrocortisol & others	18–21	• Proline analogues	47
• Medroxyprogesterone	22, 23	• α,α-Dipyridyl	47
• Heparin-steroid conjugates	24	• β-Aminoproprionitrile	47
Antibiotics		• Tricyclodecan-9-yl-xanthate (D609)	48
• Minocycline	25, 26	• GPA1734	49
• AGM-1470 and other fumagillin derivatives	27–30	• Tissue inhibitors of metalloproteinases (TIMP and TIMP-2)	50
• Herbimycin A	31	• Interferons	51–53
• 15-Deoxyspergualin	32, 33	**Miscellaneous inhibitors of angiogenesis**	
• Eponemycin	34	• Thrombospondin	54–56
Antirheumatic agents		• Site-specific mutants of angiogenin	57
• D-Penicillamine	35, 36	• α-Difluoromethylornithine (DMFO)	58
• Inhibitors of prostaglandin synthesis	37–40	• Anti-bFGF monoclonal antibody	59
• Gold compounds	41, 42		
Vitamins and derivatives			
• Vitamin D_3 analogues	43		
• Retinoids	44		

tion of the factor revealed that it is a protein with an approximate molecular weight of 24,000, potent anticollagenase properties, and a high sequence homology to a collagenase inhibitor isolated from cultured human skin fibroblasts.[14,15]

Angiostatic Steroids

The observation that heparin administered in combination with cortisone has potent antiangiogenic effects, causes tumor regression, and inhibits formation of metastases[18] led to the discovery of a new class of steroids with antiangiogenic properties. These corticosteroids inhibit angiogenesis independent of their relative

mineralocorticoid or glucocorticoid activity and have been termed "angiostatic steroids."[19]

The antitumor potential of angiostatic steroids and heparin was confirmed in a series of *in vivo* studies. Heparin and hydrocortisone administered orally inhibited angiogenesis and tumor growth in nude mice with human neurofibrosarcoma implants.[64] Heparin and cortisone, when incorporated into a biodegradable polyanhydride controlled-release polymer, had an antiangiogenic effect against tumor-induced neovascularization in the rabbit cornea model and inhibited tumor growth when implanted into 9L-gliosarcomas growing in the flanks of adult rats.[65] This demonstrated the feasibility of using implantable controlled-release polymers for the interstitial delivery of angiostatic agents[66] to inhibit tumor growth. Given the limitations imposed by the blood-brain barrier upon drug penetration into the central nervous system,[67] this drug-delivery system has become useful for studying the effects of angiogenesis inhibitors upon the growth of intracranial neoplasms.

Although it is possible that the systemic toxicity of cortisone could limit its clinical use as an inhibitor of angiogenesis, potentially less toxic alternatives have been found to have antiangiogenic properties. Tetrahydrocortisol, a major metabolite of cortisol devoid of glucocorticoid or mineralocorticoid activity, is the most potent of the known angiostatic steroids. This is only one of several examples of angiostatic steroids with no other apparent biological activity.[21]

Similar concerns that the anticoagulant effect of heparin might limit the clinical use of this potential cancer therapy prompted the discovery of heparin fragments[18] and synthetic heparin substitutes such as β-cyclodextrin tetradecasulfate[20,68] that potentiate the antiangiogenic properties of angiostatic steroids without anticoagulation. More recently, Thorpe *et al.* developed new angiogenesis inhibitors by covalently linking angiostatic steroids to a non-anticoagulant heparin derivative, thus achieving antitumor effects in mice with a compound less likely to have the side effects of heparin and cortisone.[24]

Fumagillin Derivatives

Another class of newly discovered angiogenesis inhibitors has been derived from fumagillin, an antibiotic purified from cultures of *Aspergillus fumigatus* fresenius, which inhibits endothelial cell proliferation *in vitro*.[27] Although the parent compound is systemically toxic, many less toxic and more potent antiangiogenic derivatives have been synthesized.[27,29,30] The most potent of the known fumagillin analogues, O-(chloroacetylcarbamoyl)fumagillol or AGM-1470, effectively reduces angiogenesis in several standard assays[27–30] and inhibits tumor growth and metastasis in a wide variety of mouse and rat tumors with little apparent toxicity.[27,69] When delivered systemically in nude mice, this agent inhibits tumor angiogenesis and growth of human schwannoma, neurofibroma, and neurofibrosarcoma xenografts.[70] It causes endothelial cell rounding and thus prevents endothelial proliferation. AGM-1470 is cytotoxic *in vitro* only in high concentrations; therefore its antineoplastic effects are thought to be due to inhibition of angiogenesis. This drug is currently being evaluated clinically for treatment of the highly vascular Kaposi's sarcoma in AIDS patients.

Inhibitors of Collagen Metabolism

As more is learned about the varied biological effects of different classes of antiangiogenic compounds, a common feature in their mechanisms of action is the

modulation of collagen, extracellular matrix, and basement membrane metabolism. The inhibitor of angiogenesis derived from cartilage, for example, is a protein that inhibits mammalian collagenase.[14,15] Similarly, the angiostatic steroids modulate collagen metabolism,[22,71,72] induce basement membrane dissolution,[73] and inhibit synthesis of plasminogen activator, an enzyme involved in remodeling the extracellular matrix and the capillary basement membrane.[23,74]

Capillary retraction, endothelial cell rounding, inhibition of endothelial proliferation, and vascular involution are all associated with dissolution of the basement membrane.[47,73] Regression of growing capillaries in the chick chorioallantoic membrane and potentiation of the antiangiogenic effects of heparin and steroid combinations have been achieved with a variety of agents, including proline analogues and an inhibitor of prolyl hydroxylase, that induce structural alterations in the extracellular matrix by interfering with collagen metabolism.[47] Agents that affect collagen metabolism, either anabolically or catabolically, may inhibit neovascularization and therefore have potential roles in the treatment of cancer and other angiogenic diseases. The feasibility of this therapeutic approach has been demonstrated clinically with the successful use of recombinant interferon α_{2a} to treat pulmonary hemangiomatosis, a rare angiogenic disease that is usually relentlessly progressive and fatal.[53] We have investigated other clinically available agents that modulate collagen metabolism in a search for alternative inhibitors of angiogenesis, as indicated below.

Tetracycline Derivatives

Tetracyclines, which specifically block mitochondrial protein synthesis, have long been used clinically for their broad-spectrum antibiotic properties. In 1984, Kroon *et al.* reported that tumor growth can be inhibited by tetracycline derivatives and hypothesized that this cytostatic effect was due to inhibition of mitochondrial translation in neoplastic cells.[75]

Another recently recognized characteristic of these drugs is the inhibition of extracellular matrix metalloproteinases, including type IV collagenase.[76–78] Collagenase inhibition appears to be unrelated to the antibiotic action of these compounds because chemically modified derivatives of tetracycline that lack antimicrobial activity may retain their anticollagenase properties.[79,80]

Among the commercially available tetracyclines already in clinical use as antibiotics, the semisynthetic derivative minocycline is the most lipid soluble, has the best tissue penetration,[81,82] and is a potent collagenase inhibitor.[83] We therefore began laboratory investigations to test our hypothesis that minocycline is an inhibitor of tumor angiogenesis.[25]

The rabbit cornea, normally clear and avascular, provides an important model for study of putative angiogenesis inhibitors incorporated into biocompatible polymers capable of sustained drug release because it permits direct observation and quantification of vessel growth.[12,66] This model was used to study minocycline impregnated into ethylene-vinyl acetate copolymer (EVAc), an inert nonbiodegradable controlled-release polymer well-suited for local drug delivery.[84–86] Similar polymers were fabricated with a combination of heparin and cortisone or with cortisone alone. The polymers were then implanted with rabbit VX2 carcinoma into the rabbit cornea. Minocycline significantly inhibited tumor angiogenesis with results comparable to those achieved with combinations of heparin and cortisone.

To elucidate the mechanisms of antiangiogenic action by tetracycline derivatives, we explored the *in vitro* effects of minocycline upon some of the major cell types involved in the structure and regulation of the cerebral microvasculature.[26] These

experiments demonstrated that minocycline selectively inhibits endothelial cell growth at concentrations that have minimal effect on astrocytes and pericytes. DNA and protein synthesis, as measured by incorporation of tritiated thymidine and tritiated leucine, were also inhibited selectively in endothelial cells. When other tetracycline derivatives were tested, it was found that the degree of endothelial cell growth inhibition correlated with the potency of anticollagenase activity and not antimicrobial action for each of the compounds studied.

Teicher *et al.* and Sotomayor *et al.* have reported the successful use of minocycline as an adjunct to standard cancer therapies against subcutaneously implanted Lewis lung carcinoma in mice.[87,88] In this model, the inhibition of tumor growth achieved with cyclophosphamide, melphalan, or radiation treatment was enhanced by the concurrent administration of systemic minocycline. A significant decrease in the number and size of vascularized pulmonary metastases was also demonstrated in these studies when minocycline was administered as an adjunct to cisplatin, melphalan, or cyclophosphamide. Animals treated with the combination of minocycline and cyclophosphamide showed the greatest response.

Weingart *et al.* demonstrated that interstitial administration of minocycline by controlled-release polymers for treatment of brain tumors in the rat 9L-gliosarcoma model significantly inhibited tumor growth and prolonged survival.[89] Furthermore, local delivery of minocycline, administered as an adjunct to systemic carmustine (BCNU) therapy, resulted in synergistic prolongation of survival in this brain tumor model.[90,91]

In vitro studies demonstrating that minocycline at very high concentrations is minimally cytotoxic against cultured EMT-6 murine mammary tumor cells[88] are consistent with the hypothesis that the minocycline effect is due to inhibition of angiogenesis, presumably related to its anticollagenase properties.

Further studies aimed at elucidating the mechanism of antiangiogenic action of minocycline and other chemically modified tetracycline derivatives are in progress. Minocycline shows considerable promise as a biological response modifier for use as an adjunct to cytotoxic agents in the treatment of solid tumors. If this therapeutic potential is confirmed, minocycline is an ideal candidate for clinical trials as an inhibitor of tumor neovascularization because it is readily available commercially and has for several years been safely used as a systemic antibiotic.

CONCLUSION

Antiangiogenic drugs have emerged as important potential therapeutic agents to be evaluated either independently or in combination with other approaches for the treatment of cancer and other angiogenic diseases. The variety of compounds reported to inhibit angiogenesis suggests that a broad range of antiangiogenic mechanisms exists. This diversity of therapeutic options could be exploited by using multiple different inhibitors together to more effectively control neovascularization.

SUMMARY

The exponential growth of solid tumors depends upon induction of new vessel growth, a process mediated by diffusable angiogenic factors produced by tumor cells. By inhibiting angiogenesis, it is now possible to modulate tumor growth and metastasis in laboratory animals. The first described inhibitor of angiogenesis was a

protein derived from cartilage. Other important classes of antiangiogenic agents include angiostatic steroids combined with heparin or heparin derivatives, and the synthetic derivatives of fumigallin. As the mechanisms of action of these and other angiostatic agents are being elucidated, it is becoming apparent that many modulators of collagen metabolism inhibit angiogenesis and may offer clinically useful anticancer treatments. Minocycline and other tetracycline derivatives with anticollagenase properties have been shown to be potent inhibitors of angiogenesis. These agents, when administered with other standard cancer therapies, help prolong survival in laboratory animals with solid tumors. Further studies of these biologic response modifiers of tumor progression are under way in the hope that they will offer effective new treatments for cancer in humans.

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REFERENCES

1. FOLKMAN, J. 1971. Tumor angiogenesis: Therapeutic implications. N. Engl. J. Med. **285:** 1182–1186.
2. FOLKMAN, J. 1972. Anti-angiogenesis: New concept for therapy of solid tumors. Ann. Surg. **175:** 409–416.
3. FOLKMAN, J. 1975. Tumor angiogenesis: A possible control point in tumor growth. Ann. Int. Med. **82:** 96–100.
4. FOLKMAN, J. & M. KLAGSBRUN. 1987. Angiogenic factors. Science **235:** 442–447.
5. FOLKMAN, J. 1990. What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst. **82:** 4–6.
6. FOLKMAN, M. J. 1991. Antiangiogenesis. *In* Biologic Therapy of Cancer. V. DeVita, S. Hellman & S. Rosenberg, Eds. J. B. Lippincott Co. Philadelphia, PA.
7. FOLKMAN, J. 1993. Tumor angiogenesis. *In* Cancer Medicine. J. Holland, E. Frei, R. Bast, D. Kufe, D. Morton & R. Weichselbaum, Eds. Lea and Febiger. Philadelphia, PA.
8. FOLKMAN, J. & M. HOCHBERG. 1973. Self-regulation of growth in three dimensions. J. Exp. Med. **138:** 745–753.
9. GIMBRONE, M., S. LEAPMAN, R. COTRAN & J. FOLKMAN. 1972. Tumor dormancy in vivo by prevention of neovascularization. J. Exp. Med. **136:** 261–276.
10. GIMBRONE, M., R. COTRAN, S. LEAPMAN & J. FOLKMAN. 1974. Tumor growth and neovascularization: An experimental model using the rabbit cornea. J. Natl. Cancer Inst. **52:** 413–419.
11. KNIGHTON, D., D. AUSPRUNK, D. TAPPER & J. FOLKMAN. 1977. Avascular and vascular phases of tumour growth in the chick embryo. Br. J. Cancer **35:** 347–356.
12. BREM, H. & J. FOLKMAN. 1975. Inhibition of tumor angiogenesis mediated by cartilage. J. Exp. Med. **141:** 427–439.
13. LANGER, R., H. BREM, K. FALTERMAN, M. KLEIN & J. FOLKMAN. 1976. Isolation of a cartilage factor that inhibits tumor neovascularization. Science **193:** 70–72.
14. MOSES, M. A., J. SUDHALTER & R. LANGER. 1990. Identification of an inhibitor of neovascularization from cartilage. Science **248:** 1408–1410.
15. MURRAY, J. B., K. ALLISON, J. SUDHALTER & R. LANGER. 1986. Purification and partial amino acid sequence of a bovine cartilage-derived collagenase inhibitor. J. Biol. Chem. **261:** 4154–4159.
16. PREIS, I., R. LANGER, H. BREM, J. FOLKMAN & A. PATZ. 1977. Inhibition of neovascularization by an extract derived from vitreous. Am. J. Ophthalmol. **84:** 323–328.
17. LUTTY, G. A., D. C. THOMPSON, J. Y. GALLUP, R. J. MELLO, A. PATZ & A. FENSELAU.

1983. Vitreous: An inhibitor of retinal extract-induced neovascularization. Invest. Ophthalmol. Visual Sci. **2:** 52–56.

18. FOLKMAN, J., R. LANGER, R. LINHARDT, C. HAUDENSCHILD & S. TAYLOR. 1983. Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. Science **221:** 719–725.
19. CRUM, R., S. SZABO & J. FOLKMAN. 1985. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. Science **230:** 1375–1378.
20. FOLKMAN, J., P. B. WEISZ, M. M. JOULLIE, W. W. LI & W. R. EWING. 1989. Control of angiogenesis with synthetic heparin substitutes. Science **243:** 1490–1493.
21. FOLKMAN, J. & D. E. INGBER. 1987. Angiostatic steroids. Method of discovery and mechanism of action. Ann. Surg. **206:** 374–383.
22. GROSS, J., R. AZIZKHAN, C. BISWAS, R. BRUNS, D. HSIEH & J. FOLKMAN. 1981. Inhibition of tumor growth, vascularization, and collagenolysis in the rabbit cornea by medroxyprogesterone. Proc. Natl. Acad. Sci. USA **78:** 1176–1180.
23. ASHINO-FUSE, H., Y. TAKANO, T. OIKAWA, M. SHIMAMURA & T. IWAGUCHI. 1989. Medroxyprogesterone acetate, an anti-cancer and anti-angiogenic steroid, inhibits the plasminogen activator in bovine endothelial cells. Int. J. Cancer **44:** 859–864.
24. THORPE, P. E., E. J. DERBYSHIRE, S. P. ANDRADE, N. PRESS, P. P. KNOWLES, S. KING, G. J. WATSON, Y. C. YANG & B. M. RAO. 1993. Heparin-steroid conjugates: New angiogenesis inhibitors with antitumor activity in mice. Cancer Res. **53:** 3000–3007.
25. TAMARGO, R. J., R. A. BOK & H. BREM. 1991. Angiogenesis inhibition by minocycline. Cancer Res. **51:** 672–675.
26. GUERIN, C., J. LATERRA, T. MASNYK, L. M. GOLUB & H. BREM. 1992. Selective endothelial growth inhibition by tetracyclines that inhibit collagenase. Biochem. Biophys. Res. Commun. **188:** 740–745.
27. INGBER, D., T. FUJITA, S. KISHIMOTO, K. SUDO, T. KANAMARU, H. BREM & J. FOLKMAN. 1990. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature **348:** 555–557.
28. KUSAKA, M., K. SUDO, T. FUJITA, S. MARUI, F. ITOH, D. INGBER & J. FOLKMAN. 1991. Potent anti-angiogenic action of AGM-1470: Comparison to the fumagillin parent. Biochem. Biophys. Res. Commun. **174:** 1070–1076.
29. MARUI, S., F. ITOH, Y. KOZAI, K. SUDO & S. KISHIMOTO. 1992. Chemical modification of fumagillin. I. 6-O-acyl, 6-O-sulfonyl, 6-O-alkyl, and 6-O-(N-substituted-carbamoyl)fumagillols. Chem. Pharm. Bull. (Tokyo) **40:** 96–101.
30. MARUI, S. & S. KISHIMOTO. 1992. Chemical modification of fumagillin. II. 6-Amino-6-deoxyfumagillol and its derivatives. Chem. Pharm. Bull. (Tokyo) **40:** 575–579.
31. OIKAWA, T., K. HIROTANI, M. SHIMAMURA, H. ASHINO-FUSE & T. IWAGUCHI. 1989. Powerful antiangiogenic activity of herbimycin A (named angiostatic antibiotic). J. Antibiot. **42:** 1202–1204.
32. OIKAWA, T., M. SHIMAMURA, H. ASHINO-FUSE, T. IWAGUCHI, M. ISHIZUKA & T. TAKEUCHI. 1991. Inhibition of angiogenesis by 15-deoxyspergualin. J. Antibiot. **44:** 1033–1035.
33. OIKAWA, T., M. HASEGAWA, I. MORITA, S. MUROTA, H. ASHINO, M. SHIMAMURA, A. KIUE, R. HAMANAKA, M. KUWANO, M. ISHIZUKA *et al.* 1992. Effect of 15-deoxyspergualin, a microbial angiogenesis inhibitor, on the biological activities of bovine vascular endothelial cells. Anticancer Drugs **3:** 293–299.
34. OIKAWA, T., M. HASEGAWA, M. SHIMAMURA, H. ASHINO, S. MUROTA & I. MORITA. 1991. Eponemycin, a novel antibiotic, is a highly powerful angiogenesis inhibitor. Biochem. Biophys. Res. Commun. **181:** 1070–1076.
35. MATSUBARA, T., R. SAURA, K. HIROHATA & M. ZIFF. 1989. Inhibition of human endothelial cell proliferation in vitro and neovascularization in vivo by D-penicillamine. J. Clin. Invest. **83:** 158–167.
36. BREM, S. S., D. ZAGZAG, A. M. C. TSANACLIS, S. GATELY, M. P. ELKOUBY & S. E. BRIEN. 1990. Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by penicillamine and the depletion of copper, an angiogenic cofactor. Am. J. Pathol. **137:** 1121–1142.
37. COOPER, C. A., M. V. W. BERGAMINI & I. H. LEOPOLD. 1980. Use of flurbiprofen to inhibit corneal neovascularization. Arch. Ophthalmol. **98:** 1102–1105.

38. PETERSON, H. 1983. Effects of prostaglandin synthesis inhibitors on tumor growth and vascularization: Experimental studies in the rat. Invasion Metastasis **3:** 151–159.
39. PETERSON, H. 1986. Tumor angiogenesis inhibition by prostaglandin synthetase inhibitors. Anticancer Res. **6:** 251–254.
40. MILAS, L., N. HUNTER, Y. FURUTA, I. NISHIGUCHI & S. RUNKEL. 1991. Antitumor effects of indomethacin alone and in combination with radiotherapy: Role of inhibition of tumour angiogenesis. Int. J. Radiat. Biol. **60:** 65–70.
41. MATSUBARA, T. & M. ZIFF. 1987. Inhibition of human endothelial cell proliferation by gold compounds. J. Clin. Invest. **79:** 1440–1446.
42. KOCH, A. E., M. CHO, J. BURROWS, S. J. LEIBOVICH & P. J. POLVERINI. 1988. Inhibition of production of macrophage-derived angiogenesis activity by the anti-rheumatic agents gold sodium thiomalate and auranofin. Biochem. Biophys. Res. Commun. **154:** 205–212.
43. OIKAWA, T., K. HIROTANI, H. OGASAWARA, T. KATAYAMA, O. NAKAMURA, T. IWAGUCHI & A. HIRAGUN. 1990. Inhibition of angiogenesis by vitamin D_3 analogues. Eur. J. Pharmacol. **178:** 247–250.
44. OIKAWA, T., K. HIROTANI, O. NAKAMURA, K. SHUDO, A. HIRAGUN & T. IWAGUCHI. 1989. A highly potent antiangiogenic activity of retinoids. Cancer Lett. **48:** 157–162.
45. TAYLOR, S. & J. FOLKMAN. 1982. Protamine is an inhibitor of angiogenesis. Nature **297:** 307–312.
46. MAIONE, T. E., G. S. GRAY, J. PETRO, A. J. HUNT, A. L. DONNER, S. I. BAUER, H. F. CARSON & R. J. SHARPE. 1990. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science **247:** 77–79.
47. INGBER, D. & J. FOLKMAN. 1988. Inhibition of angiogenesis through modulation of collagen metabolism. Lab. Invest. **59:** 44–51.
48. MARAGOUDAKIS, M. E., E. MISSIRLIS, M. SARMONIKA, M. PANOUTSACOPOULOU & G. KARAKIULAKIS. 1990. Basement membrane biosynthesis as a target to tumor therapy. J. Pharmacol. Exp. Ther. **252:** 753–757.
49. MARAGOUDAKIS, M. E., M. SARMONIKA & M. PANOUTSACOPOULOU. 1988. Inhibition of basement membrane biosynthesis prevents angiogenesis. J. Pharmacol. Exp. Ther. **244:** 729–733.
50. TAKIGAWA, M., Y. NISHIDA, F. SUZUKI, J. KISHI, K. YAMASHITA & T. HAYAKAWA. 1990. Induction of angiogenesis in chick yolk-sac membrane by polyamines and its inhibition by tissue inhibitors of metalloproteinases (TIMP and TIMP-2). Biochem. Biophys. Res. Commun. **171:** 1264–1271.
51. SIDKY, Y. A. & E. C. BORDEN. 1987. Inhibition of angiogenesis by interferons: Effects on tumor- and lymphocyte-induced vascular responses. Cancer Res. **47:** 5155–5161.
52. TSURUOKA, N., M. SUGIYAMA, Y. TAWARAGI, M. TSUJIMOTO, T. NISHIHARA, T. GOTO & N. SATO. 1988. Inhibition of in vitro angiogenesis by lymphotoxin and interferon-γ. Biochem. Biophys. Res. Commun. **155:** 429–435.
53. WHITE, C. W., H. M. SONDHEIMER, E. C. CROUCH, H. WILSON & L. L. FAN. 1989. Treatment of pulmonary hemangiomatosis with recombinant interferon alfa-2a. N. Engl. J. Med. **320:** 1197–1200.
54. RASTINEJAD, F., P. J. POLVERINI & N. P. BOUCK. 1989. Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. Cell **56:** 345–355.
55. BAGAVANDOSS, P. & J. W. WILKS. 1990. Specific inhibition of endothelial cell proliferation by thrombospondin. Biochem. Biophys. Res. Commun. **170:** 867–872.
56. IRUELA-ARISPE, M. L., P. BORNSTEIN & H. SAGE. 1991. Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells in vitro. Proc. Natl. Acad. Sci. USA **88:** 5026–5030.
57. SHAPIRO, R. & B. L. VALLE. 1989. Site-directed mutagenesis of histidine-13 and histidine-114 of human angiogenin. Alanine derivatives inhibit angiogenin-induced angiogenesis. Biochemistry **28:** 7401–7408.
58. TAKIGAWA, M., M. ENOMOTO, Y. NISHIDA, H. PAN, A. KINOSHITA & F. SUZUKI. 1990. Tumor angiogenesis and polyamines: α-Difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, inhibits B16 melanoma-induced angiogenesis in ovo and the proliferation of vascular endothelial cells in vitro. Cancer Res. **50:** 4131–4138.

59. HORI, A., R. SASADA, E. MATSUTANI, K. NAITO, Y. SAKURA, T. FUJITA & Y. KOZAI. 1991. Suppression of solid tumor growth by immunoneutralizing monoclonal antibody against basic fibroblast growth factor. Cancer Res. **51:** 6180–6184.
60. EISENSTEIN, R., N. SORGENTE, L. W. SOBLE, A. MILLER & K. E. KUETTNER. 1973. The resistance of certain tissues to invasion: Penetrability of explanted tissues by vascularized mesenchyme. Am. J. Pathol. **73:** 765–774.
61. HARALDSSON, S. 1962. The vascular pattern of a growing and fullgrown human epiphysis. Acta Anat. **48:** 156–167.
62. BLACKWOOD, H. J. J. 1965. Vascularization of the condylar cartilage of the human mandible. J. Anat. **99:** 551–563.
63. LANGER, R., H. CONN, J. VACANTI, C. HAUDENSCHILD & J. FOLKMAN. 1980. Control of tumor growth in animals by infusion of an angiogenesis inhibitor. Proc. Natl. Acad. Sci. USA **77:** 4331–4335.
64. LEE, J. K., B. CHOI, R. A. SOBEL, E. A. CHIOCCA & R. L. MARTUZA. 1990. Inhibition of growth and angiogenesis of human neurofibrosarcoma by heparin and hydrocortisone. J. Neurosurg. **73:** 429–435.
65. TAMARGO, R. J., K. W. LEONG & H. BREM. 1990. Growth inhibition of the 9L glioma using polymers to release heparin and cortisone acetate. J. Neuro-Oncol. **9:** 131–138.
66. LANGER, R. & J. MURRAY. 1983. Angiogenesis inhibitors and their delivery systems. Appl. Biochem. Biotechnol. **8:** 9–24.
67. TAMARGO, R. J. & H. BREM. 1992. Drug delivery to the central nervous system: A review. Neurosurg. Q. **2:** 259–279.
68. WEISZ, P. B., H. C. HERMANN, M. M. JOULLIÉ, K. KUMOR, E. M. LEVINE, E. J. MACARAK & D. B. WEINER. 1992. Angiogenesis and heparin mimics. Exs **61:** 107–117.
69. YAMAOKA, M., T. YAMAMOTO, T. MASAKI, S. IKEYAMA, K. SUDO & T. FUJITA. 1993. Inhibition of tumor growth and metastasis of rodent tumors by the angiogenesis inhibitor O-(chloroacetyl-carbamoyl)fumagillol (TNP-470; AGM-1470). Cancer Res. **53:** 4262–4267.
70. TAKAMIYA, Y., R. M. FRIEDLANDER, H. BREM, A. MALIK & R. L. MARTUZA. 1993. Inhibition of angiogenesis and growth of human nerve-sheath tumors by AGM-1470. J. Neurosurg. **78:** 470–476.
71. MARAGOUDAKIS, M., M. SARMONIKA & M. PANOUTSACOPOULOU. 1989. Antiangiogenic action of heparin plus cortisone is associated with decreased collagenous protein synthesis in the chick chorioallantoic membrane system. J. Pharmacol. Exp. Ther. **251:** 679–682.
72. HARADA, I., T. KIKUCHI, Y. SHIMOMURA, M. YAMAMOTO, H. OHNO & N. SATO. 1992. The mode of action of anti-angiogenic steroid and heparin. Exs **61:** 445–448.
73. INGBER, D., J. MADRI & J. FOLKMAN. 1986. A possible mechanism for inhibition of angiogenesis by angiostatic steroids: Induction of capillary basement membrane dissolution. Endocrinology **119:** 1768–1775.
74. BLEI, F., E. L. WILSON, P. MIGNATTI & D. B. RIFKIN. 1993. Mechanism of action of angiostatic steroids: Suppression of plasminogen activator activity via stimulation of plasminogen activator inhibitor synthesis. J. Cell. Physiol. **155:** 568–578.
75. KROON, A. M., B. H. J. DONTJE, M. HOLTROP & C. VAN DEN BOGERT. 1984. The mitochondrial genetic system as a target for chemotherapy: Tetracyclines as cytostatics. Cancer Lett. **25:** 33–40.
76. GOLUB, L. M., N. RAMAMURTHY, T. F. MCNAMARA, B. GOMES, M. WOLFF, A. CASINO, A. KAPOOR, J. ZAMBON, S. CIANCIO, M. SCHNEIR, *et al.* 1984. Tetracyclines inhibit tissue collagenase activity. A new mechanism in the treatment of periodontal disease. J. Periodontal Res. **19:** 651–655.
77. GOLUB, L. M., M. WOLFF, H. M. LEE, T. F. MCNAMARA, N. S. RAMAMURTHY, J. ZAMBON & S. CIANCIO. 1985. Further evidence that tetracyclines inhibit collagenase activity in human crevicular fluid and from other mammalian sources. J. Periodontal Res. **20:** 12–23.
78. GOLUB, L. M., N. S. RAMAMURTHY, T. F. MCNAMARA, R. A. GREENWALD & B. R. RIFKIN. 1991. Tetracyclines inhibit connective tissue breakdown: New therapeutic implications for an old family of drugs. Crit. Rev. Oral Biol. Med. **2:** 297–321.

79. GOLUB, L. M., T. F. MCNAMARA, G. D'ANGELO, R. A. GREENWALD & N. S. RAMAMURTHY. 1987. A non-antibacterial chemically-modified tetracycline inhibits mammalian collagenase activity. J. Dent. Res. **66:** 1310–1314.
80. YU, Z., M. K. LEUNG, N. S. RAMAMURTHY, T. F. MCNAMARA & L. M. GOLUB. 1992. HPLC determination of a chemically modified nonantimicrobial tetracycline: Biological implications. Biochem. Med. Metab. Biol. **47:** 10–20.
81. ZBINOVSKY, V. & G. P. CHREKIAN. 1977. Minocycline. *In* Analytical Profiles of Drug Substances. K. Florey, Ed. Academic Press. New York.
82. SANDE, M. A. & G. L. MANDELL. 1990. Antimicrobial agents: Tetracyclines, chloramphenicol, erythromycin, and miscellaneous antibacterial agents. *In* Goodman and Gilman's The Pharmacological Basis of Therapeutics. A. G. Gilman, T. W. Rall, A. S. Nies & P. Taylor, Eds. Pergamon Press. New York.
83. GOLUB, L. M., H. M. LEE, G. LEHRER, A. NEMIROFF, T. F. MCNAMARA, R. KAPLAN & N. S. RAMAMURTHY. 1983. Minocycline reduces gingival collagenolytic activity during diabetes. Preliminary observations and a proposed new mechanism of action. J. Periodontal Res. **18:** 516–526.
84. LANGER, R. & J. FOLKMAN. 1976. Polymers for the sustained release of proteins and other macromolecules. Nature **263:** 797–800.
85. RHINE, W. D., D. S. T. HSIEH & R. LANGER. 1980. Polymers for sustained macromolecule release: Procedures to fabricate reproducible delivery systems and control release kinetics. J. Pharm. Sci. **69:** 265–270.
86. LANGER, R., H. BREM & D. TAPPER. 1981. Biocompatibility of polymeric delivery systems for macromolecules. J. Biomed. Mater. Res. **15:** 267–277.
87. TEICHER, B. A., E. A. SOTOMAYOR & Z. D. HUANG. 1992. Antiangiogenic agents potentiate cytotoxic cancer therapies against primary and metastatic disease. Cancer Res. **52:** 6702–6704.
88. SOTOMAYOR, E. A., B. A. TEICHER, G. N. SCHWARTZ, S. A. HOLDEN, K. MENON, T. S. HERMAN & E. FREI. 1992. Minocycline in combination with chemotherapy or radiation therapy in vitro and in vivo. Cancer Chemother. Pharmacol. **30:** 377–384.
89. WEINGART, J., R. TAMARGO & H. BREM. 1992. Minocycline, an angiogenesis inhibitor, inhibits brain tumor growth (Abstract). Proc. Am. Assoc. Cancer Res. **33:** 75.
90. WEINGART, J. & H. BREM. 1992. Minocycline, an angiogenesis inhibitor, acts synergistically with carmustine (BCNU) to control 9L gliosarcoma growth (Abstract). Mol. Biol. Cell **3(s):** A21.
91. WEINGART, J. D., E. P. SIPOS & H. BREM. 1994. The role of minocycline in treatment of intracranial 9L glioma. J. Neurosurg. In press.