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Review

Inhibition of Angiogenesis in the Treatment of Tumors

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Abstract. Angiogenesis is essential for tumor progression, growth and metastases. Many substances present in a normal organism can inhibit or stimulate the process of new vessel formation in tumors. The use of natural or synthetic angiogenesis inhibitors as anticancer drugs is currently under intense investigation. Such agents can have lower toxicity and are less likely to generate drug resistance than conventional cytotoxic drugs. Clinical trials are now underway to develop optimum treatment strategies for antiangiogenic drugs. This paper reviews the present achievements in preclinical and clinical studies with antitumor drugs based on inhibitors of angiogenesis.

Key words: inhibitors; angiogenesis; cancer treatment.

Without new blood vessels to supply oxygen and nutrients, a tumor will not grow at all or will only reach the size of a pinhead. This is why the idea of treating the malignant tumors by inhibition of angiogenesis of vessels, promoted by Professor Judah Folkman 30 years ago, is so popular[6, 10]. His centre at the Harvard Medical School was for years the only one where serious research in this field was performed. Now many other centres around the world are deeply involved in the research on angiogenesis[9]. Some potent inhibitors of angiogenesis discovered by Folkman's co-workers are already in clinical trials and not only scientists, but media and the public are awaiting the results expected to be published in a few months.

For many years the idea of such therapy was treated as a peripheral though intellectually interesting topic which was left in the hands of experimental oncologists. After years of research and significant progress in the field of biology and the regulatory mechanisms of the blood vessel growth, no serious scientist would say that "inhibition of angiogenesis is not a realistic strategy to suppress cancer growth and metastases".

The histology of the formation of new capillaries is reasonably well known. The internal walls of blood vessels are covered by endothelial cells. These cells have the ability to divide and to migrate. Angiogenesis initiates with migration of endothelial cells from the capillary vessels to the surrounding extracellular matrix. These cells secrete enzymes which destroy the surrounding tissue substrate; then the endothelial cells start to proliferate as a solid structure of trabecular form. Finally, this trabecule formed by the growing endothelial cells is transformed into a tubular structure – the future vessel. This is the way in which a net of a new capillary vessels starts to develop[14]. Such events take part in the repair processes in normal, but also in neo-

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plastic tissue. In mature organisms the endothelial cells do not normally proliferate. A massive local multiplication of vessels is observed during wound healing. The generation of a blood vessel is controlled and balanced thanks to several different regulating factors which stimulate or inhibit angiogenesis.

The lists of angiogenesis stimulants and inhibitors constructed by Kerbel, (1999) are shown in Table 1 and 2. The lists are not complete and still growing.

Table 1. Angiogenesis stimulators

Basic fibroblast growth factor (bFGF)
Acidic fibroblast growth factor (aFGF)
Transforming growth factor α (TGF-α)
Transforming growth factor β (TGF-β)
Plateled-derived growth factor (PDGF)
Insulin-like growth factor (IGF)
Vascular endothelial growth factor (VEGF)
Plateled-derived endothelial growth factor
Hepatocyte growth factor (HGF)/SF
Angiopoietin 1
Angiogenin
Placental growth factor
Interleukin 8
Prostaglandins E_1 and E_2
Androgens
Proliferin
Estrogens

Table 2. Angiogenesis inhibitors

Platelet factor 4 (fragment)
Thrombospondin 1
Interferon αβ
Prolactin (fragment)
Angiostatin (fragment of plasminogen)
Tissue inhibitors of metalloproteinases
TIMP-1
TIMP-2
bFGF soluble receptor
Placental proliferin-related protein
Endostatin (fragment of type XVIII collagen)
Vasostatin (fragment of calreticulin)
Angiopoietin 2 (in absence of VEGF)

In experimental conditions it is relatively easy to stop angiogenesis in a tumor without damaging blood circulation in healthy organs.

An argument for searching for the substances inhibiting angiogenesis in the tumor tissue is the fact that such therapy counteracts the function of normal endothelial cells, not of the tumor cells. It is unlikely that such a kind of therapy could cause damage to bone marrow, digestive tract disorders, hair loss and other signs of toxicity normally occurring in patients given the typical chemotherapeutic treatment. The endothelial cells are genetically stable, so it is also unlikely that a clone selection or cell mutation, typical for tumor cells, will occur. Thus, a tolerance to this kind of substance should not develop.

The major difficulty is that the understanding of the basic biology causing the growth of vessels is far from complete. Until today about 15 small peptides which stimulate growth of capillary vessels are known. Among them, the most important seem to be the epidermal growth factor (EGF), angiogenin, estrogens, basic and acidic fibroblast growth factor (bFGF and aFGF) and vascular endothelial growth factor (VEGF)[18]. The factors inhibiting vascular growth are angiostatin, endostatin, platelet factor 4 (PF-4), interleukin 12 (IL-12)[16], retinoic acid and tissue inhibitors of metalloproteinases 1 and 2 (TIMP-1 and TIMP-2)[12]. Some of the vascular growth factors are synthesized in tumors in significant amounts. The most important are VEGF and bFGF. These two factors probably play a crucial role in supporting the growth of tumors. Since so many factors stimulate and inhibit the process of new vessel formation, it is almost impossible to find one substance which will solve the problem in treatment of all kinds of tumors.

A good example of the complexity of the mechanisms involved in the inhibition of angiogenesis is the successful therapy in mice with the use of monoclonal antibody against the receptors for VEGF. The nearly full regression of tumor was succeeded after 26 days by a second wave of angiogenesis and the implanted tumor continued to grow. This experiment, performed at the Massachusetts General Hospital in Boston, shows that, once we knock out the early angiogenesis, there is a second phase which will cause a new wave of angiogenesis by using other factors than VEGF. An understanding of the basic biology causing the growth of vessels is badly needed and research in this field is being highly promoted.

Nevertheless, the idea to use substances arresting the growth of blood vessels as antitumor drugs is promising and today there is a large number of synthetic and naturally occurring compounds which are already in the second and third stages of clinical trial. They act either through the inhibition of several factors or their activity is purely empirical and the mechanism is still unknown.

The two inhibitors which were discovered in the laboratory of Prof. Folkman, which are well known because of articles in the daily press – are angiostatin[23] and endostatin[22]. Both peptides are naturally occurring in tissues, angiostatin being a fragment of plasminogen and endostatin – a fragment of type XVIII collagen molecule. Experiments on tumors implanted into mice

show a high efficiency of treatment with these two peptides.

Naturally occurring inhibitors of angiogenesis, which stop the proliferation of endothelial cells, are PF-4 and IL-12. They are in the early stages of clinical tests. The factors suppressing angiogenesis in the ongoing clinical trials are: RhuMad (a monoclonal antibody binding VEGF), Su5416 (a ligand blocking the VEGF receptor)[7] and Interferon Alfa (which inhibits the secretion of VEGF). A bacterial toxin, ZD0101, bonds to the newly generated vessels and causes local inflammation. TNP-470 is a synthetic analogue of the bacterial toxin, it stops proliferation of endothelium[8, 17]. Finally, experiments on animals revealed that Thalidomide (commonly known because of its teratogenic side--effects), Suramin[5], CAI (a synthetic therapeutic agent which stops the migration of cells)[27, 19], proteasome inhibitors and IM862 (a synthetic drug of a non-specific multilateral activity) all suppress angiogenesis. The mechanism of the last group is unknown.

Research on substances inhibiting the ingrowth of small vessels into the stroma, namely inhibitors of metalloproteinases (MMPs), has made the greatest progress[32]. MMP-s form a group of 15 enzymes which digest the connective tissue matrix. These enzymes are called metalloproteinases because of the presence of Zn^{2+} in their active centre. Normally, they are responsible for the turnover of extracellular matrix, digesting collagen, laminin, fibronectin and elastin. In a primary neoplasm and in metastases, angiogenesis usually requires an MMP activity[24]. Since an increased level of MMP has been evaluated in different tumors and metastases, some pharmaceutical companies produce synthetic inhibitors of these enzymes, hoping that these substances will become a milestone in antitumor strategy[3].

Three synthetic inhibitors of MMP: Marimastat, Bay 12-9566 and Ag3340, are in clinical trials. These trials were organized because of the positive results in treatment of tumors in animal experiments and also because of large social pressure. The MMP inhibitor Ag3340, produced in LaJolla (California), passed the first and second stages of clinical trial[25]. A group of 15 patients with prostatic carcinoma and 71 patients with advanced neoplasms have been examined. The results, presented in June 1997, allowed continuation of the research within a group of 500 patients with prostatic carcinoma and 500 patients with lung cancer. The project has been carried out in 50 oncological centres in USA. The American branch of Bayer Co. also achieved the synthesis of an MMP inhibitor – Bay 12-9566. This inhibitor is specific for two enzymes present in high quantities in tumors, i.e. MMP-2 and MMP-9[11]. The first stage of clinical trial is already finished and now a group of patients for the second and third stage is organized. The head of the company in a cautious estimation of the efficacy of this drug, concluded that "patients tolerate the drug well and their clinical state is not getting worse".

Marimastat is an MMP-inhibitor of broad spectrum[21]. It has been synthesised by a British company. The third stage of clinical trial are considerably advanced and their results were to be made known in 1999. Because of its broad spectrum, this inhibitor is toxic and sometimes the signs of joint and muscle damage occurring in patients force to interruption of the treatment[30]. With the regression of symptoms, the therapy can be continued, but the dose should be reduced. In the clinical tests, patients with recognized pancreatic carcinoma[28], carcinoma of the stomach[26], glioblastoma[31] and lung cancer[29, 33] were examined. It is amazing that neither Marimastat nor Bay 12-9566 inhibited the wound healing process. Therefore, a surgical procedure is not contraindicated in patients taking one of this drugs.

It seems that the latest development in the field was achieved at the Burnham Institute in LaJolla by Pasquelini and her colleagues. They synthesised a 10-aminoacid-large circular peptide which binds precisely to two enzymes produced exclusively by tumor cells and growing blood vessels. The target enzymes are gelatinase A and gelatinase B[34]. The enzymes belong to the family of metalloproteinases. These enzymes help tumor cells to penetrate through structural tissue and promote the growth of vessels. By neutralising the enzymes, the new peptide blocks formation of new blood vessels, which are vital for nourishing tumors. The newly synthesised peptide blocks in a specific way gelatinases A and B without damaging side effects to other matrix metalloproteinases[4]. Mice implanted with three types of human tumor survived longer if treated with the new peptide. Now variants of the original peptide are being developed which will last longer in the blood circulation. The preparation for clinical trials are being organized at the M. D. Anderson Hospital in Houston. Professor Judah Folkman describes this peptide as a major breakthrough in antiangogenic treatment.

The remarkable results of experiments regarding the inhibition of angiogenesis using endostatin and angiostatin in mice, carried out in Prof. Folkman's laboratory, make one feel confident, but do not justify the exaggerated enthusiasm found in the essay in the "New York Times". However it is said that Prof. Folkman estab-

lished a prize for the one who brought him a mouse tumor which he would not be able to cure with inhibitors of angiogenesis. As explained before, angiostatin and endostatin are small fragments of naturally occurring macromolecules, more precisely one of the collagens and plasminogen. These molecules naturally regulate the vascular growth in developing and mature organisms[2, 22]. They were tested on animals with encouraging results. Tumors vanished and no drug resistance was observed[1]. It is expected that endostatin might move quickly into clinical trial in 1999 year following approval of the Food and Drug Administration. It has also been shown that human angiostatin is active against mouse tumors. Large quantities of this peptide are now being collected to proceed with clinical trials.

Three pharmaceutical companies are starting a trial to block the activity of VEGF, the peptide which stimulates the proliferation of endothelial cells. A monoclonal antibody against this factor has been obtained. Another company synthesised a ligand which binds to the VEGF receptor, blocking it competitively. The trial to use such substances in the clinic has already started.

Critical opinions concerning tumor therapy by inhibition of angiogenesis are also expressed. In the opinion of Dr Sukhatme from the Renal Division Medical Centre in Boston, the situation is complicated by the fact that the chosen anti-angiogenesis therapy "will probably not work on all tumors of the same site – all prostate or all breast tumors, for example. This is because not all prostate tumors and surrounding cells secrete the same activators and inhibitors. Some tumors will express more bFGF than VEGF and vice versa. The antigenic/anti-angiogenic profile may change with tumor type, grade or size in the same individual". There is evidence based on experiments on animals which show that any given inhibitor may not work on all tumors. A good example is that of the integrin inhibitor (integrin is a molecule present on the surface of endothelial cells). When this inhibitor was given to mice with brain tumors implanted both in the brain and subcutaneously, the tumors in the brain regressed, whereas those under the skin grew at the same rate as the control.

Anti-angiogenic therapy arrests the growth of tumors, but it does not kill the tumor cells. Most specialists in the field of oncology do not believe that one can find a single molecule which will overcome the complexity of the microenvironment of the tumor. Some advocate combinations of therapy i.e. angiogenesis inhibitors with chemo- or radiotherapy. Trials of combining the inhibitors of angiogenesis with typical cytotoxic drugs have already begun[15]. Even Folkman himself showed evidence in mice that a combination of cyclophosphamide and TNP-470 (an angiogenesis inhibitor) caused total regression of implanted tumors. Similar findings were obtained by Weichselbaum at the University of Chicago, who showed that a combination of angiostatin and radiation therapy elicits better tumor response than either alone[13, 20].

About 20 clinical trials are currently testing in phase III the combinations of different angiogenesis inhibitors combined with chemotherapy or irradiation.

The laboratory experiments and the clinical trials are promising, but do not allow the uncritical statements which give premature hope to patients and their families. These expectations cannot be verified before the stage 3 clinical trials are finished and the results accurately evaluated.

References

1. BOEHM T., FOLKMAN J., BROWDER T. and O'REILLY M. S. (1997): Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature, **390**, 404–407.
2. BOEHM T., O'REILLY M. S., KEOUGH K., SHILOACH J., SHAPIRO R. and FOLKMAN J. (1998): Zinc-binding of endostatin is essential for its antiangiogenic activity. Biochem. Biophys. Res. Commun., **252**, 190–194.
3. BROWN P. D. (1997): Matrix metalloproteinase inhibitors in the treatment of cancer. Med. Oncol., **14**, 1–10.
4. BURG M. A., PASQUALINI R., ARAP W., RHOUDAHTI E., STALLCUP R. and FOLKMAN J. (1999): NG2 proteoglicans binding peptides target tumor neovasculature. Cancer Res., **59**, 2869–2874.
5. CHESON B. D., PHILLIPS P. H., PLUDA J. M. and CHRISTIAN M. (1995): Clinical trials referral resource. Clin. Trials Suramin. Oncol., **9**, 161–163.
6. FOLKMAN J. (1972): Anti-angiogenesis: new concept for therapy of solid tumors. Ann. Surg., **175**, 409–416.
7. FONG T. A., SHAWVER L. K., SUN L., TANG C., APP H., POWELL T. J., KIM Y. H., SCHRECK R., WANG X., RISAU W., ULLRICH A., HIRTH K. P. and MCMAHON G. (1999): SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumour vascularisation, and growth of multiple tumour types. Cancer Res., **59**, 99–106.
8. FUJIMOTO J., HORI M., ICHIGO S., HIROSE R., SAKAGUCHI H. and TAMAYA T. (1997): Plausible novel therapeutic strategy of uterine endometrial cancer with reduction of basic fibroblast growth factor secretion by progestin and O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470). Cancer Lett., **113**, 187–194.
9. GIBALDI M. (1998): Regulating angiogenesis: a new therapeutic strategy. J. Clin. Pharmacol., **38**, 898–903.
10. GIMBRONE M. A. JR., LEAPMAN S. B., COTRAN R. S. and FOLKMAN J. (1972): Tumor dormancy *in vivo* by prevention of neovascularization. J. Exp. Med., **136**, 261–276.
11. GOKASLAN Z. L., CHINTALA S. K., YORK J. E., BOYAPATI V., JASTI S., SAWAYA R., FULLER G., WILDRICK D. M., NICOLSON G. L. and RAO J. S. (1998): Expression and role of matrix me-

talloproteinases MMP-2 and MMP-9 in human spinal column tumors. Clin. Exp. Metastasis, **16**, 721–728.
12. GOMEZ D. E., ALONSO D. F., YOSHIJI H. and THORGEIRSSON U. P. (1997): Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. Eur. J. Cell Biol., **74**, 111–122.
13. GORSKI D. H., MAUCERI H. J., SALLOUM R. M., GATELY S., HELLMAN S., BECKETT M. A., SUKHATME V. P., SOFF G. A., KUFE D. W. and WEICHSELBAUM R. R. (1998): Potentiation of the antitumour effect of ionising radiation by brief concomitant exposures to angiostatin. Cancer Res., **58**, 5686–5689.
14. HUDLICKA O. (1991): What makes blood vessels grow? J. Physiol., **444**, 1–24.
15. KAKEJI Y. and TEICHER B. A. (1997): Preclinical studies of the combination of angiogenic inhibitors with cytotoxic agents. Invest. New Drugs., **15**, 39–48.
16. KERBEL R. S. and HAWLEY R. G. (1995): Interleukin 12: newest member of the antiangiogenesis club. J. Int. Cancer Inst., **87**, 557–559.
17. KERR J. S., WEXLER R. S., MOUSA S. A., ROBINSON C. S., WEXLER E. J., MOHAMED S., VOSS M. E., DEVENNY J. J., CZERNIAK P. M., GUDZELAK A. JR. and SLEE A. M. (1999): Novel small molecule alpha v integrin antagonists: comparative anti--cancer efficacy with known angiogenesis inhibitors. Anticancer Res., **19**, 959–968.
18. KLAGSBRUN M. and D'AMORE P. A. (1996): Vascular endothelial growth factor and its receptors. Cytokine Growth Factor Rev., **7**, 259–270.
19. KOHN E. C., REED E., SAROSY G., CHRISTIAN M., LINK C. J., COLE K., FIGG W. D., DAVIS P. A., JACOB J., GOLDSPIEL B. and LIOTTA L. A. (1996): Clinical investigation of a cytostatic calcium influx inhibitor in patients with refractory cancers. Cancer Res., **56**, 569–573.
20. MAUCERI H. J., HANNA N. N., BECKETT M. A., GORSKI D. H., STABA M. J., STELLATO K. A., BIGELOW K., HEIMANN R., GATELY S., DHANABAL M., SOFF G. A., SUKHATME V. P., KUFE D. W. and WEICHSELBAUM R. R. (1998): Combined effects of angiostatin and ionizing radiation in antitumour therapy. Nature, **394**, 287–291.
21. NEMUNAITIS J., POOLE C., PRIMROSE J., ROSEMURGY A., MALFETANO J., BROWN P., BERRINGTON A., CORNISH A., LYNCH K., RASMUSSEN H., KERR D., COX D. and MILLAR A. (1998): Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor Marimastat on serum tumour markers in advanced cancer: selection of a biologically active and tolerable dose for longer-term studies. Clin. Cancer Res., **4**, 1101–1109.
22. O'REILLY M. S., BOEHM T., SHING Y., FUKAI N., VASIOS G., LANE W. S., FLYNN E., BIRKHEAD J. R., OLSEN B. R. and FOLKMAN J. (1997): Endostatin: an endogenous inhibitor of angiogenesis and tumour growth. Cell, **88**, 277–285.
23. O'REILLY M. S., HOLMGREN L., CHEN C. and FOLKMAN J. (1996): Angiostatin induces and sustains dormancy of human primary tumours in mice. Nat. Med., **2**, 689–692.
24. POLETTE M. and BIREMBAUT P. (1998): Membrane-type metalloproteinases in tumour invasion. Int. J. Biochem. Cell Biol., **30**, 1195–1202.
25. PRICE A., SHI Q., MORRIS D., WILCOX M. E., BRASHER P. M., REWCASTLE N. B., SHALINSKY D., ZOU H., APPELT K., JOHNSTON R. N., YONG V. W., EDWARDS D. and FORSYTH P. (1999): Marked inhibition of tumour growth in a malignant glioma tumour model by a novel synthetic matrix metalloproteinase inhibitor AG3340. Clin. Cancer Res., **5**, 845–854.
26. PRIMROSE J. N., BLEIBERG H., DANIEL F., VAN BELLE S., MANSI J. L., SEYMOUR M., JOHNSON P. W., NEOPTOLEMOS J. P., BAILLET M., BARKER K., BERRINGTON A., BROWN P. D., MILLAR A. W. and LYNCH K. P. (1999): Marimastat in recurrent colorectal cancer: exploratory evaluation of biological activity by measurement of carcinoembryonic antigen. Br. J. Cancer., **79**, 509–514.
27. QIN L. X., TANG Z. Y., LI X. M., BU W. and XIA J. L. (1999): Effect of antiangiogenic agents on experimental animal models of hepatocellular carcinoma. Ann. Acad. Med., **28**, 147–151.
28. ROSEMURGY A., HARRIS J., LANGLEBEN A., CASPER E., GOODE S. and RASMUSSEN H. (1999): Marimastat in patients with advanced pancreatic cancer: a dose-finding study. Am. J. Clin. Oncol., **22**, 247–252.
29. SHEPHERD F. A. (1997): Alternatives to chemotherapy and radiotherapy as adjuvant treatment for lung cancer. Lung Cancer, **17** (suppl. 1), 121–136.
30. STEWARD W. P. (1999): Marimastat (BB2516): current status of development. Cancer Chemother. Pharmacol., **43** (suppl.), 356–360.
31. TONN J. C., KERKAU S., HANKE A., BOUTERFA H., MUELLER J. G., WAGNER S., VINCE G. H. and ROOSEN K. (1999): Effect of synthetic matrix-metalloproteinase inhibitors on invasive capacity and proliferation of human malignant gliomas *in vitro*. Int. J. Cancer, **80**, 764–772.
32. WOJTOWICZ-PRAGA S. M., DICKSON R. B. and HAWKINS M. J. (1997): Matrix metalloproteinase inhibitors. Invest. New Drugs, **15**, 61–75.
33. WOJTOWICZ-PRAGA S., TORRI J., JOHNSON M., STEEN V., MARSHALL J., NESS E., DICKSON R., SALE M. RASMUSSEN H. S., CHIODO T. A. and HAWKINS M. J. (1998): Phase I trial of Marimastat, a novel matrix metalloproteinase inhibitor, administered orally to patients with advanced lung cancer. J. Clin. Oncol., **16**, 2150–2156.
34. YU A. E., HEWITT R. E., KLEINER D. E. and STETLER-STEVENSON W. G. (1996): Molecular regulation of cellular invasion – role of gelatinase A and TIMP-2. Biochem. Cell Biol., **74**, 823–831.

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