
Endogenous angiogenesis inhibitors

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When the FDA commissioner announced in February 2004 the approval of Avastin for the treatment of patients with colon cancer, he called angiogenesis inhibitors a fourth modality of anti-cancer therapy. Because angiogenesis inhibitors are relatively less toxic than conventional chemotherapy and have a lower risk of drug resistance, they may also represent a new class of anti-cancer agents, some of which have sufficiently reduced toxicity that they may be safely used long term. These include immunotherapy, vaccines, telomerase inhibitors, apoptosis inducers, low dose metronomic chemotherapy, novel hormonal therapies, gene therapy and others. However, at least 16 *endogenous* angiogenesis inhibitors have been discovered in the circulation, and/or in the extracellular matrix. These may become the safest and least toxic of anti-cancer therapies. Four are already being administered by injection in clinical trials for cancer. Recently, it has been reported that at least two endogenous angiogenesis inhibitors can be significantly increased in humans (endostatin), and in mice (thrombospondin), by oral administration of small molecules which themselves are already FDA approved for other uses. This finding suggests several new clinical applications for the future, including the possibility of guiding the use of angiogenesis inhibitors by blood or urinary biomarkers, currently being developed, that may detect the presence of cancer before it is symptomatic, or before it can be located by conventional methods.

Key words: Endogenous angiogenesis inhibitors; angiogenesis inhibitors; antiangiogenic therapy; metronomic chemotherapy.

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Now that angiogenesis inhibitors are receiving approval of the US Food and Drug administration for the treatment of cancer, it is timely to think about the future use of these new therapeutic agents. Of the three general types of angiogenesis inhibitors, those that target a single angiogenic mediator, such as VEGF, may be potentiated when administered together with another angiogenesis inhibitor or with low dose "metronomic" chemotherapy. Because angiogenesis inhibitors are relatively less toxic than conventional chemotherapy, and have a lower risk of drug resistance, it may become possible to

use them in therapeutic approaches that go beyond the feasibility of conventional chemotherapy. For example, as biomarkers in the blood or urine are developed that can diagnose the presence of cancer before it is symptomatic, or before it can be located by conventional methods, those angiogenesis inhibitors with the fewest side effects may become optimum therapeutic candidates. This is not to imply that preventing the disease of cancer is the exclusive province of antiangiogenic therapy. However, antiangiogenic therapy can be thought of as a representative of a new class of anti-cancer agents, some of which have sufficiently reduced toxicity that they can be used safely for long-term therapy. These agents include immunotherapy,

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vaccines, telomerase inhibitors, apoptosis inducers, novel hormonal therapies, and gene therapy, among others. The safest and least toxic of all anti-cancer therapies may be the 'endogenous' inhibitors of angiogenesis. They are the focus of this article.

ANGIOGENESIS INHIBITORS: FROM LABORATORY TO CLINICAL APPLICATION

The publication in 1971 of the hypothesis that tumor growth is angiogenesis dependent (1, 2) set in motion a study of the angiogenic process which eventually attracted thousands of investigators around the world. Endothelial cells were first passaged *in vitro* in 1973 (3, 4). This advance was followed by the development of bioassays for angiogenesis (5, 6), the discovery of angiogenic proteins, including fibroblast growth factors bFGF, aFGF (7, 8), and vascular permeability growth factor (VPF) (9), vascular endothelial growth factor (VEGF) (10), and others (6), as well as the discovery of the first angiogenesis inhibitors (11–19). The first patient to receive antiangiogenic therapy was treated in 1988 for life-threatening pulmonary hemangiomas and is healthy and free of disease today (11, 12). Throughout the 1990s, low dose daily interferon alpha began to be used to treat children with life-threatening hemangiomas and patients of all ages with angioblastomas and high grade giant cell tumors. (20–23). Thalidomide was reported to be an angiogenesis inhibitor in 1994 (19) and its first clinical success in treating multiple myeloma was reported in 1999 (24). In February 2004, the FDA approved Avastin for metastatic colon cancer, the first angiogenesis inhibitor demonstrated to prolong survival in a large, multicenter randomized, placebo controlled clinical trial in patients with advanced cancer (25). Clinical trials as well as basic research on the mechanisms of angiogenesis continue to expand. Since 1971, there have been over 20,000 publications on angiogenesis.

More than 30 angiogenesis inhibitors are in clinical trials in the US for cancer and also for macular degeneration and diabetic retinopathy, in addition to other angiogenesis inhibitors being used worldwide (Fig. 1). Taken together, these angiogenesis inhibitors can be considered

in two categories, those that are exclusively antiangiogenic, such as Avastin and VEGF-Trap, and those (called 'inclusive') that have other functions in addition to their antiangiogenic activity. In fact, several 'inclusive' angiogenesis inhibitors received FDA approval for another function before they were discovered to have antiangiogenic activity, e.g., celecoxib and zoledronate. Others were already in clinical trial before researchers found that these drugs also had antiangiogenic activity. However, at the time of their FDA approval, their antiangiogenic activity was not widely known to clinicians. Velcade, for example, is a proteasome inhibitor, but has potent antiangiogenic activity in animal models (26–28). Iressa and Erbitux, which were recently approved by the FDA, act by blocking the EGF receptor tyrosine kinase in tumor cells. This results in decreased expression by the tumor cells of VEGF, bFGF and TGF-alpha (29). Tarceva, currently in clinical trials, acts similarly. ZD6474 also inhibits the EGF receptor tyrosine kinase in endothelial cells as well as in tumor cells. The endothelial receptor is upregulated by TGF-alpha (30). For older drugs recently found to inhibit angiogenesis, the mechanism of antiangiogenic activity is still being uncovered. For example, after thalidomide was demonstrated to inhibit angiogenesis induced in the rabbit cornea by bFGF (19), subsequent reports showed that it also decreased VEGF expression and reduced overproduction of circulating precursor endothelial cells to normal levels in patients with multiple myeloma (31, 32).

TYPES OF ANGIOGENESIS INHIBITORS CURRENTLY IN THE CLINIC

In order to think about how different angiogenesis inhibitors operate in patients, we have defined three general types of angiogenesis inhibitors (Fig. 2). Type I inhibitors block a single angiogenic protein. For example, Avastin neutralizes VEGF produced by tumors and/or by stromal cells in a tumor bed. VEGF is a validated target of antiangiogenic therapy, and it is produced by approximately 60% of human tumors. However, as cancer patients live longer, there is a risk that redundant angiogenic proteins may emerge due to tumor cell mutation.

Drugs which are <u>exclusively</u> antiangiogenic	Drugs which <u>include</u> antiangiogenic activity
<p>FDA approved: Avastin (Anti-VEGF antibody) (Bevacizumab)</p> <p>In clinical trial:</p> <ul style="list-style-type: none"> * Angiostatin * Endostatin * Tetrahydrocortisol * TNP-470 Thrombospondin peptide (ABT 510) VEGF-Trap Vitaxin <p>Not yet in clinical trial:</p> <ul style="list-style-type: none"> Arresten Canstatin * Cleaved Antithrombin III * DBP-maf PEDF Tumstatin 	<p>FDA approved: Celecoxib (Celebrex) C225 (Erbix) Herceptin Iressa (Inhibits VEGF production by tumor) Rosiglitazone Taxol (Ultra low continuous dose)</p> <ul style="list-style-type: none"> * Thalidomide Velcade (proteasome inhibitor PS-341) Zoledronic acid (Bisphosphonate) <p>In clinical trial: AG013736 BAY 43-9006 CP547632 Combretastatin</p> <ul style="list-style-type: none"> * Interferon-alpha NM-3 OSI774 (Tarceva) PTK787 SU 5416 SU 6668 SU 11248 * 2-Methoxyestradiol

Fig. 1. Some angiogenesis inhibitors which are in clinical trial or already FDA approved in the U.S. The left column includes drugs for which angiogenesis inhibition is the sole function. The right column includes drugs that have other functions in addition to their antiangiogenic function. Nine of these received FDA approval for a different activity before it was discovered that the molecule was also an angiogenesis inhibitor. Examples are Celebrex (71, 72) and zoledronic acid (78).

For example, while the majority of breast cancers produce only VEGF at the time of first diagnosis, other breast cancers, especially recurrent tumors, produce up to five additional angiogenic factors (33). Therefore, some Type I angiogenesis inhibitors may over time develop the appearance of “acquired drug resistance”. However, this is likely to be the generation by the tumor, or by its associated stromal cells, of additional angiogenic proteins which the Type I inhibitor does not target. This late unresponsiveness to a Type I angiogenesis inhibitor may be avoided by combining it with other angiogenesis inhibitors, or with antiangiogenic chemotherapy (low dose or metronomic chemotherapy) (34–36) at the outset. Conventional chemotherapy may also be used together with antiangiogenic therapy, but chemotherapy at

maximum tolerated doses increases expression of tissue factor above the elevated values commonly found in cancer patients. The addition of antiangiogenic therapy to this setting may be associated with an increased risk of thromboembolic disease and myocardial infarction (37, 38).

Type II angiogenesis inhibitors block activity of two or three angiogenic proteins. For example, Iressa blocks tumor cell production of VEGF, bFGF and TGF- α , while Sugen 5416 blocks the receptor for VEGF. These inhibitors may also be augmented by administration in combination with other angiogenesis inhibitors or with metronomic low dose chemotherapy.

Type III angiogenesis inhibitors are defined as having a broad spectrum of antiangiogenic targets. For example, TNP-470, a synthetic ana-

logue of fumagillin, has perhaps the broadest anti-cancer activity of any angiogenesis inhibitor. More than 84 different types of tumors in mice, rats, hamsters and rabbits, including human tumors in mice, are inhibited from 40% to 100% in more than 400 reports. This inhibitor, which was very effective in Phase I clinical trials, was neurotoxic at higher doses and was discontinued in Phase II. However, it has now been conjugated to a polymer (hydroxypropyl methacrylamide), and is non-toxic and more effective (39). Endostatin is an endogenous angiogenesis inhibitor for which a functional receptor on vascular endothelium is $\alpha_5\beta_1$ (40, 41), but endostatin also appears to inhibit tumors that induce $\alpha_v\beta_3$ integrin on recruited endothelial cells. Endostatin has shown no toxicity in patients who have received the inhibitor by self-injection for more than 2.5 years and whose metastatic disease has remained stable or has undergone slow regression.

In summary, angiogenesis inhibitors can inhibit tumor cells from producing angiogenic proteins (e.g., Iressa, Erbitux, interferon alpha), or can block receptor interaction with these proteins (e.g., Sugen 5416 or 11248, and Tarceva), or can neutralize the protein itself, or can act directly on microvascular endothelial cells in the tumor bed to inhibit their migration and proliferation by other mechanisms (e.g., endostatin) (42).

ENDOGENOUS ANGIOGENESIS INHIBITORS

In the late 1970s a long-term effort was established in my laboratory to demonstrate the possible existence of angiogenesis inhibitors, because few scientists believed that such molecules would ever be found. From 1980 to 2003, we reported the discovery of 11 angiogenesis in-

Three types of angiogenesis inhibitors

	<i>Mechanism</i>	<i>Example</i>
I	Blocks 1 major angiogenic protein	Avastin } blocks VEGF VEGF Trap }
II	Blocks 2 or 3 angiogenic proteins	Sugen 11248 Downregulates VEGF receptor2 PDGF receptor c-kit receptor Iressa Upregulates VEGF production bFGF " " TGF- α by tumor cells
III	Blocks a broad spectrum of angiogenic regulators	Endostatin VEGF bFGF bFGF receptor HIF1 α EGF receptor ID-1 Neuropilin HPMA-TNP-470 (broadest anti-cancer spectrum) Thrombospondin-1 Maspin HIF1 α inhibitor TIMP-2

Fig. 2. An operational classification of types of angiogenesis inhibitors according to whether they inhibit one or more angiogenic proteins.

Angiogenesis inhibitors

1980	Interferon α/β , new activity (13)
1982	Platelet factor 4 (14) Protamine
1985	Angiostatic steroids (15)
1990	TNP-470, a fumagillin analogue (16)
1994	Angiostatin (17)
1994	Thalidomide (19)
1994	2-methoxyestradiol (79)
1997	Endostatin (18)
1999	Cleaved antithrombin III (43)
2002	3-amino thalidomide (44)
2003	DBP- <i>maf</i> (45)

Fig. 3. Eleven angiogenesis inhibitors discovered in the Folkman laboratory from 1980 to 2003.

hibitors. Five are novel molecules and six are molecules for which antiangiogenic activity was previously unknown (Fig. 3) (11–19, 43–45). Five of these inhibitors are currently in various phases of clinical trial and eight of them are endogenous angiogenesis inhibitors.

Interferon alpha was first shown to inhibit endothelial cell migration in a dose-dependent and reversible manner in 1980 by Bruce Zetter in my laboratory (13). This led to its subsequent characterization as an angiogenesis inhibitor (46, 47). Since 1988 (11, 12), interferon alpha has been used successfully to cause complete and durable regression of life-threatening pulmonary hemangiomatosis, hemangiomas of the brain, airway and liver in infants, recurrent high-grade giant cell tumors refractory to conventional therapy, and angioblastomas (20–23) (48 – 50). These tumors all express high levels of bFGF as their major angiogenic mediator. Interferon alpha inhibits the production of bFGF message and protein by human cancer cells (51). Interferon beta, the *in vivo* counterpart of the commercially available interferon al-

pha, was the first known endogenous angiogenesis inhibitor. There are now at least 15 such endogenous inhibitors (Fig. 4).

However, we did not immediately appreciate that these molecules could potentially function as *endogenous* angiogenesis inhibitors until the subsequent discovery of angiostatin (17). Angiostatin was the first endogenous angiogenesis inhibitor to be discovered as an internal fragment of a larger protein, plasminogen. Endostatin was the first endogenous angiogenesis inhibitor identified as an internal fragment of a matrix protein, collagen XVIII (18). For review see reference (6).

GENETIC EVIDENCE THAT ENDOGENOUS ANGIOGENESIS INHIBITORS DEFEND AGAINST PATHOLOGIC ANGIOGENESIS

There are several studies which demonstrate that angiogenesis inhibitors in the body help to suppress pathologic angiogenesis (52, 53). Raghu Kalluri and colleagues of the Beth Israel Hospital in Boston have reported compelling evidence. They discovered tumstatin, a 232 amino acid peptide in the alpha chain of collagen IV that potently inhibited angiogenesis (54).

Endogenous angiogenesis inhibitors (in plasma and / or extracellular matrix).

1. Alphastatin (83)
2. Angiostatin (17)
3. Arresten (80)
4. Anti-thrombin III (truncated) (43)
5. Canstatin (81)
6. Endostatin (18) *Phase II*
7. Fibulin-5 (84)
8. Interferon-beta (51) *Phase III*
9. 2-methoxyestradiol (79) *Phase II*
10. Pigment epithelial derived factor (PEDF) (82)
11. Platelet factor 4 (PF4) (14)
12. Tetrahydrocortisol (15) *Phase III*
13. Thrombospondin-1 (57) (& -2) (85) *Phase II*
14. TIMP-2 (86)
15. Tumstatin (55)
16. Fragment of histidine-rich glycoprotein (87)

Fig. 4. Currently known endogenous angiogenesis inhibitors.

In animals in which the alpha 3 chain was deleted, the normal blood level of tumstatin of 336 ± 28 ng/ml fell to 0 ng/ml. Tumors implanted in these tumstatin-deficient mice grew 3-fold to 4-fold faster than when grown in wild-type mice. However, if tumstatin was replaced to physiological levels, tumor growth in the alpha 3 chain-null mice was reduced to the slower rate of the wild-type mice (55). This indicated that tumors in the wild-type mice were not growing at ceiling rates, but that their growth rate was partially inhibited by endogenous tumstatin. When pharmacologic doses of tumstatin were administered, tumor growth was significantly inhibited below that of tumors in wild-type mice. This fulfills the classic paradigm of a tumor suppressor protein like p53, except that tumstatin is purely antiangiogenic and as yet has no other known function. Furthermore, circulating endothelial cells were 10-fold higher in the tumstatin deficient mice than in their wild-type counterparts, but could be reduced to normal levels by replacement of tumstatin (55). $\alpha_v\beta_3$ was found to be a functional integrin for tumstatin (42), and $\alpha_5\beta_1$ was found to be a functional integrin for endostatin (41, 42). Tumstatin inhibited DNA synthesis in endothelial cells from $\alpha_v\beta_3$ +/+ mice, but not in endothelial cells from $\alpha_v\beta_3$ -/- mice. Endostatin inhibited DNA synthesis in both mouse strains. A similar response was obtained against VEGF-induced angiogenesis in subcutaneously implanted matrigel pellets. Tumstatin suppressed angiogenesis only in β_3 positive mice, but not in β_3 negative mice, while endostatin suppressed angiogenesis in both mouse strains. In another experiment, tumstatin blood levels were decreased by approximately 40% in metalloproteinase-9 deficient mice because metalloproteinase-9 mediates cleavage of tumstatin from collagen IV (55). In these mice, implanted tumors grew significantly more rapidly, but when tumstatin was replaced to physiological levels, tumor growth returned to the slower rate of tumor growth in wild-type mice. In summary, when the angiogenesis inhibitor was knocked out, or its receptor was knocked out, or the enzyme, which releases the ligand from its matrix protein, was knocked out, all manipulations predictably showed the same effect of increased angiogenesis and increased tumor growth, phenomena

that could be returned to wild-type levels by physiological replacement of tumstatin.

Thrombospondin-1 is an endogenous angiogenesis inhibitor. Tumors grow significantly faster in thrombospondin-1 null mice than in wild-type mice (56). Therefore, endogenous angiogenesis inhibitors, like p53, may provide an additional defense against tumors under normal conditions. In fact, p53 itself inhibits angiogenesis by at least four distinct mechanisms. It (i) upregulates thrombospondin-1 expression (57); (ii) degrades hypoxia inducible factor-1 (58); (iii) suppresses expression of VEGF (59); and (iv) downregulates expression of bFGF binding protein (60).

CORRELATIVE CLINICAL EVIDENCE THAT ENDOGENOUS ANGIOGENESIS INHIBITORS MAY FUNCTION AS TUMOR SUPPRESSOR GENES

Individuals with Down syndrome have a very low incidence of solid tumors even though they now live until late middle age or older (61). In a population-based study of 17,897 individuals with Down syndrome in the US, there was less than 0.1 the expected incidence of all solid cancers except for testicular cancer, which was present at the same rate as in the normal population. There was an increased incidence of leukemia in children. Individuals with Down syndrome also have a significantly high level of circulating endostatin, attributed to the presence of three copies of collagen XVIII on chromosome 21 (62). Serum endostatin in normal subjects is 20 ± 11 ng/ml compared to 39 ± 20 ng/ml in Down individuals. The authors conclude, "an increase of about one-third of normal endostatin may represent an effective therapeutic dose to significantly inhibit many solid tumors". Diabetic retinopathy is very rare in individuals with Down syndrome (63), and so is atherosclerosis (64). Growth of atherosclerotic plaques in Apo E-null mice has been reported to be dependent upon neovascularization of these plaques. Administration of endostatin inhibits plaque growth by up to 85% of untreated controls (65). Genetic deletion of collagen XVIII with subsequent endostatin deficiency increases neovascularization and vascular permeability in atherosclerosis (66). VEGF

increases expression of calcineurin in vascular endothelial cells (67), but VEGF also increases expression of the Down syndrome critical region protein-1, which downregulates calcineurin expression and therefore tends to counteract the increased expression of calcineurin induced by VEGF. Upregulation of calcineurin is essential for endothelial proliferation. It can be speculated that endostatin contributes to the downregulation of calcineurin in endothelial cells which is also mediated in part by VEGF stimulation of the Down syndrome critical region protein-1, of which there would be an extra copy in individuals with Down syndrome.

CAN ENDOGENOUS ANGIOGENESIS INHIBITORS BE INCREASED PHARMACOLOGICALLY?

The data from individuals with Down syndrome indicate that circulating levels of endostatin can be elevated genetically. This raises the question of whether it is possible to induce the increase of any of the endogenous angiogenesis inhibitors listed in Fig. 4 by administration of small molecules, and in particular administration of orally available small molecules.

The first indication that endogenous angiogenesis inhibitors could be increased independently of administering the proteins themselves resulted from studies of low dose chemotherapy administered frequently, initiated by Timothy Browder in our laboratory (34). Browder reported that conventional chemotherapy such as cyclophosphamide administered by the traditional schedule of maximum tolerated doses interspersed with off-therapy intervals of 3 weeks to permit recovery of bone marrow led to drug resistance in all tumors when therapy was started in Lewis lung carcinomas at tumor volumes of 100 to 650 mm³ (34). In contrast, when cyclophosphamide was administered at more frequent intervals and at lower doses, it acted as an angiogenesis inhibitor. Proliferating endothelial cells in the tumor vascular bed underwent a wave of apoptosis ~4 days *before* tumor cell apoptosis began. All tumors completely regressed and animals remained tumor free for their normal lifespan (up to 657 days). When the tumors were made resistant to cyclophosphamide, there was still significant tumor inhi-

bition compared to tumors treated by the conventional (maximum tolerated dose) schedule of cyclophosphamide. When a second angiogenesis inhibitor, TNP-470, was added there was complete tumor regression which was durable throughout the normal lifespan of the animal. In contrast, all animals treated with the conventional cyclophosphamide schedule died with drug resistant tumors. This study was confirmed by Robert Kerbel's laboratory in Toronto using a different chemotherapeutic agent, vinblastine, administered in combination with a different angiogenesis inhibitor, DC101, an antibody to VEGF receptor 2 (36). In other words, "antiangiogenic chemotherapy", i.e., frequent low doses of cyclophosphamide, caused apoptosis preferentially in microvascular endothelial cells in the tumor bed, optimized the antiangiogenic capacity of this drug, reduced its toxicity, and virtually eliminated acquired resistance to cyclophosphamide. In a separate experiment, when mice received a given dose of endostatin administered continuously over 24 h by an osmotic pump, there was 10 times more efficacy in inhibiting growth of human pancreatic cancer than when the same dose was given once a day as a bolus (68). In fact, tumor regression was achieved only by continuous administration. Frequent low doses of chemotherapy may be similar to exposure of endothelial cells in a tumor bed to continuous endostatin therapy.

Browder called this approach "antiangiogenic chemotherapy" to indicate that the antiangiogenic activity of a chemotherapeutic agent could be maximized by a change in dose and schedule. In an editorial, Douglas Hanahan coined the term "metronomic" therapy to indicate the new schedule itself. This term has now become widely used. A variety of clinical trials of antiangiogenic, low dose chemotherapy are currently underway (69). Subsequently, Kerbel's lab administered low dose "metronomic" cyclophosphamide in the drinking water of tumor-bearing mice and demonstrated 95% inhibition of tumor growth associated with a significant increase in circulating thrombospondin-1 (70). The low dose "metronomic" chemotherapy was ineffective in thrombospondin-1 null mice, indicating that the low dose oral chemotherapy was in part dependent on its capacity to induce an increase in circulating thrombospondin-1. This

Orally administered small molecules which increase endogenous angiogenesis inhibitors:
An emerging pharmaceutical field?

Small molecules : Endogenous angiogenesis inhibitors

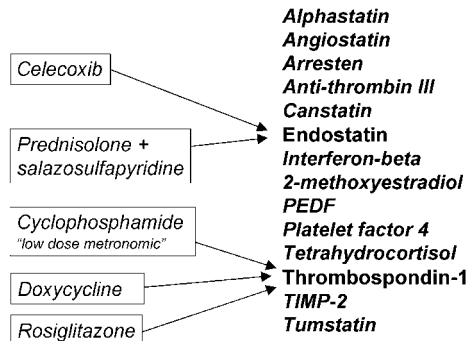


Fig. 5. A figure to illustrate the concept that certain endogenous angiogenesis inhibitors can be increased in the circulation by oral administration of small molecular weight compounds, all of which are FDA approved, and are relatively non-toxic. The figure also suggests that it may be possible to find small molecules which can increase the plasma level of other endogenous angiogenesis inhibitors on this list such as alphastatin, TIMP-2 and tumstatin.

result prompted me to ask whether any other small molecules delivered orally can induce increased circulating levels of endogenous angiogenesis inhibitors. If certain endogenous angiogenesis inhibitors could be increased in the blood, they would mimic continuous administration of the inhibitor itself. Celecoxibs were reported to be angiogenesis inhibitors (71). Subsequently, celecoxibs and rofecoxibs were reported to increase circulating endostatin by releasing it from platelets (72). Low dose doxycycline, but not tetracycline, increased thrombospondin-1 in ras transfected human cancer cells (73) and doxycycline was associated with complete regression of life-threatening pulmonary hemangioendotheliomatosis (50). Rosiglitazone, a PPAR gamma ligand, has also been reported to induce an increase in circulating thrombospondin-1 as well as an increase in its receptor, CD36 (74, 75). Furthermore, a standard therapy for arthritis, salazosulfapyridine with prednisolone, induces an increase of endostatin in the synovial joint, as well as decreased VEGF.

Taken together, at least five small molecules, all of which are FDA approved drugs in wide use for other diseases, can increase endogenous

levels of either endostatin or thrombospondin-1 (Fig. 5). We have already reported that administration of rosiglitazone to tumor-bearing mice prevents growth of lung metastases after the primary tumor was removed (75). Therefore, it may be prudent to determine if growth of human tumors in SCID immunodeficient can be reversed or prevented by elevating circulating levels of endogenous angiogenesis inhibitors.

To prevent the conversion of non-angiogenic *in situ* carcinomas to angiogenic tumors, or to treat microscopic angiogenic tumors years before they become symptomatic, increasing *endogenous* angiogenesis inhibitors may be among the safest forms of long-term anti-cancer therapy. In the near future, as biomarkers are perfected which detect the presence of cancer before it can be anatomically located (for examples of early prototypes see references (76) and (77)) it may become feasible to increase endogenous angiogenesis inhibitors. This novel form of therapy could be guided by biomarkers without waiting for a tumor to grow to a size detectable by conventional imaging methods. An analogy from cardiology would be the current use of biomarkers, such as serum cholesterol and C-reactive protein, to guide the treatment of atherosclerosis by a statin (e.g., Lipitor), without knowing the anatomical location of plaques.

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