Tumor Angiogenesis Factor
Speculations on an Approach to Cancer Chemotherapy

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Tumor angiogenesis factor (TAF) and its importance in determining a strategy for cancer chemotherapy are discussed. It is suggested that inhibition of RNA synthesis or increased RNA catabolism might interfere with the metabolism of solid tumor cells more so than in normal cells, and thus hinder angiogenesis and pursuant tumor growth by preventing the synthesis of the RNA component of TAF. An attempt is made to indicate potential models for anti-angiogenesis agents of this type. The drugs offered as initial prototypes for investigations along these lines are actinomycin D (which likely has antimetabolite and anti-angiogenesis activities), polyriboinosinic-polyribocytidylic acid (which likely has adjuvant and anti-angiogenesis activities) and ribonuclease (which in theory might be a purely anti-angiogenic agent). It is noted that these models may turn out to be less than ideal as therapeutic agents due to problems of toxicity, metabolism, potency, or distribution, but nonetheless might serve to yield insights into the design of new cancer chemotherapeutic drugs. In addition, some evidence is cited suggesting that actinomycin D may be more effective against certain tumors when employed in lower, chronic dosages rather than its present use in "loading" dosages.

The concept of anti-angiogenesis agents as fundamentally "tumorstatic" therapies is discussed, and the likelihood that such agents might be effectively "tumoricidal" in immunocompetent hosts is mentioned. The main promise of an anti-angiogenic strategy is efficacy against presently intractable slowly growing human cancers when used in combination with other treatment modalities. In summary, a strategy of cancer chemotherapy predicated upon interference with RNA synthesis or increase in RNA catabolism is offered as a potential mechanism for establishing anti-angiogenesis, and as a promising alternative and adjunct to present methods.

INTRODUCTION

This paper is an attempt to relate evidence from several disparate areas of investigation—from experiments designed to elucidate the mechanism of solid tumor

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neovascularization, and from investigations upon the anti-tumor effects of the antibiotic actinomycin D (AcD), the synthetic polyribonucleotide polyriboinosinic-polyribocytidylic acid (poly I:C) and the enzyme ribonuclease. The intent is to suggest that recent findings support the possibility of relationships between the anti-tumor activities of these agents and the substance “tumor angiogenesis factor (TAF)” (1). If any of the hypothetical relationships obtain, they have significant implications for evolving a strategy for cancer chemotherapy. It should be emphasized at the outset that the discussion, evaluation and proposals that follow are highly speculative in nature, and should not be construed to be directly supported by experimental data unless this is particularly specified. Some degree of circumstance is due to the necessity of drawing upon studies that were not designed to answer questions pertinent to the subject of this paper.

TUMOR ANGIOGENESIS FACTOR

Several years ago, Judah Folkman and his colleagues published their initial report on the “Isolation of a Tumor Factor Responsible for Angiogenesis” (1). Studies that support the existence of tumor angiogenesis factor (TAF) were later contributed by a British group (2). As summarized in these and later reports, our knowledge concerning how a tumor recruits and maintains its blood supply (neovascularization) encompasses the following principles.

1. Solid tumor growth requires neovascularization (1, 3, 4).
2. In the absence of neovascularization, solid tumors cannot grow beyond the size of a spheroid perhaps 2–3 mm in diameter (3).
3. Neovascularization of solid tumors does not require direct cell to cell contact between tumor cells and endothelial cells (1, 2).

The bioassay for TAF activity is somewhat inconvenient and requires large amounts of test material (1). However, the possibility of more convenient in vitro assays is being explored (6). TAF activity has been found in the cells of numerous animal and human tumors, including Wilm’s tumor and choriocarcinoma (1, 5). The only normal tissues in which TAF activity has been found are human placentas of 18 wk fetuses; regenerating normal tissue has been found to be TAF negative (5). The experiments proving the existence of a specific angiogenetic factor were well controlled against nonspecific inflammation and vascular proliferation (1). Confirmation of neovascularization induced by TAF preparations has been by light and electron microscopy (1, 7) and observation of radioactive nucleotide uptake in mitotic endothelial cells (4, 8).

Preliminary characterization of TAF preparations has yielded the following information.

1. TAF isolated from tumor cell cytoplasm and nuclear nonhistone protein fractions contains varying amounts of associated ribonucleic acid (RNA) and carbohydrate (1, 5, 9, 10).
2. TAF has an estimated molecular weight of 100,000 daltons or less (5, 9–12).
3. TAF activity in the bioassay is destroyed by exposure to ribonuclease and some proteases (1, 5, 10).
Folkman and his colleagues interpret these data as implying that both the protein and RNA components of TAF are essential to its biological activity. One possibility is that the protein and/or carbohydrate components act as the transport and endothelial cell receptor-specific moieties, much like a viral capsid, and that the RNA specifies or initiates the proliferative response of the endothelial cell, much like a viral genome. The sensitivity of TAF to ribonuclease suggests a crucial role for the RNA component in any case.

It has also been discovered that withdrawal of TAF is followed by the disappearance of newly formed capillaries (5, 9). This is especially relevant and hopeful from the standpoint of potential therapeutic possibilities of “anti-angiogenesis.” As a possible mode of “anti-angiogenesis” (i.e., inhibition of the activity and effects of TAF), Folkman’s laboratory has attempted to generate antibodies against TAF. Their lack of success at this time has been attributed to the seeming lack of species specificity and the RNA content of TAF (5, 9). Human tumor TAF induces endothelial cell mitoses in mice, rats, and rabbits (5).

An interesting question is how TAF is related to the only other cellular product that has been found in all solid tumors examined. Unkeless et al. (13) demonstrated that cells from animal and human solid tumors show increased fibrinolytic activity. A similar phenomenon has been reported by Yunis et al. (14). This activity has not been thoroughly biochemically characterized, but apparently it requires a cellular component and a serum component (13, 15). It would be quite fortuitous and useful if the synthesis of TAF and the cellular fibrinolytic factor were genetically linked in their synthesis or regulation. Certainly it is worth knowing if the protein component of TAF has any fibrinolytic activity.

The potential significance of TAF is best summarized by Folkman himself:

“Because endothelial cell turnover usually lags behind tumor cell growth, most solid tumors may always be on the verge of necrosis. Interference with their capillary component is likely to disturb this equilibrium and shift it toward more necrosis. If TAF is indeed the mediator for neovascularization, then blockade of TAF might prevent vascularization, i.e., anti-angiogenesis” (11).

Thus it is easy to see how anti-angiogenesis might become an important factor in cancer therapy, especially as an adjunct to surgical tumor removal, and as therapy for small, early growth tumors that have been detected, but not located. This latter situation may become a very real problem as highly sensitive immunological or biochemical tumor detection methods are developed. The microscopic tumor detected might be impossible to locate, but could be destroyed by a combination of anti-angiogenesis and other treatment modalities. Since small tumors seem to be more susceptible to chemotherapy (11), anti-angiogenesis might be able to potentiate the effects of our present therapeutic agents, and prevent development of large metastases.

Folkman and his co-workers have emphasized preparation of anti-TAF antibodies as a possible mechanism of anti-angiogenesis. However, as previously noted, there is evidence that this may be very difficult, if not impossible. I would like to suggest that an exploitable mechanism for anti-angiogenesis would be to inhibit or interfere with the synthesis of, or to increase the catabolism of TAF, through interference with the synthesis of, or increased degradation of its unusual RNA component. Anti-angiogenetic agents that affect RNA metabolism might be ex-
expected to have greater effects upon cells dependent upon TAF than on normal cells. This possibility will be discussed in the ensuing sections of this paper.

ACTINOMYCIN D

It is worthy of note prior to this discussion of actinomycin D (AcD) that many of the points made might, in most cases, apply to similar drugs which are now coming into wider clinical use, such as adriamycin, and which possess similar, but not identical properties. Actinomycin D is thought to exert its anti-tumor effects by inhibiting the DNA-dependent synthesis of RNA (16–21). AcD is believed to be selectively more toxic to certain tumor cells than to normal cells, because the tumor cells replicate faster, and thus have a greater requirement for synthesizing RNA. AcD forms a stable complex with cellular DNA, which prevents RNA polymerase from transcribing the sequences at these sites (18, 21–23). The formation of the complex requires the presence of a guanine residue, preferably in the minor groove of the DNA helix. At concentrations of AcD attainable in vivo, synthesis of ribosomal RNA is preferentially suppressed. If slightly higher AcD concentrations are attained, synthesis of all varieties of RNA is affected. In vitro, even higher concentrations of the agent can directly interfere with cellular DNA synthesis (21).

It is interesting to note that the cytotoxicity of AcD, seen primarily in proliferating cells, correlates better with tissue retention of the drug than with the degree of acute inhibition of RNA synthesis (21, 22, 24). Schluederberg et al. (25) and Benedetto and Djacenko (26) have presented evidence that cells in culture recover the ability to synthesize RNA in direct proportion to their ability to rid their intracellular milieu of AcD. It thus seems reasonable that, in the case of some tumor cells in vivo, therapeutic success would depend upon maintaining suitable intracellular AcD levels, and that intermittent AcD administration might have little anti-tumor effect.

Given AcD’s effects on RNA synthesis, and the unusual RNA content of TAF, one might predict that AcD should possess substantial anti-tumor activity against malignancies dependent upon TAF for neovascularization. If local concentrations of AcD high enough to inhibit TAF–RNA synthesis are attainable, selective toxicity against recruitment of a blood supply by tumors might be achieved, i.e., anti-angiogenesis. Not only would AcD inhibit tumor cell RNA synthesis in general, but tumor expansion and survival would be mitigated against by the inhibition of TAF synthesis. What evidence exists that AcD possesses unusual anti-tumor activity that might be attributable to such a mechanism?

Firstly, AcD is perhaps the most potent known anti-neoplastic agent on a molar basis (27). AcD inhibits by as much as 75% tumor induction by some chemical carcinogens on mouse skin (28). Hela cell tumors injected into mice that are immunosuppressed remain localized and small with AcD treatment, rather than forming large tumors as in untreated controls (29). AcD is the drug of choice for the treatment of trophoblastic neoplasms (30–32). Probable cure rates approaching 80–100% are seen with AcD as the only therapy (31). This is quite the exception in terms of efficacy for single agents employed against solid tumors. Promising responses to AcD by lung tumors (33), sarcomas (34), and testis cancer metastases (35) have also been reported. AcD is the drug of choice in the treatment of Wilm’s tumor (36–38).

Despite this evidence, it is not possible to claim a wide-spectrum anti-tumor activity for AcD compared with other drugs. However, the potential for wide-spec-
trum activity may exist. The lack of evidence is at least partially a tribute to the difficulties of screening any given drug against different tumors in vivo, and the problems of determining dosage, scheduling and route of administration optimally. At present one can only state that in the cases where an effective regimen of AcD therapy has been determined, its efficacy is rather impressive.

It has been argued that the main reason AcD and other antimetabolite drugs are effective against certain tumors is that the tumors involved are extremely quickly growing, and thus the differential drug toxicity between normal and tumor cells is correspondingly great (i.e., tumor cells are more susceptible) (39). This is certainly the major factor in the effectiveness of most “cell cycle active” antimetabolites, which depend on killing tumor cells for their success. AcD undoubtedly functions in this manner too. Such “cell cycle active” agents are usually given intermittently, in loading doses, until toxicity appears. However, if AcD can inhibit TAF–RNA synthesis, there is reason to believe that its greatest effectiveness as an anti-angiogenesis agent would be in prolonged, continual dosage at a level sufficient to establish anti-angiogenesis. Its main effect in this case would be “tumoris-tatic” rather than “tumoricidal.” This would presumably be relevant even in quickly growing tumors, because they are greatly dependent upon neovascularization for their growth. Thus, in the case of Wilm’s tumor and choriocarcinoma, AcD may exert both cell killing and anti-angiogenetic effects. It is in the majority of human tumors, which are, if anything, more slowly growing than normal cells, and thus generally insusceptible to known antimetabolite cell killing agents, that anti-angiogenesis may prove to be of critical importance. If an anti-angiogenesis agent were administered continually, a tumor would not be able to grow further, and might be expected to regress if the host were immunologically competent or if other therapies were jointly employed.

At this point it is important to note that it is possible that a potent anti-angiogenesis agent might appear to be ineffective against tumors in the type of screening systems now in use (39). This might be due to impaired immunological defences of the host animal, incapable of eradicating residual tumor cells, or to the lack of some form of adjunct therapy to destroy the bulk of any initial tumor load. An anti-angiogenetic agent would exert primarily a “tumoristic” effect, and only be “tumoricidal” if the tumor were highly dependent upon vascularization. Even then, in the immunologically incompetent host, a residual tumor of 2–3 mm in diameter would surely remain, unable to grow, but also insensitive to any further tumoricidal effects of the anti-angiogenesis drug. Thus, even if potent anti-angiogenesis agents are developed, immunotherapy and other “tumoricidal” treatment modalities will be of utmost importance.

Admittedly, much of the foregoing is speculative, or at least inferential. There is some evidence that AcD is effective at lower continual doses against more slowly growing tumors. In mouse melanoma B16 (a known TAF producer), a relatively slowly growing tumor, AcD may be most effective in prolonged, chronic usage at doses lower than those heretofore thought optimal (21). Lower dosage, chronic administration trials with AcD on slowly growing neoplasms in animals and humans have not yet been reported to any significant extent. In view of the fact that the half-life of messenger RNA in cells is on the order of hours, to at most a few days (40), it is not unreasonable to speculate that the “loading dose” scheme of AcD administration may be poor strategy. This is especially so since it is known that tumor cells may recover from AcD inhibition quite quickly (25).
POLY I:C

Interest in the synthetic, double-stranded RNA polynucleotides—polyribocytidylic acid (poly I:C) as an antitumor agent arises from reports that the substance possesses activity against the growth of several murine transplantable tumors, of both viral and nonviral origin (22, 41–46). The data of Levy et al. (41) indicated that the growth of a reticulum cell sarcoma, a lymphatic lymphoma, a fibrosarcoma and a human adenovirus-12-induced tumor was inhibited by injections of poly I:C. Leukemic malignancies were least sensitive and the reticulum cell sarcoma was most sensitive to the drug's effects. In the case of the reticulum cell sarcoma and adenovirus-induced tumors, initiation of treatment even after the malignancy had grown to moderate size led to tumor regression, and some animals were free of disease two months after therapy ceased. With the adenovirus-induced tumors, the initiation of poly I:C therapy yielded a very rapid "massive necrosis and sloughing" of tumor tissue in four of 10 animals, and marked regression in the other six. The authors mention the possibility that poly I:C may act upon blood vessels and affect the tumor blood supply. However, although many of the pathological changes attributed to poly I:C toxicity appear to be effects of the agent upon vascular endothelium (42), there is no explanation for why it might affect tumor blood vessels more than "normal" vasculature.

Data have also been published recording the apparent cure of malignant melanoma B16 in mice, employing post-transplantation treatment with poly I:C (44). Results indicating inhibition of the growth of the murine adenocarcinoma DBA in mice (45) and of two-stage skin carcinogenesis (46) by poly I:C therapy have appeared. Apparently, poly I:C is an anti-tumor agent with a surprisingly low degree of cytotoxicity (47). Assessment of the effects of poly I:C on viral and chemical oncogenesis in vitro and in vivo have, however, yielded less clear cut findings. Poly I:C enhances viral and chemical oncogenesis in certain cases and protects against it in others (22, 48–51). The general consensus is that the antitumor properties of poly I:C are unrelated to its ability to induce interferon (48–52).

It has been suggested that poly I:C's anti-tumor activity is attributable to its known activation and stimulation of the host's immunological defence system (22, 43). However, over the last few years, it has been demonstrated that poly I:C's anti-tumor activity is equivalent in both immunocompetent and thoroughly immunosuppressed animals (53–55). In addition, poly I:C has been discovered to have a variable effect on humoral immunity in normal mice, and can suppress immunity in some animals (55). This might explain the variable results in attempting to employ poly I:C against oncogenic viruses. In any case, it does not seem likely that poly I:C's anti-tumor activity is due primarily to its effects as an adjuvant.

Levy and Riley have shown that poly I:C strongly suppresses in vivo RNA and protein synthesis in several different murine tumors (56). RNA and protein synthesis in normal tissues was inhibited or enhanced depending on the mouse strain tested. The inhibitory effect of poly I:C on macromolecular metabolism was greater in tumor than non-tumor tissues. Supporting evidence suggests direct cellular toxicity of some kind as the mechanism for the anti-tumor activity of poly I:C (57–60).

It has been demonstrated that some tumors show greatly increased transfer RNA methylase enzyme activity (61–64). These studies included viral-induced (62) and
spontaneously arising tumors (63). Concurrently, investigations concerning the mechanism of anti-tumor action of poly I:C have shown that this agent significantly inhibits tumor cell ribonucleotide reductase (65) and RNA methylases (66). These latter authors suggest that “preferential inhibition of RNA methylation may be partially responsible for the anti-tumor activity of poly I:C (66).”

I would like to take this hypothesis (66) one step further. I propose that the anti-tumor activity of poly I:C, and its unusual effects on tumor vasculature are due in part to interference with the synthesis of the RNA component of TAF. Thus, poly I:C may be a potential anti-angiogenesis and “tumorstatic” agent, and is worthy of investigation as such. This is particularly so, as it appears to have low toxicity in preliminary human trials (67).

It is of interest to note, that of the two anti-angiogenesis agents thus far proposed, one (AcD) is quite potent, but has yet to demonstrate the broad-spectrum activity that would be expected, and the other, poly I:C, is less potent, but has been shown to have effects upon a number of diverse tumors. The former compound acts at the level of genetic transcription and the latter perhaps at the level of RNA “processing.” Since these agents would exert their influences at different points in the same metabolic pathway, it is to be expected that they would act synergistically. That is, by inhibiting two different stages in the synthesis of TAF–RNA, they might, used in combination, render tumor cells incapable of producing functional TAF. Employing poly I:C in mice, Pieroni et al. (68) have found that simultaneous administration of AcD increases the toxicity of poly I:C about 850-fold. Whether this cooperative effect would also obtain in tumor tissues is unknown. However, the possibility of employing lower, less toxic doses of AcD in combined chemotherapy with poly I:C is suggested by these findings.

**RIBONUCLEASE**

Since TAF by necessity functions extracellularly, it should be uniquely susceptible to chemotherapeutic or immunological attack. Difficulties in generating anti-TAF antibodies have been alluded to previously. However, TAF–RNA is sensitive to ribonuclease; therefore, the possibility of exploiting this enzyme as an antiangiogenesis agent is well worth considering. It has been known since the late 1950’s that ribonuclease administration can have an inhibitory effect on the growth of ascites type tumor cells both in vivo and in vitro, and that the enzyme may enter the cells involved (69). To the best of the author’s knowledge, no studies concerning administration of ribonuclease to humans as an antineoplastic agent have been published. This is not surprising since there was no reason to even suspect this enzyme might affect the growth of tumor tissue. Also, for reasons already discussed, since ribonuclease could be expected to be an essentially pure “tumorstatic” agent, in single agent trials, it very well might seem ineffective. However, a Russian group has reported the inhibition of lymphosarcoma growth in rats by ribonuclease therapy (70, English abstract). There is little other evidence for anti-tumor activity on the part of ribonuclease employed as a single agent.

A most interesting study is that of Sartorelli (71). In this investigation the survival of mice bearing sarcoma-180 ascites cell tumors was markedly enhanced by AcD and ribonuclease conjoint therapy at levels of each compound, which, when employed individually, displayed little or no effect. This series of experiments was carefully controlled, and therapy began 24 hr post-tumor implantation. Given the
RNA content of TAF, this cooperativity of tumor inhibition by agents interfering with RNA metabolism takes on a promising light.

Certainly there is reason to hope that it may be possible to utilize ribonuclease activity in vivo, particularly in the case of hepatomas. At least by histochemical techniques, many tumor cells do not express ribonuclease activity (72). A recent paper by Daoust is relevant (73). Briefly, he found that the development of preneoplastic liver nodules was focally accompanied by loss of ribonuclease activity. The evidence appears convincing that a significant decrease in ribonuclease activity is a prerequisite or concomitant event in the origin of chemically-induced hepatomas in the rat (73-74).

It is interesting to consider whether host serum ribonuclease levels play any role in the defence against or reaction to tumor growth. The data on serum alkaline ribonuclease levels in patients having cancer is rather variable. However, a recent study selected about 100 patients in good general health with a range of histologic types of tumors, all of which were surgically resectable (75). The observations were that the mean serum alkaline ribonuclease levels in patients with adenocarcinomas and squamous carcinomas were significantly higher than those of normal patients, or those with sarcomas or melanomas. The level of ribonuclease was not related to whether or not metastatic spread had occurred. These results, while far from conclusive, suggest that serum ribonuclease levels may possibly reflect the host’s reaction to the presence of a growing tumor.

Certainly there is reason to hope that it may be possible to utilize ribonuclease as a cancer chemotherapeutic agent, and that its postulated effects on TAF–RNA might play an important role. If this potential usefulness of ribonuclease is realized, the likelihood of its employment as a relatively nontoxic, selective, homologous anti-tumor drug is a very real one. The technology for artificial synthesis of enzymes of this type and size is now available due to the efforts of Merrifield and his colleagues (76).

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