Cancer and Metastasis Reviews **9**: 171–174, 1990.

Introduction

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The field of 'angiogenesis research' began more than two decades ago as an inquiry into the mechanisms by which tumors induce a new blood supply [1, 2]. The field has now expanded to include a diverse group of scientists who are addressing new central questions. For example, developmental biologists are asking, 'What are the rules for development of the vascular system itself?' [3]. Geneticists are studying suppressor genes which code for natural inhibitors of angiogenesis [4]. Molecular biologists are identifying specific molecules in the extracellular matrix which govern morphologic patterns such as the formation of capillary tubes [5]. Angiogenic molecules are being employed to enhance the healing of surgical wounds [6] as well as peptic ulcers [7], and experimental studies are underway to determine if blood vessel growth can be potentiated in the heart [8].

However, the field of angiogenesis research was originally based on the hypothesis that *tumor growth is angiogenesis-dependent* [9]. This hypothesis continues to be fruitful as it enlarges our understanding about tumor growth and metastasis. In this issue of *Cancer and Metastasis Reviews* seven leading investigators report new findings about the interaction between tumor cells and the vascular system. D'Amore examines the pathways by which an angiogenic peptide, basic fibroblast growth factor, can be released from tumor cells and from other cells. She raises the general question of how growth factors escape from tumor cells and reach endothelial cell targets [10]. Denekamp explores the role of current and future cancer chemotherapy in attacking tumor vasculature [11]. Gullino *et al.* show that during the spontaneous development of a tumor, onset of angiogenic activity may precede the onset of neoplasia [12]. They also review the evidence that certain naturally occuring small molecules may mediate the angiogenic process. These include prostaglandin E1, certain gangliosides and copper ions as well as copper carrier proteins. Jain provides evidence that access to tumor cells by anticancer compounds, or by immune cells is complicated by the fact that tumor vessels are subject to higher interstitial pressures and longer transport distances than are vessels within normal tissues [13]. Pauli *et al.* show that microvascular endothelial cells in different organs are heterogenous and that specific adhesion molecules on these cells may influence the site-specificity of metastases [14]. Vlodavsky *et al.* outline the experimental evidence that basis fibroblast growth factor is stored as a complex with heparan sulfate in the basement membranes of blood vessels as well as the cornea, and possibly other tissues. Because heparanase activity of certain tumors correlates with their metastatic potential, it is postulated that this enzyme may participate in both tumor cell invasion and onset of angiogenes by degradation of the heparan sulfate in extracellular matrix and release of endothelial growth factors [15]. Yayon and Klagsbrun propose a new model in which members of the fibroblast growth factor family regulate cell proliferation by both internal and external autocrine pathways [16].

When these observations are taken together with recent reports from other laboratories, some general precepts are emerging which I have assembled below in the belief that they may guide future studies. I have omitted extensive references in this preface.

(i) Considerable experimental and clinical evidence reveals that tumor growth is *angiogenesis-dependent* [17]. This phenomenon applies where

solid tumors grow in three-dimensional aggregated populations. Neovascularization is permissive for *expansion* of such a tumor beyond the volume restricted by oxygen and nutrient diffusion. In the absence of neovascularization most tumors would be limited to a volume of a few mm^3. Neovascularization is not sufficient for tumor expansion. Thus, if tumor cells are incapable of rapid proliferation, a tumor may be highly angiogenic but slowly growing, e.g., adrenal adenoma. 'Non-solid' tumor growth may be independent of angiogenesis, e.g., ascites, or tumors which spread in a thin plane along a nerve sheath or over the meninges.

(ii) Many human tumors develop through a prolonged *pre-vascular* phase followed by a *vascular phase* [18]. Pre-vascular tumors may exist for years as thin, asymptomatic lesions that shed few or no metastases, e.g., melanoma [19]. Vascularized tumors expand both locally and by metastasis, have highly permeable vessels (e.g., edema in brain tumors), and are associated with a variety of symptoms.

(iii) The demonstration of a pre-vascular and a vascular phase during the growth of human tumors, and more recently of animal tumors [20] has generated the central question, 'When and how do tumors switch to the angiogenic state?' It is becoming clear that the time of onset of angiogenic activity during tumorigenesis does not correlate with malignancy. It may occur before [12, 21] or after [19] the appearance of neoplasia. How angiogenic activity is turned on is less clear but there may be more than one mechanism. For example, a tumor suppressor gene has recently been identified which in the normal cell codes for an angiogenesis inhibitor, but is down-regulated during tumorigenesis [4, 22]. In another tumor, hyperplastic cells in the early stage of tumor development are not angiogenic, but secrete an angiogenic factor when they become malignant [20].

(iv) During the switch to the angiogenic state, microvascular endothelial cells may be the target of *more than one angiogenic molecule*. At least 7 angiogenic peptides have now been sequenced and cloned [23–26], and two non-peptide molecules have been reported [27, 28]. Some human tumor cells actually produce more than one angiogenic factor [29].

(v) Tumors may *amplify* their own angiogenic activity. For example, the onset of angiogenic activity in a tumor may be abetted by macrophages, which, when recruited into a tumor release their own angiogenic activity [30]. Certain growth factors such as basic fibroblast growth factor are now known to be stored in the extracellular matrix and could be enzymatically released by tumor cells or macrophages [15, 31].

(vii) *Metastasis may also be angiogenesis-dependent.* In human and animal tumors the probability of metastasis is low before neovascularization of the primary tumor and increases after neovascularization [19, 32–34]. The onset of angiogenic activity is only one of many requirements for a successful metastasis. However, neovascularization appears to be required for cells to escape from the primary tumor and may also be necessary for the metastatic implant to grow. Thus, angiogenesis may be important at the beginning and at the final stage of the metastatic cascade, again, a necessary but not sufficient event for a successful metastasis.

(viii) Many *clinical observations* in oncology can now be understood in terms of angiogenic mechanisms [18]. Some examples are: osteogenic sarcoma rarely invades the epiphyseal plate [inhibitor of angiogenesis in cartilage]. Retinoblastoma is often associated with iris neovascularization [diffusion of an angiogenic factor from the tumor]. When ovarian carcinoma metastasizes to the peritoneal lining, it may produce small implants nearly equivalent to each other in size [lack of neovascularization]. Bleeding is one of the most common signs of cancer [presence of neovascularization]. Early detection of cancer of the cervix by colposcopy, cancer of the bladder by cystoscopy, and cancer of the breast by mammogram, may rest in part upon the presence of neovascularization.

Finally, we can ask how new knowledge about the angiogenic process can be translated into antitumor therapy. The studies by the authors of this issue of *Cancer and Metastasis Reviews* reveal that tumor cells which reside in the extravascular space and are capable of *attracting* endothelial cells, or

tumor cells which travel in the circulation and are capable of *adhering* to endothelial cells, are inherently more dangerous than tumor cells which lack these properties. Thus, at least two general strategies for anti-tumor and anti-metastatic therapy may be considered: (i) inhibition of the *production* by tumor cells of angiogenic molecules and of molecules which may facilitate binding to endothelium; (ii) *prevention* of endothelial cells from responding to either of these types of molecules. The validity of the latter strategy is being tested in part with novel angiogenesis inhibitors [35]. The former strategy has received little attention, but hopefully the following reports will stimulate new research in this direction.

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