The Role of the Organ Microenvironment in the Biology and Therapy of Cancer Metastasis

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Abstract   By the time of diagnosis, primary neoplasms are biologically heterogeneous and contain subpopulations of cells with different metastatic potentials. The pathogenesis of a metastasis consists of many sequential steps that must be completed to produce clinically relevant lesions. During any of these steps, tumor cells interact with host factors in the microenvironment that the tumor cells can usurp. Treatment of metastasis can be directed against tumor cells and/or microenvironmental factors that support tumor growth, such as tumor-associated blood vessels. J. Cell. Biochem. 101: 927–936, 2007.   © 2006 Wiley-Liss, Inc.

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The major cause of death from cancer is metastases that are resistant to conventional therapy. Metastases can be located in different organs or in different regions of the same organ, and it is known that the anatomic location of metastases plays a critical role in determining response to therapy. Primary tumors, in general, and metastatic lesions, in particular, are biologically heterogeneous and contain multiple cell populations with diverse characteristics of growth rate, karyotype, cell-surface receptors, antigenicity, immunogenicity, enzyme profile, hormone receptor composition, sensitivity to different cytotoxic drugs, production of extracellular matrix proteins, adhesion molecule profile, angiogenic potential, invasiveness, and metastatic potential [reviewed in Fidler, 2004].

The process of metastasis is highly selective and consists of a series of sequential, interrelated steps. After the initial transformation and growth of cells, tumors must recruit a new blood supply in order to expand beyond 1 mm in diameter. The synthesis and secretion of several proangiogenic factors by tumor and infiltrating host cells lead to development of a capillary network from the surrounding host tissues. Next, local invasion of the stroma occurs as a consequence of the enhanced expression of a series of degradative enzymes (i.e., collagenases). Invading tumor cells penetrate lymphatic or small blood vessels to facilitate their spread to distal tissues. Many tumor cells form homotypic or heterotypic aggregates with platelets in order to enhance their survival in the turbulent circulation and avoid adverse encounters with components of the immune system. The tumor emboli that survive the turbulence of the circulation and immune and nonimmune defenses arrest in the microcirculation of organs where the tumor cells can proliferate within the lumen of vessels or extravasate into the organ parenchyma and then proliferate. In either case, a micrometastasis can form. The growth of these microscopic lesions likewise requires development of a vascular supply and evasion of host defenses. Metastatic lesions can shed tumor cells into the circulation to produce

metastasis of metastases [reviewed in Liotta et al., 1991; Fidler, 2004].

The outcome of metastasis is governed by multiple interactions of metastatic cells with homeostatic mechanisms, which tumor cells often usurp. At the primary metastatic sites, tumor cells interact with host cells such as endothelial cells, pericytes, epithelial cells, fibroblasts, myoepithelial cells, and leukocytes. The tissues composed by these normal cells are biologically unique, and the organ microenvironment they provide for tumor cells is unique.

THE "SEED AND SOIL" HYPOTHESIS

Clinical observations of cancer patients and studies with experimental rodent tumors have led cancer biologists to conclude that the metastatic pattern of certain tumors is organ-specific and independent of vascular anatomy, rate of blood flow, and number of tumor cells delivered to each organ [reviewed in Weiss, 2000; Fidler, 2003]. Indeed, the distribution and fate of hematogenously disseminated, radiolabeled melanoma cells in experimental animals conclusively demonstrated that tumor cells can reach the microvasculature of many organs, but growth in the organ parenchyma occurs in only specific organs [Fidler, 1970]. These findings, however, were not new.

More than 100 years earlier, Paget reached a similar conclusion. In 1889, he asked, "What is it that decides what organs shall suffer in a case of disseminated cancer?" Paget's study was motivated by the discrepancy between considerations of blood flow and the frequency of metastases in different organs. He examined the autopsy records of 735 women who died of breast cancer and many other patients with different neoplasms, and noticed the high frequency of breast cancer metastasis to the ovaries and the variations in incidence of skeletal metastases produced by different primary tumors. These findings were not compatible with the view that metastatic spread was due to "a matter of chance" or that tissues "played a passive role" in the process. Paget [1889] concluded that metastasis occurred only when certain favored tumor cells (the "seed") had a special affinity for the growth milieu provided by certain specific organs (the "soil"). The formation of metastasis required the interaction of the right cells with the compatible organ environment.

Ewing [1928] challenged Paget's seed and soil theory and hypothesized that metastatic dissemination occurs purely by mechanical factors that are a result of the anatomical structure of the vascular system. Both of these explanations have been evoked separately or together in order to explain the metastatic site preference of certain types of neoplasms. In a review of clinical studies on site preferences of metastases produced by different human neoplasms, Sugarbaker [1979] concluded that common *regional* metastatic involvements could be attributed to anatomical or mechanical considerations, such as efferent venous circulation or lymphatic drainage to regional lymph nodes, but that metastasis in *distant* organs from numerous types of cancers were indeed site-specific.

Experimental data supporting Paget's 1889 seed and soil hypothesis were provided a century later by Hart and Fidler [1980], who studied the preferential growth of B16 melanoma metastases in specific organs. Following the intravenous (i.v.) injection of B16 melanoma cells into syngeneic C57BL/6 mice, tumor growths developed in the natural lungs and in grafts of pulmonary or ovarian tissue implanted either subcutaneously (s.c.) or intramuscularly (i.m.). In contrast, neoplastic lesions failed to develop in control grafts of similarly implanted renal tissue or at the site of surgical trauma. Parabiosis experiments suggested that the growth of the B16 melanoma in ectopic lung or ovarian tissues was due to the immediate arrest of circulating neoplastic cells and not to shedding of malignant cells from foci growing in the natural lungs. Quantitative analysis of tumor cell arrest and distribution using cells labeled with [^{125}I]-5-iodo-2′-deoxyuridine indicated that the growth of tumors in the implanted organs was not due to an enhanced initial arrest of B16 cells. No significant differences in immediate tumor cell arrest were detected between implanted fragments of lungs (tumor-positive) and kidney (tumor-negative) or between organ-bearing and contralateral control limbs. These data demonstrated that the outcome of metastasis is dependent on both tumor cell properties and host factors, and supported the seed and soil hypothesis as an explanation of the nonrandom pattern of cancer metastasis.

The introduction of peritoneovenous shunts for palliation of malignant ascites provided an

opportunity to study some of the factors affecting metastatic spread in humans. Tarin et al. [1984] described the outcome in patients with malignant ascites draining into the venous circulation, with the resulting entry of viable tumor cells into the jugular veins. Good palliation with minimal complications was reported for 29 patients with various neoplasms. The autopsy findings in 15 patients substantiated the clinical observations that the shunts did not significantly increase the risk of metastasis. In fact, despite continuous entry of millions of tumor cells into the circulation, metastases in the lung (the first capillary bed encountered) were rare. These results provide compelling verification of the seed and soil hypothesis.

A clear demonstration of organ-site-specific metastasis comes from studies of experimental brain metastasis. Two murine melanomas were injected into the carotid artery to simulate the hematogenous spread of tumor emboli to the brain. The K-1735 melanoma generated lesions only in the brain parenchyma, whereas the B16 melanoma produced only meningeal growths [Schackert and Fidler, 1988]. Similarly, different human melanomas [Schackert et al., 1990] injected into the internal carotid artery of nude mice also produced unique patterns of brain metastasis. Distribution analysis of radiolabeled melanoma cells injected into the internal carotid artery ruled out the possibility that the patterns of initial cell arrest in the microvasculature of the brain predicted the eventual sites of growth. Rather, the different sites of tumor growth in the brain involved interactions between the metastatic cells and brain endothelial cells, and the response of tumor cells to local growth factors. In other words, site-specific metastases were produced by tumor cells that are receptive to their new environment. A few examples follow.

ORTHOTOPIC VERSUS ECTOPIC MURINE MODELS FOR CANCER METASTASIS

By definition, a model is an approximation of reality. Many early reports that malignant human tumors did not metastasize in the nude mouse had cast doubts on the validity of this model for studies of metastasis. It is now clear that the production of metastasis depends both on the intrinsic tumor cell properties and on host factors, the experimental technique, and the origin, health, and maintenance of the nude mouse. Today, we know that the metastatic behavior of human neoplasms can indeed be studied in athymic nude mice, but only if careful attention is paid to the experimental conditions. The neoplasms must be free of mouse pathogens, and the mice must be kept in specific pathogen-free conditions. Careful consideration must be given to the anatomic particulars of implantation because the metastatic potential of human tumor cells is dependent on both intrinsic properties of the tumor cells and host factors which, as stated earlier, vary from site to site.

Orthotopic implantation in nude mice of human tumor cells recovered from surgical specimens is mandatory for accurate assessment of metastatic potential. This is the case not only with human colon carcinomas (into the wall of the colon) and human renal cell cancers (into the kidney), but also for melanomas (into the skin), mammary carcinomas (into the mammary fat pad), bladder carcinomas (into the bladder wall), prostate carcinoma (into the prostate), pancreatic carcinoma (into the pancreas), and lung cancer (into the bronchi). Orthotopic implantations result in rapid growth of local tumors and, in several model systems, produce distant metastasis. In sharp contrast, implantation of the aforementioned human cancer cells into ectopic sites (usually subcutaneously or intramuscularly) results in slow growth of local tumors that rarely metastasize [reviewed in Fidler, 1991].

As will be discussed in subsequent sections, one possible explanation for the differences in the behavior of tumor cells in an ectopic versus orthotopic environments may well be that implantation of tumor cells into organs-tissues is invariably associated with trauma which is followed by inflammation and repair. Tissue-specific growth factors may be responsible for stimulation of tumor cells that possess the appropriate surface receptors.

THE INFLUENCE OF THE ORGAN MICROENVIRONMENT ON THE METASTATIC GENOTYPE AND PHENOTYPE

Regulation of Response to Chemotherapy by the Organ Microenvironment

Clinical observations suggest that the organ environment can influence the response of tumors to chemotherapy. For example, in women with breast cancer, lymph node and

skin metastases are more sensitive to chemotherapeutic interventions than are metastases residing in either the lung or bone [Donelli et al., 1967]. Several intrinsic properties of tumor cells can render them resistant to chemotherapeutic drugs, including increased expression of the *mdr* genes, leading to overproduction of the transmembrane transport protein P-glycoprotein (P-gp) [Bradley et al., 1988; Tsuruo, 1988]. Expression of P-gp often parallels increases in dose or duration of chemotherapy. Indeed, increased levels of P-gp can be induced by selecting tumor cells for resistance to natural product amphiphilic anticancer drugs. Nevertheless, elevated expression of P-gp, accompanied by development of the multidrug resistance (MDR) phenotype has also been found in many solid tumors of the colon, kidney, and liver that had not been previously exposed to chemotherapy [Tsuruo, 1988].

One of the most striking examples of site-specific variations in therapeutic response was observed following implantation of colon carcinoma cells into different anatomic locations of nude mice (using the highly metastatic KM12L4a human colon carcinoma cell line) or syngeneic BALB/c mice (for the CT-26 murine colon carcinoma). Mice received injections of the KM12L4a cells into either the subcutis (ectopic site), spleen (leading to experimental liver metastasis), or cecum (growth at the orthotopic site). Tumor-bearing mice were given doxorubicin and subsequently evaluated for response to treatment. Tumors grown within the subcutaneous tissue showed up to 80% inhibition of growth after two i.v. injections of doxorubicin (10 mg/kg), compared to about 40% inhibition of the intracecal tumors and less than 10% inhibition of lesions in the liver [Wilmanns et al., 1993]. These studies were expanded to use the murine tumor cell line; the subsequent studies showed that whereas subcutaneous tumors of CT-26 were sensitive to treatment with doxorubicin, lung tumors established by i.v. injection of CT-26 cells were insensitive [Dong et al., 1994]. However, the tumor cells at both of these sites were equally sensitive to 5-FU, a drug whose activity is not influenced by expression of the MDR phenotype. Northern blot analysis showed that the relative expression of the *mdr1* and *mdr3* genes was greatest in the cecum, liver, and lungs of mice, and this expression correlated with the relative resistance of tumor cells. Indeed, this expression of *mdr* was

transient and the subsequent culture of cells from a liver metastasis for 7–10 days resulted in a decrease of *mdr* expression to the level in CT-26 cells maintained in culture. Additional evidence indicated that these events were the result of organ-specific modulation of tumor cell properties. First, unlike most tumor cells selected in vitro for the MDR phenotype by exposure to anticancer drugs, CT-26 cells grown in the lung did not contain amplified *mdr1*. Second, subcutaneous implantation of CT-26 cells from lung metastases that were resistant to doxorubicin, produced tumors that were sensitive to doxorubicin. In parallel studies, doxorubicin-sensitive CT-26 cells from s.c. tumors became resistant to the drug when they were inoculated i.v. to grow in the lung parenchyma.

These findings are not restricted to experimental systems. In patients with colon carcinoma, elevated P-gp expression is found on the invasive edge of the primary tumor (growing in the colon) and in metastases located in lymph node, lung, and liver [Kitadai et al., 1995]. Whether this finding is due to selection or adaptation is unclear.

Regulation of the Invasive Phenotype by the Microenvironment

The metastatic capacity of human colon cancer cells growing in orthotopic tissues of nude mice directly correlates with the level of collagenase type IV activity [Stetler-Stevenson, 2005]. Histological examination of the human colon carcinomas growing in the subcutis, wall of the colon, or kidney of nude mice revealed a thick pseudocapsule around the subcutaneous but not cecal or kidney tumors [Fidler, 1991]. These differences suggested that the organ environment could influence the ability of metastatic cells to invade host stroma. Significant differences were found in the levels of secreted type IV collagenases between human colon cancer cells growing subcutaneously or in the cecum of nude mice. In the medium conditioned by human colon cancer cells derived from subcutaneous implants, we detected only a latent form of the 92-kDa type IV collagenase. In contrast, both latent and active forms of the 92-kDa type IV collagenase were found in culture medium conditioned by tumor cells harvested from cecal tumors. Moreover, cancer cells grown in the cecum secreted more than

twice as much enzymes as the subcutaneous tumors [Nakajima et al., 1990].

The invasive ability of human colon cancer cells is directly influenced by organ-specific fibroblasts. Primary cultures of nude mouse fibroblasts from skin, lung, and colon were established, and invasive and metastatic human colon cancer cells were cultured alone or with the fibroblasts. The cancer cells grew on monolayers of all three fibroblast cultures but did not invade through skin fibroblasts [Fabra et al., 1992]. Cancer cells growing on plastic and on colon or lung fibroblasts produced significant levels of latent and active forms of type IV collagenase, whereas colon cancer cells cocultivated with nude mouse skin fibroblasts did not. One possible explanation is that fibroblasts from the skin produce IFN-β, whereas those from the kidney or colon do not. The incubation of human colon cancer cells [Fabra et al., 1992] and human renal cancer cells [Gohji et al., 1994] with IFN-β, significantly reduced the expression and activity of collagenase type independently of antiproliferative activity.

Regulation of Angiogenesis by the Organ Microenvironment

The survival and growth of normal cells and tumor cells are dependent on an adequate supply of oxygen and nutrients and on the removal of toxic molecules. Oxygen can diffuse from capillaries for only 150–200 μm, and cells located beyond this barrier undergo cell death [Jain, 2005]. Thus, the expansion of tumor masses beyond 1 mm in diameter depends on neovascularization, that is, angiogenesis [Folkman and Klagsbrun, 1987]. Angiogenesis consists of multiple, interdependent steps. It begins with local degradation of the basement membrane surrounding capillaries, followed by invasion of the surrounding stroma and migration of endothelial cells in the direction of the angiogenic stimulus. Proliferation of endothelial cells occurs at the leading edge of the migrating column, and the endothelial cells begin to organize into three-dimensional structures to form new capillary tubes [Auerbach and Auerbach, 1994]. Differences in cellular composition, vascular permeability, blood vessel stability, and growth regulation distinguish vessels in neoplasms from those in normal tissues. Unlike the vascularization that accompanies highly regulated physiologic processes, angiogenesis associated with tumor growth is incessant. This observation led Dvorak [1986] to characterize tumors as "wounds that do not heal."

The induction of angiogenesis is a consequence of an imbalance between multiple inhibitor and stimulator molecules [Bergers and Benjamin, 2003]. Normal tissues are exposed to an excess of inhibitor molecules that maintain the vascular endothelium in a quiescent, nonproliferating state. Measurements of cell proliferation in nondiseased tissues indicate that the turnover time of endothelial cells may be measured in years [Hobson and Denekamp, 1984]. Induction of angiogenesis may occur at any stage of tumor development but it is usually synchronized with increasing metabolic pressures, oncogene activation, or mutation of tumor suppressor genes [Bergers et al., 2003]. For example, loss of the wild-type allele of the p53 tumor suppressor gene results in reduced production of the angiostatic factor thrombospondin-1 [Dameron et al., 1994], and activation of the ras oncogene [Kerbel, 2006] or inactivation of the von Hippel–Lindau tumor suppressor gene [Siemeister et al., 1996] increases expression of vascular endothelial cell growth factor (VEGF)/vascular permeability factor (VPF), a potent proangiogenic cytokine. Many other angiogenic molecules, including members of the fibroblast growth factor family, IL-8, epidermal growth factor (EGF), angiogenin, and others have been identified [Folkman and Klagsbrun, 1987]. These proteins regulate endothelial cells' directional migration, invasion, cell division, proteolysis, expression of antiapoptotic proteins, and ultimately, new capillary formation [Ferrara et al., 2003].

The intensity of the angiogenic response varies considerably among different types of tumors and within different zones of a single tumor [Eberhard et al., 2000]. The rate of tumor cell division is still several orders of magnitude greater than the rate of neovascularization. As tumors expand, their microenvironment is often hypoxic [Vaupel et al., 2004]. Hypoxia is often associated with the activation of the transcription factor hypoxia-inducible factor-1 alpha (HIF-1α) to initiate the transcription of genes, for example, VEGF/VPF [Ferrara et al., 2003]. VEGF/VPF increases the permeability of blood vessels by stimulating the functional activity of vesicular-vacuolar organelles, clusters of cytoplasmic vesicles, and vacuoles located in microvascular endothelial cells [Dvorak et al., 1995]. VEGF also induces migration, protease

production, and endothelial cell proliferation [Ferrara et al., 2003]. In addition, VEGF/VPF regulates endothelial cell survival by activating the phosphatidylinositol-3 kinase/Akt signal transduction pathway and stimulating expression of the antiapoptotic proteins Bcl-2 and A1 [Hicklin and Ellis, 2005].

HIF-1α-signaling also encodes the polypeptide chains of platelet-derived growth factor (PDGF) [Harris, 2002]. The functional activity of PDGF is, to a large extent, determined by the anatomical location of a specific tumor. For example, in pancreatic tumors, PDGF has been shown to stabilize developing vascular networks by recruiting pericytes to support the immature blood vessel walls [Bergers et al., 2003]. In tumors of the central nervous system, PDGF stimulates the release of VEGF from the tumor-associated endothelium [Guo et al., 2003]. In contrast, tumors in the skin rely on PDGF signaling to regulate the level of interstitial fluid pressure in the tumor [Pietras et al., 2001]. In prostate cancer bone metastasis (see Contribution of Host MMP-9 to Tumor Growth and Vascularization section), PDGF functions as a survival factor for tumor endothelial cells by activating the intracellular effectors MAPK and Akt [Langley et al., 2004].

The vascular endothelium is regarded as structurally and functionally heterogeneous [Gerritsen, 1987]. To examine this diversity, we generated a broad panel of microvascular endothelial cells from various organs of H-$2K^b$-tsA58 transgenic mice [Langley et al., 2003]. cDNA expression profiles generated on the endothelial cells predicted significant organ-specific differences in expression levels of tyrosine kinase receptors, chemokine receptors, and proteins that regulate the efflux of toxic substrates; these were confirmed at the protein level. Endothelial cells derived from the mouse brain expressed measurable levels of PDGF-Rβ, the chemokine receptor CXCR-2, and P-glycoprotein, whereas endothelial cells from the pulmonary circulation did not express detectable levels of these proteins. The organ-derived endothelial cells also exhibited vast differences in response to stimulation with endothelial cell mitogens. Endothelial cells originating from the brain and liver showed the greatest increase in cell division in response to basic fibroblast growth factor, while EGF was the most potent mitogen for endothelial cells derived from the lung and uterus.

Contribution of Host MMP-9 to Tumor Growth and Vascularization

The expression level of matrix metalloproteinases (MMPs) in ovarian cancer cells is directly associated with invasion and metastasis. To examine the contribution of mouse stromal MMP-9 to the progressive growth of human ovarian cancer cells, we generated a strain of nude mice that have a homozygous null mutation in the MMP-9 gene (i.e., MMP-9$^{-/-}$ mice). We implanted human ovarian cancer cells into the peritoneal cavities of MMP-9$^{-/-}$ nude mice and mice with intact MMP-9 genes (MMP-9$^{+/+}$ nude mice), and measured tumor incidence, angiogenesis, and progressive growth. Blood vessel density and the pattern of macrophage infiltration into the lesions were determined by immunohistochemistry of excised tumors. Tumor growth was also studied in MMP-9$^{+/+}$ or MMP-9$^{-/-}$ nude mice reconstituted with spleen cells collected from either MMP-9$^{+/+}$ or MMP-9$^{-/-}$ nude mice. Regardless of the expression level of MMP-2 and -9 by ovarian tumor cells, progressive tumor growth and carcinomatosis were statistically significantly lower in MMP-9$^{-/-}$ mice than in MMP-9$^{+/+}$ mice. MMP-9$^{-/-}$ mice injected with human ovarian cancer cells displayed impaired tumor angiogenesis, which was associated with decreased macrophage infiltration into the lesions and decreased microvessel formation. Reconstitution of MMP-9$^{-/-}$ mice with spleen cells collected from postnatal MMP-9$^{+/+}$ mice led to increased angiogenesis and tumorigenicity of human ovarian cancer [Huang et al., 2002].

The results clearly demonstrate that host-derived MMP-9 plays an important role in angiogenesis, tumor growth, and the formation of ascites by human ovarian cancer cells implanted into the peritoneal cavities of nude mice. In mice that expressed MMP-9, intraperitoneally injected human ovarian cancer cells produced highly vascularized and rapid-growing tumors, whereas in mice that did not express MMP-9, the human ovarian cancer cells produced slow growing and poorly vascularized tumors. The decrease in angiogenesis in mice that lacked MMP-9 expression was associated with a decrease in macrophage infiltration into the ovarian tumors. In mice that had received spleen cells from mice that expressed MMP-9, enhanced vascularity and tumorigenesis were associated with the expression of MMP-9 in

macrophages, suggesting that tumor-infiltrating macrophages played a major role in the angiogenesis and growth of the human ovarian tumors in the animal model.

Activated macrophages influence the angiogenic process by secreting enzymes that can breakdown the extracellular matrix and by secreting angiogenic molecules and growth factors, such as bFGF, TGF-α and -β, insulin-like growth factor-I, PDGF, and VEGF/VPF [Koch et al., 1992; Mantovani et al., 1992; Sunderkotter et al., 1994]. These factors induce endothelial cells to migrate and proliferate. The number of macrophages that infiltrate human ovarian cancers has been shown to directly correlate with the microvessel density [Orre and Rogers, 1999], and macrophages isolated from ascitic fluid aspirated from women with advanced ovarian cancer exhibit significant angiogenic potential in vitro and in vivo [Sheid, 1992]. Collectively, the clinical and experimental data demonstrate that host cells (macrophages) in the organ microenvironment are responsible for robust angiogenesis and growth of ovarian carcinomas.

Organ-Dependent Expression of Interferon Influencing Angiogenesis

As mentioned above, the expression of bFGF was higher in experimental renal cancers growing in the kidney than in those growing in the subcutaneous tissues. In sharp contrast, the expression of IFN-β was high in epithelial cells and fibroblasts surrounding the subcutaneous tumors, whereas no IFN-β was found in or around tumors growing in the kidney. These findings prompted us to investigate whether IFNs could modulate the expression of the angiogenic molecule bFGF. We found that IFN-α and -β, but not IFN-γ, downregulated the expression of bFGF mRNA and protein in HRCC as well as in human bladder, prostate, colon, and breast carcinoma cells [Singh et al., 1995]. The inhibitory effect of IFN-α and -β on bFGF expression was cell-density-dependent and independent of the antiproliferative effects of IFNs. We recently confirmed that IFN can inhibit bFGF production in an in vivo model system. Systemic administration of human IFN-α downregulated the in vivo expression of bFGF, decreased blood vessel density, and inhibited tumor growth of a human bladder carcinoma implanted orthotopically in nude mice [Slaton et al., 1999]. Under both in vitro and in vivo conditions, the downregulation of bFGF required a long exposure of cells to low concentrations of IFNs. In addition, when IFN was withdrawn, cells resumed production of bFGF.

The body's first lines of defense against external challenge are the epithelial cells that line the skin and the respiratory, digestive, and genitourinary tracts. Inasmuch as IFN-β participates in host defense against viral, bacterial, and parasitic infections and tumors, we hypothesized that this secreted protein is expressed in various murine epithelial cell types that line portals of entry to the body. We used immunohistochemistry and ISH techniques to measure IFN-β expression in the various epithelial cell types and in internal murine organs sheltered from environmental stimuli. The epithelial cell types lining the skin, digestive tract, urinary tract, reproductive tract, and upper respiratory tract constitutively expressed IFN-β. Indeed, all differentiated epithelial cells at risk of environmental exposure expressed IFN-β (protein and mRNA) with the exception of the ciliated epithelial cells lining the lower respiratory tract. Epithelial cells of internal organs that are not directly exposed to external pathogens did not express IFN-β [Bielenberg et al., 1998].

ANTI-VASCULAR THERAPY OF PROSTATE CANCER BONE METASTASIS

In clinical specimens of human prostate cancer bone metastasis and in experimental models of orthotopic human prostate cancers in the long bones of nude mice, the expression of PDGF and PDGF-R correlates with the growth of metastatic tumor cells in the bone parenchyma. Specifically, prostate cancer cells growing adjacent to bone tissue express high levels of the PDGF protein and the PDGF-R, which is phosphorylated, and tumor-associated endothelial cells express high levels of phosphorylated PDGF-R [Kim et al., 2004]. Oral administration of the PDGF-R tyrosine kinase inhibitor imatinib (STI571) to nude mice blocks the PDGF-signaling pathway by inhibiting phosphorylation of its receptors [Uehara et al., 2003]. Oral administration of STI571 or STI571 plus injectable taxol to male nude mice reduced the incidence and size of primary tumors and bone lesions and prevented bone lysis as measured by digital radiography. Immunohistochemical

analysis of control, untreated bone lesions, demonstrated that human prostate cancer cells growing adjacent to the bone, expressed high levels of PDGF and activated (phosphorylated) PDGF-R, whereas tumor cells growing in the adjacent musculature following lysis of the bone did not. Treatment with STI571 and more so with STI571 plus taxol significantly inhibited phosphorylation of PDGF-R on tumor cells and endothelial cells, decreased tumor cell proliferation, and induced significant apoptosis in tumor cells and tumor-associated endothelial cells. These data indicate that targeting PDGF-R phosphorylation can produce significant therapeutic effects against prostate cancer bone metastasis [Kim et al., 2004].

To determine whether the PDGF-R expressed on tumor-associated endothelial cells could be the primary target of imatinib, we selected a multidrug-resistant variant of the human PC-3MM2 prostate cancer. These cells were implanted into the tibia of nude mice, and when the lesions were growing progressively, the mice were randomized to different treatment regimens. The bone tumors were resistant to taxol, whereas treatment with imatinib and taxol produced a significant decrease in tumor incidence, bone lysis, and incidence of lymph node metastasis. Initially, this treatment produced apoptosis of tumor-associated endothelial cells (but not tumor cells), followed 1 week later by apoptosis of the tumor cells. Collectively, these studies demonstrated that the primary targets for imatinib treatment are the tumor-associated endothelial cells, that is, an anti-vascular therapy [Kim et al., 2006].

CONCLUSIONS

A current definition of the seed and soil hypothesis consists of three principles. First, neoplasms are biologically heterogeneous and contain subpopulations of cells with different properties. Second, the process of metastasis is selective for cells that succeed in all steps of the process. Although some of the steps in this process contain stochastic elements, as a whole, metastasis favors the survival and growth of a few subpopulations of cells that pre-exist within the parent neoplasm. Thus, metastases can have a clonal origin, and different metastases can originate from the proliferation of different single cells. Third, the outcome of metastasis depends on multiple interactions ("cross-talk")

of metastatic cells with homeostatic mechanisms, which the tumor cells can usurp.

Several years ago, we likened the metastatic cell to a decathlon champion who must excel in all ten events rather than just one or two. In the context of this article, we wish to add that the decathlon champion cannot reach his goal of winning independently of his gear and adequate conditions, that is, no mud or sand on the running track. Since the outcome of metastasis depends on multiple interactions of metastatic cells with homeostatic mechanisms in the organ microenvironment, therapy for metastasis can be targeted not only against tumor cells, but also against the homeostatic factors that helps metastatic cells grow and survive.

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