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Animal Models and the Tumor Microenvironment: Studies of Tumor–Host Symbiosis

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Abstract

The contributions of murine models to elucidation of processes central to tumor growth are reviewed. Localized acidosis, increased interstitial pressure, perturbations in structure and function of the extracellular matrix, hypoxia, angiogenesis, and co-optation of the immune response are all phenomena that promote tumor survival and metastasis. The use of animal models is critical to understanding the pathophysiology of these processes and the development of more effective cancer therapies.

It has been 100 years since C.C. Little first used inbred mouse strains to study carcinogenesis in vivo.¹ The subsequent development of syngeneic, allogeneic, and xenogeneic tumor models has advanced our understanding of the tumor microenvironment, permitting direct measurement of physico-chemical parameters such as cell metabolism with resultant acidosis, elevation of extracellular interstitial oncotic pressure, as well as formation and degradation of the extracellular matrix (ECM). Advances in multiphoton in vivo imaging technology now permit time-lapse video analysis of host cell and tumor cell migration and invasion in anesthetized tumor-bearing animals. For the past 40 years, animal models have proven invaluable in deciphering the molecular mechanisms that underlie tumor–host symbiosis, tumor-induced angiogenesis, and the immune response. While an intact immune system and normal cellular microenvironment in a vertebrate can inhibit malignant cell growth and on occasion even mediate spontaneous tumor regression, functional aberrations in the microenvironment can actually promote tumor cell proliferation. As tumors proliferate, intimately interfaced with host stroma, they select for a local microenvironment that is symbiotic and beneficial for both tumor and host tissue. A review by Polyak et al² notes that “although the importance of an altered microenvironment in tumorigenesis is no longer disputed, the nature of the molecular alterations underlying these changes remains unclear.” Ultimately, successful cancer therapies must disrupt the symbiotic relationship between tumor and stroma. Three-dimensional in vitro culture systems continue to evolve in their sophistication and complexity but are not yet able to accurately model the complex pathophysiology of the tumor–host interface as faithfully as that which occurs in intact animal models.

METABOLISM AND ACIDOSIS

As tumor cells proliferate, the microenvironment becomes hypoxic and acidotic, as lactic acid accumulates due to anaerobic glycolysis (Warburg effect).³ The acidic extracellular environment can inhibit the efficacy of alkaline chemotherapeutic drugs. Tumor vasculature is morphologically and functionally abnormal, containing dysmorphic sprouts, defective endothelial monolayers, and intercellular gaps that render the vessels hyperpermeable.⁴ This results in accumulation of proteins, lymphatic fluid, and elevation of interstitial oncotic pressure within the tumor mass. Inside the tumor, the low pH of the extracellular microenvironment and the high interstitial oncotic pressure become effective chemical and hydrostatic barriers to drug delivery. The distorted architecture of tumor extracellular matrix is an additional physical barrier that protects tumor cells from chemotherapeutic drugs and inhibits contact with immune cells. Hypoxia promotes tumor cell survival by enhancing genomic instability and selecting for a more aggressive tumor phenotype.^{5,6} We have used direct invasive methods to measure intratumoral pH in syngeneic mouse models via microelectrodes, and have shown that changes in extracellular pH can predict tumor response to chemotherapy.⁷ The baseline intratumoral pH of B16F10 murine melanoma tumors was approximately 6.5 (Figure 1). Mice bearing doxorubicin (Adriamycin)-sensitive B16F10 tumors treated with doxorubicin (SA, Figure 1) exhibited a further drop in intratumoral pH to approximately 5.5, probably as a result of tumor lysis and release of lysosomal contents. As tumors regressed, pH returned to 6.5. Thus, the transient extracellular acidosis in drug-sensitive tumors translated into prolonged survival. In doxorubicin-resistant tumors (RA) no pH drop occurred after drug treatment, and mice did not survive past day 24. Similarly, mice bearing doxorubicin-sensitive B16F10 melanoma demonstrated normalization of interstitial oncotic tumor pressure and displayed tumor regression following drug administration (Figure 2). Recent advances in magnetic resonance imaging technology may provide noninvasive methods to assess extracellular tumor pH using chemical exchange saturation transfer (CEST), and thus provide a physiologically relevant biomarker for tumor response to therapy.^{8,9}

EXTRACELLULAR MATRIX AND CANCER-ASSOCIATED FIBROBLASTS

Many different murine tumor models have been used to study the interaction between tumor cells and the extracellular matrix (ECM). The ECM surrounding blood vessels is a proteinaceous basement membrane that is rich in collagens, laminin, and fibronectin, and that can signal to endothelial cells (ECs) via integrins expressed on the ECs surface.¹⁰ Stromal elements provide a supportive microenvironment for tumor growth, angiogenesis, and metastasis, and in turn undergo structural change due to the influence of tumor cells.² Tumors recruit ECs, fibroblasts, inflammatory cells, and pericytes; these are cells that surround and support capillaries, and which all contribute to generation of the ECM. Stromal cells provide both tumor-promoting and tumor-suppressive signals. In a syngenic murine model of pancreatic cancer, inhibition of the Hedgehog signaling pathway reduced the proliferation of tumor-associated stroma and improved the delivery of gemcitabine.¹¹ Cancer-associated fibroblasts (CAFs) are not malignant, in that they are not tumorigenic in athymic nude mice, but rather they promote angiogenesis and tumor cell proliferation. CAFs secrete oncogenic growth factors and cytokines. In breast cancer patients, CAFs promoted

tumor growth more vigorously than normal fibroblasts¹² and promoted angiogenesis via expression of stromal cell–derived factor-1 (SDF-1 or CXCL12). SDF-1 recruits circulating endothelial progenitor cells (EPCs) to tumors. Tumor cells express CXCR4, the receptor for SDF-1, thus permitting stromalSDF-1 to directly promote angiogenesis and tumor growth. The recruited EPCs then differentiate into tumor-associated vascular ECs and become tumor capillaries. Circulating EPCs can migrate to other tissues and form a pre-metastatic niche for the later colonization of circulating tumor cells.¹³

Biomarkers for CAFs have been identified recently. One such marker is fibroblast activation protein (FAP), a post-prolyl endopeptidase that is expressed chiefly in CAFs.¹⁴ Recently, the endopeptidase function of FAP was exploited therapeutically by synthesis of a prodrug that was activated by FAP,¹⁵ which released the active drug (thapsigargin) exclusively in the region of the CAF. In vitro, the prodrug killed human tumor cells at an IC50 of 3 nmol/L. In mice, MCF-7 breast and LNCaP prostate xenografts were inhibited in their growth by 75% after 3 weeks. Selective knockdown of FAP-expressing stromal cells in transgenic C57Bl/6 mice caused rapid hypoxic necrosis of both stromal (CAF) cells and tumor cells (Lewis lung carcinoma). Additionally, FAP depletion in a pancreatic carcinoma model enabled mice to respond to a tumor-associated antigen peptide (mesothelin) and reject tumor growth. Only when FAP depletion was combined with immunization did tumors regress.¹⁶ Transplanted bone marrow cells introduced into tumor-bearing mice comprised 25% of the stromal fibroblasts surrounding the tumor.^{17,18} One might predict that CAFs would exhibit a normal genotype, identical to normal host stroma. However, several studies have described extensive genetic changes present in CAFs, including gene amplification, loss of heterozygosity, and missense mutations. Inoculation of PC3 C4-2 human prostate carcinoma in an athymic nude mouse xenograft model resulted in peri-tumoral mouse stromal cells (CAFs) that bore multiple copies of mouse chromosome 15, the location of *c-myc*.¹⁹ Giemsa-banding and fluorescence in situ hybridization (FISH) analysis confirmed that these stromal cells were indeed of murine and not human origin; neither were they murine/human chimeras. The mechanism for this chromosomal amplification phenomenon in CAFs is unknown. Concomitant with chr15 amplification, loss of the Y chromosome was also noted in CAFs. Microcell-mediated chromosome transfer of the Y chromosome into PC3 cells suppressed the tumorigenicity of these cells when inoculated into athymic nude mice,²⁰ suggesting the presence of a gene required for prostate tumorigenesis on the Y chromosome.

Growth factors secreted by the tumor into the ECM also recruited cells to the tumor microenvironment. Transforming growth factor- β (TGF- β) recruited EPC to the tumor and mediated activation of fibroblasts to CAFs, while platelet-derived growth factor (PDGF) recruited fibroblasts and stimulated their proliferation. Vascular endothelial growth factor (VEGF) promoted aberrant vascularization that permitted additional leakage of plasma proteins that in turn attracted more fibroblasts.¹² Thus, the microenvironment may convert recruited stromal cells into CAFs through epigenetic changes, including DNA methylation and chromatin remodeling, as changes in histone modification and DNA methylation have been found in CAFs.

Proteins secreted by the tumor include not only growth factors, but also proteases that degrade the ECM, thus affecting cell adhesion and motility. Stromal cells secrete ECM

structural proteins, cytokines, growth factors, proteases, protease inhibitors, and endoglycosidases such as heparinase. Transforming growth factor- β (TGF- β), osteopontin, galectin-3, and matrix metalloproteinases (MMPs) are secreted proteins implicated in carcinogenesis.²¹ MMPs are overexpressed in tumor-associated epithelial cells compared to normal epithelial cells. MMP remodel the ECM by degrading the basement membrane and other ECM proteins via proteolysis. Increased proteolytic activity can promote tumor growth. Conversely, protease inhibition by plasminogen activation inhibitor-1 (PAI-1) can exert an anti-angiogenic effect in mouse ECs and stromal cells.²² Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of MMP and thus also function to inhibit angiogenesis.

ANGIOGENESIS

Tumor-induced angiogenesis is one of the most striking morphologic changes associated with tumor progression, and plays a major role in the function of the tumor microenvironment. The origin of the tumor vasculature is more complex than was originally proposed. Tumor endothelial cells (TECs) do not simply arise by mitosis, budding, and sprouting from existing capillaries in the same manner that occurs during wound repair or regeneration of normal tissues. Qualitatively, these processes do occur in tumor angiogenesis, but they are modified by other influences. There is evidence that stem-like tumor cells themselves can differentiate to generate endothelial-like structures.²³ Mesenchymal stem cells that have characteristics of ECs, can give rise to both endothelium and pericytes in hemangiomas in immunodeficient athymic nude mice.²⁴ Hence, with contributions from these various host sources, tumor vasculature is not a homogenous endothelial surface but rather consists of a mosaic of cells with different structure and function.

Ischemia and hypoxia are drivers of both physiological and pathological angiogenesis. During tumor angiogenesis the angiogenic cascade is unresolved, driven by tumor-secreted factors and tumor hypoxia. The interstitial hypertension of the tumor limits delivery of drugs.²⁵ Anti-angiogenic agents such as anti-VEGF-A antibodies (bevacizumab) or VEGFreceptor tyrosine kinase inhibitors (sunitinib, pazopanib, axitinib), or anti-HER2 antibodies (trastuzumab) can transiently normalize function of the tumor vasculature, resulting in improved delivery of oxygen and drugs into the tumor microenvironment.²⁶ However, after an initial response, tumors generally become resistant to these agents. The chimeric structure of tumor ECs, including cells with cytogenetic abnormalities, aneuploidy, and multiple chromosomes, contributes to resistance to antiangiogenic therapy.²⁷

ENDOGENOUS ANTI-ANGIOGENIC AGENTS

There have been many attempts to selectively target the tumor vasculature. Type I interferons (IFNs) were among the first biologic therapeutics to be used as anti-angiogenic agents. Twenty years ago, the efficacy of IFN- α 2a was demonstrated by dramatic regression of life-threatening hemangiomas of infancy.²⁸ Administration of IFN- α / β to mice bearing Friend erythroleukemia tumors induced necrosis of ECs within tumors while sparing normal vessels, resulting in decreased metastases to liver and spleen.²⁹ Inhibition of angiogenesis

occurred before antiproliferative effects on tumor cells and was identified *in vivo* within 24 hours of tumor cell inoculation.³⁰ In syngeneic tumors comprised of malignant murine ECs (SVR and MS1-VEGF angiosarcomas), IFN- α/β inhibited tumor growth by 70% *in vivo* and inhibited tumor-induced angiogenesis by 50%.³¹ Gene array studies identified IFN-stimulated genes (ISG) that inhibited angiogenesis, including IP-10 (CXCL10). Recombinant IP-10 inhibited EC proliferation, budding, and vessel formation in the fibrin gel assay. Thus, the combination of gene array studies and syngeneic tumor studies identified ISGs responsible for the anti-angiogenic activity of IFNs.

HIF-1 α , a transcription factor induced by hypoxia, plays an important and complex role in the tumor microenvironment. VEGF is one of the target genes induced by HIF-1 α . Depending on the cell type in which it occurs, HIF-1 α activation can either promote or repress tumorigenesis.³² In breast carcinoma cells it acts as a tumor-suppressor, but in CAFs it acts as a tumor promoter. Deletion of HIF-1 α in host T cells resulted in their activation *in vitro* and *in vivo*.^{33,34} Thus, HIF-1 α expression in the tumor microenvironment directly impacts the immune response. Mice transplanted with HIF-1 α -deficient bone marrow displayed stronger anti-tumor response than mice with wild-type marrow.³⁵ Hypoxia and subsequent HIF-1 α induction promoted differentiation of myeloid-derived suppressor cells into immune-suppressive tumor-associated macrophages.³⁵

ANTI-TUMOR AND ANTI-ANGIOGENIC ACTIVITY OF APOLIPOPROTEIN A1

Within the tumor microenvironment, dysregulated interactions of immune cells, including myeloid-derived suppressor cells and tumor-associated macrophages, with T and B cells, promote tumor cell immune evasion, migration, tumor-associated angiogenesis, and metastasis.^{36–38} High-density lipoprotein (HDL) is a ubiquitous plasma lipoprotein with atheroprotective functions mediated via its cholesterol efflux activity.^{39,40} HDL has been proposed to also have anti-inflammatory, anti-parasitic, anti-apoptotic, and innate immune activities.^{39,41,42} HDL anti-inflammatory activity is mediated by interaction with the endothelium and with inflammatory cells.^{39,41–43} Apolipoprotein A1 (apoA1), the major structural protein of HDL, when expressed or injected *in vivo*, spontaneously lipidates forming HDL. ApoA1 has antiviral activity toward DNA and RNA viruses,^{44,45} bactericidal and bacteriostatic effects,⁴⁶ and modulatory effects on regulatory T cells.⁴⁷ ApoA1 binds and neutralizes bacterial endotoxin and lipoteichoic acid.^{48,49}

Recently, we have shown that genetic manipulation of apoA1 levels in mice, or administration of purified apoA1, resulted in anti-tumor activity against syngeneic melanoma and lung carcinoma.⁵⁰ Tumor growth was examined in transgenic C57Bl/6 mice that overexpressed human apoA1 (A1Tg+/-) and compared to wild-type and apoA1 knockout mice (A1KO). Tumor growth was reduced 10-fold in A1Tg+/- mice compared to A1KO (Figure 3A, $P < .05$). Metastasis was monitored by *in vivo* bioluminescence imaging in mice inoculated with B16F10L cells that stably expressed Renilla luciferase. Similarly, A1Tg+/- mice exhibited a 410-fold reduction in metastasis compared to A1KO mice. The anti-neoplastic effect of apoA1 was not unique to malignant melanoma. The growth of Lewis lung tumors was also inhibited in A1Tg+/- mice, and even further inhibited in animals homozygous for the human apoA1 gene (A1Tg+/, Figure 3B, $P < .05$). Exogenous

apoA1 injections also provided protection from tumor growth and metastasis in both tumor types.

Because tumors harvested from A1KO mice seemed more hemorrhagic, tumor-induced angiogenesis was assessed. Analyses of blood vessels supplying day 7 primary mouse melanoma tumors revealed a significant ($P < .005$) > 2 -fold decrease in the number of vessels in tumors from A1Tg $^{+/+}$ mice compared to A1KO hosts (Figure 4), consistent with apoA1 having an inhibitory effect on angiogenesis in the tumor microenvironment. Tumors in A1Tg $^{+/+}$ versus A1KO mice displayed reduction in vessel area, vessel length density, and size of vessels ($P < .005$ for each; Figure 4). Thus, primary tumors from animals with the same initial tumor burden exhibited decreased angiogenesis in the presence of apoA1. Angiogenesis can be VEGF-A– dependent or –independent.^{51,52} VEGFa mRNA expression and protein levels from tumor tissue harvested 7 days post inoculation in A1Tg animals exhibited a modest increase relative to that observed in A1KO animals. Thus, reduction in angiogenesis observed within the A1Tg animals did not appear to be VEGF-related. MMP-9 protein levels were 1.8-fold lower ($P < .05$) in tumors from A1Tg mice, and displayed > 2 -fold less enzymatic activity. ApoA1 appears to inhibit the level and activation of MMP-9 in the tumor microenvironment. Survivin, an inhibitor of apoptosis, was expressed at a 2.7-fold lower level in A1Tg mice compared to A1KO mice.

ANTI-ANGIOGENIC ACTIVITY OF THROMBOSPONDIN-1

Thrombospondin-1 (TSP-1) is a glycoprotein subunit of a disulfide-linked homotrimer that mediates cell-to-cell and cell-to-matrix interactions. TSP-1, an important component of the ECM, binds to fibrinogen, fibronectin, laminin, type V collagen, and integrins $\alpha V/\beta 1$. TSP-1 modulates platelet aggregation, tumorigenesis, cell proliferation, adhesion, migration, and tumor metastasis through interaction with several different proteins and cell receptors.⁵³ Decreased expression of TSP1 can occur in primary tumors. Aberrant methylation of TSP1 has been postulated as associated with angiogenesis in gastric cancer, colorectal cancer, glioblastoma multiforme, and neuroblastoma.^{54–57} Recently we have confirmed that TSP1 expression is suppressed by methylation in melanoma, and shown that treatment with the demethylating agent 5-Aza-deoxycytidine (5-Aza-dC) restores TSP1 expression; the ultimate effect of this restored expression is a potent antiangiogenic and antiproliferative effect.⁵⁸ Using a xenograft model, TSP1 levels were knocked down using shRNA in A375 human melanoma cells. TSP1 concentration was fivefold higher in SCR-A375 cells (expressing scrambled shRNA) compared to shTSP1- A375 cells (TSP-1 knockdown by specific shRNA) (Figure 5A). The majority of TSP1 appeared to be secreted, as levels were 10-fold higher in conditioned medium compared to cell lysates. To assess the contribution of TSP1 suppression to drug sensitivity, mice were inoculated with shTSP1-A375 cells or SCR-A375 cells and treated with vehicle or 5-Aza-dC as above. A375-shTSP1 tumors grown in mice receiving vehicle were 38% larger by day 21 compared to SCR-TSP1 tumors ($P = .021$) (Figure 5B). Treatment with 5-Aza-dC inhibited the growth rate of both tumor types: A375-shTSP1 by 38% and SCR-TSP1 by 37% ($P = .00017$ and $P = .00032$, respectively). These results confirm the role of 5-Aza-dC as an inhibitor of both angiogenesis and tumor growth. Furthermore, increased expression of TSP1 in tumor cells might play a major role in angiogenesis suppression in vivo. Our finding further supports other studies that the

expression level of endogenous TSP1 is important in the regulation of the onset of tumor angiogenesis.^{59,60}

INFLAMMATION AND CANCER

“If genetic damage is the match that lights the fire, inflammation may provide the fuel that feeds the flames.”

Fran Balkwill—Barts & The London School of Medicine

Tumors were described by Dvorak as wounds that never heal.⁶¹ Sites of chronic inflammation and the tumor microenvironment have similar humoral and cellular components. It has been well appreciated that chronic inflammatory conditions can lead to carcinogenesis in many tissues, including skin, lung, stomach, liver, gallbladder, pancreas, and colon (Table 1).

The acute inflammatory response is normally terminated when the inciting process has been resolved. Mechanisms that serve to terminate inflammation include the intrinsic short half-life of inflammatory mediators, production of TGF- β by macrophages,^{62–64} local interleukin (IL)-10 production,⁶⁵ lipoxin production,⁶⁶ downregulation of leukotrienes, upregulation of IL-1 receptor antagonist and soluble tumor necrosis factor (TNF) receptor, apoptosis of inflammatory cells,⁶⁷ desensitization of receptors, cleavage of chemokines by MMP and production of anti-inflammatory factors,⁶⁸ and production of resolvins,⁶⁹ protectins,⁷⁰ and maresins.⁶⁹

The initiation of an inflammatory response contains genetically programmed instructions for its termination.⁷¹ Within hours after entering the inflammatory site, granulocytes switch from production of arachidonic acid–derived prostaglandins and leukotrienes to lipoxins, which initiate the termination sequence. During the resolution phase, further neutrophil recruitment ceases and existing neutrophils undergo apoptosis, promoted by resolvins and protectins, which are synthesized from omega-3 polyunsaturated fatty acids. Apoptotic neutrophils are then phagocytosed by macrophages, followed by release of anti-inflammatory cytokines such as TGF- β 1. The resolution phase ends with the departure of macrophages through the lymphatics.

The hallmarks of inflammation in Latin are: color (heat), dolor (pain), rubor (redness), turgor (swelling), and functio laesa (loss of function). Of these hallmarks, turgor is the most pernicious. Comparison of the tumor microenvironment with a venous stasis ulcer is illustrative. If interstitial swelling (lower leg edema) is not treated (by limb elevation, compression stockings) a venous ulcer may take months to heal, or may never heal at all. A metaanalysis of 17 randomized controlled trials (1,034 patients) determined that the application of autografts or allografts to a venous ulcer was of minimal benefit.⁷² This is because skin grafting the ulcer does nothing to address the presence of pro-inflammatory agents that linger in the wound and cannot drain via the venous or lymphatic system that have impaired outflow. Elevation of the limb and wet-to-dry dressing changes debride the wound. As the saline-moistened gauze is allowed to dry, it adheres to the open wound, and dressing changes physically remove inflammatory cells along with the exudate. Eventually

the ulcer shrinks as the epithelium heals from the periphery of the wound inwards and the wound circumference decreases. Hence, dressing changes act as a functional surrogate for the obstructed veins and lymphatics.

To explore further the inflammation–carcinogenesis connection in different tissues, specific animal models have been created. Clinically, exposure to silica dust is associated with lung cancer development in humans. A silica-induced multistep lung carcinogenesis model driven by chronic inflammation in Fisher F344/NCr rats has allowed identification of molecular markers and genetic alterations.⁷³ Following a single intra-tracheal instillation of silica solution, the DNA damage response pathway was activated in preneoplastic lesions, associated with inducible nitric oxide synthase (iNOS) and p53 induction. Early tumorigenic progression was characterized by p16 induction, followed by p16 inactivation in bronchiolar dysplasias and tumors. Tumors induced in the silica model contained epigenetic alterations in p16(CDKN2A), CDH13, and APC but not in Rassf1 and Nore1A. Silica-induced tumors also lacked Ras, EGFR, and Tp53 mutations, suggesting that these tumors shared molecular signatures with only a subset of human lung carcinomas.

Inflammatory bowel disease, such as ulcerative colitis and Crohn's disease, predisposes for development of colorectal cancer (CRC). Multi-step carcinogenesis including production of free radicals, reactive oxygen and nitrogen species, upregulation of inflammatory enzymes in arachidonic acid biosynthesis, upregulation of cytokines, and intestinal immune system dysfunction are likely required for development of CRC. Various carcinogens have been used to induce CRC in rat animal models, including azoxymethane (AOM)/dextran sodium sulfate (DSS),^{74,75} methylazoxymethanol (MAM) acetate, and 1,2-dimethylhydrazine (DMH).⁷⁶ Using these preclinical cancer carcinogenesis models, Tanaka et al have demonstrated the chemopreventative activity of agents such as morin (a potent flavonoid inhibitor of fatty acid synthase), bezafibrate (peroxisome proliferator-activated receptor [PPAR]- α agonist used in treatment of hyperlipidemia), and valproic acid (an anticonvulsant and histone deacetylase inhibitor). In these models, inflammatory T-helper 1, T-helper 2, and T-helper 17 cytokines derived from CD4+ T cells, including TNF- α and IL-1 β , were elevated during carcinogenesis.⁷⁷ Agents such as valproate, bezafibrate, and morin suppressed these pro-inflammatory cytokines and resulted in lower incidence of CRC tumor formation.

Treatment of C57Bl/6 mice with infliximab (a chimeric anti-TNF- α monoclonal antibody) suppressed CRC formation in the DSS model.⁷⁸ Multiple CRC tumors developed in 80% of control mice, whereas only 16% of mice treated with infliximab developed CRC. Reduction in CRC incidence was accompanied by a decrease in tumor TNF- α level, reduction in mast cell number, reduction in inflammatory cytokine expression, reduction in the expression and activity of MMP-9 and MMP-11, and reduction of β -catenin–accumulated crypts.

CONCLUSION

Great strides have been made in identifying the pathophysiologic processes that lead to carcinogenesis and tumor proliferation. When applied to animal tumor models, analytic and genetic tools have allowed for extensive cataloguing of aberrations present in the physico-

chemical, metabolic, proteomic, and immunological makeup of the tumor microenvironment. Current clinically approved chemotherapeutics achieve complete remission in only a minority of stage IV patients. A far more detailed understanding of these aberrations is required to identify which are critical for maintenance of tumor–host symbiosis and to establish methods for disrupting the relationship.

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Conflicts of interest:

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Figure 1.

Survival of C57Bl/6 mice bearing B16-BL6 tumors and kinetics of pH response. (A) Mice inoculated with tumors were monitored for survival following treatment with doxorubicin (ADR; 2 mg/kg/d intraperitoneally, days 6–12) or PBS. PBS treatment designations SP (sensitive, PBS), RP (resistant, PBS), SA (sensitive, ADR), RA (resistant, ADR) indicate cell subline and treatment (n = 16 mice per group). (B) A second series of C57Bl/6 mice (n = 6 per group) bearing B16-BL6 tumors with indwelling intratumoral guide tubes received ADR or PBS treatment (days 6–12) and underwent repetitive pH measurement on days 6 (pre- and post-treatment), 7, and 16. Adapted with permission.⁷

Figure 2.

Intratumoral pressure (ITP) measurements in C57Bl/6 mice bearing B16F10 subcutaneous tumors. PBS or doxorubicin 2 mg/kg/day given intraperitoneally on days 1–2. On day 4, a 25-g needle was inserted into the center of 6-mm diameter tumors and ITP determined by manometer. N = 6 for each group.

Figure 3.

Anti-tumor activity of apoA1. Syngeneic C57BL/6J mice of indicated genotypes were inoculated subcutaneously in the flanks with 10^5 tumor cells in a volume of 0.1 mL (A), B16F10L melanoma (B), or Lewis lung carcinoma. Tumor volume at site of inoculation was monitored by caliper measurements. Adapted with permission.⁵⁰

Figure 4.

ApoA1 inhibits tumor angiogenesis. Blood vessels feeding day-7 B16F10L melanomas in apoA1KO and apoA1Tg^{+/-} mice were counted under a dissecting microscope (12.5× objective). Every vessel touching the circumference of the tumor was scored as a single vessel. Two measurements were taken to assess tumor area (the largest diameter coplanar with the skin, and a second diameter perpendicular to the first). The product of these measurements was the tumor area index. Photographs were subject to digital analysis using VESGEN software, where the central region of interest (white) represented the tumor mass defined the perimeter of the tumor. The output was a series of color Generation Maps (colored vessels on black background) in which the largest diameter vessels were defined as G1 (red), with each subsequent smaller generation represented as G2–G6. Number of blood vessels was expressed based on total vessel area, vessel length density, and vessel diameter (n = 14 per group). Adapted with permission.⁵⁰

Figure 5.

Antiproliferative activity of thrombospondin-1. A375 human melanoma cells were stably transfected with SCR (scrambled shRNA) or sh-TSP1 (shRNA specific for TSP1). (A) TSP1 concentration was determined in conditioned medium (CM) and cell lysates. (B) Both cell types were inoculated into nude mice that were subsequently treated with vehicle (veh) or 5-Aza-dC (5aza). A375 tumors expressing sh-TSP1 displayed increased growth rate compared to SCR-A375 tumors, suggesting a direct tumor-suppressive role for TSP1 in this model. Both transfectants were further suppressed in their growth by 5-Aza-dC

treatment, suggesting that additional tumor-suppressor genes other than TSP1 are also activated by 5-Aza-dC treatment.
Adapted with permission.⁵⁸

Table 1

Provide title

Inflammation	Acute	Chronic
Cell types	Neutrophils, basophils, eosinophils, monocytes, macrophages	Monocytes, macrophages, lymphocytes, plasma cells, fibroblasts
Mediators	Vasoactive amines, eicosanoids	Interferon- γ , other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes