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

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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.
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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 al[2] 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.

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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

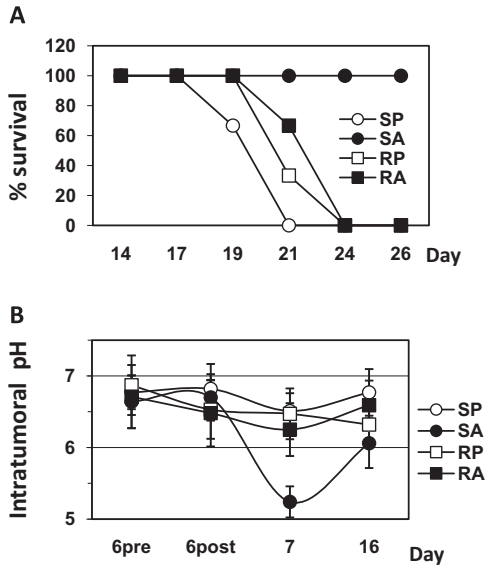

Figure 1. Survival of C57Bl/6 mice bearing B16-BL6 tumors and kinetics of pH response. (A) Mice inoculated with tumors were monitored for survival following treatment with doxorubicin (ADR; 2 mg/kg/d intraperitoneally, days 6–12) or phosphate-buffered saline (PBS). PBS treatment designations SP (sensitive, 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]

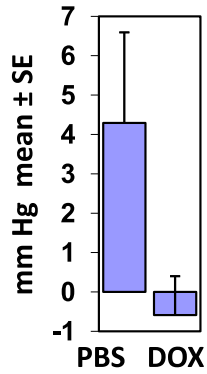

Figure 2. Intratumoral pressure (ITP) measurements in C57Bl/6 mice bearing B16F10 subcutaneous tumors. PBS or doxorubicin 2 mg/kg/day given intraperitoneally on days 1–2. On day 4, a 25-g needle was inserted into the center of 6-mm diameter tumors and ITP determined by manometer. N = 6 for each group.

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 fibroblasts[12] 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 stromal