Differences in organization of metastatic and nonmetastatic tumors initiated by the same B16 melanoma clone in mature and young mice

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Subcutaneous transplants of mouse B16 melanoma clone G3.26 grow more slowly, and are markedly more metastatic to the lungs, in mature (> 12-month-old) mice than in young (2-month-old) mice. Previous studies suggested that tumors in young mice fail to disseminate viable tumor cells into the hematogenous circulation. To determine if changes in intratumor organization might accompany this altered tumor behavior, G3.26 tumors growing in young and mature mice were examined comparatively at progressive sizes relative to the onset of metastatic dissemination in the older mice. Although the degree of necrosis was comparable in both groups of tumors, vascular density, measured morphometrically in histological sections, was significantly lower in tumors from mature mice at a size when dissemination would be occurring. With the onset of reduced vascular density in tumors in mature mice, there was a substantial increase in the proportion of viable tumor cells that was hypoxic, based on radioresistance and incorporation of the hypoxic cell sensitizer, misonidazole. Quiescent tumor cells, identified by flow cytometry, were also more numerous in tumors from mature mice than in tumors from young mice. Although the importance of these differences in tumor organization to enhanced metastatic behavior is unclear, increased intratumor hypoxia might promote generation of metastatic variants. Alternately, dissemination of tumor cells might be facilitated through a reduced and possibly defective vasculature.

Introduction

Metastasis of a solid tumor is generally considered to depend on the presence of tumor cells expressing special phenotypic characteristics necessary for effecting successful dissemination, as well as on appropriately receptive host conditions [9, 24]. Much less attention has been given to the potential role of the developing tumor, or tumor transplant, in determining or modulating metastatic activity [6, 32]. There are a variety of ways in which host components of developing tumors might either promote or suppress metastatic activity under certain conditions. Macrophages or lymphocytes infiltrating into developing tumors could contribute angiogenic stimuli or proliferative growth factors [12, 21], or alternately, kill disseminating cells [8, 23]. Intravascular invasion of tumor cells may be enhanced by tumor cell stimulation of proteolytic activities of stromal fibroblasts or capillary endothelial cells [3, 14]. Fibroblasts within tumors might also produce factors contributing to the generation of tumor cell metastatic potential [28]. Developing tumor vasculatures may even differ in structural integrity [31], which could influence vascular invasion by tumor cells.
There is some indirect evidence that the manner in which tumors develop can dramatically influence metastatic activity. Transplantation of the same tumor at different anatomical sites has significantly influenced metastasis of a number of experimental tumors [17]. Increased metastasis of mouse B16 melanoma transplants has resulted from experimental reduction of fibroblast-generated desmoplasia associated with tumor development [2]. Inhibition of angiogenesis in mice bearing Lewis lung carcinoma or B16 melanoma transplants, and in rats with Walker carcinosarcoma transplants, significantly reduced tumor growth rates and metastasis [10, 29]. Anti-metastatic activity of the drug ICRF-159 for the same rodent tumors has been attributed to normalization of structural defects in the developing tumor vasculature [13, 16], whereas hyperthermia may promote metastasis by causing vascular damage [20]. Transplantation of several mouse sarcoma and carcinoma lines into preirradiated tumor beds resulted in formation of tumors that were more metastatic, and more necrotic, than tumors in control mice [18]. Whether such changes in tumor growth or organization are responsible for, or merely accompany, altered metastatic behavior is unclear.

We have observed that cultured B16 melanoma cells which formed rapidly growing but virtually nonmetastatic tumors in normal young (2-month-old) mice grew more slowly and were highly metastatic to the lungs in mature-to-aged (> 10-month-old) mice, in young mice immunized against tumor-associated antigens, and in young mice maintained on a calorie-restricted diet [1, 25, 27]. Expression of metastatic activity was strictly dependent on reduced tumor growth rates but not on prolonged host survival, with dissemination beginning at relatively small tumor sizes. This evidence suggested that the basis for greater metastasis in mature, immune, and calorie-restricted mice might be an altered tumor organization conducive to generation of metastatic variants or to dissemination of pre-existing variants [1]. To begin assessing this possibility, the general organization of tumors initiated in young and mature mice with a single B16 melanoma clone was investigated comparatively.

Materials and methods

Animals

Female C57BL/6 mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, at 6–8 weeks of age or as 10-month-old retired breeders. Mice were received and maintained free of disease and subclinical infection with bacterial, mycoplasma, and viral pathogens [1]. Mice designated as 'young' were 2–3 months old at the time of tumor initiation, and 'mature' mice were 12–20 months old.

Subcutaneous tumors

B16 melanoma subclone G3.26 was propagated in monolayer culture, cells were dissociated by trypsinization, and tumors were initiated by injecting 2.5 × 10^5 cells subcutaneously in the flank midway between the front and hind legs, as described previously [1, 25]. Identical aliquots of G3.26 cells, frozen after initial isolation and expansion in culture, were grown only for 1–2 weeks before each experiment, to minimize phenotypic diversification [25]. When tumors became externally visible ('palpable'), mice were housed separately during subsequent tumor growth. Tumor mean geometric diameter (MGD) was calculated as the cubed root of the product of maximum tumor length, width and depth, with measurements made in orthogonal