Characterization of growth and radiation response of KHT tumor cells metastatic from lung to ovary and kidney

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Female C3H/HeJ mice were inoculated intravenously with KHT sarcoma cells. Once macroscopic colonies were established in the lungs, the thoraces of the animals were locally irradiated. Despite significant lung nodule regression following treatment, animals were observed to die with ovarian and renal metastases. By irradiating the lungs at various times after intravenous tumor cell injection, it was demonstrated that ovarian and renal metastases arose only from established lung colonies and were not a consequence of the initial cell inoculum. Incidence of metastases increased from 0 to 100 per cent when the time between cell inoculation and thoracic irradiation was increased from 4 to 16 days. Once established, ovarian and renal metastases grew with a doubling time of approximately 1–2 days. Metastatic tumors in the ovaries were found to be refractory to radiation therapy because of a large component of radiation-resistant hypoxic cells. Parallel experiments utilizing male C3H/HeJ mice demonstrated metastases only in the kidneys and these grew at a growth rate similar to that seen for renal metastases in female mice. This system may serve as a model for the study of factors influencing the dissemination of tumor cells to these anatomical sites and their response to treatment.

Introduction

A major problem in current cancer therapy is the dissemination of tumor cells from the primary tumor site [12, 16, 18]. Consequently, considerable efforts have been spent on evaluating the process of metastatic spread, growth, and response to therapies [2, 5, 7, 8, 10, 14, 19, 20]. More recently, many studies have focused on clones of tumor cell subpopulations with various metastatic properties and target selectivity [1–4, 11]. Traditionally, the emphasis in studies of metastases has been to evaluate and compare the biological properties of select tumor cell clones relative to the original tumor. Less information is available on the role that organ-specific factors play in the subsequent development of cells as metastases and in their therapeutic response [19, 20]. Acquisition of such information has been hampered by the lack of model systems in which metastases can be studied in multiple internal organs without prior clonal selection.

The KHT sarcoma may represent such a tumor model since cells growing as lung colonies were observed to spread directly to the ovaries and kidneys [14]. These ovarian and renal metastases were evident only when animal mortality resulting from

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lung colony growth was inhibited by local radiation treatment of the thorax. This paper reports on studies aimed at characterizing this model system by the evaluation in detail of the dissemination and establishment of these secondary metastases.

Materials and methods

Animal and tumor system. Experiments were performed using female and male C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) bearing the KHT sarcoma [7, 9]. This tumor is propagated in vivo every 2 weeks by injecting 1–2 × 10⁵ KHT sarcoma cells intramuscularly. The preparation of single-cell suspensions used to passage the tumor has already been described in detail [17].

KHT sarcoma lung colonies. Suspensions of single KHT sarcoma cells were prepared from intramuscularly growing tumors using a combination mechanical and enzymatic (trypsin + DNAse) procedure [17]. A known number of tumor cells (5 × 10²–1 × 10⁵) were then mixed with lethally irradiated tumor cells (2 × 10⁶) and 15 μm-diameter plastic microspheres (6 × 10⁵) and injected intravenously into mice. After 12 days, discrete lung colonies were recognizable. The lung colony plating efficiency using this procedure is 1–4 per cent [6, 8, 14]. Once established, KHT sarcoma lung colonies grow with a doubling time of 1–2 days [8, 14].

Irradiation. The mice were irradiated using a 13⁷Cs source operating at a dose rate of 413 rad/min [14]. The mice were not anesthetized during irradiation. Instead, they were confined to plastic jigs so that the thoraces of the animals were in radiation fields defined by lead collimators. Ten mice could be irradiated simultaneously. Following irradiation, the mice were kept under filter caps five or six per cage and supplied with standard laboratory diet and acidified water ad libitum.

Morphology. Tissue specimens were taken from the lungs, ovaries and kidneys and immediately immersed in Bouin’s fixative and imbedded in paraffin. Six to eight micrometer sections were stained by hematoxylin and eosin and examined by light microscopy.

Metastases growth rate. KHT sarcoma cells were inoculated i.v. and sixteen days later, at a time when macroscopic lung colonies were established, the lungs were irradiated. At various times after irradiating the KHT sarcoma lung colonies, groups of mice were killed and their ovaries and kidneys removed and weighed. Tumor weight was determined from the weight difference of these organs and those of comparable untreated control animals. This weight difference, although primarily the result of tumor growth, is partly due to non-neoplastic host cells (see below).

Clonogenic cell survival

To determine the radiation response of tumor cells growing as ovarian metastases, the mice were irradiated whole-body using the 13⁷Cs source described above. At the time of irradiation the ovaries weighed between 0.5 and 0.8 g. The mice were killed either 10 min before tumor irradiation (anoxic condition) or immediately after irradiation (air-breathing condition). Their tumors were removed and dissociated into single cell suspensions using a combined mechanical plus enzymatic (trypsin + DNAse) procedure [17]. This procedure results in a cell recovery of between 0.2 and 2.0 × 10⁸ cells/g of tumor tissue, with greater than 95 per cent of the cells being dye excluding [7]. Cells were mixed with lethally irradiated cells and plated into 24-well dishes containing alpha-minimum essential medium with 10 per cent fetal calf serum plus 0.2 per cent agar. After a 2-week incubation at 37°C, the number of colonies formed was counted with the aid of a dissecting microscope. At