Loss of spontaneous metastasizing potential in mouse mammary tumors†

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Among 25 C3H/He and C3Hf/He spontaneous mammary tumors that all had produced pulmonary metastases in the autochthonous mice, and also produced pulmonary metastases during subsequent sequential syngeneic intramammary passages, three were observed to stop producing metastases. The specific investigation of the observed changes in behavior is described. When the tumors were re-started in serial intramammary passage from early, cryopreserved transplant generations, the loss of metastasizing potential reoccurred in the same, or in nearly the same, transplant generations. In one tumor from which three transplant lines had been established, the metastasizing potential was lost in all transplant lines during closely related transplant generations. It seems that a spontaneous reduction of malignant behavior in mouse mammary tumors may be an occasional neoplastic characteristic, and that the observed losses of metastasizing potential may have been determined by a programmed genetic change.

Introduction

It is frequently observed, and generally held to be the rule, that cancer is a disease that tends to progress towards greater malignancy. A tendency to revert spontaneously would be considered an improbable neoplastic characteristic. Since the most malignant aspect of cancer is its tendency to metastasize, it follows that the loss of metastasizing potential would represent a reversal to a less malignant state. It was therefore considered of potential interest when a permanent loss of the ability to metastasize spontaneously from intramammary implants was occasionally observed in mouse mammary tumors during their serial intramammary transplantation in syngeneic mice.

To determine whether the observations had been haphazard or if some tumors can indeed lose the ability to metastasize during orthotopic growth and without intentional selection, the metastasizing behavior of several tumors was retraced by starting the transplantation sequence again from tissue cryopreserved in earlier transplantation generations. Other mouse mammary tumor characteristics, such as immunogenicity [13] and growth rate and i.v. transplantability [14] have also been seen to change reproducibly (from cryopreserved primary tumors) at predictable generations during serial transplantations.

In other tumor systems, using intentional selection procedures, tumor sublines with mostly increased but in two cases with decreased spontaneous metastasizing abilities, have been isolated by in vivo [16] and in vitro [6] selection procedures. A spontaneous and complete loss of non-artificial metastasizing potential has not been described before. The subject of phenotypic variability in metastasizing tumor lines was reviewed recently [16].

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Materials and methods

Mice

The tumor-producing mice were breeding or retired females of two pedigreed sublines of the C3H strain, the mouse mammary tumor virus (MMTV)-infected C3H/He and the MMTV-free C3Hf/He. To ensure genetic similarity between the two sublines, the C3H/He line had been re-derived from pedigreed C3Hf/He mice in 1979 by foster-nursing on C3H/He mice.

The mice used for tumor implantation were 10-week-old line-bred female C3H/He and C3Hf/He mice. All of the mice in this study were raised and kept in air-filter covered cages.

Tumors

Tumor 1 was a mammary sarcoma that developed spontaneously in a C3Hf/He mouse and tumors 2 and 3 were mammary adenocarcinomas that developed spontaneously in C3H/He mice. Pulmonary metastases removed from the autochthonous mice were used to start intramammary transplant lines in syngeneic mice. In each assay of metastasis, the tumor implants grew until they measured 20–25 mm in diameter. Before showing signs of cachexia, the mice were killed by CO₂ asphyxiation and necropsied. Metastatic nodules were dissected from the lungs and implanted into the mammary glands of 5–10 mice depending on the number of metastases found. The intramammary growth of implanted metastatic tissue was used for the next transplant generation if gross metastases were not found. Evidence that primary mouse mammary carcinomas and their metastases maintain parallel metastatic potentials during both pulmonary and intramammary growth in serial transplantations has been published [15]. The use of intramammary tumor tissue was therefore not a factor in the loss of metastasizing potential. Tissue from each primary spontaneous tumor was cryopreserved, as was tissue from each successive intrammary transplant generation.

Tumor implantation

Pulmonary metastatic nodules or tissue from intramammary tumor implants were cut into 1 mm pieces and rinsed once in cold culture medium before intramammary implantation of randomly selected pieces. The rinsing reduced the chance of dissemination of loosely adherent tumor cells at the time of implantation. The selection of pieces of tissue from an excess of prepared pieces made a non-random sampling error unlikely. Two pieces of tissue were implanted into each of the No. 4 mammary glands with the use of a very sharp 18-gauge biopsy trocar through incisions in the skin of mice anesthetized with Pentrane (Abbot, North Chicago, IL). The incisions were closed with wound clips.

Histology

The lungs of all tumor hosts, and other organs when suspected of containing metastases, were examined histologically. The specimens were formalin-fixed and paraffin-embedded. Three or four stepwise 3 μm sections were taken of each of the five lobes of the lungs. Some of the smaller micrometastases may therefore have been missed and may have generated false negatives, but the lungs were code-numbered and the statistical comparisons were not affected. Organs which were only rarely the site of metastasis (ovaries, adrenals, liver and draining lymphnodes) were in most