Studies of mammary carcinoma metastasis in a mouse model system. I: Derivation and characterization of cells with different metastatic properties during tumour progression in vivo

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The biological and metastatic properties of cells from a murine mammary adenocarcinoma, MT1, were studied during serial transplantation in syngeneic hosts. Over 35 generations the tumour progressed from a well-differentiated, poorly metastatic neoplasm to an anaplastic highly metastatic state. At early passages the tumour yielded uniform cultures of cuboidal epithelial cells, at passage 17 both epithelioid and spindle type cells were present, and by passage 30 only spindle type cells were obtained. Epithelioid cell lines and clones when injected intravenously into syngeneic hosts produced lung colonies only, whereas spindle cell lines were capable of extensive extrapulmonary colonisation. Similar patterns of dissemination and growth were seen in spontaneous metastasis assays. In spite of the marked phenotypic differences in these 'subpopulations', their comparable ultrastructural features, oestrogen receptor levels, expression of MMTV antigens, DNA content and lectin binding profiles suggested a common cell lineage. It is proposed that these cell lines will be of use in the determination of tumour and host factors influencing tumour progression and the evolution of metastatic potential.

Introduction

Heterogeneity associated with mouse mammary adenocarcinomas is now a well recognized phenomenon. The extreme variability in histological type of tumours (even those induced in the same mouse strain by the same viral agent) has been well described by Dunn [8] and differences between individual primary tumours in terms of growth characteristics and host response [43] and organ colonization potential [39] have been documented. Superimposed upon the heterogeneity evident between individual tumours, there is also the possibility of intratumour cellular heterogeneity. Heppner and her colleagues have isolated clones of cells from cultures of a primary murine mammary adenocarcinoma differing in karyotype, morphology, surface antigen expression, in vitro growth characteristics and in vivo tumorigenicity [14, 6].

In addition to population heterogeneity examined at a single point in a tumour's life history, we must also consider variation in an individual tumour's behaviour as a function of time, i.e. progression [13]. At its first manifestation a tumour may be at any stage of progression, and this process does not always reach an endpoint within the lifetime of the host [13, 10]. In a large series of squamous cell carcinomas and mammary carcinomas, primary tumours ranging from benign to highly metastatic were obtained [10]. Most of the former, if their lifespans were extended by serial
transplantation, progressed to less well differentiated phenotypes (as shown for other carcinomas by Begg [2], and Moore and Dixon [28]), although the rate of change varied considerably. Of particular interest to us were examples of tumours undergoing rapid progression, where within a single tumour-host system changes occurred in cell morphological and behavioural characteristics. In the present communication we introduce one such tumour, the CBA/Ca mammary adenocarcinoma MT1, and describe the process of progression and the acquisition of enhanced metastatic capacity in parallel with an analysis of the tumour cell population characteristics.

Materials and methods

Tumour origin and maintenance

The mammary adenocarcinoma MT1 arose in 1979 in a female CBA/Ca carrying murine mammary tumour virus (MMTV). The tumour was routinely transplanted every 2–3 weeks in the subcutis of syngeneic MMTV-free 10–12 week-old mice using a trocar needle. At each passage, tissue samples were taken for histological examination and adjacent samples cryopreserved in 95 per cent foetal calf serum (FCS) + 5 per cent DMSO at −170°C.

Tumour cell cultures

The cell lines described in this study were derived from different in vivo transplant generations of MT1. Tumour tissue was minced finely and disaggregated for 1 hour at room temperature using an enzyme mixture of 0.05 per cent trypsin, 0.05 per cent collagenase and 0.01 per cent DNAse in T.C. medium 199. The resulting cell suspension was filtered through sterile gauze, centrifuged, resuspended and plated in Falcon tissue culture flasks in DMEM supplemented with 10 per cent FCS, penicillin and streptomycin. All cultures were kept at 37°C in a humidified atmosphere of 5 per cent CO₂ in air. Initially high cell inocula (1–2 × 10⁶ cells per 25 cm² flask) were employed, but gradually as the lines became established the cell numbers were reduced to 3 × 10⁵ cells per 25 cm² flask. From such inocula confluent monolayers were obtained in about 7 days. The cells were refed three times a week, and when required were removed from the flasks using 0.1 per cent trypsin in PBS-EDTA. Cell lines were routinely cryopreserved in ampoules containing 1 × 10⁶ cells in 95 per cent FCS + 5 per cent DMSO at −170°C.

Cloning of cell lines

Culture lines derived from a single parental cell were obtained by limiting dilution. Two hundred cells were inoculated into the first row of 96-well microtitre plates (Nunc) and serial doubling dilutions made across the remaining rows. Wells containing single cells were determined by inspection under an inverted microscope; those producing viable clones were picked 14 days later and transferred to 6-well plates, and finally 25 cm² flasks.

Growth rate and saturation density determinations

3 × 10⁵ cells were inoculated into 25 cm² flasks and at various time intervals cells were harvested from duplicate flasks and counted in a haemocytometer. Results were plotted on semilogarithmic graph paper, and doubling times calculated. The saturation densities were estimated from the total number of cells obtained at the