In vitro modulation of the metastatic phenotype.
I. Analysis of differentiation forms of the B16 melanoma expressing Met-72 determinants and metastatic activity

JIANHUA XIANG and ARTHUR K. KIMURA†
Department of Pathology, Box J-275, University of Florida College of Medicine, Gainesville, Florida 32610, U.S.A.

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In vitro cultures of a highly metastatic B16 melanoma clone (BL6-10) were found to undergo dramatic changes in morphology and differentiation upon transfer to another culture medium. Specifically, BL6-10 melanoma cells which had been originally selected and adapted for growth in Eagles' Hanks' amino acid supplemented media with 10 per cent newborn calf serum were amelanotic and epitheliod in shape. When these cells were shifted into Dulbecco's modified Eagles medium with 10 per cent fetal calf serum, they became highly melanotic and of spindle/dendritic morphology within 4 days of culture. These morphological changes as well as other parameters were all characteristic of established criteria of melanoma differentiation. Alterations in the differentiation state of our highly metastatic variant, BL6-10, did not result in any change in tumorigenicity but did have profound effects on metastatic potential. All of the morphological and functional characteristics of the differentiated melanoma were found to be reversible by re-plating the cells in their original growth medium and 4 days of in vitro growth.

These studies have allowed us to follow and more firmly establish Met-72 antigen expression as a surface marker for metastatic cells of the B16 melanoma, and have provided direct experimental evidence that the less differentiated, Met-72 positive melanoma form is the dominant cell type capable of metastatic potential.

Introduction

Tumor cell differentiation has long been used as an essential parameter in a grading system for pathologic diagnosis of tumors. Evaluation of the malignancy and metastatic potential of tumors is to a large part based on morphological classification of the differentiation forms present [31]. Specifically in the case of melanoma, a number of cell lines derived from and thought to represent 'blocked' stages of melanoma differentiation have been isolated and studied [20–22, 40]. It is anticipated that analysis of characteristics and surface markers of the various differentiation forms will permit a more precise definition of the biological and clinical outcome associated with cell types present in malignant melanoma.

Recent studies have now shown that although many cell lines are considered representative of a given differentiation form, a number of culture conditions can influence the growth, differentiation and phenotype of a variety of different cell lines [5, 35], including melanoma [4, 19, 28, 29]. These variable conditions along with the continual tendency of tumor cell populations to undergo cellular diversification and phenotypic drift [16, 36] have complicated efforts to assign biological behavior to each of the multiple morphological forms obtained from primary tumors and their metastases.

† To whom correspondence should be addressed.
In the original investigations leading to the present study, several monoclonal antibodies (mAbs) were produced which defined a 72,000 dalton cell-surface determinant, Met 72. Levels of Met 72 antigen expression in every case directly correlated with the metastatic potential of clones derived from the B16 melanoma [26]. In the present study, recent in vitro manipulations described herein have enabled us to modulate the differentiation state of a cultured melanoma clone and define more precisely the differentiation form(s) of the B16 melanoma which expresses Met 72 on their cell surface and possess highly metastatic potential.

**Materials and methods**

**Animals**

Inbred 4–8-week-old female C57BL/6 mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, U.S.A., quarantined and maintained in the Health Center Animal Resources Facility at the University of Florida.

**Tumor cell lines and culture conditions**

An in vitro selected, highly invasive metastatic variant, BL6, derived from the B16-F10 melanoma line was obtained from the Division of Cancer Treatment Tumor Bank (EG & G Mason Research Institute, 57 Union St, Worcester, MA, U.S.A.), where they were deposited by Dr I. J. Fidler. A highly metastatic clone, BL6-10, was derived from the BL6 line by micromanipulation [26]. Since obtaining these cells in 1982, they and clones derived from them have been maintained in vitro at 37°C in a humidified incubator containing 8 per cent CO₂/air in Eagles' Hanks amino acid supplemented medium (EHAA) [12] containing 10 per cent newborn calf serum (NCS; Gibco Laboratories, Life Technologies, Inc., Chagrin Falls, Ohio, U.S.A.), penicillin and streptomycin (100 μg/ml). For continuous passage of cells, monolayers of cells were detached from the culture dish by brief incubations (5 min, room temp.) with 0.5 mM EDTA in Ca²⁺ and Mg²⁺-free phosphate-buffered saline (PBS) supplemented with 10 g/l glucose (cPEG) [32], washed and replated at a concentration of 5 × 10⁵/10 cm petri dish in a total of 10 ml media. Experimental assays were carried out with cells which had been maintained in culture for no more than 10 passages from the frozen stocks.

**Monoclonal antibodies**

Anti-Met 72 mAbs were produced through syngeneic immunizations of C57BL/6 mice with clones of metastatic variants derived from the B16 melanoma [26]. These anti-Met 72 mAbs (K88.146-1-2 and K88.151-11-2) have been shown to react strongly with highly metastatic variants derived from the B16 melanoma. Correlation coefficients comparing Met-72 expression and metastatic potential of all 17 clones thus far tested are r = 0.94 and 0.93 for the two anti-Met 72 mAbs described above.

An anti-IA^b^ mAb, K14.83-11, was produced in this laboratory through immunizations of B10.D2 mice with C57BL/10 spleen cells. Details of the fusion and specificity profiles are published elsewhere [25].

**Radioimmunoassay of cell surface antigens**

Both direct and indirect radioimmunoassays were performed to determine the extent of antibody binding to cell surfaces. In the direct assays, monoclonal antibodies were first purified from ascites by affinity chromatography through