Malignant melanoma cell lines selected in vitro for increased homotypic adhesion properties have increased experimental metastatic potential

TIMOTHY V. UPDYKE and GARTH L. NICOLSON

Department of Tumor Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030, U.S.A.

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Using unselected and selected B16 melanoma cell lines, we examined the relationship between homotypic aggregation properties and the potential to form experimental metastatic lung colonies. The B16 sublines were selected in vitro from a line with relatively low homotypic aggregation kinetics and experimental metastatic potential (B16-F1) by successive steps of cell aggregation, followed by separation of cell aggregates from single cells. The selected sublines possessed significantly higher rates of aggregation than did the parental cell line and, when injected intravenously as single cells, formed greater numbers of lung tumor colonies. The aggregation kinetics of the parental and selected cell lines were dependent on divalent cations, with the following order of selectivity: Ca^{2+} > Mn^{2+} >> Mg^{2+}. Syngeneic and xenogeneic serum components and the protease inhibitor leupeptin enhanced the aggregation kinetics of various B16 cell lines. The results support the proposal that a positive correlation exists between increased homotypic adhesion and experimental metastatic potential.

Introduction

Metastasis is a multistep process in which the adhesive interactions of malignant tumor cells with host cells (heterotypic adhesion) or with themselves (homotypic adhesion) are thought to be important in determining the success of the process at several steps [5, 7]. Studies with homotypically aggregated B16-F1 cells showed that the frequency of lung colonies per cell number after intravenous (i.v.) injection of tumor cells was significantly higher than with a single-cell inoculum of B16-F1 cells [2]. Several B16 melanoma sublines have been selected both in vivo and in vitro for various properties, and these B16 sublines show correlations between homotypic or heterotypic adhesiveness and experimental metastatic potential [4, 6, 9, 10, 13, 14]. The selection of a subline of B16-F1 with reduced homotypic aggregation properties resulted in lower lung-colonizing potential than that seen with the parental line [4]. A prediction from such results is that B16 cells selected in vitro for greater aggregation kinetics should produce larger numbers of lung tumor colonies per cell after i.v. injection as single cells than the parental cell line does.

This report describes the selection of a series of B16-F1-homotypic adhesion variants and compares their aggregation properties in vitro to their abilities to colonize the lungs of syngeneic mice after i.v. injection as single cells. The data demonstrate a positive correlation between homotypic adhesion properties and experimental metastatic potential.
Materials and methods

Cell lines and culture conditions

The parental B16-F1 and B16-F10 melanoma cell lines (from Dr I. J. Fidler) were maintained in antibiotic-free DME/F12 [Dulbecco’s modified Eagle’s medium (with low glucose and pyruvate)/Ham’s F-12 medium (1:1, v/v); GIBCO, NY, U.S.A.] plus 5 per cent fetal bovine serum (FBS, HyClone, Logan, UT, U.S.A.) in 100 mm tissue culture dishes (Costar, Cambridge, MA, U.S.A.).

Selection procedures

The selection of B16-F1 aggregation variants (B16-F1-Agg) was performed with antibiotic-free, sterile solutions and equipment. Subconfluent monolayers of parental B16-F1 cells (and subsequent selected variants) were rinsed twice with filtered (0.22 μm, Millipore, Bedford, MA, U.S.A.) Ca²⁺- and Mg²⁺-free HEPES-buffered saline (CMFH: 7.54 g NaCl, 0.37 g KCl, 0.09 g Na₂HPO₄·7H₂O, 0.084 g NaHCO₃, 0.9 g glucose, 5.96 g HEPES (N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid) in 11 of H₂O, pH 7.4) containing 0.1 per cent bovine serum albumin (BSA: Bovuminar, Armour, Kankakee, IL, U.S.A.) (CMFH.1) and incubated at 37°C for 5 min in CMFH.1 plus 2 mM ethylenediaminetetraacetic acid (EDTA) (4 ml/100 mm dish). Any cells still adhering to the dish were removed by gently tapping the sides of the dish, adding 6 ml HEPES-buffered DME plus 0.1 per cent BSA [DH12-1: DME (without NaHCO₃), 2.86 g HEPES, 0.876 g NaCI, 1.0 g BSA, in 11 H₂O, pH 7.4, 300 mosmol/kg, 25°C], and pipetting the cells into a single-cell suspension. Cell numbers and viabilities were determined in a hemacytometer using the Trypan blue dye exclusion technique. After the cells were centrifuged once at 150 g for 3 min, they were resuspended in DH12-1 at 10 times the cell concentration used in the aggregation-selection assay (see table 1). In a tissue culture hood, 0.10 ml of the cell suspension was added to 0.90 ml of DH12-1 (37°C) in an autoclaved Poly Q plastic scintillation vial (Beckman, Fullerton, CA, U.S.A.), and the vial was capped and rotated in an Orbit Envron-shaker (Lab-line, Melrose Park, IL, U.S.A.) at 165 r.p.m. (2 inch orbit; RCF = 0.295 g) at 37°C for various times (table 1). The resulting cell aggregates were separated from single cells in a tissue culture hood using sterile (autoclaved) filtermesh nylon discs (mesh opening = 20 μm, disc diameter = 55 mm; Filtron, Santa Barbara, CA, U.S.A.) placed over the opening of a sterile 50 ml polypropylene centrifuge tube. Prior to filtering the cell suspension, the filtermesh was wetted with DH12-1 to ensure free flow of medium into the tube. To ensure that the cell aggregates were not disturbed, the cell-aggregate suspension was carefully withdrawn from the vial with a 1 ml plastic pipet and gently transferred to the filtermesh with the pipette tip close to the filtermesh surface. The vial and filtermesh were rinsed twice with 2 ml DH12-1, and the filtermesh was then turned over with sterile forceps and placed over the opening of a new 50 ml centrifuge tube. The cell aggregates were carefully washed off the filtermesh with 30 ml CMFH.1 plus 2 mM EDTA and resuspended before centrifuging at 100 g for 3 min. The cells were resuspended and cultured in 12 ml DME/F12 plus 10 per cent FBS. This procedure was repeated sequentially to obtain the various B16-F1-Agg-selection sublines (table 1).

Cell aggregation assays

Subconfluent cell monolayers were washed twice with CMFH.1 and incubated in 4 ml/100 mm dish of CMFH.1 plus 2 mM EDTA at 37°C for 5 min. Cells still