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Adhesion defect of ascites cells corrected with membrane-bound attachment molecules

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Adhesion is obligatory for cell proliferation in most types of cells. This function becomes defective after malignant transformation. An extreme example is ascites cells which proliferate in suspension. The nature of their defects remains obscure. Here we show that the linking of biotin molecules to the Ehrlich ascites carcinoma cells enables these cells to spread normally on an avidin-coated substrate. The spreading was the result of specific avidin-biotin interaction. The morphology of the spread cells and sensitivity to different inhibitors are similar to those of normal epithelia. Thus it is enough to supply appropriate substrate-adhesive molecules to the ascites cell surface to normalize their adhesion.

Adhesion; Spreading; Avidin; Biotin; (Ascites)

1. INTRODUCTION

Adhesion is one of the most important cellular functions, obligatory for cell proliferation in most types of cells. In vitro normal fibroblasts and epithelial cells attach in the rounded state via short (1–2 μm) processes, develop longer pseudopodia and lamellae and finally spread upon a substrate [1]. Malignant transformation is followed by the defects in the mechanism of cell adhesion [2]. An extreme case of such a defect is exhibited by ascites cells which grow and proliferate in suspension. The nature of their defects remains obscure. Here we show that linking of substrate-adhesive molecules to the plasma membrane of Ehrlich ascites carcinoma cells enables these cells to spread normally on an appropriate substrate.

2. MATERIALS AND METHODS

Ehrlich ascites cells were grown i.p. in C3HA mice. On the 6–7th day of growth, the cells were removed, washed with Hank's balanced solution (HBSS) and suspended (1×10^7 cells/ml) in 20 mM Hepes (Calbiochem) containing 0.15 M NaCl, 7 mM glucose (pH 7.4, buffer A). The cell suspension (0.5 ml) was mixed with an equal volume of *N*-hydroxysulfosuccinimidyl (6-biotinylamido) hexanoate (1 mg/ml) for 20 min at 4°C.

Biotinylated cells [3] were washed 5–6 times by sedimentation through 30% Ficoll-Plaque (Pharmacia) solution in HBSS. Modified cells were resuspended in HBSS at a concentration of 4×10^5 cells/ml and placed over the solid substrate for 1–3 h at 37°C. For scanning electron microscopy, the cells were fixed in 1% glutaraldehyde, dehydrated in acetone and dried at critical point of CO_2.

Avidin-covered substrates were prepared in two ways: (i) glass coverslips were treated with 4% HF in 50% HNO_3 for 10 s, washed with water and dehydrated in acetone. The coverslips were incubated in a solution of carbonyldiimidazole in dimethylformamide (100 mg/ml) for 1 h under argon. Activated coverslips were stirred in avidin solution (2 mg/ml, 50 mM $Na_2B_4O_7$, pH 7.8) overnight at 25°C. (ii) Glass coverslips were treated with 7% 3-(triathoxysilyl)-propylamine in benzene (1.5 h at 80°C) and washed with *n*-hexane. The coverslips were incubated in a solution of biotin-ϵ-aminocaproic acid *N*-hydroxysuccinimide ester (1 mg/ml in buffer A) for 2 h at 20°C, washed with water and incubated in avidin solution (1.5 mg/ml HBSS) for 1 h at 20°C.

3. RESULTS AND DISCUSSION

To promote cell adhesion we have taken advan-

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tage of avidin-biotin high-affinity binding. The surface molecules of Ehrlich ascites carcinoma cells were biotinylated while the surface of the coverslip was covered with avidin.

Untreated control cells when put either on the free coverslips or on the coverslips covered with avidin remain round as seen in phase-contrast microscopy. These cells do not spread. They are readily washed away. The cells which bear biotin molecules on their surface firmly attach to the avidinylated substrate and spread upon it producing large thin lamellae (fig.1a,b).

The spreading was the result of specific avidin-biotin interactions. Biotinylated cells spread neither upon non-modified substrate nor upon bovine serum albumin-covered coverslips. If avidin-covered substrate was preincubated with free biotin (so that the accessible avidin-binding sites become occupied) the biotinylated cells do not spread either (fig.1c).

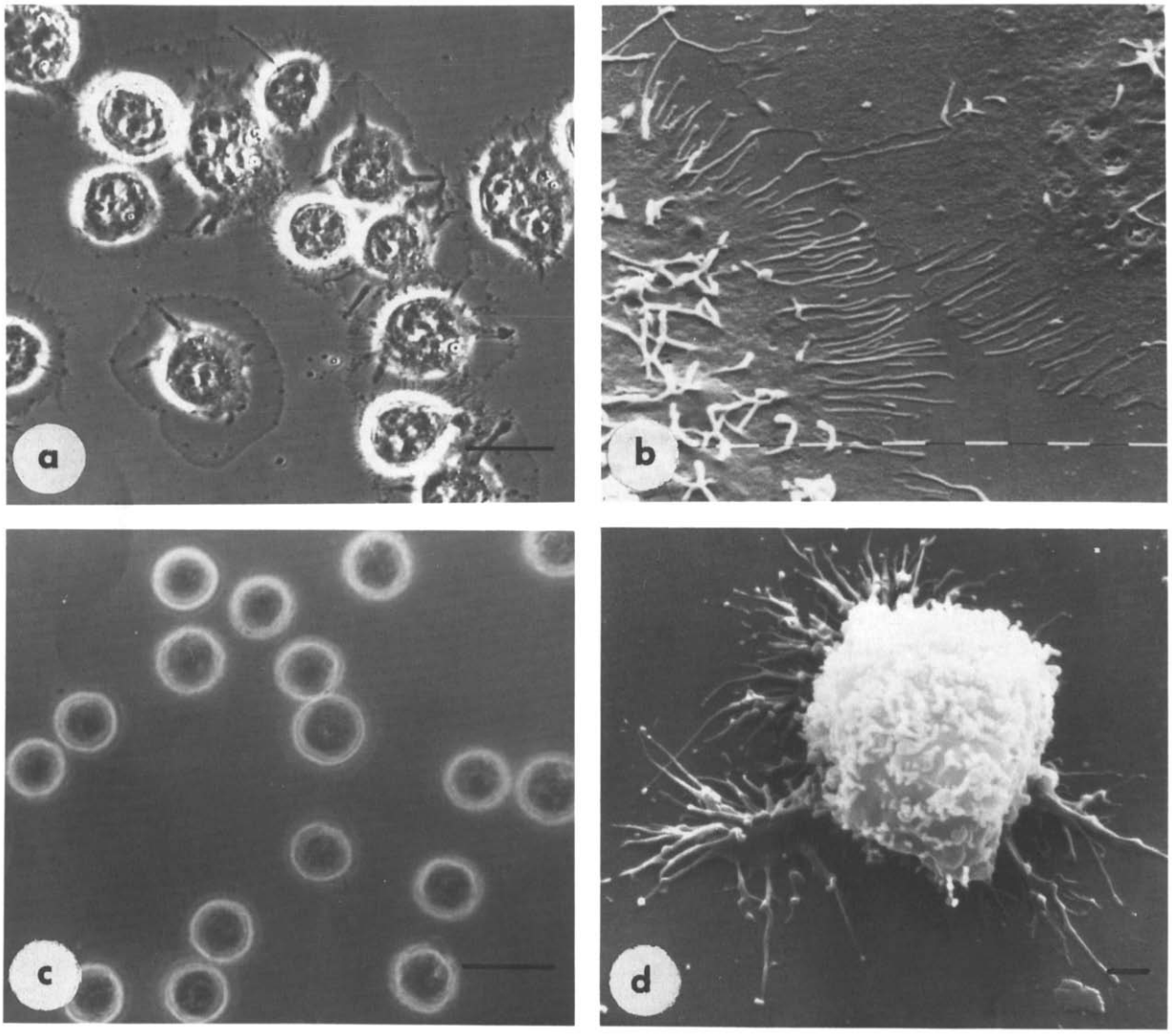

Fig.1. Spreading of biotinylated Ehrlich ascites cells upon avidinylated substrates. (a,b) Biotin-bearing cells spread upon the avidin-covered coverslip for 120 min; (c) control, the substrate was preincubated in biotin solution; (d) cells spread in cytochalasin B (10 μg/ml) for 60 min. (a,c) Phase contrast microscopy. Bar, 20 μm. (b,d) Scanning electron microscopy. Bar, 1 μm.

To determine whether the observed spreading is a normal physiological process we have tested several general inhibitors of cell activity. Sodium azide (20 mM) and low temperature (4°C) inhibit the spreading of biotinylated ascites cells upon the avidin-covered substrate. When the spread cells were treated with cytochalasin B (CB, 10 μg/ml), their lamellae retract and only arborized retraction fibers remain. If CB was applied to suspended cells, they produce upon attachment only narrow elongated processes (fig.1d). This effect was reversible: when CB is washed away, the cells produce ruffles and begin to spread.

The morphology of the cells upon spreading resembles that of normal epithelia, from which Ehrlich ascites carcinoma originates. Interference reflection microscopy reveals black contacts along the margin of the spread cells, typical of normal epithelial cells [4]. Thus it seems that binding of substrate-adhesive molecules allows the ascites cells to enter the normal physiological spreading.

We conclude that it is enough to supply appropriate adhesive molecules to the ascites cells surface to normalize their spreading. It is reasonable to suppose that in the course of tumor progression cells become ascitic due to the loss of the adhesive surface molecules rather than to any defects in the intracellular machinery.

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