F-Actin Patterns Quantitated with FI-Phalloidin in Skin Fibroblasts of Individuals Genetically Predisposed to Colon Cancer

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Abstract

FI-phalloidin is a chemical probe for F-actin distribution in fixed cultured cells. Skin fibroblast cells from an individual can be distributed into four classes according to number and size of structures containing F-actin visualized with fluorescence microscopy after FI-phalloidin staining. The distribution shifts significantly when skin fibroblasts of persons with the inherited colonic neoplasm ACR are compared with cells from unaffected individuals. Skin fibroblasts from cancer patients of non-ACR colon cancer-prone families, and from cancer patients in families with multiple primary tumors, show normal F-actin distribution. These data confirm earlier studies which used antibody to actin (Kopelovich et al. 1980). Skin fibroblasts from one child of an ACR patient have an abnormal F-actin distribution, even though this child is currently free of symptoms of the disease. FI-phalloidin permits a prospective study of such individuals to determine whether F-actin distribution detected by a chemical probe can be used prognostically.

Introduction

Most human cancers are of unknown etiology. The relative weight of environmental factors and host genetic susceptibility is thought usually to be tipped toward the environment. In rare cases however, the disease occurs as the result of a host mutation. In such cases it is reasonable to hope that all cells of an affected individual might reveal an altered phenotype when appropriately examined. Detection of such an abnormality in easily cultured cells might permit prognosis of asymptomatic children of individuals affected by an inherited cancer.

Methods

Cell and Culture Conditions

Biopsies were obtained from patients and their relatives seen at Memorial Sloan-Kettering Cancer Center and were processed as previously described (Kopelovich et al.
Skin fibroblasts were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum (FCS, Gibco) and 100 units per ml of penicillin and streptomycin, and were kept at 37 °C in an atmosphere of 10% CO₂, 90% air, and 100% humidity. All cultures were routinely checked for mycoplasma contamination (Chen 1977) and were found to be negative.

**Fixation and Staining**

Cells were plated on coverslips at a density of 2-4x10³ cells/cm² in DME 10% FCS. One day later the medium was changed to 1% FCS. The following day the cells were fixed in 10% formalin in phosphate-buffered saline pH 6.8 (PBS) for 20 min at room temperature, rinsed with PBS and extracted with 1% NP40 in PBS for 20 min. After three rinses in PBS, 5 min each, the cells were stained with 10 µl of Fl-phalloidin (1 µg/ml in PBS) at 37° for 20 min. The coverslips were rinsed three times in PBS and mounted on microscope slides with Aquamount (Verderame et al. 1980).

**Fluorescence Microscopy**

Stained coverslips were examined with a Leitz Orthoplan microscope using a Zeiss Planapo 63× oil immersion objective coupled to a Leitz I2 wide band exciter-barrier filter cube.

**Results**

**Method of Scoring**

Well-spread flat cells were scored for their actin cable content (Verderame et al. 1980). Each cell was placed in one of four distinct categories. Class I cells had heavy distinct cables crossing more than 90% of the central area of the cell, 50% of which span the breadth of the cell. Class II cells had fine cables and at least 2 heavy cables within the central half of the cell, extending more than half of its breadth. Class III cells had only fine cables and class IV cells had no detectable cables in the central area, but only a diffuse fluorescence; some class IV had fine cables solely at the periphery. 200 cells were scored for each culture by at least two individuals, using blind-coded slides.

**Comparison of Actin Pattern in Different Groups**

Six groups of subjects were examined for their actin cable patterns: (1) normal subjects, either from the general population or spouses from the cancer families; (2) patients diagnosed with adenomatosis of colon and rectum (ACR); (3) asymptomatic children of ACR patients who have a 50% probability of developing the disease; (4) persons from colon cancer-prone families who have colon cancer (CCP+), (5) persons from colon cancer-prone families who have no symptoms but a 50% risk of developing colon cancer (CCP−), and (6) one patient with multiple primary colonic tumors.

Table 1 shows the percent of skin fibroblast cells at three different thresholds of cytoskeletal organization. The percent of cells with the largest cables are in category I. The percent of cells with fine cables but also at least some large cables are in categories I+II. The percent with any detectable cables are in catagories I+II+III. Published data with anti-actin are based on a single threshold (+/-) score for the presence of cables (for example, Pollack et al. 1975). The percent (+) cells reported earlier in studies with anti-actin (Kopelovich et al. 1977; Kopelovich et al. 1980) is also given in Table 1. The general correlation of Fl-phalloidin distribution with α-actin data is good in that population with more cells in categories I and II also have higher (+) scores.

By Student's t-test normal and ACR individuals have significantly different percentages of cells when scored by I+II or I+II+III thresholds (Table 2). In contrast, when cells are scored for the presence of only large cables (Category I) there is no statistical difference between normal and ACR individuals. The threshold of any detectable cables (I+II+III) depends upon the capacity of