Attachment of Human Pancreatic Tumor Cell Lines to Collagen in Vitro

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ABSTRACT

The attachment to purified collagens of three cell lines established from human pancreatic carcinomas was investigated. PANC-1 and CAPAN-1 cells attached to type I, III, and IV collagens in the absence of fetal bovine serum. MIA PaCa-2 cells, on the other hand, attached only to type IV collagen. In the case of MIA PaCa-2 cells, the attachment occurs more slowly and to a lesser extent. Increasing concentrations of fetal bovine serum had no effect on the attachment of PANC-1 and CAPAN-1 cells to the collagens. However, the attachment of MIA PaCa-2 cells to all the collagen types was greatly enhanced by 10% fetal bovine serum. This enhancement was shown to be due to the fibronectin present in the serum.

INTRODUCTION

Following malignant transformation, many changes in the in vitro properties of animal cells can be detected (9, 21, 31). A number of attempts have been made to link these changes with the ability of cells to form tumors in vivo, most commonly in the nude mouse (1, 9, 27). The in vitro property which has been found to correlate best with tumor formation in vivo is that of anchorage-independent growth, which is defined as the ability of cells to proliferate in a semisolid medium that prevents their attachment to the tissue culture plate (1, 9, 27). These results indicate that the interaction with a substratum is an important event in the control of cell proliferation.

One of the most extensively studied components in cell attachment has been fibronectin, a large glycoprotein found in the extracellular matrices of many normal cells in culture (12, 32). Fibronectin is present in a variety of connective tissues and basement membranes (30). An immunologically identical protein is found in serum (21). In many cases, surface-associated fibronectin is absent or reduced in virally or chemically transformed cells and in cells isolated from naturally occurring tumors (12, 32).

A second widespread component of extracellular matrices and basement membranes is collagen (13, 19, 20). Collagen occurs in several chemically and genetically distinct forms. Type I and III collagens are found in the stroma of many tissues including skin and tendon (19), type II collagen is found in cartilage (20), and type IV is in basement membranes (13). In vitro systems have been developed to examine the interaction of cells with collagen (14, 16, 26). A variety of cells use fibronectin to attach to collagen substrates in vitro (14). In addition, several cell types have been shown to be capable of attachment in the absence of fibronectin (10, 23). These studies have been extended recently to investigate the relationship between malignant potential and attachment to collagen (16, 22). However, very few of the cell lines examined thus far have been of human origin.

Several cell lines have been established from human pancreatic carcinomas (8, 17, 34). The attachment of such cell lines to purified collagens and the involvement of fibronectin in the interaction were studied as part of an investigation of the molecular events involved in invasion and metastasis in pancreatic cancer. This study is also significant because, although most cancers occur in epithelial tissue, very little work has been done on the mechanism of attachment of established epithelial cell lines to collagen substrates.

MATERIALS AND METHODS

Cell Lines. MIA PaCa-2 cells were kindly provided by Dr. Adel A. Yunis (University of Miami School of Medicine, Miami, Fla.). The PANC-1 cells were obtained from Dr. Walter A. Nelson-Rees and were produced with support from the National Cancer Institute, Viral Oncology Program, under the auspices of the Office of Naval Research and the Regents of the University of California. The CAPAN-1 cells were supplied by Dr. Jorgen Fogh (Sloan-Kettering Institute for Cancer Research, Rye, N. Y.). The cell lines were all maintained as monolayer cultures in DME supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 /g/ml). For attachment assays, the cells were harvested using a solution of 0.05% trypsin-0.02% EDTA in Hanks' balanced salt solution without calcium and magnesium. They were then washed twice with DME containing 200 /g bovine serum albumin per ml and resuspended in the same medium at 2 to 4 X 10^6 cells/ml.

Attachment Assay. Thirty-five-mm bacteriological plastic Petri dishes (Falcon Plastics, Cockeysville, Md.) were coated with collagen as described previously (16). One ml of DME containing 200 /g bovine serum albumin per ml with or without fetal bovine serum or fibronectin was added to the dish, which was incubated at 37° in 95% air-5% CO2 for 60 min. One hundred /l of the cell suspension were then added, and the dish was incubated once again. At the end of the incubation period, unattached cells were removed by careful washing with phosphate-buffered saline. The attached cells were removed with 0.05% trypsin-0.02% EDTA, and the number of cells was determined using an electronic cell counter (Coulter Electronics, Inc., Hialeah, Fla.). The type I collagen used as a substrate was isolated from the skin of lathyritic rats (2), and type II was from a rat chondrosarcoma (28). Type III collagen was purified from fetal calf skin (7), and type IV was from a murine sarcoma (25).

Affinity Chromatography. Fibronectin was removed from serum by affinity chromatography on a collagen-Sepharose 4B column (6, 11). The column was equilibrated with 0.05 M Tris-HCl (pH 7.4) containing 0.025 M 6-aminoheptonic acid. Fetal bovine serum was added to the column and incubated for 1 hr. The unbound serum was then collected, the column was washed with buffer, and the bound fibronectin was

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3 The abbreviation used is: DME, Dulbecco's modified Eagle's medium.
eluted with buffer containing 1 M KBr. The fibronectin was dialyzed against DME prior to use.

RESULTS

Pancreatic Cell Attachment. The rate of attachment of the pancreatic cell lines to different collagen types was investigated. The results obtained are shown in Chart 1. The overall patterns of binding of CAPAN-1 and PANC-1 cells were similar, although there were differences in the initial rates of attachment. In both cases, the cells attached rapidly to type I, III, and IV collagens and attached very slowly or not at all to type II collagen. Control plates with no collagen on them showed minimal binding with any of the cell lines studied. The attachment of MIA PaCa-2 cells to the various types of collagen was very different from that of the CAPAN-1 and PANC-1 cells. In the case of MIA PaCa-2 (Chart 1C), the cells attached only to type IV collagen, and after 21 hr, less than 50% of the cells had bound to this type of collagen. These results suggest that there are 2 very different mechanisms of attachment to collagen substrates in human pancreatic cancer cell lines.

Effect of Serum. One of the important aspects in investigating the attachment of cultured cells to collagen substrates has been the involvement in the process of factors contained in serum, especially fibronectin (10, 14, 22). The effect on attachment to different collagen types of increasing amounts of fetal bovine serum in the assay buffer is shown in Chart 2. With all cell lines, the assays were carried out for 3 hr. In the case of CAPAN-1 cells (Chart 2A) and PANC-1 cells (Chart 2B), increasing the serum concentration to a maximum of 10% had no effect on the number of cells which attached to each type of collagen. This was in marked contrast to the effect of serum on the attachment of MIA PaCa-2 cells (Chart 2C). An increase in the concentration of fetal bovine serum in the assay buffer above 0.5% led to an increase in the attachment of MIA PaCa-2 cells to all the collagen types but with most binding occurring to type IV collagen. With all the cell lines studied, increasing concentration of serum made no difference to the lack of binding observed when control plates, with no collagen, were used. These results once more emphasize the difference in attachment to collagens between CAPAN-1 and PANC-1 cells, on the one hand, and MIA PaCa-2 cells, on the other.

Effect of Fibronectin. The results indicate that there is a factor present in fetal bovine serum which greatly enhances the attachment of MIA PaCa-2 cells to collagen substrates. A strong candidate for such a factor, from previous studies (14, 16, 22, 26), is fibronectin. In order to examine this possibility, the attachment of MIA PaCa-2 cells to collagen was measured in the presence of increasing concentrations of serum from which fibronectin had been removed. The experiment was also carried out using equivalent concentrations of partially purified fibronectin (Chart 3). The results show that fibronectin is indeed the factor in fetal bovine serum which appears to be involved in the attachment of MIA PaCa-2 cells to collagen substrates.

DISCUSSION

When human pancreatic cancer cell lines were studied, 2 distinct patterns of attachment to different collagen types were discerned. In the first case, PANC-1 and CAPAN-1 cell lines attached rapidly to type I, III, and IV collagens but not to type II. Increasing concentrations of fetal bovine serum had no effect on the attachment of these cells to any of the collagens tested. On the other hand, MIA PaCa-2 cells attached much more slowly and to a lesser extent to collagen and showed a marked preference for type IV collagen. The attachment of MIA PaCa-
arises from the fact that pancreatic tissue is composed of several cell types, mainly acinar cells with some ductal and endocrine cells. A large percentage of pancreatic carcinomas is thought to originate in the ductal cells (4), but it is sometimes difficult to identify the cell of origin of the tumor (18). For this reason, it is possible that the 2 types of attachment to collagen are due to the fact that the carcinomas arose from 2 different cell types. Previous studies have shown that different cell types have different patterns of attachment to collagens (15).

A second possible reason for the 2 patterns of attachment to different collagens could be that it is a reflection of the metastatic potential of the original tumor. A recent study has shown that, with mouse fibroblasts, metastatic cells differ from normal and transformed cells in their attachment properties (22). Although the results are not directly comparable, it was found that, in the case of normal or transformed cells, attachment to type I or IV collagens was stimulated by fibronectin. This also occurred with metastatic cells attaching to type I collagen, but in the case of type IV collagen, the metastatic cell line was not affected by fibronectin. A similar mechanism could be operating in the attachment of PANCl- and CAPAN-1 cells to all the collagen types except type II. It is known that CAPAN-1 cells are derived from the metastasis of a pancreatic carcinoma (8) and that the tumor from which the PANCl-1 cell line was established had metastasized (17). There is no information about this aspect of the tumor from which MIA PaCa-2 cells were cultured. It should, however, be noted that most pancreatic carcinomas have undergone metastasis by the time they are diagnosed (5).

Other workers (3, 24, 29) have studied the correlation between cell-associated fibronectin and the metastatic potential of cultured cells. Although the results have been conflicting, a recent study of human epithelial cells in culture (29) suggested that cells from metastases produce little or no fibronectin compared with cells from primary carcinomas.

The fact that PANCl- and CAPAN-1 cells did not require exogenous fibronectin suggests that they may use some other molecule in attachment to collagen. One possible candidate for this role would be laminin, a component of basement mem-

Potential errors:  
- Chart 3. The effect of fibronectin-free serum (B) and fibronectin (C) on the attachment of MIA PaCa-2 cells to type I collagen. The assays were carried out for 3 hr in the presence of increasing concentrations of fibronectin on fibronectin-free serum. Each point represents the mean of duplicate measurements which did not differ by more than 10%. The concentrations of fibronectin used were adjusted so that they were equivalent to the amounts of fibronectin removed from each concentration of fibronectin-free serum, FBS, fetal bovine serum.
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