Establishment of a Human Fetal Small Intestinal Epithelial Cell Line

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Key Words

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Introduction

The manifestations of gastrointestinal disorders often result from a failure of the intestinal epithelium to interact beneficially with the luminal milieu. The function of the enterocyte can radically alter according to its state of differentiation. However, the mechanisms which control this process are ill defined. This is, in part, because small intestinal epithelial cells have proven awkward to grow in vitro, making the external influences on enterocyte differentiation difficult to study. Furthermore, intestinal epithelial cells are increasingly recognized to be an integral part of the mucosal immune system. They express class II MHC molecules [1, 2] and may secrete cytokines [3]. The mechanisms underlying this activity in the human could be studied more effectively in isolated enterocyte cell lines.

To date, the only human epithelial cells that have remained viable in culture for indefinite periods have been derived from colonic carcinomas [4]. However, nonmalignant cell lines (IEC-6) have been successfully developed from the rat [5], which can differentiate when grown in the presence of connective tissue elements [6, 7]. Clearly, a nontransformed human cell line in culture would increase

our ability to study epithelial cell function, particularly with regard to genetic control when carcinoma cell lines may be abnormal.

Results

We have established epithelial cell lines from human fetal intestine. Morphologic, immunochemical and molecular techniques suggest that these cells originated from intestinal epithelial cells and retain features of small intestinal enterocytes. Furthermore, extracellular basement matrix induces brush border enzyme activity. Characterization has been extensive in one particular line, H-4. Examination of the chromosomes of the H-4 cell line demonstrated a normal 46 XY karyotype. The morphology of the viable cell lines was similar to that of rat IEC-6

cells. They, like IEC-6 cells, consist of a homogeneous population of epithelial-like cells with large, oval nuclei growing as tight colonies of polygonal, closely apposed cells. Growth to confluence resulted in a sheet of cells that covered the surface in which they grew. Staining with antikeratin antibody demonstrated strands of cytokeratin in H-4 cells. Similar staining was

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found in the human colonic carcinoma cell line (Caco-2). However, no staining was seen in human fibroblasts. Growth of H-4 cells reached confluence by day 7. There was a reduction in cell number 24 h after plating. Cells became more compact on confluence. However, the monolayers were not completely sealed by tight junctions. As in IEC-6 cells, transepithelial resistance remained low after confluence. Brush border enzyme activity was low, as in H-4 cells grown on plastic. They were therefore plated onto Matrigel to determine whether extracellular matrix induced expression of brush border enzymes. The expression of alkaline phosphatase in cells plated onto plastic did not vary with time. However, when plated on Matrigel, expression of alkaline phosphatase increased significantly ($p < 0.05$). Under these conditions, however, proliferation of H-4 cells is halted. Extracellular matrix therefore appears to be important in the differentiation of these isolated human cells.

Ionic carcinoma cell lines have enhanced our understanding of epithelial function [4], their disadvantage is the unknown degree to which their properties have been affected by malignant transformation. We have now isolated a nonmalignant human small intestinal cell line from fetal tissue. The properties of this line are similar to those seen in the rat IEC-6 cell line. The similarity of H-4 to IEC-6 cells is probably a consequence of the similarity in isolation techniques adopted in the isolation of the two cell lines.

In addition to their use as a tool to study epithelial cell differentiation, isolated human cell lines will be used to examine the role of the epithelium as a component of the mucosal immune system. Experiments are underway to test whether H-4 cells express genes for cytokines and immunologically active surface molecules.

Acknowledgments

Discussion

The study of small intestinal cell differentiation has been hampered by the lack of appropriate cell lines. Although co-

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