Epithelial Cells and Von Gierke's Disease

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Summary

Epithelial cells and not fibroblasts from human liver and amniotic fluid contain inducible glucose-6-phosphatase (G-6-Pase) activity. The diagnosis of Von Gierke's disease has been made in a patient with hepatomegaly utilizing cultured epithelial cells grown from a liver biopsy. G-6-Pase activity in epithelial cells from this patient could not be induced by dibutyl cyclic AMP and theophylline. This is the first use of epithelial cells for diagnosis of a metabolic disease. G-6-Pase activity in cloned epithelial cells from amniotic fluid increases 2- to 3-fold after 24-hr exposure to dibutyl cyclic AMP and theophylline. The prenatal diagnosis of Von Gierke's disease may be possible in a laboratory experienced with these techniques if epithelial cell growth is obtained from amniotic fluid.

Speculation

Epithelial cell culture may add a new dimension to the study of metabolic disease in man.

The cultured skin fibroblast has played an important role in diagnosis and research in human biochemical genetics for over 10 years. Many enzyme activities, however, are not present in skin fibroblasts so that some metabolic problems are not amenable to study in cell culture. The epithelial cell, with different properties, enzyme constitution, and physical appearance, is potentially of use in the investigation of metabolic disease. We describe the first application of epithelial cell culture to diagnosis of a metabolic enzyme deficiency in man.

METHODS

Epithelial cells from human liver were grown in F12 medium with 2X concentrated amino acids (10) and 50 mg/liter hydrocortisone and dialyzed 15% fetal calf serum. Control liver biopsies were obtained at operation when the liver was invaded for a different purpose or within 2 hr of death. Informed consent was obtained for this procedure. Informed consent was obtained for liver biopsy in a patient with hepatomegaly, growth failure, hypoglycemia, hypertriglyceridemia, and hyperlacticacidemia. Liver samples were dissociated with dilute trypsin (10, 18), and cells sequentially plated in three flasks (20). Epithelial cells predominated in the third flask. Epithelial cell cultures without fibroblasts were obtained by placing a sterile stainless steel nut over an epithelial cell clone, detaching the cells in 0.05% trypsin in pH 7.2-7.4 Hanks' solution, and subculturing into a tissue culture plate with a Pasteur pipette. Fetal calf sera were pre-tested to eliminate toxic batches.

Amniotic fluid cultures were initiated by placing 2-3 cc uncentrifuged amniotic fluid in a tissue culture dish. After 10-15 min 5 cc F12 media with 30% fetal calf serum were added. Dishes were incubated at 37° with 5% CO₂ for 4 days before the medium was changed and F12 media with 15% fetal calf serum added. Epithelial cell clones were isolated from mixed cultures with a sterile stainless steel nut as described above.

Glucose-6-phosphatase was induced in epithelial cell cultures by the addition of dibutyl cyclic AMP, 9 mg/100 ml, and theophylline, 18 mg/100 ml, to the culture media (19). Cells were scraped from the dish with a rubber policeman, ruptured by the sudden release of nitrogen under pressure (16), and assayed for G-6-Pase activity by the method of Erickson *et al.* (4). The method of Chen *et al.* (3) was used to determine inorganic phosphate. Duplicate assay reactions were used to selectively inactivate G-6-Pase and correct for nonspecific phosphatase (17). Tyrosine aminotransferase (TAT) activity was measured as previously described (8).

RESULTS

Epithelial cells from human liver resemble amniotic fluid epithelial cells as shown in Figure 1. Hydrocortisone increases epithelial cell growth from both sources (14) and facilitates the isolation of epithelial clones in mixed cultures. We have maintained human liver epithelial cells for up to 6 months in mixed cultures, but have generally only been able to maintain amniotic fluid epithelial cell growth for 2-5 subcultures, as has been noted by others (11). Epithelial cell cultures can be obtained from about half of amniotic fluid samples, but this is variable and may depend on the cell composition of the sample, the media, and the batch of fetal calf serum.

Four epithelial cell clones tested from human liver had G-6-Pase activity of 8-62 nmol Pi/mg protein/hr. Enzyme activity was induced 2- to 3-fold with dibutyl cyclic AMP and theophylline. Similar G-6-Pase activity and induction was found in control epithelial cell clones from amniotic fluid, as shown in Figure 2. One of four epithelial cell strains tested from amniotic fluid had almost no G-6-Pase activity; however, activity could be demonstrated after induction with dibutyl cyclic AMP and theophylline as shown in Figures 2 and 3. Bissell *et al.* (1) have noted that G-6-Pase in human liver and primary nonproliferating monolayer cultures of liver has a higher specific activity than the values reported here. Figure 3 shows the G-6-Pase response of epithelial cells and fibroblasts from liver and amniotic fluid to dibutyl cyclic acid and theophylline. It can be seen in Figure 3 that fibroblasts from amniotic fluid and liver do not have G-6-Pase activity when cell cultures are exposed to dibutyl cyclic AMP and theophylline. Epithelial cells from a patient with hepatomegaly, hypoglycemia, and hyperlactic acidemia, with no G-6-Pase activity in a liver biopsy sample, did not have G-6-Pase activity in cultured epithelial cells with or without dibutyl cyclic AMP and theophylline, as shown in Figure 3. These cells had a TAT activity of 14 nmol HPP/mg protein/hr after a 24-hr exposure to 5 mg/100 ml hydrocortisone in F12 media with 15% fetal calf serum. Epithelial cell culture confirmed the diagnosis of Von Gierke's disease in this patient.

DISCUSSION

Liver biopsy and assay of G-6-Pase activity are usually employed to make the diagnosis of Von Gierke's disease. Once thought to be an enzyme specific to liver, G-6-Pase activity is also found in small intestine (5) and platelets (13). We found cyclic AMP-inducible G-6-Pase activity in epithelial cells from

Fig. 1. Epithelial cells from cultured liver (A) and amniotic fluid (B), $\times 600$.

liver and amniotic fluid, as shown in Figures 2 and 3. Failure to find inducible activity in epithelial cells from a patient with hepatomegaly, hypoglycemia, hyperuricemia, and hyperlipidemia confirmed the diagnosis of Von Gierke's disease in our patient. This is the first example of an enzyme diagnosis made in a patient with a metabolic disease by the failure of an enzyme to respond to an inducing agent in cultured cells, and the first use of epithelial cells for enzyme diagnosis. It has been suggested that decrease in G-6-Pase activity in affected patients may be failure of enzyme induction (15), rather than a primary structural defect in the enzyme. However, half-normal enzyme activity in the intestinal mucosa of patients of affected children (5) suggests that a structural mutation rather than a regulatory mutation is involved. Studies of immunoprecipitable G-6-Pase enzyme activity in liver or liver epithelial cells of affected patients are needed to determine this point.

A new but difficult treatment is now available for Von Gierke's disease (7), and exact diagnosis is necessary. Enzyme assays of epithelial cell cultures are confirmatory and may be useful since some patients with high glycogen or fat content may have low levels of G-6-Pase activity in a biopsy sample but do not have Von Gierke's disease. Tyrosine aminotransferase, another epithelial cell specific enzyme, is not found in fibroblasts (2). The presence of 14 nmol HPP/mg protein/hr of hydrocortisone-inducible TAT activity in cloned cells from the patient with Von Gierke's disease confirmed that these cells, without G-6-Pase activity, were epithelial. It is likely that epithelial cells will be useful in the study of other disorders where the normal enzyme or metabolic function is expressed in epithelial cells and not fibroblasts.

Amniotic fluid variably yields epithelial or fibroblastic type cells or both in cell culture (6, 11). Epithelial cells from liver and amniotic fluid have similar morphology, as shown in Figure 1, and possibly similar enzymic constitution. We therefore examined amniotic fluid cells for G-6-Pase activity. Cyclic AMP-inducible G-6-Pase activity is present in epithelial cells from amniotic fluid, as shown in Figure 2, but not fibroblasts, as shown in Figure 3. Von Gierke's disease has not previously been listed among diseases diagnosable before birth (12). Although it now appears feasible, great caution should be used in genetic counseling. If fibroblast cell types predominate, the prenatal diagnosis of Von Gierke's disease could not be attempted since

Fig. 2. Induction of glucose-6-phosphatase in four epithelial cell clones from amniotic fluid. Glucose-6-phosphatase activity was measured in epithelial cell homogenates 24 hr after exposure to control media and media with 9 mg/100 ml dibutyl cyclic AMP and 18 mg/100 ml theophylline in F12 media with 15% calf serum.

Fig. 3. Induction of glucose-6-phosphatase activity in cultured cells. Epithelial cells from control human liver culture (▲—▲), cultured liver from a patient with Von Gierke's disease (■—■), amniotic fluid (●—●), and control fibroblasts from liver and amniotic fluid (△—△) were exposed to 9 mg/100 ml dibutyl cyclic AMP and 18 mg/100 ml theophylline in F12 media and 15% fetal calf serum for different periods of time.

the diagnosis depends upon lack of enzyme response to induction, a property shared by amniotic fluid fibroblasts, as shown in Figure 3. TAT activity is present in cloned amniotic fluid epithelial cells (14). TAT enzyme assays should be performed in cells from amniotic fluid from a fetus at risk for Von Gierke's disease to confirm that the cells are epithelial. Cloning of epithelial cells from fibroblastic cells may not be necessary if cultures are obtained which are predominantly epithelial. A G-6-Pase/TAT ratio might be helpful and could be established in advance of examining cells from a fetus at risk. Although it is likely that the G-6-Pase enzyme proteins in liver and amniotic epithelial cells are the same, this is not proven at the present time, and the diagnostic accuracy in a potentially affected fetus has not been established.

Several enzymes, particularly the lysosomal hydrolases, have a wide quantitative range of specific activities in amniotic fluid cultures (9, 21). This variability is much greater than found in fibroblast cell cultures, and may depend upon whether fibroblasts or epithelial cells predominate. Qualitative differences exist as well. Cystathionine synthetase activity is found primarily in fibroblasts, and histidase activity in epithelial cells (6). We show that G-6-Pase activity is another enzyme activity specific to epithelial cells, and report the first application of this cell type to a genetic disorder of metabolism in man. Unfortunately, epithelial cells are short lived in tissue culture, and tolerate subculturing poorly (6, 11). It may not be possible to extensively study this cell type until properties of epithelial cell growth are better understood.

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DNA
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Peroxidase Technique for the Detection of Photochemical Lesions in Intracellular Deoxyribonucleic Acid

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Summary

The widespread use of phototherapy for the prevention and treatment of neonatal hyperbilirubinemia has generated some concern as physiologic substances other than bilirubin may be photoactivated. Little information is available on the long term toxicity of these photodecomposition products. Recent observations of the *in vitro* DNA-modifying activity of phototherapy lights has encouraged us to develop laboratory procedures which can identify and quantitate these light-induced alterations. The purpose of the present study was to develop a technique capable of detecting photochemical changes in the genetic material of human cells in tissue culture. The results demonstrate that the antinucleoside peroxidase staining procedure is capable of detecting changes in the DNA of human cells exposed to physiologic (riboflavin) and nonphysiologic (methylene blue) photosensitizing agents in the presence of light with a fluence rate (450 nm) of 141 $\mu\text{W} \cdot \text{cm}^2$.

Speculation

In view of the known relationship between the ability of a number of environmental agents to modify DNA on the one hand and their potential to induce mutations and cancer on the other, the present study, which documents the DNA-modifying potential of phototherapy, raises questions regarding the safety of this therapeutic maneuver. The further development of techniques described here to identify and quantitate photochemical alterations in the genetic material of irradiated infants would enable us to intelligently define the risk-benefit ratio of phototherapy and to identify an *at risk* population for long term follow-up studies.

The effects of environmental agents on child health is a subject of considerable interest at the present time (1). Of special concern are the long term effects of such agents and more specifically their carcinogenic, mutagenic, and teratogenic po-