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Review

Cell culture systems in the elucidation of cellular and molecular mechanisms associated with intestinal adaptation

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Cell cultures provide a unique in vitro model, a tool or a "test tube" system to establish the relationship between brush border membrane lipid composition, enterocyte microsomal activities of key lipid metabolizing enzymes, the adaptation of intestinal transport, and to identify transcriptional and post-transcriptional events which lead to the adaptation of transport. Transfection of cells in culture with cDNA not normally present will permit the study of the phenotypic expression of a specific hydrolytic or transport function. Enrichment of the culture medium with nutrient modifiers of transport (such as polyamines or fatty acids) will permit better disection of the events controlling phenotype expression. In this way, clinical problems of nutrient absorption may be better understood and eventually modified in a therapeutically important manner. (J. Nutr. Biochem. 6:240–245, 1995.)

Introduction

The morphology and functions of the intestine are not static but are subject to adaptation in a number of situations in health and disease, such as in response to starvation/refeeding or a change in diet.[1] Cultured cell lines have been used in studies of intestinal differentiation and function and have provided a useful model system to explore many of the cellular processes seen in intestinal adaptation.[2] Primary culture of intestinal epithelial cells is not possible beyond several days but has been used to study cholesterol uptake.[3] The establishment of stable cell lines in culture is an important technique for the maintenance and propagation of cells ex vivo in an isolated and defined environment. Cell culture systems allow for manipulation of the culture environment, the ability to study complex physiological phenomena in both differentiated and undifferentiated cells in a simplified environment, and the ability to manipulate genetic makeup in these cells.[4] Cell culture systems have proven invaluable in the elucidation of the fundamental biochemical pathways and regulatory steps involved in the metabolism of lipids and lipoproteins. The relative ease with which subcellular fractions of cultured cells are obtained allows for the localization of different lipid pools and the intracellular metabolism and targeting of lipids to various organelles. It may be difficult to select an appropriate animal model that closely resembles characteristics of the pathogenesis of disease processes in humans, and this must be done with considerable thought and compromise. In the future, it is anticipated that the combination of molecular biology techniques, experimental systems for the coculture of cells, and the availability of cells from humans with genetic abnormalities will facilitate the elucidation of the molecular defects underlying specific diseases. These systems will also provide a framework for the development of pharmacological and genetic approaches to cure or to improve the phenotypic expression in certain human diseases.

The identification of the signals for and the mechanisms of adaptation of intestinal sugar and lipid transport may now be examined using cell culture systems.[5] The study of cellular and molecular events associated with intestinal glucose transport has been examined in a variety of cultured cell lines.[6] This has provided model systems to study the adaptive responses to nutrients and other possible signals that lead to up- or down-regulation of transport. Some of the cell lines that have been used to examine intestinal absorption will be discussed.

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IEC, RIE1, and IRD 98

Cell lines originating from fetal or postnatal small intestine (IEC, RIE1, or IRD 98) have been shown on morphological grounds, and on the basis of immunological characterization of cell surface markers, to behave like intestinal crypt cells.[7,8] Under the influence of mesenchyme, a proportion of IEC cells is able to differentiate into absorptive, goblet, endocrine, and Paneth cells. Extensive studies of hydrolytic or transport function have not yet been reported in this cell line.

HT-29 cells

The human colon adenocarcinoma cell lines Caco-2 and HT-29 are able to express typical enterocyte differentiation. These are of interest in studies of regulation of the Na^+-dependent glucose transporter ($SGLT_1$), the sodium-independent fructose transporter ($GLUT_5$) or of lipid absorption and metabolism. The first demonstration of the capacity for differentiation of HT-29 cells was reported a decade ago, with the observation that the substitution of galactose for glucose in the culture medium results in the emergence of an enterocyte-like differentiated population.[9,10] The cellular events associated with differentiation of HT-29 cells have been examined in studies of changes in the levels of activity of brush border membrane (BBM) markers such as alkaline phosphatase, sucrase-isomaltase, aminopeptidase N, and dipeptidylpeptidase IV. Also, HT-29 cells are an interesting in vitro model for studying the formation and regulation of tight junctions.

A number of subpopulations and clones have been isolated from HT-29 cells. Some of these populations are enterocyte-like, others are mucus-secreting, while others form domes (a characteristic associated with ion transport properties).[11] Previously, it was assumed that the parental cell line was undifferentiated, but this assumption is not entirely correct.[12,13] For example, antibodies against villin, BBM-associated hydrolases, and gastrointestinal mucins have been developed and allow for screening of the differentiated phenotype. Using such probes, postconfluent cultures of HT-29 cells do not contain just undifferentiated cells but also a small proportion of differentiated cell types. The HT-29 cell line, therefore, is a heterogeneous cell line that under some culture conditions contains a majority of undifferentiated or differentiated cells. For example, it is possible to obtain the emergence of totally differentiated populations by replacing glucose with galactose in the culture medium,[9,14–16] with glutamine deprivation,[17] by treatment of the cell line with sodium butyrate,[18,19] or with exposure of the cells to methotrexate or to 5-fluorouracil.[12,13] The emergence of differentiated HT-29 cell populations may be the result of a process of selection, in which a small proportion of differentiated cells already present in the parental line would also possess an advantage enabling them to cope with adverse metabolic conditions or biochemical hazards.

HT-29 cells form a polarized monolayer with tight junctions, express hydrolases normally associated with the BBM of enterocytes, and actively transport by $SGLT_1$ the nonmetabolized sugar analog methyl-D-glucoside (AMG) in a manner that is inhibited by the replacement of sodium, by phlorizin, and by glucose.[20] When HT-29 cells are grown in culture medium in which glucose is replaced by galactose, a greater proportion of differentiated cells appear which express active glucose transport.[21–23] HT-29 cells absorb D-glucose and galactose (as would be expected, since like AMG they are transported by $SGLT_1$), as well as fructose (Thomson et al., unpublished observations). When HT-29 cells are grown in standard culture conditions with 25 m*M* glucose and serum, they are undifferentiated and only express facilitating glucose transport,[24] and sucrase-isomaltase activity is abolished.[25] This suggests that HT-29 cells contain both the sodium-dependent BBM transporter $SGLT_1$ as well as the sodium-independent fructose transporter $GLUT_5$. The hydrolytic and transport function of the HT-29 cells can be modified by changing the composition of the culture medium. For example, the expression of the protein of the BBM sucrase-isomaltase has been shown to be modulated by the presence of glucose in the culture medium.[25–27] Similarly, HT-29 cells cultured in late confluency in a glucose-free medium containing inosine exhibit enterocyte differentiation, with the presence of tight junctions and BBM.[19,28] Finally, the glucose concentration in the culture medium can regulate the expression of $SGLT_1$ in HT-29 cells through sugar metabolism.[29]

HT-29 cells grown under standard culture conditions have high activities of pyruvate kinase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase.[30] Fructose 2,6-diphosphate is an allosteric activator of phosphofructokinase-1, which likely plays an important role in the control of carbohydrate metabolism in these cells. Insulin receptors have been characterized in HT-29 cells and are efficient in stimulating cell proliferation[31] as well as in controlling the carbohydrate metabolism of these cells.[32] Galactose is poorly metabolized by enterocytes; galactose increases the doubling time of colonic carcinoma HT-29 cells[9] while inducing the expression of B-glucosidase, microvillus length, dipeptidylpeptidase-IV, alkaline phosphatase, and aminopeptidase N.[9,33]

There are distinct Cl^-- and mucin-secreting subclones of the HT-29 cells that allow assessment in differentiated and nondifferentiated cells of a number of enterocyte functions.[34] The mucin-secreting subclone HT-29-18N2 has provided a useful model system to examine the signal transduction pathways mediating mucin secretion from intestinal Goblet cells.[14] Cholinergic stimulation of these cells does not lead to exocytosis of mucin granules, whereas activation of protein kinase C by phorbol myristate acetate (a phorbol ester) increases mucin secretion by the exocytic pathway.[35] Similar findings have been observed in the T84 adenocarcinoma cell line.[36]

Caco-2 cells

Caco-2 cells undergo typical enterocyte differentiation, with the onset of differentiation occurring at confluence. This confluence is complete within 20 days, with the establishment of a polarized layer with tight junctions, microvilli, and BBM enzymes.[10,22,23] Structural proteins associated with the BBM of Caco-2 cells include villin, the 110 kD polypeptide, and the 140 kD glycoprotein,[37,38] together with hydrolases normally associated with the entero-

cyte BBM. These have been demonstrated by immunofluorescence, in synthetic labeling studies using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting, and by measurement of hydrolase activities in cellular extracts.[10,22,26,39,40]

The Caco-2 cell line is spontaneously differentiated, and the degree of differentiation may be varied by changing the duration of culture.[10] Caco-2 cells can be stimulated to become differentiated to mature villus-like enterocytes by adding forskolin to the culture medium, thereby increasing adenylate cyclase synthesis, enhancing cytosolic cAMP, up-regulating the activity of BBM sucrase-isomaltase (SI) and its mRNA, as well as enhancing the expression of the BBM fructose transporter protein, $GLUT_5$.[41,42] The forskolin-dependent decrease in SI expression results from a decrease in the rate of transcription of the gene,[43] and there is a close correlation between the sucrase activities and the SI mRNA levels.[6] The uptake of biotin is up-regulated by growing confluent Caco-2 cells in a biotin-deficient environment,[44] whereas glucose interferes with the expression of SI in Caco-2 cells at the mRNA level.

Lipid uptake and cell cultures

The intestinal uptake of fatty acids and cholesterol is thought to occur by passive diffusion.[1,45] Recent evidence suggests that a fatty acid binding protein in the BBM ($FABP_{pm}$) may contribute to uptake.[46] Also, an amiloride-inhibitable carrier-mediated step facilitates fatty acid uptake by the exchange of sodium (Na^+) for hydrogen (H^+). This provides for and maintains an acid microclimate adjacent to the BBM. This acidic microclimate enhances the release of fatty acids for the luminal bile salt micelles as well as increases their protonation and subsequent permeation of the BBM.[46] Autoradiographic[47] and cell fractionation studies[48] have suggested that lipid uptake is greater into the differentiated enterocytes on the upper portion of the villus than into the less differentiated cells near the intestinal crypts. Caco-2 cells have also been shown to synthesize apolipoproteins,[5] although it is unknown whether forskolin-stimulated differentiation alters apolipoprotein synthesis, lipoprotein secretion, or lipid uptake. In Caco-2 cells the transport of the bile acid cholytaurine across the apical BBM occurs by a Na^+-dependent process in a manner similar to isolated ileal enterocytes.[49]

Differentiated and undifferentiated HT-29 cells are a useful tool to assess the uptake of lipids and the effect of altering intracellular cAMP (Thomson et al., unpublished observations).

Sorting and recycling: Membrane lipid composition

The adaptation of lipid uptake occurs in a host of animal models, such as in streptozotocin-induced diabetes mellitus, after ileal resection, following abdominal irradiation, with chronic ingestion of ethanol, or with isocaloric changes of dietary lipids.[1] Variations in nutrient uptake are associated with changes in the type of phospholipids and their fatty acids in the enterocyte BBM.[50–54] These alterations in BBM lipids are associated with changes in the activity of key enterocyte microsomal lipid metabolizing enzymes, such as phosphatidylcholine transferase, phosphatidylethanolamine methyl transferase, and desaturases.[55–60] The use of cultured cells of varying degrees of cellular differentiation will permit the careful definition of the appropriate time-course studies to determine whether the activities of the enterocyte microsomal enzymes precede the alterations in BBM lipid composition, and whether altered levels of activity of these lipid metabolizing enzymes are associated with increased abundance of enzyme protein and of their respective mRNAs.

Numerous attempts have been made to correlate changes in phospholipid patterns with variations in the organization and functioning of cellular membranes.[61] The enterocyte BBM has a high sphingomyelin content, whereas the basolateral membrane (BLM) is enriched in phosphatidylcholine.[62–65] The relative rate of translocation (and not the synthetic step) is the most important factor controlling the phospholipid segregation between BBM and BLM in rat renal proximal tubular cells.[66] Similar studies have not yet been reported on for intestinal tissue. The phospholipid composition of the plasma membrane is a function of the state of cell differentiation. This suggests that the polarized distribution of the phospholipids between the exoplasmic and cytoplasmic leaflets of the antipodal domains in epithelial cells is altered in undifferentiated HT-29 cells but is restored in their enterocytic-differentiated counterparts. When comparing the differentiated versus the undifferentiated HT-29 plasma membranes, there is an increased sphingomyelin content, higher ratios of monounsaturated: polyunsaturated fatty acids in phosphatidylethanolamine, and concomitant decreases in phosphatidylserine and cardiolipin.

Sorting and recycling are well known phenomena in intracellular protein trafficking, occurring during biosynthesis and endocytosis.[67] The sorting of sphingolipids in the endocytic pathways has been studied in HT-29 cells and occurs only in undifferentiated cells.[68] There is also different expression and distribution of other cell surface molecules in undifferentiated as compared with differentiated HT-29 cells.[15,16,69–71] This difference between the two cell types suggests that lipid sorting phenomena are related to the state of cellular differentiation. Thus, these cell culture lines may be useful in determining whether BBM transporters are transferred directly from the Golgi to the BBM, or whether they are transferred indirectly via transcytosis to the BBM from the BLM as they establish their polarity during cell growth and differentiation.[72] Caco-2 cells use a mixture of the direct and transcytotic strategies for dipeptidyl peptidase IV and aminopeptidase N to reach their ultimate destinations. By contrast, the vast majority of newly synthesized sucrase-isomaltase is sorted intracellularly and is delivered directly to the BBM domain.[73]

Δ^9-desaturase is responsible for the conversion of saturated (16:0 and 18:0) to monounsaturated (16:1 and 18:1) fatty acids. Δ^6- and Δ^5-desaturases are mainly involved in the metabolism of essential fatty acids, i.e., 18:2(6) and 18:3(3):[74,75] Δ^6-desaturase converts 18:2(6) to 18:3(6) and is considered to be a rate-limiting step.[76,77] 18:3(3) is also desaturated by the same Δ^6-desaturase, and the presence of

18:3(3) in the diet has been shown to inhibit the desaturation of 18:2(6) and the subsequent formation of 20:4(6).[78] The intestinal mucosa possesses desaturase activities[55–60] and can synthesize significant amounts of 20:4(6) from 18:2(6). Feeding diets containing fish oil rich in eicosapentaenoic (20:5[3]) and docosahexaenoic (22:6[3]) acids inhibits Δ^5- and Δ^6-desaturase activities.[55–60] Dietary cholesterol supplementation also impairs the desaturation of 18:2(6) and decreases 20:4(6) levels in plasma and tissue lipids when fed in combination with saturated or omega-6 fatty acids.[79] Glucagon, epinephrine, glucocorticoids, and thyroxine depress while insulin increases the activity of Δ^6-desaturase.[80] Other physiologic changes such as aging and fasting, as well as some pathological processes (e.g., diabetes mellitus, chronic zinc deficiency, and chronic alcohol consumption) have also been associated with reduced Δ^6-desaturase activity (80).

Assessing degrees of differentiation

Forskolin, an activator of adenylate cyclase,[81] influences glycogenolysis and glucose consumption.[22] Treatment of Caco-2 cells with forskolin inhibits the migration of the complex fully glycosylated form of the hydrolases to the BBM.[22] The induction of modifications of glucose utilization (by means of glucose deprivation or by the action of drugs which act on glucose metabolism) may provide useful information on the potential role of glucose in the biosynthesis and glycosylation of the BBM hydrolases as well as information on the role of glucose metabolism on the control of transport.

Differentiated cells accumulate 10–20 times more villin than do undifferentiated cells, and the total amount of villin expressed in differentiated HT-29 cells is close to the value observed for normal, freshly isolated colonocytes.[38] mRNA for villin is more abundant in differentiated as compared with undifferentiated cells.[82] This suggests that during the course of differentiation, the villin gene is more actively transcribed, or that mRNA for villin is stabilized. Interestingly, sucrase-isomaltase expression is highly heterogeneous among differentiating Caco-2 cells, as suggested on the basis of in situ immunostaining patterns.[83] Further analysis of this phenomenon is consistent with the model where Caco-2 cells undergo structural and functional differentiation according to a transient mosaic pattern.[84]

Potential limitations to the use of cell cultures

Differentiated HT-29 and Caco-2 cells cannot be compared with the cells of normal colon since they develop the digestive, absorptive, and morphological characteristics of the enterocytes of the small intestine. On the other hand, these cell lines are not completely identical with enterocytes of the small intestine: for example, lactase is absent from HT-29 cells, maltase-glucoamylase is absent from both HT-29 and Caco-2 cell lines,[39,85] and the ion transport properties of HT-29[18] and Caco-2 cells[86,87] are different from those observed in the small intestine. In addition, the molecular form of sucrase-isomaltase from HT-29 and Caco-2 cells is different from that found in the adult small intestine but is similar to that observed in the fetal colon.[25,85,88]

Notwithstanding the differences between these cell lines and enterocytes from the small intestine, HT-29 and Caco-2 cells are useful for observing the onset of structural and molecular events leading to cell polarity and for studying the time course of changes in functional parameters of the BBM, such as digestion and absorption. These cell lines will prove to be useful tools for the study of intestinal adaptation. For example, in the intact intestine most uptake occurs in the more differentiated cells at the upper third of the villus, as compared with the less differentiated cells in the lower portion of the villus, or in the crypt cells.[47] The use of cell cultures will provide a unique opportunity to study the mechanisms of altered nutrient transport which occurs with variations in enterocyte differentiation.

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