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Gene Expression Profiles of Normal Proliferating and Differentiating Human Intestinal Epithelial Cells: A Comparison With the Caco-2 Cell Model

Eric Tremblay,[1,2] Joëlle Auclair,[1,2] Edgar Delvin,[1,3] Emile Levy,[1,3] Daniel Ménard,[1,2] Alexey V. Pshezhetsky,[3] Nathalie Rivard,[1,2] Ernest G. Seidman,[1,3] Daniel Sinnett,[4] Pierre H. Vachon,[1,2] and Jean-François Beaulieu[1,2]*

[1]CIHR Group on Functional Development and Physiopathology of the Digestive Tract, Sherbrooke, Québec, Canada
[2]Département d'anatomie et de biologie cellulaire, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada
[3]Service de gastroentérologie, Centre de Recherche, Hôpital Ste-Justine, Université de Montréal, Québec, Canada
[4]Division d'Hématologie-Oncologie, Centre de Recherche, Hôpital Ste-Justine, Université de Montréal, Québec, Canada

Abstract cDNA microarray technology enables detailed analysis of gene expression throughout complex processes such as differentiation. The aim of this study was to analyze the gene expression profile of normal human intestinal epithelial cells using cell models that recapitulate the crypt-villus axis of intestinal differentiation in comparison with the widely used Caco-2 cell model. cDNA microarrays (19,200 human genes) and a clustering algorithm were used to identify patterns of gene expression in the crypt-like proliferative HIEC and tsFHI cells, and villus epithelial cells as well as Caco-2/15 cells at two distinct stages of differentiation. Unsupervised hierarchical clustering analysis of global gene expression among the cell lines identified two branches: one for the HIEC cells versus a second comprised of two sub-groups: (a) the proliferative Caco-2 cells and (b) the differentiated Caco-2 cells and closely related villus epithelial cells. At the gene level, supervised hierarchical clustering with 272 differentially expressed genes revealed distinct expression patterns specific to each cell phenotype. We identified several upregulated genes that could lead to the identification of new regulatory pathways involved in cell differentiation and carcinogenesis. The combined use of microarray analysis and human intestinal cell models thus provides a powerful tool for establishing detailed gene expression profiles of proliferative to terminally differentiated intestinal cells. Furthermore, the molecular differences between the normal human intestinal cell models and Caco-2 cells clearly point out the strengths and limitations of this widely used experimental model for studying intestinal cell proliferation and differentiation. J. Cell. Biochem. 99: 1175–1186, 2006.

Key words: genomics; human intestine; growth and differentiation

The epithelium of the small intestine is continuously and rapidly renewed by a process involving cell generation and migration from the multipotential stem cells, housed in specific "niches" within the crypts, to the extrusion of the terminally differentiated cells at the tips of the villi, 3–5 days later [Leblond, 1981; Potten and Loeffler, 1990; Babyatsky and Podolsky,

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*Correspondence to: Jean-François Beaulieu, Département d'anatomie et de biologie cellulaire, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.
E-mail: Jean-Francois.Beaulieu@USherbrooke.ca

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1999]. Epithelial cell proliferation, migration, and differentiation must be tightly regulated by various mechanisms controlled by growth factors and cytokines as well as cell–cell and cell–matrix interactions [Podolsky, 1993; Gordon and Hermiston, 1994; Beaulieu, 1999; Montgomery et al., 1999]. Most of the current knowledge of human intestinal cell regulation has been derived from studies conducted on cell cultures generated from experimental animals and human colon cancers [Zweibaum and Chantret, 1989; Evans et al., 1994; Whitehead and Watson, 1997; Pageot et al., 2000]. Human cancer cell lines such as the Caco-2, which can undertake a spontaneous and complete intestinal-like program of differentiation [Pinto et al., 1983; Vachon and Beaulieu, 1992], have been useful to investigate intestinal cell function and regulation [Beaulieu and Quaroni, 1991; Jumarie and Malo, 1991; Levy et al., 1995; Vachon and Beaulieu, 1995]. However, theoretical limitations of this model lie in its cancerous nature as well as its colonic origin [Ménard and Beaulieu, 1994; Pageot et al., 2000].

In the last decade, new cell models have been established for the study of human intestinal cell functions without the limitations mentioned above. Among these are the non-transformed human intestinal epithelial crypt (HIEC) cells [Perreault and Beaulieu, 1996], a human intestinal cell line with typical crypt cell characteristics. Recent studies have shown the usefulness of the HIEC for the analysis of the regulation of cell proliferation and differentiation [Deschenes et al., 2001; Escaffit et al., 2005], the mechanism of cell survival [Francoeur et al., 2004; Harnois et al., 2004], the functional basis of cell matrix interactions [Desloges et al., 1998; Simoneau et al., 1998; Basora et al., 1999] as well as lipid and lipoprotein metabolism [Levy et al., 2000]. The tsFHI is another well-characterized intestinal cell model with crypt cell characteristics [Quaroni and Beaulieu, 1997; Tian and Quaroni, 1999]. Finally, a procedure based on the use of a non-enzymatic solution was described for the isolation of pure human villus epithelial (HVE) cell fractions isolated from the human small intestine [Perreault and Beaulieu, 1998a; Perreault et al., 1998b]. Taken together, the features of these models provide an in vitro recapitulation of the entire crypt-villus axis of the normal human intestine [Pageot et al., 2000].

Recently, cDNA microarray technologies have been used to characterize global changes in gene expression during intestinal epithelial differentiation [Mariadason et al., 2002; Fleet et al., 2003; Sugiyama et al., 2005]. Herein, we have analyzed the gene expression profiles of the HIEC, tsFHI, and HVE cell models in comparison with proliferative and fully differentiated Caco-2 cells in order to acquire additional knowledge of enterocyte cell state progression along the crypt-villus axis. We have identified 272 genes as being differentially expressed across all of the cell models, which by supervised clustering analysis, were found to fall into four distinct expression patterns correlating with the phenotype of each cell type. Pertaining to the Caco-2 cells, our results demonstrate that these cells share many characteristics with the normal differentiated villus cell population (HVE) when in their differentiated state but have little in common with normal crypt cells (HIEC and tsFHI) in their proliferative state.

MATERIALS AND METHODS

Tissues

Four specimens of small intestine (ileum) from four fetuses ranging from 16 to 20 weeks of age (postfertilization) were obtained after legal abortion. Only specimens obtained rapidly (<60 min) were used. The project was in accordance with a protocol approved by the Institutional Human Research Review Committee for the use of human material. Fetal small intestinal epithelia were separated from the mesenchyme using Matrisperse (Collaborative Biomedical Products, Becton Dickenson Labware, Mississauga, ON) as described previously [Perreault et al., 1998b] allowing for the isolation of pure HVE cells.

Cell Culture

Three epithelial cell lines, HIEC, tsFHI, and Caco-2/15, which together recapitulate the entire crypt-villus axis of the fetal intestine [Pageot et al., 2000], were used in this study. The HIEC cell line was generated from normal fetal human intestine [Perreault and Beaulieu, 1996]. These cells express a number of crypt cell markers but no villus cell markers [Perreault and Beaulieu, 1996; Desloges et al., 1998; Basora et al., 1999] and appear to be unable to differentiate. HIEC cells are thus considered to

be intestinal stem-like cells [Perreault and Beaulieu, 1996; Quaroni and Beaulieu, 1997; Perreault et al., 1998b; Pageot et al., 2000; Escaffit et al., 2005]. The HIEC cells (n = 4) were used at passages 14–17 and grown as described [Perreault and Beaulieu, 1996]. The tsFHI cell line was derived from fetal human intestinal epithelial cells conditionally immortalized with a temperature-sensitive large T antigen [Quaroni and Beaulieu, 1997] and is phenotypically similar to the HIEC cell line when grown under permissive conditions (32°C). The tsFHI cells (n = 4) were used at passages 14–22 and grown as described [Pinto et al., 1983]. Finally, the Caco-2/15 cell line, a stable clone of the parent Caco-2 cell line [Pinto et al., 1983], has been characterized elsewhere [Beaulieu and Quaroni, 1991; Vachon and Beaulieu, 1992]. Cells between passages 64 and 72 were cultured in plastic dishes as described [Vachon and Beaulieu, 1992] and analyzed at subconfluence (n = 4), and 25 days of postconfluence (n = 4).

Reference Pool

A reference pool was produced from a mixture of equimolar aliquots of RNA from the colon adenocarcinoma cell lines Caco-2/15 (at −1, 10, and 25 days of confluence) and HT29 [Basora et al., 1998], the lung cancer A549 cell line (obtained from Dr. Jacques Bérard, Département d'anatomie et de biologie cellulaire, Université de Sherbrooke) and the ovarian cancer SKOV3 [Lane et al., 2004] cell line (a kind gift of Dr. Claudine Rancourt, Département de microbiologie et d'infectiologie, Université de Sherbrooke). These cell lines were chosen to obtain a more complete representation of the genes spotted on the arrays.

RNA Extraction

RNA was extracted with TRIzol (Invitrogen) according to the manufacturer's protocol and stored at −80°C. Quality of RNA was verified on agarose gel and by spectrophotometric assay. Purification of mRNA from 100 μg of total RNA using Oligotex resin (Qiagen) following the manufacturer's directions yielded 2–4 μg poly(A)$^+$-enriched RNA which was aliquoted in 1 μg fractions and used for probe preparation.

Probe Preparation

First-strand cDNA synthesis from 1 μg of poly(A)$^+$-enriched RNA was primed with 6 μg random hexamers (Invitrogen) by heating at 70°C for 10 min, snap-cooling on ice for 30 s, and incubating at room temperature for an additional 5–10 min. Reverse transcription was performed in the presence of 500 μM dATP, dCTP, and dGTP, 300 μM 5-aminoallyl-dUTP (Sigma) and 200 μM dTTP, 1× first-strand buffer, 10 mM dithiothreitol, and 400 U Superscript II (Invitrogen) in a volume of 40 μl at 42°C for 3 h to overnight. Reactions were quenched by the addition of 10 μl of 0.5 M EDTA, and the RNA template was hydrolyzed by the addition of 10 μl of 1M NaOH followed by heating at 65°C for 10 min. Reactions were neutralized with 10 μl of 1M HCl, and the cDNA was purified on QIAquick columns (Qiagen) according to the manufacturer's directions except for the substitution of 75% ethanol for the PE buffer, and phosphate elution buffer (4 mM potassium phosphate, pH 8.5) for the EB buffer. The cDNA was lyophilized and resuspended in 8 μl of 0.1 M sodium carbonate, pH 9.0. An aliquot of the NHS ester of Cy3 or Cy5 (Amersham Pharmacia) was resuspended in 2 μL of DMSO (dye from each tube had been previously dissolved in 72 μl DMSO, divided into 4.5 μL aliquots and lyophilized), added to the reactions and incubated at room temperature in the dark for 1 h. For all microarray experiments, the reference pool was labeled with Cy3 dye, while test samples were labeled with Cy5 dye. Coupling reactions were quenched by the addition of 35 μl of 0.1M sodium acetate, pH 5.2, and unincorporated dye was removed using QIAquick columns. The labeling efficiency was determined by analyzing the whole undiluted sample in a spectrophotometer using a 50 μL microcuvette (Beckman).

Hybridization and Image Processing

cDNA microarrays were obtained from the University Health Network of Toronto, Ontario (Canada). A total of 40 slides (4 independent biological samples of each of the five cell models: HIEC, tsFHI, proliferative and differentiated Caco-2 and HVE; 2 slides per biological sample), composed of two different slide sets, SS-H19k6 and SS-H19k7, representing 19,200 human cDNA clones were used for the experiments. Slides were prehybridized in 0.1% BSA, 5× SSC, 0.1% SDS for 45 min, washed by dipping in MilliQ water twice and 2-propanol once, and air-dried. Fluorescent cDNA probes were lyophilized and resuspended in 24 μl of hybridization buffer (50% formamide, 5× SSC, 0.1% SDS). To

the combined Cy3 and Cy5 samples, 20 μg Cot1 DNA and 20 μg poly(A)$^+$ DNA were added and the samples were denatured at 95°C for 5 min, followed by snap cooling on ice for 1 min. Room-temperature probes were applied to a prehybridized array, covered with another slide rather than a glass coverslip, and placed in a humidified hybridization chamber (Corning). Hybridizations were carried out at 42°C for 16–20 h, followed by 5 min washings in: 1× SSC, 0.2% SDS at 42°C, 0.1× SSC, 0.2% SDS at room temperature, and 0.1× SSC at room temperature, twice. Arrays were scanned using a ScanArray Express dual-color confocal laser scanner (Perkin Elmer). Data were collected in Cy3 and Cy5 channels and stored as paired TIFF images.

Data Analysis

Spots were identified and local background subtracted using the TIGR_Spotfinder 2.2 software [Saeed et al., 2003]. A quality control (QC) filter was used to remove questionable array features. Two criteria for spot rejection were a spot shape that deviated from a circle and a low signal-to-noise ratio. Hybridization intensity data were normalized using iterative mean-$\log_2$(ratio)-centering (data range for mean centering ±3 SD) and Lowess procedures (smoothing parameter was set to 33%) using the native Java function of the TIGR MIDAS 4.0 software (Microarray Data Analysis System) [Saeed et al., 2003]. Statistical significance was assessed by one-way ANOVA and an adjusted Bonferroni correction was applied for controlling the false-positive rate ($P < 0.0005$ was considered significant), and hierarchical clustering analysis was performed using the TMEV 3.0 software (TIGR_MultiExperiment Viewer) [Saeed et al., 2003]. All software are available at The Institute for Genomic Research (TIGR) website, http://www.tigr.org/.

Data Validation by Quantitative RT-PCR

Several genes showing various patterns of expression in the microarray analysis were re-examined by real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to validate the changes observed in an independent manner. We selected six genes that showed significant variation among the cell models [caveolin 1 (CAV1), v-ets erythroblastosis virus E26 oncogene homolog 2 (ETS2), interleukin enhancer binding factor 3 (ILF3), ras suppressor protein 1 (RSU1), transforming growth factor beta-induced (TGFBI), and tissue inhibitor or metalloproteinase 3 (TIMP3)] and also randomly selected 14 non-differentially expressed genes [cyclin D1 (CCDN1), v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS1), fibroblast growth factor receptor 4 (FGFR4), frizzled homolog 8 (FZD8), homeodomain-only protein (HOP), inhibitor of DNA binding 2 (ID2), mannosidase α, class 1B, member 1 (MAN1B1), 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), mutL homolog 1 (MutL), MAX interacting protein 1 (Mxi1), proliferating cell nuclear antigen (PCNA), protein tyrosine kinase 2 beta (PTK2B), transporter 1 ATP-binding cassette sub-family B (TAP1), and transferrin receptor (TFRC)] to be investigated on three independent biological samples. We used MAN1B1 as a normalizing gene [Dydensborg et al., 2006] and sucrase-isomaltase (SI) as a reference for intestinal differentiation. We performed a statistical analysis of each test gene (independent t-test, $P < 0.05$) to compare the microarray and real-time qPCR data. First, cDNA was prepared from each cell line and tissue using random primers and Superscript II (Invitrogen). Primers for each gene (Supplementary Table 2) were designed at exon-exon junctions using the Primer3 [Rozen and Skaletsky, 2000] software. Each PCR was carried out in triplicate on each biological sample in an Mx3000P (Stratagene, Cedar Creek, TX) as previously described [Escaffit et al., 2005]. Briefly, each PCR reaction contained 1 μL of RT product and 0.15 μM of each primer in the Brilliant SYBR Green QPCR Master Mix (Stratagene). Following a 95°C denaturation for 10 min, the reactions were cycled 40 times at 95°C for 30 s, 55-60°C (depending on the primer set) for 1 min, and 72°C for 1 min. To verify that only the specific product was amplified, a melting point analysis was performed after the last cycle by cooling the samples to 55°C and then increasing the temperature to 95°C at 0.2°C/s. A single product at a specific melting temperature was found for each gene target. Specific amplification was also confirmed by electrophoresis of the PCR products on agarose gel. Results for each sample were expressed in fold of variation versus reference pool after correction by the amplification efficiency of each PCR reaction and normalization with mannosidase α, class 1B (MAN1B1).

RESULTS

Cluster Analysis of Cell Lines

To identify genes associated with human intestinal cell proliferation and/or differentiation, we used the two normal intestinal cell models HIEC (proliferative and undifferentiated) and HVE (non-proliferative and fully differentiated) as well as Caco-2 cells under both proliferative and differentiated states to compare their global gene expression profiles. The tsFHI cell model was excluded from the statistical analysis in order to eliminate the effect of the SV40 large T antigen. From the 19,200 genes present on the array, 11,222 had sufficient data for comparison (genes present three times out of four tested samples were included in the analysis). The one-way ANOVA test with adjusted Bonferroni correction ($P < 0.0005$) was applied to the data set and 272 genes (2.4% of the total number of filtered genes analyzed) were found to be differentially expressed across all cell lines, including 34 genes with no Unigene match (Supplementary Table I). First, the global gene expression profiles of all cell samples were examined by an unsupervised hierarchical clustering algorithm (average linkage clustering) irrespective of the cell phenotype in order to determine the degree of similitude between each cell model. The most notable property of the cell model-dendogram was that each biological sample was clustered into the independent terminal branch specific to its respective cell type (Fig. 1A). Classification of all samples revealed two major branches (A and B): the first major branch contained exclusively the normal undifferentiated epithelial cell line (HIEC) while the second was separated into two secondary branches, containing the proliferative Caco-2 (SC) in one sub-branch and the two very closely related differentiated Caco-2 (25PC) and HVE cells in the other. This classification, based on global gene expression patterns, demonstrates that undifferentiated Caco-2 cells are significantly distinct from the proliferative crypt-like HIEC cells, being in fact

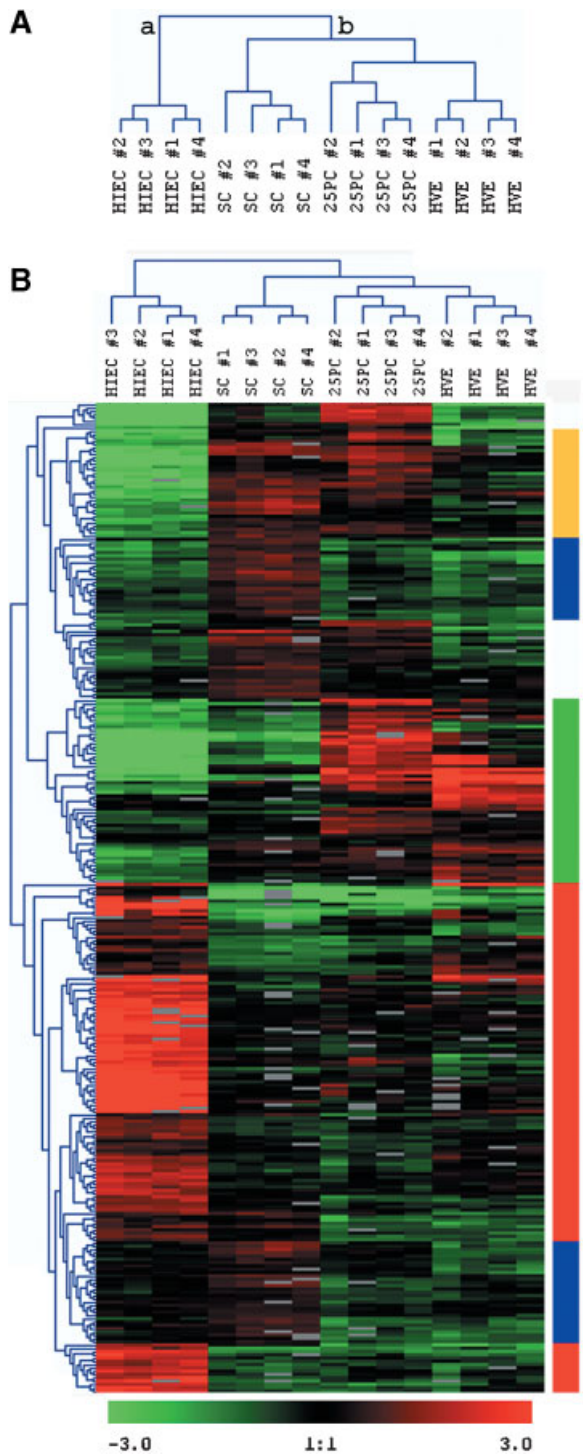

Fig. 1. Profiles of gene expression in human intestinal cells. **A**: Unsupervised hierarchical clustering of the cell samples based on mRNA expression levels. Samples were classified into two major clusters (A and B). **B**: Supervised hierarchical clustering of the different cell models using the 272 differentially expressed genes (adjusted Bonferroni correction $P < 0.0005$). Microarray profile showing four clusters of genes with distinct expression patterns between each cell type. Each row represents a gene and each column represents an independent biological sample. For each gene, a green signal represents under-expression, a black signal denotes similarly expressed genes, a red signal represents over-expressed genes, and a gray signal denotes missing data. Dendograms of samples (above matrix) and genes (to the left of matrix) represent overall similarities in gene expression profiles. Colored bars to the right indicate the locations of the four gene clusters of interest.

more related to differentiated Caco-2 cells and HVE cells. Moreover, the high similarity found between differentiated Caco-2 cells and the HVE indicated that at this stage, postconfluent Caco-2 cells appear to be relatively well representative of normal differentiated enterocytes.

Then, a supervised hierarchical clustering analysis was performed using the 272 differentially expressed genes and expression patterns characteristic to the four cell models were distinguished (Fig. 1b). For example, two clusters of 102 genes were highly upregulated in the HIEC (red clusters in Fig. 1b). This set of genes included those involved in the regulation of cell proliferation (AF1Q, CDKN1A, ELK3, ETS2, PDGFA, etc.), intracellular signaling (PTPRN2, RASA1, RSU1, TGFBI), and cell adhesion and extracellular matrix interactions (CD44, COL6A3, CLECSF2, SRPX). These are summarized in Figure 2 (panel Ai–iv). A second set of clusters containing approximately 60 genes, upregulated exclusively in the proliferative Caco-2 cells, was characterized (blue clusters in Fig. 1b). These clusters included genes known to regulate cell proliferation, DNA replication and transcription (such as BCCIP, CSE1L, E2F6, MCMs, MYC, SKB1), and RNA processing (DCP2, DDX21, SSB), suggesting different regulatory machinery for proliferating Caco-2 cells (Fig. 2Bi–iv) as compared with the normal proliferating intestinal cells. A third cluster of ~50 genes (green cluster in Fig. 1b) was found to be strongly expressed in differentiated cells. A large number of genes within this cluster (Fig. 2, panel C) were involved in lipoprotein biology (APOB, APOC3) and transport (AFP, TTR). The fourth cluster, including ~33 genes (yellow cluster in Fig. 1b) contained genes upregulated in both the proliferative and differentiated Caco-2 cells. Within this cluster we found genes

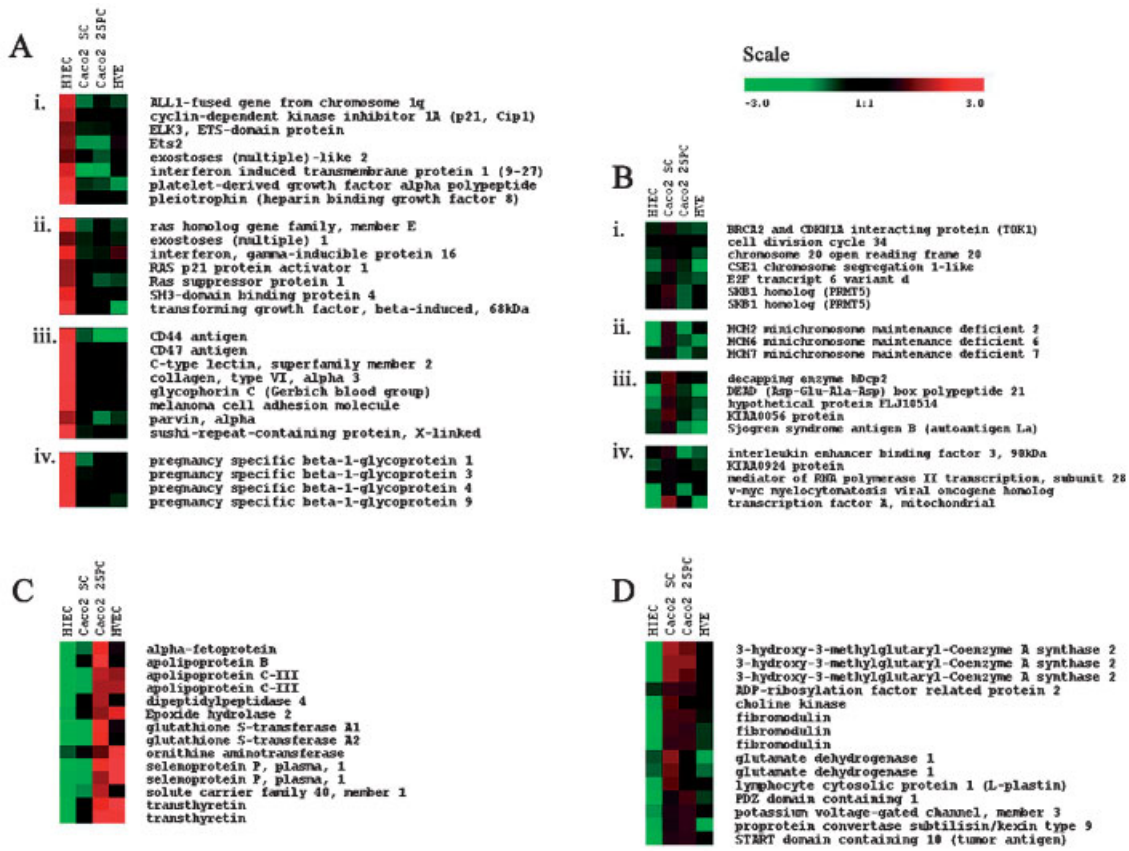

Fig. 2. Cluster analysis of gene expression in human intestinal cells. Expression profiles of upregulated genes in HIEC (**A**), proliferative Caco-2 (**B**), differentiated cells (**C**), or cancer cells (**D**). A: **i**: cell proliferation; **ii**, signal transduction; **iii**, cell adhesion and extracellular matrix; iv, defense and immunity activity; B: **i**, cell proliferation and apoptosis; **ii**, DNA replication; **iii**, RNA processing; **iv**, transcription; C: genes involved in lipid biology, response to oxidative stress and transport; D: genes upregulated in colon cancer or involved in cholesterol biosynthesis and TGFβ signaling. Values shown are the mean of four independent biological samples.

(Fig. 2, panel D) known to be upregulated in colon cancer (CHK, LCP1, PDZK1), involved in the TGFβ pathway (FMOD) and in the regulation of cholesterol levels (PDZK1, PCSK9).

Validation of Microarray Results

We examined 20 genes (6 differentially (Table I) and 14 non-differentially (Table II) expressed) by qRT-PCR to compare the changes observed in the microarray analysis. The differentially expressed genes examined in each cell model showed a relative change in expression which was not statistically different from that found in the array data (Table I). Further analysis of the data using the correlation coefficient (r^2) confirmed this validation (Fig. 4). The lower concordance observed with the HIEC (r^2:0.65) could be explained by the higher ratio measured for CAV1 in qRT-PCR probably due to the higher sensitivity of this technique compared to microarray analysis.

TABLE I. Relative Levels of Differentially Expressed Genes in Cell Models Versus Reference Pool, as Determined by Microarray Hybridization and Validation by Real Time Reverse Transcription-Polymerase Chain Reaction

Gene	Cell line	Microarray (mean ± SEM)	RT-PCR (mean ± SEM)
CAV1	HIEC	3.78 ± 0.53	8.20 ± 0.43
	Caco-2 SC	0.09 ± 0.12	0.05 ± 0.002
	Caco-2 25PC	0.10 ± 0.40	0.03 ± 0.003
	HVE	0.18 ± 0.52	0.06 ± 0.02
ETS2	HIEC	2.64 ± 0.32	1.88 ± 0.65
	Caco-2 SC	0.46 ± 0.06	0.35 ± 0.03
	Caco-2 25PC	0.44 ± 0.10	0.26 ± 0.08
	HVE	1.63 ± 0.81	2.58 ± 0.69
ILF3	HIEC	0.99 ± 0.14	0.70 ± 0.49
	Caco-2 SC	1.85 ± 0.22	2.09 ± 1.02
	Caco-2 25PC	0.45 ± 0.05	0.30 ± 0.10
	HVE	0.64 ± 0.19	0.81 ± 0.58
RSU1	HIEC	2.87 ± 0.19	2.29 ± 0.45
	Caco-2 SC	1.23 ± 0.05	1.21 ± 0.16
	Caco-2 25PC	0.56 ± 0.08	0.46 ± 0.11
	HVE	0.58 ± 0.24	0.44 ± 0.10
TGFBI	HIEC	6.69 ± 1.79	3.95 ± 2.57
	Caco-2 SC	1.10 ± 0.04	1.37 ± 0.39
	Caco-2 25PC	0.77 ± 0.03	0.91 ± 0.14
	HVE	0.32 ± 0.10	0.13 ± 0.09
TIMP3	HIEC	4.90 ± 0.19	3.07 ± 0.56
	Caco-2 SC	1.23 ± 0.10	1.59 ± 0.20
	Caco-2 25PC	2.13 ± 0.19	1.94 ± 0.25
	HVE	0.42 ± 0.11	0.21 ± 0.06[a]
SI	HIEC	n/a	n.d.
	Caco-2 SC	n/a	0.03 ± 0.005
	Caco-2 25PC	n/a	2.50 ± 1.60
	HVE	n/a	32.14 ± 6.45

SI, sucrase-isomaltase (used as reference for intestinal differentiation); n/a, not applicable; n.d., not detected.
Values shown are the mean of three independent biological samples.
[a]Statistically significantly different (*t*-test, $P < 0.05$).

TABLE II. Relative Levels of Randomly Selected Genes in Cell Models Versus Reference Pool, as Determined by Microarray Hybridization and Validation by Real Time Reverse Transcription-Polymerase Chain Reaction

Gene	Cell line	Microarray (mean ± SEM)	RT-PCR (mean ± SEM)
CCND1	HIEC	2.97 ± 0.26	8.37 ± 0.78
	Caco-2 SC	1.05 ± 0.02	1.77 ± 0.20
	Caco-2 25PC	1.16 ± 0.20	1.39 ± 0.02
	HVE	0.94 ± 0.01	0.78 ± 0.08
ETS1	HIEC	2.83 ± 0.07	4.31 ± 1.20
	Caco-2 SC	0.60 ± 0.31	0.03 ± 0.01
	Caco-2 25PC	0.84 ± 0.46	0.01 ± 0.001
	HVE	1.15 ± 0.14	2.31 ± 0.95
FGFR4	HIEC	0.63 ± 0.03	0.10 ± 0.003[a]
	Caco-2 SC	1.78 ± 0.50	2.22 ± 0.19
	Caco-2 25PC	1.17 ± 0.12	1.04 ± 0.19
	HVE	2.39 ± 0.29	1.24 ± 0.22
FZD8	HIEC	3.09 ± 0.13	5.98 ± 1.18
	Caco-2 SC	0.61 ± 0.14	0.16 ± 0.02[a]
	Caco-2 25PC	0.63 ± 0.33	0.02 ± 0.003[a]
	HVE	1.12 ± 0.24	0.06 ± 0.01[a]
HOP	HIEC	2.62 ± 0.22	33.7 ± 5.27[a]
	Caco-2 SC	1.04 ± 0.08	0.02 ± 0.003[a]
	Caco-2 25PC	1.67 ± 0.49	0.004 ± 0.0005[a]
	HVE	1.69 ± 0.07	95.6 ± 26.3[a]
ID2	HIEC	0.93 ± 0.21	0.16 ± 0.07[a]
	Caco-2 SC	1.07 ± 0.08	0.98 ± 0.08
	Caco-2 25PC	1.71 ± 0.27	1.70 ± 0.30
	HVE	1.91 ± 0.36	2.51 ± 1.19
MAN1B1	HIEC	1.29 ± 0.09	1.20 ± 0.17
	Caco-2 SC	0.88 ± 0.13	0.99 ± 0.15
	Caco-2 25PC	1.25 ± 0.28	0.84 ± 0.01[a]
	HVE	1.00 ± 0.20	0.63 ± 0.06
MTR	HIEC	1.04 ± 0.09	0.92 ± 0.13
	Caco-2 SC	1.07 ± 0.04	1.10 ± 0.15
	Caco-2 25PC	1.03 ± 0.21	0.41 ± 0.11[a]
	HVE	0.94 ± 0.11	1.12 ± 0.16
MutL	HIEC	1.57 ± 0.09	1.32 ± 0.08
	Caco-2 SC	1.60 ± 0.09	2.29 ± 0.25[a]
	Caco-2 25PC	0.62 ± 0.18	0.69 ± 0.04
	HVE	0.65 ± 0.32	1.07 ± 0.08
Mxi1	HIEC	0.29 ± 0.22	0.12 ± 0.02[a]
	Caco-2 SC	0.50 ± 0.19	0.27 ± 0.06
	Caco-2 25PC	1.46 ± 0.20	1.82 ± 0.37
	HVE	0.61 ± 0.42	0.56 ± 0.04
PCNA	HIEC	0.55 ± 0.32	0.39 ± 0.09
	Caco-2 SC	1.19 ± 0.13	1.60 ± 0.30
	Caco-2 25PC	0.29 ± 0.15	0.21 ± 0.04
	HVE	0.35 ± 0.33	0.32 ± 0.02
PTK2B	HIEC	0.77 ± 0.14	0.11 ± 0.01[a]
	Caco-2 SC	1.03 ± 0.04	0.73 ± 0.03[a]
	Caco-2 25PC	1.10 ± 0.13	1.75 ± 0.24
	HVE	1.61 ± 0.07	8.44 ± 1.05[a]
TAP1	HIEC	2.55 ± 0.21	2.94 ± 0.33
	Caco-2 SC	0.67 ± 0.23	0.04 ± 0.002[a]
	Caco-2 25PC	0.44 ± 0.27	0.15 ± 0.02[a]
	HVE	0.91 ± 0.26	1.12 ± 0.06
TFRC	HIEC	0.52 ± 0.35	0.07 ± 0.02[a]
	Caco-2 SC	0.84 ± 0.09	0.84 ± 0.03
	Caco-2 25PC	2.66 ± 0.19	2.41 ± 0.07
	HVE	0.50 ± 0.34	0.15 ± 0.04

Values shown are the mean of three independent biological sample.
[a]Statistically significantly different (*t*-test, $P < 0.05$).

Indeed, microarray analysis provides a maximum of three orders of magnitude of dynamic range between non-specific hybridization noise and the highest intensity gene-specific signal

compared to six to seven orders of magnitude for qRT-PCR. Among the non-differentially expressed genes, the observed differences in magnitude of gene expression in qRT-PCR were overall both quantitatively and statistically in agreement with the cDNA array (Table II: 78.6%, 11/14). Indeed, of the 14 genes tested, only the homeodomain-only protein (HOP) gene showed a significant discrepancy between the microarray and qRT-PCR data. In fact, the microarray data clearly underestimated the change in HOP gene expression, suggesting a lack of specificity in the primer designed to discriminate between gene family members or splice variants. Two other genes, FZD8 and PTK2B, also showed a lower concordance between microarray and qRT-PCR analysis. For the FZD8 gene, we observed a higher microarray expression ratio probably due to non-specific hybridization noise while the higher qRT-PCR expression ratio for PTK2B observed in HVE was likely the result of the sensitivity of the PCR technique compared to microarray analysis. Sucrase-isomaltase (SI) was used as a reference for the intestinal cell differentiation level in the four models.

Genes Enriched or Depleted in Undifferentiated Cell Lines

To further analyze the gene expression profile common to the undifferentiated intestinal cell types, we performed analyses of HIEC, tsFHI, and proliferative Caco-2 cells. For this qualitative experiment, genes with expression levels of more or less than twofold compared to the reference pool were arbitrarily defined as being enriched or depleted, respectively. Figure 3 shows the Venn diagrams of the overlapping genes enriched (Fig. 3A) or depleted (Fig. 3B) in each cell line. Of the 19,200 genes present on the arrays, 16,593 had sufficient data for this gene analysis. We found 901 genes to be enriched in the HIEC, 854 in the tsFHI, and 125 in the proliferative Caco-2 cells. Surprisingly, only two genes were found to be common to the three cell lines, six to the HIEC and Caco-2, and eight to the tsFHI and Caco-2. However, we did find a quite high overlap (31.2%) between the HIEC- and the tsFHI-enriched genes emphasizing a similar origin for these two normal undifferentiated cell lines. For the depleted gene analysis, we observed a significant number of depleted genes for each cell line (HIEC: 500, tsFHI: 624, and Caco-2: 227). The number of genes common to all cell lines increased to 66. Examination of gene function revealed that an important proportion of these common genes were involved in metabolism and transport. Unsurprisingly, the proportion of genes common (36.7%) to the HIEC and tsFHI was also high for these two normal crypt cell lines.

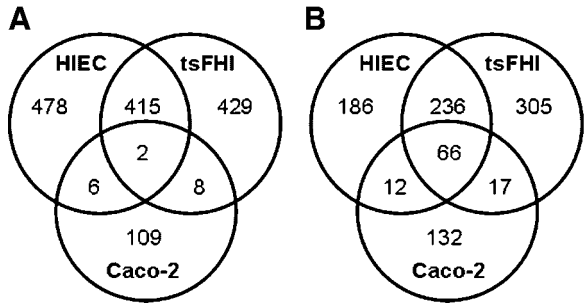

Fig. 3. Genes enriched or depleted in undifferentiated cell lines. **A**: Intersection between HIEC-, tsFHI-, and proliferative Caco-2-enriched genes. Numbers represent highly expressed genes (ratio $> +2$:1 relative to the reference pool) in each cell line. **B**: Intersection between HIEC-, tsFHI-, and proliferative Caco-2-depleted genes. Numbers represent poorly expressed genes (ratio < -2:1 relative to the reference pool) in each cell line.

DISCUSSION

Microarray technology and clustering algorithms are powerful methods to explore global gene expression. The goal of the present study was to exploit these techniques to analyze the gene expression profiles of different cell models recapitulating the crypt-villus axis of the human small intestine [Pageot et al., 2000] and compare them with the Caco-2 model. Our strategy was to generate high quality microarray data, use a hierarchical clustering algorithm to identify groups of genes reflecting various molecular features of each cell type and then validate the data by measuring the relative gene expression ratios by qRT-PCR on a subset of test genes and determine the statistical significance. In general, there was excellent agreement between our microarray and qRT-PCR data, both qualitatively and quantitatively.

The unsupervised hierarchical clustering tree that was generated clearly showed that the HIEC cell line has little in common with either the proliferative or differentiated Caco-2, or with the HVE cells. At first sight, albeit their

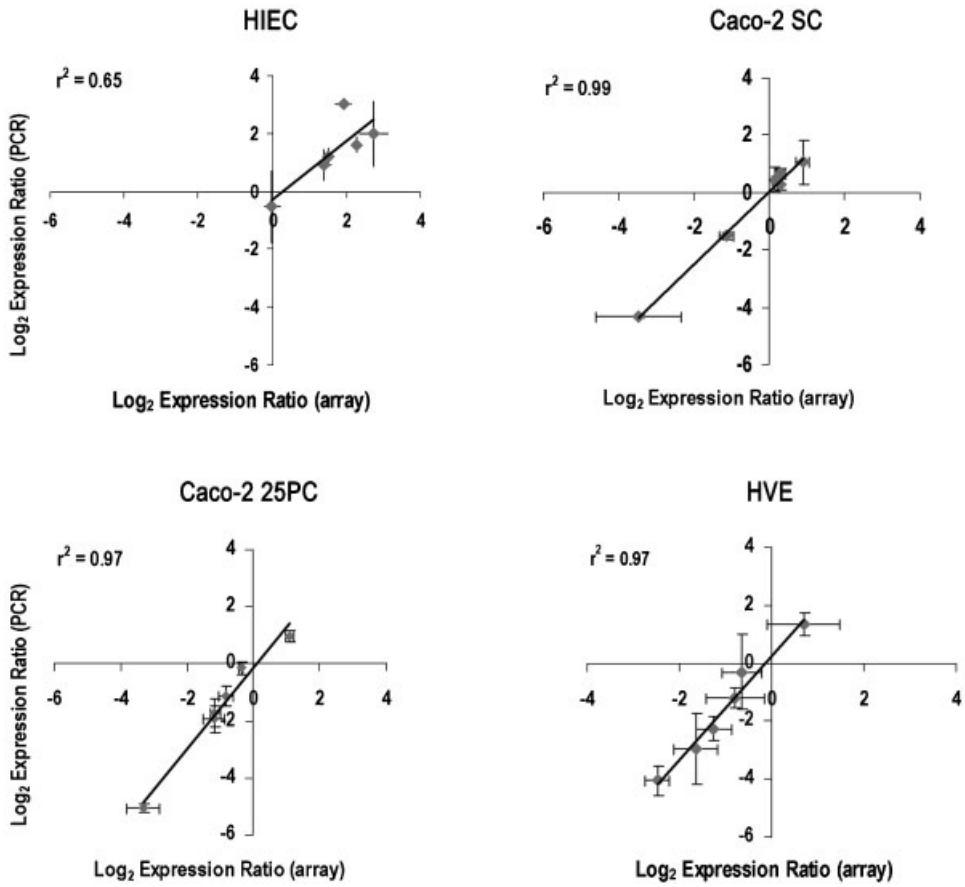

Fig. 4. A comparison of log_2 gene expression ratios measured by microarray analysis (X-axis) versus qRT-PCR (Y-axis). The data are derived from the results of Table I, shown here as scatter plots. r^2 values for HIEC, Caco2 SC, Caco2 25PC, and HVE were 0.65, 0.99, 0.97, and 0.97, respectively.

cancerous nature [Zweibaum and Chantret, 1989; Ménard and Beaulieu, 1994], we may have expected to find the proliferative Caco-2 cells to be more closely related to their normal proliferating counterparts, the HIEC cells. However, on the contrary, the gene expression profile of the proliferating Caco-2 cells was found to be more interrelated with the differentiated Caco-2 and villus epithelial cells. At the gene level, we have identified four clusters corresponding to the different cell phenotypes of our cell models. One of the clusters contains a large number of genes that are upregulated exclusively in HIEC cells. Many of these genes are involved in cell growth, cell signaling, and immunity but very few transporter and metabolic enzymes are represented, reflecting the crypt-like status of the HIEC cells. Interestingly, several pregnancy-specific beta-1 glycoproteins (PSG) were found to be upregulated in the HIEC. PSG transcripts have already been identified in the human intestine [Shupert and Chan, 1993]. These genes belong to the CEA family and are involved in intercellular cell adhesion [Benchimol et al., 1989] and immune defense [Hammarstrom and Baranov, 2001]. Incidentally, recent studies have reported that interferon-gamma modulates the expression of different CEA family members in colonic mucosa [Fahlgren et al., 2003]. In this context, our observations that molecules involved in interferon signaling (such as interferon gamma-inducible 16 (IFI16) and interferon-induced transmembrane protein 1 (IFITM1)) are expressed at high levels in HIEC cells are well supportive of previous data indicating that this pathway is active in crypt cells [Francoeur et al., 2004]. In addition to the CEA members we noted an upregulation of CD44 in HIEC compared to Caco-2 cells. CD44 has been shown to be normally expressed in the lower crypt epithelium of the intestinal mucosa [Wielenga et al., 1999; Lakshman et al., 2005]. Interestingly, the phenotype observed in Tcf-4 knockout mice

included both the loss of CD44 and the absence of a proliferative stem cell compartment in the crypt region [Wielenga et al., 1999] suggesting a potential stem-like cell phenotype for the undifferentiated intestinal epithelial cells HIEC.

In the second cluster we found genes exclusively enriched in proliferative Caco-2 cells. Interestingly, we identified several upregulated genes involved in cell-cycle and/or apoptosis suggesting a particular molecular regulatory pathway in cell proliferation. Among these genes was CSE1L/CAS, known to be implicated in the regulation of proliferation and apoptosis [Behrens et al., 2003], in addition to being over-expressed in several cancers including colon cancer [Brinkmann, 1998; Seiden-Long et al., 2005]. We also identified MCM 2, 6, and 7 proteins that are known substrates of cell-cycle kinases [Cortez et al., 2004] involved in the initiation and elongation of replication. In addition to the above-mentioned genes, two p21-interacting proteins, BCCIP (BCRA2 and CDKN1A interacting protein, also known as TOK1), and SKB1 were also identified. Recent reports have demonstrated that these two genes interact directly with p21 to modulate mitosis [Gilbreth et al., 1998; Ono et al., 2000]. Finally, one member of the MYC family (v-myc) was also identified in this group. MYC is an important regulator of intestinal epithelial cells [Mariadason et al., 2005; Ni et al., 2005]. By linking our list of differentially expressed genes to the MYC target gene database (www.myccancer.org), we identified 40 genes among the 272 differentially expressed genes that are known targets of the MYC transcription factor. Taken together, these results suggest that proliferation in Caco-2 cells is regulated by a particular group of genes involved either in the progression of cell-cycle or apoptosis.

Unsurprisingly, within the third cluster we identified several upregulated genes that are found mainly in differentiated Caco-2 as well as in villus epithelial cells. Among them, we noted genes involved in lipoprotein metabolism (such as APOB, APOC3), in selective transport/exchange (such as ABCC2, CLCN5, SEPP1, TTR), or in different metabolic pathways (such as ASAH1, FMO5, OAT, SULT1A1). Of note, we did not observe an upregulation of genes involved in apoptosis in differentiated epithelial cells. These observations are in agreement with previous reports [Watson and Pritchard, 2000; Mariadason et al., 2005] mentioning that apoptotic cells are rarely seen in the villus epithelial cells of the normal intestine, and also suggests that mechanisms other than apoptosis could be responsible for cell shedding at the villus tip of the small intestine.

Within the last cluster were the genes upregulated almost exclusively in proliferative and differentiated Caco-2 cells. We identified some genes known to be over-expressed in colorectal cancer, such as choline kinase (CK) and L-plastin (LCP1). Over-expression of CK is a frequent feature of human colorectal cancer [Nakagami et al., 1999; Ramirez de Molina et al., 2002], while the expression of LCP1 has been shown to correlate with the progression of cancer staging [Otsuka et al., 2001].

We next established transcriptional profiles for the undifferentiated intestinal cell models to identify genes enriched (increase of twofold) or depleted (decrease of twofold) in each cell population and then compared these sets of genes to one another. We observed a great overlap between both enriched and depleted genes in HIEC and tsFHI. Indeed, a third of both the enriched and depleted genes in the HIEC cells were shared with the tsFHI cells, an observation consistent with the crypt-like status of both of these cell lines [Perreault and Beaulieu, 1996; Quaroni and Beaulieu, 1997; Pageot et al., 2000; Escaffit et al., 2005]. However, we found that proliferative Caco-2 cells have a weak similarity to the two crypt-like cell lines sharing only 16 genes with HIEC and tsFHI, confirming that proliferative Caco-2 cells have little in common with normal proliferative intestinal epithelial cells.

In conclusion, gene expression profiles from these various well-characterized cell models have identified known and unknown genes that participate in different cell processes such as proliferation, differentiation, apoptosis, and immune defense. While further studies of the delineated genes are required to improve our understanding of the molecular mechanisms involved in the maintenance of human small intestinal function, we have successfully demonstrated major molecular distinctions between proliferating Caco-2 cells and the more normal human intestinal cell models HIEC and tsFHI while good similarities were displayed between postconfluent Caco-2 cells and normal human differentiated villus cells.

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REFERENCES

Babyatsky M, Podolsky D. 1999. Growth and development of the gastrointestinal tract. In: Yamada T, editor. Textbook of gastroenterology. Philadelphia: J.B. Lippincott. pp 547–584.

Basora N, Desloges N, Chang Q, Bouatrouss Y, Gosselin J, Poisson J, Sheppard D, Beaulieu JF. 1998. Expression of the alpha9beta1 integrin in human colonic epithelial cells: Resurgence of the fetal phenotype in a subset of colon cancers and adenocarcinoma cell lines. Int J Cancer 75:738–743.

Basora N, Herring-Gillam FE, Boudreau F, Perreault N, Pageot LP, Simoneau M, Bouatrouss Y, Beaulieu JF. 1999. Expression of functionally distinct variants of the beta(4)A integrin subunit in relation to the differentiation state in human intestinal cells. J Biol Chem 274: 29819–29825.

Beaulieu JF. 1999. Integrins and human intestinal cell functions. Front Biosci 4:D310–D321.

Beaulieu JF, Quaroni A. 1991. Clonal analysis of sucrase-isomaltase expression in the human colon adenocarcinoma Caco-2 cells. Biochem J 280(Pt 3):599–608.

Behrens P, Brinkmann U, Wellmann A. 2003. CSE1L/CAS: Its role in proliferation and apoptosis. Apoptosis 8:39–44.

Benchimol S, Fuks A, Jothy S, Beauchemin N, Shirota K, Stanners CP. 1989. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell 57:327–334.

Brinkmann U. 1998. CAS, the human homologue of the yeast chromosome-segregation gene CSE1, in proliferation, apoptosis, and cancer. Am J Hum Genet 62:509–513.

Cortez D, Glick G, Elledge SJ. 2004. Minichromosome maintenance proteins are direct targets of the ATM and ATR checkpoint kinases. Proc Natl Acad Sci USA 101: 10078–10083.

Deschenes C, Vezina A, Beaulieu JF, Rivard N. 2001. Role of p27(Kip1) in human intestinal cell differentiation. Gastroenterology 120:423–438.

Desloges N, Basora N, Perreault N, Bouatrouss Y, Sheppard D, Beaulieu JF. 1998. Regulated expression of the integrin alpha9beta1 in the epithelium of the developing human gut and in intestinal cell lines: Relation with cell proliferation. J Cell Biochem 71:536–545.

Dydensborg AB, Herring E, Auclair J, Tremblay E, Beaulieu JF. 2006. Normalizing genes for quantitative RT-PCR in differentiating human intestinal epithelial cells and adenocarcinomas of the colon. Am J Physiol Gastrointest Liver Physiol 290:G1067–G1074.

Escaffit F, Perreault N, Jean D, Francoeur C, Herring E, Rancourt C, Rivard N, Vachon PH, Pare F, Boucher MP, Auclair J, Beaulieu JF. 2005. Repressed E-cadherin expression in the lower crypt of human small intestine: A cell marker of functional relevance. Exp Cell Res 302: 206–220.

Evans GS, Flint N, Potten CS. 1994. Primary cultures for studies of cell regulation and physiology in intestinal epithelium. Annu Rev Physiol 56:399–417.

Fahlgren A, Baranov V, Frangsmyr L, Zoubir F, Hammarstrom ML, Hammarstrom S. 2003. Interferon-gamma tempers the expression of carcinoembryonic antigen family molecules in human colon cells: A possible role in innate mucosal defence. Scand J Immunol 58:628–641.

Fleet JC, Wang L, Vitek O, Craig BA, Edenberg HJ. 2003. Gene expression profiling of Caco-2 BBe cells suggests a role for specific signaling pathways during intestinal differentiation. Physiol Genomics 13:57–68.

Francoeur C, Escaffit F, Vachon PH, Beaulieu JF. 2004. Proinflammatory cytokines TNF-alpha and IFN-gamma alter laminin expression under an apoptosis-independent mechanism in human intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 287:G592–G598.

Gilbreth M, Yang P, Bartholomeusz G, Pimental RA, Kansra S, Gadiraju R, Marcus S. 1998. Negative regulation of mitosis in fission yeast by the shk1 interacting protein skb1 and its human homolog, Skb1Hs. Proc Natl Acad Sci USA 95:14781–14786.

Gordon JI, Hermiston ML. 1994. Differentiation and self-renewal in the mouse gastrointestinal epithelium. Curr Opin Cell Biol 6:795–803.

Hammarstrom S, Baranov V. 2001. Is there a role for CEA in innate immunity in the colon? Trends Microbiol 9: 119–125.

Harnois C, Demers MC, Bouchard V, Vallee K, Gagne D, Fujita N, Tsuruo T, Vezina A, Beaulieu JF, Cote A, Vachon PH. 2004. Human intestinal epithelial crypt cell survival and death: Complex modulation of Bcl-2 homologs by Fak, PI3-K/Akt-1, MEK/Erk, and p38 signaling pathways. J Cell Physiol 198:209–222.

Jumarie C, Malo C. 1991. Caco-2 cells cultured in serum-free medium as a model for the study of enterocytic differentiation in vitro. J Cell Physiol 149:24–33.

Lakshman M, Subramaniam V, Wong S, Jothy S. 2005. CD44 promotes resistance to apoptosis in murine colonic epithelium. J Cell Physiol 203:583–588.

Lane D, Cartier A, L'Esperance S, Cote M, Rancourt C, Piche A. 2004. Differential induction of apoptosis by tumor necrosis factor-related apoptosis-inducing ligand in human ovarian carcinoma cells. Gynecol Oncol 93: 594–604.

Leblond CP. 1981. The life history of cells in renewing systems. Am J Anat 160:114–158.

Levy E, Mehran M, Seidman E. 1995. Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion. FASEB J 9:626–635.

Levy E, Beaulieu JF, Delvin E, Seidman E, Yotov W, Basque JR, Menard D. 2000. Human crypt intestinal epithelial cells are capable of lipid production, apolipoprotein synthesis, and lipoprotein assembly. J Lipid Res 41:12–22.

Ménard D, Beaulieu J. 1994. Human intestinal brush border membrane hydrolases. In: Bkaily G, editor. Membrane physiopathology. Norwell, MA: Kluwer. pp 319–341.

Mariadason JM, Arango D, Corner GA, Aranes MJ, Hotchkiss KA, Yang W, Augenlicht LH. 2002. A gene expression profile that defines colon cell maturation in vitro. Cancer Res 62:4791–4804.

Mariadason JM, Nicholas C, L'Italien KE, Zhuang M, Smartt HJ, Heerdt BG, Yang W, Corner GA, Wilson AJ, Klampfer L, Arango D, Augenlicht LH. 2005. Gene expression profiling of intestinal epithelial cell maturation along the crypt-villus axis. Gastroenterology 128: 1081–1088.

Montgomery RK, Mulberg AE, Grand RJ. 1999. Development of the human gastrointestinal tract: Twenty years of progress. Gastroenterology 116:702–731.

Nakagami K, Uchida T, Ohwada S, Koibuchi Y, Suda Y, Sekine T, Morishita Y. 1999. Increased choline kinase activity and elevated phosphocholine levels in human colon cancer. Jpn J Cancer Res 90:419–424.

Ni H, Dydensborg AB, Herring FE, Basora N, Gagne D, Vachon PH, Beaulieu JF. 2005. Upregulation of a functional form of the beta4 integrin subunit in colorectal cancers correlates with c-Myc expression. Oncogene 24: 6820–6829.

Ono T, Kitaura H, Ugai H, Murata T, Yokoyama KK, Iguchi-Ariga SM, Ariga H. 2000. TOK-1, a novel p21Cip1-binding protein that cooperatively enhances p21-dependent inhibitory activity toward CDK2 kinase. J Biol Chem 275:31145–31154.

Otsuka M, Kato M, Yoshikawa T, Chen H, Brown EJ, Masuho Y, Omata M, Seki N. 2001. Differential expression of the L-plastin gene in human colorectal cancer progression and metastasis. Biochem Biophys Res Commun 289:876–881.

Pageot LP, Perreault N, Basora N, Francoeur C, Magny P, Beaulieu JF. 2000. Human cell models to study small intestinal functions: Recapitulation of the crypt-villus axis. Microsc Res Tech 49:394–406.

Perreault N, Beaulieu JF. 1996. Use of the dissociating enzyme thermolysin to generate viable human normal intestinal epithelial cell cultures. Exp Cell Res 224:354–364.

Perreault N, Beaulieu JF. 1998a. Primary cultures of fully differentiated and pure human intestinal epithelial cells. Exp Cell Res 245:34–42.

Perreault N, Herring-Gillam FE, Desloges N, Belanger I, Pageot LP, Beaulieu JF. 1998b. Epithelial vs. mesenchymal contribution to the extracellular matrix in the human intestine. Biochem Biophys Res Commun 248: 121–126.

Pinto M, Robine-Leon S, Appay M, Kedinger M, Triadou N, Dussaulx E, Lacroix B, Simon-Assmann P, Haffen K, Fogh J, Zweibaum A. 1983. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Biol Cell 47:323–330.

Podolsky DK. 1993. Regulation of intestinal epithelial proliferation: A few answers, many questions. Am J Physiol 264:G179–G186.

Potten CS, Loeffler M. 1990. Stem cells: Attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development 110:1001–1020.

Quaroni A, Beaulieu JF. 1997. Cell dynamics and differentiation of conditionally immortalized human intestinal epithelial cells. Gastroenterology 113:1198–1213.

Ramirez de Molina A, Rodriguez-Gonzalez A, Gutierrez R, Martinez-Pineiro L, Sanchez J, Bonilla F, Rosell R, Lacal J. 2002. Overexpression of choline kinase is a frequent feature in human tumor-derived cell lines and in lung, prostate, and colorectal human cancers. Biochem Biophys Res Commun 296:580–583.

Rozen S, Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386.

Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich E, Borisovsky I, Liu Z, Vinsavich A, Trush V, Quackenbush J. 2003. TM4: A free, open-source system for microarray data management and analysis. Biotechniques 34:374–378.

Seiden-Long IM, Brown KR, Shih W, Wigle DA, Radulovich N, Jurisica I, Tsao MS. 2005. Transcriptional targets of hepatocyte growth factor signaling and Ki-ras oncogene activation in colorectal cancer. Oncogene (advanced online publication).

Shupert WL, Chan WY. 1993. Pregnancy specific beta 1-glycoprotein in human intestine. Mol Cell Biochem 120: 159–170.

Simoneau A, Herring-Gillam FE, Vachon PH, Perreault N, Basora N, Bouatrouss Y, Pageot LP, Zhou J, Beaulieu JF. 1998. Identification, distribution, and tissular origin of the alpha5(IV) and alpha6(IV) collagen chains in the developing human intestine. Dev Dyn 212:437–447.

Sugiyama Y, Farrow B, Murillo C, Li J, Watanabe H, Sugiyama K, Evers BM. 2005. Analysis of differential gene expression patterns in colon cancer and cancer stroma using microdissected tissues. Gastroenterology 128:480–486.

Tian JQ, Quaroni A. 1999. Involvement of p21(WAF1/Cip1) and p27(Kip1) in intestinal epithelial cell differentiation. Am J Physiol 276:C1245–C1258.

Vachon PH, Beaulieu JF. 1992. Transient mosaic patterns of morphological and functional differentiation in the Caco-2 cell line. Gastroenterology 103:414–423.

Vachon PH, Beaulieu JF. 1995. Extracellular heterotrimeric laminin promotes differentiation in human enterocytes. Am J Physiol 268:G857–G867.

Watson AJ, Pritchard DM. 2000. Lessons from genetically engineered animal models. VII. Apoptosis in intestinal epithelium: Lessons from transgenic and knockout mice. Am J Physiol Gastrointest Liver Physiol 278:G1–5.

Whitehead R, Watson N. 1997. Gastrointestinal cell lines as a model for differentiation. In: Halter FWD, Wright NA, editors. The gut as a model in cell and molecular biology. Dordrecht: Kluwer. pp 275–290.

Wielenga VJ, Smits R, Korinek V, Smit L, Kielman M, Fodde R, Clevers H, Pals ST. 1999. Expression of CD44 in Apc and Tcf mutant mice implies regulation by the WNT pathway. Am J Pathol 154:515–523.

Zweibaum A, Chantret I. 1989. Human colon carcinoma cell lines as in vitro models for the study of intestinal cell differentiation. In: Smith MW, Sepulveda FV, editors. Adaptation and Development of the Intestinal Functions. Manchester: Manchester University Press. pp 103–112.