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Laboratory of Comparative Genomics

Comparative Analyses of Villus and Crypt Small Intestinal Cell Gene Expression Profiles

K. N. Kuzmuk, L. A. Rund, K. S. Swanson, L. B. Schook

Department of Animal Sciences, University of Illinois, Urbana

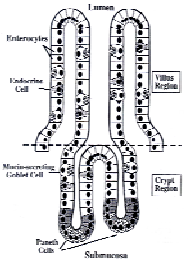
http://www.ansci.uiuc.edu/labs/schook

Abstract

The objective of this study was to compare gene expression profiles of villus and crypt intestinal cell populations within and between species. Laser capture microdissection (LCM) was used to isolate individual villus and crypt epithelial cells from swine, canine, and murine ileal samples. RNA was isolated and amplified using the PicoPure™ RNA Isolation Kit and RiboAmp® RNA Amplification Kit (Arcturus), respectively. Gene expression profiles were generated by hybridizing amplified RNA from a 12 wk-old C57Bl/6 mouse with the NIA 15K Mouse cDNA microarray, amplified RNA from a white crossbred sow with the 13K Porcine Oligo Array (Qiagen) and amplified RNA from a 1 yr-old beagle dog with the Affymetrix Human U133A microarray. Preliminary results show that >1000 genes were more highly expressed in the crypt epithelial cells than in villus cells. This list includes many genes related to apoptosis, cell cycle, DNA replication, and energy/metabolism. Genes (13%) more highly expressed in villus than crypt were associated with matrix or structural proteins. The use of LCM provides a cell-specific gene expression profile of distinct intestinal cell populations. While villus cells are composed of differentiated cell populations possessing specific functions, crypt cells are primarily composed of undifferentiated stem cells. Research in this area may identify factors associated with cellular differentiation and lead to the development of therapies for intestinal disease and may be used as a screening tool for gene targets associated with growth promotion. Supported by Pyxis Genomics, Inc. and the Critical Research Initiative (CRI) at the University of Illinois.

Introduction

- Intestinal villi are composed of differentiated cell populations possessing specific functions, whereas crypts are primarily composed of undifferentiated stem cells.
- Research comparing villus and crypt epithelial cells may identify factors associated with cellular differentiation and lead to the development of therapies for intestinal disease.
- Microarrays provide a method for measuring the interaction of thousands of genes simultaneously, providing a global vision of gene expression. Gene expression profiling may be used to investigate the effects of specific nutrients or therapies and provides an approach for establishing biomarkers for health assessment.
- Laser Capture Microdissection (LCM) isolates a pure population of intestinal epithelial cells from a heterogenous tissue. In combination with microarrays, LCM can be used to generate cell-specific gene expression profiles without contamination from unwanted cell populations.



Objectives

- Isolate individual villus and crypt epithelial cells from swine, canine, and murine ileal samples
- Compare gene expression profiles of villus and crypt intestinal cell populations within and between species

Materials and Methods

Sample collection and preparation:

- Intestinal samples collected from: 1) white crossbred sow; 2) 12 wk-old C57Bl/6 mouse; and 3) 1 yr-old beagle dog
- Cryopreserved frozen samples cut to 8 μm thickness and placed on slide
- To preserve RNA, samples stained and dehydrated using Histogene™ LCM Frozen Section Staining Kit (Arcturus)

Laser capture microdissection:

- PixCell® IIe LCM Instrument (Arcturus) used to harvest 200 to 400 crypt and villus epithelial cells
- Steps for collecting tissue (adapted from Arcturus; www.arctur.com):


A. Locate target on slide


B. Set slide position


C. Set LCM cap position

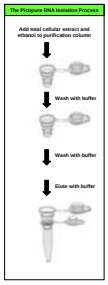

D. Harvest cells using laser

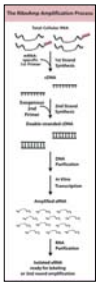

E. Remove cap with adhered target cells

Laser settings used:
- Spot size: 7.5 μm
- Power: 25-30 mW
- Duration: 2 ms

RNA isolation and amplification:

- RNA was isolated using the PicoPure™ RNA isolation kit (Arcturus)
- RNA amplified using RiboAmp® RNA amplification kit (Arcturus)
- For oligonucleotide microarrays (used with pig and dog), ENZO® BioArray™ High Yield™ RNA Transcript Labeling Kit (T7) was used to perform RNA transcript labeling.





Microarray slides/chips:

Mouse: NIA 15K Mouse cDNA microarray

Pig: 13K Porcine Oligo Array (Qiagen)

Dog: Affymetrix Human U133A microarray

- Genes considered differentially expressed if 2-fold greater than other tissue

Results

Crypt and villus cells successfully harvested from murine (Fig. 1), swine (Fig. 2), and canine (Fig. 3) intestinal tissue using laser capture microdissection.

Figure 1. Laser capture of murine ileal crypt cells

Figure 2. Laser capture of swine ileal crypt cells

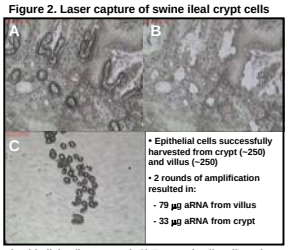


Figure 3. Laser capture of canine ileal villus cells

Figures 1-3: A) Initial tissue sample; B) Tissue after targeted epithelial cells removed; C) Targeted cells adhered to cap; D) Crypt epithelial cells hybridized with NIA 15K Mouse cDNA microarray.

Table 1. Genes Differentially Expressed in Crypt Over Villus

Function	Number	% of total
Apoptosis	6	0.6
Cell Cycle	15	1.5
DNA Replication	4	0.4
Energy/ Metabolism	41	4.0
Heat Shock/ Stress	10	1.0
Matrix/Structural Proteins	47	4.6
Protein Synthesis	51	5.0
Signal Transduction	36	3.5
Transcription/ Chromatin	23	2.3
Total	1022	

Table 2. Genes Differentially Expressed in Villus Over Crypt

Function	Number	% of total
Apoptosis	2	0.5
Cell Cycle	1	0.3
DNA Replication	1	0.3
Energy/ Metabolism	13	3.3
Heat Shock/ Stress	1	0.3
Matrix/Structural Proteins	51	13.0
Protein Synthesis	6	1.5
Signal Transduction	37	9.4
Transcription/ Chromatin	9	2.3
Total	393	

Table 3. Functional Characteristics of Genes Differentially Expressed in Crypt

Gene	Abbr.	Functional Category	Description	Implications
Cytochrome c, somatic	Cycs	Metabolism	• Electron transporter activity • Caspase activation via cytochrome c	Only water-soluble component of e⁻ transfer chain; responsive to DNA damage and cell stress, being released from mitochondria, activating caspases and inducing apoptosis; anticancer therapy targeting the apoptotic pathway.
Programmed cell death 4	Pdcd 4	Apoptosis	• DNA topoisomerase activity • Isomerase activity	Inhibits neoplastic transformation, playing role as a tumor suppressor; loss of expression is a prognostic factor in many cancers and is correlated with tumor progression.
Cyclin D1	Ccnd1	Cell Cycle	• Protein binding and kinase activity • Amino acid phosphroylation • Cell cycle regulation	Major role in controlling G1 phase of cell cycle; inhibition delays/prevents S phase of cell cycle; elevated expression found in esophageal, gastric, small intestinal, and large intestinal tumors.
Heat shock 70kD protein 5	Hspa5	Heat Shock; Stress response	• ATP, ribosome, and protein binding • Response to ER overload • Heat shock protein activity	Member of superfamily of molecular chaperones that stabilize proteins and polypeptides in cells; minimize protein misfolding and aggregation; present under normal conditions and induced by environmental stressors; shown to be a chaperone for tumor-derived peptides and is a candidate for personalized cancer vaccines.
Ubiquitin-conjugating enzyme	Ube2c	Protein Synthesis; Translational Control	• Ubiquitin-protein ligase activity • Positive regulation of cell proliferation • Ubiquitin-dependent protein catabolism	Ubiquitination is key in dislocation of proteins from ER to cytosol for degradation through ubiquitin-proteasome pathway; one of 3 enzymes that catalyzes ubiquitination reaction, playing role in several biological processes; ubiquitin ligases are over-expressed in several cancers and are targets for cancer therapy.
Adenylate kinase 2	Ak2	Signal Transduction	• Adenylate kinase activity • ATP binding • Phosphotransferase activity, phosphate group as acceptor	Important role in energy metabolism; catalyzes reaction: ATP + AMP ↔ 2 ADP; regulates cellular adenine and guanine nucleotide pools; transfers high-energy phosphates from sites of ATP production to those of ATP utilization; during apoptosis, is released from mitochondria and accumulates in cytosol along with cytochrome c; up-regulation is signal of apoptosis.

Table 4. Functional Characteristics of Genes Differentially Expressed in Villus

Gene	Abbr.	Functional Category	Description	Implications
Phosphodiesterase 8a	Pdea8a	Matrix/ Structural Proteins	• Catalytic activity • Signal Transduction	High affinity for cyclic nucleotides (cAMP), regulating their activity as protein kinases.
Lactotransferrin	Ltf	Matrix/ Structural Proteins	• Iron ion transport/ homeostasis	Iron binding protein with role in its transport; possesses antimicrobial activity.
Glutathione peroxidase 3	Gpx3	Energy/ Metabolism	• Catalysis of redox reactions • Response to oxidative stress	Member of family of selenoproteins that catalyze inactivation of reactive oxygen and nitrogen species that cause oxidative damage to membrane lipids, DNA, and proteins; redox status crucial for cellular viability; oxidative stress involved with cell signalling and gene regulation and is thought to be a major contributor of cancer.
Glutathione S-transferase	Gstm1	Energy/ Metabolism	• Transferase activity	Member of superfamily that catalyze conjugation of glutathione with toxic compounds targeted for excretion; polymorphisms shown to increase susceptibility to colon cancer.
Transforming growth factor, beta 2	Tgfb2	Signal Transduction	• Regulation of cell cycle • Growth • Extracellular organization and biogenesis	One of 3 isoforms of TGF-β; this form key in regulating cellular differentiation and growth; because of its dual role as tumor suppressor and oncogene, it may be pro- or anti-carcinogenic; mutation in TGF-β2 receptor associated with colorectal polyps and gastric tumors.
Insulin-like growth factor binding protein 5	Igfbp2	Signal Transduction	• Regulation of cell growth • Growth factor binding	IGF crucial in normal growth and development, regulating cell growth, survival and differentiation; IGF also crucial in development and progression of cancers; IGF binding proteins control IGF action; IGFBP-5 affects proliferation, migration, and sensitivity to apoptosis and may influence colon cancer incidence.

Conclusions

- Undifferentiated crypt cells had more differentially expressed genes (1022) than differentiated villus cells (393).
- Functional categories associated with protein synthesis, matrix/structural proteins, energy/metabolism, and signal transduction had several genes differentially expressed in crypt cells.
- Several genes associated with matrix/structural proteins, signal transduction, and energy/metabolism were differentially expressed in villus cells.
- Numerous genes important in the growth, differentiation, and maintenance of gastrointestinal tissue were identified and may be the focus of future projects.
- Future experiments in this area may further our understanding of cellular differentiation and may be used for the development of therapies designed to prevent or treat gastrointestinal diseases and cancers.

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