Differential expression in histologically normal crypts of ulcerative colitis suggests primary crypt disorder

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Abstract. Ulcerative colitis is characterized by crypt infiltration particularly of neutrophils. However, it is not known whether it reflects a primary crypt disorder or a secondary inflammatory response. In this study, we analyzed the expression profiles of histologically normal crypts microdissected from formalin-fixed biopsies of early stage ulcerative colitis. Total RNAs were extracted, amplified, and applied to Affymetrix GeneChip® X3P Array. For the control, similar crypts from nonspecific colitis biopsies were applied. A total of 353 (4.3%) and 111 (1.4%) genes were >3 times up-, and down-regulated in ulcerative colitis. Up-regulated genes included *FCGBP* (Fc fragment of IgG binding protein), cyclophilin A, chemokine (C-X-C motif) ligand 3, and genes associated with lipid metabolism. Down-regulated genes included *APOA4* (apolipoprotein A-IV), cylindromatosis, BCL2-like 10, claudin 8, and numerous transcriptional regulators. *FCGBP* and *APOA4* have been implicated in ulcerative colitis previously. Our data show differential expression in the crypt epithelia of ulcerative colitis before active inflammation is initiated, suggesting primary crypt abnormalities that might be implicated in the pathogenesis of ulcerative colitis.

Introduction

Ulcerative colitis is a chronic inflammatory bowel disease of unknown pathogenesis (1). Diverse genetic backgrounds and aberrant immune regulations have been associated with ulcerative colitis (2). Ulcerative colitis consists of mucosal inflammation with characteristic crypt abscesses (3-8). The infiltration of neutrophils has been taken as a parameter of disease activity (9-12).

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In ulcerative colitis, neutrophils appear to infiltrate specifically into the crypts, suggesting that they might target the crypt epithelium. It is particularly evident in early stage biopsies, often showing scattered crypt infiltrates in otherwise unremarkable mucosa. The crypt targeted infiltration is consistent with previous studies of impaired crypt epithelial barriers in ulcerative colitis (13,14). However, it is not clear whether the barrier disruption is a primary disorder or secondary to inflammation (15). Inflammatory cytokines have been shown to down-regulate tight junction proteins secondarily (16,17). Thus, a critical question remains whether the crypt epithelia have pre-existing abnormalities before active infiltration and/or regenerative changes occur.

Expression profiling analyses have reported candidate genes which might be associated with ulcerative colitis (18-25). However, DNA microarray data using whole tissue samples of gastrointestinal tracts is difficult to interpret because of considerable noise from various inflammatory infiltration, stromal reaction, and regenerative epithelial change (26). Recently, a few studies of expression profiling or pattern analysis of isolated inflammatory cells have been reported (27,28). However, no crypt-specific expression profiling has been reported in ulcerative colitis.

Colonoscopic biopsies are routinely fixed in formalin and processed for histopathologic examination. Formalin-fixation provides excellent preparations for the recognition of subtle pathologic changes of crypt epithelium. Furthermore, it would make retrospective studies possible using remnant paraffin blocks in pathology files. However, it causes extensive base modification of nucleic acids (29), which makes it difficult to recover RNAs for expression profiling. The emerging technology has improved considerably the expression profiling of formalin-fixed tissue samples (30,31). Recently, we reported a simple and reproducible procedure of RNA extraction and amplification which successfully detected sequential expression changes in the gastric adenoma/carcinoma sequence (32).

In this study, we analyzed the expression profiles of histopathologically normal crypts microdissected from formalin-fixed, paraffin-embedded biopsies of ulcerative colitis, and a comparison was made with the controls of similar normal crypts in non-specific colitis. Our data show considerable differential expression suggesting that aberrant crypt regulation is implicated in the pathogenesis of ulcerative colitis.

Materials and methods

Patients and biopsies. Three biopsies of ulcerative colitis were selected following guidelines of the Institutional Review Board of Asan Medical Center, Seoul, Korea. They were from a 34-year-old female and 46-, and 55-year-old male patients who were either first diagnosed as having ulcerative colitis or examined for recurrent clinical symptoms. Clinical and laboratory diagnostic criteria for ulcerative colitis were met in all cases as follows (33): i) a typical history of diarrhea or blood and pus, or both, in the stool for longer than 4 weeks; ii) a typical colonoscopic picture with diffusely granular, friable, or ulcerated mucosa without rectal sparing or skip lesions. Biopsies were diagnostic of ulcerative colitis of early stage showing typical crypt abscesses in mild chronic inflammation. No crypt distortion or regenerative change was evident. For the control, 3 biopsies of nonspecific colitis showing mild inflammation of similar degree were selected. Biopsies were taken from 31-, 49-, and 53-year-old males who had abdominal discomfort and intermittent diarrhea, which did not meet the above criteria. No specific colonoscopic features of ulcerative colitis were present.

Microdissection of crypts. Biopses were fixed immediately in 10% buffered-formalin and routinely processed for paraffin-embedding. After pathological examinations, additional step sections of 8-μm thickness were prepared. To avoid RNase contamination, slides and instruments were autoclaved and all solutions were freshly made using DEPC-treated water. From each biopsy, 100 histologically normal crypts were microdissected using the AutoPix laser capture microscope system (Arcturus Bioscience, Inc., Mountain View, CA), as described previously (32). No inflammatory cells were included. To ensure the quality of RNA samples and minimize individual variations, microdissected samples of ulcerative colitis and controls were pooled respectively.

RNA extraction. Microdissected samples were suspended in 200 μl digestion buffer [2% SDS, 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA], and incubated at 70˚C for 1 h to relieve the formalin-induced modifications. Then, 3 μl proteinase K (30 μg/μl, Intron Biotech., Songnam, Korea) was added, and incubated again at 55˚C for 1 h. RNAs were extracted twice using TRIzol reagents (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The extracted RNAs were precipitated in isopropanol with 5 μg linear acrylamide (Ambion, Austin, TX), and the RNA pellets were resuspended in 10 μl nuclease-free water (Ambion). The quality of extracted RNAs was checked using denaturing agarose gel. The amount of extracted RNA samples and/or amplified RNA was measured using RiboGreen RNA quantitation kit (Molecular probes, Eugene, OR) according to the manufacturer's protocol. Each measurement was duplicated, and the average values were taken.

Affymetrix GeneChip analysis. Amplifications were performed using Affymetrix two-cycle cDNA synthesis kit (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. To synthesize the first-strand cDNA, 15 ng total RNA was incubated with T7-oligo(dT) primer and SuperScript II reverse transcriptase at 42˚C for 1 h. After incubating the mixture at 70˚C for 10 min, *E. coli* DNA polymerase I and RNase H were added and incubated at 16˚C for 2 h to produce the second-strand cDNAs. First-cycle cRNA synthesis was made by MEGAscript T7 kit (Ambion) by incubating the sample with reagents at 37˚C for 16 h. cRNAs were purified by GeneChip cleanup module (Affymetrix) according to manufacturer's instructions. Purified cRNAs were used for the second-cycle cDNA synthesis. After the incubation with random primer and SuperScript II at 42˚C for 1 h, RNase H was added to the mixture and incubated at 37˚C for 20 min additionally. *E. coli* DNA polymerase I was added and incubated at 16˚C for 2 h. Then, T4 DNA polymerase was added and incubated at 16˚C for an additional 10 min.

Purified cDNAs were used for the subsequent *in vitro* transcription (IVT) labeling reaction using GeneChip IVT labeling kit (Affymetrix) according to manufacturer's instruction. cRNAs were fragmented by incubation at 94˚C for 35 min. Fragmented cRNAs were hybridized to Affymetrix GeneChip® Human X3P Array at 45˚C for 16 h. Washing and staining the array was performed by GeneChip Fluidics 450 according to the manufacturer's instructions. Scanning of the array was done using Affymetrix GeneChip Scanner 3000. Expression signals were analyzed using Affymetrix GeneChip software MAS5.0 and Microsoft Excel (Microsoft Corp., Redmond, WA). The complete data set is publicly available in the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) through the accession number: GSE3801.

Immunohistochemistry. Five ulcerative colitis and control biopsies were immunostained as described previously (34). Immunohistochemical staining was done using a Benchmark Autostainer (Ventana Medical Systems Inc., Tucson, AZ), according to manufacturer's instructions. For the antigen retrieval, slides were heated at 100˚C for 30 min. Rabbit polyclonal anti-cyclophilin A (Santa Cruz Biotechnology, Santa Cruz, CA) was applied at the dilution of 1:100. The ABC complex was developed using diaminobenzidine as chromogen, and the slides were counterstained with hematoxylin.

Results

Histopathology and microdissection. Mucosal biopsies of ulcerative colitis showed typical pathologic features with scattered crypt abscesses with epithelial damage (Fig. 1A). However, adjacent uninvolved crypts did not show infiltration or regenerative change (Fig. 1B). In nonspecific colitis, no crypt abscess was present. There were minimal chronic inflammatory infiltration in lamina propria of both ulcerative colitis and nonspecific colitis control. Histopathologically normal crypts were microdissected from ulcerative colitis (Fig. 1C and D); they were indistinguishable from those of nonspecific colitis (Fig. 1E and F). No inflammatory cells were included in the microdissected crypts.

RNA extraction and amplification. From total 300 crypts of ulcerative colitis crypts and controls, 81 and 89 ng total RNAs were extracted, respectively, then 15 ng RNA was applied to the amplification as described in Materials and methods, yielding 7.4 and 6.9 μg of aRNA for ulcerative

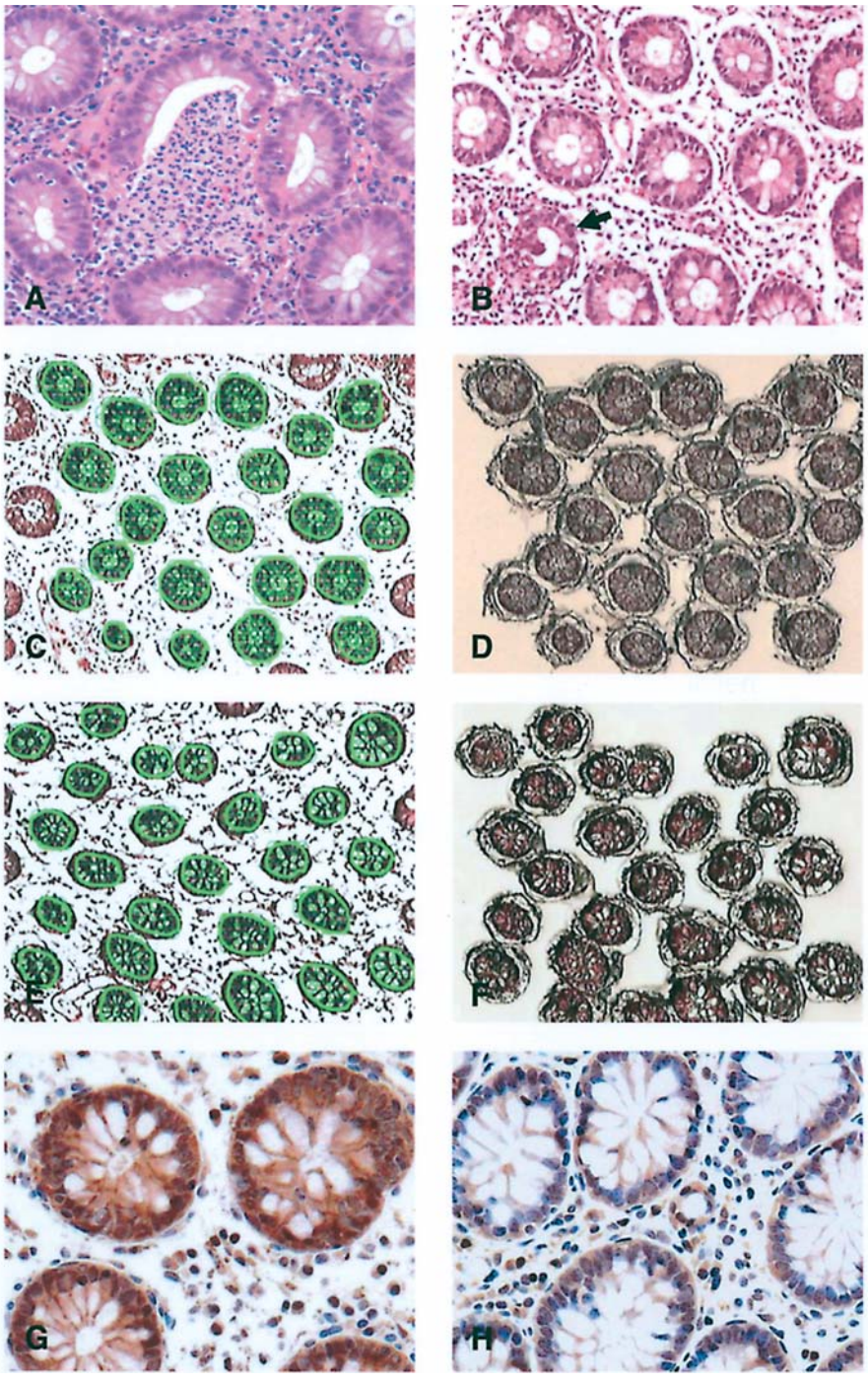

Figure 1. (A) Ulcerative colitis. A typical crypt abscess with dense inflammatory infiltration and epithelial damage is shown (H&E, x150). (B) Preparation for microdissection without cover slip. A small crypt abscess is recognized (arrow), but adjacent crypts are histologically normal without infiltration or reactive change (H&E, x100). (C) Histopathologically normal crypts in ulcerative colitis under the laser capture microscope. Crypts to be captured are marked by green circles (x50). (D) Microdissected crypts. Ruffles around crypts reflect capture membranes melted by laser beam (x50). (E) Crypts in nonspecific colitis before microdissection (x50). (F) Microdissected crypts from E, histopathologically indistinguishable from D. (G) Strong immunostaining for cyclophilin A in crypts of ulcerative colitis. Chronic inflammatory cells are similarly immunostained in G and H (ABC method, x200). (H) Nonspecific colitis showing mild immunostaining for cyclophilin A in crypt epithelium (x200).

colitis and control, respectively. For the expression profiling, labeled aRNAs were applied to Affymetrix GeneChip Human X3P Arrays.

DNA microarray analysis. Affymetrix GeneChip Human X3P Array was specifically designed for whole-genome expression profiling of formalin-fixed, paraffin-embedded samples. To detect amplified signals efficiently, the majority of probe sets on the arrays were selected from the 300 bases at the extreme 3'-end of the transcripts.

Among 61,359 gene spots in the microarray, 14,429 (23.5%) and 10,036 (16.4%) spots were hybridized significantly for ulcerative colitis and control, respectively (detection $p<0.05$). A total of 8,198 gene spots (13.4%) was hybridized significantly in both ulcerative colitis and controls. After sorting out redundant spots for a given gene, 353 (4.3%) and

Table I. Up-regulated genes in ulcerative colitis.

Rank	Symbol	Gene title	GenBank ID	Unigene ID	Ratio
1	XIST	X (inactive)-specific transcript	NR_001564	Hs.529901	55.525
2	CLCA1	Chloride channel, calcium activated, family member 1	NM_001285	Hs. 194659	18.931
3	-	EST	AK129631	Hs.433995	17.823
4	HIST1H4C	Histone 1, H4c	NM_003542	Hs.46423	11.709
5	FCGBP	Fc fragment of IgG binding protein	NM_003890	Hs.111732	10.798
6	ACTG1	Actin γ-1	NM_001614	Hs.514581	9.425
7	MS4A12	Membrane-spanning 4-domains, subfamily A, member 12	NM_017716	Hs.272789	9.365
8	SLC25A5	Solute carrier family 25, member 5	NM_001152	Hs.522767	9.015
9	CXCL3	Chemokine (C-X-C motif) ligand 3	NM_002090	Hs.89690	8.992
10	PDIA4	Protein disulfide isomerase family A, member 4	NM_004911	Hs.93659	8.284
11	CEACAM7	Carcinoembryonic antigen-related cell adhesion molecule 7	NM_006890	Hs.74466	7.754
12	IFITM3	Interferon induced transmembrane protein 3 (1-8U)	NM_021034	Hs.374650	7.475
13	PLAC8	Placenta-specific 8	NM_016619	Hs.546392	7.397
14	SNRPG	Small nuclear ribonucleoprotein polypeptide G	NM_003096	Hs.6454	7.333
15	IFI27	Interferon, α-inducible protein 27	NM_005532	Hs.532634	7.262
16	SFRS10	Splicing factor, arginine/serine-rich 10	NM_004593	Hs.533122	7.246
17	PSMA7	Proteasome (prosome, macropain) subunit, α type, 7	NM_002792	Hs.233952	7.075
18	RHOT1	Ras homolog gene family, member T1	NM_018307	Hs.462742	7.004
19	FGFBP1	Fibroblast growth factor binding protein 1	NM_005130	Hs.1690	6.945
20	PDE8A	Phosphodiesterase 8A	NM_002605	Hs.306330	6.885
21	SIPA1L3	Signal-induced proliferation-associated 1 like 3	NM_015073	Hs.157259	6.596
22	HECTD1	HECT domain containing 1	NM_015382	Hs.210850	6.579
23	CEACAM6	Carcinoembryonic antigen-related cell adhesionmolecule 6	NM_002483	Hs.73848	6.522
24	ZG16	Zymogen granule protein 16	NM_152338	Hs.184507	6.347
25	TSPAN3	Tetraspan 3	NM_005724	Hs.100090	6.335
26	CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	NM_004360	Hs.194657	6.329
27	S100A6	S100 calcium-binding protein A6	NM_014624	Hs.275243	6.287
28	FDFT1	Famesyl-diphosphate farnesyltransferase 1	NM_004462	Hs.546253	6.191
29	THRAP2	Thyroid hormone receptor associated protein 2	NM_015335	Hs.4084	6.175
30	LOC389765	Similar to KIF27C	XM_372122	Hs.160561	6.093
31	ACSL3	Acyl-CoA synthetase long-chain family member 3	NM_004457	Hs.471461	6.055
32	HK2	Hexokinase 2	NM_000189	Hs.198427	6.047
33	ZWINT	ZW10 interactor	NM_007057	Hs.42650	6.006
34	SFPQ	Splicing factor proline/glutamine-rich	NM_005066	Hs.355934	5.993
35	ZNF562	Zinc finger protein 562	NM_017656	Hs.531179	5.972
36	SRP14	Signal recognition particle 14 kDa	NM_003134	Hs.533732	5.946
37	S100A14	8100-type calcium binding protein A14	NM_020672	Hs.288998	5.936
38	C7orf24	Chromosome 7 open reading frame 24	NM_024051	Hs.288649	5.845
39	HDHD1A	Haloacid dehalogenase-like hydrolase domain containing 1A	NM_012080	Hs.226469	5.802
40	SUM02	SMT3 suppressor of mif two 3 homolog 2 (yeast)	NM_006937	Hs.380973	5.770
41	SNRPE	Small nuclear ribonucleoprotein polypeptide E	NM_003094	Hs.334612	5.697
42	TARDBP	TAR DNA binding protein	NM_007375	Hs.300624	5.598
43	C10orf45	Chromosome 10 open reading frame 45	NM_031453	Hs.103378	5.569
44	TSPAN8	Tetraspanin 8	NM_004616	Hs.170563	5.567

Table I. Continued.

Rank	Symbol	Gene title	GenBank ID	Unigene ID	Ratio
45	GALNT2	UDP-N-acetyl-α-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	NM_004481	Hs.130181	5.531
46	SRP9	Signal recognition particle 9 kD	NM_003133	Hs.511425	5.439
47	FEN1	Flap structure-specific endonuclease 1	NM_004111	Hs.4756	5.401
48	VPS35	Vacuolar protein sorting 35 (yeast)	NM_018206	Hs.447547	5.398
49	CCT2	Chaperonin containing TCP1, subunit 2 (ß)	NM_006431	Hs.6456	5.374
50	TOP2B	Topoisomerase (DNA) IIß 180 kDa	NM_001068	Hs.75248	5.295

111 (1.4%) genes were >3 times up-, and down-regulated in ulcerative colitis (Tables I and II).

Among up-regulated genes, the top was X (inactive)-specific transcript (XIST) (Table I). XIST is expressed only in inactive X chromosomes, indicating a female origin (35). There was a female patient included in our ulcerative colitis group, while the control group consisted of only males. Thus, the 'up-regulation' of XIST served as an internal control in this study.

Some normally expressed genes in colon were up-regulated considerably: chloride channel calcium activated family member 1 (CLCA1), Fc fragment of IgG binding protein (FCGBP), γ-actin (ACTG1), trefoil factor 3 (TFF3), carcino-embryonic antigen-related cell adhesion molecule 7 (CEACAM7), and annexin 4 (ANXA4). FCGBP has been reported to be up-regulated in ulcerative colitis (36). It is a mucin-like, non-Fc receptor protein which was implicated in immunological defense of mucosa (37). Annexin 4 is expressed by only epithelial cells.

Genes implicated in inflammation were up-regulated: peptidylprolyl isomerase A (cyclophilin A) (PPIA), nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor α (NFκBIα), chemokine (C-X-C motif) ligand 3 (CXCL3), CXCL1, and CXCL2. Cyclophilin A has been reported to be a chemotactic factor for neutrophils (38,39). The up-regulation suggested an implication in the neutrophil-dominant targeted crypt infiltration in ulcerative colitis.

Many lipid metabolism-associated genes were also up-regulated: Farnesyl-diphosphate farnesyltransferase 1 (FDFT1), acyl-CoA synthetase long-chain family member 3 (ACSL3), fatty acid binding protein 1, liver (FABP1), low density lipoprotein receptor (LDLR), and fatty acid binding protein 5 (FABP5). Anti-apoptotic genes baculoviral IAP repeat-containing 6 (BIRC6) and ADP-ribosylation factor-like 6 interacting protein (ARL6IP), and cell junction/cytoskeleton genes E-cadherin (CDH1), tight junction protein 2 (TJP2), and keratin 20 (KRT20) were also up-regulated.

Down-regulated genes included the ones implicated in lipid metabolism such as apolipoprotein A-IV(APOA4) and membrane-bound transcription factor protease site 1 (MBTPS1) (Table II). APOA4 has been implicated in the protection against inflammatory bowel disease in mice suggesting an anti-inflammatory role. Vowinkel *et al* reported that APOA4-knockout mice were susceptible to experimentally induced colitis, which was protected by exogenous administration of APOA4 (40). Cylindromatosis (CYLD) negatively regulates NF-κB signaling by deubiquitination (41). Down-regulation of CYLD might also be implicated in enhanced inflammation. Tight junction protein claudin 8 was down-regulated, suggesting an implication in the crypt epithelial barriers in ulcerative colitis. Apoptosis-related genes, APG12 autophagy 12-like (APG12L), BCL2-like 10 (BCL2L10) were also down-regulated.

Immunohistochemistry. We investigated the expression of cyclophilin A using immunohistochemistry. In nonspecific colitis control, crypts in the basal compartment were mildly immunostained for cyclophilin A (Fig. 1G). Under the same staining conditions, crypt epithelial cells were strongly immunostained in ulcerative colitis (Fig. 1H), concurring with the expression data. Goblet cells were not immuno-stained. Chronic inflammatory cells were immuno-stained similarly in ulcerative and nonspecific colitis.

Discussion

We have shown differential expressions in the crypts of ulcerative colitis before the infiltration. We were able to obtain reliable gene expression profiling of microdissected crypts from formalin-fixed, paraffin-embedded tissue using a relatively simple procedure. Our RNA extraction method including sequential heating and proteinase K digestion has been shown to provide quality samples for DNA microarray analysis (32). Results are significantly better in terms of the amount and quality of amplified signals than the ones, for instance, obtained using a commercially available Paradise™ Reagent System (Arcturus Bioscience) which is proposed for the application to formalin-fixed, paraffin-embedded tissue (Lee I, *et al*, unpublished data). Using our method, ~100 cross-sectioned crypts were enough to provide RNA for the hybridization to a microarray.

Because morphologically normal crypts from early stage ulcerative colitis were microdissected from well-prepared sections, data noise from inflammatory cell contamination and/or secondary regenerative epithelial changes were largely eliminated. Mitoses in crypts were minimal in both ulcerative colitis and control, suggesting that nonspecific expressions related to cell proliferation were virtually excluded. The control

Table II. Down-regulated genes in ulcerative colitis.

Rank	Symbol	Gene title	GenBank ID	Unigene ID	Ratio
1		EST	AK097792	Hs.518244	0.085
2		EST	R98018	Hs.35533	0.088
3	HLA-C	Major histocompatibility complex, class I, C	NM_002117	Hs.277477	0.101
4	RNPC3	RNA-binding region (RNP1, RRM) containing 3	NM_017619	Hs.512635	0.112
5	LOC51315	Hypothetical protein LOC51315	NM_016618	Hs.469254	0.120
6	APG12L	APG12 autophagy 12-like *(S. cerevisiae)*	NM_004707	Hs.264482	0.121
7		EST	BC033966	Hs.2.271478.1	0.126
8	FM05	Flavin containing monooxygenase 5	NM_001461	Hs.303476	0.141
9	VPS13C	Vacuolar protein sorting 13C (yeast)	NM_001018088	Hs.511668	0.158
10	DKFZP586 A0522	DKFZP586A0522 protein	NM_014033	Hs.288771	0.165
11	MBNL1	Muscleblind-like *(Drosophila)*	NM_021038	Hs.478000	0.166
12		EST	BC053686		0.168
13	CDC42	Cell division cycle 42 (GTP binding protein, 25 kDa)	AF086337	Hs. 384596	0.172
14	GOSR1	Golgi SNAP receptor complex member 1	BC012620	Hs.8868	0.178
15	LOC253039	Hypothetical protein LOC253039	BF476087	Hs.432834	0.183
16	FAM55A	Family with sequence similarity 55, member A	NM_152315	Hs.374720	0.193
17	SLC26A8	Solute carrier family 26, member 8	NM_052961	Hs.20969	0.200
18	APOA4	Apolipoprotein A-IV	NM_000482	Hs.1247	0.203
19		EST	AI082479	Hs.271381	0.212
20	KIAA1970	KIAA1970 protein	NM_33451	Hs.559239	0.217
21	CYLD	Cylindromatosis (turban tumor syndrome)	NM_015247	Hs.432993	0.223
22	HRMT1L1	HMT1 hnRNP methyltransferase-like 1 *(S. cerevisiae)*	U79286	Hs.235887	0.224
23	PTPN18	Protein tyrosine phosphatase, non-receptor type 18	NM_014369	Hs.516390	0.229
24	KIAA0804	KIAA0804	NM_001009921	Hs.269263	0.230
25	BCL2L10	BCL2-like 10 (apoptosis facilitator)	NM_020396	Hs.283672	0.231
26	RNU17D	RNA, U17D small nucleolar	AJ006834	Hs.469723	0.232
27	XKR5	X Kell blood group precursor-related family, member 5	NM_207411	Hs.369771	0.233
28	NBR1	Neighbor of BRCA1 gene 1	NM_005899	Hs.277721	0.234
29	HOXD10	Homeo boxD10	AW299531	Hs.188023	0.236
30	CPM	Carboxypeptidase M	NM_001005502	Hs.484551	0.237
31	SNRP70	Small nuclear ribonucleoprotein 70 kD polypeptide	NM_001009820	Hs.467097	0.243
32	HCG18	HLA complex group 18	AK056160	Hs.301985	0.243
33	SUZ12	Suppressor of zeste 12 homolog *(Drosophila)*	NM_015355	Hs.462732	0.244
34	THUMPD3	THUMP domain containing 3	NM_015453	Hs.443081	0.249
35	RAB11B	RAB11B, member RAS oncogene family	NM_004218	Hs.433888	0.250
36		EST	AK024994	Hs.306738	0.252
37		EST	BC020857	Hs.554307	0.254
38	ZNF16	Zinc finger protein 16 (KOX 9)	NM_006958	Hs.23019	0.255
39		EST	AW002073	Hs.236524	0.257
40	C21orf88	Chromosome 21 open reading frame 88	AF426267	Hs.375120	0.261
41		EST	BC022384	Hs.352171	0.262
42	LR8	LR8 protein	NM_014020	Hs.521295	0.263
43	CROP	Cisplatin resistance-associated overexpressed protein	BE887449	Hs.32112	0.265

Table II. Continued.

Rank	Symbol	Gene title	GenBank ID	Unigene ID	Ratio
44	C19orf33	Chromosome 19 open reading frame 33	NM_033520	Hs.348553	0.268
45		EST	BC004382	Hs.267319	0.270
46	SLC17A4	Solute carrier family 17 (sodium phosphate), member 4	NM_005495	Hs.282931	0.270
47	SMARCE1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	NM_003079	Hs.233354	0.271
48	FLJ30313	Hypothetical protein FLJ30313	NM_152757	Hs.444743	0.272
49	FLJ90396	Hypothetical protein FLJ90396	NM_153358	Hs.346875	0.275
50	RPS4Y1	Ribosomal protein S4, Y-linked 1	NM_001008	Hs.180911	0.277

was taken similarly from nonspecific colitis to eliminate potential effect of 'inflammatory environment' that might not have been reflected morphologically.

For this study, a standard linear amplification method was adopted using an Affymetrix kit. However, the procedure including two-cycle amplification would inevitably induce deviation of data the extent of which is not clear in this study. Nevertheless, there is evidence that support the reliability of our data. The fact that female-origin XIST was the most up-regulated gene strongly suggests the fidelity of our data serving as an internal control. The immunohistochemical staining of cyclophilin A also supported the expression data. Among the differentially expressed genes, those being implicated in ulcerative colitis such as APOA4 and FCGBP were included. Another indirect evidence of the reproducibility is the redundancy of signals. In the microarray, some genes are represented by redundant spots in which seeded oligonucleotides are not necessarily identical. For instance, 10 γ-actin and 6 cyclophilin A spots were included in the differential expression, suggesting the reproducibility. Together, it is suggested that reliable expression profiling data could be obtained using a reasonable number of micro-dissected cells from routinely processed biopsies, although there is considerable loss of signals in quantity. The amount of data obtained from formalin-fixed biopsies appear to be reduced to about half of the fresh sample counterparts using our procedure (Lee I, *et al*, unpublished data). The obtainable data may be extended by increasing the load and/or repeated applications.

The up-regulation of cyclophilin A in normal crypts might suggest an implication in the pathogenesis. Cyclophilin A is an intracellular protein that is known as a receptor for the immunosuppressive drug, cyclosporine A (38,39). Cyclophilin A is elevated in inflammation and may induce neutrophil chemotaxis *in vivo* and *in vitro*. The neutrophil chemotaxis concurs with the crypt-specific infiltration in ulcerative colitis. The pathobiological role of Cyclophilin A and other inflammation-associated genes needs further characterization in ulcerative colitis.

Numerous genes associated with lipid metabolism were differentially expressed, suggesting a pathogenic association of aberrant lipid regulation with ulcerative colitis. It has been shown that APOA4 has a protective effect against experimentally induced inflammatory bowel disease in mice (40). The up-regulation of FABP1 was concurrent with a previous expression profiling study (20).

Down-regulation of claudin 8 might be consistent with previous studies of altered epithelial barriers of colonic mucosa in ulcerative colitis (13-15). Claudin 8 is a member of a large family of tight junction integral membrane protein, and is suggested to have a cation barrier function (42). In our data, claudins 4, 12, and 15 were not differentially expressed, and claudin 7 was up-regulated <3 times. Other claudins were not significantly hybridized. Tight junction protein 2 (zona occludens 2) and adherent junction protein E-cadherin were up-regulated. It is possible that only a limited number of tight junction protein(s) might be down-regulated in ulcerative colitis while other junction proteins show compensatory up-regulation.

Taken together, the differential expression in uninflamed crypt epithelia suggests innate disorders of crypt regulation in ulcerative colitis. Differentially expressed genes of various functions might suggest a complex pathogenesis encompassing abnormal epithelial barrier and aberrant immune regulation (Kim *et al*, unpublished data). As diagnostic biopsies may be applied readily, we expect that the expression profiling will be extended for various purposes such as sequential progression of subtle diseases and treatment effects.

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References

1. Fiocchi C: Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 115: 182-205, 1998.
2. Bouma G and Strober W: The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 3: 521-533, 2003.
3. Nostrant TT, Kumar NB and Appelman HD: Histopathology differentiates acute self-limited colitis from ulcerative colitis. Gastroenterology 92: 318-328, 1987.
4. Goldman H: Acute versus chronic colitis: how and when to distinguish by biopsy. Gastroenterology 86: 199-201, 1984.

5. Seldenrijk CA, Morson BC, Meuwissen SG, Schipper NW, Lindeman J and Meijer CJ: Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease: diagnostic implications. Gut 32: 1514-1520, 1991.
6. Jenkins D, Balsitis M, Gallivan S, Dixon MF, Gilmour HM, Shepherd NA, Theodossi A and Williams GT: Guidelines for the initial biopsy diagnosis of suspected chronic idiopathic inflammatory bowel disease. The British Society of Gastroenterology Initiative. J Clin Pathol 50: 93-105, 1997.
7. Tanaka M, Riddell RH, Saito H, Soma Y, Hidaka H and Kudo H: Morphologic criteria applicable to biopsy specimens for effective distinction of inflammatory bowel disease from other forms of colitis and of Crohn's disease from ulcerative colitis. Scand J Gastroenterol 34: 55-67, 1999.
8. Warren BF: Classic pathology of ulcerative and Crohn's colitis. J Clin Gastroenterol 38 (Suppl 5): S33-S35, 2004.
9. Gomes P, du Boulay C, Smith CL and Holdstock G: Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. Gut 27: 92-95, 1986.
10. Ina K, Kusugami K, Yamaguchi T, Imada A, Hosokawa T, Ohsuga M, Shinoda M, Ando T, Ito K and Yokoyama Y: Mucosal interleukin-8 is involved in neutrophil migration and binding to extracellular matrix in inflammatory bowel disease. Am J Gastroenterol 92: 1342-1346, 1997.
11. Elliott SN and Wallace JL: Neutrophil-mediated gastrointestinal injury. Can J Gastroenterol 12: 559-568, 1998.
12. Geboes K, Riddell R, Ost A, Jensfelt B, Persson T and Lofberg R: A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut 47: 404-409, 2000.
13. Schmitz H, Barmeyer C, Fromm M, Runkel N, Foss HD, Bentzel CJ, Riecken EO and Schulzke JD: Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. Gastroenterology 16: 301-309, 1999.
14. Gassler N, Rohr C, Schneider A, Kartenbeck J, Bach A, Obermuller N, Otto HF and Autschbach F: Inflammatory bowel disease is associated with changes of enterocytic junctions. Am J Physiol Gastrointest Liver Physiol 281: G216-G228, 2001.
15. Kucharzik T, Walsh SV, Chen J, Parkos CA and Nusrat A: Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. Am J Pathol 159: 2001-2009, 2001.
16. Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM and Nusrat A: Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. J Immunol 171: 6164-6172, 2003.
17. Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Burgel N, Fromm M, Zeitz M, Fuss I, Strober W and Schulzke JD: Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 129: 550-564, 2005.
18. Okahara S, Arimura Y, Yabana T, Kobayashi K, Gotoh A, Motoya S, Imamura A, Endo T and Imai K: Inflammatory gene signature in ulcerative colitis with cDNA macroarray analysis. Aliment Pharmacol Ther 21: 1091-1097, 2005.
19. Langmann T, Moehle C, Mauerer R, Scharl M, Liebisch G, Zahn A, Stremmel W and Schmitz G: Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes. Gastroenterology 127: 26-40, 2004.
20. Dooley TP, Curto EV, Reddy SP, Davis RL, Lambert GW, Wilborn TW and Elson CO: Regulation of gene expression in inflammatory bowel disease and correlation with IBD drugs: screening by DNA microarrays. Inflamm Bowel Dis 10: 1-14, 2004.
21. Masuda H, Takahashi Y, Asai S and Takayama T: Distinct gene expression of osteopontin in patients with ulcerative colitis. J Surg Res 111: 85-90, 2003.
22. Dieckgraefe BK, Crimmins DL, Landt V, Houchen C, Anant S, Porche-Sorbet R and Ladenson JH: Expression of the regenerating gene family in inflammatory bowel disease mucosa: Reg I alpha upregulation, processing, and antiapoptotic activity. J Investig Med 50: 421-434, 2002.
23. Uthoff SM, Eichenberger MR, Lewis RK, Fox MP, Hamilton CJ, McAuliffe TL, Grimes HL and Galandiuk S: Identification of candidate genes in ulcerative colitis and Crohn's disease using cDNA array technology. Int J Oncol 19: 803-810, 2001.
24. Lawrance IC, Fiocchi C and Chakravarti S: Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes. Hum Mol Genet 10: 445-456, 2001.
25. Dieckgraefe BK, Stenson WF, Korzenik JR, Swanson PE and Harrington CA: analysis of mucosal gene expression in inflammatory bowel disease by parallel oligonucleotide arrays. Physiol Genomics 4: 1-11, 2000.
26. Kim B, Bang S, Lee S, Kim S, Choi K, Lee C, Lee SG, Lee K, Lee Y, Kim Y, Kim S, Yeom Y, Yu H, Song K and Lee I: Expression profiling and subtype-specific expression of stomach cancer. Cancer Res 63: 8248-8255, 2003.
27. Schreiter K, Hausmann M, Spoettl T, Strauch UG, Bataille F, Schoelmerich J, Herfarth H, Falk W and Rogler G: Glycoprotein (gp) 96 expression: induced during differentiation of intestinal macrophages but impaired in Crohn's disease. Gut 54: 935-943, 2005.
28. Ten Hove T, The Olle F, Berkhout M, Bruggeman JP, Vyth-Dreese FA, Slors JF, Van Deventer SJ and Te Velde AA: Expression of CD45RB functionally distinguishes intestinal T lymphocytes in inflammatory bowel disease. J Leukoc Biol 75: 1010-1015, 2004.
29. Masuda N, Ohnishi T, Kawamoto S, Monden M and Okubo K: Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples. Nucleic Acids Res 27: 4436-4443, 1999.
30. Bibikova M, Talantov D, Chudin E, Yeakley JM, Chen J, Doucet D, Wickham E, Atkins D, Barker D, Chee M, Wang Y and Fan JB: Quantitative gene expression profiling in formalin-fixed, paraffin-embedded tissues using universal bead arrays. Am J Pathol 165: 1799-1807, 2004.
31. Paik S, Kim CY, Song YK and Kim WS: Technology insight: Application of molecular techniques to formalin-fixed paraffin-embedded tissues from breast cancer. Nat Clin Pract Oncol 2: 246-254, 2005.
32. Lee C, Bang S, Lee S, Song K and Lee I: Gene expression profiling reveals sequential change in gastric tubular adenoma and carcinoma *in situ*. World J Gastroenterol 11: 1937-1945, 2005.
33. Yang SK, Hong WS, Min YI, Kim HY, Yoo JY, Rhee PL, Rhee JC, Chang DK, Song IS, Jung SA, Park EB, Yoo HM, Lee DK and Kim YK: Incidence and prevalence of ulcerative colitis in the Songpa-Kangdong District, Seoul, Korea, 1986-1997. J Gastroenterol Hepatol 15: 1037-1042, 2000.
34. Jang J, Lee S, Jung Y, Song K, Fukumoto M, Gould VE and Lee I: Malgun (clear) cell change in *Helicobacter pylori* gastritis reflects epithelial genomic damage and repair. Am J Pathol 162: 1203-1211, 2003.
35. Vawter MP, Evans S, Choudary P, Meador-Woodruff J, Tomita H, Molnar M, Li J, Lopez JF, Myers R, Cox D, Watson SJ, Akil H, Jones EG and Bunney WE: Gender-specific gene expression in post-mortem human brain: localization to sex chromosomes. Neuropsychopharmacology 29: 373-384, 2004.
36. Kobayashi K, Blaser MJ and Brown WR: Identification of a unique IgG Fc binding site in human intestinal epithelium. J Immunol 143: 2567-2574, 1989.
37. Kobayashi K, Ogata H, Morikawa M, Iijima S, Harada N, Yoshida T, Brown WR, Inoue N, Hamada Y, Ishii H, Watanabe M and Hibi T: Distribution and partial characterisation of IgG Fc binding protein in various mucin producing cells and body fluids. Gut 51: 169-176, 2002.
38. Xu Q, Leiva MC, Fischkoff SA, Handschumacher RE and Lyttle CR: Leukocyte chemotactic activity of cyclophilin. J Biol Chem 267: 11968-11971, 1992.
39. Arora K, Gwinn WM, Bower MA, Watson A, Okwumabua I, MacDonald HR, Bukrinsky MI and Constant SL: Extracellular cyclophilins contribute to the regulation of inflammatory responses. J Immunol 175: 517-522, 2005.
40. Vowinkel T, Mori M, Krieglstein CF, Russell J, Saijo F, Bharwani S, Turnage RH, Davidson WS, Tso P, Granger DN and Kalogeris TJ: Apolipoprotein A-IV inhibits experimental colitis. J Clin Invest 114: 260-269, 2004.
41. Trompouki E, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A and Mosialos G: CYLD is a deubiquitinating enzyme that negatively regulates NF-kappa-B activation by TNFR family members. Nature 424: 793-796, 2003.
42. Yu AS, Enck AH, Lencer WI and Schneeberger EE: Claudin-8 expression in Madin-Darby canine kidney cells augments the paracellular barrier to cation permeation. J Biol Chem 278: 17350-17359, 2003.