Original Article

Expression Profiling of Inflammatory and Immunological Genes in Collagenous Colitis

Qingqing Liu and Noam Harpaz

The Department of Pathology, Molecular and Cell-based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Corresponding author: Noam Harpaz, MD, PhD, Department of Pathology, Annenberg 15-38E, Icahn School of Medicine at Mount Sinai, 1468 Madison Avenue, New York, NY 10029, USA. Tel: [212] 241-6692; Fax: [212] 828-4188; Email: noam.harpaz@mountsinai.org

Abstract

Background: Collagenous colitis [CC] is a common idiopathic cause of chronic watery diarrhoea. We investigated its pathogenesis by means of gene expression analysis.

Methods: We analysed the expression of genes implicated in immunological and inflammatory pathways in paired colonic biopsies of histologically involved and uninvolved mucosa from five patients with histologically patchy CC, in pooled colonic biopsies of eight other patients with diffuse CC, and in pooled biopsies of eight normal controls. Analyses were performed with the Nanostring nCounter system. Expression ratios were generated and confirmed by quantitative reverse transcription PCR.

Results: CC mucosa was characterized by enhanced expression of nitric oxide synthase 2; of matrix metalloproteinases 3 and 9 and tissue inhibitor of metalloproteinase 1, but not transforming growth factor β 1; of mediators of T-helper 1 immunity including interleukins 12A [*IL12A*], 12B, IL12 receptor B1 and interferon γ ; of immune mediators of the leukocyte immunoglobulin-like receptor subfamily B; and of multiple T cell cytokines and their receptors. The mitogen-activated protein kinase signalling pathway was unchanged. There were no increases in *IL22*, *IL22RA2* or tumour necrosis factor α , which are reportedly elevated in chronic inflammatory bowel disease. In four of five patients with patchy CC, similar gene expression profiles were observed in histologically involved and uninvolved mucosa.

Conclusions: CC is characterized by altered expression of a limited repertoire of genes involved in nitric oxide synthesis, extracellular matrix remodelling, T-helper 1 immunity and immune modulation. The abnormal gene expression in patchy CC may be expressed in mucosa with and without histological disease manifestations.

Key Words: Basic science; experimental models and pathophysiology; pathology

1. Introduction

Collagenous colitis [CC] is a form of microscopic colitis that is characterized clinically by chronic watery diarrhoea, a predominance in older women and minimal endoscopic abnormalities. Microscopically, it features abnormal subepithelial collagen deposition, lamina propria lymphoplasmacytosis and, in some cases, increased intra-epithelial lymphocytes.¹

The aetiopathogenesis of CC is obscure but is thought to be multifactorial, including dysregulated mucosal immune responses to

unknown luminal factors and individual susceptibility factors. The disease is strongly associated with coeliac disease and other autoimmune disorders² and exposure to certain medications such as proton pump inhibitors and selective serotonin reuptake inhibitors.^{3,4}

No specific genetic mutations have as yet been associated with CC, but allelic variations in certain genes have been implicated in defining a complex genetic risk profile for the disease. For instance, carriage of allele GG in *MMP-9* exon 6 significantly increased the risk for CC with an odds ratio of 1.9.⁵ Specific human leukocyte

antigen [HLA-DQ2, -DQ1/3, -DR3-DQ2] haplotypes that correlate with susceptibility to coeliac disease are also more frequent in CC.^{6,7} The carriage rates of tumour necrosis factor 2 [*TNF2*] allele are increased in CC, although this may reflect linkage equilibrium with DQ2.⁶ Recently, a genome-wide study of Swedish and German populations implicated HLA 8.1 haplotype alleles including DQ2.5 as markers of increased risk for CC.⁸ Interestingly, this association was not seen in patients with lymphocytic colitis, a clinically similar disorder with overlapping histology.⁹

To gain further insight into the pathogenesis of CC we carried out a gene expression analysis of the mucosa with a focus on genes related to inflammatory and immunological pathways. To our knowledge this kind of analysis has not been reported previously.

2. Materials and Methods

2.1. Study populations, patient characteristics and study design

We identified 13 patients with CC by means of a text-based search of the surgical pathology records of the Department of Pathology, Icahn School of Medicine at Mount Sinai, for February–July, 2016. The electronic medical records were reviewed for demographic data, clinical presentation and medications taken at the time of biopsy, and anatomical location of the biopsies. These data are provided in [Supplementary Table 1](#). Inclusion criteria included clinical symptoms of chronic watery diarrhoea, little or no inflammatory mucosal changes based on endoscopic examination, and fulfilment of the histological criteria for CC [see below]. Exclusion criteria included a history of coeliac disease, and lack of access to histological slides and paraffin blocks. The median age was 60 years [range 22–74] and 11 patients were females.

2.2. Histopathology

The biopsy slides were stained with haematoxylin–eosin and the diagnoses of CC were confirmed by staining with Sirius red.¹⁰ In some cases, Masson's trichrome staining was performed in parallel and showed precise concordance with Sirius red [Figure 1]. The histological criteria for CC included lamina propria lymphoplasmacytosis, relatively preserved crypt density and architecture, 'basket weave' subepithelial fibrosis and intraepithelial lymphocytosis [a, H&E; b, Sirius red, 20×]. Paired biopsy samples of involved [•] [thickened subepithelial collagen band marked by arrows] and uninvolved mucosa [★] were selected for analysis [c, H&E; d, Sirius red, 4×]. Higher magnification of the framed box area marked in d is shown in e [involved mucosa, Sirius red, 40×] and f [uninvolved mucosa, Sirius red, 40×]. Masson Trichrome staining on the same area is shown in g [involved mucosa, 40×] and h [uninvolved mucosa, 40×].

2.3. RNA extraction and Nanostring gene expression analysis

Total RNA was extracted from 50-µm-thick formalin-fixed, paraffin-embedded [FFPE] colonic tissue using a Maxwell 16 LEV RNA FFPE kit [Promega] according to the manufacturer's instructions. RNA concentration and purity were determined using a Nanodrop spectrophotometer [Thermo Fisher Scientific]. Equal quantities of RNA from each of the CC-diffuse cases [$n = 8$] and of the controls [$n = 8$] were pooled^{11–14} and designated pooled CC and pooled N, respectively. In total, 100 ng of RNA from the matched 'involved' and 'uninvolved' pairs of CC-patchy cases [$n = 5$ each], from the pooled CC-diffuse cases and from the pooled N controls was subjected to Nanostring gene expression analysis with the Nanostring nCounter system.

The relative mRNA copy number of 778 human genes, 594 genes that are differentially expressed in immunological disorders [

Figure 1. Collagenous colitis featuring lamina propria lymphoplasmacytosis, preserved crypt density and architecture, 'basket weave' subepithelial fibrosis and intraepithelial lymphocytosis [a, H&E; b, Sirius red, 20×]. Paired biopsy samples of involved [•] [thickened subepithelial collagen band marked by arrows] and uninvolved mucosa [★] were selected for analysis [c, H&E; d, Sirius red, 4×]. Higher magnification of the framed box area marked in d is shown in e [involved mucosa, Sirius red, 40×] and f [uninvolved mucosa, Sirius red, 40×]. Masson Trichrome staining on the same area is shown in g [involved mucosa, 40×] and h [uninvolved mucosa, 40×].

hdmzlive.nanostring.com/products/gene-expression-panels/ncounter-immunology-panels] and 184 genes that are differentially expressed in inflammatory disorders [<https://www.nanostring.com/products/gene-expression-panels/ncounter-inflammation-panels>], was quantified as per the manufacturer's recommendations [NanoString Technologies]. The raw expression data were normalized first to the geometric mean of positive controls, and then to the geometric mean of reference genes using nSolver Analysis Software 3.0 [NanoString Technologies]. Datasets of gene expression ratios for 'involved' vs 'uninvolved' or CC-diffuse vs controls were then generated. For each sample, $\log_2$ expression of selected genes was hierarchically clustered in Cluster3 and the heat map was created in Java Treeview [<http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm>]. The $\log_2$ gene expression levels were used to calculate Euclidean distances with the R distribution function. Samples were clustered hierarchically with complete linkage to estimate distances for pseudo-items, and a sample to sample heat map was created with R Pheatmap software [<https://github.com/ravikolde/pheatmap>].

2.4. Quantitative reverse transcription PCR

Real-time PCR assays were carried out selectively based on differential gene expression levels between CC and controls and their potential functional importance. Although not included in the Nanostring

panel, we also performed quantitative reverse transcription PCR [qRT-PCR] on the *TIMP1* gene. These analyses were performed on 2 ng aliquots of total RNA after reverse transcription using an ABI prism 7900HT sequence Detector [Applied Biosystems] with β -actin as the internal control. Data were analysed by the relative quantification [$\Delta\Delta C_t$] method. The sequences of the oligonucleotide primers are provided in [Supplementary Table 2](#). Statistical analysis was performed via Student's *t* test with significance set at $p < 0.05$.

3. Results

3.1. Cases for analysis

Eight cases of CC were classified as CC-diffuse [[Figure 1a, b](#)]. The median age of this patient group was 58 years [range, 22–74; six females, two males]. Five cases were designated CC-patchy [[Figure 1c–h](#)]. The median age of this group was 69 years [range 46–74; all females]. Control biopsies of normal mucosa were obtained from eight patients who had undergone colonoscopic examinations for non-inflammatory disorders [median age 50.5 years, range 25–76; five females, three males].

Our analysis compared the expression levels of genes implicated in inflammatory and immunological functions in paired mucosal biopsies from patients with histologically patchy CC [CC-patchy, $n = 5$], in

pooled samples from patients with histologically diffuse CC [CC-diffuse, $n = 8$], and in pooled samples from normal controls [$n = 8$]. We then performed validation by means of qRT-PCR analysis of genes of potential interest. [Figure 2a](#) shows the fold changes in expression levels of these genes based on Nanostring analysis of the pooled diffuse CC vs pooled normal controls. [Figure 2b](#) shows the corresponding gene expression levels determined by qRT-PCR. As shown, there was good consistency between the two data sets. [Figure 2c](#) shows the expression levels of these genes in all samples as a heat map; the corresponding data are provided in [Supplementary Table 3](#). [Figure 3a](#) shows the differential expression levels of genes determined by Nanostring analysis but not confirmed by qRT-PCR and [Figure 3b](#) is the corresponding heat map, which also includes the individual patchy CC samples. The fold change data are provided in [Supplementary Table 4](#). [Supplementary Table 5](#) shows the fold changes of those additional genes which were up- or down-regulated by greater than 1.5-fold.

3.2. Expression of genes involved in collagen metabolism

The CC samples showed increased expression levels of matrix-metalloproteinases [MMP] 3 and 9, which are involved in connective tissue remodelling [MMP-3, 7.84-fold, $p = 0.008$ by qRT-PCR; MMP-9, 3.50-fold, $p = 0.029$ by qRT-PCR, [Figure 2a](#),

Figure 2. Representative genes differentially expressed in pooled diffuse collagenous colitis by Nanostring nCounter gene expression assay, confirmed by qRT-PCR. [a] The fold changes [FC] of gene expression in pooled diffuse-CC samples [CC] compared with pooled normal colonic controls [N]. Positive FC indicates upregulation and negative FC indicates downregulation. [b] qRT-PCR validation of gene expression. Results are presented as mean $\pm$ SD. The *p* values [unpaired *t* tests] are shown. * $0.01 < p < 0.05$, ** $0.001 < p < 0.01$. NS, not statistically significant. [c] Expression levels of these genes in individual patchy CC samples were provided for comparison. These genes were median-centred, with changes in expression level colour coded as indicated. At the top of the expression data, a hierarchical clustering tree indicates the relationship among samples based on expression patterns.

b] and tissue inhibitor of metalloproteinase [*TIMP1*, $p = 0.027$ by qRT-PCR, [Figure 2a, b](#)]. There was no increase, however, in the level of transforming growth factor beta 1 [*TGF-β1*], a cytokine that promotes extracellular matrix production following inflammatory responses [1.33-fold, $p = 0.664$ by qRT-PCR, [Figure 2a, b](#)].

3.3. Upregulation of genes involved in the T-helper 1 pathway

The CC cases demonstrated upregulation of multiple genes involved in T-helper 1 [Th1] immune responses. Among them were interleukin [IL]12A [2.03-fold, [Figure 3a](#)], *IL12B* [4.14-fold, [Figure 3a](#)] and their receptor *IL12RB1* [1.95-fold, [Figure 3a](#)], effector cytokine interferon gamma [*IFNγ*, 31-fold, $p = 0.007$ by qRT-PCR, [Figure 2a, b](#)], T cell immunoreceptor *TIGIT* [2.98-fold, [Figure 3a](#)], Th1 transcription factor *TBX21* [1.73-fold, [Figure 3a](#)] and nitric oxide synthase 2 [*NOS2*, 7.48-fold, $p = 0.049$ by qRT-PCR, [Figure 2a](#) and [b](#)]. In addition to upregulation of *IFNγ*, there were corresponding increases in the expression of multiple downstream genes including *STAT1* of the JAK-STAT pathway [3.98-fold, $p = 0.012$ by qRT-PCR, [Figure 2a, b](#)], interferon-induced proteins *IFIT3* [3.84-fold, $p = 0.001$ by qRT-PCR, [Figure 2a, b](#)] and *IFIT4* [2.51-fold, [Figure 3a](#)],¹⁵ and others [range, 1.74- to 7.95-fold, [Figure 3a](#)]. Interestingly, *FOXP3*, a master regulator in regulatory T cells, was also upregulated in CC [6.53-fold, [Figure 3a](#)].

3.4. Altered expression of inflammatory chemokines and their receptors

Multiple members of the chemoattractant chemokine family were differentially upregulated in CC, including *CCL11* [3.38-fold, $p = 0.031$ by qRT-PCR, [Figure 2a, b](#)], *CCL20* [3.65-fold, $p = 0.061$ by qRT-PCR, [Figure 2a, b](#)], *CXCL5* [43.39-fold, $p = 0.042$ by qRT-PCR, [Figure 2a, b](#)], *CXCL8* [30.52-fold, $p = 0.049$ by qRT-PCR, [Figure 2a, b](#)], *CXCL9* [10.97-fold, $p = 0.011$ by qRT-PCR, [Figure 2a, b](#)] and others [range, 1.55- to 16.78-fold, [Figure 3a](#)]. In addition, we also observed increased expression of several chemokine receptors [[Figure 3a](#)]: *CCR10* [2.07-fold], *CCR3* [2.37-fold], *CCR5* [1.63-fold], *CCR7* [1.85-fold], *CCR8* [11.05-fold], *CXCR2* [5.71-fold], *CXCR3* [2.54-fold] and *CXCR6* [2.79-fold]. A small number of chemokines was downregulated in CC [[Figure 3a](#)], including *CCL16* [-1.59-fold], *CCL7* [-1.97-fold], *CCL8* [-1.59-fold], *CXCL12* [-1.76-fold], *CXCL13* [-1.92-fold] and *XCL1* [-5.16-fold].

3.5. Some cytokines and members of signalling pathways implicated in inflammatory bowel disease were not upregulated in CC

In contrast to the significant upregulation of *IL22* and *IL22RA2* that is reported in inflammatory bowel disease [IBD]^{16,17} and the critical roles of these cytokines in its pathogenesis, neither cytokine showed significant differential expression in CC. Thus, the

Figure 3. Genes differentially expressed [up- or downregulated greater than 1.5-fold] in collagenous colitis [CC] by Nanostring nCounter gene expression assay and not confirmed by qRT-PCR. [a] The fold changes [FC] of gene expression in CC compared with normal colonic controls [N]. Positive FC indicates upregulation and negative FC indicates downregulation. [b] Expression levels of these genes in individual patchy CC samples are provided for comparison. These genes were median-centred, with changes in expression level colour coded as indicated. At the top of the expression data, a hierarchical clustering tree indicates the relationship among samples based on expression patterns.

expression levels of *IL22* [3.19-fold, $p = 0.377$ by qRT-PCR, Figure 2a, b] and *IL22RA2* [5.66-fold, $p = 0.194$ by qRT-PCR, Figure 2a, b] in CC were increased when compared with controls but the increases did not attain statistical significance. By the same token, expression of *TNFA* remained unchanged in CC [1.28-fold, $p = 0.377$ by qRT-PCR, Figure 2a, b] in contrast to its reported overexpression in IBD.¹⁷ Expression levels of other members of the TNF family are summarized in Figure 4a.

We also examined components of the NOD1/2-NF- κ B and mitogen-activated protein kinase [MAPK] signalling cascades, in which various extracellular stimuli converge to initiate inflammation in IBD.^{18,19} No significant differential expression of genes that are critical in NOD1/NOD2 signalling was observed, including *NOD1* [1.01-fold, Figure 4a], *NOD2* [1.51-fold, $p = 0.377$ by qRT-PCR, Figure 2a, b], *RIPK2* [1.26-fold, Figure 4a], *NLRP3* [−1.35-fold, Figure 4a], *CARD9* [−1.12-fold, Figure 4a], *ATG5* [−1.30-fold, Figure 4a], *ATG12* [1.15-fold, Figure 4a] and *ATG16L1* [−1.18-fold, Figure 4a]. Expression levels of these genes in patchy CC are given in Figure 4b and Supplementary Table 6.

Since *NOD1* and *NOD2* mediate activation of the nuclear factor-kappa B [NF- κ B] family of transcriptional regulators in response to specific peptidoglycans, we also determined that the expression of individual components of I κ B kinase, which inactivates NF- κ B.

CHUK [*IKK α* , 1.06-fold], *IKBKB* [*IKK β* , −1.02-fold] and *IKBKG* [*IKK γ* , −1.02-fold] [Figure 4a] were not significantly altered in CC. However, we noted increased expression of multiple downstream effectors of NF- κ B signalling, including *CASP1* [2.47-fold], *Eomes* [17.47-fold] and *MUC1* [3.27-fold] [Figure 4a].

There was no significant upregulation of multiple members of the MAPKs, including MAP3Ks (*MAP3K1* [−1.09-fold], *MAP3K5* [−1.06-fold], *MAP3K7* [−1.13-fold] and *MAP3K9* [−1.21-fold]), MAP2Ks (*MAP2K1* [1.03-fold], *MAP2K4* [−1.13-fold] and *MAP2K6* [1.04-fold]) and MAPKs [*MAPK1* [−1.27-fold], *MAPK3* [−1.46-fold], *MAPK8* [−1.25-fold] and *MAPK14* [−1.18-fold] [Figure 4a)].

3.6. Leukocyte immunoglobulin-like receptor family members in CC

Multiple members of leukocyte immunoglobulin-like receptor sub-families A and B [*LILRA* and *LILRB*, respectively] were differentially expressed in CC. We observed upregulation of *LILRA3* [26.99-fold, $p = 0.031$ by qRT-PCR, Figure 2a, b], *LILRA5* [3.32-fold, Figure 3a] and *LILRA6* [2.07-fold, Figure 3a], and downregulation of *LILRA2* [−2.83-fold, Figure 3a] and *LILRA4* [−10.74-fold, Figure 3a]. We also observed upregulation of *LILRB2* [2.06-fold, $p = 0.032$ by

Figure 4. Expression of representative genes of TNF family, NOD1/2, MAPK and NF- κ B signalling cascades in collagenous colitis [CC] by Nanostring nCounter gene expression assay. The gene expression was not confirmed by qRT-PCR. [a] The fold change [FC] of gene expression in CC compared with normal colonic controls [N]. Positive FC indicates upregulation and negative FC indicates downregulation. [b] Expression levels of these genes in individual patchy-CC samples were provided for comparison. These genes were median-centred, with changes in expression level colour coded as indicated. At the top of the expression data, a hierarchical clustering tree indicates the relationship among samples based on expression patterns.

qRT-PCR, Figure 2a, b], *LILRB3* [2.02-fold, $p = 0.031$, Figure 2a, b] and *LILRB4* [2.83-fold, Figure 3a].

3.7. Pairwise comparison of mucosa with and without subepithelial collagen

The degree of similarity among all the samples is shown in the heat map in Figure 5. In four of five patients with patchy CC, comparison of the gene expression patterns of paired mucosal samples with and without subepithelial collagen revealed similarly altered gene expression profiles in both [patients 1, 2, 3 and 5]. Differential expression between the paired samples was observed in one patient [patient 4]. In this case, the profile of the sample with subepithelial collagen was very similar to that of the pooled CC sample, whereas that of the sample without subepithelial collagen was similar to the pooled normal sample.

4. Discussion

This gene expression analysis of CC is the most comprehensive of its kind. Using biopsy tissues, we evaluated the expression levels of 594 and 184 genes that are differentially expressed in immunological and inflammatory disorders, respectively.

The subepithelial collagen deposition which defines CC has been ascribed both to increased immature collagen matrix synthesis and to impaired matrix degradation, but inconsistencies exist among individual studies in the literature. We detected enhanced mRNA expression levels both of the fibrogenic gene *TIMP1* and of the fibrolytic genes *MMP3* and *MMP9* but not of *TGF-β1*. Günther *et al.*, based on variable expression of *MMP1* and undetectable expression of *MMP13*,

reported that increased expression of immature interstitial collagenous matrix components in CC, including *TIMP1*, is accompanied by impaired fibrolysis.²⁰ However, Aigner *et al.* reported reduced degradation but no increase in collagen matrix synthesis.²¹ Lakatos *et al.* reported that *MMP9* protein levels in CC are not increased.²² Inconsistencies between studies, including discrepancies between mRNA and protein levels,^{23–25} are recognized in the CC literature. They may result in part from dynamic imbalances between enhanced collagen synthesis and breakdown in different phases of the disease.

Our results showing unaltered expression of *TGF-β1* are in agreement with those of Carrasco *et al.*²⁵ Stähle-Bäckdahl *et al.*, however, reported increased expression of eosinophil-derived *TGF-β1* in CC,²⁶ possibly reflecting variations in eosinophil density in individual biopsies,²⁷ dilution effects in whole biopsy analyses or independence of *TGF-β1* as an obligatory fibrogenic factor in CC. Other candidate fibrogenic cytokines which were upregulated in our study and may play a role in the pathogenesis of CC include *INF-γ*, *IL1β* and *IL-6*.^{28–31}

We observed upregulation of *NOS2*, an established cause of increased intestinal permeability, which is consistent with the results of a prior study.³² *NOS2* can be induced in inflammatory cells and enterocytes by *IFNγ* and *IL1-β*,^{33–35} which we also found to be upregulated.

Our study revealed upregulation of multiple inflammatory chemotactic proteins CCLs and CXCLs and their receptors CCRs and CXCRs, respectively, in agreement with a previous report²⁴ and reflecting an enhanced inflammatory milieu in CC. Similar elevations are well recognized in other inflammatory disorders such as in IBD¹⁷ where they are implicated in critical functions such as lymphocyte homing, neutrophil activation and MMP production.^{36,37}

Figure 5. Heatmap of similarities of gene expression pattern between individual patchy collagenous colitis [CC] involved and uninvolved mucosa, and mixed CC and mixed normal colonic controls [N]. Similarities between gene expression patterns of different samples were calculated based on Euclidean distance and samples were hierarchically clustered. Stronger relationships between samples are indicated by shorter distances in the dendrograms and darker blue colour in the heatmap. Asterisks designate paired involved and uninvolved specimens. Note that 4 of the 5 matched pairs showed high degrees of similarity.

In contrast with data reported in IBD,^{17,38} expression of *IL-22*, *IL-22RA2* [also termed *IL-22BP*] and *TNF α* were not increased in CC. The observation that anti-TNF α agents can have a beneficial role in managing some patients with refractory CC³⁹ might reflect the involvement of other inflammatory pathways in CC or heterogeneity within the disease entity itself.⁴⁰

NF- κ B has been shown to be activated in colonic mucosa from patients with CC,⁴¹ implying intact NOD1/2-NF- κ B signalling. Our observation of unaltered gene expression levels of components involved in NOD2-NF- κ B signalling, together with upregulation of multiple downstream effectors of NF- κ B, support an intact NOD1/2 – NF- κ B cascade in CC. In agreement with this, neither mutations nor polymorphisms of NOD1 or NOD2 have yet been identified in patients with CC.⁴²

MAPK signalling is known to be involved in the pathogenesis of IBD. We did not observe increased expression of MAPK cascade members in CC, including MAP3Ks, MAP2Ks and MAPKs. Nonetheless, alternative mechanisms that regulate MAPK signalling, such as phosphorylation vs de-phosphorylation, cannot be excluded. Taken together, our results highlight several distinctions between the pathogenesis of CC and of IBD.

We observed upregulation of multiple critical Th1 genes in CC, including 31-fold expression of *IFN γ* , supporting the pathogenic role of the Th1 pathway in this disease and in agreement with previous studies.^{23,25,43} Kumawat *et al.* reported that the protein levels of IFN γ are unaltered in CC.²³ Rapid degradation and consumption of IFN γ protein by T lymphocytes are among possible causes of low protein levels, although mRNA levels have been shown to correlate more closely with clinical activity of CC.²³ Another Th1 gene that we found to be upregulated is *FOXP3*, a master regulator of immune tolerance and specific marker of regulatory T cells.

One of the new findings of our study was differential regulation of multiple members of LILRA and LILRB, including 27-fold upregulation of *LILRA3*, a secreted soluble component of the LILRA family. *LILRA3* is thought to suppress the action of LILRB, which is essential to immune tolerance.^{44–49} High serum levels of LILRA3 have been correlated with disease severity in other disorders, including multiple sclerosis and rheumatoid arthritis,^{50,51} and investigation of its serum concentrations in CC patients may therefore be warranted.

A novel feature of our study is the comparison of gene expression profiles between mucosa with classical histological features of CC and adjacent mucosa with normal histology. Despite the conventional impression that CC is histologically diffuse, patchy mucosal distribution has been well documented in the pathology literature.^{52–55}

We used this property to identify molecular changes that are differentially expressed in accordance with histological variations. Unexpectedly, the gene expression profiles in the paired samples were similar in all cases except one, suggesting that the underlying pathogenetic factors may be distributed more diffusely than the histological changes. One biopsy pair, by contrast, demonstrated disparate gene expression profiles, the biopsy with characteristic CC histology resembling the pooled CC results and the histologically normal biopsy resembling the pooled normal controls. Taken together, it appears that the pathogenesis of CC involves factors that are not necessarily faithfully reflected by the tissue histology.

In addition to confirming certain key results reported previously, our study provides new information regarding upregulation of several potentially important factors, including members of LILRA and LILRB as a novel class of immunomodulators in CC and of the fibrolytic genes *MMP3* and *MMP9* in CC. In addition, our results highlight certain distinguishing features between CC and IBD.

Our study has several limitations. First, the expression profiling platform which we utilized does not provide comprehensive data regarding metabolic and signalling pathways related to fibrogenesis and fibrolysis, although it does investigate those which are considered most critical. Second, these analyses do not provide direct measurements of gene function or signalling pathway activity. Third, confirmations of gene expression levels by qRT-PCR were carried out selectively rather than comprehensively.

In summary, we have shown that the colonic mucosa in CC is characterized by altered expression of a limited repertoire of genes involved in nitric oxide synthesis, extracellular matrix remodelling, Th1 immunity and immune modulation. The abnormal gene expression in patchy CC may be similarly expressed in mucosa with and without histological disease manifestations.

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Conflict of Interest

None declared.

Author Contributions

QL: substantial contribution to the study design, study execution, analysis and interpretation of data and drafting of manuscript. NH: substantial contribution to the study design, study execution, data analysis and drafting the manuscript. Both authors approved the manuscript, including the authorship lists.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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