Fibrosis Mediators in the Colonic Mucosa of Acute and Healed Ulcerative Colitis

Mona Dixon Gundersen, MBChB[1,2], Rasmus Goll, MD, PhD[1,2], Christopher Graham Fenton, PhD[3], Endre Anderssen, PhD[3], Sveinung Wergeland Sørbye, MD, PhD[4], Jon Ragnar Florholmen, MD, PhD[1,2] and Ruth Hracky Paulssen, PhD[1,3]

OBJECTIVES: **A healed intestinal mucosa is the aim of therapy in acute ulcerative colitis (UC). Disruption of mucosal wound healing may lead to severe complications including intestinal fibrosis. This study examined mucosal gene expression in the healing process of acute UC with a special focus on known mediators of fibrosis.**

METHODS: **Endoscopic biopsies from patients with acute, moderate to severe UC were analyzed with a quantitative polymerase chain reaction array for 84 genes involved in fibrosis pathways. All patients were treated with infliximab (anti– tumor necrosis factor). Biopsies were taken before therapy and when disease remission was reached, defined as a Mayo score of ≤2, with an endoscopic subscore of 0 or 1. A healthy control group was included. Immunostaining of matrix metallopeptidase 9 and smooth muscle actin was performed.**

RESULTS: **Mucosal biopsies from acute UC (n = 28), remission UC (n = 28), and healthy controls (n = 13) were analyzed. Fibrosis and extracellular matrix-associated genes were upregulated in the endoscopically healed UC mucosa vs controls, with collagen type III alpha 1 chain, actin alpha 2, lysyl oxidase, TIMP metallopeptidase inhibitor 3, and caveolin 1 uniquely showing no overlap with acute disease. Pro- and antifibrotic mediators (interleukin [IL]13 receptor subunit alpha 2, IL1B, IL10, tumor necrosis factor, snail family transcriptional repressor 1, and C-C motif chemokine ligand 2) were upregulated in both acute and healed UC compared with controls. An attenuated pattern of the canonical transforming growth factor beta (TGFB) pathway was observed in acute UC and to a lesser extent in the healed mucosa, except for TGFB2, which was enhanced.**

DISCUSSION: **The endoscopically healed mucosa of UC showed a persisting dysregulation of fibrosis-associated mediators compared with controls, including extracellular matrix remodeling, profibrotic cytokines, and TGFB signaling pathways.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A103

Clinical and Translational Gastroenterology 2019;10:e00082. https://doi.org/10.14309/ctg.0000000000000082

INTRODUCTION

Ulcerative colitis (UC) is a chronic, relapsing inflammatory disease affecting the colon (1). The pathogenesis is complex involving dysregulated immune responses to mucosal injury, with persistent inflammation and disruption of wound healing (2,3). The proinflammatory cytokine tumor necrosis factor (TNF) plays a pivotal role in mediating inflammation in UC. Antibodies targeting TNF induce mucosal healing in over 60% of patients with inflammatory bowel disease (IBD) (4,5). Achieving mucosal healing is the current goal of treatment in IBD as associated with clinical improvement and longer relapse-free periods (6).

There is an increasing need for knowledge of which mediators are involved in mucosal healing. This is emphasized by the fact that 10%–30% of patients with IBD are unresponsive to anti-TNF therapy, as well the lack of therapies targeting intestinal fibrosis in IBD (7,8). Intestinal fibrosis is a severe complication in IBD, causing excessive scar tissue formation in the bowel wall, with distortion of tissue architecture and intestinal function as sequelae (9,10). In UC, up to 11% experience fibrostenotic complications, vs over 50% in Crohn's disease (9). Recent studies suggested that the complications of intestinal fibrosis may be severely underestimated in UC, indicating that fibrosis is more prominent in the pathogenesis of UC than previously attributed (11,12).

[1]Research Group of Gastroenterology and Nutrition, Department of Clinical Medicine, Faculty of Health Sciences, University of Tromsø-The Arctic University of Norway, Tromsø, Norway; [2]Gastroenterology Unit, Department of Medicine, University Hospital of North Norway, Tromsø, Norway; [3]Genomics Support Centre Tromsø (GSCT), Department of Clinical Medicine, UiT-The Arctic University of Norway, Tromsø, Norway; [4]Department of Clinical Pathology, University Hospital of North Norway, Tromsø, Norway.
Correspondence: Mona Dixon Gundersen, MD. E-mail: mgu039@uit.no.
Received May 31, 2019; accepted August 16, 2019; published online October 3, 2019

Following injury to the intestinal barrier, the body is dependent on executing a swift and effective response to prevent pathogen invasion (13). This is a complex process involving hemostasis, followed by fibrogenesis, epithelial regeneration, scar tissue remodeling, and eventually restoration of the intestinal barrier (13,14). Mesenchymal cell activation by transforming growth factor beta (TGFB) is central for production of extracellular matrix (ECM) proteins and wound contraction (ECM) (15). Degradation and turnover of the ECM is tightly regulated by matrix metallopeptidases (MMPs) and their inhibitors (TIMPs) (16). The canonical TGFB pathway is central in fibrosis progression and implicated in IBD (17,18). TGFB binds to the membrane-bound TGFB receptors, which activate intracellular SMAD signaling cascades. Mediators of the TGFB–SMAD pathway are therefore of interest as target for antifibrotic therapy (19–21).

Currently, no methods exist for detecting early stages of intestinal fibrosis (9,10). In this study, we applied a PCR array of fibrosis-associated mediators in a well-stratified cohort of patients with acute UC that have been treated with anti-TNF until disease remission. The differential expression of fibrosis-associated mediators in the healed mucosa of UC may give insights into active pathways and potential therapeutic targets for fibrosis.

MATERIALS AND METHODS

Ethical considerations

The study and storage of biological samples was approved by the Regional Committee for Medical and Health Research Ethics North (Reference no: REK1349/2012) and performed in accordance with the Declaration of Helsinki principles. Written and informed consent was obtained from all study participants.

Patient population

Patients were included from the IBD Biobank at the University Hospital of North Norway; the IBD cohort has previously been described (22). Patients aged 18 years or older, with moderate to severe UC defined as Mayo score ≥6, were included. All patients had been treated with an induction course of infliximab 5 mg/kg intravenously at 0, 2, and 6 weeks followed by maintenance therapy every 4–8 weeks. Only patients who achieved endoscopic remission after infliximab therapy were included. We defined endoscopic remission as a Mayo score ≤2 with no individual subscore >1 (23). Geboes score was assessed on available hematoxylin and eosin-stained slides at endoscopic remission by an experienced pathologist (S.W.S.), with histological remission defined as Geboes score <3.1 (24). Primary sclerosing cholangitis, pregnancy, lactation, and a history of cancer were exclusion criteria.

Tissue samples

Endoscopic biopsies were obtained from the most inflamed region of colonic mucosa before infliximab therapy and a new biopsy obtained from the same region at the time point of disease remission. Biopsies were taken with standard endoscopic forceps and immediately immersed in RNA later (Ambion Austin, TX) or 10% formalin for quantitative polymerase chain reaction (qPCR) or histological analysis, respectively. RNA was extracted using the Promega method (Promega Madison, WI) following manufactures' instructions and stored at −70 °C.

Control group

A healthy control group referred for colonoscopy was included if the following criteria were met: age 18 years or above, a complete colonoscopy examination with normal macroscopic and histological findings, and no diarrhea in the presenting history. Pregnancy or lactation, previous or current cancer, systemic inflammatory disease, and presence or a history of colonic polyps were exclusion criteria. Endoscopic biopsies were taken from the sigmoid or rectal mucosa.

qPCR analysis

RNA concentration was determined with Nanodrop (Thermo Fisher Scientific, Waltham, MA). Reverse transcriptase was performed with Quantitect 2 step Kit (Qiagen, Hilden, Germany). The RT^2 profiler PCR Array Human Fibrosis PAHS-120Z (product nr 330213 Qiagen, Hilden, Germany) was used according to the manufacturer's instructions with RT^{2}SYBER Green mastermix (Qiagen, Hilden, Germany). Real-time qPCR was performed with Bio-Rad CFX96 (Roche Diagnostics, Rotkreuz, Switzerland). A computed tomography (CT) cutoff for lower limit of detection was set at 40 (see Supplementary Information, Supplementary Digital Content 1, http://links.lww.com/CTG/A103).

Immunostaining

Four-μm sections of formalin-fixed paraffin-embedded samples were deparaffinized and rehydrated through a series of xylene to alcohol. DAKO antigen retrieval buffer (pH6) (DAKO, Glostrup Denmark) was used in a waterbath for 20 minutes at 100 °C and 20 minutes of cooling at room temperature. Sections were blocked with 10% goat serum. Primary antibodies were incubated overnight at 4 °C. Secondary goat antibodies conjugated with Alexa 555 (Cell Signaling Technologies, Danvers, MA) or Alexa 647 (Invitrogen, Thermo Scientific, Waltham, MA) were incubated for 90 minutes at room temperature, and cell nuclei stained with Hoechst 33258. Negative isotype- and concentration-matched controls were used (antibody details listed in Supplementary Table 1, see Supplementary Digital Content 1, http://links.lww.com/CTG/A103). Images were obtained with a Zeiss LSM780 CLSM microscope (Carl Zeiss Microscopy, Zena, Germany) using Zen 2012 black edition software. Three nonoverlapping, representative images at ×200 magnification were used for quantification with Volocity 6.3 software (PerkinElmer Waltham, MA) with positive fluorescence area given as a ratio over total nuclei area for each slide. Images were processed in Adobe Photoshop CC 2019 (Adobe Systems Software, Ireland Ltd, Dublin, Ireland), and adjustments to image histograms were applied to whole image only. The Human Protein Atlas database (www.proteinatlas.org) was used to identify validated antibody staining with a high reliability score ("enhanced score").

Statistics

Data were analyzed using R Statistical Environment (https://www.r-project.org) with Bioconductor R software for principal component analysis and impute package for missing values. Normfinder (https://moma.dk/normfinder-software) software was used for evaluating reference genes used for normalization (25).

For comparative gene analysis, we used the linear modeling framework from the R Stats Package with the (lm() function using normalized CT values (delta CT). Independent samples and paired samples were taken into account. Correction for sex and endoscopic remission status was performed. P values were adjusted for multiple testing using the Benjamini Hochberg method, with a significant adjusted $P < 0.05$ set as threshold (26). Comparison of gene expression between groups was given as log2 fold

change. Quantification of immunostaining was performed with *t* tests for independent groups and a paired *t* test when comparing acute and remission disease, all after control of normality with IBM SPSS Statistics version 25.0 (Armonk, NY). Plot and bar charts were visualized with GraphPad prism v. 7 (La, Jolla, CA).

RESULTS

Patient population

Twenty-eight patients with moderate to severe acute UC and 13 healthy controls met the study inclusion criteria. Five patients with UC were newly diagnosed, whereas the majority had well-established disease. Patient demographics are shown in Table 1. At endoscopic remission, 16 patients had a Mayo endoscopic subscore of 0. For endoscopic remission, histology was available for n = 7 patients with UC, of these, n = 6 were in histological remission (Geboes score <3.1) (See Supplementary Table 2, Supplementary Digital Content 1, http://links.lww.com/CTG/A103). Biopsies from all healthy controls (n = 13) showed normal histological findings, with no inflammation.

Unique expression pattern in the healed mucosa of UC

All samples passed qPCR quality control, and interleukin 5 (IL5) was excluded because of >25% of CT values over 40 and not included in further analyses. Principal component analysis showed separation of acute UC, remission UC, and healthy control samples in the first principal component, with 44.5% of the variance explained (Figure 1a). Gene expression analysis revealed a significant number of differently expressed genes in all sample groups with a total of 41 upregulated and 38 downregulated genes. A Venn diagram (Figure 1b) depicts the overlap of differentially expressed genes between the comparisons of groups with active UC (A), remission (R), and control samples (N). We noted that 6 genes were uniquely expressed in the endoscopically healed mucosa of UC vs controls (differentially expressed compared with control samples with no overlap to active UC). These genes actin alpha 2, smooth muscle (ACTA2), caveolin 1 (CAV-1), collagen type III alpha 1 chain (COL3A1), lysyl oxidase (LOX), and TIMP metallopeptidase inhibitor 3 (TIMP3), were all upregulated compared with healthy controls, whereas integrin subunit beta 6 (ITGB6) was significantly downregulated. The latter 4 genes (COL3A1, LOX, TIMP3, and ITGB6) are associated with the ECM remodeling. Angiotensinogen (AGT) was the only gene with an expression pattern showing upregulation in acute disease and downregulation in healed mucosa compared with controls. The entire gene expression pattern is shown in Figure 2a. Gene table and pathway annotations are given in Supplementary Table 3 and Supplementary Figure 1 (see Supplementary Digital Content 1, http://links.lww.com/CTG/A103).

Attenuation of the TGFB canonical pathway in UC

The profibrotic TGFB-SMAD pathway (illustrated in Figure 3) showed a significant dysregulated signaling pattern in acute UC and to a lesser extent in endoscopic remission (Figure 2b).

Table 1. Clinical characteristics at study inclusion time for patients with acute UC and control group

	UC (n = 28)	Controls n = 13	*P*-value[a]
Sex, male/female (n)	18/10	7/6	0.73
Age at diagnosis (median, [range])	51 (18–69)	39 (20–82)	0.05
Smoking status (n) non-/ex-/current smoker	16/1/11	—	NA
Disease duration mo (median, [range])	52.5 (0–372)	—	NA
Disease extent (n)			
Proctitis	6	—	NA
Left sided	15	—	NA
Extensive	7	—	NA
Biopsy location endoscopy (n) (rectum/sigmoid)	13/15	5/8	0.5
Mayo score baseline	10 (7–12)	—	NA
Endoscopic subscore	3 (1–3)[c]	—	NA
Medication at baseline[b]			
5-ASA	19	0	NA
Steroids po/iv	12/0	0	NA
AZA or MTX	11	0	NA
Anti-TNF	0	0	NA

Variables are given as median with range, if not otherwise indicated. No significant differences ($P < 0.05$) were found between the UC and the control group with regard to age, sex, or biopsy location (independent samples *t* test and Fisher exact test for continuous and categorical variables were used, respectively). Disease extent was defined using the Montreal Classification as recommended by ECCO guidelines (6). Patient medication at baseline included no anti-TNF therapy within the last 3 months.
5-ASA, 5-aminosalicylate; AZA, azathioprine; MTX, methotrexate; NA, not applicable; TNF, tumor necrosis factor; UC, ulcerative colitis.
[a]Comparison between UC and healthy controls.
[b]Patients can be on >1 medication at baseline.
[c]Two patients had an endoscopic subscore of 1, however met the inclusion criteria as a full Mayo score was >6, both with high rectal bleeding scores and histologically active disease with cryptitis and crypt abscesses.

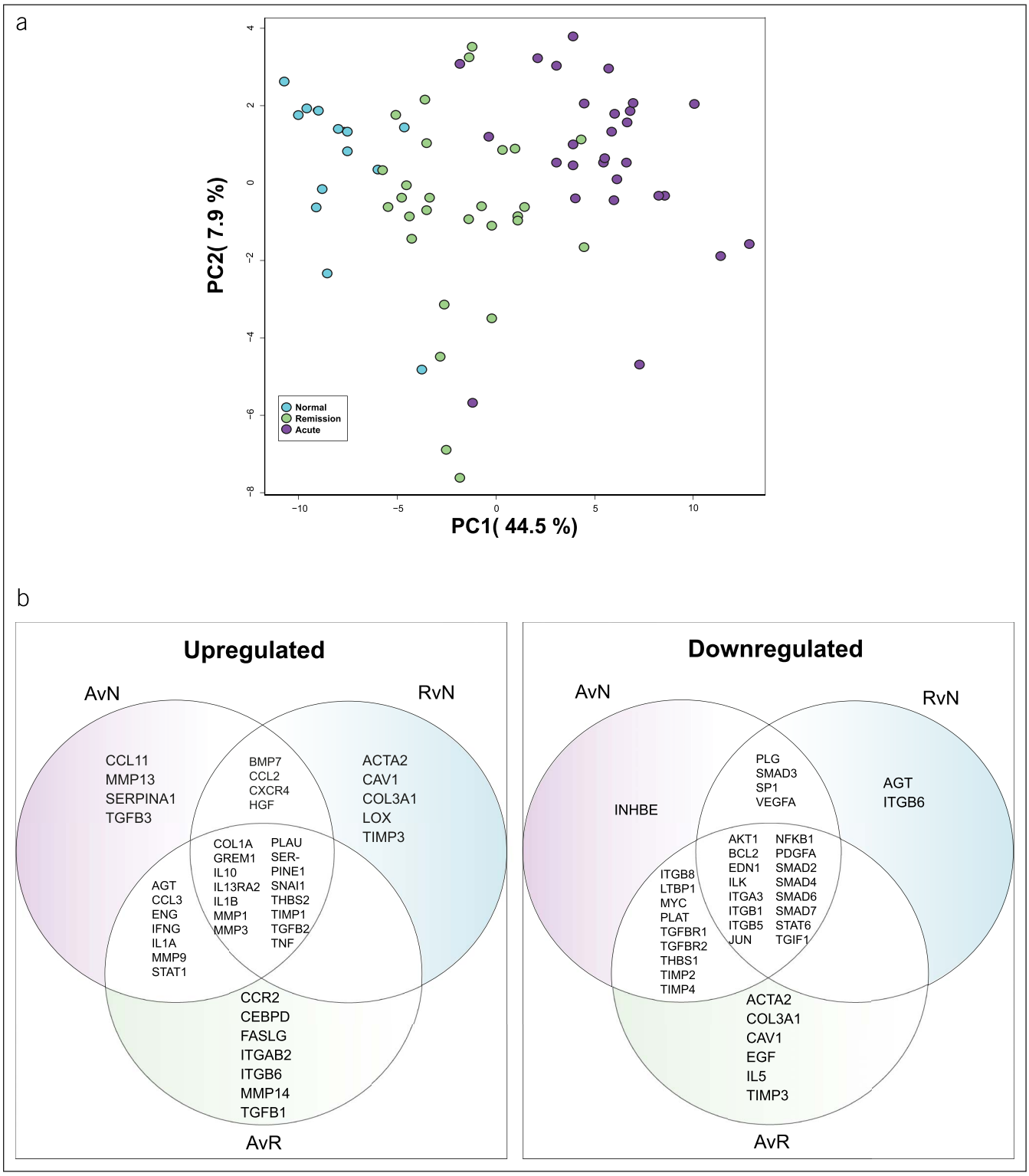

Figure 1. Principal component plot and Venn diagrams. (**a**) PCA with separation of groups seen along the x-axis. Of note, 44.5% of the variance is explained by PCA1. (**b**) Venn diagrams showing overlap of significantly expressed genes (adjusted $P < 0.05$) between groups: acute UC vs healthy control group (AvN), remission UC vs healthy control group (RvN), and acute UC vs remission UC (AvR). PCA, principal component analysis; UC, ulcerative colitis.

Comparison of acute UC and healthy controls revealed an upregulation of TGFB2 and TGFB3, but not TGFB1. Furthermore, the corresponding receptors for these ligands, TGFB receptor 1 (TGFBR1) and TGFB receptor 2 (TGFB2), were downregulated along with intracellular SMAD signaling mediators SMAD2, SMAD3, SMAD4, SMAD6, and SMAD7. Inhibitors of the

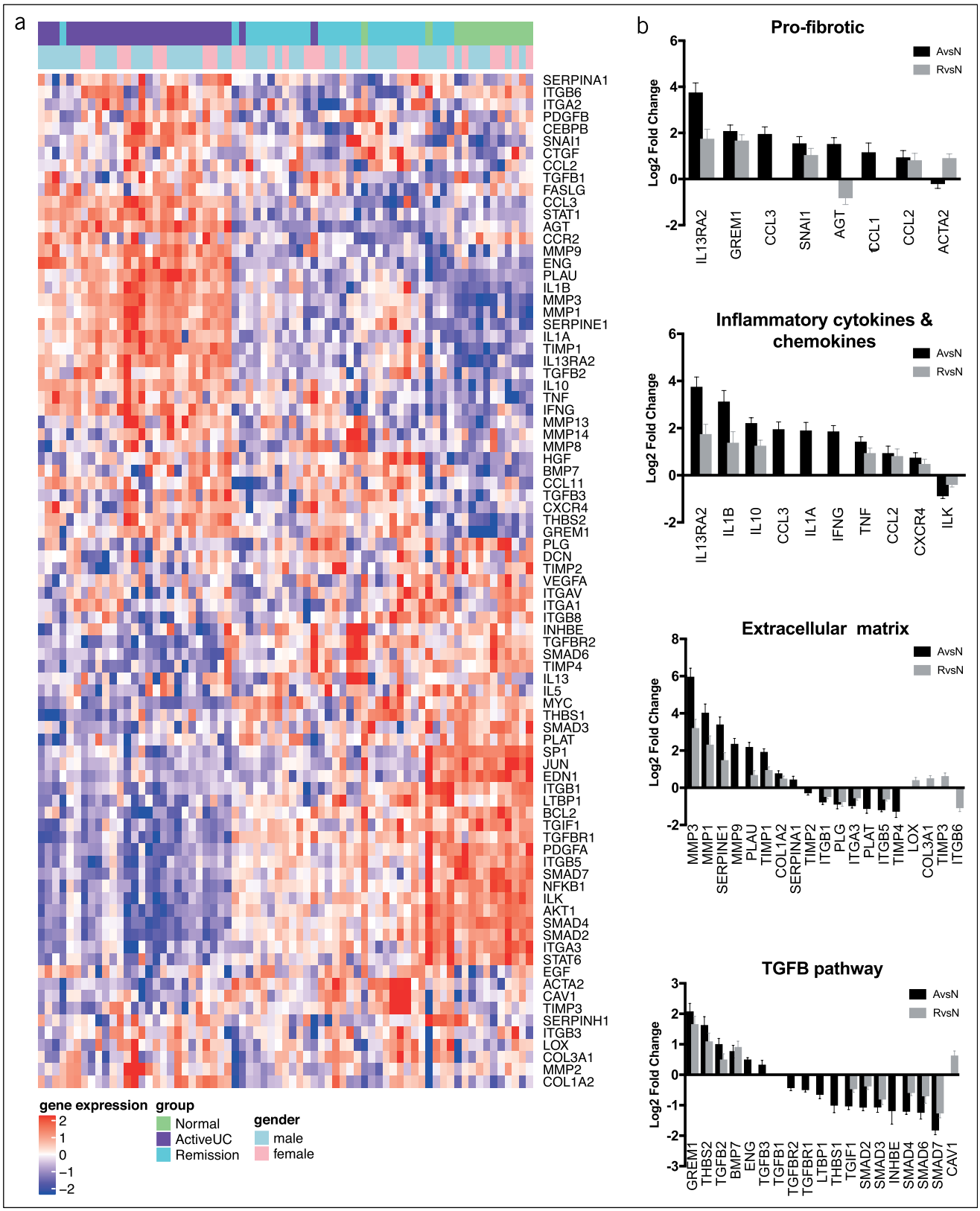

Figure 2. Gene expression analysis. (**a**) Heatmap of all genes examined in the PCR array with an unsupervised cluster analysis, with study groups and sex given. (**b**) Pathway analysis was performed using gene annotations provided by RT^2 Profiler Human Fibrosis array (Qiagen). Selected bar charts show log2 fold changes with SE given for significantly differentially expressed genes (adj. $P < 0.05$). Group comparisons are given as follows: acute UC vs healthy control group (AvsN) and remission UC vs healthy control group (RvsN).UC, ulcerative colitis.

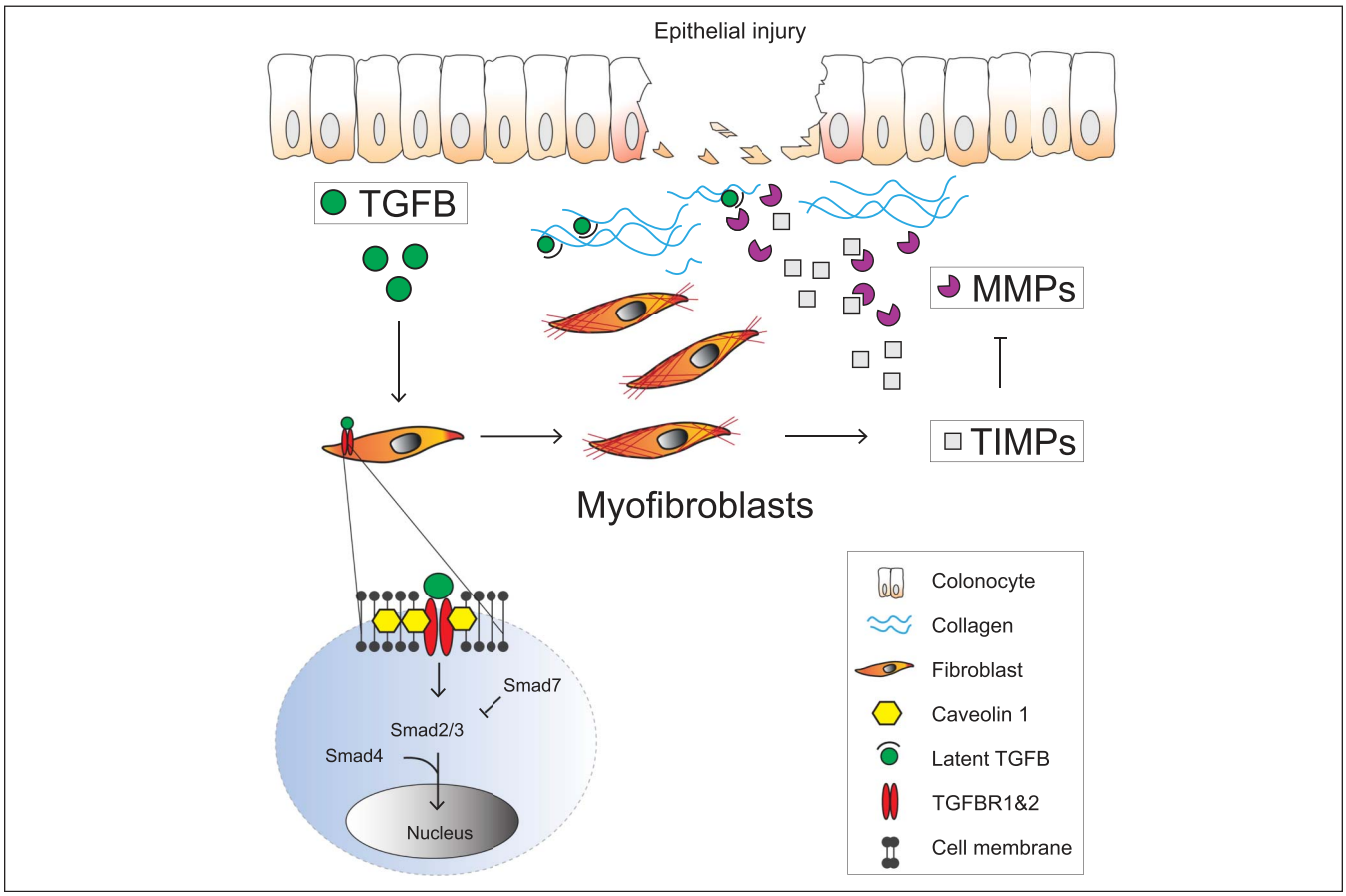

Figure 3. TGFB signaling in the colonic mucosa. Following injury to the epithelial barrier, activity of matrix metallopeptidases (MMPs) is increased. TGFB is bound in a latent form in the extracellular matrix. MMPs degrade the collagen matrix, also releasing and activating TGFB (19). The canonical TGFB pathway involves activation of membrane-bound TGFB receptors 1 and 2, which phosphorylates SMAD 2/3 and together with SMAD4 before translocating to the nucleus (21). TGFB promotes recruitment and differentiation of fibroblasts into myofibroblasts, increasing production of collagen and TIMPs. Caveolin 1, a glycoprotein in the cell membrane, inhibits this signaling by internalizing the TGFB receptors (36). TIMP, tissue inhibitor of metallopeptidase.

TGFB-SMAD pathway, THBS2 and BMP7, were increased in both the acute and endoscopically healed UC mucosa. Furthermore, TGFB3 normalized, whereas a persistent overexpression of TGFB2 was observed at the time of endoscopic remission.

Other relevant fibrotic mediators in UC

ECM remodeling enzymes MMP3 and MMP1 showed the most prominent log2 fold changes in acute and healed UC compared with controls. Proinflammatory cytokines and chemokines were strongly upregulated during acute disease, including IL 1 beta (IL1B), interferon gamma (IFNG), and TNF (Figure 2b). Comparison of the healed UC mucosa with the healthy control group revealed profibrotic genes IL 13 receptor subunit alpha 2 (IL13RA2) and Gremlin-1 (GREM 1) among the top overexpressed genes. Several upregulated inflammatory mediators normalized completely in the healed mucosa including MMP9, IFNG, IL 1alpha (IL1A), and C-C motif chemokine ligand 3 (CCL3). Immunostaining was consistent with the gene expression pattern of MMP9 (shown in Figure 4). In contrast, several fibrosis mediators showed little or no change in gene expression following anti-TNF therapy (BMP7, GREM1, CCL2, and CXCR4). In this cohort, TNF was still found to be upregulated in the mucosa following anti-TNF therapy. Comparison of endoscopic subscore 0 and 1 in disease remission revealed significant differences in 12 genes with log2 fold changes <1. Of these genes, IL10 and MMP14 were upregulated, whereas the remaining genes were downregulated (integrin subunit alpha 1 (ITGA1), integrin subunit beta 1 (ITGB1), integrin subunit beta 6 (ITGB6), SMAD4, signaling transducer and activator of transcription 6 (STAT6), epidermal growth factor, specificity protein 1 (SP1), and latent transforming growth factor–binding protein 1 (LTBP1). The remaining genes ($n = 31$) were not affected by endoscopic remission score 0 or 1. Correction for sex revealed only CCL3 to show a significant sex difference (log2 fold change male vs female: −0, 15).

DISCUSSION

We have clearly demonstrated that genes involved in ECM remodeling and fibrosis development do not all normalize in the endoscopically healed mucosa of UC (defined as Mayo endoscopic subscore of 0 or 1). Over half of the investigated fibrosis-associated genes were differentially expressed in the healed UC mucosa. This supports the notion that fibrosis is indeed an important component in the pathophysiology of UC. We found an increased TGFB2 and TGFB3 ratio in acute UC, with TGFB3 expression not normalizing in endoscopic

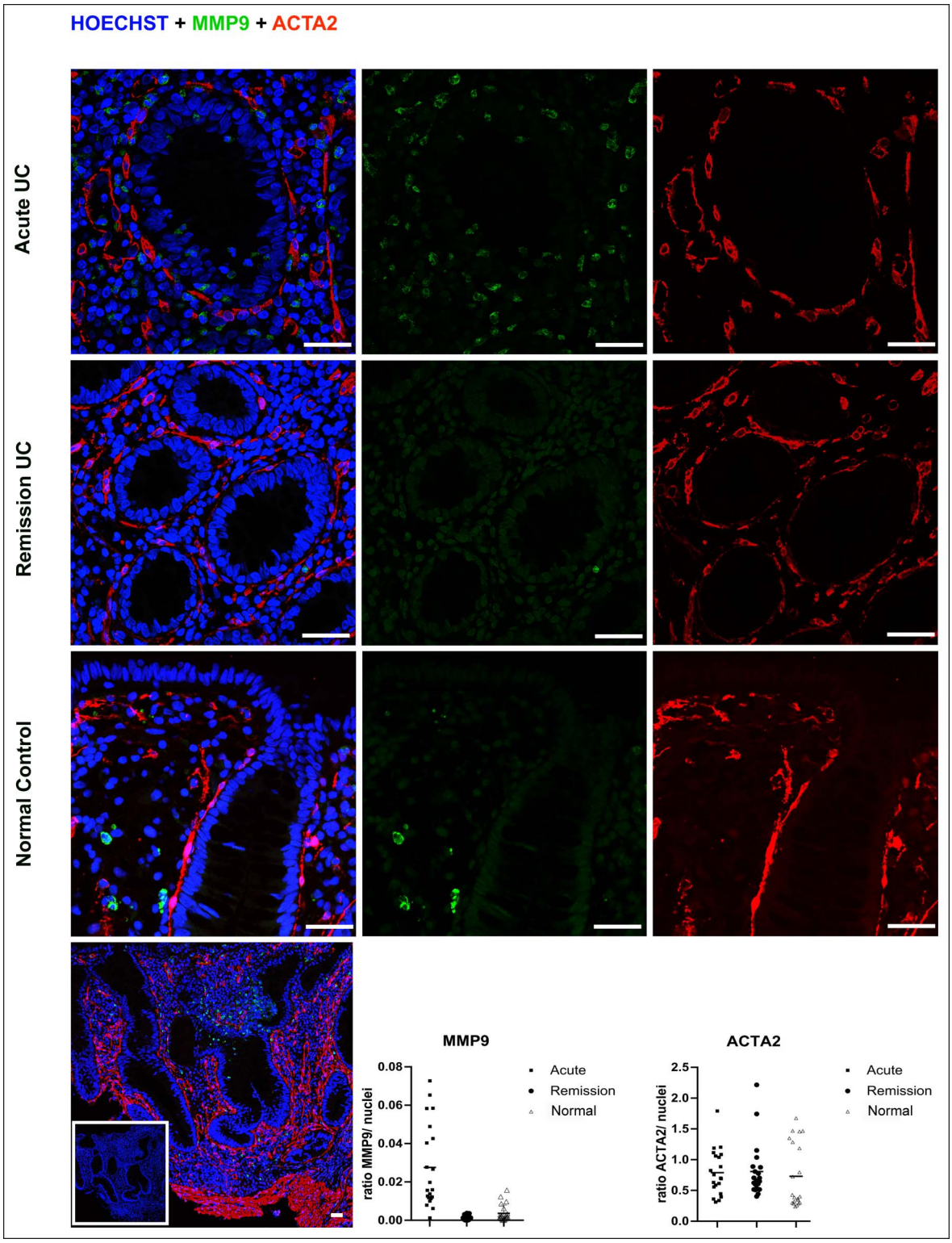

Figure 4. Immunostaining of MMP9 and ACTA2. Antibodies directed at MMP9 and ACTA2 had the highest upregulated and downregulated gene patterns in our study with enhanced antibody scores. Panels show representative immunostaining of formalin-fixed, paraffin-embedded colonic endoscopic biopsies from acute UC, remission UC, and a healthy control group at ×400 magnification. Scale bars = 35 μm. Dual staining with monoclonal rabbit antibody against MMP9 ([1/400], Cell Signaling Technologies) and monoclonal mouse antibody against ACTA2/SMA (DAKO, [0.35 μg/mL]) were used. Cell nuclei are stained with Hoechst (blue). Positive cytosolic staining of stromal cells was observed for MMP9 (green), whereas a membranous/cytoplasmic signal was seen in ACTA2-positive cells (red). A significant decrease ($P < 0.05$) of MMP9 was seen comparing acute UC with remission UC or normal controls, but not for ACTA2. Further antibody details are given in Supplemental Table 1(see Supplementary Digital Content, http://links.lww.com/CTG/A103). A negative control is shown in the white frame for acute UC (bottom left panel). ACTA2, actin alpha 2; UC, ulcerative colitis.

remission. In contrast, the well-known profibrotic TGFB1 was present, but not significantly expressed in the UC mucosa. We believe that the balance of TGFB isoforms in different UC disease phases may be important for achieving mucosal healing and a potential factor to modulate. Indeed, others have reported differences in the TGFB ratio between patients with UC who respond or not to anti-TNF therapy (27). Furthermore, intestinal myofibroblasts isolated from healthy controls and patients with IBD have been shown to secrete different TGFB isoform ratios (28). All 3 TGFB isoforms are present in the intestine; however, their role in UC is unclear (19,21). Looking to other organ systems, TGFB1 and TGFB2 have been found to promote ECM deposition during skin wound healing, whereas TGFB3 reduces scarring (19,29). In sum, knowledge of the TFB isoforms role in UC is incomplete, warranting further research.

Although we found an upregulation of the TGFB2 and TGFB3, the canonical TGFB pathway showed an attenuated response with downregulated TGFB receptors (TGFBR1 and TGFBR2), together with intercellular SMAD signaling mediators. This might be an appropriate response in the acute phase of inflammation, as TGFB is recognized to be released from its latent bound form in the ECM by MMPs and other ECM–degrading factors (19). Indirectly, our findings support this with overexpression of MMPs and extracellular remodeling enzymes in acute UC, suggesting an abundance of released and activated TGFB. However, the canonical TGF pathway does not normalize in the endoscopically healed UC mucosa, suggestive of a persistent dysregulation.

As expected, proinflammatory genes showed a pattern of reduction, and even normalization following anti-TNF therapy in agreement with other studies (27,30–33). In contrast, several fibrosis mediators showed little or no change in gene expression following anti-TNF therapy including BMP7, GREM1, CCL2, and CXCR4. Specific to the endoscopically healed mucosa, a set of genes associated with collagen synthesis and degradation (ACTA2, COL3A1, LOX, and TIMP3) were upregulated compared with controls. In summary, our findings show that fibrosis-associated mediators are still dysregulated in the endoscopically healed UC mucosa compared with healthy controls. This is in keeping with other reports that the healed UC mucosa remains dysregulated (27,32). A recent study by Arijs et al. found a large overlap of mucosal genes that were persistently dysregulated in patients with UC responding to infliximab (anti-TNF) or vedolizumab (anti-$\alpha 4\beta b7$ integrin), suggesting that unidentified pathways are yet to be targeted (34). Furthermore, vedolizumab was found to influence a unique set of mucosal genes independent of anti-TNF therapy. It is exceedingly interesting whether the new biological therapies, including vedolizumab and ustekinumab (inhibitor of p40 subunit of IL12 and IL23), will target additional pathways and influence fibrosis. Our findings highlight the current knowledge gap of fibrosis-associated pathways in UC, also evident in the clinics as no effective antifibrotic therapies exist.

Our finding of an upregulated CAV-1 in the healed UC mucosa may reflect a fibroprotective mechanism, as reported in murine studies (35). The protein Cav-1 acts as the scaffolding in caveolae of the cell membrane, important for endocytosis and cell signaling (36). However, to our knowledge, the role of CAV-1 in IBD is little explored. One study supports the location of CAV-1 and -2 expression in the colonic mucosa (37). Interestingly, the literature reports CAV-1 to exhibit antifibrotic properties by binding and internalizing TGFB receptors on the cell membrane, effectively attenuating TGFB signaling and reducing collagen production (38). A low gene expression of CAV-1 has been reported in other fibrotic disorders including idiopathic pulmonary fibrosis and scleroderma (38). In addition, we found an interesting gene expression pattern for AGT, being upregulated in acute disease and downregulated in the healed UC mucosa. Mediators of the renin-angiotensin-aldosterone system are found in many tissues including the colon (39). Inhibitors of the renin-angiotensin-aldosterone system are well-established therapies for prevention of end-organ damage in cardiac and renal disease, and also of interest in IBD as therapeutic targets for inflammation and fibrosis.

IL13RA2 was among the top DEGs in our study. Interestingly, high mucosal expression of this receptor in IBD has been linked to anti-TNF unresponsiveness and impaired restoration of the intestinal barrier in a murine Dextran sulfate sodium colitis model (40). Both profibrotic and antifibrotic properties have been described in mouse models of colitis and pulmonary fibrosis, respectively; thus, tissue and cellular context is important (41,42). Taken together, IL13RA2 is emerging as an interesting future target both for refractory UC and in modulating fibrosis.

A limitation to the study is the lack of a general consensus on what constitutes a "truly healed" mucosa. The widely used Mayo score includes endoscopic subgrades 0 and 1 in disease remission, with a score of 1 allowing for the presence of mild erythema, decreased vascular pattern, and mild friability. The definitions of mucosal healing are currently being debated, with endoscopic subscore 0 and histological remission being associated with improved clinical outcomes (43). However, histological remission does not necessary imply endoscopic remission and *vice versa*. In a recent study, Magro et al. (44) showed that histological indexes could be associated with endoscopic outcomes with a high sensitivity when the Mayo endoscopic subscore was set at 1. Of the 7 patients in our study with available histological assessment, 1 patient met the criteria for histological remission with an endoscopic subscore of 1. This raises the questions of how do we distinguish between transcription patterns of inflammation, tissue restitution, and those representing the underlying disease? Some overlap is likely. Is the UC mucosa ever truly inactive? Arguably, we could have used stricter remission criteria; on the other hand, most genes did not show any significant difference when corrected for endoscopic score of 0 and 1 in disease remission. Although the paired samples design is a strength reducing interpatient variation, our sample size was limited by available biopsies in the IBD Biobank and at risk of type II error. Another limitation is the use of a preselected gene panels associated with general human fibrosis, thus not comprehensive. The heterogeneity of cell populations across biopsies is also important to take into account when interpreting results (45).

Apart from CCL3, we did not uncover any sex-dimorphic patterns for our gene set. In our study, most patients with UC had well-established UC; however, the role of disease duration on fibrosis development is not clear. A large histopathology study of fibrosis in UC found that the degree of fibrosis was linked to the severity ad chronicity of disease and, interestingly, not to disease duration (46). Thus, intestinal fibrosis is also a complication occurring in short-standing UC. Although different disease entities, we and others have previously found that the mucosal gene expression of inflammatory cytokines between UC and Crohn's disease is remarkably similar (47,48). In view of this, our findings could be relevant for future research in Crohn's disease; however, this remains to be explored. Comparison of mucosal fibrosis mediators in patients with UC with established fibrotic disease was not within the scope of the present

study, but of interest for future work. Combining knowledge of mucosal fibrosis mediators between anti-TNF responders and nonresponders may be useful as therapeutic biomarkers, providing clinicians with tools to personalize therapy.

In conclusion, we have seen a significant modulation of genes associated with both inflammation and fibrosis in patients treated to a clinical and endoscopically verified healed mucosa. The mucosa of UC in disease remission showed a persistent dysregulation of fibrosis-associated genes, with an attenuated pattern of TGFB signaling mediators in UC. We identified mucosal TGFB isoforms, CAV-1 and AGT as potential antifibrotic targets in UC, of interest for future research.

CONFLICTS OF INTEREST

Guarantor of the article: Mona Dixon Gundersen, MD.

Specific author contributions: M.D.G: planning, conducting the study, analysis, interpretation, drafting the manuscript, and approved the final submitted draft. R.G.: planning the study, manuscript review and writing, and approved the final submitted draft. C.G.F: statistical analysis and data interpretation, review of the manuscript, and approved the final submitted draft. E.A: statistical analysis and data interpretation, visualization and editing/writing of the manuscript, and approved the final submitted draft. S.W.S.: analysis of the study material, interpretation, review of the manuscript, and approved the final submitted draft. J.R.F.: planning the study, interpretation of data, manuscript review and editing, and approved final submitted draft. R.H.P: planning and conducting the study, interpretation and data analysis, manuscript review and editing, and approved the final submitted draft.

Financial support: This work was supported by the Northern Norway Regional Health Authority (SFP-1134-13).

Potential competing interests: None to report.

ACKNOWLEDGEMENTS

We thank colleagues at the Gastroenterology Department, University Hospital North Norway, for inclusion and follow-up of study participants; Hagar Taman and Kristine Osther for excellent assistance with qPCR array; and the Imaging Core Facility at UiT—The Arctic University of Norway for assistance with image acquisition.

Study Highlights

WHAT IS KNOWN

- ✓ Intestinal fibrosis is a severe complication in IBD.
- ✓ No effective antifibrotic therapies exist for intestinal fibrosis.
- ✓ Recent studies report that intestinal fibrosis is underestimated in UC.

WHAT IS NEW HERE

- ✓ Profibrotic mediators are dysregulated in the endoscopically healed mucosa of UC, including transforming growth factor beta 2 (TGFB2).
- ✓ Genes involved in ECM remodeling were uniquely upregulated in the endoscopically healed mucosa of UC compared with healthy controls.

TRANSLATIONAL IMPACT

- ✓ Identification of mucosal markers warranting further exploration as potential antifibrotic targets in UC therapy.

REFERENCES

1. Magro F, Gionchetti P, Eliakim R, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and Ileo-anal pouch disorders. J Crohn's Colitis 2017;11(6):649–70.
2. Friedrich M, Pohin M, Powrie F. Cytokine networks in the pathophysiology of inflammatory bowel disease. Immunity 2019;50(4): 992–1006.
3. Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014;14(5):329–42.
4. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353(23): 2462–76.
5. Pineton de Chambrun G, Peyrin-Biroulet L, Lemann M, et al. Clinical implications of mucosal healing for the management of IBD. Nat Rev Gastroenterol Hepatol 2010;7(1):15–29.
6. Harbord M, Eliakim R, Bettenworth D, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 2: Current management. J Crohns Colitis 2017;11(7):769–84.
7. Rieder F, de Bruyn JR, Pham BT, et al. Results of the 4th scientific workshop of the ECCO (group II): Markers of intestinal fibrosis in inflammatory bowel disease. J Crohns Colitis 2014;8(10):1166–78.
8. Roda G, Jharap B, Neeraj N, et al. Loss of response to anti-TNFs: Definition, epidemiology, and management. Clin Transl Gastroenterol 2016;7:e135.
9. Rieder F, Fiocchi C, Rogler G. Mechanisms, management, and treatment of fibrosis in patients with inflammatory bowel diseases. Gastroenterology 2017;152(2):340–50 e346.
10. Lenti MV, Di Sabatino A. Intestinal fibrosis. Mol Aspects Med 2019;65: 100–9.
11. Latella G, Rieder F. Time to look underneath the surface: Ulcerative colitis-associated fibrosis. J Crohns Colitis 2015;9(11):941–2.
12. de Bruyn JR, Meijer SL, Wildenberg ME, et al. Development of fibrosis in acute and longstanding ulcerative colitis. J Crohns Colitis 2015;9(11): 966–72.
13. Leoni G, Neumann PA, Sumagin R, et al. Wound repair: Role of immune-epithelial interactions. Mucosal Immunol 2015;8(5):959–68.
14. Latella G, Rogler G, Bamias G, et al. Results of the 4th scientific workshop of the ECCO (I): Pathophysiology of intestinal fibrosis in IBD. J Crohns Colitis 2014;8(10):1147–65.
15. Lawrance IC, Rogler G, Bamias G, et al. Cellular and molecular mediators of intestinal fibrosis. J Crohns Colitis 2017;11(12):1491–503.
16. O'Sullivan S, Gilmer JF, Medina C. Matrix metalloproteinases in inflammatory bowel disease: An update. Mediators Inflamm 2015;2015: 964131.
17. Kotlarz D, Marquardt B, Barøy T, et al. Human TGF-β1 deficiency causes severe inflammatory bowel disease and encephalopathy. Nat Genet 2018; 50(3):344–8.
18. Sedda S, Marafini I, Dinallo V, et al. The TGF-beta/smad system in IBD pathogenesis. Inflamm Bowel Dis 2015;21(12):2921–5.
19. Morikawa M, Derynck R, Miyazono K. TGF-beta and the TGF-beta family: Context-dependent roles in cell and tissue physiology. Cold Spring Harb Perspect Bio 2016;8(5):a021873.
20. Ihara S, Hirata Y, Koike K. TGF-beta in inflammatory bowel disease: A key regulator of immune cells, epithelium, and the intestinal microbiota. J Gastroenterol 2017;52(7):777–87.
21. Yun SM, Kim SH, Kim EH. The molecular mechanism of transforming growth factor-beta signaling for intestinal fibrosis: A mini-review. Front Pharmacol 2019;10(162):162.
22. Gundersen MD, Goll R, Hol J, et al. Loss of interleukin 33 expression in colonic crypts—A potential marker for disease remission in ulcerative colitis. Sci Rep 2016;6:35403.
23. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987;317(26):1625–9.
24. Geboes K, Riddell R, Ost A, et al. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut 2000; 47(3):404–9.
25. Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 2004;64(15): 5245–50.

26. Benjamini Y, Hochberg Y. Controlling the false discovery rate—A practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 1995;57(1):289–300.
27. Toedter G, Li K, Sague S, et al. Genes associated with intestinal permeability in ulcerative colitis: Changes in expression following infliximab therapy. Inflamm Bowel Dis 2012;18(8):1399–410.
28. McKaig BC, Hughes K, Tighe PJ, et al. Differential expression of TGF-beta isoforms by normal and inflammatory bowel disease intestinal myofibroblasts. Am J Physiol Cell Physiol 2002;282(1):C172–182.
29. So K, McGrouther DA, Bush JA, et al. Avotermin for scar improvement following scar revision surgery: A randomized, double-blind, within-patient, placebo-controlled, phase II clinical trial. Plast Reconstr Surg 2011;128(1):163–72.
30. Arijs I, Li K, Toedter G, et al. Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis. Gut 2009;58(12):1612–9.
31. Toedter G, Li K, Marano C, et al. Gene expression profiling and response signatures associated with differential responses to infliximab treatment in ulcerative colitis. Am J Gastroenterol 2011;106(7):1272–80.
32. Planell N, Lozano JJ, Mora-Buch R, et al. Transcriptional analysis of the intestinal mucosa of patients with ulcerative colitis in remission reveals lasting epithelial cell alterations. Gut 2013;62(7):967–76.
33. de Bruyn M, Machiels K, Vandooren J, et al. Infliximab restores the dysfunctional matrix remodeling protein and growth factor gene expression in patients with inflammatory bowel disease. Inflamm Bowel Dis 2014;20(2):339–52.
34. Arijs I, De Hertogh G, Lemmens B, et al. Effect of vedolizumab (anti-alpha4beta7-integrin) therapy on histological healing and mucosal gene expression in patients with UC. Gut 2018;67(1):43–52.
35. Weiss CR, Guan Q, Ma Y, et al. The potential protective role of caveolin-1 in intestinal inflammation in TNBS-induced murine colitis.PLoS One 2015;10(3):e0119004.
36. de Almeida CJG. Caveolin-1 and caveolin-2 can be antagonistic partners in inflammation and beyond. Front Immunol 2017;8:1530.
37. Andoh A, Saotome T, Sato H, et al. Epithelial expression of caveolin-2, but not caveolin-1, is enhanced in the inflamed mucosa of patients with ulcerative colitis. Inflamm Bowel Dis 2001;7(3):210–4.
38. Gvaramia D, Blaauboer ME, Hanemaaijer R, et al. Role of caveolin-1 in fibrotic diseases. Matrix Biol 2013;32(6):307–15.
39. Romero CA, Orias M, Weir MR. Novel RAAS agonists and antagonists: Clinical applications and controversies. Nat Rev Endocrinol 2015;11(4):242–52.
40. Verstockt B, Perrier C, De Hertogh G, et al. Effects of Epithelial IL-13Rα2 Expression in Inflammatory Bowel Disease. Front Immunol 2018;9:2983.
41. Fichtner-Feigl S, Strober W, Kawakami K, et al. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med 2006;12(1):99–106.
42. Lumsden RV, Worrell JC, Boylan D, et al. Modulation of pulmonary fibrosis by IL-13Rα2. Am J Physiol Lung Cell Mol Physiol 2015;308(7):L710–8.
43. Sturm A, Maaser C, Calabrese E, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD Part 2: IBD scores and general principles and technical aspects. J Crohns Colitis 2019;13(3):273–84.
44. Magro F, Lopes J, Borralho P, et al. Comparison of different histological indexes in the assessment of UC activity and their accuracy regarding endoscopic outcomes and faecal calprotectin levels. Gut 2019;68(4):594–603.
45. Taman H, Fenton CG, Hensel IV, et al. Transcriptomic landscape of treatment-naive ulcerative colitis. J Crohns Colitis 2018;12(3):327–36.
46. Gordon IO, Agrawal N, Willis E, et al. Fibrosis in ulcerative colitis is directly linked to severity and chronicity of mucosal inflammation. Aliment Pharmacol Ther 2018;47(7):922–39.
47. Olsen T, Rismo R, Cui G, et al. TH1 and TH17 interactions in untreated inflamed mucosa of inflammatory bowel disease, and their potential to mediate the inflammation. Cytokine 2011;56(3):633–40.
48. Granlund Av, Flatberg A, Østvik AE, et al. Whole genome gene expression meta-analysis of inflammatory bowel disease colon mucosa demonstrates lack of major differences between Crohn's disease and ulcerative colitis. PLoS One 2013;8(2):e56818.