substrates also generated via a fatty acid (FA) biosynthetic cascade. Dietary studies using fish oil-derived EPA have been disappointing in UC; we hypothesised that the PUFA biosynthetic pathway in inflamed tissue is altered. This study evaluated PUFA profile in inflamed and non-inflamed mucosa from UC patients and compared to matched controls.

Methods Ethical approval was obtained. Patients were prospectively recruited from outpatients' clinics. Mucosal biopsies at flexible sigmoidoscopy (FS) were taken from UC patients within inflamed and normal proximal mucosa. Age-sex matched control patients undergoing FS for functional symptoms were compared. Inflammation was scored endoscopically and histologically. Membrane bound FA (MBFA): Biopsies were spiked with deuterated internal standard, followed by liquid-liquid extraction and quantitative gas chromatography mass spectrometry (MS). Free Fatty Acid (FFA): Biopsies were homogenised, followed by solid phase extraction and liquid chromatography orbitrap MS. Data were expressed as percentage abundance. Dietary fatty acid analysis was undertaken. Wilcoxon signed rank pair and Spearman's correlation analysis were employed.

Results 69 active UC patients (54 paired normal/inflamed mucosa) and 69 controls were compared. No biologically significant differences were noted between endoscopically normal mucosa from UC patients and controls other than DPA ($p<0.0025$). Inflamed mucosa compared to non-inflamed mucosa demonstrated highly significant reduction in LA and αLNA ($p<0.0001$) and increased AA, DPA, and DHA ($p<0.0001$); EPA was reduced ($p<0.005$). The ratio of AA/EPA was increased in inflamed mucosa ($p<0.0001$). These findings are consistent between MBFA and FFA and correlate with severity of inflammation.

Conclusion Mucosal PUFA bioavailability is altered in active UC, with significant elevation of AA and reduction of LA, αLNA and EPA. This suggests modification of the FA biosynthetic pathway with elevated delivery of AA as a precursor of pro-inflammatory eicosanoids in active UC. These findings may explain the lack of efficacy of supplemental fish oil and suggests new alternative therapeutic targets.

Competing interests None declared.

PMO-249 QUANTITATIVE PROTEOMIC ANALYSIS OF INTERMEDIATE FILAMENT PROFILE IN ULCERATIVE COLITIS REVEALS INCREASED LEVELS OF KERATINS 8, 18 AND 19 IN PATIENTS WITH LONGSTANDING PAN COLITIS WHICH ARE REDUCED WITH DEVELOPMENT OF DYSPLASIA

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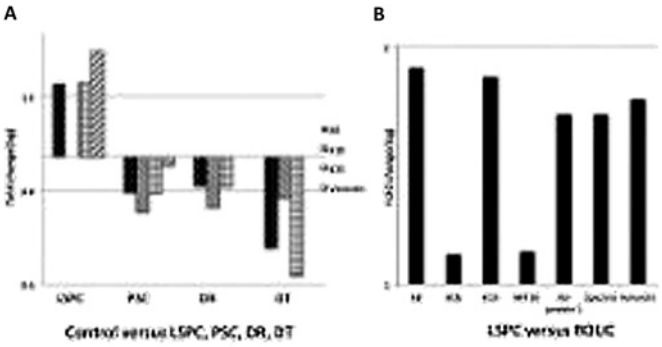
Introduction Intermediate filaments (IF), principally keratins (K), are key components of epithelial cytoskeleton. K8, 18 and 19 are expressed in intestinal epithelial cells and play a role in cell-death signalling pathways, in particular apoptosis mediated by tumour necrosis factor-α. Reduced K8 and K20 expression is linked to epithelial-to-mesenchymal transition indicative of increased tumour aggressiveness. We investigated the change in levels of insoluble IF proteins in well-characterised groups of patients at differing risk of UC-associated cancer.

Methods Rectal biopsies were obtained from patients with inactive UC with: (1) Long-standing (20–40 years) pancolitis (LSPC) (n=10); (2) Recent onset (<5 years) UC (ROUC) (n=8); (3) UC with primary sclerosing cholangitis (PSC) (n=7); (4) pancolitis with dysplasia (n=4) and 10 controls, with additional biopsies from dysplastic/neoplastic lesions and snap frozen. An iTRAQ (isobaric tagging for relative and absolute quantification)-compatible extraction and solubilisation protocol for IF proteins was developed. Labelled peptides from pooled patients were analysed by SCX-LC-MS/MS (strong cation exchange-reverse phase HPLC tandem mass spectrometry) and data reconstituted in GeneBio Phenyx. Intergroup comparisons were made using in-house algorithms based on t-testing with multiple test correction.



Abstract PMO-249 Figure 1 Tandem mass spectrometry results showing significant log fold changes ($p<0.05$) in IF levels.

Results Tandem mass spectrometry (MS/MS) identified 52 proteins; 32 (61.5%) were matched by two or more peptides. Abstract PMO-249 figure 1A shows the log fold change in IF levels compared to control, with significant increase in levels of K8, K19 and vimentin in those with LSPC, but marked reduction in IF levels in areas of dysplasia (DT) and rectal mucosa distant from this (DR). Marked increase in levels of keratins was noted in patients with LSPC compared to those with ROUC (Abstract PMO-249 figure 1B), suggesting an effect of disease duration on IF levels.

Conclusion This is the first study using a quantitative proteomic approach with an iTRAQ based proteomic workflow to analyse changes in IF levels in patients with UC with differing colon cancer risk. LSPC is associated with enhanced mucosal levels of keratins, spectrin and xin, which is reduced in dysplasia and in distant rectal mucosa of those with dysplasia—suggesting a field change. These changes need further characterisation including of post-translational modifications, which may help better understanding of the pathogenesis of colitis associated cancer.

Competing interests None declared.

PMO-250 QUANTITATIVE PROTEOMICS IN ULCERATIVE COLITIS REVEALS MUCOSAL INFLAMMATION REDUCES LEVELS OF KERATINS IN THE INSOLUBLE FRACTION OF THE INTERMEDIATE FILAMENT PROTEOME

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Introduction Keratins (K) are a key component of intermediate filaments (IF), primarily composed of K8, 18 and 19 in the intestinal epithelia. Apart from a structural role, they may play a role in moderating TNF effects, including cytotoxicity. K8-null mice develop colitis, a subset of patients with IBD have missense mutation in K8 gene. Colonic expression of K8/K18 has been shown to be regulated by IL-6. In order to examine the relationship between acute inflammation and alteration in levels of insoluble IFs in mucosa of patients with ulcerative colitis (UC), we undertook a quantitative proteomic approach using an iTRAQ (isobaric tagging for relative and absolute quantification)-based proteomic workflow.

Methods Endoscopic biopsies were obtained from patients with UC, from actively inflamed rectum and from non-inflamed proximal