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Proteomic Approaches for Biomarker Discovery in Ulcerative Colitis

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1. Introduction

Ulcerative colitis (UC) as well as Crohn's disease (CD) is one of the major inflammatory bowel diseases (IBD). Although genetic (1), infectious (2), and immunological (3, 4) factors have been reported to be involved in the pathogenesis of UC, the precise etiology remains unclear. UC is now diagnosed based on clinical, radiologic, endoscopic and histopathological findings. Thus, biomarkers for UC have been vigorously explored to diagnose UC accurately and non-invasively. The most clinically useful biomarker for UC at present is perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) which is detected in 50-80% of UC patients (5). However, p-ANCA is also detected in 10-40% of CD patients, 30-80% of patients with microscopic polyangiitis, 30-75% of patients with Churg-Strauss syndrome and 50% of patients with rapid progressive glomerulonephritis (5-7). A more sensitive and specific biomarker for UC should be established.
Recently, there have been great advances in proteomics, the science dealing with the comprehensive analysis of protein expression. Proteomics have been applied to search of biomarkers in various diseases (8-10). In this paper, we introduced proteomic studies which explored biomarkers for UC by analyzing proteins in various clinical samples such as sera, peripheral blood mononuclear cells (PBMCs) and colonic mucosa. The comprehensive study can detect unexpected and sometimes novel molecules as a biomarker, which may also lead to elucidation of the pathogenesis of UC.

1.1 Representative methods for proteomics

As an assembly of genes is called as genome (gene + ome), an assembly of proteins is named as proteome (protein + ome). An assembly of low molecular proteins (peptides) is specifically called as peptidome (peptide + ome) (11). Proteomics are the study to comprehensively analyze proteome. There are two major methods for proteomics, 2-dimensional electrophoresis (2DE) and shotgun method (Fig.1).

A. 2-dimensional electrophoresis (2DE)

Extraction of whole proteins from cells or tissue
↓
Isoelectric focusing
↓
Sodium dodecyl sulfate-polyaclylamide gel electrophoresis (SDS-PAGE)
↓
Detection of individual proteins as spots
↓
Comparison of individual protein spot intensity between disease A and disease B
↓
Cut out the gel of the protein spots of interest
↓
In gel digestion of the proteins by a protease
↓
Identification of the proteins by mass spectrometry (MS) and protein database search

B. Shotgun method

Extraction of whole proteins from cells or tissue
↓
Digestion of the mixture of proteins with a protease
↓
Fractionating the obtained peptides by liquid chromatography
↓
Analysis of individual peptides by MS/MS method to identify the original proteins

Fig. 1. Representative methods for proteomics.
The outlines of 2DE and shotgun method are described

2DE is the method to separate cell- or tissue-derived proteins into protein spots by isoelectric focusing and subsequent sodium dodecyl sulfate-polyaclylamide gel electrophoresis (SDS-PAGE) (Fig.2). After the 2DE, protein spots of interest are cut out, and the proteins contained in the spots are identified by mass spectrometry (MS). Both matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF/MS) and liquid chromatography-mass spectrometer (LC-MS) are mainly used for the identification. One advantage of 2DE is to visualize the proteome of targeted cells or tissue as protein spots. A representative case is 2-dimensional differential image gel electrophoresis (2D-DIGE) which displays 2 kinds of proteome with different fluoresceines on the same gel (9). 2D-DIGE can visualize and compare proteome of two different samples, for examples, between a patient and a healthy donor, and before and after treatment with a drug. The other advantage of 2DE is to detect at least a part of the difference of post-translational modification, amino acid mutation, and isotypes of one protein as different spots. Disadvantages of 2DE are that it is laborious requiring many manual procedures, and that number of detectable proteins is limited because proteins with low expression levels

and with extremely high or low molecular weights/isoelectric points are not visualized and separated, respectively. However, automation of 2DE has been recently developed, and use of a longer isoelectric focusing gel has increased number of detected proteins to achieve more comprehensiveness.

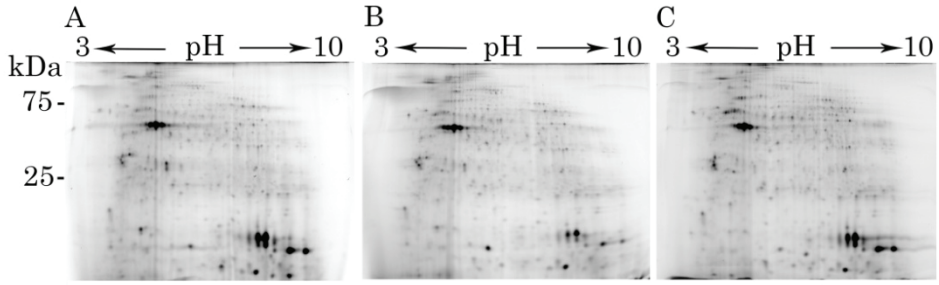

Fig. 2. 2DE analysis of a UC patient.
PBMCs were obtained from patients with UC, CD, and from a healthy subject, and proteins were extracted from the cells to be separated by 2DE. Representative results of a UC patient (A), a CD patient (B), and a healthy subject are shown. pI, isoelectric points; MW, molecular weights

In the shotgun method, mixture of proteins extracted from cells or tissue is digested with a protease. When protein profiles of several disease groups are compared, proteins are sometimes labeled by isotopes such as iTRAQ and ICAT. The obtained peptide mixture was fractionated by liquid chromatography (LC) to be finally analyzed by MS/MS method (LC-MS/MS). To fractionate minutely, 2D-HPLC is useful, for example, using a combination of strong cation exchange column and reverse-phase column. Surface enhanced laser desorption/ionization (SELDI)-TOF/MS is also used, in which proteins/peptides are trapped by radicals and molecules immobilized on protein chips to be directly measured by the MS system. The shotgun method has advantages to detect proteins with low expression levels, to achieve high comprehensiveness because of no limitation of isoelectric points, molecular weights, and hydrophilicity, and to automatize the procedures from fractionation to mass spectrometry. However, the proteome is not visualized, and nature of the original proteins remains unknown in this method.

1.2 Discovery of biomarkers for UC

Proteomic studies using sera, PBMCs, and colonic mucosa have found biomarker candidates for UC by comparison of UC, CD, other colitis, and healthy condition. Comprehensive analysis of a number of proteins makes multivariate analysis possible, which raises not only one protein but a combination of multiple proteins as a biomarker for UC.

2. Serum proteomics

A serum sample is one of the most frequently used clinical samples, which is obtained with low invasiveness. It contains a number of proteins which are physiologically and pathologically important. Serum samples would be an excellent source for the surveillance of biomarker candidates.

As a conventional proteomic study using MALDI-TOF/MS, Nanni et al analyzed serum protein profiles of UC patients, CD patients, and healthy subjects (12). The profiles of 20 peptides extracted based on hydrophobic interaction completely classified all the cases into the original three groups, and UC was predicted with 96.3% prediction ability by cross validation of the classification model. In this study, the 20 peptides were not identified. In contrast, three studies analyzing serum proteins by SELDI-TOF-MS identified the biomarker candidates (Table 1) (13-15). Subramanian et al analyzed sera from UC and CD patients, and detected 12 discriminative peaks with both specificity and sensitivity of approximately 95% (13). Six out of the 12 proteins were identified, including inter alpha trypsin inhibitor 4, apolipoprotein C1, and platelet activated factor 4 variants. Meuwis et al generated classification models by multivariate analysis, whose sensitivity and specificity to discriminate UC from CD were approximately 80% and 90%, respectively (14). Four biomarkers with important diagnostic values were identified as platelet aggregation factor 4 (PF4), myeloid related protein 8 (MRP8), fibrinopeptide A (FIBA), and haptoglobin α2 (Hpα2). Kanmura et al selected human neutrophil peptides 1-3 (HNP 1-3) from the 27 proteins with significantly different concentration between UC and healthy sera (15). In a larger cohort, concentration of HNP 1-3 were significantly higher in active UC patients compared to that in UC patients in remission, CD patients, patients with infectious colitis, and healthy subjects. Levels of HNP 1-3 decreased after corticosteroid therapy in responders for the drug, whereas the levels were not changed in non-responders. As a new method, Haas et al analyzed serum samples from UC and CD patients by Fourier Transform Near-Infrared Spectroscopy (FT-NIR) (16). The cluster and Artificial Neural Networks (ANN) analyses of the results correctly identified 80% and 69.8% of UC, respectively, suggesting a usefulness of this technology. In addition, a pilot study compared sera between corticosteroid-resistant and -responsive UC patients, and detected 19 proteins with significantly different concentration, which may predict response to the treatment in UC (17).

Proteomics include studies using various kinds of protein arrays. Kader et al used antibody arrays containing 78 cytokines, growth factors and soluble receptors to screen sera from UC and CD patients (18). In UC, only IL-12p40 was significantly upregulated in the remission stage compared to in the active stage ($p<0.02$). On the other hand, in CD, significantly elevated levels of 4 cytokines including IL-12p40 were found in the remission stage compared to in the active stage ($p<0.01$). The other 3 cytokines were placenta-derived growth factor, IL-7, and TGF-β1. In another study using protein arrays, Escherichia coli-derived proteome was served to screen serum antibodies (19). A set of antibodies distinguished UC patients from healthy subjects with 66% accuracy ($p<0.05$). The other antibody set distinguished UC patients from CD patients with 80% accuracy ($p<0.01$). The latter set consists of only two kinds of antibodies which recognized YidX and Frv X, suggesting that immune reaction to the 2 proteins from E. coli would be useful for discrimination of UC from CD.

It seems to be difficult to find serum autoantibodies which are more powerful than p-ANCA even using proteomic techniques. Vermeulen et al analyzed serum autoantibodies against commercial human protein arrays (20). 75 proteins reacted more strongly with sera from IBD patients than those from healthy subjects, while 88 proteins showed the opposite pattern. One of the identified proteins as an autoantigen for IBD was pleckstrin homology-like domain, family A, member 1 (Phla1). In a large cohort, 42.8% of the UC patients, 50.0% of the CD patients, (taken together, 46% of the IBD patients), 33.3% of the patients with non-IBD gastrointestinal diseases, and 28.7% of the healthy subjects were positive for the anti-

Phla1 antibodies. Thus, discriminative power between UC and CD, and IBD and controls remained low. The same research group also analyzed serum autoantibodies against α-enolase in IBD by a classic proteomic approach (21). The anti-α-enolase antibodies were detected in 49.0% of the UC patients, 50.0% of the CD patients, 37.8% of the patients with autoimmune hepatitis, 34.0% of the patients with ANCA-positive vasculitis, and 31.0% of the patients with the other gastrointestinal diseases, showing the only limited diagnostic value.

References	Samples	Methods	Identified proteins
Subramanian et al (13)	sera	SELDI-TOF MS	inter alpha trypsin inhibitor 4 apolipoprotein C1 platelet activated factor 4 variants
Meuwis et al (14)	sera	SELDI-TOF MS	platelet aggregation factor 4 (PF4) myeloid related protein 8 (MRP8) fibrinopeptide A (FIBA) haptoglobin α2 (Hpα2)
Kanmura et al (15)	sera	SELDI-TOF MS	Human neutrophil peptide (HNP) 1-3
Kader et al (18)	sera	antibody array	IL-12p40 (upregulated in the remission stage than in the active stage in UC)
Chen et al (19)	sera	E. coli-protein array	YidX Frv X
Vermeulen et al (20)	sera	Human protein arrays	autoantibodies against pleckstrin homology-like domain, family A, member 1 (Phla1)
Vermeulen et al (21)	sera		autoantibodies against α-enolase
Hatsugai et al (9)	PBMCs	2DE, MALDI-TOF MS	cyclophilin A (PPIA) protein S100-A9 (S100A9) peroxiredoxin-2 (PRDX2) carbonic anhydrase 2 (CA2) β-actin (ACTB) annexin A6 (ANXA6) α/β Hydrolase domain-sontaining protein 14B (ABHD14B)

Table 1. Blood biomarker candidates for UC

3. Proteomics of PBMCs

PBMCs, relatively easily prepared from the peripheral blood, contain a number of proteins different from serum proteins. Because UC is considered as an autoimmune disease, analysis of PBMCs which include lymphocytes and monocytes is useful not only for the biomarker surveillance but also for the elucidation of the pathogenesis of UC. However, little has been known about the protein profile of PBMCs in UC.

We comprehensively analyzed proteins in PBMCs from UC, focusing on discrimination of UC from CD (9). PBMC-derived proteins from UC patients, CD patients, and healthy

subjects were separated by 2DE, and intensity of individual protein spots was subjected to multivariate analysis to generate differential diagnostic models between UC and CD. As a result, 547 protein spots were detected in the 2DE results. Two diagnostic models were generated using intensity of selected 276 protein spots and further selected 58 protein spots, both of which completely discriminated between UC and CD (sensitivity and specificity were 100% in these models). Eleven out of the 58 protein spots were identified, which were functionally related to inflammation (cyclophilin A, PPIA; protein S100-A9, S100A9), oxidation/reduction (peroxiredoxin-2, PRDX2; carbonic anhydrase 2, CA2), cytoskeleton (β-actin, ACTB), endocytotic trafficking (annexin A6, ANXA6), and transcription (α/β Hydrolase domain-sontaining protein 14B, ABHD14B). Interestingly, the PBMC protein profiles were useful for prediction of disease activity in the UC and the CD patients, and prediction of severity and responses to treatments in the UC patients. Especially, some clinical parameters were predicted by intensity of a few protein spots, for example, intensity of only 2 protein spots for disease activity of the UC. Proteins associated with the activity of UC may be extremely restricted. PBMC protein profile would be a potent biomarker for differential diagnosis of UC from CD, and investigation of the proteins contributing to the discrimination may elucidate the different pathophysiology of UC from CD.
As an antigen-specific model, an IBD model was established using male SD rats by colonic administration of trinitrobenzene sulfonic acid (TNBS) in 50% ethanol (22). Lymphocyte-derived protein profiles from the model rats and the control rats receiving 50% ethanol were compared by 2DE and MALDI-TOF/MS, which revealed different expression of 26 proteins (17, upregulated; and 9, downregulated) included regulators of the cell cycle and cell proliferation, signal transduction factors, apoptosis-related proteins and metabolic enzymes.

4. Proteomic analyses of colonic mucosa from UC

Proteomic analysis of colonic mucosa has demonstrated multiple biomarker candidates. The analyses using clinical samples of the disease-affected sites may highly contribute to elucidation of the pathophysiology of UC, indicating functional difference of various proteins from the other gastrointestinal diseases.
Comparing protein profiles between the UC-affected mucosa and normal mucosa both from UC patients by 2DE and subsequent LC-MS, protein spots showing higher intensity in the UC-affected mucosa than in the normal mucosa were identified (23). They were protocadherin, α-1 antitrypsin, tetratrico-peptide repeat domains, caldesmon, and mutated desmin, associated with inflammation and cell repair (Table 2). Especially, a mutated form of desmin was detected in all the examined UC-affected mucosa, suggesting its potential as a UC biomarker. Another study comparing colonic mucosa from UC patients and healthy subjects by 2DE and MALDI-TOF/MS showed 13 downregulated and 6 upregulated proteins in UC (24), which were involved in mitochondrial function (heat shock protein 70, HSP70; HSP60; H+-transporting two sector ATPase, ATP5B; prohibitin, PHB; malate dehydrogenase, MDH2; voltage-dependent anion-selective channel protein 1, VDAC1; thioredoxin peroxidase 1, PRDX1; PRDX2), energy generation (ATP5B, MDH2, triosephosphate isomerase), cellular antioxidants (PRDX1; PRDX2; selenium binding protein 1, SELENBP1), and stress-response (HSP70, HSP60, PRDX1, PRDX2, PHB, VDAC1). Aberrant activation of nuclear factor of activated T cell (NFAT), and ectopic expression of tumor rejection antigen 1 and poliovirus receptor related protein 1 were detected in the UC-affected colonic mucosa.

References	Samples	Methods	Identified proteins
Fogt et al (23)	colonic mucosa	2DE, LC-MS	protocadherin, α-1 antitrypsin, tetratrico-peptide repeat domains, caldesmon, mutated desmin
Hsieh et al (24)	colonic mucosa	2DE, MALDI-TOF/MS	heat shock protein 70 (HSP70), HSP60, H+-transporting two sector ATPase (ATP5B), *prohibitin (PHB)*, malate dehydrogenase 2 (MDH2), voltage-dependent anion-selective channel protein 1 (VDAC1), thioredoxin peroxidase 1 (PRDX1), *PRDX2*, triosephosphate isomerase, selenium binding protein 1 (SELENBP1), nuclear factor of activated T cell (NFAT), tumor rejection antigen 1, poliovirus receptor related protein 1
Shkoda et al (25)	colonic mucosa	2DE, MALDI-TOF/MS	programmed cell death protein 8, annexin 2A (Both increased in inflamed regions)
Shih et al (26)	colonic mucosa	2DE, IHC	Translocation of NFAT2 into nuclei
Berndt et al (27)	T cells in colon	MELC	Colocalization of NF-kB and poly (ADP-ribose)-polymerase
Naito et al (30)	mouse intesitinal mucosa	2DE, MALDI-TOF/MS	3-Hydroxy-3- methlglutaryl-coenzyme A synthase 2, serpin b1a, protein disulfide-isomerase A3, PRDX6, vimentin

Table 2. Biomarker candidates for UC identified from colonic mucosal cells

Comparison with colonic mucosal proteins between UC and CD revealed their specific characters of UC and common features to IBD (25-27). Intestinal epithelial cells (IECs) from patients with UC, CD, and colon cancer, analyzed by 2DE and MALDI-TOF/MS, showed 21 protein spots with at least 2-fold change between inflamed tissue from the IBD (UC and CD) patients and non-inflamed tissue from the patients with colonic cancer (25). The identified proteins were functionally related to signal transduction, stress response, and energy metabolism. Specifically, Rho-GDP dissociation inhibitor α, which inhibits cell cycle progression, was upregulated in IBD and sigmoid diverticulitis, possibly involving with the destruction of IEC homeostasis under the condition of chromic inflammation. On the other hand, 40 proteins were significantly altered between inflamed and noninflamed regions in the UC patients. The proteins included programmed cell death protein 8 and annexin 2A, both of which were increased in the inflamed regions. In addition, localization of the proteins may indicate the pathophysiological difference of UC and CD (26, 27). NFAT2, increased in the UC-affected colon tissue in the 2DE results, was specifically translocated into nuclei of the UC colonic mucosa, whereas NFAT2 was located exclusively in cytoplasm in the normal and the CD mucosa (26). A modified proteomic method, Multi-Epitope Ligand Cartography (MELC), showed that only CD4+ T cells co-expressing NF-kB were caspase-8+ and poly(ADP-ribose)-polymerase+ in the UC colonic mucosa (27). The colocalization of NF-kB+ and poly(ADP-ribose)-polymerase+ would be the base motif that

discriminates UC from CD. Interestingly, the number of CD4+CD25+ T cells was elevated only in the UC mucosa, but not in the CD mucosa and the normal mucosa from patients with colonic cancer, suggesting the specific activation of regulatory T cells in UC.

Other modified methods including cellular or subcellular analyses have brought useful information (28, 29). Effects of inflammatory cytokines of IFNγ, IL-1β, and IL-6 on IBD were investigated human adenocarcinoma cells by 2DE and MALDI-TOF/MS (28). Tryptophanyl tRNA synthetase, indoleamine-2,3-dioxygenase (IDO), heterogenous nuclear ribonucleoprotein JKTBP, IFN-induced p35, proteasome subunit LMP2, and arginosuccinate synthetase were identified as the cytokine-regulated proteins. Overexpression of IDO in IECs was found in the UC and CD mucosa, but not in the diverticulitis and normal mucosa, suggesting that the specific response of IDO to the inflammatory cytokines may be a character of IBD. As a subcellular fractionation analysis, expression levels of 5′ nucleotidase (plasma membrane), malate dehydrogenase (mitochondria), catalase (peroxisomes), LDH (ER), N-acetyl-β-glucosaminidase (lysosomes), and neutral-α-glucosidase (ER) in rectal biopsy homogenates from the UC, CD, and non-rectal CD patients were assayed (29). Reduction of both cytosolic and particulate N-acetyl-β-glucosaminidase was found in the UC patients, whereas a selective reduction in particulate activity was found in the non-rectal CD patients, demonstrating lysosomal alterations in these diseases.

IECs from UC or IBD model mice have been analyzed by proteomics (30-33). Intestinal mucosa from a UC mouse model, made by oral administration of 8.0% dextran sodium sulfate, was analyzed by 2DE and MALDI-TOF/MS (30). Comparison of mucosa from the UC model with that from normal mice revealed 7 altered protein spots. Five proteins were identified from the spots, which were 3-Hydroxy-3-methlglutaryl- coenzyme A synthase 2, serpin b1a, protein disulfide-isomerase A3, PRDX6 and vimentin. To investigate response of IECs against a pathogen, Caco-2 IEC line was co-cultured with Enteropathogenic E. coli (EPEC) to be injected the bacterial proteins through bacterial type III secretion system (TTSS) (31). Among 2,090 host proteins identified by LC-MS, 264 proteins (approximately 13%) were differentially expressed between WT EPEC-cocultured IECs and TTSS-deficient EPEC-cocultured IECs, suggesting that host proteins were potentially involved in EPEC-induced colitis.

Based on an interesting idea that endoplasmic reticulum (ER)-mediated stress responses in IECs may contribute to chronic intestinal inflammation, IECs from Enterococcus faecalis-monoassociated IL-10-deficient mice and WT mice were analyzed by 2DE and MALDI-TOF/MS (32). Increased expression of glucose-regulated ER stress protein (grp)-78 was found in the IL-10-deficient mice. In human, the increased expression of grp-78 was also found in the inflamed colonic tissue from patients with UC, CD and sigmoid diverticulitis. IL-10 was found to inhibit inflammation-induced ER stress response by modulating nuclear recruitment of activating transcriptional factor (ATF)-6 to the grp-78 gene promoter. Another interesting idea is raised from the field of neutrinogenomics, in which environmental factors would contribute to the chronic intestinal inflammation in the genetically susceptible hosts (33, 34). In this respect, TNFDeltaARE/WT mice were prepared, which showed impaired regulation of TNFα synthesis by deletion of an AU-rich motif in the 3′-untranslated region of the TNF gene (35). WT and TNFDeltaARE/WT mice were fed with adequate and low amount of iron, and the adequate iron-fed TNFDeltaARE/WT mice were found to develop severe ileal inflammation. Comparison of IEC-derived proteins between adequate iron-fed WT and TNFDeltaARE/WT mice (inflamed conditions), and that between adequate iron- and low iron-fed

TNFDeltaARE/WT mice (absence of inflammation), by 2DE and MALDI-TOF/MS showed 4 contrarily regulated proteins including aconitase 2, catalase, intelectin 1, and fumarylacetoacetate hydrolase (FAH). These proteins are associated with energy homeostasis, host defense, oxidative, and ER stress responses.

5. Prediction of colorectal cancer associated with UC

UC shows an increased risk of colorectal cancer compared to other inflammatory intestinal diseases. In UC patients, occurrence of colorectal cancer is periodically examined by colonoscopy throughout their lives. To avoid this invasive and expensive examination, a biomarker which predicts occurrence of colorectal cancer in UC will be useful. Further, although UC-associated colon cancer is known to develop from dysplastic lesions caused by chronic inflammation, the molecular mechanism how inflammation leads to carcinogenesis should be elucidated.

Brentnall et al analyzed protein profiles of epithelium from normal colon, nondysplastic colon of UC patients without dysplasia (UC nonprogressors), nondysplastic colon of UC patients with high grade dysplasia or cancer (UC progressors), and high grade dysplastic colon of UC progressors by LC-MS subsequent to strong cation exchange (36). Proteins related to mitochondria, oxidative activity, and calcium-binding proteins were associated with the neoplastic progression in UC. In the early and late stages, Sp1 and c-myc may play roles in UC neoplastic progression, respectively (Table 3). Carbamoyl-phophate synthase 1 (CPS1) and S100P were overexpressed in nondysplastic colon tissue from the UC progressors. The overexpression may be useful for the prediction of dysplasia in UC.

In another study from the same research group, differently expressed proteins between nondysplastic and dysplastic tissue from the UC progressors were detected by LC-MS (37). They were mitochondrial proteins, cytoskeletal proteins, RAS superfamily, proteins related to apoptosis and metabolism, suggesting their importance in the early stages of neoplastic progression in UC. Among such proteins, both TNF receptor-associated protein 1 (TRAP1) and CPS1 were increased in nondysplastic and dysplastic tissue in the UC progressors than in the nonprogressors. Rectal CPS1 staining predicts dysplasia or cancer in the colon with 87% sensitivity and 45% specificity, indicating its feasibility as a biomarker to predict colonic dysplasia or cancer. On the other hand, comparison of UC-associated and sporadic colon cancer cell lines by 2DE and LC-MS showed that the expression of heat shock protein (HSP47) was significantly higher in UC-associated colon cancers, the increase of which was correlated to the progression of neoplastic lesions (38). HSP47 was co-expressed with type I collagen in the cytoplasm, and both of them were released from culture cells into the medium, suggesting the possibility of HSP47 as a biomarker for UC-associated cancer.

Analysis of colonic mucosa by MELC study showed significant increase of NF-kB+ HLA-DR+ cells in CD4+ and CD8+ cell populations in UC patents and patients with colorectal cancer compared to healthy subjects (39). This suggested increase of activated T cells and an altered antigen presentation. In the UC group, NF-kB+ cells were significantly increased in CD45RO+ cell populations, but not in CD45RA+ cell population, suggesting the activation in memory T cells. CD4+CD25+NF-kB+ cells were also specifically increased in the UC group, which indicated the increase of regulatory T cells. The specific activation of such subpopulations of T cells would play protective roles in UC, and loss of the activation may play a role in the progression of colorectal cancer. In an animal model for UC, which was

established by repeatedly exposing B6 mice to dextran sodium sulfate (DSS), proteins in colonic mucosa were analyzed by 2DE and MALDI-TOF/MS (40). 38 protein spots were found to be differently expressed in colon tumors compared to normal colon, 27 of which were identified. They included glucose-regulated protein (GRP) 94, HSC70, emolase, PHB and transgelin. Transgelin was found to be significantly reduced in human colon tumors compared with adjacent nontumorous tissues, suggesting that low expression of this protein may be a candidate biomarker of colitis-associated colon cancer.

References	Samples	Methods	Identified proteins
Brentnall et al (36)	colonic mucosa	LC-MS	Sp1, *Carbamoyl-phophate synthase 1 (CPS1)*, S100P
May et al (37)	colonic mucosa	LC-MS	TNF receptor-associated protein 1 (TRAP1), *CPS1*
Araki et al (38)	colon cancer cell lines	2DE, LC-MS	heat shock protein (HSP47)
Berndt et al (39)	T cells in colon	MELC	(Increase of CD45RO+NFkB+ cells and increase of CD4+CD25+NFkB+ cells in UC than in colorectal cancer)
Yeo et al (40)	Mouse colonic mucosa	2DE, MALDI-TOF/MS	transgelin (GRP94, HSC70, enolase, *PHB*, and transgelin were differently expressed in colon tumors in the UC-model mice from those in normal colon)

Table 3. Biomarker candidates to predict complication of colorectal cancer in UC

6. Subproteomic analyses – metabolomics and other studies

As subproteomic analyses, metabolomics which comprehensively analyze metabolites have been performed in IBD patients and also in UC model mice. Because metabolites are easily obtained from urine or fecal samples, use of biomarkers detected by metabolomics may be less-invasive compared to those derived from blood and colonic tissue. In addition to MS analysis, nuclear magnetic resonance (NMR) spectroscopy is frequently used in metabolomics. Metabolomics, which analyze different molecular profiles from proteomics, should also contribute to unraveling the pathophysiology of UC.

Fecal extracts from patients with CD and UC were analyzed by 1H NMR spectroscopy (41). The levels of butyrate, acetate, methylamine, and trimethylamine were found to be lower in both diseases than in healthy subjects. The results may indicate changes of microbial community in gut. In contrast, elevated quantities of amino acids were demonstrated in both diseases, implying malabsorption caused by inflammation. Interestingly, the decreased amounts of amino acids and glycerol, and the increase of butyrate and acetate, in the feces of UC patients contributed to the discrimination of UC from CD (Table 4). A conventional metabolic analysis, in which utilization of n-butyrate, glucose, and glutamine in isolated colonic epithelial cells were evaluated, showed that oxidation of butyrate to CO2 and ketones was significantly suppressed in UC colonic mucosa compared to normal mucosa (42). The failure of n-butyrate oxidation in UC suggests that UC may be an energy-deficiency disease of the colonic mucosa. To specifically distinguish UC from CD,

exoprotease activity was assayed using 2 synthetic peptides as substrates, which were fibrinopeptide A without the N-terminal alanine and complement 3f (43). The two peptides were spiked into serum samples from 3 UC patients, 3 CD patients, and 3 healthy subjects, and the metabolite pattern was analyzed by MALDI-MS and chemometric analysis. Although 100% discrimination of the UC patients from the CD patients and the healthy subjects was achieved, the diagnostic power should be verified with more number of subjects.

References	Samples	Methods	Identified metabolites
Marchesi et al (41)	fecal extracts	NMR	decreased amounts of amino acids and glycerol, and increase of *butyrate* and acetate, compared to those in CD
Roediger et al (42)	IECs of colon	Metabolic analysis	decreased oxidation of *butyrate* to CO2 and ketones

Table 4. Biomarker candidates for UC identified by metabolomics

As an IBD model study, time course of urine metabolites from IL-10-deficient mice were compared with those from control mice by NMR analysis (44). Both groups initially had similar metabolic profiles, then diverged substantially with the onset of IBD. The levels of trimethylamine and fucose changed dramatically in 8wk IL-10-deficient mice, at the timeline of histological injury. In addition, bacterial signaling molecules involved in their communication may serve as potential biomarkers for IBD (45). Profiles of N-acyl homoserine lactones (AHLs), the chemical signaling molecules in Gram-negative bacteria, in saliva from healthy donors and patients with gastrointestinal disorders were analyzed by LC-MS. The levels of AHLs may correlate with the health status of subjects.

7. Conclusion

Novel approaches by proteomics and subproteomics for biomarker discovery of UC, including those of the complication of colorectal cancer, were introduced. Many proteins have been identified and considered to be candidates for UC biomarkers, however, most of them have not established, indicating the broad range of functional abnormality in UC. PRDX2, PHB, CPS1, and butyrate were identified in multiple different studies, suggesting their usefulness as a biomarker for UC or the associated colonic cancer. The candidate biomarkers should be validated with more number of patients with UC, CD, other control diseases, and healthy subjects. Even though simple and less-invasive biomarkers are desirable for clinical examination, if the biomarkers are sensitive and specific enough for the UC diagnosis, examination of colonic mucosa obtained by endoscopy and combination of multiple proteins as the biomarker are also acceptable. Further advances in these approaches would be useful to establish biomarkers for the accurate diagnosis and the disease course prediction, and may be useful to elucidate the complicated disease mechanisms of UC.

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9. References

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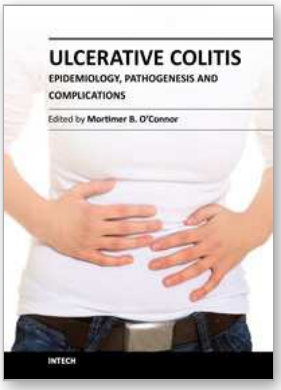

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This book is intended to act as an up-to-date reference point and knowledge developer for all readers interested in the area of gastroenterology and in particular, Ulcerative Colitis. All authors of the chapters are experts in their fields of publication, and deserve individual credit and praise for their contributions to the world of Ulcerative Colitis. We hope that you will find this publication informative, stimulating, and a reference point for the area of Ulcerative colitis as we move forward in our understanding of the field of medicine.

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