Dig Dis Sci (2017) 62:2266–2276
DOI 10.1007/s10620-017-4673-0

REVIEW

Proteomics in Inflammatory Bowel Disease: Approach Using Animal Models

Fadi H. Mourad[1,2] · Yunki Yau[2] · Valerie C. Wasinger[3] · Rupert W. Leong[2]

Received: 29 April 2017 / Accepted: 4 July 2017 / Published online: 17 July 2017

Abstract Recently, proteomics studies have provided important information on the role of proteins in health and disease. In the domain of inflammatory bowel disease, proteomics has shed important light on the pathogenesis and pathophysiology of inflammation and has contributed to the discovery of some putative clinical biomarkers of disease activity. By being able to obtain a large number of specimens from multiple sites and control for confounding environmental, genetic, and metabolic factors, proteomics studies using animal models of colitis offered an alternative approach to human studies. Our aim is to review the information and lessons acquired so far from the use of proteomics in animal models of colitis. These studies helped understand the importance of different proteins at different stages of the disease and unraveled the different pathways that are activated or inhibited during the inflammatory process. Expressed proteins related to inflammation, cellular structure, endoplasmic reticulum stress, and energy depletion advanced the knowledge about the reaction of intestinal cells to inflammation and repair. The role of mesenteric lymphocytes, exosomes, and the intestinal mucosal barrier was emphasized in the inflammatory process. In addition, studies in animal models revealed mechanisms of the beneficial effects of some therapeutic interventions and foods or food components on intestinal inflammation by monitoring changes in protein expression and paved the way for some new possible inflammatory pathways to target in the future. Advances in proteomics technology will further clarify the interaction between intestinal microbiota and IBD pathogenesis and investigate the gene-environmental axis of IBD etiology.

Keywords Proteomics · Inflammatory bowel disease · Biomarkers · Animal models of colitis, inflammation

✉ Fadi H. Mourad
fmourad@aub.edu.lb

Yunki Yau
yunki.yau@sswahs.nsw.gov.au

Valerie C. Wasinger
v.wasinger@unsw.edu.au

Rupert W. Leong
rupert.leong@sswahs.nsw.gov.au

[1] Department of Internal Medicine, Faculty of Medicine, American University of Beirut, P.O. Box 113-6044, Hamra, Beirut 110 32090, Lebanon

[2] Gastroenterology and Liver Services, Concord Repatriation General Hospital, Hospital Road, Concord, NSW 2137, Australia

[3] Bioanalytical Mass Spectrometry Facility, Mark Wainwright Analytical Centre, The University of NSW Australia, Kensington, NSW 2052, Australia

Introduction

Proteomics refers to the total protein complement able to be encoded by a given genome [1]. Unlike genes, the number of proteins in the human proteome is difficult to measure, since proteome is a dynamic mixture of proteins that contains subproteomes restricted to certain cells [2]. The proteome owes its complexity mainly to posttranslational modifications as modulation in proteins activity depends on posttranslational modification rather than the expression of their genes [2, 3]. The goal of proteomics has been described as a "comprehensive, quantitative description of protein expression and its changes under the influence of biological perturbations" [3] leading to

the construction of proteomic maps of functions in an organism, and the understanding of its response to environmental changes [4, 5]. Proteomics is now used in many fields such as signal transduction, structural biology, protein interactions, and biomarker discovery, thus providing a deeper understanding of the role of specific proteins in health and disease [2]. At the time being, the application of proteomics in inflammatory bowel disease (IBD) is at its discovery stage. Several approaches have been taken in the past several years that rely mainly on the idea of assessing the differences in proteins between normal controls and certain diseased states [6]. Recent proteomics research has shed light on some aspects of the pathogenesis [7] and pathophysiology [8–11] of IBD and helped in the search for some biomarkers for disease activity as well as for differentiation between ulcerative colitis (UC) and Crohn's disease (CD) [12–14]. Studies used blood or tissue samples to look at the difference in protein expression between control and IBD patients using the techniques of 2D electrophoresis and mass spectrometry [2, 6]. Other studies relied on intestinal cells removed from the colon [8, 10, 11] or on colonic epithelial cell lines [15–17] to study the effect of inflammation on protein production. Due to the multiple factors in the pathogenesis and the pathophysiology of IBD, proteomics studies in humans are faced with many difficulties related to the huge number of proteins to be tested, the small number of patients included in the studies, the environmental and genetic heterogeneity, and the biological variation of the populations studied and their metabolic state [12, 13]. Animal models may provide an alternative approach that could control for confounding environmental, genetic, and metabolic factors. In addition, a large number of specimens could be collected from multiple sites that will help understand the importance of different proteins at different stages of the disease and unravel the different pathways that are activated or inhibited during the inflammatory process. Furthermore, animal models may help discover markers of disease [13] and study the mechanism of the beneficial effects of some therapeutic interventions and foods or food components on intestinal inflammation by monitoring changes in protein expression [18].

Animal Models of Colitis

With nearly 60 animal models of intestinal inflammation having been developed, animal models of IBD have been useful in the identification of those immune responses uniquely involved in IBD pathogenesis and in defining the important roles of environmental influences, such as normal luminal bacterial flora and the genetic composition of the host, in modifying IBD-associated inflammation [19]. This has provided new insights into the pathogenetic mechanisms and the development of new therapies. Although these models do not represent the complexity of human disease and do not replace studies with patient material, they are valuable tools for studying many important disease aspects in controlled and reproducible in vivo systems. In proteomics studies, a defined genetic mutation or a defined pathogenesis of inflammation would help decipher the exact proteins produced during a specific immune activation or cascade and abrogate the inconsistent results obtained from human studies, most likely secondary to activation of different pathways reflecting the multiple pathogenetic mechanisms for IBD development.

Animal models of IBD could be divided into four broad categories: (i) inducible colitis models, (ii) spontaneous colitis models, (iii) adoptive transfer models, and (iv) genetically engineered models such as knockout (KO) models and transgenic models. (For excellent reviews on the subject see references [20–23].) The most widely used models in proteomics research are the IL-10-deficient mice, the multi-drug-resistant Mdr1a mice, dextran sulfate sodium (DSS)-induced colitis, and trinitrobenzene sulfonic acid (TNBS)-induced colitis.

Interleukin-10-Knockout (IL-10−/−) Mouse Model

IL-10 is produced by T cells, B cells, macrophages, thymic cells, and keratinocytes. It downregulates the function of T helper (Th)-1 cells, NK cells, and macrophages. It inhibits the production of inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor α (TNF-α) by stimulated macrophages. IL-10−/− mice spontaneously develop chronic inflammation in the whole intestine, but mainly in the duodenum, proximal jejunum, and ascending colon. The activation of $CD4^+$ Th1 cells and the depletion of their inhibitor, the regulatory T cells, are presumed to be the cause of the inflammation [24]. As the development of colitis is quite predictable, this model can be used to study the longitudinal production of serum and intestinal proteins during various stages of colonic inflammation and help look for disease markers in IBD, and investigate the effect of diet on inflammation. Moreover, because colitis develops only after exposure to commensal bacteria, this model is useful in studying the complex interaction between colonic epithelium and intestinal microbiota. The potential to control and compare different bacterial exposures, and cross-check simultaneous human IBD microbiome profiles, makes the spontaneous colitis–metaproteome analysis a unique platform for uncovering host–bacteria interactions in human IBD.

Multi-Drug Resistance Targeted Mutation (Mdr1a −/−) Model

In this model, mice lack a functional MDR1a gene which encodes for P-glycoprotein, a membrane drug efflux pump expressed in several cell types, including the apical surface of colonic epithelial cells. These mice spontaneously develop intestinal inflammation with pathology similar to human Crohn's disease [25]. The gene expression changes in inflamed colon tissue overlap with genes modulated in IBD patients [26] making this model an appropriate model for IBD studies. Similarly to IL-10-deficient model, this model has also the potential to study the interaction between microbiota and IBD pathogenesis as colitis occurs after exposure to commensal bacteria.

DSS Model

The administration of DSS dissolved in water to mice or rats causes hematochezia, body weight loss, shortening of the intestine, mucosal ulcers, and infiltration of neutrophils. This model can be used for both acute and chronic colitis. Chronic colitis is considered to be caused by CD4+ T cells that are activated by cytokines secreted by activated macrophages [27, 28]. The advantage of this model is that it is accessible, inexpensive, highly reproducible, and relatively easy to induce and handle. However, the resulting inflammation is generally less representative of the specific immunohistopathology present in the inflamed colons of patients with UC or Crohn's colitis. Yet, this model can be quite useful for the study of acute colonic tissue injury and repair mechanism as the acute inflammation is considered to be driven by the innate immune system.

TNBS Model

Colitis would also occur in mice by treatment with a TNBS enema after destruction of the mucosal barrier with an ethanol enema [22]. Granulomas with infiltration of inflammatory cells in all layers of the intestine can be seen in this model. The isolated macrophages and lymphocytes produce large amounts of IL-12, IFN-γ, and IL-2. This evidence suggests that colitis seen in this model is induced by a Th type-1 response, constituting a CD model [29]. In this model, it is easy to induce an inflammation in the colon that pathologically resembles Crohn's colitis but may not be a good representative of human CD. Therefore, the trigger for inflammation and the upregulated or downregulated proteins may not be identical to the ones encountered in CD. However, it may give a good idea about the different activated pathways during inflammation.

Application of Proteomics in Exploring Molecular Pathogenesis and Biomarkers According to Animal Models

Proteomics studies in animal models of colitis were able to describe the proteins expressed at different stages of the disease even before inflammation has started. This provided important information on the activated pathways in animals predisposed to inflammation. Expressed proteins related to inflammation, cellular structure, endoplasmic reticulum stress, and energy depletion helped understand the reaction of intestinal cells to inflammation and repair. The role of mesenteric lymphocytes, exosomes, and the intestinal mucosal barrier was emphasized in the inflammatory process. Furthermore, the differential expression of proteins during some therapeutic interventions and various food administrations provided some explanation on the mode of action of these agents and paved the way for new possible inflammatory pathways to target in the future (Table 1).

Markers of Disease at Different Stages of Inflammation

In a longitudinal study of protein markers in IL-10 (−/−) animal model of colitis, a total of 15 proteins were identified and confirmed by ELISA and Western blot to differentially accumulate in serum samples from mid-stage to late stages of colonic inflammation as compared to early non-inflamed IL-10 (−/−) mice [30]. Some proteins like alpha 1B glycoprotein (A1BG) were decreased, while others were increased throughout the establishment of the disease (pregnancy zone protein (PZP), haptoglobin (HP), and hemopexin (HPX)), and some others were transiently increased (peroxiredoxin-2 (PRDX2)) or increased later in disease progression (transferrin (TF)). To investigate the specificity of these findings and to come up with a protein signature for the different stages of inflammation, protein profiles were repeated in another model of acute colitis (DSS) and in a model of arthritis. PZP and PRDX2 were not increased in mice treated with DSS suggesting that these markers are specific for a chronic inflammatory state. On the other hand, HP and HPX were increased in both colitis models implying that these markers are non-specific for chronic colitis and may be general markers of any form of intestinal inflammation. Additionally, some proteins were found to be specific for the development of arthritis (serum amyloid P component and transthyretin), while other proteins were found to have an altered expression in any inflammatory condition (HP, HPX, C3, and A1BG).

Serum protein profiling of early-stage CD in humans was also investigated in order to improve the comprehension of the very early pathologic mechanisms. Inflammatory

Table 1 Results of proteomic studies in different animal models of colitis

Animal model	Source of specimen	Findings	Reference
IL-10 (−/−) mice DSS	Serum	15 proteins preferentially increased in mid-stage to late stages of inflammation in IL-10 (−/−) mice. Unique protein signature that may be used to discriminate between colitis and non-colitis, and between different stages of colitis	Viennois [30]
IL-10 (−/−) mice Monoassociated with Enterococcus fecalis	Intestinal epithelial cells	14 proteins preferentially increased in chronic infection. Increased expression levels of Grp78 and cleaved caspase-3. Decreased hydroxymethylglutaryl-CoA synthase 2, creatine kinase, pyruvate kinase, and cadherin	Shkoda [35]
IL-10 (−/−) mice	Colonic tissue	Increased expression of cellular stress and immune response proteins. Decreased expression of proteins from pathways of metabolism and digestion/absorption/excretion of nutrients/ions	Knoch [37] Cooney [38]
Mdr1a (−/−) mice	Colonic tissue	33 proteins upregulated related to immune/inflammatory response, heat shock proteins, PI3 K pathway. Downregulated xenobiotic metabolism and cytoskeletal/structural proteins	Cooney [39]
DSS in mice	Colonic tissue	Upregulated: hydroxymethylglutaryl-CoA synthase 2 and serpinb1a. Downregulated: disulfide isomerase A3, peroxiredoxin-6, and vimentin	Naito [40]
IL-10 (−/−) mice Monoassociated with Enterococcus fecalis	Intestinal epithelial cells	Composition of differentially expressed proteins 2 weeks after colonization is completely different as compared to wild mice despite the absence of colonic inflammation	Werner [36]
TNBS colitis is rats	Colonic mucosa	Increased expression of ubiquitinated-Grp75 Decreased expression of ubiquitinated-Grp78	Bertrand [17]
TNBS colitis is rats	Lymphocytes from mesenteric lymph nodes	26 differentially expressed proteins. 17 upregulated and 9 downregulated. Involved in inflammation, apoptosis, metabolism, regulation of cell cycle and cell proliferation, and signal transduction	Liu [43]
DSS in mice	Exosomes from mice serum	56 proteins differentially expressed in exosomes related to complement and coagulation cascades	Wong [47]
TNBS in rats	Dorsal root ganglia (DRG) Spinal cord (SC)	DRG: 12 proteins upregulated and 14 downregulated. SC: 9 proteins upregulated and 10 downregulated. Altered proteins involved in inflammatory/immune responses, cell signaling, sulfate transport, redox homeostasis, and cellular metabolism	Zhang [49]
IL-10 (−/−) mice	Colonic tissue	Polyunsaturated fatty acids (PUFA) treatment showed anti-inflammatory activity; n-3 eicosapentaenoic acid (EPA) acts via the PPARα pathway, whereas n-6 arachidonic acid (AA) increases energy metabolism and cytoskeletal organization and reduces cellular stress responses	Knoch [37] Cooney [38]
Mdr1a (−/−) mice	Colonic tissue	Increased proteins associated with immune and inflammatory response and fibrogenesis pathways Green tea polyphenols decreased these proteins and increased proteins associated with xenobiotic metabolism pathways	Barnett [50]
Mdr1a (−/−) mice	Colonic tissue	Curcumin increased remodeling/barrier repair, alpha-catenin signaling and xenobiotic metabolism	Cooney [39]
TNBS colitis is rats	Colonic mucosa	Eight proteins less ubiquitinated and four proteins more ubiquitinated. Glutamine enema restored the latter four proteins to normal	Bertrand [51]

proteins such as alpha 1-antitrypsin, alpha 1-antichymotrypsin, and complement 3 chain C (C3c) were found to be over-expressed in the serum during early stage of CD, whereas clusterin, retinol-binding protein, alpha 1-microglobulin, and transthyretin were under-expressed compared to controls. The under-expressed proteins returned to normal in the late stage of CD [31]. Like animal studies, these findings reflect the different inflammatory pathways activated during different stages of CD.

In addition, there is a differential expression of proteins in pediatric compared to adult CD. Vaiopoulou et al. [32] provided evidence that there is a differential serum protein expression of apolipoprotein B-100 and ceruloplasmin in children compared to adults with CD, whereas the protein clusterin was significantly upregulated in adult CD patients compared to pediatric CD patients. These findings suggest that children and adult CD patients may exhibit different immunological responses to the disease manifesting in

different proteins expression and identifying potential biomarkers associated with children CD onset using proteomics.

The statistical analysis of a combination of several specific and non-specific biomarkers of inflammation (including global inflammatory markers, intestinal inflammation specific markers, and chronic intestinal inflammation markers) in the above animal model of colitis allowed the investigators to define a unique protein signature that may be used to discriminate between colitis and non-colitis as well as between different stages of colitis. This study emphasized the concept that a panel of proteins rather than only one protein should be used as biomarker of disease severity, progression, or response to treatment. A similar conclusion was reached in humans. For example, the Brignola score using multiple markers including alpha 2 globulin, alpha 1 glycoprotein, and alpha 2-antitrypsin may predict relapse in asymptomatic Crohn's disease patients [33]. Recently, Wasinger et al. [34] reported a panel of protein markers differentially expressed in active and quiescent UC and CD. Two proteins [phosphoprotein 24 (SPP24) and α-1 microglobulin] were reported to be able to differentiate IBD patients and healthy controls, while guanylin and secretogranin-1 differentiated UC and CD. Furthermore, three of these proteins (secretogranin-1, SPP24, and α-1 microglobulin) were able to distinguish between active and quiescent disease in UC and CD.

Pathogenesis and Pathophysiology

Expression of Inflammatory and Stress Proteins

Using the chronically inflamed IL-10 (−/−) mice monoassociated with Enterococcus fecalis model, Shkoda et al. [35] showed preferential expression of 14 proteins in primary intestinal epithelial cells as compared to wild mice. These proteins are involved in signal transduction, stress response, and cellular homeostasis. Specifically, there was an increased expression level of the glucose-regulated endoplasmic reticulum (ER) stress protein (Grp)-78, a finding that was confirmed in intestinal cells from patients with IBD. In addition, TNF-α induced an increase in Grp78 in primary intestinal cells from IL-10 (−/−) mice with a maximal response after 60 min. The investigators came to the conclusion that IL-10 modulates ER stress response and in the absence of adequate control, ER stress response may lead to a loss of epithelial function and play a role in the pathogenesis and progression of IBD. To further examine the relationship between bacterial colonization and epithelial response, the same group studied protein expression at an early stage (two weeks) after monoassociated Enterococcus fecalis colonization [36]. Despite the absence of colonic inflammation, the composition of differentially regulated proteins was completely different in primary intestinal epithelial cells from wild and IL-10 (−/−) mice two weeks after colonization. Therefore, the absence of IL-10 may have primed the epithelium toward a completely different response leading to its failure to counteract the effect of bacteria in inducing chronic colitis.

In proteomics and transcriptomics studies, Knoch et al. [37] and Cooney et al. [38] identified 172 spot/feature changes that were consistently detected in the colon of IL-10 (−/−) mice as compared to C57 mice. There was an increased abundance of 120 proteins of cellular stress and inflammatory responses. Interestingly, there was a limited concordance between proteomics and transcriptomics profile for the two different genotypes supporting the need for multi-omics to study complex diseases such as IBD.

Cooney et al. [39] identified 33 unique proteins differentially expressed in colonic tissue of Mdr1a(−/−) mice as compared to wild FVB mice. There was an increased expression of proteins associated with cellular stress response and inflammation. Using a DSS model of colitis, Naito et al. [40] identified five differentially expressed proteins of which two were upregulated and three downregulated in the intestinal mucosa of mice with colitis in comparison with control. The proteins hydroxymethylglutaryl-CoA synthase 2 (HMGCS2) and serpinb1a were upregulated, whereas disulfide isomerase A3 (PDIA3), peroxiredoxin-6 (PRDX6), and vimentin were downregulated. HMGCS2 plays an important role in ketogenesis, an undesirable metabolic characteristic of the proliferating cell [41]. Serpinb1a is localized in neutrophils and monocytes and contributes to innate immunity [42]. It is important to note that the deregulated proteins were not identical in these three models emphasizing different pathophysiologic mechanisms and inflammatory pathways that were induced. Thus, this proteome analysis might provide important, novel clues for understanding IBD biology and reveal candidates for different therapeutic targets.

The importance of Grp and the role of the ubiquitin proteasome system (UPS) were also studied by Bertrand et al. [17]. Using proteomics approach, the investigators identified differential expression of ubiquitinated proteins in human intestinal HCT-8 cells during inflammatory conditions and then the ubiquitination states of identified proteins were evaluated in an animal model of TNBS-treated rats. Thus, the role of UPS in the regulation of Grp75 and Grp78 in the colonic mucosa during intestinal inflammation was investigated. Compared to controls, rats with colitis had a significant increased expression of ubiquitinated-Grp75 (associated with a decrease in free Grp75), whereas ubiquitinated-Grp78 was less expressed (associated with an increase in free Grp78). These changes were not observed in acute restraint stressed rats.

Therefore, ubiquitination of these proteins is modulated during the inflammatory response and thus could participate in pathological intestinal epithelium injury by regulating apoptosis and disrupting UPR mechanism. Further studies are needed to identify which ubiquitin ligase E3 enzymes are responsible for this protein ubiquitination.

To get more information on the pathogenesis of IBD, protein profile of lymphocytes isolated from mesenteric lymph nodes of colitic rats treated with TNBS was studied [43]. Twenty-six proteins with at least a twofold difference in abundance between colitis and control groups were identified, 17 were upregulated, and 9 were downregulated. The altered proteins included inflammatory factors (such as myeloid-related protein 14, a potential mediator of p38 mitogen-activated protein kinases-dependent functional responses), apoptosis-related proteins, metabolic enzymes (such as ATP citrate synthase), regulators of cell cycle and cell proliferation, and signal transduction factors (such as nucleoside diphosphate kinases and ubiquitin-conjugating enzyme E2N).

Role of Exosomes

Proteomics studies in animal models have also contributed to our understanding of the role of exosomes in IBD. Exosomes are 40–150-nm microvesicles of endosomal origin released from different cell types and are found in a variety of physiological fluids, including serum, and can participate in intercellular communication and mediate the immune response [44]. It was recently shown that exosomes activate macrophages [45] and thus may play a role in IBD pathogenesis. In addition, a recent study found that serum levels of annexin A1-containing exosomes were elevated in IBD patients and were correlated with the inflammatory activity of the disease [46]. Wong et al. [47] studied the effect of exosomes isolated from DSS-induced colitis on macrophage activation and identified exosome proteins that could play a role in this activation. Treatment of macrophages with exosomes isolated from serum of DSS-treated mice induced phosphorylation of p38 and extracellular signal regulated kinases (ERK) and production of tumor necrosis factor when compared to treatment with exosomes isolated from control mice. Proteomics analysis of these exosomes identified 56 differentially expressed proteins, a majority of which were acute-phase proteins (14) and immunoglobulins (16) that were mainly involved in the complement and coagulation cascade. These findings provided a better understanding of the role of circulating serum exosomes in acute colitis and the role of macrophage activation in the pathogenesis of IBD. Serum exosome concentration and specific exosome proteins are potential inflammatory markers of IBD. Recently, it was found that salivary exosomal proteasome subunit alpha type-7 (PSMA7) differed significantly between healthy individuals and patients with UC and CD. This could be an ideal biomarker of IBD [48]. Surely, further studies on the importance of exosomes and their proteins in patients with IBD are needed.

Cytoskeletal and Structural Proteins

Many animal studies showed a disturbance of cytoskeletal and structural proteins during intestinal inflammation that can explain the "leaky gut." An increased level of cleaved caspase-3 and a decreased level of cadherin [35] as well as VIL 1 and KRT8 [38] in IL-10-deficient mice pointed to a disturbed intestinal mucosal barrier. In addition, a decreased expression of cytoskeletal and structural proteins associated with cellular assembly and organizational processes was identified in Mdr1a (−/−) mice. For example, multiple cytoskeletal assembly proteins important for barrier integrity, including gelsolin (GSN) and vinculin (VCL), were expressed at lower levels in the inflamed colon of Mdr1a (−/−) mice compared with non-inflamed FVB mice emphasizing the importance of the gut barrier in the pathogenesis of colitis [39].

Cell Metabolism Proteins

Multiple proteins related to cell metabolism were found to be decreased in colitis. A decrease in hydroxymethylglutaryl-CoA synthase 2, creatine kinase, pyruvate kinase was described in IL-10-deficient mice [35]. Using the same animal model, a decrease in 52 metabolism-related proteins including carbohydrate, lipid, amino acid, and energy metabolism was identified emphasizing the fact that chronic intestinal inflammation represents an energy deficiency state [37, 38] and may contribute to ER stress [35]. Similarly, in human studies performed by Shkoda et al. [11], 32% of all detected differentially regulated proteins associated with IBD were involved in energy metabolism. Proteins involved in energy generation like H+-transporting two-sector ATPase (ATP5B), mitochondrial malate dehydrogenase (MDH2), and triosephosphate isomerase were found to be downregulated in colonic mucosa of UC patients [7].

Mechanism of Extraintestinal Manifestation of IBD

In an attempt to understand the mechanism of extraintestinal manifestations of IBD like neuropathic pain, cerebrovascular events, white matter lesions, and visceral pain, Zhang et al. [49] studied the differential expression of proteins in the dorsal ganglia (DRG) and spinal cord (SC) in TNBS-induced colitis in rats. In the DRG, 26 proteins were differentially expressed, 12 of which were

upregulated and 14 were downregulated, whereas in the SC a total of 19 proteins were differentially expressed, 9 of which were upregulated and 10 were downregulated. The altered proteins are involved in a number of biological functions including inflammatory/immune responses like proteasome subunit α type-1 (PSMA1); cell signaling like adenylylcyclase-associated protein 1 (Cap1); sulfate transport; redox homeostasis like superoxide dismutase (Sod2); and cellular metabolism. These findings provided useful proteins for further investigation on the neurological manifestations of IBD.

Use of Proteomics to Investigate the Effect of Food on Intestinal Inflammation

Animal models of colitis have been also used to investigate the mechanisms by which some food components may have a beneficial effect on intestinal inflammation using the omics technology [18]. Such food components included polyunsaturated fatty acids [37, 38], polyphenol-rich green tea extract [50], curcumin [39], and glutamine [51].

Knock et al. [37] and Cooney et al. [38] used IL-10-deficient mice to study proteomics profile after administration of monounsaturated (OA) or polyunsaturated fatty acid diets (n-3 eicosapentaenoic acid (EPA) and n-6 arachidonic acid (AA)) and described their possible functional pathways in attenuating inflammation. Whereas EPA modulated the expression of the heat shock protein HSP90AB1 and therefore exerted its anti-inflammatory effects via PPARα, AA increased proteins involved in energy metabolism and cytoskeletal assembly and organization. These findings support studies suggesting that dietary polyunsaturated fatty acids supplementation, or consumption of foods rich in these compounds may reduce IBD symptoms and therefore may play a part in an overall IBD treatment regime. However, care must be taken when extrapolating data to a human clinical situation. Further research is required to confirm the beneficial effects of polyunsaturated fatty acid on the outcome of patients with IBD.

Barnett et al. [50] studied the effect of a diet rich in green tea extract in polyphenols (GrTP) on colonic inflammation and protein expression in the multi-drug resistance targeted mutation Mdr1a (−/−) mouse model of IBD. The GrTP-rich diet resulted in a decrease in colonic inflammation and a differential expression of 33 unique proteins. Proteins involved with cellular stress, immune response, and inflammation like albumin, heat shock proteins, and macrophage migration inhibitory factor were downregulated. On the other hand, upregulated proteins were those associated with cellular assembly like desmin, or with energy metabolism like ATP synthase.

Dietary supplementation of Mdr1a (−/−) mice with curcumin resulted in modulation of 35 unique proteins involved with cellular stress, immune response, and inflammation [39]. Curcumin, a food additive extracted from the medicinal plant Curcuma longa, has been previously found to significantly ameliorate diarrhea, decrease severity of colitis, and decrease MPO activity in TNBS-induced colitis in mice [52]. This effect was associated with a decrease in mucosal level of IFN-γ and IL-12 and inhibition of NF-κB. The downregulated proteins included those associated with negative acute-phase response like albumin and apolipoprotein A1; endoplasmic reticulum stress response like heat shock proteins; and inflammation and inflammatory response like macrophage migrating inhibitory factor. A newly discovered activation of α-catenin resulting in upregulation of multiple cytoskeletal assembly proteins important for barrier integrity, including actin, desmin, and gelsolin, was described. Therefore, curcumin's anti-inflammatory activity in Mdr1a (−/−) mouse colon may be mediated through decreased neutrophil migration by inhibition of PI3 K complex and p38 MAPK and increased remodeling of epithelial adherin junctions/repair of barrier mediated through inhibition of IFN-γ and activation of α-catenin.

The conditionally essential amino acid glutamine was found to have a beneficial effect on intestinal inflammation in experimental animal models by increasing defense systems and maybe by regulating the ubiquitination process of other proteins. Bertrand et al. [51] evaluated the result of intestinal inflammation on differential proteins ubiquitination in colonic mucosa and the effect of rectal glutamine enema in TNBS-treated rats. Eight proteins were less ubiquitinated, and four proteins were more ubiquitinated in TNBS group compared with controls. Glutamine restored the latter four proteins to normal. Twelve ubiquitinated proteins, mostly involved in metabolic pathways, were only affected by glutamine. Although glutamine did not affect histological inflammatory grade in the colon, this study demonstrated that glutamine can affect proteasome-mediated protein degradation in health and disease.

Advances in Technology and Tools for Proteomics Research

The technological basis of modern translational proteomics is a hybrid instrument that couples a liquid chromatograph (LC) with a tandem mass spectrometer (MS/MS). With this analytical system (LC–MS/MS), a pre-proteolyzed biological sample can have its constituent peptides identified and quantified by the collection of a number of critical characteristics such as chromatography retention time, mass, ion abundance, and fragmentation pattern, that is

matched with empirical and in silico entries in curated LC–MS/MS databases [53].

One of the primary challenges for complete proteome coverage is the resolving power of LC–MS/MS to sequence all peptides in complex biologically derived mixtures [54]. Technological developments in this field have thus worked to overcome this hurdle through advances in chromatographic separation and MS/MS hardware, employing multi-dimensional chromatography columns to separate peptides based on two or more properties, such as hydrophobicity, charge, and size, and through new MS/MS systems with ever-increasing mass accuracy and sensitivity. Among these advances, recent innovations in MS/MS data-independent acquisition (DIA) workflows such as Sequential Window Acquisition of all Theoretical Mass Spectra (SWATH-MS) and MS^E have added novel tools for increasing proteome coverage.

DIA is a MS methodology that aims to increase the number of peptides sequenced per sample subject to LC–MS/MS. While in traditional data-dependent acquisition (DDA) MS ions with strong signals (high abundance within the sample) are biased for fragmentation selection, in DIA, all ions within a specified time or m/z window (irrespective of abundance) are sent for fragmentation and can, hence, be sequenced using proteomic databases [55].

MS^E is a DIA method that sequences all peptides irrespective of time or m/z window, utilizing a dynamic switching of high and low collision energies to simultaneously survey all ions within a full mass range (typically 300–2000 m/z) and fragmenting them for sequencing information [54, 56].

SWATH-MS is a DIA method that has been enabled by advances in MS/MS hardware giving instruments unprecedented mass accuracy and scan speed capabilities for producing complex, high-resolution datasets. In SWATH-MS, complete MS/MS is performed on all ions in a sample in overlapping 25 m/z windows across a set mass range of interest [54]. The resulting high-resolution, comprehensive a priori dataset that contains information for highly confident identifications and quantification (superior to multiple reaction monitoring, the gold standard for peptide and small molecule identification and quantification in the chemical and pharmacological laboratory) can then be mined by the proteomics scientist for peptides and proteins of interest. The crux of this technique is a novel data analytics principle—the ability to interrogate a highly accurate and rich proteome dataset ad libitum [57].

Besides these advances in label-free quantitative mass spectrometry, there have also been vast improvements to imaging MS, which allows scrutiny of the spatial distribution of proteins on tissue surfaces, making it particularly applicable to investigations of the gastrointestinal mucosal barrier. Matrix-assisted laser desorption ionization (MALDI) is the most widely used form of imaging MS, using a chemical matrix to assist in the desorption of proteins from sample tissue as it gets exposed to an ultraviolet (UV) laser and ionizes [58]. The UV laser scans the dimensions of the sample tissue, generating a mass spectrum for unique X, Y coordinates on the sample plane [58]. Conventionally, the spatial resolution of MALDI imaging MS is in the range of 20–100 μm and there has been considerable interest in the biomedical scientific community to develop sub-10-μm resolution to achieve cellular-level protein imaging. Some of the novel methods used for this end include the development of ultrafine matrix coatings by electrospray and vibrational means, and decreasing laser beam focal diameters [59].

One notable method of resolution enhancing MALDI imaging MS is the scanning microprobe MALDI (SMALDI) platform, which utilizes five large-diameter stacked lenses in close proximity (as opposed to the conventional three-lens setup) to focus the laser beam to a minimum effective spot diameter of close to 1 μm [59, 60]. This allows the high-resolution imaging of proteins and peptides on tissue surfaces to a lateral resolution of 5–10 μm [59, 60], bringing the imaging capability of MALDI to unprecedented levels of cellular-level detail.

While the technological advances of new mass spectrometry methodologies such as MS^E, SWATH-MS, and SMALDI are on the verge of transforming translational proteomics for biomedical research, their usefulness hinges tremendously on the tandem development of algorithms to accurately interpret the highly complex data in which they generate. Specifically, the ability to deconvolute and assign the correct product ion spectra with their corresponding precursor is required in order to make new DIA methodology data compatible with the wealth of MS/MS knowledge bases currently available for peptide and protein identification [54, 61].

Future Role of Animal Models in Proteomics Studies

Animal models of colitis provide IBD researchers a number of options for studying the different pathogenic and therapeutic aspects of IBD. Combined with the unprecedented resolving power of new DIA technologies to decipher the proteome, there are several important opportunities in IBD research specific to this partnership.

Spontaneous colitis models (i.e., IL-10 and Mdr1a) are ideal platforms to study the interaction between the microbiota and IBD pathogenesis, as colitis occurs after exposure to commensal bacteria in these settings. Using metaproteomic profiling (the identification of the protein complement of microbial communities) [62] to compare pre- and post-colitis onset in these models could potentially

identify important pathogenic phylotypes for colitis, given key host immunodeficiencies. This may be achieved by daily sampling of metaproteomic content up to and beyond spontaneous colitis development. The potential to control and compare different bacterial exposures, and cross-check simultaneous human IBD microbiome profiles, makes the spontaneous colitis–metaproteome analysis a unique platform for uncovering host–bacteria interactions in human IBD [63, 64].

Another intriguing prospect of animal models of colitis–proteomic partnership is in deciphering the mechanistic pathways of IBD susceptibility genes. It is well established that IBD-associated genes confer only a small percentage of IBD risk—owing to the heterogeneity of the disease and the number of possible polymorphisms per gene that result in varied protein production. The development of "knock-in" mice, a model of a specific polymorphism [65], combined with targeted proteomic analysis (DIA or MRM), could explicitly define the mechanism of a pathogenic pathway. For example, while Mdr1a−/− mice reliably develop spontaneous colitis, MDR1 association studies in human IBD have returned with variable results [66–69]. The complexity lies both in the number of polymorphisms possible to the gene and in the undefined role of its product, P-glycoprotein 170, in regulating host–bacteria interactions [67]. Direct profiling of P-glycoprotein 170 and simultaneous metaproteomic analysis of specific MDR1 SNP "knock-in" mice could elucidate this pathway. However, it must be noted that this would require the specific SNP in question to be conserved between human and mouse.

The animal colitis–proteomics experimental paradigm has a unique capacity to investigate the gene-environmental axis of IBD etiology, which may unlock the full spectrum of causes that manifest in these difficult heterogeneous diseases.

Conclusion

The use of animal models of colitis has significantly contributed to proteomics research in various ways. The knowledge acquired through studying proteins deregulation during the different stages of inflammation has helped in understanding some aspects of the pathogenesis and pathophysiology of IBD. Getting information about the different inflammatory pathways activated or inhibited secondary to a specific genetic mutation or colitis induction may explain the heterogeneous results in human studies reflecting the complex nature of the disease. Animal studies have also contributed to improving the tools used in protein determination, and the information gathered has helped in defining pathways of inflammation and construct a network of inflammatory bowel disease interaction between genes, RNAs, proteins, and metabolites leading to a user-friendly software like Ingenuity Pathway Analysis (IPA) which is essential in all proteomics research [5]. The effect of some nutrients on intestinal inflammation and their effect on proteins secretion will open the way to the wider use of therapeutic intervention targeting specific inflammatory pathways. Despite the difference between human IBD and experimental colitis, the use of animal models may facilitate the identification of disease biomarkers or at least prioritize the candidate biomarkers to be tested in clinical studies [70].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. No financial support for this work.

References

1. Wasinger VC, Cordwell SJ, Cerpa-Poljak A, et al. Progress with gene-product mapping of the mollicutes: *Mycoplasma genitalium*. *Electrophoresis*. 1995;16:1090–1094.
2. Hoehn GT, Suffredini AF. Proteomics. *Crit Care Med*. 2005;33:S444–S448.
3. Anderson NL, Anderson NG. Proteome and proteomics: new technologies, new concepts, and new words. *Electrophoresis*. 1998;19:1853–1861.
4. Arsene-Ploetze F, Bertin PN, Carapito C. Proteomic tools to decipher microbial community structure and functioning. *Environ Sci Pollut Res Int*. 2015;22:13599–13612.
5. Polytarchou C, Koukos G, Iliopoulos D. Systems biology in inflammatory bowel diseases: ready for prime time. *Curr Opin Gastroenterol.* 2014;30:339–346.
6. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol*. 2006;24:971–983.
7. Hsieh SY, Shih TC, Yeh CY, Lin CJ, Chou YY, Lee YS. Comparative proteomic studies on the pathogenesis of human ulcerative colitis. *Proteomics*. 2006;6:5322–5331.
8. Barcelo-Batllori S, Andre M, Servis C, et al. Proteomic analysis of cytokine induced proteins in human intestinal epithelial cells: implications for inflammatory bowel diseases. *Proteomics*. 2002;2:551–560.
9. Felley-Bosco E, Andre M. Proteomics and chronic inflammatory bowel diseases. *Pathol Res Pract*. 2004;200:129–133.
10. Fogt F, Jian B, Krieg RC, Wellmann A. Proteomic analysis of mucosal preparations from patients with ulcerative colitis. *Mol Med Rep.* 2008;1:51–54.
11. Shkoda A, Werner T, Daniel H, Gunckel M, Rogler G, Haller D. Differential protein expression profile in the intestinal epithelium from patients with inflammatory bowel disease. *J Proteome Res*. 2007;6:1114–1125.
12. Bennike T, Birkelund S, Stensballe A, Andersen V. Biomarkers in inflammatory bowel diseases: current status and proteomics identification strategies. *World J Gastroenterol*. 2014;20:3231–3244.
13. Chan PP, Wasinger VC, Leong RW. Current application of proteomics in biomarker discovery for inflammatory bowel disease. *World J Gastrointest Pathophysiol.* 2016;7:27–37.
14. Soubieres AA, Poullis A. Emerging role of novel biomarkers in the diagnosis of inflammatory bowel disease. *World J Gastrointest Pharmacol Ther.* 2016;7:41–50.

15. Buhrke T, Lengler I, Lampen A. Analysis of proteomic changes induced upon cellular differentiation of the human intestinal cell line Caco-2. *Dev Growth Differ*. 2011;53:411–426.
16. Kaulmann A, Serchi T, Renaut J, Hoffmann L, Bohn T. Carotenoid exposure of Caco-2 intestinal epithelial cells did not affect selected inflammatory markers but altered their proteomic response. *Br J Nutr*. 2012;108:963–973.
17. Bertrand J, Tennoune N, Marion-Letellier R, et al. Evaluation of ubiquitinated proteins by proteomics reveals the role of the ubiquitin proteasome system in the regulation of Grp75 and Grp78 chaperone proteins during intestinal inflammation. *Proteomics*. 2013;13:3284–3292.
18. Barnett M, Young W, Cooney J, Roy N. Metabolomics and proteomics, and what to do with all these 'Omes': insights from nutrigenomic investigations in New Zealand. *J Nutrigenet Nutrigenomics.* 2014;7:274–282.
19. Sartor RB. Review article: Role of the enteric microflora in the pathogenesis of intestinal inflammation and arthritis. *Aliment Pharmacol Ther*. 1997;11 Suppl 3:17–22; discussion 22–13.
20. Blumberg RS, Saubermann LJ, Strober W. Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. *Curr Opin Immunol*. 1999;11:648–656.
21. Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. *Annu Rev Immunol*. 2002;20:495–549.
22. Hibi T, Ogata H, Sakuraba A. Animal models of inflammatory bowel disease. *J Gastroenterol*. 2002;37:409–417.
23. Wirtz S, Neurath MF. Animal models of intestinal inflammation: new insights into the molecular pathogenesis and immunotherapy of inflammatory bowel disease. *Int J Colorectal Dis*. 2000;15:144–160.
24. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell*. 1993;75:263–274.
25. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. *J Immunol.* 1998;161:5733–5744.
26. Dommels YE, Butts CA, Zhu S, et al. Characterization of intestinal inflammation and identification of related gene expression changes in mdr1a(−/−) mice. *Genes Nutr.* 2007;2:209–223.
27. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology*. 1990;98:694–702.
28. Shintani N, Nakajima T, Okamoto T, Kondo T, Nakamura N, Mayumi T. Involvement of CD4+ T cells in the development of dextran sulfate sodium-induced experimental colitis and suppressive effect of IgG on their action. *Gen Pharmacol*. 1998;31:477–481.
29. Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med*. 1995;182:1281–1290.
30. Viennois E, Baker MT, Xiao B, Wang L, Laroui H, Merlin D. Longitudinal study of circulating protein biomarkers in inflammatory bowel disease. *J Proteomics.* 2015;112:166–179.
31. Piras C, Soggiu A, Greco V, et al. Serum protein profiling of early and advanced stage Crohn's disease. *EuPa Open Proteom.* 2014;3:48–59.
32. Vaiopoulou A, Gazouli M, Papadopoulou A, et al. Serum protein profiling of adults and children with Crohn disease. *J Pediatr Gastroenterol Nutr*. 2015;60:42–47.
33. Brignola C, Campieri M, Bazzocchi G, Farruggia P, Tragnone A, Lanfranchi GA. A laboratory index for predicting relapse in asymptomatic patients with Crohn's disease. *Gastroenterology*. 1986;91:1490–1494.
34. Wasinger VC, Yau Y, Duo X, et al. Low mass blood peptides discriminative of inflammatory bowel disease (IBD) severity: a quantitative proteomic perspective. *Mol Cell Proteomics*. 2016;15:256–265.
35. Shkoda A, Ruiz PA, Daniel H, et al. Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. *Gastroenterology*. 2007;132:190–207.
36. Werner T, Shkoda A, Haller D. Intestinal epithelial cell proteome in IL-10 deficient mice and IL-10 receptor reconstituted epithelial cells: impact on chronic inflammation. *J Proteome Res*. 2007;6:3691–3704.
37. Knoch B, Barnett MP, Cooney J, et al. Dietary oleic acid as a control fatty acid for polyunsaturated fatty acid intervention studies: a transcriptomics and proteomics investigation using interleukin-10 gene-deficient mice. *Biotechnol J*. 2010;5:1226–1240.
38. Cooney JM, Barnett MP, Brewster D, et al. Proteomic analysis of colon tissue from interleukin-10 gene-deficient mice fed polyunsaturated Fatty acids with comparison to transcriptomic analysis. *J Proteome Res*. 2012;11:1065–1077.
39. Cooney JM, Barnett MP, Dommels YE, et al. A combined omics approach to evaluate the effects of dietary curcumin on colon inflammation in the Mdr1a(−/−) mouse model of inflammatory bowel disease. *J Nutr Biochem*. 2016;27:181–192.
40. Naito Y, Takagi T, Okada H, et al. Identification of inflammation-related proteins in a murine colitis model by 2D fluorescence difference gel electrophoresis and mass spectrometry. *J Gastroenterol Hepatol*. 2010;25(Suppl 1):S144–S148.
41. Camarero N, Mascaro C, Mayordomo C, Vilardell F, Haro D, Marrero PF. Ketogenic HMGCS2 Is a c-Myc target gene expressed in differentiated cells of human colonic epithelium and down-regulated in colon cancer. *Mol Cancer Res*. 2006;4:645–653.
42. Benarafa C, Priebe GP, Remold-O'Donnell E. The neutrophil serine protease inhibitor serpinb1 preserves lung defense functions in Pseudomonas aeruginosa infection. *J Exp Med*. 2007;204:1901–1909.
43. Liu BG, Cao YB, Cao YY, et al. Altered protein profile of lymphocytes in an antigen-specific model of colitis: a comparative proteomic study. *Inflamm Res*. 2007;56:377–384.
44. Choi DS, Kim DK, Kim YK, Gho YS. Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. *Proteomics*. 2013;13:1554–1571.
45. Singh PP, Smith VL, Karakousis PC, Schorey JS. Exosomes isolated from mycobacteria-infected mice or cultured macrophages can recruit and activate immune cells in vitro and in vivo. *J Immunol.* 2012;189:777–785.
46. Leoni G, Neumann PA, Kamaly N, et al. Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. *J Clin Invest.* 2015;125:1215–1227.
47. Wong WY, Lee MM, Chan BD, et al. Proteomic profiling of dextran sulfate sodium induced acute ulcerative colitis mice serum exosomes and their immunomodulatory impact on macrophages. *Proteomics*. 2016;16:1131–1145.
48. Zheng X, Chen F, Zhang Q, et al. Salivary exosomal PSMA7: a promising biomarker of inflammatory bowel disease. *Protein Cell*. 2017. doi:10.1007/s13238-017-0413-7.
49. Zhang XJ, Leung FP, Hsiao WW, et al. Proteome profiling of spinal cord and dorsal root ganglia in rats with trinitrobenzene sulfonic acid-induced colitis. *World J Gastroenterol*. 2012;18:2914–2928.
50. Barnett MP, Cooney JM, Dommels YE, et al. Modulation of colonic inflammation in Mdr1a(−/−) mice by green tea polyphenols and their effects on the colon transcriptome and proteome. *J Nutr Biochem*. 2013;24:1678–1690.
51. Bertrand J, Marion-Letellier R, Azhar S, et al. Glutamine enema regulates colonic ubiquitinated proteins but not proteasome

activities during TNBS-induced colitis leading to increased mitochondrial activity. *Proteomics*. 2015;15:2198–2210.
52. Ukil A, Maity S, Karmakar S, Datta N, Vedasiromoni JR, Das PK. Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. *Br J Pharmacol*. 2003;139:209–218.
53. Wenner BR, Lynn BC. Factors that affect ion trap data-dependent MS/MS in proteomics. *J Am Soc Mass Spectrom*. 2004;15:150–157.
54. Law KP, Lim YP. Recent advances in mass spectrometry: data independent analysis and hyper reaction monitoring. *Expert Rev Proteom.* 2013;10:551–566.
55. Doerr A. DIA mass spectrometry. *Nat Meth.* 2015;12:35.
56. Plumb RS, Johnson KA, Rainville P, et al. UPLC/MS(E); a new approach for generating molecular fragment information for biomarker structure elucidation. *Rapid Commun Mass Spectrom*. 2006;20:1989–1994.
57. Gillet LC, Navarro P, Tate S, et al. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. *Mol Cell Proteom.* 2012;11(O111):016717.
58. Angel PM, Baldwin HS, Gottlieb Sen D, et al. Advances in MALDI imaging mass spectrometry of proteins in cardiac tissue, including the heart valve. *Biochim Biophys Acta*. 2017. doi:10.1016/j.bbapap.2017.03.009.
59. Dreisewerd K. Recent methodological advances in MALDI mass spectrometry. *Anal Bioanal Chem*. 2014;406:2261–2278.
60. Spengler B, Hubert M. Scanning microprobe matrix-assisted laser desorption ionization (SMALDI) mass spectrometry: instrumentation for sub-micrometer resolved LDI and MALDI surface analysis. *J Am Soc Mass Spectrom*. 2002;13:735–748.
61. Wasinger VC, Zeng M, Yau Y. Current status and advances in quantitative proteomic mass spectrometry. *Int J Proteom.* 2013;2013:180605.
62. Hettich RL, Pan C, Chourey K, Giannone RJ. Metaproteomics: harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities. *Anal Chem*. 2013;85:4203–4214.
63. Wilk JN, Bilsborough J, Viney JL. The mdr1a−/− mouse model of spontaneous colitis: a relevant and appropriate animal model to study inflammatory bowel disease. *Immunol Res*. 2005;31:151–159.
64. Fiebiger U, Bereswill S, Heimesaat MM. Dissecting the interplay between intestinal microbiota and host immunity in health and disease: lessons learned from germfree and gnotobiotic animal models. *Eur J Microbiol Immunol (Bp).* 2016;6:253–271.
65. Ladiges WC. Mouse models of XRCC1 DNA repair polymorphisms and cancer. *Oncogene*. 2006;25:1612–1619.
66. Oostenbrug LE, Dijkstra G, Nolte IM, et al. Absence of association between the multidrug resistance (MDR1) gene and inflammatory bowel disease. *Scand J Gastroenterol*. 2006;41:1174–1182.
67. Ardizzone S, Maconi G, Bianchi V, et al. Multidrug resistance 1 gene polymorphism and susceptibility to inflammatory bowel disease. *Inflamm Bowel Dis*. 2007;13:516–523.
68. Krupoves A, Seidman EG, Mack D, et al. Associations between ABCB1/MDR1 gene polymorphisms and Crohn's disease: a gene-wide study in a pediatric population. *Inflamm Bowel Dis*. 2009;15:900–908.
69. Zintzaras E. Is there evidence to claim or deny association between variants of the multidrug resistance gene (MDR1 or ABCB1) and inflammatory bowel disease? *Inflamm Bowel Dis*. 2012;18:562–572.
70. Whiteaker JR, Lin C, Kennedy J, et al. A targeted proteomics-based pipeline for verification of biomarkers in plasma. *Nat Biotechnol*. 2011;29:625–634.