Proteome profiling of spinal cord and dorsal root ganglia in rats with trinitrobenzene sulfonic acid-induced colitis

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AIM: To investigate proteomic changes in spinal cord and dorsal root ganglia (DRG) of rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis.

METHODS: The colonic myeloperoxidase (MPO) activity and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) level were determined. A two-dimensional electrophoresis (2-DE)-based proteomic technique was used to profile the global protein expression changes in the DRG and spinal cord of the rats with acute colitis induced by intracolonic injection of TNBS.

RESULTS: TNBS group showed significantly elevated colonic MPO activity and increased TNF-\(\alpha\) level. The proteins derived from lumbosacral enlargement of the spinal cord and DRG were resolved by 2-DE; and 26 and 19 proteins that displayed significantly different expression levels in the DRG and spinal cord were identified respectively. Altered proteins were found to be involved in a number of biological functions, such as inflammation/immunity, cell signaling, redox regulation, sulfate transport and cellular metabolism. The overexpression of the protein similar to potassium channel tetramerisation domain containing protein 12 (Kctd12) and low expression of proteasome subunit \(\alpha\) type-1 (psma) were validated by Western blotting analysis.

CONCLUSION: TNBS-induced colitis has a profound impact on protein profiling in the nervous system. This result helps understand the neurological pathogenesis of inflammatory bowel disease.

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Key words: Inflammatory bowel disease; Trinitrobenzene sulfonic acid; Two-dimensional electrophoresis-based proteomic technique; Dorsal root ganglia; Spinal cord

INTRODUCTION

Inflammatory bowel disease (IBD) is defined as a group
of inflammatory conditions in the colon and small intestine, mainly including ulcerative colitis and Crohn’s disease. The cause of IBD is suggested to be a nebulous combination of not only host genetic factors, but also immune dysfunction, dysbiosis, cellular oxidative stress and leakage of intestinal barrier[2]. Fundamental therapy for this condition has not yet been established because its etiology remains obscure. Unfortunately, the prevalence of IBD is continuing to increase in both Eastern and Western countries, causing enormous medical costs[2,3]. Beside intestinal disorders, many organs outside the gastrointestinal tract, such as the central nervous system, are involved in IBD[3]. Neuropathies, cerebrovascular events, white matter lesions, and visceral pain are common neurological manifestations[4]. These alterations may help explain some of the underlying comorbidities, such as hyperalgesia, seizure and anorexia[5,6]. Unfortunately, the exact mechanism for IBD needs further investigations.

This study focuses on the spinal cord and dorsal root ganglia (DRG) to reveal the neurological dimension in a trinitrobenzene sulfonic acid (TNBS)-induced active colitis model. Unlike previous studies that were based mainly on investigations of specifically selected gene/proteins, proteomic approach was applied in this study to reveal the global changes of proteins. The two-dimensional electrophoresis (2-DE) in combination with matrix-assisted laser desorption-time-of-flight/time-of-flight mass spectrometer (MALDI-TOF/TOF MS) have been widely used to probe into changes of protein profiles accompanied with diseases like cancer and hyperalgesia[7,8]. In the present study, this approach was applied to analyze the proteomic differences in lumbar enlargement of spinal cord and DRG in the rat model of TNBS-induced colitis. This study aimed to investigate whether changed protein profiles in the nervous system are in any way associated with neurological dimensions in IBD animal model.

**MATERIALS AND METHODS**

**Animals and tissue processing**

Male Sprague-Dawley rats (180-200 g in weight) were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong. Rats were kept at room temperature 23 ℃ ± 2 ℃ with an alternating 12 h light-dark cycle, and were allowed access to food and water ad libitum. All of the experimental protocols were carried out with the approval of the Committee on Use of Human and Animal Subjects in Teaching and Research of Hong Kong Baptist University and according to the Regulations of the Department of Health, Hong Kong, China.

**Induction of colitis**

Induction of colitis was adapted from the previously reported methods[9,10]. Briefly, under chloral hydrate (350 mg/kg, ip) anesthesia, colitis was induced in overnight-fasted rats (n = 5) by intra-colonic administration of 30 mg/kg of TNBS (Sigma, St. Louis, United States) dissolved in 50% ethanol solution at 8 cm from the anal verge using a rubber catheter. The rats were kept upside-down for 1 min to ensure that the TNBS solution was not expelled immediately. The rats in control group (n = 4) received intra-colonic injection of saline.

**Tissue preparation**

On the 7th day after TNBS instillation, the rats were anesthetized with chloral hydrate (350 mg/kg, ip). Distal colon tissue was excised in two pieces. One piece was fixed in 4% paraformaldehyde, routinely embedded in paraffin, cut into 5 μm sections, mounted on glass slides and stained with hematoxylin and eosin to reveal structural features. The other piece of colon sample was frozen in liquid nitrogen and stored at -80 ℃ for measurement of myeloperoxidase (MPO) activity and tumor necrosis factor-α (TNF-α) level. The rat was then perfused with ice-cold normal saline. The spinal cord and DRG of the lumbo-sacral enlargement were dissected, immediately frozen and stored at -80 ℃ until use. Samples were firstly lysed in buffer (8 mol/L urea, 2 mol/L thiourea, 2% 3-[3-cholamidopropyl] dimethylammonio-1-propanesulfonate (CHAPS), 1% NP-40, 2 mmol/L trichloroacetic acid (TBP), 1 × protease inhibitor mix, 1 × nuclease mix, 1 mmol/L phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluoride (PMSF), and 2% immobilized pH gradient (IPG) buffer, and then incubated on ice for 45 min. The lysed mixtures were centrifuged at 14 000 × g for 15 min at 4 ℃. The supernatant samples were determined by Bradford protein assay (BioRad, California, United States) and stored at -80 ℃.

**Two-dimensional gel electrophoresis and image analysis**

2-DE and image analysis were performed as previously described with some modifications[11]. Isoelectric focusing (IEF) was performed using IPGphor II apparatus (Amerham, Sweden). Samples (150 μg protein/group, containing an equal amount of protein from each animal) were diluted in 250 μL rehydration solution (8 mol/L urea, 2% CHAPS, 0.4% dithiothreitol (DTT), 0.5% IPG buffer, 0.002% bromophenol blue) and loaded onto the IPG strips (13 cm, pH 3-10, NL) by 10 h rehydration at 30 V. The strips were incubated in the equilibration buffer (6 mol/L urea, 2% SDS, 30% glycerol, 0.002% bromophenol blue, 50 mmol/L Tris-HCl, pH 6.8) containing 1% DTT for 15 min with gentle agitation. The strips were then transferred to the equilibrating solution containing 2.5% iodoacacetamide and agitated for 15 min, and subsequently were placed on top of a 12.5% uniform SDS-PAGE gel (150 mm × 158 mm × 1.5 mm). Separation in the second dimension was performed in Tris-glycine buffer (25 mmol/L Tris, 0.2 mol/L glycine, 0.1% SDS) at a constant current setting of 15 mA/gel initially for 30 min and 30 mA/gel thereafter. SDS-PAGE was terminated when the bromophenol blue dye front reached the lower ends of the gels. After 2-DE, gels were visualized using silver-staining[12]. All the raw images were digitalized using
Protein spots of interest were manually excised from the 2-D gels, and digested as previously described with small modification\[12-14\]. Briefly, the gel plugs were washed in 30 mmol/L potassium ferricyanide and 100 mmol/L sodium thiosulfate (1:1 v/v) for 5 min, and then washed in water twice. Subsequently, the gel plugs were equilibrated in 50 mmol/L ammonium bicarbonate for 20 min, then in 25 mmol/L ammonium bicarbonate and 50% acetonitrile (ACN), and finally soaked in 100% ACN until gel plugs became opaque. Thereafter, vacuum-dried gel plugs were rehydrated with 10 mg/mL of trypsin in 25 mmol/L ammonium bicarbonate (pH 8.0). Proteolysis of proteins was performed at 37 °C for 16-18 h. Supernatants were transferred into a new tube, and mixed with 1/2 volume of 1% trifluoroacetic acid to stop digestion. The samples were then vacuum dried at 45 °C for 1-2 h.

**Protein identification by MS/MS**

Protein identification was performed using a Autoflex III MALDI-TOF/TOF mass spectrometer (Bruker, Germany) equipped with a 200 Hz N2 laser operating at 337 nm. Data were acquired in the positive ion reflector mode over a mass range of 800-4000 m/z using Bruker calibration mixture as an external standard. Bruker calibration mixture consists of the following peptides (monoisotopic mass of the singly protonated ion is given in parenthesis in Da): bradykinin (757.3992), angiotensin II (1046.5420), angiotensin I (1296.6853), substance P (1347.7361), bombesin (1619.8230), renin substrate (1758.9326), ACTH clp 1-17 (2093.0868), ACTH clp 18-39 (2465.1990) and somatostatin 28 (3147.4714). Keratin contamination peaks, matrix ion peaks and trypsin ion peaks were excluded from spectra. Typically 400 shots were accumulated per spectrum in MS mode and 2000 shots in MS/MS mode. The spectra were processed using the FlexAnalysis 3.0 and BioTools 3.1 software tools (Bruker, Germany). Protein identification was performed using Mascot (2.2.04, http://www.matrixscience.com) to search the international protein index (IPI) database. Peptide masses were matched with the theoretical peptides of all proteins in the IPI database using the Mascot search program. The following parameters were used for database searches: monoisotopic mass accuracy < 100 ppm, missed cleavages 1, carbamidomethylation of cysteine as fixed modification, oxidation of methionine as variable modifications. In MS/MS mode, the fragment ion mass accuracy was set at < 0.5 Da.

**In-gel digestion**

**Protein identification by MS/MS**

Protein identification was performed using a Autoflex III MALDI-TOF/TOF mass spectrometer (Bruker, Germany) equipped with a 200 Hz N2 laser operating at 337 nm. Data were acquired in the positive ion reflector mode over a mass range of 800-4000 m/z using Bruker calibration mixture as an external standard. Bruker calibration mixture consists of the following peptides (monoisotopic mass of the singly protonated ion is given in parenthesis in Da): bradykinin (757.3992), angiotensin II (1046.5420), angiotensin I (1296.6853), substance P (1347.7361), bombesin (1619.8230), renin substrate (1758.9326), ACTH clp 1-17 (2093.0868), ACTH clp 18-39 (2465.1990) and somatostatin 28 (3147.4714). Keratin contamination peaks, matrix ion peaks and trypsin ion peaks were excluded from spectra. Typically 400 shots were accumulated per spectrum in MS mode and 2000 shots in MS/MS mode. The spectra were processed using the FlexAnalysis 3.0 and BioTools 3.1 software tools (Bruker, Germany). Protein identification was performed using Mascot (2.2.04, http://www.matrixscience.com) to search the international protein index (IPI) database. Peptide masses were matched with the theoretical peptides of all proteins in the IPI database using the Mascot search program. The following parameters were used for database searches: monoisotopic mass accuracy < 100 ppm, missed cleavages 1, carbamidomethylation of cysteine as fixed modification, oxidation of methionine as variable modifications. In MS/MS mode, the fragment ion mass accuracy was set at < 0.5 Da.

** Immunoblotting analysis**

Two identified proteins: (1) similar to potassium channel tetramerisation domain containing protein 12 (Kctd12); and (2) proteasome subunit α type-1 (Psma1), were selected for the confirmation study. For Western-blot analysis, protein lysates were diluted in sample buffer and denatured at 100 °C for 5 min. Proteins (15 μg/lane) of interest were separated by 12% SDS-PAGE, and transferred onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad). Nonspecific binding sites were blocked with 5% nonfat milk for 1 h at room temperature, then the blots were incubated at 4 °C overnight with rabbit antibody against mouse antibody against Psma1 (1:250 in TBST, Santa Cruz) or Kctd12 (1:500 in TBST, Santa Cruz). After washing, the membranes were incubated in horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000) in Tris-Buffered Saline and Tween 20 (TBST) with 5% nonfat milk against the primary antibody species for 1 h at room temperature. The immunoreaction was detected with the enhanced chemiluminescence (ECL) Western blotting kit (Invitrogen). Bands were visualized on Bio-max X-ray film (Kodak, Japan) and captured by a scanner. The optical densities of protein blots were analyzed using Image J software. The results were presented as the ratio of optical density of Kctd12 or Psma1 standardized to optical density of β-actin.

**RESULTS**

**Establishment of IBD model**

Rats developed hypomotility and diarrhea 1 d after TNBS treatment. Hematoxylin and eosin staining of distal colon revealed incarcassation and edema in the mucosa and submucosa, hyperaemia and dilation of the blood vessel, and prominent neutrophilic infiltrates in the submucosal layer, indicating severe colonic inflammation (Figure 1A). TNBS group showed significantly elevated MPO activity (Figure 1B) and TNF-α level (Figure 1C), suggesting increased granulocyte recruitment and macrophage activation in the acute phase of inflammation.

**Identified proteins by 2-DE-based proteomic technique**

The representative 2-DE images of protein profiling changes in DRG and spinal cord is shown in Figure 2. Overall, a total of 26 spots differentially expressed in the DRG of these two groups were identified by the mass spectrometry (MS) analysis, 12 of which were up-regulated and 14 of which were down-regulated (Table 1). A total of 19 spots differentially expressed in the spinal cord of the two groups were identified by MS analysis, 9 of which...
were up-regulated and 10 of which were down-regulated (Table 2). In particular, we found altered expression of (1) proteins involved in inflammatory/immune responses, such as Isoform B0a of heterogeneous nuclear ribonucleoproteins A2/B1 (Hnrnpa2b1) and proteasome subunit α type-1 (Psma1); (2) signal-related proteins, such as adenylyl cyclase-associated protein 1 (Cap1) and voltage-dependent anion-selective channel protein 2 (Vdac2); (3) proteins involved in sulfate transport, thiosulfate sulfurtransferase (Tst); (4) cellular enzymes involved in cell redox homeostasis, such as glutathione S-transferase P (Gstp1) and superoxide dismutase (Sod2); (5) metabolic enzymes, such as fructose-bisphosphate aldolase C (Aldoc); (6) structure protein Lamin C2 (Lmna); and (7) chaperonins, such as heat shock cognate 71 kDa protein (Hspa8, or HSC70) and stress-induced phosphoprotein 1 (Stip1). Although previous studies have reported the contribution of a few proteins, such as down-regulated glutathione peroxidase-1 (Prdx1) and malate dehydrogenase (Mdh2) [12], most of the proteins were first identified in TNBS-induced colitis (Table 3). Most importantly, the samples analyzed in previous studies were usually mucosa or submucosa of the colon, whereas this paper firstly investigated the global protein expression changes in the spinal cord and DRG of...
Table 1  Significantly regulated proteins after trinitrobenzene sulfonic acid-induced colitis in dorsal root ganglia

| Function                                      | Cell component                  | Protein name                                                                 | Abbreviation | Accession No. | Theoretical PI/Mr (kDa) | Sequence coverage (%) | MASSOT score | Change (TNBS) |
|------------------------------------------------|---------------------------------|-------------------------------------------------------------------------------|--------------|---------------|------------------------|-----------------------|--------------|-------------- |
| Proteins involved in inflammatory/immune response |                                |                                                                              | Hnrnpa2b1    | IPI00923129   | 8.74/32.572            | 16                     | 220          | ↑            |
| Autoantigen in many autoimmune diseases        | Cytoplasm, nucleus spliceosome | Isform B6a of heterogeneous nuclear ribonucleoproteins A2/B1                | Hpx          | IPI00195316   | 7.58/52.060            | 22                     | 597          | ↑            |
| Hemopexin                                      | Secreted                        | Hemopexin                                                                    | Ppia         | IPI00387771   | 8.34/18.091            | 32                     | 363          | ↓            |
| 1. Accelerate the folding of protein           | Cytosol, nucleus                | Peptidyl-prolyl cis-trans isomerase A                                       |              |               |                        |                       |              |              |
| 2. Immunosuppression                            |                                 |                                                                               |              |               |                        |                       |              |              |
| Proteins involved in cell signaling            | Membrane                        | Adenyl cyclase-associated protein 1                                         | Capl         | IPI00555187   | 7.16/51.899            | 5                      | 145          | ↑            |
| Growth protein                                 |                                 | Similar to potassium channel tetramerisation domain containing protein 12   | Kctd12       | IPI00359734   | 8.95/47.077            | 16                     | 192          | ↑            |
| Cytoplasmal tetramerisation domain of voltage-gated K+ channel | | Voltage-dependent anion-selective channel protein 2 | Vdac2 | IPI00198327   | 7.44/32.333            | 9                      | 76           | ↓            |
| Proteins involved in redox regulation          | Mitochondria                    | Dihydroxypropion dehydrogenase, mitochondrial                              | Dld          | IPI00365545   | 7.96/54.574            | 5                      | 141          | ↑            |
| Cell redox homeostasis                         | Mitochondria                    | Superoxide dismutase [Mn], mitochondrial                                   | Sod2         | IPI00211593   | 8.96/24.887            | 22                     | 130          | ↓            |
| Antioxidant                                    | Mitochondria                    | Glutathione                                                                  | Gstp1        | IPI00231229   | 6.89/23.652            | 12                     | 255          | ↓            |
| Xenobiotic metabolism and cellular defense     | Nucleus                         | S-transferase P                                                             | Prdx1        | IPI00211779   | 8.27/22.323            | 9                      | 108          | ↓            |
| Eliminating peroxides                          | Mitochondria, cytosol           | Peroxiredoxin-1                                                            |              |               |                        |                       |              |              |
| Proteins involved in chaperone function        | Mitochondria                    | Heat shock cognate 71 kDa protein                                           | Hspa8        | IPI00208205   | 5.37/71.055            | 18                     | 304          | ↓            |
| Chaperonins/heat shock proteins                | Nucleus, cytoplasm               | Stress-induced-phosphoprotein 1                                            | Stip1        | IPI00213013   | 6.40/63.158            | 9                      | 156          | ↑            |
| Proteins involved in cellular structure        | Insoluble fraction, lamin filament, nuclear matrix                          | Lamina C2                                                                   | Lmna         | IPI00188879   | 6.22/52.661            | 16                     | 136          | ↑            |
| Component of the nuclear lamina                |                                 |                                                                               |              |               |                        |                       |              |              |
| Proteins involved in cellular metabolism       | Mitochondria                    | Methylmalonate-semialdehyde dehydrogenase [acylating],mitochondrial         | Aldh6a1      | IPI00205018   | 8.44/58.396            | 4                      | 68           | ↓            |
| Oxidoreductase in valine and pyrimidine catabolic pathways | |                                                                               | AldoC        | IPI00231734   | 8.31/39.783            | 11                     | 148          | ↓            |
| Glycolytic enzyme                              | Mitochondria                    | Fructose-bisphosphate aldolase A                                           | Aldoc        | IPI00561972   | 8.64/16.666            | 14                     | 88           | ↓            |
| Glycolytic enzyme                              | Axon, mitochondria              | Dihydrolipoyllysine-residueacyltransferase component of pyruvate dehydrogenase complex | Dlat | IPI00231714   | 8.76/67.637            | 7                      | 79           | ↑            |
| Glycolytic enzyme                              | Mitochondria                    | Phosphoglycerate kinase 1                                                  | Pgk1         | IPI00231426   | 8.02/44.909            | 16                     | 184          | ↓            |
| Glycolytic enzyme                              | Cytoplasm                       | Similar to pyruvate kinase 3                                               |              | IPI00561880   | 7.15/58.264            | 17                     | 231          | ↓            |
| Glycolytic enzyme                              | Nucleus cytoplasm               | Isoform M1 of pyruvate kinase isozymes M1/ M2                              | Plk2         | IPI00231929   | 6.63/58.452            | 22                     | 491          | ↑            |
| Glycolytic enzyme                              |                                 |                                                                               |              |               |                        |                       |              |              |
| 1. Glycolytic enzyme                            |                                 |                                                                               |              |               |                        |                       |              |              |
| 2. Transcription activation                     |                                 |                                                                               |              |               |                        |                       |              |              |
| 3. Initiation of apoptosis                      |                                 |                                                                               |              |               |                        |                       |              |              |
| ATP energy transduction                         | Cytoplasm                       | Similar to glycosyldehyde-3-phosphate dehydrogenase                        | RGD1565368   | IPI00554039   | 8.44/36.045            | 14                     | 347          | ↑            |
| ATP transducing                                 | Mitochondria                    | Creatine kinase M-type                                                      | Ckm          | IPI00211053   | 6.58/43.246            | 19                     | 361          | ↑            |
|                   |                                 | Creatine kinase, mitochondrial 1, ubiquitous                               | Ckm1         | IPI00555166   | 8.58/47.331            | 21                     | 231          | ↓            |
rats with TNBS-induced acute colitis.

Validation by Western blotting analysis
In order to validate the proteomic data, two of the protein spots: Psma1, a protein involved in immunity, and Kctd12, a protein involved in voltage-gated potassium channel activity, were chosen for validation by Western blotting analysis. The comparison of samples derived from TNBS rats (lanes 1-4) and samples derived from saline control (lanes 5-8) revealed down-regulation of Psma1 (Figure 3A) in the spinal cord and up-regulation of Kctd12 (Figure 3B) in the DRG. These results confirmed the changed protein levels concluded from 2-DE.

DISCUSSION
Colitis persists for at least 28 d after TNBS colonic administration[17,18]. For the study of TNBS-induced colitis, rats were usually sacrificed 7 d after TNBS treatment[19], since TNBS-caused changes of gene expression profile were maximal at day 5 and day 7 after induction[20]. Consistent with previous studies, on day 7 after TNBS intraluminal treatment, we observed macroscopic and microscopic damage of the distal colon, demonstrating the presence of acute colitis. By using 2-DE in combination with MALDI-TOF/TOF MS based proteomic approach, we observed changed expression profiles not only in proteins participating in the immune/inflammatory response, but also in a broad range of proteins involved in cell signaling, sulfate transport, redox homeostasis, and cell metabolism. This result is consistent with previous studies revealing that in addition to inflammation/immunity response, TNBS-colitis affects the gene expression related to a numerous biological functions, such as signal transduction and cell metabolism[21,22]. The identified proteins from the spinal cord and DRG could be categorized into seven groups as follows.

Group 1: Proteins involved in inflammatory/immune responses
Consistent with previous results[23,24], the current study

| TCA cycle | Mitochondria | Isoform mitochondrial of fumarate hydratase, mitochondrial |
|-----------|--------------|-----------------------------------------------------------|
| 1. TCA cycle | Mitochondria | Malate dehydrogenase, mitochondrial |
| 2. Gluconeogenesis | Mitochondria | 3-ketoacid-coenzyme A transferase 1, mitochondrial |
| 3. Antioxidant | Mitochondria | Succinyl-CoA |

†: Elevated protein expression in TNBS group compared with saline group; ↓: Decreased protein expression in TNBS group in comparison with saline group. DRG: Dorsal root ganglia; TNBS: Trinitrobenzene sulfonic acid; Hnrnpa2b1: Heterogeneous nuclear ribonucleoproteins A2/B1; Cap1: Cyclase-associated protein 1; Kctd12: Potassium channel tetramerisation domain containing protein 12; Vdac2: Voltage-dependent anion-selective channel protein 1; Dld: Dihydrolipoyl dehydrogenase; Sod2: Superoxide dismutase; Gstl1: Glutathione S-transferase P; Prdx1: Peroxiredoxin-1; Stip1: Stress-induced phosphoprotein 1; Lmna: Lamin C2; Ckm: Creatine kinase M-type; Ckmt1: Creatine kinase, mitochondrial 1, ubiquitous; Fh1: Fumarate hydratase; Mdh2: Malate dehydrogenase; Oxct1: Succinyl-CoA 3-ketoacid-coenzyme A transferase 1.

Figure 3 Immunoblotting analyses to validate the differential expression of proteasome subunit \( \alpha \) type-1 (A, 0.53 ± 0.14 vs 1.81 ± 0.53) and potassium channel tetramerisation domain containing protein 12 (B, 1.21 ± 0.20 vs 0.56 ± 0.07) between trinitrobenzene sulfonic acid treated group and saline control group. The relative expression ratio standardized to \( \beta \)-actin. \( P < 0.05 \) vs saline group. TNBS: Trinitrobenzene sulfonic acid; Psma1: Proteasome subunit \( \alpha \) type-1; Kctd12: Potassium channel tetramerisation domain containing protein 12.
demonstrated immune regulation in the nervous system following peripheral inflammation. Hnrnpa2b1 hinders the double strand DNA break repair process by binding the DNA-dependent protein kinase complex\(^{[25]}\), and is an autoantigen in autoimmune diseases such as rheumatoid arthritis and autoimmune hepatitis\(^{[26,27]}\). Thus, the up-

### Table 2 Proteins significantly regulated after trinitrobenzene sulfonic acid-induced colitis in spinal cord

| Function | Cell component | Protein name | Abbreviation | Accession No. | Theoretical PI/Mr (kDa) | Sequence coverage (%) | MASSOT score | Change (TNBS) |
|----------|----------------|--------------|--------------|---------------|-------------------------|-----------------------|--------------|---------------|
| Proteins involved in inflammatory/immune response | Cytoplasm | Isoform 1 of cytosolic acyl coenzyme A thioester hydrolyase α-erase | Acof7 | IPI00213571 | 7.16/37 936 | 14 | 167 | ↓ |
| 1. Fatty acid catabolic process | Cell membrane/cytoplasm | Pro tease subunit α-1 | Psmα1 | IPI00191748 | 6.15/29 784 | 17 | 193 | ↓ |
| 2. Play a role in eicosanoid synthesis and inflammation | Cytoplasm, nucleus | Proteome subunit α-1 | Psmα1 | IPI00191748 | 6.15/29 784 | 17 | 193 | ↓ |
| 3. Autoantigen | | | | | |
| Immunity | Cytoplasm | Dihydroxyindinease-related protein 2 | Dpsα2 | IPI00870112 | 5.95/62 638 | 16 | 270 | ↑ |
| 1. Growth protein | Cytoplasm | Trophinin C-like protein | gL223036 | 4.12/6 166 | 9 | 91 | ↓ |
| 2. Modulate calcium influx | | | | | |
| Calcium ion signaling | Cytoplasm, cytoskeleton | Heat shock cognate 71 kDa protein | Hspa8 | IPI00208205 | 5.37/71 055 | 11 | 172 | ↑ |
| 1. Cell division | Melanosome, cytoplasm | 14-3-3 protein epsilon | Ywhaε | IPI003025135 | 4.63/29 326 | 15 | 165 | ↑ |
| 2. Apoptosis | | | | | |
| 3. Regulate insulin sensitivity | | | | | |
| Proteins involved in cell signaling | Mitochondrial matrix | Thiosulfate sulfurtransferase | Tst | IPI00366290 | 7.71/33 614 | 25 | 333 | ↓ |
| Proteins involved in redox regulation | Nucleus | Glutathione S-transferase P | Gstp1 | IPI00251229 | 6.89/23 652 | 20 | 263 | ↓ |
| Proteins involved in chaperone function | Cytoplasm | T-complex protein 1 subunit 7 | Cct3 | IPI00372388 | 6.23/61 179 | 8 | 122 | ↑ |
| Assist protein folding | Mitochondria | Heat shock cognate 71 kDa protein | Hspα | IPI00208205 | 5.37/71 055 | 11 | 172 | ↑ |
| Chaperonins/heat shock proteins | Cytoplasm, nucleus | Stress-induced-phosphoprotein 1 | Stip1 | IPI00213013 | 6.4/63 158 | 7 | 143 | ↑ |
| Chaperonins/heat shock proteins | | | | | |
| Proteins involved in cellular metabolism | Axon, mitochondria | Fructose-bisphosphate aldolase C | Aldoc | IPI00231736 | 6.67/39 658 | 10 | 189 | ↓ |
| Glycolytic enzyme | Cytoplasm | Similar to phosphoglycerate kinase 1 | RGD1560402 | IPI00372910 | 6.15/43 644 | 10 | 151 | ↑ |
| 1. Glycolytic enzyme | | | | | |
| 2. Transcription activation | | | | | |
| 3. Initiation of apoptosis | | | | | |
| ATP transducing | Mitochondria | Creatine kinase, mitochondrial 1, ubiquitous | Ckm1 | IPI0055166 | 8.58/47 331 | 12 | 155 | ↑ |
| Proteins involved in cell signaling | Mitochondria | NADH dehydrogenase (ubiquinone) flavoprotein 2 | Ndufβ2 | IPI00367152 | 6.23/27 703 | 24 | 253 | ↑ |
| 1. Electron transport in respiratory chain | Mitochondria | NADH dehydrogenase (ubiquinone) flavoprotein 2, mitochondrial | Ndufβ2 | IPI00367152 | 6.23/27 703 | 24 | 253 | ↑ |
| 2. Oxidoreductase | Membrane, mitochondria | NADH dehydrogenase (ubiquinone) 1 β subcomplex 10 | Ndufβ10 | IPI00202238 | 7.57/21 131 | 32 | 306 | ↓ |
| Electron transport in respiratory chain | Mitochondrial | Cytochrome b-c1 complex subunit 2, mitochondrial | Uqcrβ2 | IPI00188924 | 9.16/48 423 | 8 | 222 | ↓ |
| 1. Electron transport in respiratory chain | | | | | |
| 2. Mitochondrial dysfunction | | | | | |
| Amino-acid (serine) biosynthesis | Mitochondria | | | | |
| Proteins involved in sulfate transport | Mitochondrial matrix | | | | |
| Glutathione | Nucleus | Glutathione S-transferase P | Gstp1 | IPI00251229 | 6.89/23 652 | 20 | 263 | ↓ |
| Proteins involved in redox regulation | Mitochondria | Similar to glyceraldehyde-3-phosphate dehydrogenase | RGD1565368 | IPI00554039 | 6.4/36 045 | 18 | 454 | ↑ |
| Proteins involved in chaperone function | | | | | |
| 1. Glycolytic enzyme | | | | | |
| 2. Transcription activation | | | | | |
| 3. Initiation of apoptosis | | | | | |
| Proteins involved in cellular metabolism | Axon, mitochondria | | | | |
Table 3  Differentially expressed proteins identified in cellular and animal models of inflammatory bowel disease and in clinical samples of inflammatory bowel disease patients

| Ref. | Animal model | Cell Sample | Analytical technique ($) | No. of regulated proteins | Major findings | Protein name | Change |
|------|--------------|-------------|--------------------------|---------------------------|---------------|--------------|--------|
| [68] | Human adenocarcinoma cells Dld-1 exposed to interferon-γ, interleukin-1 and interleukin-6 | 2D PAGE-MALDI-TOF-MS/MS | 5 | Protein biosynthesis | Tryptophanyl-tRNA synthetase | Up |
| [14] | HT29 Cl.16E cell treated with interferon-γ | 1D PAGE-LC ESI/QTOF-MS/MS | 7 | Redox regulation | Indoleamine-2,3-Dioxygenase | Up |
|      | CD Intestinal epithelial patients | Structure protein | Histone H2A type-1B | Up |
|      |  | Metabolic enzymes | Adenosylhomocysteinase | Down |
|      |  | Redox regulation | Peroxiredxin-1 | Up |
|      |  | Structure protein | Histone H1, H2A, H2B | Down |
|      |  | Type 1-C/F/G/I, H3-like, H4 | | |
|      |  | Chaperone | Heat shock 70 kDa protein 5 | Up |
| [69] | CD and UC patients | Endoscopic biopsies of colonic mucosa | Multi-epitope-ligand-cartographic immunofluorescence microscopy | 14 | Apoptosis | Caspase-3 | Down |
|      |  | Transcription regulation | NF-κB | Up |
| [12] | UC patients | Colon biopsies | 2D-MALDI-TOF-MS/MS | 19 | Negative regulation of cell proliferation and DNA replication | Prohibitin (PHB) | Down |
|      |  | 1. TCA Cycle | Mitochondrial malate dehydrogenase (Mdh2) | Down |
|      |  | 2. Gluconeogenesis | | |
|      |  | 3. Antioxidant | Thioredoxin peroxidase (Prdx1) | Down |
|      |  | Eliminating Peroxides | Voltage-dependent antion-selective channel protein 1 (Vdac1) | Down |
|      |  | 1. Ion Channel | Nuclear factor of activated T-cells cytoplasmic (NFAT C1) | Up |
|      |  | 2. Apoptosis | | |
|      |  | Host-virus interaction | | |
| [70] | CD and UC patients | Serum | SELDI-TOF-MS/MS | 4 | Cytokine-mediated signaling | Platelet factor 4 | Up |
|      |  |  | Chronic inflammation | Myeloid related protein 8 | Up |
|      |  |  | Fibrin producing and inflammation | FIBA | Up |
| [71] | CD and Intestinal UC epithelial cells | 2D PAGE-MALDI-TOF-MS | 17 | Regulation of GTPase activity | Haptoglobin alpha2 | Up |
|      |  |  | Calcium ion binding | Myosin regulatory light chain 2, nonsarcomeric | Up |
|      |  |  | Immunity | Complement C3 | Up |
|      |  |  | Blood coagulation | Fibrinogen α chain | Up |
| [72] | CD patients | Serum | RP NANO-LC ESI/Q-TOF MS | 8 | Lipid transport | Apolipoprotein E | Down |
regulated Hnmpa2b1 in DRG of TNBS rats may suggest reduced DNA repair efficiency of neurons and activated autoimmunity in DRG. Ppia (also known as cyclophilin A) contributes to the pathogenesis of inflammation-mediated diseases by directly inducing leukocyte chemotaxis and expression of proinflammatory cytokine/chemokines through a NF-κB dependent pathway. Ppia is a novel paracrine and autocrine modulator of endothelial cell functions in immune-mediated diseases. The down-regulated expression of Ppia in DRG of TNBS rats might associate with impaired immune modulation following acute colitis. Eno1 is a multifunctional enzyme that plays a part in various processes such as glycolysis, growth control and allergic responses. It is an autoantigen in many diseases, however, its diagnostic value in IBD is still controversial. The underexpressed Eno1 may suggest that glycolysis is blocked and gluconeogenesis is dominant, which may associate with diarrhea and emaciation caused by colitis. Psma1 and Acot7 may be associated with the anti-inflammatory effect of macrophages. Psma1 mediates lipopolysaccharide-induced signal transduction in the macrophage proteasome. Acot7 hydrolyzes the CoA thioester of palmitoyl-CoA, an important precursor for proinflammatory and immunosuppressive eicosanoids. Acot7 gene is highly expressed in macrophages and up-regulated by lipopolysaccharide. The down-regulated Psma1 and Acot7 expression in the spinal cord of TNBS rats may be associated with inhibited anti-inflammatory responses.

**Group 2: Proteins involved in cell signaling**

Group 2 consists of proteins involved in ion channel signaling. These proteins play crucial roles in the function of neurons and may be implicated in autoimmune diseases. The table below summarizes the proteins found in the spinal cord of TNBS rats, highlighting their involvement in various cellular processes and signaling pathways.

| Group 2: Proteins involved in cell signaling |
|---------------------------------------------|

| Protein | Function | Expression |
|---------|----------|------------|
| Psma1   | Proteasome activator complex subunit 2 | Down |
| Acot7   | Lipolysis of coenzyme A | Down |

**Summary**

The proteome profiling of TNBS rats reveals significant differences in protein expression patterns associated with inflammation and autoimmunity. Proteins implicated in cell signaling, such as Psma1 and Acot7, play critical roles in immune modulation and may be targets for therapeutic interventions in autoimmune and inflammatory diseases.
function, cell growth and division. Potassium channels are important in shaping the action potential, excitability and plasticity of neurons. Changes in the properties of potassium channels at the soma accompanied with hyperexcitability in nociceptive DRG neurons in animal with TNBS-induced ileitis. We observed overexpressed kctd12 protein in DRG of the TNBS rats. This might be related to altered function of potassium channel in DRG and hyperalgesia in colitis rats. Voltage-dependent anion-selective channel protein 2 (Vdac2) inhibits mitochondrial apoptosis. It is interesting that a significantly down-regulated Vdac2 protein expression was observed in the DRG of TNBS rats, indicating an up-regulated apoptosis signaling. Adenyl cyclase-associated protein 1 (Cap1) is a growth protein involved in the cyclic AMP (cAMP) pathway. Inflammatory signals can activate cAMP-protein kinase A pathways, which correlates with electrophysiological hyperexcitability of DRG neuron. And, cAMP plays a role in DRG axon regeneration. The up-regulated Cap1 in DRG of TNBS rats is probably a sign of neuronal hyperexcitability and/or axon regeneration. The 14-3-3 protein epsilon (Ywhae) is a splice variant of the 14-3-3 protein, which modulates cell division and apoptosis. Elevated Ywhae expression was observed in the spinal cord of injury rats. Consistent with down-regulated Vdac2 expression in DRG, the elevated Ywhae expression in spinal cord of TNBS rats may also indicate enhanced apoptosis signaling. Dihydrolipinidase-related protein 2 (Dpysl2) plays a role in axon guidance, neuronal growth cone collapse and cell migration. In rat brain after ischemic stroke, up-regulated Dpysl2 indicates an early neuronal defense mechanism involving active neuronal repair, regeneration and development. The up-regulated Dpyls2 in the spinal cord of TNBS rats may be related to the neurite outgrowth/plasticity associated with immunoreaction.

**Group 3: Proteins involved in sulfate transport**
Thiosulfate sulfurtransferase (Tst) can detoxify H.S. Dysregulation of Tst expression associates with inability to detoxify detrimental H.S and could be a factor in cell loss and inflammation. The down-regulated Tst expression in spinal cord of TNBS rats may indicate dysfunction of the Tst detoxification that is possibly related to cell damage and inflammation in acute colitis.

**Group 4: Proteins involved in cell redox homeostasis**
Glutathione S-transferase P (Gstp1) functions in xenobiotic metabolism and plays a central role in the cellular defense against harmful endogenous compounds and xenobiotics. Gstp1 is distributed in neuronal perikarya and oligodendrocytes in the central nervous system (CNS), and in neuronal perikarya and satellite cells of the DRG. Reduced level of Gstp1 indicates a declined antioxidant capacity which may contribute to the damage to motor neurons in the process of immune-mediated motor neuron injury. The underexpression of Gstp1 in both spinal cord and DRG of TNBS rats might suggest oxidative stress and damage in neuronal cells. Prdx1 may participate in the signaling cascades of growth factors and TNF-α by regulating the intracellular concentrations of H2O2. A recent study revealed that in dextran sulfate sodium (DSS) mice, the inflamed intestinal mucosa has a down-regulated Prdx6 expression in comparison with normal mucosa. Consistently, down-regulated Prdx1 in DRG of rats with TNBS colitis suggesting oxidative stress occurred in DRG. Superoxide dismutase (Mn), mitochondrial (Sod2) is an important antioxidant defender in nearly all cells exposed to oxygen. Ulcerative colitis involves intestinal mucosal damage driven by reactive oxygen species (ROS), in particular, superoxide anion. At the stage of severe inflammation, suppression of superoxide dismutase activity and elevation of nitrous oxide systems activity occur concomitantly. The underexpression of Sod2 protein indicates oxidative stress existing in the DRG of TNBS rats. Taken together, the down-regulated Gstp1 expression in spinal cord and DRG of TNBS rats, along with decreased expression of Prdx1 and Sod2 in DRG of TNBS rats, suggest that TNBS rats may have a significantly declined antioxidative and cellular defense capacity in the nervous system. Interestingly, another protein involved in cell redox homeostasis, dihydrolipoyl dehydrogenase (Dld), has a higher expression in the DRG of TNBS rats. Dld is a source of ROS, capable of scavenging nitric oxide, and can serve as an antioxidant by protecting other proteins against oxidative inactivation.

**Group 5: Chaperonins**
In both the spinal cord and DRG of TNBS rats, we observed significant up-regulation of Stip1. It is a chaperone that mediates the association of the molecular chaperones, heat shock cognate 71 kDa protein (Hspa8) and heat shock protein 90. Hspa8 is a key component of stress responses induced by various noxious conditions. It regulates protein biosynthesis and refolding of denatured proteins, and plays an essential role in protecting cells in intestinal mucosal inflammation, potentially by lessening the extent and severity of injury. The up-regulated Stip1 expression was observed in both the DRG and spinal cord of TNBS rats, indicating stress responses in primary afferent and CNS. Hspa8 is up-regulated in the spinal cord and down-regulated in DRG of TNBS rats. As Hspa8 exhibits both protective and antigenic properties, and the Hspa8 expression may stem from neuron, satellite or immune cells, the conflicting Hspa8 expression in spinal cord and DRG merits further investigation.

**Group 6: Structure protein**
Lamins are components of the nuclear lamina, providing a framework for the nuclear envelope. The mechanical properties of the cytoskeleton and cytoskeleton-based processes (such as cell motility and cell polarization), depend critically on the integrity of the nuclear lamina. The overexpressed Lmna protein was observed in DRG of TNBS rats, which may suggest altered cytoskeleton.
Proteins involved in glycolysis: Significant down-regulation of glycolysis enzymes, for example, Aldoc 17 kDa protein, fructose-bisphosphate aldolase A (Aldoa), and a third enzyme similar to pyruvate kinase 3, was observed in the DRG of TNBS group. Consistent with this, decreased expression of Aldoc was observed in spinal cord of TNBS rats. Aldoc and Aldoa regulate the stability of light-neurofilament mRNA, and Aldoc provides marked neuroprotection to Purkinje cells after trauma and AMPA-

**Group 7: Cell metabolic enzymes**

**Proteins involved in glycolysis:** Significant down-regulation of glycolysis enzymes, for example, Aldoc 17 kDa protein, fructose-bisphosphate aldolase A (Aldoa), and a third enzyme similar to pyruvate kinase 3, was observed in the DRG of TNBS group. Consistent with this, decreased expression of Aldoc was observed in spinal cord of TNBS rats. Aldoc and Aldoa regulate the stability of the light-neurofilament mRNA, and Aldoc provides marked neuroprotection to Purkinje cells after trauma and AMPA-

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**Structure protein**

**Proteins regulate redox homeostasis**

**Fh1**

**Ndufv2**

**Cell metabolic enzymes**

**Glycolysis**

**DRG**

**Aldoa**

**Aldoc**

**Did**

**Gstp1**

**Pdx1**

**Sod2**

**Lmna**

**Gstp1**

**Structure protein**

**DRG**

**Aldoa**

**Aldoc**

**Did**

**Gstp1**

**Pdx1**

**Sod2**

**Lmna**

**Energy metabolism dysfunction**

**ATP transduction**

**DG**

**Ckm**

**Spinal cord**

**Ckm**

**Spinal cord**

**Ckmt1**

**Mitochondrial respiratory chain**

**Spinal cord**

**Ndufv2**

**Ndufb10**

**Uqcr2**

**Ketone body catabolism:**

**DRG**

**Oxct1 succinyl-CoA**

**Valine and pyrimidine catabolism:**

**DRG**

**Aldh6a1**

**CCA cycle**

**DRG**

**Fh1**

**Mdh2**

**Serine biosynthesis:**

**Spinal cord**

**Psat1**

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**Figure 4** Schematic drawing summarizes the major findings that might associate with pathophysiological changes in rat nervous system caused by trinitrobenzene sulfonic acid-induced colitis. TNBS: Trinitrobenzene sulfonic acid; DRG: Dorsal root ganglia; Hnrnpa2b1: Heterogeneous nuclear ribonucleoproteins A2/B1; Gsp1: Glutathione S-transferase P; Pxd1: Peroxiredoxin-1; Sod2: Superoxide dismutase; Lmna: Lamin C2; Aldoa: Aldolase A; Aldoc: Aldolase C; Ckm: Creatine kinase M-type; Ndufv2: NADH dehydrogenase (ubiquinone) flavoprotein 2; Ndufb10: NADH dehydrogenase (ubiquinone) 1 β subcomplex 10; Oxct1 succinyl-CoA: 3-ketoacid-coenzyme A transferase 1; Fh1: Fumarate hydratase; Mdh2: Malate dehydrogenase; Psat1: Phosphoserine aminotransferase; Cap1: Cyclase-associated protein 1; Kctd12: Potassium channel tetramerisation domain containing protein 12; Vdac2: Voltage-dependent anion-selective channel protein 2; Ywhae: The 14-3-3 protein epsilon; Tst: Thiosulfate sulfurtransferase; Stip1: Stress-induced phosphoprotein 1; Hspa8: Heat shock cognate 71 kDa protein.
mediated excitotoxicity\textsuperscript{[55]}. The down-regulated Aldoc in spinal cord and DRG together with decreased Aldoa expression in DRG may suggest down-regulated neuroprotection in TNBS rats. In addition, inhibition or activation of other glycolysis enzymes may result in speeding up or slowing down certain steps in the glycolysis pathway, and reflecting adjustment to physiological/pathological changes compensating for the cellular energy or neuron apoptosis\textsuperscript{[49]}.

**Proteins involved in adenosine triphosphate transduction:** Creatine kinase serves as an energy reservoir for the rapid buffering and regeneration of adenosine triphosphate (ATP) to sites with high, fluctuating energy demands, such as the synapse\textsuperscript{[59]}. In the present study, expression of creatine kinase, mitochondrial 1, ubiquitous (Ckm1) in the spinal cord was significantly up-regulated in TNBS rats. In contrast, Ckm1 expression was decreased but the expression of creatine kinase M-type (Ckm) was up-regulated in the DRG of TNBS rats. Given that selective localization of Ckm in neurons was postulated to reflect the specific energy requirements of the specialized cells, these alterations may indicate enhanced phosphocreatine energy shuttles in spinal cord and remodelled energy shuttles/circles in the DRG\textsuperscript{[26,57]}.

**Proteins involved in mitochondrial respiratory chain:** Cytochrome b-c\textsubscript{1} complex subunit 2, mitochondrial (Uqcr2c) is implicated in mitochondrial ROS generation and inflammation. Uqcr2c deficiency causes mitochondrial dysfunction and exacerbates allergic airway inflammation\textsuperscript{[58]}. The down-regulated Uqcr2c expression in the spinal cord of TNBS rats might associate with inflammatory responses. Nicotinamide adenine dinucleotide (NADH) dehydrogenase is a potent source of reactive oxygen species such as superoxide and hydrogen peroxide\textsuperscript{[59]}. It is interesting that, in contrast to the up-regulated protein expression of NADH dehydrogenase (ubiquinone) flavoprotein 2 (Nduf2) in the spinal cord of the TNBS group, the NADH dehydrogenase (ubiquinone) 1 β subcomplex 10 (Ndufb10) was down-regulated. As the specific cellular functions of these subcomplexes are still not well known, further investigation is warranted.

**Proteins involved in ketone body catabolism:** 3-ketoacid-coenzyme A transferase 1 (Oxct1 Succinyl-CoA) is a mitochondrial matrix enzyme that plays a central role in extrahepatic ketone body catabolism. In the DRG of TNBS rats, Oxct1 Succinyl-CoA showed a 4-fold higher expression than in control rats. This observation is consistent with accelerated hepatic gluconeogenesis as well as ketogenesis in patients with chronic IBD, which probably is a consequence of the altered hormonal milieu\textsuperscript{[64]}. Similarly, the down-regulated Mdh2 and isoform mitochondrial of fumarate hydratase (Fh1) expression in DRG of TNBS rats may indicate a TCA metabolic dysregulation under disease condition.

**Proteins involved in serine biosynthesis:** Phosphoserine aminotransferase (Psat1) is an active serine biosynthesis enzyme in the mammal brain. Patients with Psat1 deficiency present with intractable seizures and psychomotor retardation\textsuperscript{[62]}. The significantly down-regulated Psat1 expression in spinal cord of the TNBS group may indicate dysregulation of serine biosynthesis and may be associated with seizure susceptibility in TNBS rats.

Our analysis provides an overview profiling the proteomic changes in the spinal cord and DRG of rats with TNBS-induced colitis. As summarized in Figure 4, intracolonic instillation of TNBS not only induces inflammatory/immune responses in the DRG and spinal cord, but also triggers broad alterations of protein involving cell signaling, cellular metabolism, redox regulation, stress response \textit{etc.} The TNBS-induced colitis in rodents is an immunologically mediated colitis that accompanies with an increase in proinflammatory factors and systemic en-dotoxaemia\textsuperscript{[63]}. Besides colonic and systemic changes, a series of alterations in the nervous system have been described, such as transient disruption of blood-brain-barrier to small molecules\textsuperscript{[64]}; a marked inflammatory response within the CNS\textsuperscript{[22]}; and neurosignalling activation in rodent primary afferent nerve as well as in DRG and spinal cord neurons\textsuperscript{[20,65,66]}. These neurological alterations may relate to intrinsic neuronal excitability and help explain some of the underlying comorbidities, such as hyperalgesia, seizure and anorexia\textsuperscript{[5,6]}. The neurologic manifestations of IBD are most likely primary immune-mediated disorders, possibly caused by proinflammatory cytokines that diminish neuron proliferation, increase apoptosis, increase neuronal excitability, exacerbate sickness and/or result in psychological symptoms\textsuperscript{[21,67]}. Our results delineated a dramatic deviation of protein profiling from the carefully orchestrated physiological balance in the DRG and spinal cord of TNBS rats. These findings provide useful proteins for further investigation on the neurological manifestations of IBD.

**COMMENTS**

**Background**

Inflammatory bowel disease (IBD) is a systematic illness, whose etiology and pathophysiology is incompletely understood. Many organs outside the gastrointestinal tract are involved in IBD, including the nervous system (neuropathies, cerebrovascular events, white matter lesions). These pathological changes may associate with a variety of comorbidities, such as hyperalgesia, seizure, and anorexia, but the underlying mechanism remains poorly understand.

**Research frontiers**

Proteomics keeps a rapidly expanding field, with a wealth of reports regularly appearing on technology enhancements and scientific studies using these new instruments.
Innovations and breakthroughs

This study demonstrated that trinitrobenzene sulfonic acid (TNBS) colitis profoundly changed expression of not only proteins involved in inflammatory/immune responses, but also proteins involved in cell signaling, sulfate transport, redox homeostasis and cell metabolism.

Applications

This study provides an overview profiling the proteomic changes in the DRG and spinal cord in rats with acute colitis induced by TNBS using a two-dimensional electrophoresis-based proteomic technique. They found that altered proteins were involved in a number of biological functions including inflammation/immunity, cell signaling, redox regulation, sulfate transport and cellular metabolism. Although the number of the samples examined in this study was small, this paper is well written and the results are interesting.

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