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ORIGINAL ARTICLE

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

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Abstract

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-α (TNF-α) 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-α 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 (Kctd 12) and low expression of proteasome subunit α 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.

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

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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[1]. 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[4]. 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 lumbosacral 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 tribromophenol (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 (Amersham, 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. Proteins were focused by using a step-wise voltage ramp: 500 V for 1 h, 1000 V for 1 h, and finally 8000 V for 6 h. The IPG strips were then 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% iodoacetamide 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[11]. All the raw images were digitalized using

a scanner (GS-800 calibrated densitometer, BioRad) and the Quantity One software (BioRad). Further analysis was completed using PDQuest (version 8.0, BioRad) mainly for spots' detection and quantification. The protein spots where the peak-volume ratio in the TNBS group changed more than 3-folds in comparison with the matched spots in the control group, were considered as differentially expressed, and were picked out for identification by tandem mass spectrometer (MS-MS).

In-gel digestion

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 ℃ 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 ℃ 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 clip 1-17 (2093.0868), ACTH clip 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.

Determination of MPO activity and TNF-α level

The MPO activity was measured following the method as previously described[15,16]. Colonic TNF-α was determined using an enzyme-linked immunosorbent assay kit (Leinco Technologies, United States). The protein was quantified using a bicinchoninic acid protein assay kit (Thermo Scientific, United States).

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 ℃ 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 ℃ 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 incrassation 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

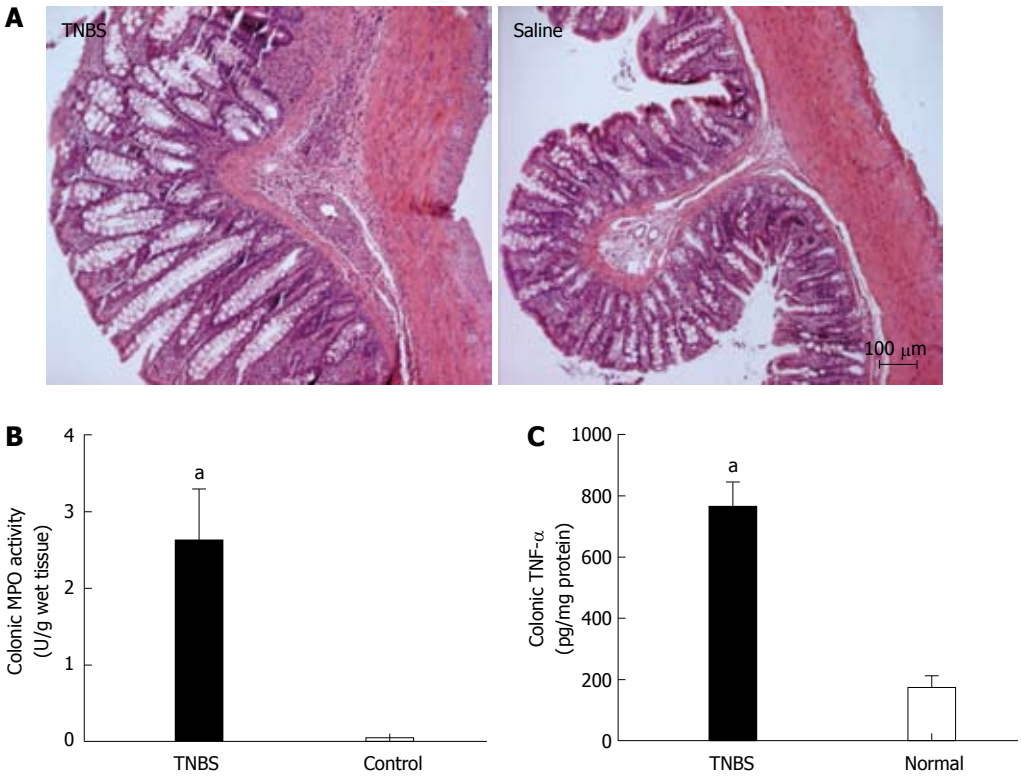

Figure 1 The establishment of colitis in trinitrobenzene sulfonic acid rats. Representative hematoxylin and eosin microscopic photos of the colon tissue (A) revealed inflammation in the sub-mucosa layer of trinitrobenzene sulfonic acid (TNBS) rats; measurement of myeloperoxidase (MPO) activity in wet colon tissue (B, 2.61 ± 2.47 *vs* 0.03 ± 0.01) and tumor necrosis factor-α (TNF-α) level in colonic total protein (C, 759.80 ± 81.07 *vs* 174.00 ± 31.92) revealed significantly elevated MPO activity and TNF-α level in TNBS treated group in comparison with saline control group. $^{a}P < 0.05$ *vs* saline group.

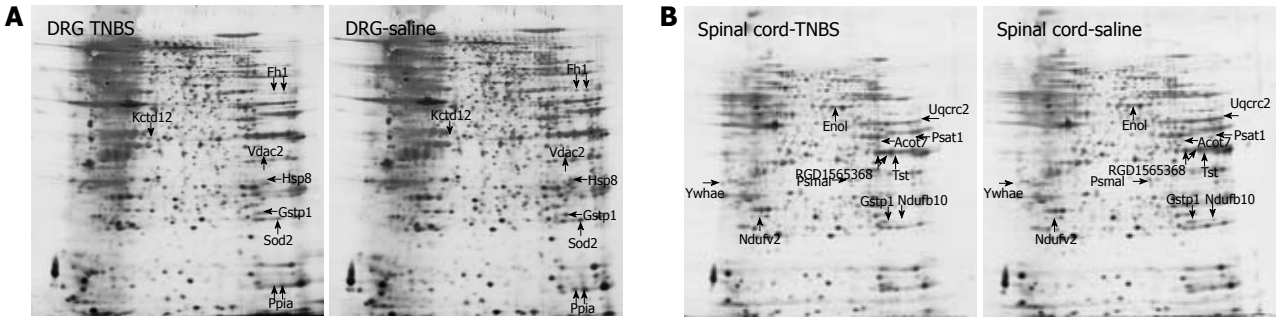

Figure 2 Representative examples of the silver-stained two-dimensional electrophoresis gels show expression maps of proteins in dorsal root ganglia (A) and spinal cord (B) of trinitrobenzene sulfonic acid colitis group and saline control groups. TNBS: Trinitrobenzene sulfonic acid; DRG: Dorsal root ganglia; Gstp1: Glutathione S-transferase P; Sod2: Superoxide dismutase; Ndufv2: NADH dehydrogenase (ubiquinone) flavoprotein 2; Ndufb10: NADH dehydrogenase (ubiquinone) 1 β subcomplex 10; Fh1: Fumarate hydratase; Psat1: Phosphoserine aminotransferase; 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; Hspa8: Heat shock cognate 71 kDa protein.

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 peroxiredoxin-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								
Autoantigen in many autoimmune diseases	Cytoplasm, nucleus spliceosome	Isoform B0a of heterogeneous nuclear ribonucleoproteins A2/B1	Hnrnpa2b1	IPI00923129	8.74/32 572	16	220	↑
Hemopexin	Secreted	Hemopexin	Hpx	IPI00195516	7.58/52 060	22	597	↑
1. Accelerate the folding of protein 2. Immunosuppression	Cytosol, nucleus	Peptidyl-prolyl cis-trans isomerase A	Ppia	IPI00387771	8.34/18 091	32	363	↓
Proteins involved in cell signaling								
Growth protein	Membrane	Adenylyl cyclase-associated protein 1	Cap1	IPI00555187	7.16/51 899	5	145	↑
Cytoplasmic tetramerisation domain of voltage-gated K+ channel		Similar to potassium channel tetramerisation domain containing protein 12	Kctd12	IPI00359734	8.95/47 077	16	192	↑
1. Ion channel 2. Mitochondrial apoptosis	Mitochondrial outer membrane	Voltage-dependent anion-selective channel protein 2	Vdac2	IPI00198327	7.44/32 353	9	76	↓
Proteins involved in redox regulation								
Cell redox homeostasis	Mitochondria	Dihydrolipoyl dehydrogenase, mitochondrial	Dld	IPI00365545	7.96/54 574	5	141	↑
Antioxidant	Mitochondria	Superoxide dismutase [Mn], mitochondrial	Sod2	IPI00211593	8.96/24 887	22	130	↓
Xenobiotic metabolism and cellular defense	Nucleus	Glutathione S-transferase P	Gstp1	IPI00231229	6.89/23 652	12	255	↓
Eliminating peroxides	Mitochondria, cytosol	Peroxiredoxin-1	Prdx1	IPI00211779	8.27/22 323	9	108	↓
Proteins involved in chaperone function								
Chaperonins/heat shock proteins	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								
Component of the nuclear lamina	Insoluble fraction, lamin filament, nuclear matrix	Lamin C2	Lmna	IPI00188879	6.22/52 661	16	136	↑
Proteins involved in cellular metabolism								
Oxidoreductase in valine and pyrimidine catabolic pathways	Mitochondria	Methylmalonate-semialdehyde dehydrogenase [acylating],mitochondrial	Aldh6a1	IPI00205018	8.44/58 396	4	68	↓
Glycolytic enzyme	Mitochondria	Fructose-bisphosphate aldolase A	Aldoc	IPI00231734	8.31/39 783	11	148	↓
Glycolytic enzyme	Axon, mitochondria	Aldoc 17 kDa protein	Aldoc	IPI00561972	6.84/16 666	14	88	↓
Glycolytic enzyme	Mitochondria	Dihydrolipoyllysine-residueacetyltransferase component of pyruvate dehydrogenase complex	Dlat	IPI00231714	8.76/67 637	7	79	↑
Glycolytic enzyme	Cytoplasm	Phosphoglycerate kinase 1	Pgk1	IPI00231426	8.02/44 909	16	184	↓
Glycolytic enzyme		Similar to pyruvate kinase 3		IPI00561880	7.15/58 264	17	231	↓
Glycolytic enzyme	Nucleus cytoplasm	Isoform M1 of pyruvate kinase isozymes M1/M2	Pkm2	IPI00231929	6.63/58 452	22	491	↑
1. Glycolytic enzyme 2. Transcription activation 3. Initiation of apoptosis	Cytoplasm	Similar to glyceraldehyde-3-phosphate dehydrogenase	RGD1565368	IPI00554039	8.44/36 045	14	347	↑
ATP energy transduction	Cytoplasm	Creatine kinase M-type	Ckm	IPI00211053	6.58/43 246	19	361	↑
ATP transducing	Mitochondria	Creatine kinase, mitochondrial 1, ubiquitous	Ckmt1	IPI00555166	8.58/47 331	21	231	↓

TCA cycle	Mitochondria	Isoform mitochondrial of fumarate hydratase, mitochondrial	Fh1	IPI00231611	9.06/54 714	5	115	↓
1. TCA cycle 2. Gluconeogenesis 3. Antioxidant	Mitochondria	Malate dehydrogenase, mitochondrial	Mdh2	IPI00197696	8.93/36 117	11	90	↓
Extrahepatic ketone body catabolism	Mitochondria	3-ketoacid-coenzyme A transferase 1, mitochondrial	Oxct1 Succinyl-CoA	IPI00766788	8.7/56 624	6	116	↑

↑: 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 2; Dld: Dihydrolipoyl dehydrogenase; Sod2: Superoxide dismutase; Gstp1: 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.

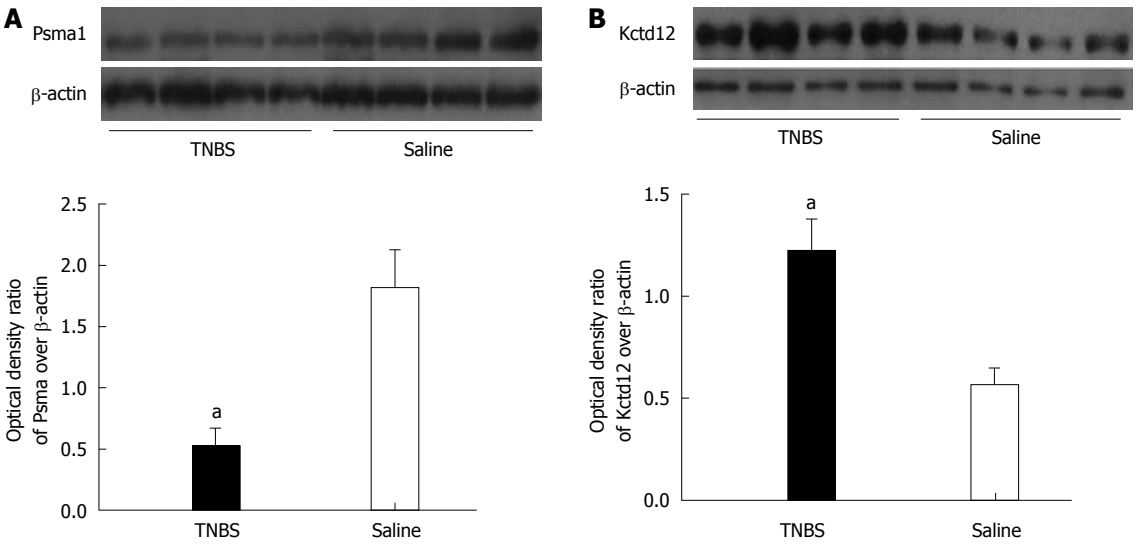

Figure 3 Immunoblotting analyses to validate the differential expression of proteasome subunit α type-1 (A, 0.53 ± 0.14 *vs* 1.81 ± 0.53) and potassium channel tetramerisation domain containing protein 12 (B, 1.21 ± 0.20 *vs* 0.56 ± 0.07) between trinitrobenzene sulfonic acid treated group and saline control group. The relative expression ratio standardized to β-actin. $^{a}P < 0.05$ *vs* saline group. TNBS: Trinitrobenzene sulfonic acid; Psma1: Proteasome subunit α type-1; Kctd12: Potassium channel tetramerisation domain containing protein 12.

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

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								
1. Fatty acid catabolic process 2. Play a role in eicosanoid synthesis and inflammation	Cytoplasm	Isoform 1 of cytosolic acyl coenzyme A thioester hydrolase	Acot7	IPI00213571	7.16/37 936	14	167	↓
1. Glycolytic enzyme 2. Autoantigen	Cell membrane, cytoplasm	α-enolase	Eno1	IPI00464815	6.16/47 440	13	183	↓
Immunity	Cytoplasm, nucleus	Proteasome subunit α type-1	Psma1	IPI00191748	6.15/29 784	17	193	↓
Proteins involved in cell signaling								
1. Growth protein 2. Modulate calcium influx 3. Regulate the release of ICGRP	Cytoplasm	Dihydropyrimidinase-related protein 2	Dpysl2	IPI00870112	5.95/62 638	16	270	↑
Calcium ion signaling	Cytoplasm, cytoskeleton	Troponin C-like protein		gi223036	4.12/16 696	10	91	↓
1. Cell division 2. Apoptosis 3. Regulate insulin sensitivity	Melanosome, cytoplasm	14-3-3 protein epsilon	Ywhae	IPI00325135	4.63/29 326	15	165	↑
Proteins involved in sulfate transport								
Transferase detoxification	Mitochondrial matrix	Thiosulfate sulfurtransferase	Tst	IPI00366293	7.71/33 614	25	333	↓
Proteins involved in redox regulation								
Xenobiotic metabolism and cellular defense	Nucleus	Glutathione S-transferase P	Gstp1	IPI00231229	6.89/23 652	20	263	↓
Proteins involved in chaperone function								
Assist protein folding	Cytoplasm	T-complex protein 1 subunit γ	Cct3	IPI00372388	6.23/61 179	8	122	↑
Chaperonins/heat shock proteins	Mitochondria	Heat shock cognate 71 kDa protein	Hspa8	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	↑
Proteins involved in cellular metabolism								
Glycolytic enzyme	Axon, mitochondria	Fructose-bisphosphate aldolase C	Aldoc	IPI00231736	6.67/39 658	10	189	↓
Glycolytic enzyme	Cytoplasm	Similar to phosphoglycerate kinase 1	RGD1560402	IPI00372910	6.15/43 604	10	151	↑
1. Glycolytic enzyme 2. Transcription activation 3. Initiation of apoptosis	Cytoplasm	Similar to glyceraldehyde-3-phosphate dehydrogenase	RGD1565368	IPI00554039	8.44/36 045	18	454	↑
ATP transducing	Mitochondria	Creatine kinase, mitochondrial 1, ubiquitous	Ckmt1	IPI00555166	8.58/47 331	12	155	↑
1. Electron transport in respiratory chain 2. Oxidoreductase	Mitochondria	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	Ndufv2	IPI00367152	6.23/27 703	24	253	↑
Electron transport in respiratory chain	Membrane, mitochondria	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 10	Ndufb10	IPI00202238	7.57/21 131	32	306	↓
1. Electron transport in respiratory chain 2. Mitochondrial dysfunction	Mitochondrial	Cytochrome b-c1 complex subunit 2, mitochondrial	Uqcrc2	IPI00188924	9.16/48 423	8	222	↓
Amino-acid (serine) biosynthesis		Phosphoserine aminotransferase	Psat1	IPI00331919	7.57/40 943	8	97	↓

↑: Elevated protein expression in TNBS group compared with saline group; ↓: Decreased protein expression in TNBS group in comparison with saline group; TNBS: Trinitrobenzene sulfonic acid; NADH: Nicotinamide adenine dinucleotide; Psma1: Proteasome subunit α type-1; Dpysl2: Dihydropyrimidinase-related protein 2; Ywhae: The 14-3-3 protein epsilon; Tst: Thiosulfate sulfurtransferase; Gstp1: Glutathione S-transferase P; Hspa8: Heat shock cognate 71 kDa protein; Stip1: Stress-induced phosphoprotein 1; Aldoc: Aldolase C; Ckmt1: Creatine kinase mitochondrial 1 ubiquitous; Ndufv2: NADH dehydrogenase (ubiquinone) flavoprotein 2; Ndufb10: NADH dehydrogenase (ubiquinone) 1 β subcomplex 10; Uqcrc2: Cytochrome b-c1 complex subunit 2; Psat1: Phosphoserine aminotransferase.

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 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	Patient	Sample	Analytical technique (S)	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
							Redox regulation	Indoleamine-2, 3-Dioxygenase	Up
[14]		HT29 Cl.16E cell treated with interferon-γ			1D PAGE-LC ESI/QTOF-MS/MS	7	Structure protein	Histone H2A type-1B	Up
							Metabolic enzymes	Adenosylhomocysteinase	Up
							Redox regulation	Peroxiedonxin-1	Down
			CD patients	Intestinal epithelial cells		14	Structure protein	Histone H1.2, H2A.V, H2B Type 1-C/E/F/G/I, H3 Like, H4	Up
							Chaperone	Heat shock 70 kDa protein 5	Up
							Annexin	Annexin A1	Down
[69]			CD and UC patients	Endoscopic biopsies of colonic mucosa	Multi-epitope-ligand-cartographic immunofluorescence microscopy		Apoptosis	Caspase-3	Down
							Transcription regulation	NF-κ B	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 2. Gluconeogenesis 3. Antioxidant	Mitochondrial malate dehydrogenase (Mdh2)	Down
							Eliminating Peroxides	Thioredoxin peroxidase (Prdx1)	Down
							1. Ion Channel 2. Apoptosis	Voltage-dependent anion-selective channel protein 1 (Vdac1)	Down
							1. Intracellular signal transduction 2. Regulation of transcription, DND-dependent	Nuclear factor of activated T-cells cytoplasmic (NFAT C1)	Up
							Protein folding	Tumor rejection antigen 1 (TRA1)	Up
							Cell adhesion Host-virus interaction	Poliovirus receptor related protein 1 (PVRL1)	Up
[70]			CD and UC patients	Serum	SELDI-TOF-MS	4	Cytokine-mediated signaling	Platelet factor 4	Up
							Chronic inflammation	Myeloid related protein 8	Up
							Fibrin producing and inflammation	FIBA	Up
							Transferase	Haptoglobin alph2	Up
[71]			CD and UC patients	Intestinal epithelial cells	2D PAGE-MALDI-TOF-MS	17	Regulation of GTPase activity	Rho gdp dissociation inhibitor (GDI) α chain	Up
							Differentiation	Nicotinamide phosphor-ribosyltransferase	Up
							Calcium ion binding	Myosin regulatory light chain 2, nonsarcomeric	Up
[72]			CD patients	Serum	RP NANO-LC ESI/Q-TOF MS	8	Immunity	Complement C3	Up
							Blood coagulation	Fibrinogen α chain	Up
							Lipid transport	Apolipoprotein E	Down

[73]		UC patients	Mucosa/ submucosa	2D PAGE-LC-MS/MS	7	Cell adhesion	Protocadherin 17 precursor	Up
						Acute-phase response	α-1-Antitrypsin (precursor)	Up
						Muscle protein	Caldesmon	Up
						Structural molecule activity	Mutant desmin	Up
[74]	DSS treated Balb/C mice		Mucosa	2D-DIGE-MALTI-TOF	5	Isomerase	Protein disulfide isomerase family A, member 3	Down
						Redox regulation	Peroxiredoxin 6 (Prx6)	Down
[13]	TNBS treated SD rats		Lymphocytes	2D-MALDI-TOF-MS/MS	42	Apoptosis-related proteins	PYD and Card domain containing protein	Down
						DNA damage response	Proteasome activator complex subunit 2	Down
						Glycolysis	Phosphoglycerate mutase type B subunit	Down
						Xenobiotic metabolism and cellular defense	Glutathione S-transferase, Pi 2	Down
						Cytokine	Interleukin-12 p40 precursor	Up
						Proteins involved in cell growth, differentiation and signal trransduction	Nucleoside diphosphate kinases β isoform	Up
						Inflammatory factors	Myeloid-related protein	Up
						ATP transduction	ATP-citrate synthase	Up
						Redox regulation	Dismutase	Up
[49]	TNBS treated SD Rats		DRG	2D-MALDI-TOF-MS/MS	26	Xenobiotic metabolism and cellular defense	Glutathione S-transferase P (Gstp1)	Down
						Eliminating peroxides	Peroxiredoxin-1 (Prdx1)	Down
						1. Accelerate the folding of protein 2. Immunosuppression	Peptidyl-prolyl cis-trans isomerase A (Ppia)	Down
						1. Ion channel 2. Mitochondrial apoptosis	Voltage-dependent anion-selective channel protein 2 (Vdac2)	Down
						Cytoplasmic tetramerisation domain of voltage-gated K+ channel	similar to Potassium channel tetramerisation domain containing protein 12	Up
			Spinal cord		19	Xenobiotic metabolism and cellular defense	Glutathione S-transferase P (Gstp1)	Down
						1. Glycolytic enzyme 2. Autoantigen in many diseases	α-enolase (Eno1)	Down
						Immunity	Proteasome subunit α type-1 (Psma1)	Down

DRG: Dorsal root ganglia; TNBS: Trinitrobenzene sulfonic acid; CD: Crohn's disease; UC: Ulcerative colitis; DSS: Dextran sulfate sodium; NF: Nuclear factor.

regulated Hnrnpa2b1 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[28,29]. In addition, Ppia is a novel paracrine and autocrine modulator of endothelial cell functions in immune-mediated diseases[30]. 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[31]. 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[32]. Acot7 hydrolyzes the CoA thioester of palmitoyl-CoA, an important precursor for proinflammatory and immunoactive eicosanoids. Acot7 gene is highly expressed in macrophages and up-regulated by lipopolysaccharide[33]. 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

function, cell growth and division. Potassium channels are important in shaping the action potential, excitability and plasticity of neurons[34]. Changes in the properties of potassium channels at the soma accompanied with hyperexcitability in nociceptive DRG neurons in animal with TNBS-induced ileitis[35]. 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[36]. 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. Adenylyl 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[37]. And, cAMP plays a role in DRG axon regeneration[38]. 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[39]. Elevated Ywhae expression was observed in the spinal cord of injury rats[40]. Consistent with down-regulated Vdac2 expression in DRG, the elevated Ywhae expression in spinal cord of TNBS rats may also indicate enhanced apoptosis signaling. Dihydropyrimidinase-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[41]. The up-regulated Dpysl2 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_2S. Dysregulation of Tst expression associates with inability to detoxify detrimental H_2S and could be a factor in cell loss and inflammation[42]. 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[43,44]. Gstp1 is distributed in neuronal perikarya and oligodendrocytes in the central nervous system (CNS), and in neuronal perikarya and satellite cells of the DRG[45]. Reduced level of Gstp1 indicates a declined antioxidative capacity which may contribute to the damage to motor neurons in the process of immune-mediated motor neuron injury[46]. 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 H_2O_2. 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[13]. 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 defenser in nearly all cells exposed to oxygen[47]. 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[48].

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[49]. 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[50,51]. 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[49,52], 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[53]. The overexpressed Lmna protein was observed in DRG of TNBS rats, which may suggest altered cytoskeleton.

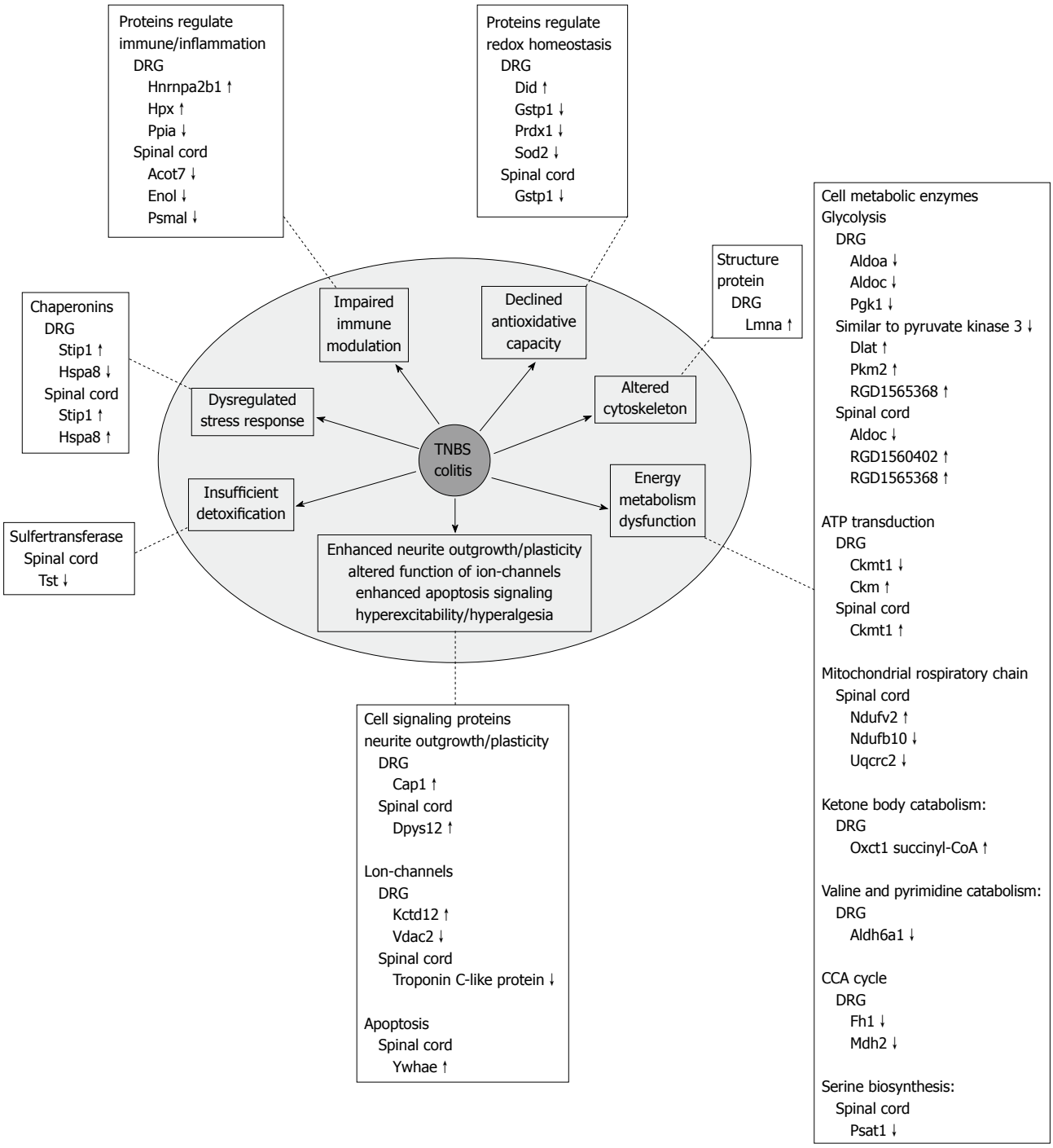

Figure 4 Schematic drawing summarizes the major findings that might associate with pathophysiological changes in rat nervous system caused by trinitrobenzene sulfonic acid-induced colitis. TNBS: Trinitrobenzene sulfonic acid; DRG: Dorsal root ganglia; Hnrnpa2b1: Heterogeneous nuclear ribonucleoproteins A2/B1; Gstp1: Glutathione S-transferase P; Prdx1: Peroxiredoxin-1; Sod2: Superoxide dismutase; Lmna: Lamin C2; Aldoa: Aldolase A; Aldoc: Aldolase C; Ckmt1: Creatine kinase, mitochondrial 1, ubiquitous; 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.

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[54], and Aldoc provides marked neuroprotection to Purkinje cells after trauma and AMPA-

mediated excitotoxicity[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[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[55]. In the present study, expression of creatine kinase, mitochondrial 1, ubiquitous (Ckmt1) in the spinal cord was significantly up-regulated in TNBS rats. In contrast, Ckmt1 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[56,57].

Proteins involved in mitochondrial respiratory chain: Cytochrome b-c1 complex subunit 2, mitochondrial (Uqcrc2) is implicated in mitochondrial ROS generation and inflammation. Uqcrc2 deficiency causes mitochondrial dysfunction and exacerbates allergic airway inflammation[58]. The down-regulated Uqcrc2 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[59]. It is interesting that, in contrast to the up-regulated protein expression of NADH dehydrogenase (ubiquinone) flavoprotein 2 (Ndufv2) 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 subcomplexs are still not well known, further investigation is warranted.

Proteins involved in ketone body catabolism: 3-keto-acid-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[60].

Proteins involved in tricarboxylic acid cycle: Malate dehydrogenase, mitochondrial (Mdh2) is a cellular antioxidant, an enzyme in the tricarboxylic acid (TCA) cycle and gluconeogenesis. Mdh2 was down-regulated in experimental autoimmune uveitis oxidative stress, and in colon mucosa of ulcerative colitis patients[12,61]. 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[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, intracolonal 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 *etc*. The TNBS-induced colitis in rodents is an immunologically mediated colitis that accompanies with an increase in proinflammatory factors and systemic endotoxaemia[63]. Besides colonic and systematic changes, a series of alterations in the nervous system have been described, such as transient disruption of blood-brain-barrier to small molecules[64]; a marked inflammatory response within the CNS[22]; and neurosignaling activation in rodent primary afferent nerve as well as in DRG and spinal cord neurons[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[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[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 spinal cord and dorsal root ganglia (DRG) of rats with TNBS-induced experimental colitis. These findings provide useful proteins for further investigation on the neurological manifestations of IBD.

Peer review

In this study, the authors profiled the global protein expression 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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