

# Identification of acute spinal cord injury and autophagy-related potential key genes pathways, and targeting drugs through bioinformatics analysis

Wei-long Xu

Inner Mongolia Medical University

Yan Zhao (✉ [zy1994957145@163.com](mailto:zy1994957145@163.com))

Inner Mongolia Medical University <https://orcid.org/0000-0002-1494-9917>

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## Research article

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# Abstract

**Background:** Acute spinal cord injury (ASCI) is considered a form of severe central nervous system damage. At present, research in the fields of spinal surgery and neurology has highlighted the complex mechanisms underlying ASCI, among which autophagy is considered to play a crucial role.

**Objectives:** We aimed to identify the genes and molecular pathways associated with ASCI and autophagy using computational tools and publicly available data, and to identify drugs targeting the relevant genes associated with ASCI and autophagy.

**Materials and Methods:** We used text mining to detect the ASCI and autophagy-associated genes, and the intersection of the two gene sets was selected for gene ontology analysis using the DAVID program. We then constructed protein–protein interaction networks, followed by a functional enrichment analysis, from which we obtained two significant gene modules. Finally, the final list of genes was queried against the Drug Gene Interaction database to find drug candidates targeting the relevant ASCI and autophagy genes.

**Results:** Our analysis identified 156 genes common to both the “ASCI” and “Autophagy” text mining concepts. Gene enrichment analysis yielded two significant gene modules (20 genes), which represent six significant signal pathways and could be targeted by 28 Food and Drug Administration (FDA)-approved drug molecules, and identified the drug–gene interactions.

**Conclusion:** In conclusion, we presented a method to explore the potential key genes, molecular pathways and candidate drugs associated with ASCI and autophagy. As a result, in this method, we identified a total of 20 potential genes, six significant pathways and 28 candidate drugs, which could provide a basis for new trials and the development of novel targeted therapies as potential treatments for ASCI.

## Introduction

Acute spinal cord injury (ASCI) is considered a form of severe central nervous system damage, which often leads to a partial or complete motor, sensory, and autonomous function loss below the injured segment, and results in areflexia. Common causes of ASCI include high-altitude falls, traffic accidents, heavy pound injury, and sports injuries [1]. Since the second half of the 20th century, with the rapid development of China's economy, the annual incidence of spinal cord injuries (SCIs) in Beijing, the capital of China, has increased every year, reaching 60.6 /1 million, and the global annual incidence rate has reached 15–40 /1 million [2, 3]. However, an effective treatment for ASCI remains to be found. At present, the research in the fields of spinal surgery and neurology has highlighted the complex mechanisms underlying ASCI [4, 5], with autophagy being considered as one of the important processes involved [6].

Autophagy is an essential lysosome-dependent cellular catabolic pathway that degrades cytoplasmic proteins, protein aggregates, and organelles. Although under certain conditions pathologically increased autophagy has been implicated in cell death, it is considered cytoprotective under most circumstances.

Basal levels of autophagy are important for homeostasis in all types of cells, and are especially crucial in terminally differentiated cells, such as neurons and oligodendrocytes [7].

Many gene expression profiling studies have focused on ASCI and autophagy in the last decade, and hundreds of candidate genes have been identified. These genes have different functions and are involved in a variety of processes[8, 9]. This study was designed to identify the potential key genes and molecular pathways associated with ASCI and autophagy via bioinformatics methods, and to explore drugs targeting the relevant genes associated with ASCI and autophagy. First, we made a preliminary list of related genes by mining the literature. Subsequently, we performed functional and signaling pathway analyses using the online bioinformatics resource DAVID. Next, we constructed PPI networks of the common genes and identified two significant gene modules. Finally, based on the drug–gene interaction analysis of the final genes, we identified candidate drugs. Using this approach, we identified some potentially important ASCI and autophagy-related genes, significant pathways and candidate drugs, which could provides a basis for new trials and the development of novel targeted therapies as potential treatments for ASCI.

## Materials And Methods

### Text mining

The web-based service GenCLip3 was used to perform text mining (<http://ci.smu.edu.cn/genclip3/analysis.php>). When a query is performed, GenCLip3 extracts all the gene names found in the available literature related to the search concepts [10]. We performed two queries: one for the concept termed acute spinal cord injury (ASCI), and one for the concept termed autophagy. We then extracted all the unique gene hits from each result. The intersection of these two gene sets was used in the subsequent analyses.

### Gene Ontology (GO) Enrichment And Pathway Analysis

Gene ontology [11] is a structured vocabulary of terms describing gene products according to their biological process (BP), molecular function (MF), and cellular component (CC). The Kyoto Encyclopaedia of Genes and Genomes (KEGG) [12] provides data resources of known biological metabolic pathways. We used DAVID [13], a web-accessible program that integrates functional genomic annotations with intuitive graphical summaries, to view the GO and KEGG enrichment of the common genes. A p value of < 0.05 was considered statistically significant.

### PPI networks and module analysis

The Search Tool for the Retrieval of Interacting Genes (STRING, Version 11.0) [14] database was used to retrieve the common genes, encoded proteins and PPI network information. This database contains over 24.6 million proteins and 2 billion interactions observed in 5,090 organisms. We uploaded the common genes to the STRING database and set the interaction score to > 0.900 (highest confidence) as the significance threshold. Following this, PPI networks were constructed using the Cytoscape software [15].

The Molecular Complex Detection (MCODE) built in Cytoscape is an automated method that was used to analyse highly interconnected modules as molecular complexes or clusters. The analysis parameters were set to default. The functional enrichment analysis was executed for the common genes, from which two significant gene modules were identified with  $p < 0.05$  set as the significance threshold.

### **Drug-gene Interaction**

We used the Drug Gene Interaction Database (DGIdb, <http://www.dgldb.org>) to explore drug–gene interactions in the final list of genes, which were used as the potential targets in a search for existing drugs [16]. Our criteria for drug selection required FDA-approval and the presence of defined drug–gene interactions. These candidate drugs targeting the genes/pathways relevant to ASCI and autophagy may represent potential treatment strategies.

## **Results**

### **Text mining**

Based on the data mining strategy, 4178 genes identified were related to autophagy, 210 genes were related to ASCI, and 156 genes were common to both lists (Fig. 1 and Table 1).

Table 1. 156 common genes were identified

The common genes					
AKT1	NFKB1	HDAC9	CASR	EGR3	CALCA
BCL2	FAS	DCN	CAST	PRDX1	NTF3
CASP3	JAK2	AR	FNDC5	LIN28A	KDM6B
MAPK1	TNF	SMAD2	MIR210	F2RL1	FOXA1
MAPK8	NOS2	FADD	SMURF1	THBS1	MIR411
BAX	IL1B	NGF	MPO	GRASP	SERPINC1
PIK3CA	MALAT1	RAC1	TRAF3	GAP43	KLK7
HMGB1	GJA1	HGF	CSF3	PDE5A	ADORA1
MAPK14	CCL2	CASP12	CST3	KITLG	PRKCG
SOD1	MMP9	EPO	CSF2	NTRK1	EMILIN1
MAPT	PTGS2	FUS	TNFRSF1A	DUSP1	NEFH
NLRP3	RIPK3	FGF2	AAVS1	DPYSL2	MIR199B
MAPK3	MET	CDKN1B	IFNB1	SIAH1	SERPINI1
STAT3	VIM	MAP3K5	GRIN2B	LATS1	NTF4
FOXO3	LEP	PPP1R1A	ENO2	DNMT3B	ARTN
TLR4	ALB	GRN	MIR214	PTPRC	C5AR1
CASP9	MMP2	VHL	GDNF	MT2A	KLK8
APP	MIF	CD34	MBP	MIR182	ALG1
HMOX1	GHRL	ZC3H12A	TUG1	S100B	BMP7
IL6	MIR21	AURKA	BMP4	AQP4	TNFRSF1B
HSPA4	CDC6	AMH	MAP2	CHAT	CORD1
VEGFA	CXCR4	RHOA	BDNF-AS	MIR31	OPRK1
HIF1A	BDNF	FASLG	CRP	TAC1	PROC
RELA	GFAP	FOS	TPT1	JAG1	SENP3
CAT	TGFB1	TSPO	PTH	GJA8	KLK6
PSEN1	MAP3K7	TP63	KCNA3	SLC30A1	KCNB1

## GO enrichment and pathway analysis

To further explore the potential targets of these common genes in ASCI and autophagy, we performed GO and pathway analyses on these common genes with the criterion of having a p value of < 0.05 (Fig. 2). Figure 3 shows the top six significant terms for each of the following: the BP, CC, MF, and KEGG pathways of the common genes, respectively.

We also show the annotation of the common genes. As shown in Table 2, in the BP group, the common genes were mainly enriched for genes involved in the regulation of programmed cell death and regulation of the apoptotic process. In the CC group, the common genes were mainly enriched for genes associated with the extracellular space, axon, neuron projection, cell body, neuron part, and membrane raft. In the MF group, the common genes were primarily enriched for genes associated with cytokine receptor binding, receptor binding, growth factor activity, enzyme binding, cytokine activity, and identical protein binding. In the KEGG pathway group, the enrichment was observed for genes in the tumour necrosis factor (TNF)

signaling pathway, cancer pathways, as well as hepatitis B, Chagas disease, neurotrophin, and hypoxia-inducible factor-1 (HIF-1) signaling pathways.

Table 2. The top six pathways in GO and KEGG enrichment analysis of the common genes

Category	Term	Count	PValue
GOTERM_BP_FAT	GO:0008219~cell death	91	1.52E-48
GOTERM_BP_FAT	GO:0010941~regulation of cell death	81	2.42E-46
GOTERM_BP_FAT	GO:0043067~regulation of programmed cell death	79	3.26E-46
GOTERM_BP_FAT	GO:0012501~programmed cell death	87	4.45E-46
GOTERM_BP_FAT	GO:0006915~apoptotic process	85	6.82E-46
GOTERM_BP_FAT	GO:0042981~regulation of apoptotic process	76	3.93E-43
GOTERM_CC_FAT	GO:0005615~extracellular space	50	9.94E-16
GOTERM_CC_FAT	GO:0030424~axon	24	1.37E-11
GOTERM_CC_FAT	GO:0043005~neuron projection	35	2.87E-11
GOTERM_CC_FAT	GO:0044297~cell body	24	2.17E-10
GOTERM_CC_FAT	GO:0097458~neuron part	39	7.26E-10
GOTERM_CC_FAT	GO:0045121~membrane raft	17	1.13E-08
GOTERM_MF_FAT	GO:0005126~cytokine receptor binding	27	2.77E-19
GOTERM_MF_FAT	GO:0005102~receptor binding	53	1.26E-18
GOTERM_MF_FAT	GO:0008083~growth factor activity	20	5.30E-16
GOTERM_MF_FAT	GO:0019899~enzyme binding	49	2.63E-12
GOTERM_MF_FAT	GO:0005125~cytokine activity	17	2.29E-10
GOTERM_MF_FAT	GO:0042802~identical protein binding	39	3.18E-10
KEGG_PATHWAY	hsa04668:TNF signaling pathway	26	2.10E-22
KEGG_PATHWAY	hsa05200:Pathways in cancer	39	1.24E-19
KEGG_PATHWAY	hsa05161:Hepatitis B	26	5.81E-19
KEGG_PATHWAY	hsa05142:Chagas disease (American trypanosomiasis)	22	1.68E-17
KEGG_PATHWAY	hsa04722:Neurotrophin signaling pathway	21	5.73E-15
KEGG_PATHWAY	hsa04066:HIF-1 signaling pathway	19	1.70E-14

GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

### PPI network and module analysis

All the common genes were uploaded to the STRING website and were analysed using the Cytoscape software. A total of 146 nodes with 451 edges with scores > 0.900 (highest confidence) were selected to construct the PPI networks (Fig. 3). Two significant modules were selected using the MCODE plug-in. Module 1 consisted of 12 nodes/genes and 32 edges (Fig. 4), which were mainly associated with response to the peptide (BP), cytosol (CC), phosphatase binding (MF), and the mammalian target of rapamycin (mTOR) signaling pathway (KEGG) (Table 3). Module 2 consisted of 8 nodes/genes and 18

edges (Fig. 5), which were mainly associated with the apoptotic process (BP), extracellular space (CC), protease binding (MF), and Toll-like receptor signaling pathway (KEGG) (Table 4).

Table 3. Functional and Pathway Enrichment of Module 1 Genes

Category	Term	Count	PValue	Genes
GOTERM_BP_FAT	GO:1901652~response to peptide	10	3.30E-13	<i>AKT1, CDKN1B, MAPK14, RELA, MAPK3, IL1B, NFKB1, FOXO3, TGFB1, STAT3</i>
GOTERM_BP_FAT	GO:0010243~response to organonitrogen compound	11	5.69E-13	<i>AKT1, APP, CDKN1B, MAPK14, RELA, MAPK3, IL1B, NFKB1, FOXO3, TGFB1, STAT3</i>
GOTERM_BP_FAT	GO:0071222~cellular response to lipopolysaccharide	8	1.28E-12	<i>AKT1, MAPK14, RELA, MAPK3, IL1B, NFKB1, NLRP3, TGFB1</i>
GOTERM_CC_FAT	GO:0005829~cytosol	11	4.22E-06	<i>AKT1, APP, CDKN1B, MAPK14, RELA, MAPK3, IL1B, NFKB1, FOXO3, NLRP3, STAT3</i>
GOTERM_CC_FAT	GO:0005654~nucleoplasm	8	0.002364082	<i>AKT1, CDKN1B, MAPK14, RELA, MAPK3, NFKB1, FOXO3, STAT3</i>
GOTERM_CC_FAT	GO:0033256~I-kappaB/NF-kappaB complex	2	0.005289539	<i>RELA, NFKB1</i>
GOTERM_MF_FAT	GO:0019902~phosphatase binding	5	2.95E-06	<i>AKT1, CDKN1B, MAPK14, MAPK3, STAT3</i>
GOTERM_MF_FAT	GO:0019899~enzyme binding	9	3.75E-06	<i>AKT1, APP, CDKN1B, MAPK14, RELA, MAPK3, FOXO3, TGFB1, STAT3</i>
GOTERM_MF_FAT	GO:0008134~transcription factor binding	6	1.52E-05	<i>MAPK14, RELA, NFKB1, FOXO3, NLRP3, STAT3</i>
KEGG_PATHWAY	hsa04151:PI3K-Akt signaling pathway	6	1.11E-04	<i>AKT1, CDKN1B, RELA, MAPK3, NFKB1, FOXO3</i>
KEGG_PATHWAY	FoxO signaling pathway	7	2.08E-08	<i>AKT1, CDKN1B, MAPK14, MAPK3, FOXO3, TGFB1, STAT3</i>
KEGG_PATHWAY	hsa04621:NOD-like receptor signaling pathway	6	1.33E-08	<i>MAPK14, RELA, MAPK3, IL1B, NFKB1, NLRP3</i>

Table 4. Functional and Pathway Enrichment of Module 2 Genes

Category	Term	Count	PValue	Genes
GOTERM_BP_FAT	GO:0006915~apoptotic process	7	8.44E-06	<i>MAP3K7, BMP4, IL6, ALB, CST3, TLR4, PROC</i>
GOTERM_BP_FAT	GO:0012501~programmed cell death	7	1.19E-05	<i>MAP3K7, BMP4, IL6, ALB, CST3, TLR4, PROC</i>
GOTERM_BP_FAT	GO:0008219~cell death	7	1.65E-05	<i>MAP3K7, BMP4, IL6, ALB, CST3, TLR4, PROC</i>
GOTERM_CC_FAT	GO:0005615~extracellular space	6	1.70E-04	<i>BMP4, IL6, ALB, CST3, SERPINC1, PROC</i>
GOTERM_CC_FAT	GO:0044421~extracellular region part	6	0.017228348	<i>BMP4, IL6, ALB, CST3, SERPINC1, PROC</i>
GOTERM_CC_FAT	GO:0005576~extracellular region	6	0.037110279	<i>BMP4, IL6, ALB, CST3, SERPINC1, PROC</i>
GOTERM_MF_FAT	GO:0002020~protease binding	2	0.051738674	<i>CST3, SERPINC1</i>
GOTERM_MF_FAT	GO:0097367~carbohydrate derivative binding	4	0.067437364	<i>MAP3K7, BMP4, SERPINC1, TLR4</i>
GOTERM_MF_FAT	GO:0008201~heparin binding	2	0.070168181	<i>BMP4, SERPINC1</i>
KEGG_PATHWAY	hsa04620:Toll-like receptor signaling pathway	3	0.003388652	<i>MAP3K7, IL6, TLR4</i>
KEGG_PATHWAY	hsa04621:NOD-like receptor signaling pathway	2	0.047878013	<i>MAP3K7, IL6</i>
KEGG_PATHWAY	hsa04610:Complement and coagulation cascades	2	0.058714834	<i>SERPINC1, PROC</i>

## Drug–gene interactions

Using the final list of 20 genes, which were identified as the potential targets by the two significant modules in the drug–gene interaction analysis, 28 autophagy-regulating drugs were selected as possible molecules that can be repurposed for ASCI treatment (Table 5). Potential gene targets of the drugs in this list are *STAT3* (1 drug), *AKT1* (3 drugs), *TGFB1* (1 drug), *MAPK3* (2 drugs), *IL6* (1 drug), *IL1B* (2 drugs), *NFKB1* (1 drugs), *SERPINC1* (9 drugs), *PROC* (2 drugs), *ALB* (3 drugs), *APP* (3 drugs). Common previously approved uses for these drugs include the treatment of cancer, inflammation, angiopathy disease, diabetes, and Alzheimer disease.

Table 5. Candidate drugs targeting genes

Number	Drug	Gene	Interaction type	Score	Approved?	Reference (PubMed ID)
1	Acitretin	<i>STAT3</i>	inhibitor	1	Yes	None found
2	Ardeparin sodium	<i>SERPINC1</i>	activator	7	Yes	7632944
3	Arsenic trioxide	<i>AKT1, MAPK3</i>	inducer	7	Yes	12472888
4	Bismuth Subsalicylate	<i>ALB</i>	binder	1	Yes	None found
5	Canakinumab	<i>IL1B</i>	inhibitor	7	Yes	19169963
6	Dalteparin sodium	<i>SERPINC1</i>	activator	5	Yes	8707165
7	Danaparoid sodium	<i>SERPINC1</i>	activator	2	Yes	None found
8	Enoxaparin sodium	<i>SERPINC1</i>	activator	1	Yes	None found
9	Everolimus	<i>AKT1</i>	inhibitor	3	Yes	None found
10	Florbetaben	<i>APP</i>	binder	1	Yes	None found
11	Florbetapir	<i>APP</i>	binder	2	Yes	19837759
12	Flutemetamol	<i>APP</i>	binder	2	Yes	27031469
13	Fondaparinux	<i>SERPINC1</i>	activator	3	Yes	None found
14	Fondaparinux sodium	<i>SERPINC1</i>	activator	6	Yes	12501872
15	Gadobenate dimeglumine	<i>ALB</i>	binder	1	Yes	None found
16	Gallium nitrate	<i>IL1B</i>	inhibitor	3	Yes	16122880
17	Heparin calcium	<i>SERPINC1</i>	activator	1	Yes	None found
18	Heparin sodium	<i>SERPINC1</i>	activator	1	Yes	None found
19	Hyaluronidase	<i>TGFB1</i>	Inhibitor	5	Yes	9435505
20	Iodipamide	<i>ALB</i>	binder	1	Yes	None found
21	Menadione	<i>PROC</i>	activator	5	Yes	17215245
22	Nelfinavir	<i>AKT1</i>	inhibitor	1	Yes	None found
23	Rilonacept	<i>IL1B</i>	inhibitor	5	Yes	23319019
24	Siltuximab	<i>IL6</i>	inhibitor	6	Yes	8823310
25	Sodium tetradecyl sulfate	<i>PROC</i>	inhibitor	8	Yes	11752352
26	Sulindac	<i>MAPK3</i>	inhibitor	6	Yes	15548677
27	Thalidomide	<i>NFKB1</i>	antagonist	5	Yes	11752352
28	Tinzaparin sodium	<i>SERPINC1</i>	activator	7	Yes	19888521

## Discussion

We classified and summarised the final list of 20 genes, their targeted drugs, and the signaling pathways involved.

□ Genes, gene-targeted drugs, and gene-mediated signaling pathways associated with neuroinflammation in ASCI.

Seven significant genes, *NFKB1*, *RelA*, *IL1B*, *NLRP3*, *TLR4*, *IL6* and *STAT3*, are associated with neuroinflammation in ASCI.

*NFKB1* and *RelA*—*NFKB1* and *RelA* are important members of the NFKB family. NF-κB is a rapidly acting primary transcription factor found in all cell types. It is involved in the cellular responses to stimuli, such as cytokines and stress, and plays a key role in regulating the immunological response to infections. In terms of candidate drugs targeting genes, thalidomide is a specific antagonist of NFKB1.

*IL1B*—The protein encoded by *IL1B* is a member of the interleukin 1 cytokine family. This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. In terms of candidate drugs targeting genes, *canakinumab*, *gallium* and *nitrate rilonacept* are its inhibitors.

*NLRP3*—Jiang et al. demonstrate that pharmacologic suppression of NLRP3 inflammasome activation controls neuroinflammation, attenuates mitochondrial dysfunction, alleviates the severity of spinal cord damage, and improves neurological recovery after SCI[17].

*TLR4*—The protein encoded by *TLR4* is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. They recognize pathogen-associated molecular patterns that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity.

*IL6*—*IL6* encodes a cytokine that functions in inflammation and the maturation of B cells. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. In terms of candidate drugs targeting genes, *siltuximab* is a specific inhibitor of this gene.

*STAT3*—The protein encoded by *STAT3* is a member of the STAT protein family. In response to cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a crucial role in many cellular processes such as cell growth and apoptosis. In terms of candidate drugs targeting genes, *acitretin* is a specific inhibitor of this gene.

Two significant signaling pathways, the nucleotide-binding oligomerisation domain-like receptor (NLR) and Toll-like receptor (TLR)/Nuclear factor-κB (NF-κB) signalling pathways, are associated with neuroinflammation and autophagy in ASCI.

The prognosis of spinal cord injury (SCI) is closely related to secondary injury, which is dominated by neuroinflammation. Considerable studies have shown that autophagy can be activated through the NLR and TLR/NF-κB signaling pathways after SCI. However, activation of autophagy can inhibit the activity of

these pathways, thus playing a role in reducing the inflammatory response and protecting the injured spinal cord [18,19,20,21,22].

### ▣ **Genes, gene-targeted drugs, and gene-mediated signaling pathways associated with complement and coagulation cascades after ASCI**

Spinal cord ischemia, due to thrombosis after SCI, is one of the important mechanisms of secondary SCI. The complement and coagulation cascades involve two major genes, *SERPINC1* and *PROC*.

**SERPINC1:** The protein encoded by this gene, antithrombin III (ATIII), is a plasma protease inhibitor and a member of the serpin superfamily. In terms of candidate drugs targeting genes, activatorheparin calcium is a specific activator of it.

**PROC**▣ This gene encodes a vitamin K-dependent plasma glycoprotein. The encoded protein is cleaved to its activated form by the thrombin-thrombomodulin complex. This activated form contains a serine protease domain and functions in degradation of the activated forms of coagulation factors V and VIII.

### ▣ **Albumin (ALB) reduces oedema of the injured area after ASCI**

ALB gene encodes the most abundant protein in human blood. This protein functions in the regulation of the blood plasma colloid osmotic pressure and acts as a carrier protein for a wide range of endogenous molecules, including hormones, fatty acids, and metabolites, as well as exogenous drugs.

Following acute SCI, 5% ALB is often used in the clinic to increase the plasma colloid osmotic pressure, to reduce the oedema of the injured area. ALB is also routinely used in neurosurgical procedures and often combined with mannitol 20% (MAN). Palmaers et al., demonstrated that clinically relevant dilutions of MAN+ALB showed significant inhibition of blood coagulation and platelet function [23].

### ▣ **Three significant signaling pathways, which include five key genes, reduce apoptosis and neuroinflammation in ASCI by enhancing autophagy.**

The AMPK/mTOR signaling pathway

Zhou et al. showed that SPT ameliorates the AMPK/mTOR signaling-induced autophagy and thereby improves functional recovery in SCI-induced rats [24]. Furthermore, Meng et al. and Wang et al. showed that resveratrol promoted functional recovery and inhibited neuroinflammation through the activation of autophagy, mediated by the AMPK/mTOR pathway following SCI [25,26]. In general, the activation of this pathway enhances autophagy to protect the injured spinal cord.

The phosphatidylinositol-3-kinase (PI3K)/protein kinase B(Akt)/mTOR signaling pathway

Wang et al. showed that autophagy protects spinal cord neurons against PI3K/Akt/mTOR-mediated apoptosis after mechanical injury [27]. Furthermore, Li et al. showed that melatonin (MT) can improve the recovery of locomotor function by enhancing autophagy as well as reducing apoptosis after SCI in rats,

probably via the inhibition of the PI3K/Akt/mTOR signaling pathway [28]. In conclusion, the inhibition of this pathway can enhance autophagy to protect the injured spinal cord.

The AMPK- forkhead box O-3 (FOXO3) signaling pathway

Zhang et al. demonstrated that liraglutide was therapeutically beneficial in treating spinal contusion injury and its underlying mechanism was the activation of autophagic responses through the AMPK-FOXO3 signaling pathway [29]. Thus, the activation of this pathway can enhance autophagy to protect the injured spinal cord.

Five significant genes, *AKT1*, *FOXO3*, *CDKN1B*, *MAPK3* and *MAPK14*, are involved in three significant signaling pathways.

**AKT1**—The serine-threonine protein kinase encoded by the *AKT1* gene is catalytically inactive in serum-starved primary and immortalized fibroblasts. *AKT1* and the related *AKT2* are activated by the platelet-derived growth factor. It was shown that the activation occurs through phosphatidylinositol 3-kinase. In the developing nervous system, *AKT* is a critical mediator of growth factor-induced neuronal survival. In terms of candidate drugs targeting genes, arsenic trioxide is a specific inducer of this gene. Everolimus and Nelfinavir are specific inhibitors of it.

**FOXO3**: *FOXO3* belongs to the forkhead family of transcription factors, which are characterized by a distinct forkhead domain. This gene likely functions as a trigger for apoptosis through the expression of genes necessary for cell death.

**CDKN1B**: *CDKN1B* encodes a cyclin-dependent kinase inhibitor, which shares a limited similarity with CDK inhibitor *CDKN1A/p21*. The encoded protein binds to and prevents the activation of cyclin E-CDK2 or cyclin D-CDK4 complexes, and thus controls the cell cycle progression at G1. The degradation of this protein, which is triggered by its CDK dependent phosphorylation and subsequent ubiquitination by SCF complexes, is required for the cellular transition from quiescence to the proliferative state.

**MAPK3 and MAPK14**: The proteins encoded by *MAPK3* and *MAPK14* are the members of the mitogen-activated protein (MAP) kinase family. This kinase plays a significant role in stress-related transcription and cell cycle regulation, as well as in genotoxic stress responses. In terms of candidate drugs targeting genes, sulfonic acid is a specific inhibitor of *MAPK3* and arsenic trioxide is its specific inducer.

**There are five significant genes, which can be used as important directions for future research in ASCI**

Beta/A4 protein precursor (*APP*)

Although a precursor of Alzheimer amyloid, substantial evidence suggests that *APP* is involved in the regulation of neuronal growth and survival. Recently, Bowes et al. have demonstrated that the trophic properties of *APP* are completely preserved in a 17-amino acid sequence. If *APP* is neurotrophic, then it would be anticipated that the administration of the growth-promoting segment of the *APP* 17-mer peptide

might attenuate the neuronal dysfunction or loss or behavioural deficits associated with neuronal injuries, such as those accompanying CNS ischemia [30].

### Cystatin C (*CST3*)

Cystatin C, which belongs to the type II cystatin gene family, is a potent inhibitor of lysosomal proteinases. Zhang et al. showed that CysC levels are increased in patients with acute SCI, possibly as a direct result of the injury. Serum CysC is a potential biomarker of SCI [31].

### Bone morphogenic protein 4 (*BMP4*)

BMP4 is a key morphogen in neurodevelopment. Hart et al. showed that the beneficial effects of BMP inhibition in acute SCI are related to the ability of BMP4 to directly induce caspase-3 mediated apoptosis in neurons and oligodendrocytes in vitro [32].

### Transforming Growth Factor Beta 1 (*TGFB1*)

Lagord et al. showed that the cellular localization and temporal pattern of expression of TGFbeta after spinal cord injury suggest that TGFbeta1 modulates the inflammatory and neuronal responses, while TGFbeta2 regulates glial/collagen scarring[33]. What's more, Kohta et al. demonstrated that inhibition of TGF-beta1 promotes functional recovery after spinal cord injury[34].

### Mitogen-Activated Protein Kinase Kinase Kinase 7 (*MAP3K7*)

The protein encoded by MAP3K7 is a member of the serine/threonine protein kinase family. This kinase mediates the signaling transduction induced by TGF beta and morphogenetic protein (BMP), and controls a variety of cell functions including transcription regulation and apoptosis.

There are two limitations in our study: Firstly, the information on the functions or roles of the final list of 20 genes have not been verified through experiments but via databases used. Thus, further molecular biological experiments are required to confirm the function of these identified genes. Secondly, not all existing gene interactions are known for a given drug. Therefore, it is possible that drugs which could potentially be useful were missed or ignored because their gene interactions have not yet been fully elucidated.

## Conclusion

In conclusion, the mechanisms underlying secondary SCI, usually include inflammation, ischemia, oedema, and apoptosis. Autophagy could regulate these injury mechanisms through different genes and their signaling pathways, thus playing a role in protecting the damaged spinal cord. In this article, we presented a method to explore the potential key genes, molecular pathways and candidate drugs associated with ASCI and autophagy. As a result, in this method, we identified a total of 20 potential genes, six significant pathways and 28 candidate drugs, which could provides a basis for new trials and

the development of novel targeted therapies as potential treatments for ASCI. However, further molecular biological experiments are required to confirm the function of these identified genes, molecular pathways and candidate drugs in ASCI and autophagy.

## Abbreviations

ASCI: Acute spinal cord injury; BP: Biological processes; CC: Cellular components; MF: Molecular function; PPI: Signal pathways and protein–protein interaction; GO: Gene ontology; KEGG: Kyoto encyclopaedia of genes and genomes; STRING: Search tool for the retrieval of interacting genes; MCODE: Molecular complex detection; DGldb: Drug gene interaction database; MAP: Mitogen-activated protein; BMP4: Bone morphogenic protein 4; TGFB1: Transforming Growth Factor Beta 1; MAP3K7: Mitogen-Activated Protein Kinase Kinase Kinase 7

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

All authors read the final manuscript and approved for publication

### Availability of data and materials

All data generated or analyzed during this study are included in this published article

### Competing interests

The authors declare that they have no competing interests.

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### Author contributions

Both authors contributed to the preparation of the manuscript and approved the final manuscript.

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## Figures

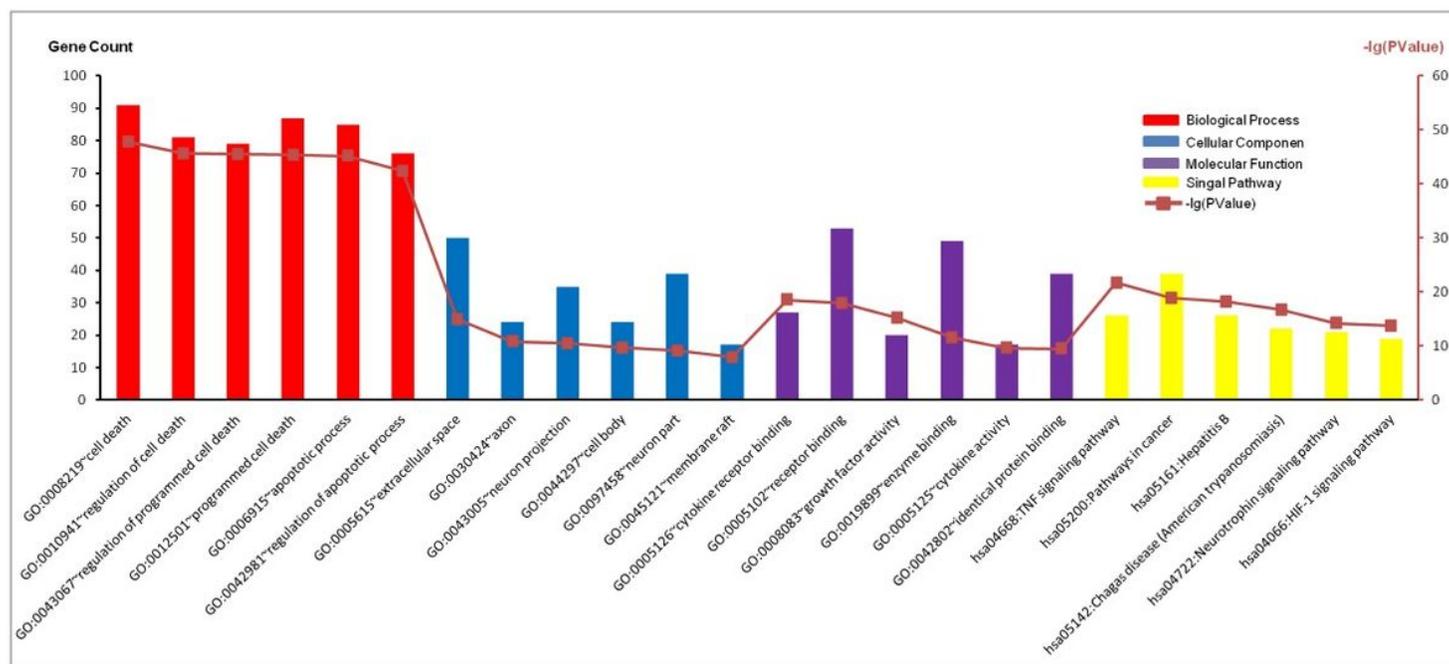
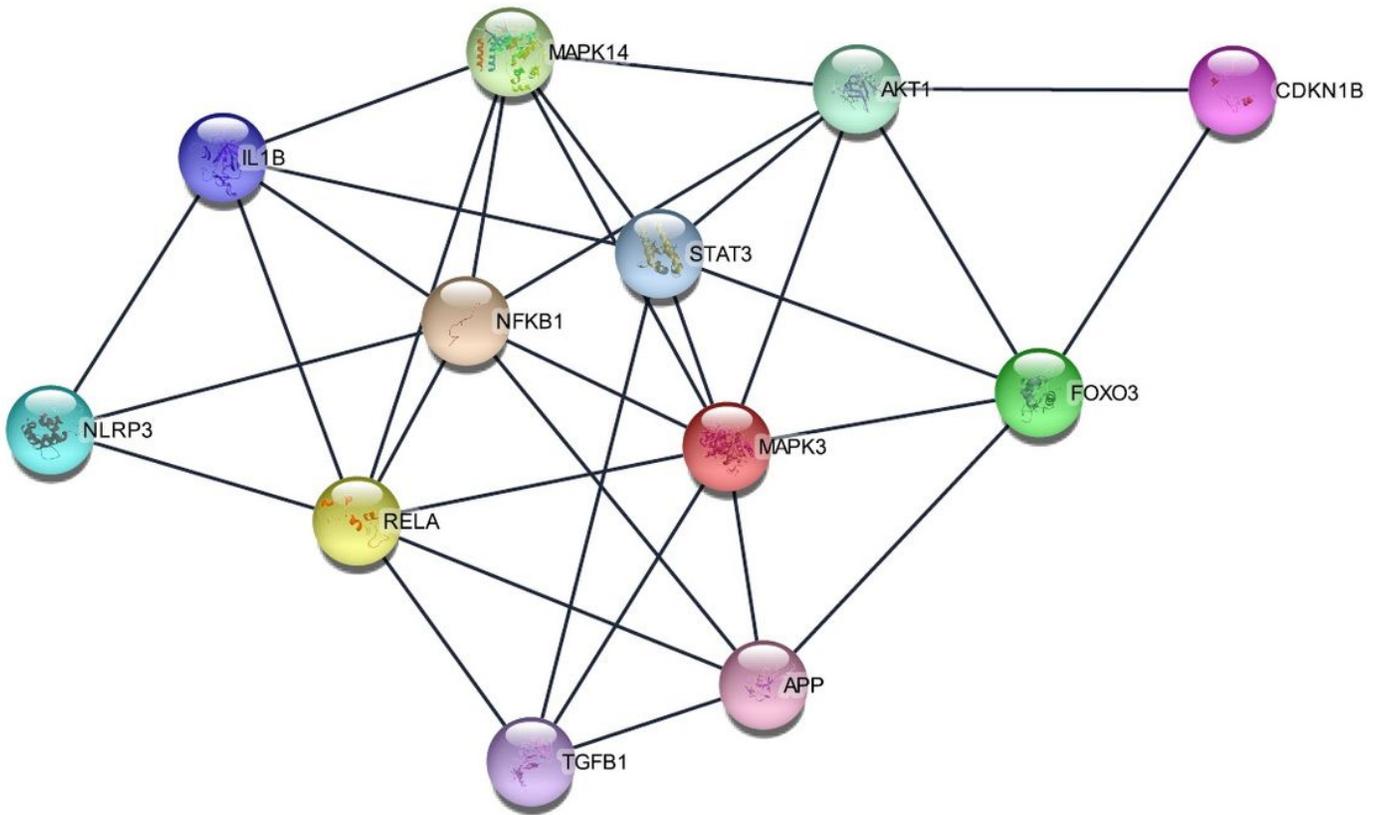


Figure 2

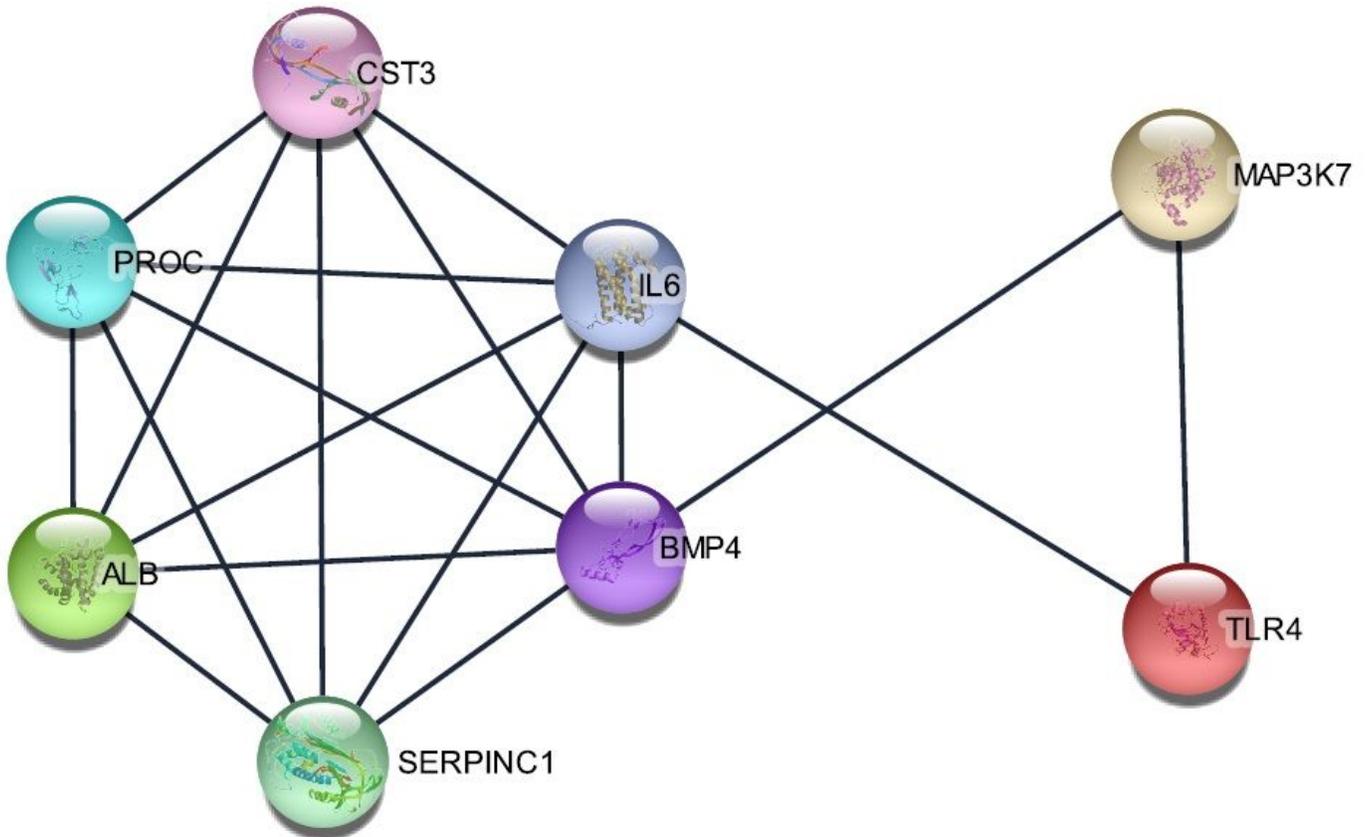
GO terms and KEGG pathways of the common genes.





**Figure 4**

The most significant module 1 from the PPI network



## Figure 5

The second significant module 2 from the PPI network