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Research paper

Identifying signature genes and their associations with immune cell infiltration in spinal cord injury

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ABSTRACT

Background: Early detection of spinal cord injury (SCI) is conducive to improving patient outcomes. In addition, many studies have revealed the role of immune cells in the progression or treatment of SCI. The objective of this study was to identify the early signature genes and clarify how they are related to immune cell infiltration in SCI.
Methods: We analysed and identified early signature genes associated with SCI via bioinformatics analysis of the GSE151371 dataset from the GEO database. These genes were subsequently verified in the GSE33886 dataset and qRT—PCR. Finally, the CIBERSORT algorithm was used to examine the immune cell infiltration in SCI and its relationship with signature genes.
Results: Seven SCI-related signature genes, including ARG1, RETN, BPI, GGH, CCNB1, HIST1H2AC, and HIST1H2BJ, were identified, and their expression was verified via an external validation cohort and qRT—PCR. Moreover, the ROC curves revealed the diagnostic value of these genes. In addition, on the basis of immune cell infiltration analysis, plasma cells, M0 macrophages, activated CD4+ memory T cells, γδ T cells, naive CD4+ T cells, and resting CD4+ memory T cells may participate in the progression of SCI.
Conclusion: This study identified seven early signature genes of SCI that may serve as biomarkers for the early diagnosis of SCI and contribute to our understanding of immune changes during the pathology of SCI.

1. Introduction

Spinal cord injury (SCI) occurs when an external force causes temporary or permanent damage to the spinal cord that can lead to motor and sensory dysfunction and even paralysis (Huang et al., 2021). According to statistics, approximately 0.9 million total cases of SCI were reported in 2019. The age-standardized incidence rate of SCI is 12 cases per 100,000 (Ding et al., 2022). Early detection of SCI is conducive to clinical intervention and improves the outcomes of patients (Sterner and Brooks, 2022). At present, the International Neurological Classification of Spinal Cord Injury (ISNCSCI) is the most common tool used to determine and evaluate the severity of SCI in clinical practice. However, owing to its subjectivity, the results are not completely reliable (Franz et al., 2022). In addition, conventional MRI can reveal the changes in intramedullary macrostructures such as haemorrhage and oedema after injury and is used as the gold standard for the diagnosis of SCI. Unfortunately, the results are sometimes affected by metal implants. Although quantitative MRI, such as diffusion imaging, can reveal the changes in the microstructure of neuraxis, its clinical application is limited by imaging technology (Freund et al., 2019). Like the study of cancer biomarkers, the detection of SCI biomarkers is helpful for the diagnosis and evaluation of the disease and can reflect the efficacy of treatment from a biological point of view, compensating for the shortcomings of previous diagnostic methods (Li et al., 2024; Kwon et al., 2019). In addition, the discovery of biomarkers can provide a new direction for the research of therapeutic targets. For example, Liu discovered that the majority of pyroptosis-related genes (PRGs) exhibit elevated expression levels in SCI patients through bioinformatics methods and that inhibiting the expression of PRGs contributes to recovery after SCI (Liu et al., 2024). The development of suitable biomarkers is therefore needed to improve the early diagnosis of SCI.

Currently, the treatments for SCI include drug therapy (Karsy and Hawryluk, 2017), early surgical decompression (Ma et al., 2020), cell therapy (Assinck et al., 2017), tissue engineering, and gene therapy (Gong et al., 2022). However, these methods do not significantly improve the sensory and motor functions of patients (Badhiwala et al.,

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2019). In fact, it is challenging to develop new medications or treatments because there is no obvious molecular cause of SCI. Hence, determining the precise molecular mechanism underlying the pathophysiology of SCI is necessary.

Complicated pathological events are involved in SCI, in which the inflammatory cascade plays a central role. Inflammatory cells worsen the injury by producing reactive oxygen species and releasing proinflammatory factors (Chen et al., 2017). Notably, the immune system is essential for controlling inflammation (Li et al., 2022a,2022b). Many studies have revealed the role of immune cells in the progression or treatment of SCI. For example, the inhibition of astrocyte hemichannels can reduce gliosis and improve neuronal survival (Zhang et al., 2021). Leukocyte protease inhibitors, which are secreted by neutrophils, can reduce inflammation and aid in axonal regeneration (Ghasemlou et al., 2010). Macrophages control tissue remodelling, which can accelerate spinal cord healing (Hong et al., 2017). C-C motif chemokine ligand 20 (CCL20) aggravates neuroinflammation after SCI by regulating Th17 cell recruitment, indicating that CCL20 may emerge as a novel therapeutic target (Hu et al., 2016). Therefore, the progression of SCI must be studied for any potential mechanisms involving different immune cells, which is crucial for identifying new immunotherapeutic targets.

Here, we first identified SCI signature genes via bioinformatics methods, which may serve as novel markers for the early diagnosis of SCI. In addition, these genes were confirmed via an external dataset and molecular experiments. Next, we evaluated the infiltration of 22 immune cell subsets in SCI. Finally, we analysed the relationships between signature genes and immune cells with significant differences in infiltration to understand the molecular immune mechanisms involved in the pathological process of SCI.

2. Material and methods

2.1. Data sources and identification of DEGs

Two datasets, namely GSE151371 and GSE33886, were downloaded from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo). The training dataset, GSE151371 include 38 SCI and 20 non-SCI blood samples, whereas the validation dataset, GSE33886, include 3 SCI and 4 non-SCI muscle samples.

Differentially expressed genes (DEGs) between SCI and non-SCI samples in GSE151371 were analysed via the "limma" R package, with an adjusted p value < 0.05 and a $|$ log2-fold change (FC)$| > 1$. Volcano plot and heatmaps were generated to display these DEGs.

2.2. Functional and pathway enrichment analyses of DEGs

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed via DAVID 6.8 (http://david-d.ncifcrf.gov/). GO terms and KEGG pathways with a p value < 0.05 and a count ≥ 2 were considered as significant enrichment thresholds. To display the enrichment results more intuitively, we used the "ggplot2" R package.

2.3. Identification and diagnostic efficacy analysis of signature genes

A protein-protein interaction (PPI) network with confidence = 0.9 was constructed from the DEGs via the STRING database (https://string-db.org/) and visualized via Cytoscape software. The CytoNCA plugin of Cytoscape software was used to analyse the degree of connectivity of the genes in the PPI network. Candidate genes were screened according to a degree of connectivity$\geq$ 30. The "glmnet" R package with 20-fold cross-validation was subsequently used for the least absolute shrinkage and selection operator (LASSO) regression analysis to further determine signature genes. Finally, the receiver operating characteristic (ROC) curves were used to evaluate the diagnostic performance of these signature genes in the training dataset and the validation dataset respectively.

2.4. Signature gene enrichment analysis

To explore the relationships between signature genes and signaling pathways, the ClueGo plug-in of Cytoscape software was used to perform functional enrichment analysis of signature genes. A p value < 0.05 was considered a significant enrichment threshold.

2.5. Immune cell infiltration analysis

The CIBERSORT algorithm was used to analyse the immune cell infiltration. First, we calculated the proportions of 22 immune cells in the SCI group and non-SCI group. We subsequently analysed the differences in immune cells between the two groups and identified immune cells with significant differences in infiltration. Finally, Spearman analysis was used to calculate the correlation between signature genes and immune cells with significant differences in infiltration.

2.6. Sample collection and quantitative real-time PCR

Peripheral blood samples were collected from 11 SCI patients and 11 healthy controls. The study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital. All the participants provided written informed consent.

Total RNA from the SCI and non-SCI samples was extracted via TRIzol reagent (Cat. No.15596–026, Ambion, USA). Next, HiScript II Q RT SuperMix for qPCR (Cat. No. R233–01, VazymE, Nanjing, China) was used for cDNA synthesis of total RNA. The expression level of each signature gene was subsequently determined by qRT–PCR using SYBR Green Master Mix (Cat. No. Q111–02, VazymE, Nanjing, China) and calculated via the $2^{-\Delta\Delta CT}$ method. The results were normalized to GADPH expression. The primer sequences are listed in Table 1.

2.7. Statistical analysis

All the statistical analyses were performed with R software (version 4.0.0). Comparisons between the two groups were performed via the Wilcoxon test. A p value < 0.05 was considered statistically significant unless otherwise specified.

3. Results

3.1. Identification of DEGs

Fig. 1 shows the flow chart of this research. Between the SCI and non-SCI samples, 1159 DEGs were found, including 791 upregulated genes and 368 downregulated genes. Figs. 2A and 2B display the volcano plots and heatmaps of the DEGs, respectively.

3.2. Functional and pathway enrichment analyses of DEGs

The GO terms (BP, CC, and MF) are displayed in Fig. 3**A**. In the BP analysis, telomere organization, chromatin silencing at rDNA, and DNA replication-dependent nucleosome assembly were significantly enriched. The CC analysis revealed that these DEGs were associated mainly with nuclear chromosomes, extracellular regions, and extracellular exosomes. In addition, protein heterodimerization activity, histone binding, and arachidonic acid binding play critical roles in MF. The KEGG pathway analysis revealed that the DEGs were related mainly to inflammation and immune-related diseases, including systemic lupus erythematosus, haematopoietic cell lineage, complement and coagulation cascades, inflammatory bowel disease (IBD), the NOD-like receptor signalling pathway, and the T cell receptor signalling pathway (Fig. 3B).

Table 1
Primer sequences for qRT-PCR.

Genes	Forward	Reverse
GAPDH	TCAAGAAGGTGGTGAAGCAGG	TCAAAGGTGGAGGAGTGGGT
ARG1	TGGCAGAAGTCAAGAAGAACG	TACAGGGAGTCACCCAGGAGA
RETN	CTCCCTGTCCTGGGGCTGTTGGTGT	ACTGGCAGTGACATGTGGTCTCGGC
BPI	TGCTTCAGCCTCACCAGAAC	TGCAGCCTTAGCCCTTGAAA
GGH	TGCTGATTAGTGGAGAGTGCTT	ATCTTGCCATCTGTATTTGTAG
CCNB1	TTTTGGTTGATACTGCCTC	ATTTTGGTCTGACTGCTTG
HIST1H2AC	CCTGGCGGCGGTGTTAGAGTA	CAGAAGCACGGCCTGGATGTT
HIST1H2BJ	TCTGCTCCCGCCCCGAAAAAG	CCTGCGATGCGCTCGAAAATG

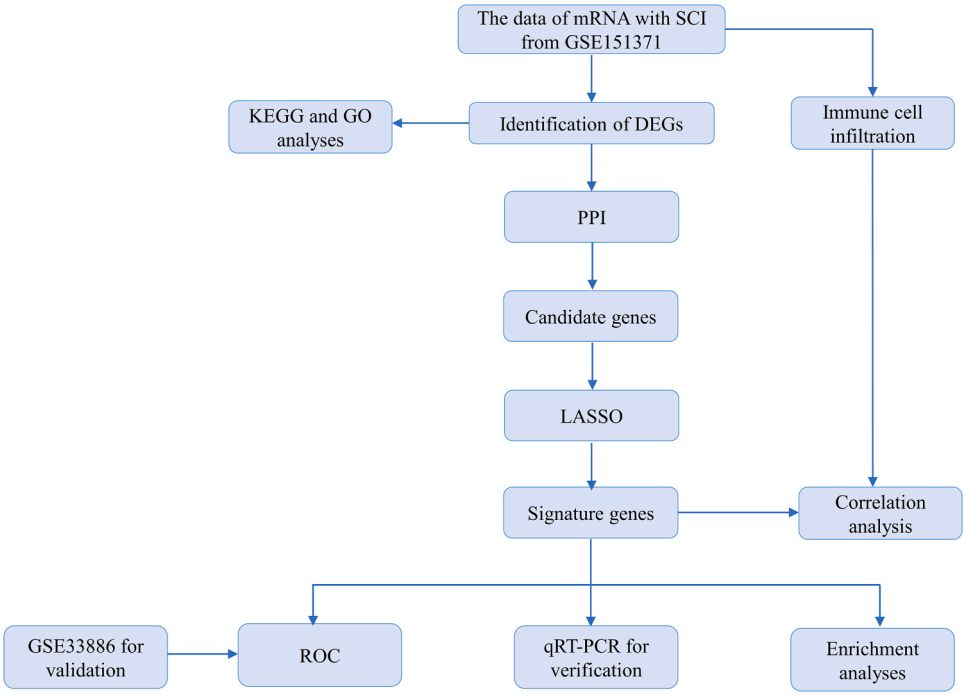

Fig. 1. The flow chart for this study.

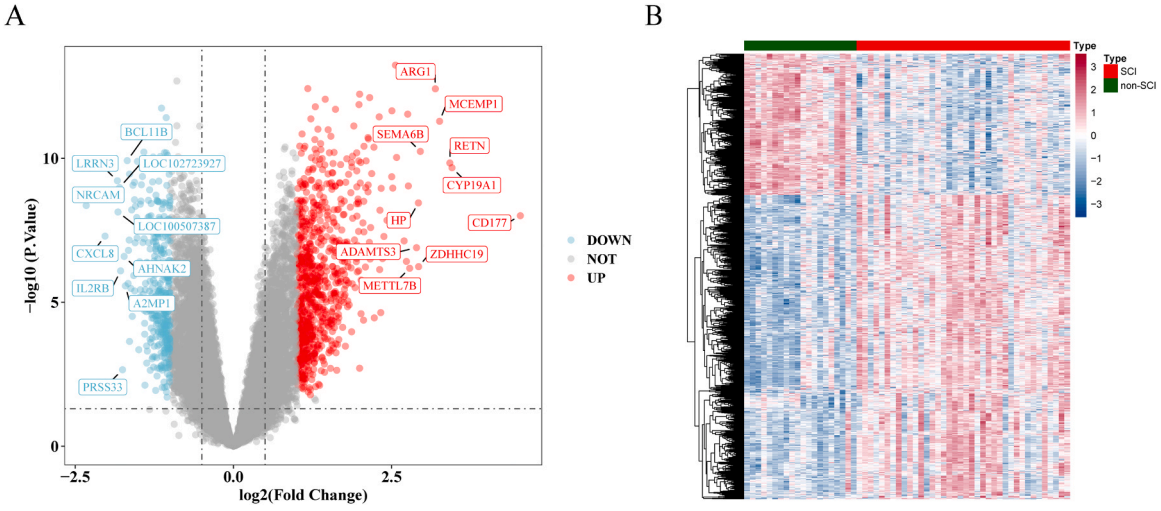

Fig. 2. Identification of the DEGs. (A) Volcano plot showed 791 upregulated (red) and 368 downregulated (blue) DEGs were determined between the SCI and non-SCI groups. (B) The heatmap showed all DEGs.

3.3. Identification and diagnostic efficacy analysis of signature genes

The PPI network, composed of 1914 interaction pairs and 416 nodes, was visualized by Cytoscape software (Fig. 4A). with the CytoNCA plug-in, 20 genes were subsequently identified as candidate genes associated with SCI, and seven signature genes were subsequently obtained from

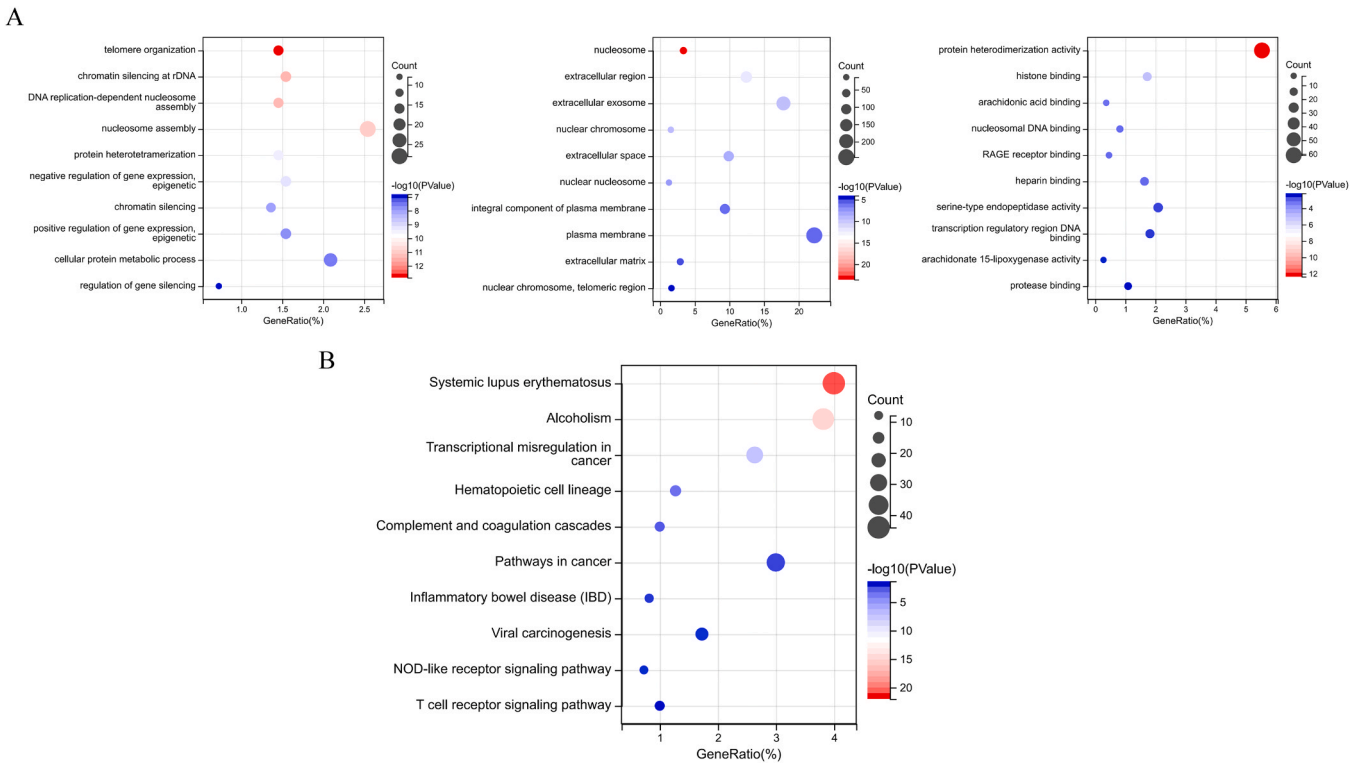

Fig. 3. Functional and pathway enrichment analyses of DEGs. (A) The top 10 functional enrichment terms in the BP, CC, and MF analyses, respectively. (B) The top 10 results of the KEGG analysis.

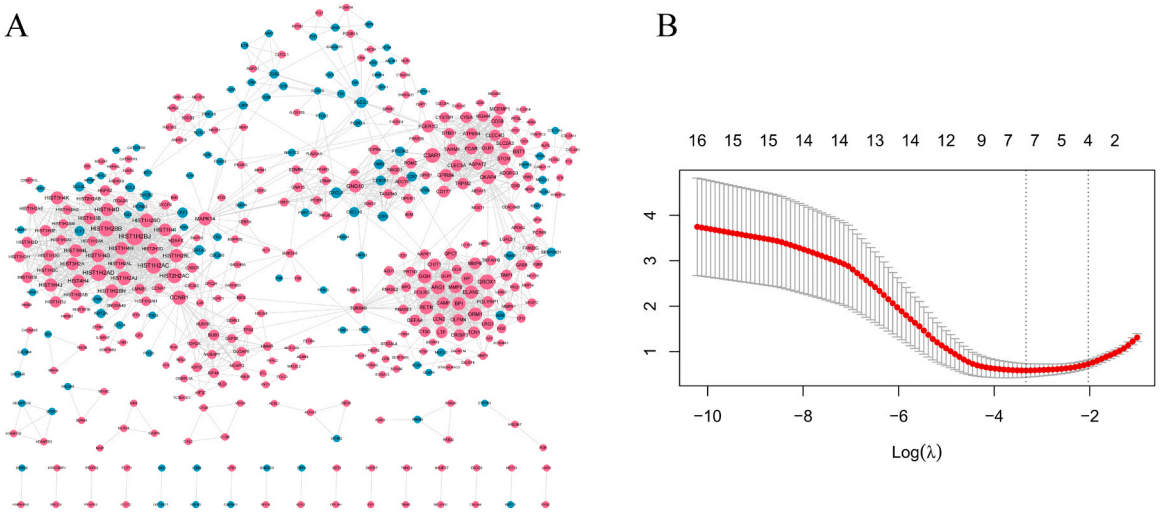

Fig. 4. Identification of signature genes through PPI network and LASSO algorithm. (A) Identifying candidate genes through PPI network. (B) Identifying signature genes using LASSO algorithm.

those candidate genes via the LASSO algorithm (Table 2, Fig. 4B).

Compared with those in the non-SCI group, these signature genes were significantly overexpressed in the SCI group, which implies that these genes might potentially be involved in SCI (Fig. 5A-G). In addition, these genes had areas under the ROC curves (AUCs) of the ROC that were as follows: 0.946 for ARG1, 0.914 for RETN, 0.916 for BPI, 0.899 for GGH, 0.821 for CCNB1, 0.820 for HIST1H2AC, and 0.768 for HIST1H2BJ (Fig. 5H-N). Moreover, the expression of ARG1, RETN, BPI, GGH, and CCNB1 in the external validation dataset and the AUC values of the ROC curve were mostly consistent with those in the training dataset (Fig. 6).

Table 2
The list of candidate genes and signature genes.

Analysis	Genes
PPI	HIST1H2BJ, HIST1H2AD, HIST1H2AC, HIST1H2BB, QSOX1, C3AR1, HIST2H2AC, CCNB1, CKAP4, GGH, HIST1H2BO, HIST1H2AJ, ORM1, HIST1H2BN, HIST1H2BL, ARG1, ELANE, RETN, BPI, MAPK14
LASSO	ARG1, RETN, BPI, GGH, CCNB1, HIST1H2AC, HIST1H2BJ

3.4. *Signature gene enrichment analysis*

To further understand the role of signature genes in SCI, we

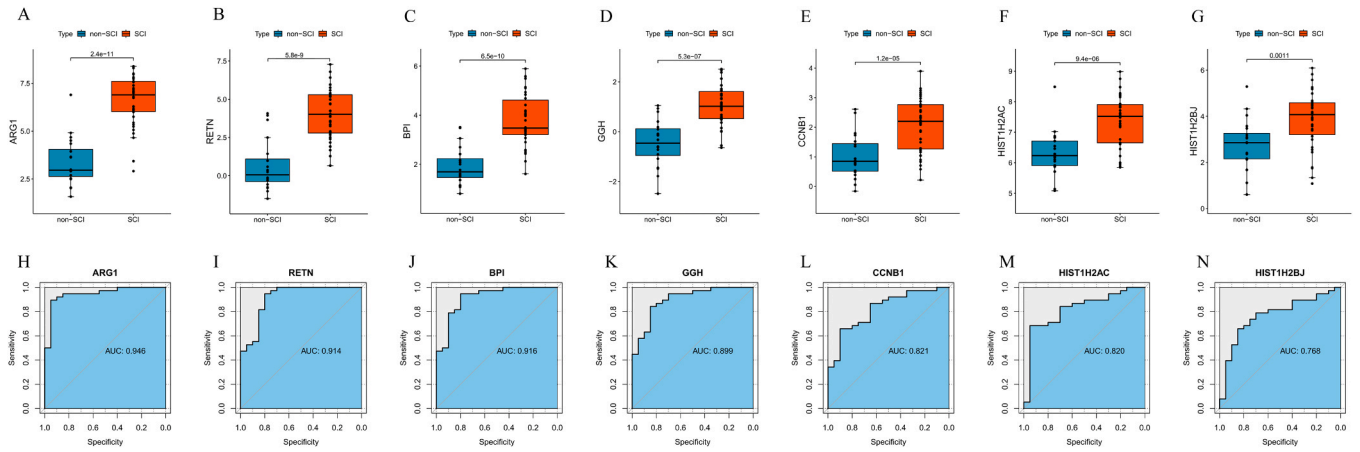

Fig. 5. The diagnostic value of the signature genes in GSE151371. (A-G) The expression of signature genes between the SCI and non-SCI groups. (H-N) ROC showed the diagnostic value of the signature genes.

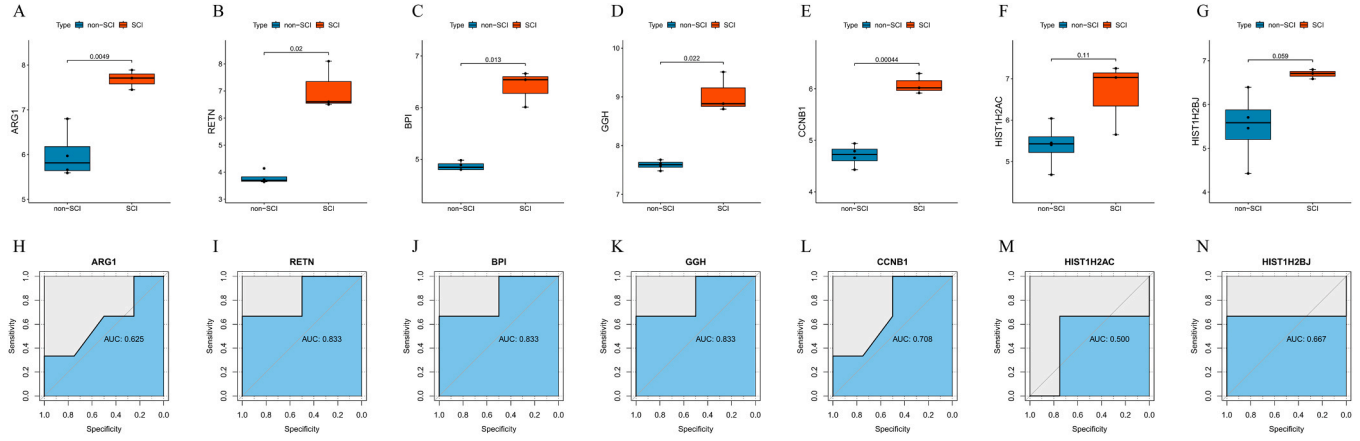

Fig. 6. The diagnostic value of the signature genes in GSE33886. (A-G) The expression of signature genes between the SCI and non-SCI groups. (H-N) ROC showed the diagnostic value of the signature genes.

performed functional enrichment with the ClueGO plug-in (Fig. 7, Supplementary Table 1). The results revealed that a total of 33 functional pathways were enriched. For ARG1, the GO-BP terms included "regulation of type 2 immune response", "positive regulation of neutrophil-mediated cytotoxicity", "and negative regulation of T-helper 2 cell cytokine production". RETN, BPI and GGH all relate to GO-CC terms "azurophil granule lumen" and "specific granule lumen". The GO-BP terms for CCNB1 included "histone H3-S10 phosphorylation", "response to DDT", "positive regulation of cell cycle G2/M phase transition", and "cellular response to iron (III) ion". HIST1H2BJ was involved in the innate immune response in the mucosa according to the GO-BP analysis.

3.5. Immune cell infiltration analysis

We evaluated the immunological characteristics of SCI according to the infiltration of immune cells. Fig. 8A shows the percentages of 22 types of immune cells in all the samples. Furthermore, compared with the non-SCI group, the SCI group presented greater numbers of plasma cells, activated CD4+ memory T cells, gamma delta (γδ) T cells, and M0 macrophage infiltration, and lower naive CD4+ T cell, and resting memory CD4+ T cell infiltration (Fig. 8B). All signature genes were positively correlated with the infiltration of M0 macrophages. ARG1, RETN, BPI, and GGH were negatively correlated with naive CD4+ T cells and resting CD4+ memory T cells and positively correlated with plasma cells, activated CD4+ memory T cells, and γδ T cells (Fig. 8**C**).

3.6. Validation of signature genes

We determined the expression of these signature genes via qRT-PCR analysis. These seven biomarkers were expressed at high levels in the SCI group (Fig. 9), which was consistent with our previous bioinformatics results.

4. Discussion

Dynamic biomarkers are objective and easily available tools for SCI diagnosis (Stukas et al., 2023). They can provide additional diagnostic information and are an effective supplement to clinical information and MRI images. Furthermore, a previous study indicated that neuroinflammation and the immune response are closely related to SCI (Alizadeh et al., 2018). In this study, we screened SCI-related signature genes derived from blood samples (ARG1, RETN, BPI, GGH, CCNB1, HIST1H2AC, and HIST1H2BJ). We subsequently validated these genes in external validation cohorts and molecular experiments.

Among these genes, ARG1, RETN, BPI, GGH and CCNB1 in the SCI group were all significantly different in the training dataset, validation dataset and molecular experiments. ARG1 is an enzyme that regulates cellular nitrogen homeostasis. In addition, it has increasingly been investigated as a crucial immune system component associated with inflammation (Li et al., 2022a,2022,b; Zhu et al., 2015). In our study, we observed high expression of ARG1 in the SCI group, which is consistent with the findings of other studies (Zhang et al., 2020). Previous studies

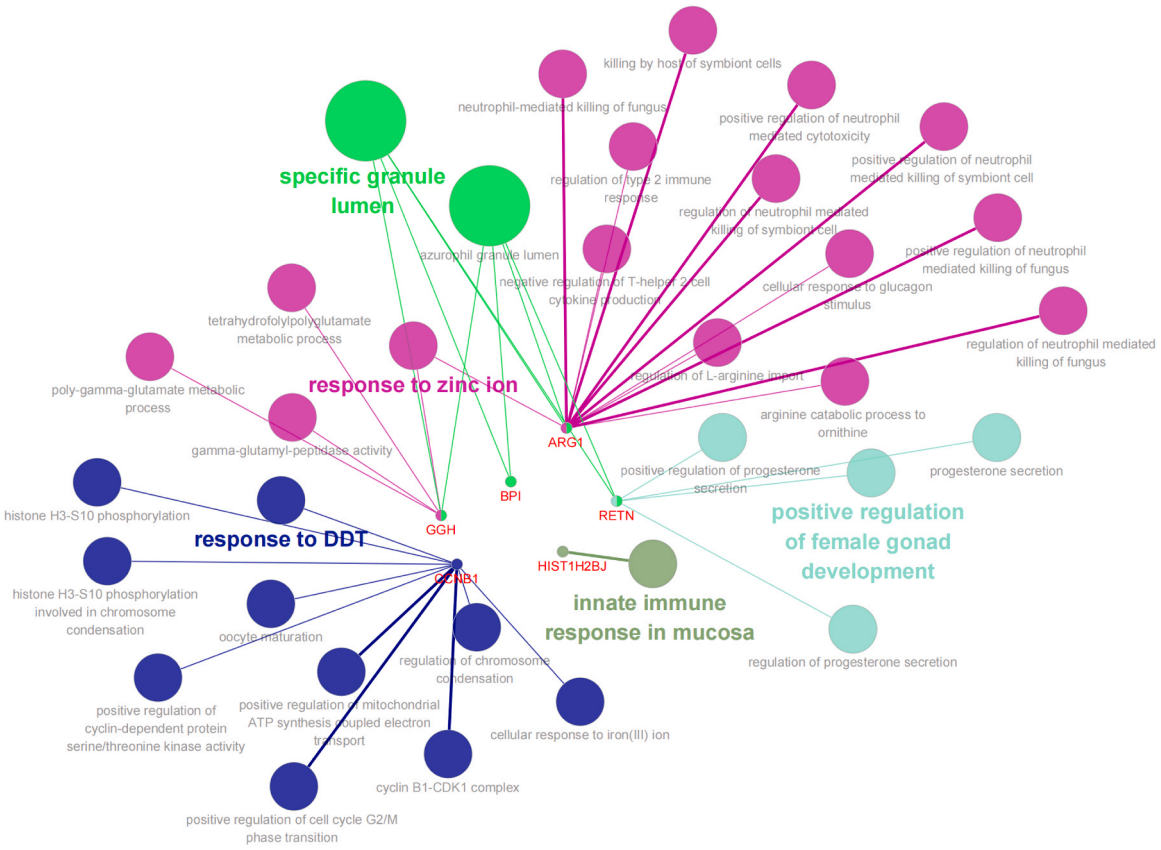

Fig. 7. Signature genes enrichment analysis.

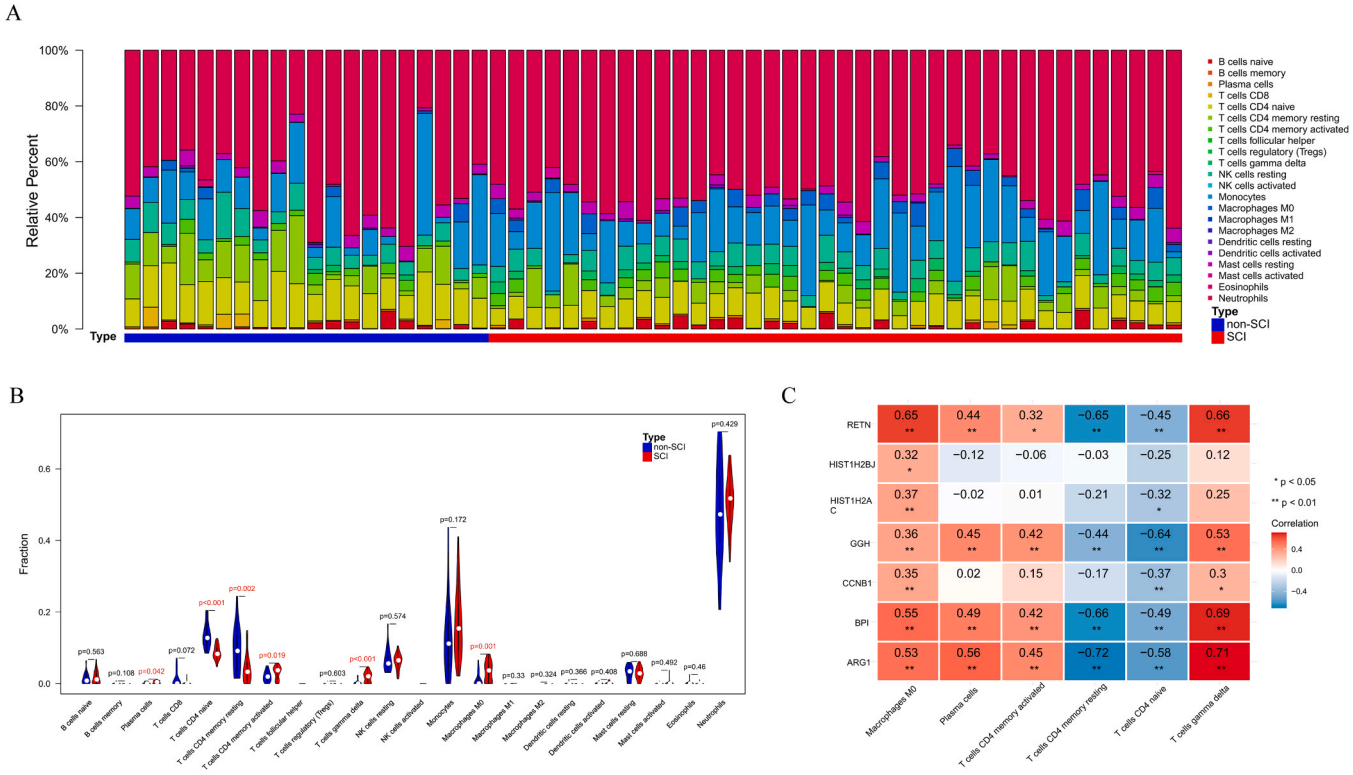

Fig. 8. Immune cell infiltration in SCI. (A) The relative percentage of 22 types of immune cells. (B) The immune cell infiltration between the SCI and non-SCI groups. (C) The correlation between signature genes and immune cells with significant differences in infiltration. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

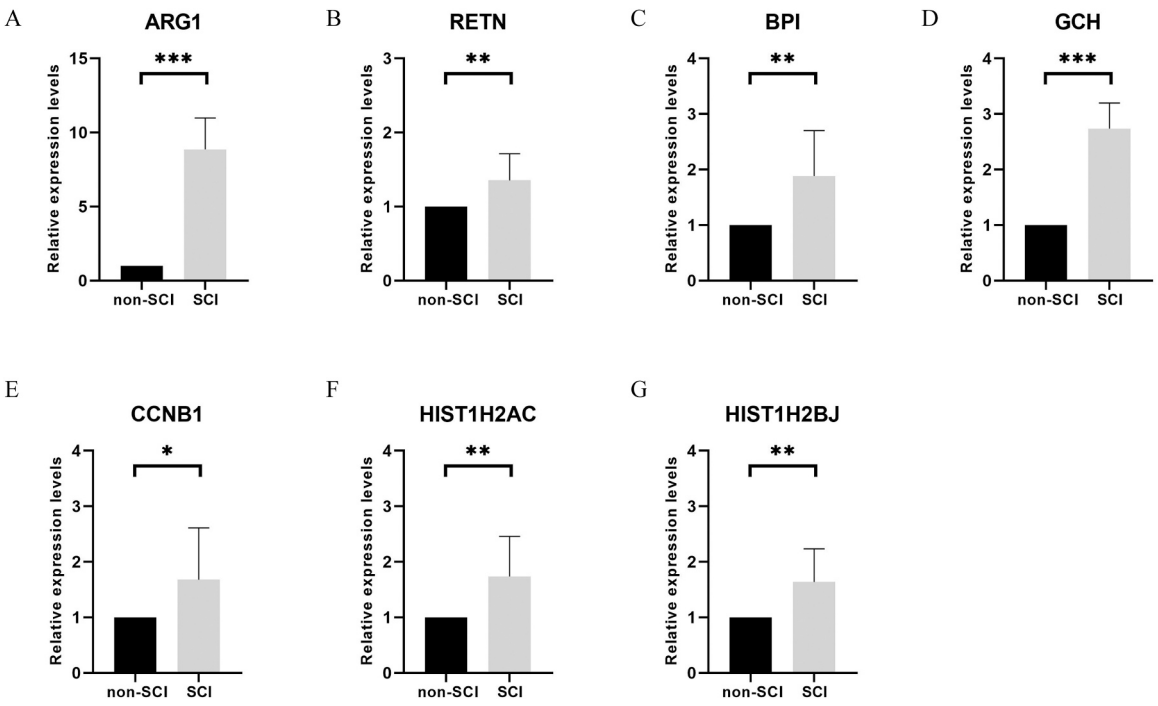

Fig. 9. Validation of signature genes. The expression of seven signature genes between the SCI and non-SCI groups was measured by qRT-PCR. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

have shown that increased levels of ARG1 induce M2 microglia/macrophage polarization and that M2 microglia/macrophage can play a neuroprotective role in SCI through anti-inflammatory effects and tissue repair (Lund et al., 2022; Cai et al., 2019). These findings suggest that ARG1 is a neuroprotective factor in the development of SCI. In addition, ARG1 can promote structural and functional recovery of the injured spinal cord by restoring axonal regeneration (Fu et al., 2022). However, the specific mechanism by which ARG1 plays a protective role in SCI by regulating inflammation and the immune response is unclear. Our study revealed that ARG1 was associated with the regulation of the type 2 immune response, the positive regulation of neutrophil-mediated cytotoxicity, and the negative regulation of T-helper 2 cell cytokine production.

In the past, RETN was generally considered as an adipokine that links obesity with diabetes (Gao et al., 2021). Research has showen that it is also an inflammatory cytokine, that is mainly secreted by macrophages (Sudan et al., 2020). For example, high levels of RETN are associated with sepsis and intrahepatic inflammation (Wang et al., 2022; Meng et al., 2017). BPI is an antimicrobial protein (Theprungsirikul et al., 2021). It has been reported that BPI can reduce the inflammatory reaction in patients with colitis and arthritis (Kong et al., 2021; Scanu et al., 2022). GGH is a widely expressed enzyme that controls the metabolism of folate inside cells, and its high expression is often associated with poor cancer prognosis (Melling et al., 2017; Yu et al., 2022). In fact, to date, the potential functions and expression levels of these three genes in SCI have not been reported. In our study, their expression was increased in SCI patients, and they were enriched in specific granule lumens and azurophil granule lumens. Azurophil granules and specific granules are primary and secondary granules, respectively, in the subcellular fraction of neutrophils, which contain a large amount of granular protein that orchestrates the inflammatory response by interacting with innate and adaptive immune cells (Cassatella et al., 2019; Othman et al., 2022).

CCNB1 is a protein that regulates the transition of the cell cycle from the G2 phase to mitosis (Zhang et al., 2018). A previous study revealed that CCNB1 participates in the acute phase of SCI (Shi et al., 2019). In addition, CCNB1 expression was significantly increased in patients with SCI compared with healthy individuals (Baek et al., 2019). The present study revealed that CCNB1 was involved mainly in histone H3-S10 phosphorylation, the response to DDT, the cellular response to iron (III) ions, the regulation of chromosome condensation, and the positive regulation of mitochondrial ATP synthesis coupled with electron transport, similarly, it was highly expressed in the SCI group.

Interestingly, in our results, two SCI-related genes were upregulated only in the training dataset and molecular experiments, whereas the expression of these genes was not different between the validation dataset and the non-SCI group. HIST1H2AC and HIST1H2BJ belong to histone members. At present, there are few reports about HIST1H2AC and HIST1H2BJ. It was reported that high levels of HIST1H2AC and HIST1H2BJ are related to monkeypox infection and poor cancer prognosis (Xuan et al., 2022; Liu et al., 2020). In addition, HIST1H2BJ can participate in wound healing through an immune response mechanism (Wang et al., 2019). In our study, HIST1H2AC and HIST1H2BJ were highly expressed in the SCI group, and HIST1H2BJ was involved in innate immune response in the mucosa.

In immune infiltration analysis, we observed that the infiltration of plasma cells, activated CD4+ memory T cells, γδ T cells, and M0 macrophages was increased in the SCI group, which means that there is a certain relationship between them and SCI. In fact, although plasma cell infiltration is very rare in SCI, a study observed CD138+ IgG + plasma cells in the lesioned spinal cord of some SCI patients (Zrzavy et al., 2021). γδ T cells, a subset of T cells, are essential for regulating the immune response in the central nervous system (Wo et al., 2020). In a previous study, it was discovered that T lymphocytes were detectable in the cerebrospinal fluid of SCI patients as well as in the lesion sites in SCI model mice one day after damage (Sun et al., 2018). Another study showed that γδ T cells were recruited to the SCI site through CCL2/CCR2 signalling, which promotes inflammatory response and aggravates neurological injury (Xu et al., 2021). M0 macrophage is an inactive macrophage subtype (Tang et al., 2021). One study found that the infiltration of M0 macrophages was increased in SCI model mice (Cao and Li, 2022. At present, there is no report on the link between activated CD4+ memory T cells and SCI. It is worth mentioning that immune cells have dynamic characteristics at different stages of SCI. Therefore, the

molecular mechanism of immune cell infiltration in SCI needs further study.

Concerning the connections between immune cells and signature genes, ARG1, RETN, BPI, and GGH were positively correlated with M0 macrophages, plasma cells, activated CD4+ memory T cells, and γδ T cells and negatively correlated with naive CD4+ T cells and resting CD4+ memory T cells. In addition, M0 macrophages and γδ T cells exhibited positive correlations with CCNB1, while naive CD4+ T cells exhibited negative correlations. HIST1H2AC was positively correlated with M0 macrophages and negatively correlated with naive CD4+ T cells. HIST1H2BJ was positively correlated with M0 macrophages. A previous study showed that the functional connection between CD4+ T cells and myeloid ARG1, that is, inhibition of either of them can inhibit tumour formation (Van de Velde et al. 2021). In addition, ARG1 inhibits the proliferation of CD4+ T cells by depleting L-arginine (Salminen 2021; Soileau et al., 2022). It was reported that RETN stimulated CD4+ T cells chemotaxis through activated Src- and phosphoinositol 3 -kinase (Walcher et al., 2010). In addition, RETN promotes the expression of COX-2 in macrophages, regulating inflammatory response (Zhang et al., 2010). A study showed that the interaction between BPI and gram-negative bacteria can enhance the phagocytosis of macrophages (Balakrishnan et al., 2016). Additionally, in clinical samples, the expression of GGH was positively associated with CD4+ T cell infiltration in colon cancer (Chen et al. 2022). It was reported that the high expression of CCNB1 was related to the increased proliferation of macrophages (Wang et al., 2008). The increased expression of HIST1H2BJ may promote the phagocytosis of macrophages (Wang et al., 2019). Due to the limited information about the complicated interaction mechanism between signature genes and immune cells, further studies should be carried out based on the above bioinformatics analysis results.

Our study also has some limitations. On the one hand, there are fewer publicly available datasets on acute SCI, and we were only able to select two datasets for analysis and validation. On the other hand, the diagnostic value of several signature genes in the validation dataset is limited. We hypothesize that there are three reasons for this result: first, the SCI samples in the training and validation datasets were collected at different times. The samples in the training dataset were collected at 30.3 ± 18.9 h post-injury (Kyritsis et al., 2021), whereas those in the validation dataset were samples with SCI lesions more than 4 years old (Lammers et al., 2012). Second, the tissue sources for the training dataset and validation dataset were also different. Since the genetic information of the SCI subjects in the validation dataset was only present in the muscle samples, we ultimately chose the muscle samples instead of the blood samples consistent with the training dataset (Lammers et al., 2012). Finally, the sample size of the validation dataset is small, thus the validation efficacy is limited. Notably, our clinical samples may have confirmed our analysis to some extent. Hence, more acute SCI data are needed for our future studies. Additionally, the specific mechanism of these signature genes in SCI should be further confirmed using in vivo and in vitro experimental validation. This will be the main topic of our upcoming research.

5. Conclusion

In summary, we identified seven early signature genes of SCI through bioinformatics and molecular experiments, namely ARG1, RETN, BPI, GGH, CCNB1, HIST1H2AC, and HIST1H2BJ. These genes may serve as biomarkers for the early diagnosis of SCI and contribute to our understanding of immune changes during the pathology of SCI.

Ethics statement

The patients involved in the GEO database have obtained ethical approval. Our experimental research with human subjects has been formally approved by the Human Subjects Review Committee of Shaanxi Provincial People's Hospital.

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CRediT authorship contribution statement

Zhengchao Gao: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Su'e Chang:** Software. **Yingjie Zhao:** Writing – original draft, Methodology. **Meng Lv:** Writing – original draft, Methodology, Conceptualization.

Declaration of Competing Interest

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

Acknowledgments

The authors would like to express their appreciation to GEO dataset for providing data.

Consent for publication

We confirm that the manuscript has not been published elsewhere and is not under consideration by other journals. All authors have contributed significantly to the work and have read and agreed on the content of the final version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2024.09.002.

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