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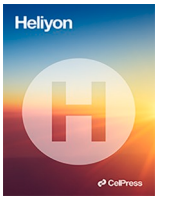

Exploration of the effect and potential mechanism of quercetin in repairing spinal cord injury based on network pharmacology and *in vivo* experimental verification

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A R T I C L E I N F O

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A B S T R A C T

Spinal cord injury (SCI) is a highly complex neurological disease, but there is no effective repair method. Quercetin is a flavonol drug and has a variety of biological activities, such as scavenging oxygen free radicals in the body to resist oxidation, inhibiting inflammation, and so on. In this study, quercetin was firstly demonstrated to reduce tissue damage, promote neuron survival and repair motor function after SCI in rats through *in vivo* experiments. Then, 293 potential targets of quercetin repair for SCI were predicted by network pharmacology. GO analysis revealed that the biological processes of potential targets focused mainly on signal transduction, negative regulation of the apoptotic process, protein phosphorylation, drug response, and so on. Similarly, KEGG analysis suggested that these potential targets were involved in cell growth regulation, differentiation, apoptosis, and a few metabolic pathways. PPI network analysis predicted that the key genes were *EP300, CREBBP, SRC, HSP90AA1, TP53, PIK3R1, EGFR, ESR1*, and *CBL*. Further, the molecular docking showed that quercetin binds well with these proteins. Finally, RT-qPCR and Western blotting experiments verified that quercetin downregulated the expression levels of PIK3R1 and EGFR. It is suggested that quercetin can repair SCI in rats through PI3K-AKT signaling pathway and EGFR/MAPK pathway, which may provide a new theoretical basis for the repair of spinal cord injury.

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1. Introduction

Spinal cord injury (SCI) often leads to permanent motor and sensory dysfunction [1,2], it can be categorized into primary and secondary injury phases based on the complex pathophysiology of this disease [3]. Microenvironment changes after SCI include increased inflammatory factors, apoptosis, oxidative stress, etc., which are the key to the difficulties of SCI recovery [4–6]. According to previous studies, it has been observed that inhibiting the microenvironment deterioration after SCI can reduce secondary injury and may improve the recovery of neurological functions [7,8]. However, there is a lack of effective drugs to treat SCI.

Quercetin is a flavonol drug, which is widely distributed in the roots and shoots of some plants [9]. It performs a variety of biological activities, such as the scavenging of oxygen free radicals to resist oxidation and inhibiting the proliferation of inflammatory cells to play an anti-inflammatory role [10]. Further studies also reported that quercetin enhances neuronal longevity and neurogenesis in neurodegenerative diseases, such as Alzheimer's disease (AD) [11], Huntington's disease (HD) [12], and Parkinson's disease (PD) [13]. It has been widely discussed that quercetin protects neurons through numerous signaling pathways, for example, it protects neurons from neurotoxicity by activating the NRF2-ARE signaling pathway [14]; Quercetin significantly reduces oxidative stress [15], reduces lipid peroxidation, promotes anti-apoptotic signal, and attenuates the cell damage through PI3K pathway [16]. In addition, it suppresses acute inflammation by inhibiting the TNF-α pathway in human umbilical vein endothelial cells [17].

Although some reports showed that quercetin is effective in the treatment of spinal cord injury [18], the key pathways and genes involved in the repair of spinal cord injury are still unclear. Based on the complex interactions and influences between drug molecules and different intracellular pathways in SCI, network pharmacology analysis was used to explore the targets and potential mechanisms of quercetin in SCI repair.

In our study, we verified the therapeutic effect of quercetin with SCI and found the key regulatory proteins PIK3R1 and EGFR may provide a new theoretical basis for quercetin repair of spinal cord injury on rats.

2. Materials and methods

2.1. Animals

Adult female Wistar rats, 8 weeks old, weighing 200 ± 20 g, were used in this study. The rats were maintained in a humidity – and temperature – controlled environment with a 12:12 light-dark cycle, and were allowed free access to food and water. The Ethics Committee of Tianjin Medical University General Hospital approved the experiments on February 21, 2022. Ethical Approval No. IRB2022-DWFL-154. A total of 144 rats were used in this study.

2.2. Reagents

Quercetin was purchased from Shanghai yuanye Bio-Technology Co., Ltd, (B20527, Shanghai, China). 2 g quercetin was dissolved in 1 L of 5 wt % CMC-NA aqueous solution (A501427, Sangon Biotech, Shanghai, China). The antibodies used in the immunofluorescence are listed as follows: anti-NeuN antibody (1:500, ab177487, Abcam), Cy3 Donkey Anti-Rabbit IgG (1:500, 711-165-152, Jackson ImmunoResearch). The antibodies used in Western blotting are listed as follows: Anti-PI3 Kinase P85 Alpha antibody (1:1000, ab191606, Abcam), Anti-EGFR antibody (1:1000, ab52894, Abcam), Anti-GAPDH antibody (1:1000, ABL1021, Abbkine), Anti-rabbit IgG, HRP-linked antibody (1:1000, 7074S, Cell Signaling Technology).

2.3. Establishment of the SCI model

For each *in vivo* experiment, rats were randomly divided into 3 groups of 6 rats. The SCI model was performed as per previously published reports [19], on Feng's Standard SCI coaxial platform [20]. Briefly, rats were anesthetized with isoflurane and the spinal cord was exposed after T10 laminectomy. The spinal cord was hit with a 10 g × 25 mm free dropped node. After the hit, the obvious hematoma on the surface of the spinal cord and the convulsions of the hind limbs and tail were considered successful contusions. Each injured rat received exquisite care, including assisting urination to avoid hematuria. The sham group was only exposed to the spinal cord without spinal cord injury, and the solvent (5 wt % CMC-Na aqueous solution) was orally administered twice a day. The SCI group was treated with solvent (5 wt % CMC-Na aqueous solution) by oral gavage twice a day after spinal cord injury, and the administration group (Qu) was treated with quercetin solution (10 mg kg^{-1}) by oral gavage twice a day after spinal cord injury. The intervention dose of quercetin was determined based on previous experimental studies, and finally, 10 mg kg^{-1} of quercetin was orally administered to rats daily after SCI.

2.4. Motor function analysis

The locomotor function of rats after SCI was evaluated by Basso Beattie Bresnahan's (BBB) score and Catwalk Analysis. The BBB test score ranged from 0 to 21 to assess hindlimb movement. Scoring was based on the accurate observation of hind limb movement during a period of 5 min in the open field. Catwalk analysis was tested on the Catwalk XT system (Noldus, Wageningen, NLD). The device has a tactile screen as a track that collects the footprints of rats, as well as the strength and residence time of footprints, etc. Also, it can record the actions of rats through a camera lens. We released the rats on track one at a time and put the feed in the goal box to induce the rat to run toward it. Then during this period, the rat's footprints were recorded to analyze their characteristics of locomotion and

behavior.

2.5. Hematoxylin and Eosin (H&E) staining

The rats were perfused with 4 °C PBS, followed by 4% paraformaldehyde. The tissue was resected and dehydrated, embedded in paraffin, and cut into 8 μm serial sections. After rehydration, the sections were stained with an H&E staining kit (Beyotime, Shanghai, China), and sealed with neutral resin.

2.6. Immunofluorescence

The protocol of the immunofluorescence refers to the previous study of the team [21]. The rats were perfused with 4 °C PBS, followed by 4% paraformaldehyde. 0.5 cm spinal cord segment was resected from the level of epicenter and postfixed in 4% paraformaldehyde solution overnight. The samples were dehydrated through increasing concentration of sucrose solution (10%, 20%, and 30%). After embedding with Tissue-Tek OCT compound (4583; SAKURA, Torrance, CA, USA), the tissue was frozen in −80 °C or in liquid nitrogen for long-time preservation. Tissues were sliced into 8 μm. Slices were incubated with the anti-NeuN antibody (1:500) overnight at 4 °C. The Cy3 Donkey Anti-Rabbit IgG was staining at room temperature for 1 h. A Leica fluorescence microscope (Leica DMi8, Germany) was used to acquire images. Acquired images were quantified by the software of Fiji (Fiji is just ImageJ, National Institutes of Health, USA).

2.7. Quercetin target genes prediction

The structural information about quercetin was obtained from the PubChem platform (https://pubchem.ncbi.nlm.nih.gov/). Quercetin targets were predicted from three Targets fishing databases viz. Swiss Target Prediction Database (www.swisstargetprediction.ch/), PharmMapper Database (www.lilab-ecust.cn/pharmmapper/), and STITCH (stitch.embl.de). In all searches, the attributes were set to "Homo sapiens", and the predicted targets had normal fit scores ≥0.4 in PharmMapper, probability score was greater than 0 in SWISS, and interaction score ≥0.4 in STITCH. The SCI-related genes were accessed from two databases: GeneCards (www.genecards.org/), and OMIM (www.omim.org). Venny2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) was used to determine the targets of quercetin on SCI.

2.8. Protein-protein interaction (PPI) network

The common targets were input into the STRING database (https://string-db.org/), the minimum required interaction score was set to 0.9, and outliers were deleted. The Cytoscape 3.9.1 software was used to import PPI data, build PPI network, and obtain hub targets. CytoHubba plugin was executed to obtain the degree of interaction between nodes. All the nodes of the PPI are presented in degree values, with the higher values being closer to the core.

2.9. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The DAVID bioinformatics resource (david.ncifcrf.gov) was used to perform GO and KEGG pathway analysis of the common targets. The GO and KEGG were ranked by Count and *P*-value <0.05. The top 10 pathways were visualized as bubble plots using the SRplot (www.bioinformatics.com.cn).

2.10. RT-qPCR

For quantitative real-time PCR (RT-qPCR), total RNA was extracted from spinal cord tissues with Trizol (Invitrogen, Thermo, USA). A FastKing cDNA First Strand Synthesis Kit (Tiangen, Beijing, China) was used to synthesize cDNA from total RNA, and then RT-qPCR was carried out using SYBR Green PCR Master Mix (Tiangen, Beijing, China). Three replicate wells were set up for each reaction.

Table 1
RT-qPCR primers.

Gene name	Forward 5′-3′	Length (bp)	Reverse 5′-3′	Length (bp)
GAPDH	GCAAGTTCAACGGCACAG	18	GCCAGTAGACTCCACGACAT	20
EP300	CCTGGTGGACATTTGGAT	18	GTAAGGGAGAACTGGGAGAC	20
CREBBP	TACCGAGAAATGGTGAGAAGAC	22	GGGAGATGTGAAGCCTGTG	19
SRC	CATCCAAGCCTCAGACCC	18	GACACCACGGCATACAGC	18
HSP90AA1	ATTGCCCAGTTAATGTCC	18	ATAGTGAGGGTTCGGTCT	18
CBL	GAGGATGATGGCTATGATGT	20	TTGAAGTTTGTGGGTTCG	18
TP53	GCGTTGCTCTGATGGTGA	18	CAGCGTGATGATGGTAAGGA	20
PIK3R1	ACAAAGCCGAGAACCTAT	18	GACTTCGCCATCTACCAC	18
EGFR	GCAGAACCCAGTCTATCAC	19	CAAACCCACTACTGAGGC	18
ESR1	ATGAAAGGCGGGATACGA	18	TGCCAGGTTGGTCAATAAGC	20

Table 1 lists the primer sequences (Sangon Biotech, Shanghai, China). Light Cycler96 (Roche, Shanghai, China) was used to perform the reaction. The data were analyzed by the $2^{-\Delta\Delta CT}$ method by researchers blinded to the experimental groups of the animals.

2.11. Western Blotting

The protocol of the Western Blotting experiment refers to the previous study of our team [21,22]. Injured spinal cord epicenters 0.5 cm in size were harvested from 6 individual rats and lysed by homogenization in RIPA Lysis Buffer (P0013B, Beyotime) (100 μL/10 mg spinal cord) with PhosSTOP (04906837001; phosphatase inhibitor) and cOmplete (04693132001; protease inhibitor) from Roche (Mannheim, Germany). The protein concentration of each sample was measured using the bicinchoninic acid assay (BCA) (BL52A, Biosharp, China). After SDS-PAGE and PVDF membrane transfer, antibodies were used to probe different proteins. Image analysis was performed by Fiji (Fiji is just ImageJ, National Institutes of Health, USA).

2.12. Molecular docking technology

The chemical structures were obtained from the PubChem platform (https://pubchem.ncbi.nlm.nih.gov/). The three-dimensional

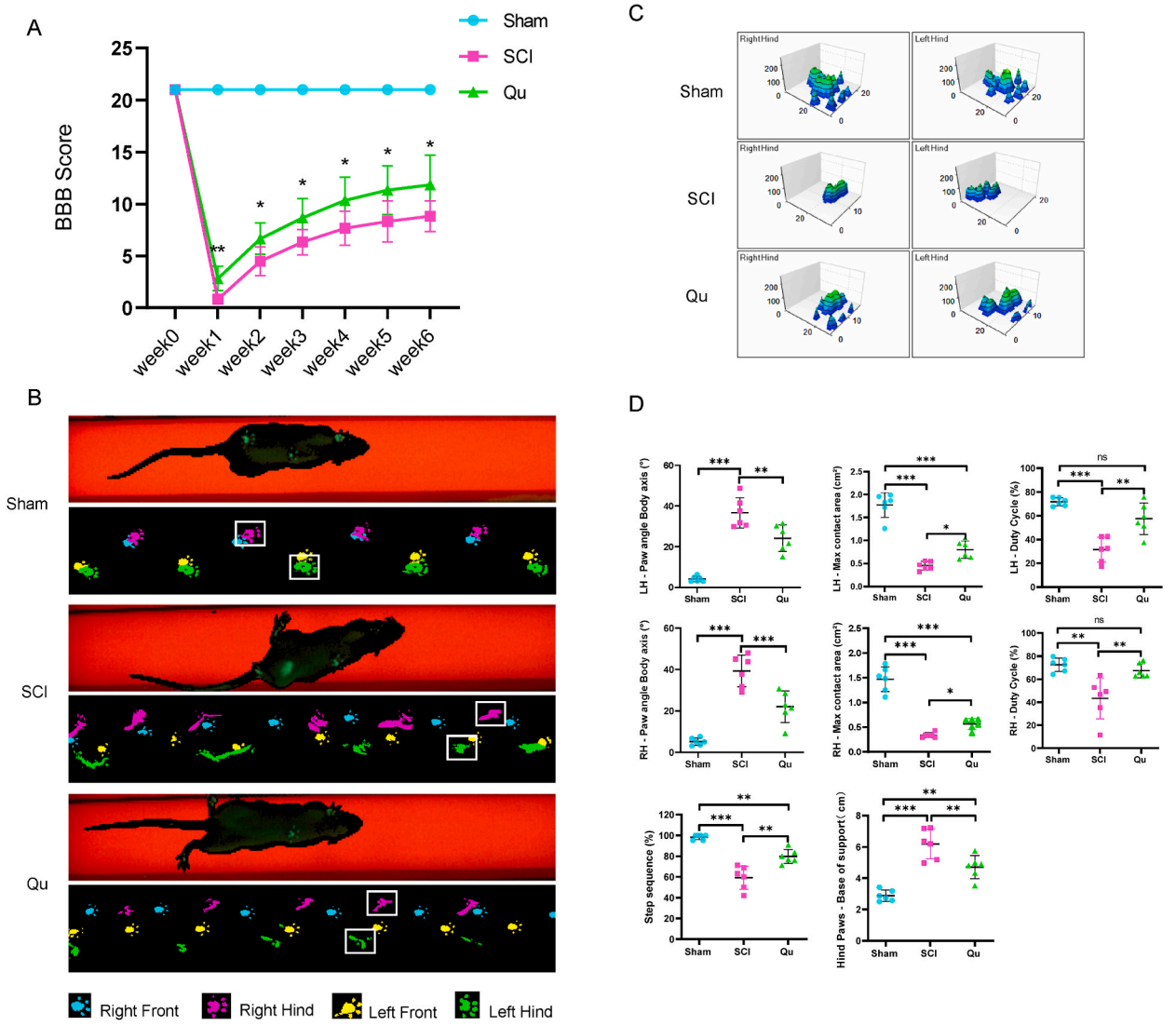

Fig. 1. Quercetin promotes the repair of SCI in rats. (A) BBB score to evaluate the locomotor ability. (B–D) Catwalk gait analysis to determine the locomotor recovery after quercetin treatment. (B) Representative images of footprints and body position after quercetin treatment. (C) 3D footprint intensity of hind limbs of rats in each group. (D) Step sequence, base of support, duty cycle, paw angle body axis, and maximum contact area of hind limbs of rats in each group. One-way ANOVA and Tukey's multiple comparisons test were used to analyze differences among groups. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n = 6 in each group.

(3D) structures of the proteins were downloaded from the Protein Data Bank (PDB) databases (www.rcsb.org). Target proteins were dehydrated and dephosphorylated by PyMOL software. AutodockTools 1.5.7 was used to dock the target protein with the ligand quercetin molecule, binding energy analysis was performed, and the output was PDBQT format. Finally, the results of molecular docking were visualized by PyMOl software.

2.13. Statistical analysis

All the data were presented as mean ± standard error of the mean (SEM). Multiple different groups were analyzed with a one-way analysis of variance (ANOVA), and then the Bonferroni post hoc test was performed for equal variances data. The Mann-Whitney *U* test was used instead when the variance was not equal. A P *value* < 0.05 was considered statistically significant.

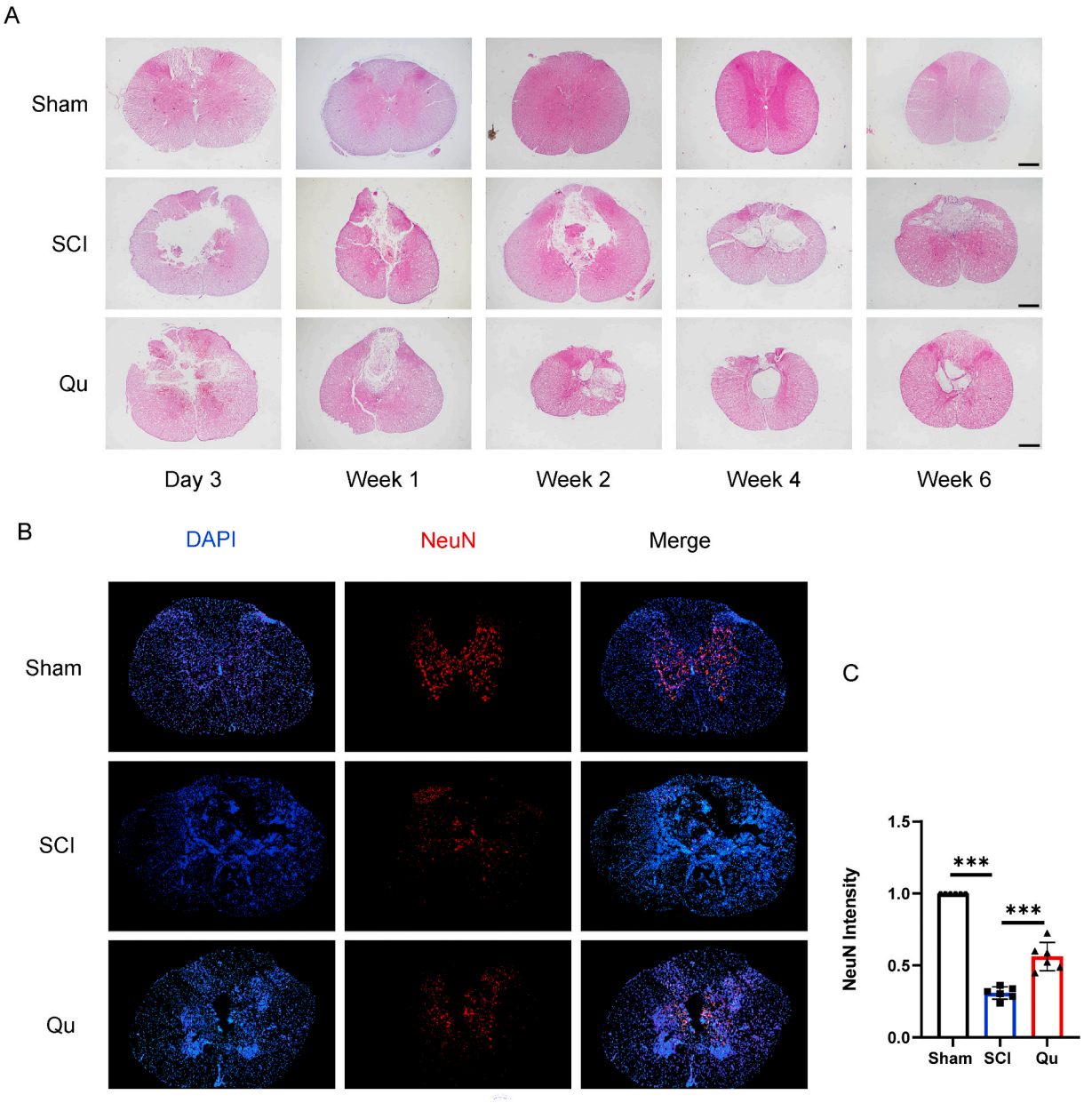

Fig. 2. Quercetin promotes tissue recovery after SCI. (A) Representative images showing H&E staining after SCI. Scale bar = 0.2 mm. (B) Representative images showing immunofluorescence staining (2 weeks after SCI). Scale bar: 0.2 mm. (C) Quantitative analysis of relative fluorescence intensity. One-way ANOVA and Tukey's multiple comparisons test were used to analyze differences among groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n = 6 in each group.

3. Results

3.1. Quercetin promotes the functional recovery of SCI rats

To observe the effect of quercetin treatment on functional recovery, we performed BBB scores from day 1–6 weeks after SCI to evaluate the recovery of locomotor functions. The difference between the SCI and Qu groups was statistically significant from week 1, indicating that quercetin could improve functional recovery (Fig. 1A). Furthermore, we used the Catwalk XT system to verify the recovery of motor function in rats as well. The Catwalk experiment was applied 6 weeks post-SCI. As shown in Fig. 1B, the sham group is the body morphology and ordered footprint of normal rats. After SCI, the body morphology of the rats showed hind limb weakness and disordered gait. After quercetin treatment, a certain support effect of the hind limbs could be observed in the body morphology of the rats, and the gait sequence was restored to a certain extent, but it still did not return to the normal condition. Fig. 1C shows the pressure analysis of the footprints of the selected boxes in Fig. 1B, and the 3D images also demonstrate the increased pressure of the hind limbs after treatment with quercetin, illustrating the enhanced support of the hind limbs. Further analysis of the footprints (Fig. 1D) showed that the quercetin-treated group had a smaller body-paw angle (Paw Angle Body Axis), increased maximum hind limb contact area (max contact area), increased proportion of time spent on the same paw to the ground (duty cycle), greater step sequence order (step sequence) and greater distance between feet (base of support). The above results indicated that quercetin treatment improved the locomotor ability of SCI rats.

3.2. Quercetin reduces tissue damage and promotes neuronal survival after SCI

In order to explore the effect of quercetin on SCI repair at the histological level, we performed H&E staining to observe the tissue damage in sections from the middle of lesions (Fig. 2A). The cells in the spinal cord tissue were stained pink, and the nuclei were stained blue-purple. The staining objectively showed that within 1 week after the injury, there was no significant difference between

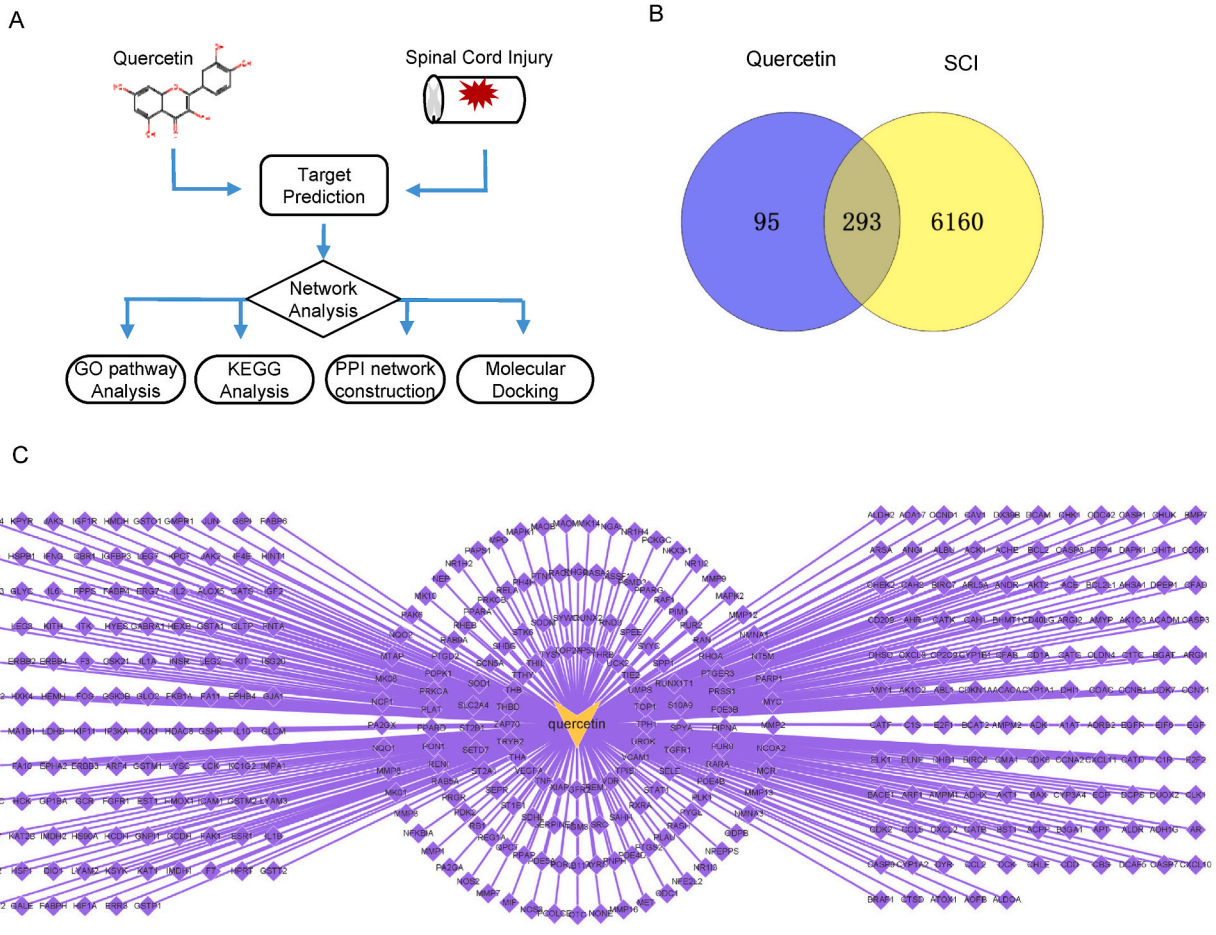

Fig. 3. Network pharmacology workflow and quercetin anti-SCI target prediction. (A) Network pharmacology workflow. (B) Diagram of quercetin targets and SCI-related genes. (C) Schematic representation of the network of common targets of quercetin and SCI.

the cavity area of the SCI group and the Qu group, but from the second week, the tissue damage of the Qu group was significantly alleviated than that of the SCI group. In addition, we examined the neuroprotective effect of quercetin through immunofluorescence. We labeled all nuclei with DAPI (blue) and neuronal nuclei with anti-NeuN antibody (red). We chose the time point when the difference in cavity area first appeared in H&E staining, which is the second week. The results showed that compared with the SCI group, the number and fluorescence intensity of neurons in the Qu group were significantly increased (Fig. 2B–C).

3.3. Identification of the potential targets of quercetin

To investigate the mechanism of quercetin treating SCI, we predicted the targets of quercetin in SCI by network pharmacology, and its analysis flow is shown in Fig. 3A. Firstly, the target genes of quercetin were searched in Swiss, Stitch, and PharmMapper databases, we obtained 388 potential targets of quercetin in total. And then 6453 SCI-related genes were obtained from the GeneCards and OMIM. We identified 293 target genes between SCI and quercetin, which could be considered as potential targets of quercetin to treat SCI (Fig. 3B). We then established visualized drug-target networks for quercetin and SCI target genes (n = 293) via Cytoscape 3.9.1 software (Fig. 3C).

3.4. GO and KEGG pathway enrichment analysis

For a better understanding of the biological effects and potential mechanisms of quercetin treating SCI, DAVID was used to perform GO and KEGG pathway enrichment analysis of 293 targets. According to the protein count involved in the pathway, the biological processes (BP) is mainly involved in signal transduction, negative regulation of the apoptotic process, RNA polymerase II promoter transcription, protein phosphorylation, proteolysis, response to xenobiotic stimulus, positive regulation of cell proliferation, positive regulation of transcription, DNA-templated (Fig. 4A, left). Similarly, the cellular components (CC) included cytosol, cytoplasm, plasma membrane, nucleus, extracellular exosome, extracellular region and space, membrane, and mitochondrion (Fig. 4A, middle). Protein, protein kinase, DNA, ATP, enzyme and zinc ion binding, protein homodimerization, and kinase activities, were among molecular functions (Fig. 4A, right). Also, 171 KEGG enrichment entries were enriched in the cell growth, differentiation, and apoptosis, such as metabolic, the PI3K-Akt, the MAPK, the Ras signaling pathways, and so on (Fig. 4B). Finally, the top 10 KEGG enrichment samples were screened by bubble graph, and genes related to these top 10 enriched pathways are shown in Tables S1 and S2, respectively.

3.5. The PPI network analysis

To investigate the relationship of potential target genes, protein-protein interactions were constructed. In Fig. 5, the 293 target genes had complex interactions with each other, which were represented as 4 concentric circles. Among these genes, *EP300, CREBBP, SRC, HSP90AA1, TP53, PIK3R1, EGFR, ESR1*, and *CBL* were considered to be the core genes in potential targets of quercetin against SCI

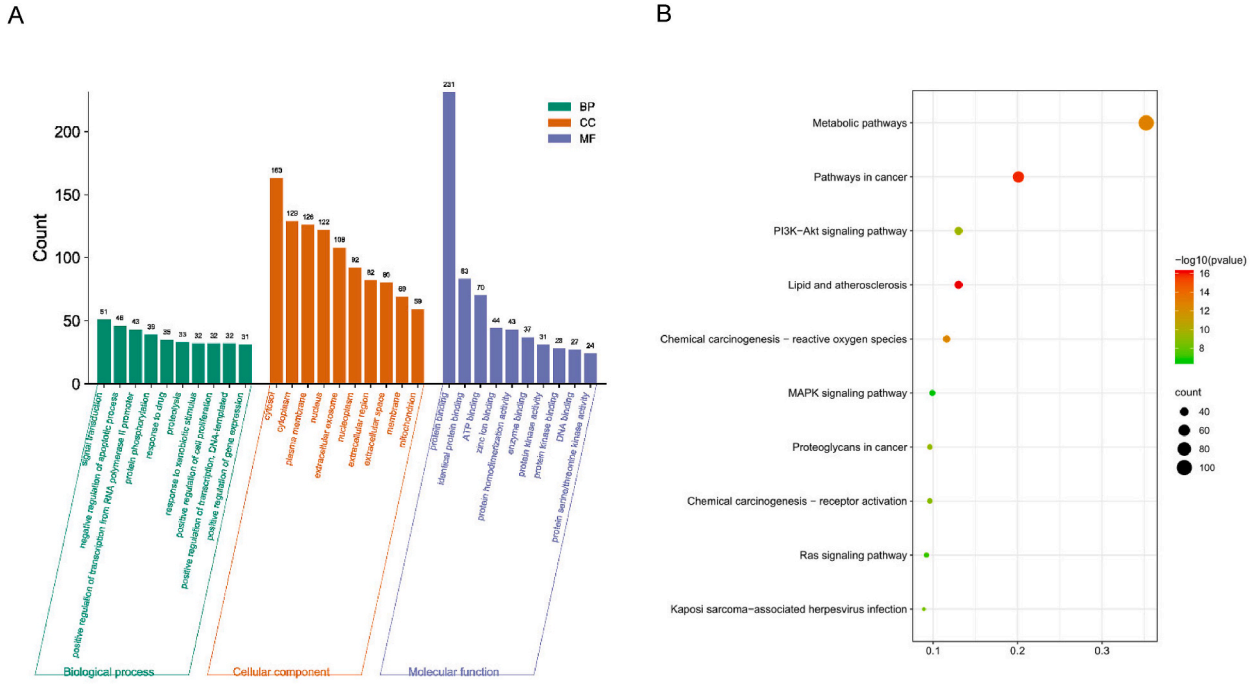

Fig. 4. GO and KEGG analysis. (A) GO analyses of target genes. (B) The top 10 enriched KEGG pathways of target genes.

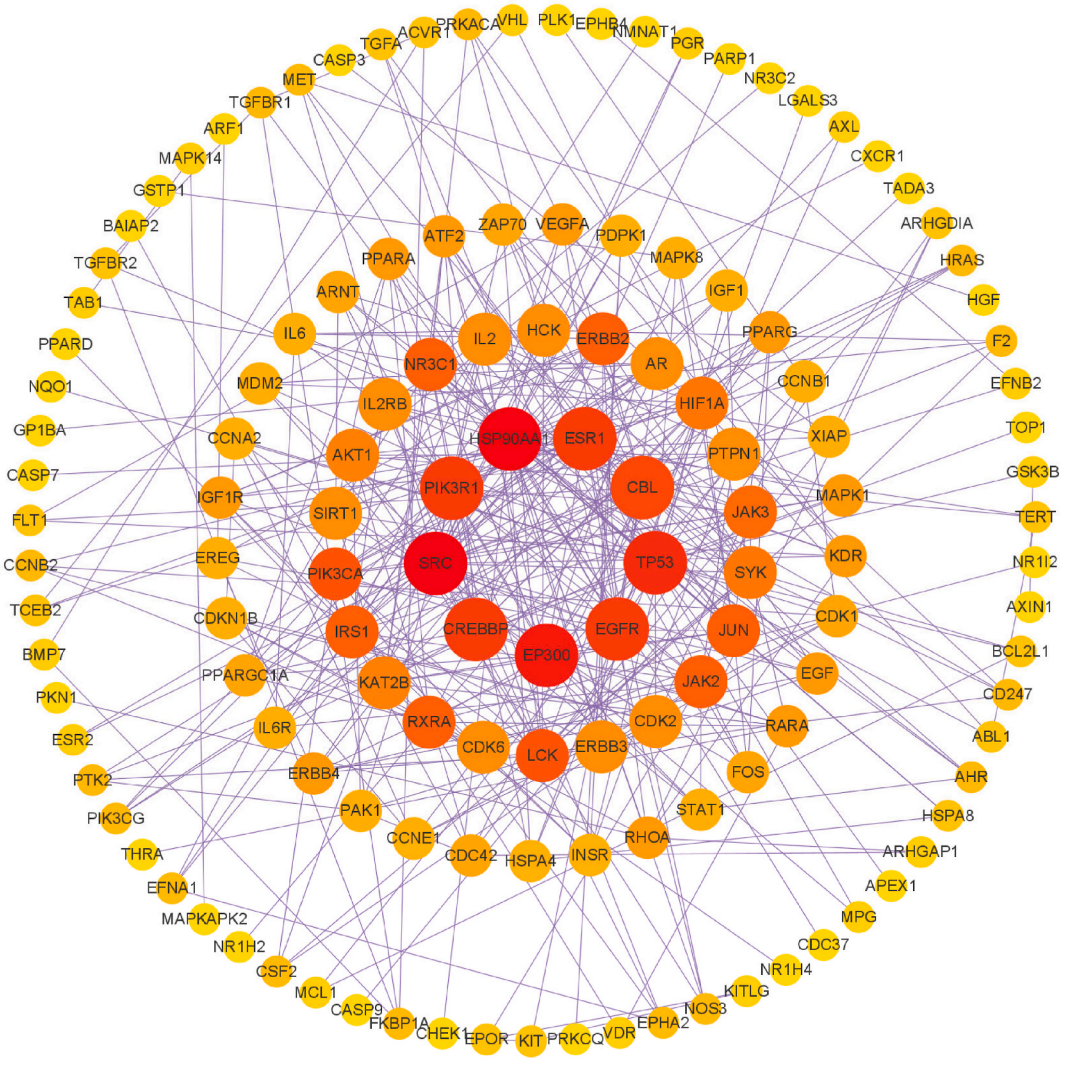

Fig. 5. PPI network of quercetin and SCI co-targets.

(Fig. 5).

3.6. Molecular docking of quercetin and core proteins

The molecular docking of the proteins expressed by the 9 core genes with quercetin was done to evaluate the binding ability of compounds to target the molecules at the molecular level. The results showed a high combining ability between quercetin and the selected proteins: EP300, CREBBP, SRC, HSP90AA1, TP53, PIK3R1, EGFR, ESR1, and CBL (Fig. 6A–I). The binding energies of quercetin and target proteins are presented in Fig. 6J, the binding energy lower than -5 kcal mol^{-1} indicates quercetin is easy to bind to the target protein.

3.7. Quercetin regulates PI3K and EGFR/MAPK pathways in SCI repair

To verify the quercetin target genes in SCI screened by network pharmacology's prediction, RT-qPCR was used to detect transcriptional changes of EP300, CREBBP, SRC, HSP90AA1, TP53, PIK3R1, EGFR, ESR1, and CBL in the spinal cord tissue after SCI (Fig. 7A-I). Statistical analysis showed that compared with the sham group, the transcriptional expression of PIK3R1, EGFR, and ESR1 increased after injury in the SCI group, and significantly decreased after quercetin treatment in the Qu group. ESR1 was involved in the cancer pathways, which does not seem to be related to nerve repair. PIK3R1 is involved in the PI3K/AKT1/mTOR pathway, which participates in the apoptosis. EGFR is involved in the EGFR/MAPK signaling pathway, which can reduce inflammatory response. Therefore, protein expression levels of PIK3R1 and EGFR were detected by Western blotting. The results showed that the expression of

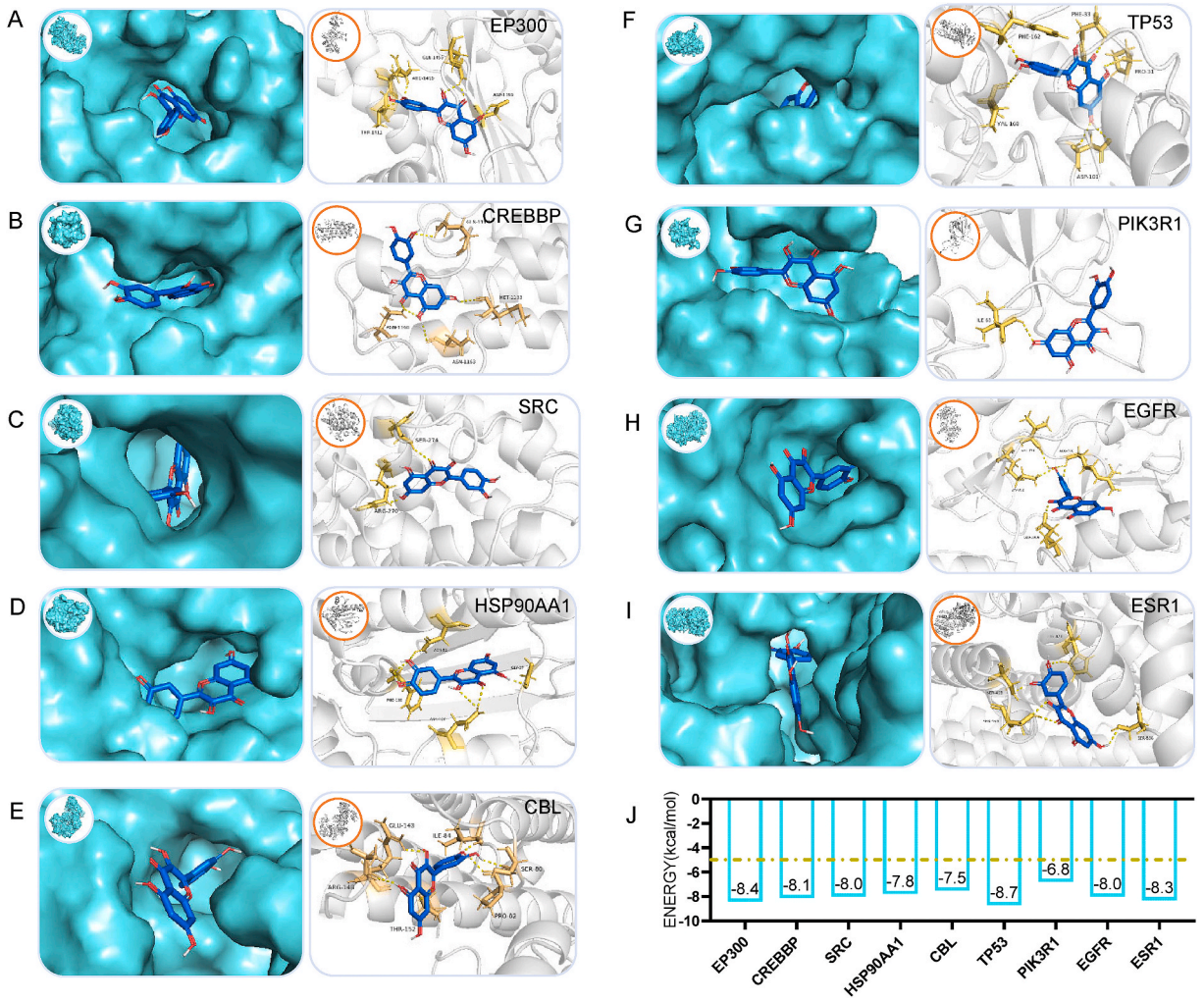

Fig. 6. Molecular docking results of quercetin with key targets. (A) EP300; (B) CREBBP; (C) SRC; (D) HSP90AA1; (E) TP53; (F) PIK3R1; (G) EGFR; (H) ESR1; (I) CBL; (J) Binding energy of quercetin to each target.

PIK3R1 and EGFR at the early injury site 2 days after injury was significantly higher in the SCI group than in the sham group. However, their expression levels recovered significantly in the Qu group (Fig. 8A–B). The results confirmed that quercetin can indeed regulate the key genes and proteins of these pathways, indicating that quercetin can intervene in the repair of SCI by participating in PI3K and EGFR/MAPK pathways.

4. Discussion

SCI often causes motor and sensory dysfunction [23]. Due to the poor plasticity of the central nervous system and the limited neuronal regeneration ability, SCI treatment has become a key medical problem globally [24], and still, there is no effective treatment available in this regard despite its increasing global incidence rate. In this study, we verified the therapeutic effects of quercetin on SCI, and explored the targets and pathways of quercetin in treating the SCI, and the underlying mechanism for the repair effect was further verified by *in vivo* experiments.

We observed that PIK3R1, EGFR, HSP90AA1, and TP53 were jointly involved in the PI3K-AKT signaling pathway, which involves controlling the cell viability, inhibits apoptosis, and promotes cell cycle progression. Previous studies suggest that the PI3K/AKT1/mTOR pathway participates in the apoptosis of spinal cord neurons and glial scar formation after SCI, and inhibiting this pathway can alleviate these negative effects [25,26]. Similarly, EGFR activation is involved in the destruction of the blood-spinal cord barrier (BSCB) and secondary injury after SCI. Studies have confirmed that inhibiting the EGFR/MAPK signaling pathway can reduce inflammatory response [27] and inhibiting the EGFR/NF-κB pathway can reduce the damage to BSCB after SCI [28]. These two signal pathways are of particular interest to us, and the PPI network suggests a correlation between these two genes, so we have verified them not only at the transcriptional level but also at the protein level. The results confirmed that quercetin can regulate the key genes and

proteins of these pathways, indicating that quercetin can intervene in the repair of SCI by participating in PI3K and EGFR pathways.

Results from KEGG analysis revealed that ESR1 was involved in the cancer pathways. Because previous studies have reported that ESR1 mutations can lead to breast cancer [29,30]. At present, the relationship between ESR1 and SCI is rarely reported, suggesting that ESR1 may be a new target for SCI repair.

Also *in vivo* experiments with RT-qPCR, we found that quercetin did not change the expression levels of some genes (Fig. 7E-I). We considered that the way they participate in SCI may be that the protein they express directly combines with quercetin, thereby acting as an inhibitor or agonist. To verify this, we performed molecular docking. The binding energies of quercetin to EP300, CREBBP, SRC, HSP90AA1, CBL, TP53, PIK3R1, EGFR, and ESR1 were all lower than -5 kcal mol^{-1}, indicating quercetin has the ability to stable bind with proteins expressed by these genes. Also, we confirmed that quercetin can reduce tissue damage, promote survival of neurons and promote motor functional recovery in rats with SCI, combined with the molecular experiment above, suggesting that quercetin may have the ability of treating SCI through the pathways above. In this study, the potential mechanisms of quercetin in treating SCI were studied with the aspects of target and signal pathway prediction, gene-protein expression, protein binding, histological and behavioral analysis, but further study of this pathway is still needed. This paper still has some limitations. First of all, it is not clear how quercetin functions when directly interacted with EP300, CREBBP, SRC, etc. Secondly, how quercetin protects neurons through PI3K and EGFR/MAPK remains to be further studied.

Taken together, we investigated the repair effect of quercetin on SCI by histological examination and motor behavioral analysis experiments. The potential pathways and key genes of quercetin in the treatment of spinal cord injury were predicted by network pharmacological analysis and molecular docking techniques. It was found that the key genes *PIK3R1* and *EGFR* may play a major role in quercetin therapy of SCI through PI3K-AKT and EGFR/MAPK pathways. Overall, our study brings a new and comprehensive

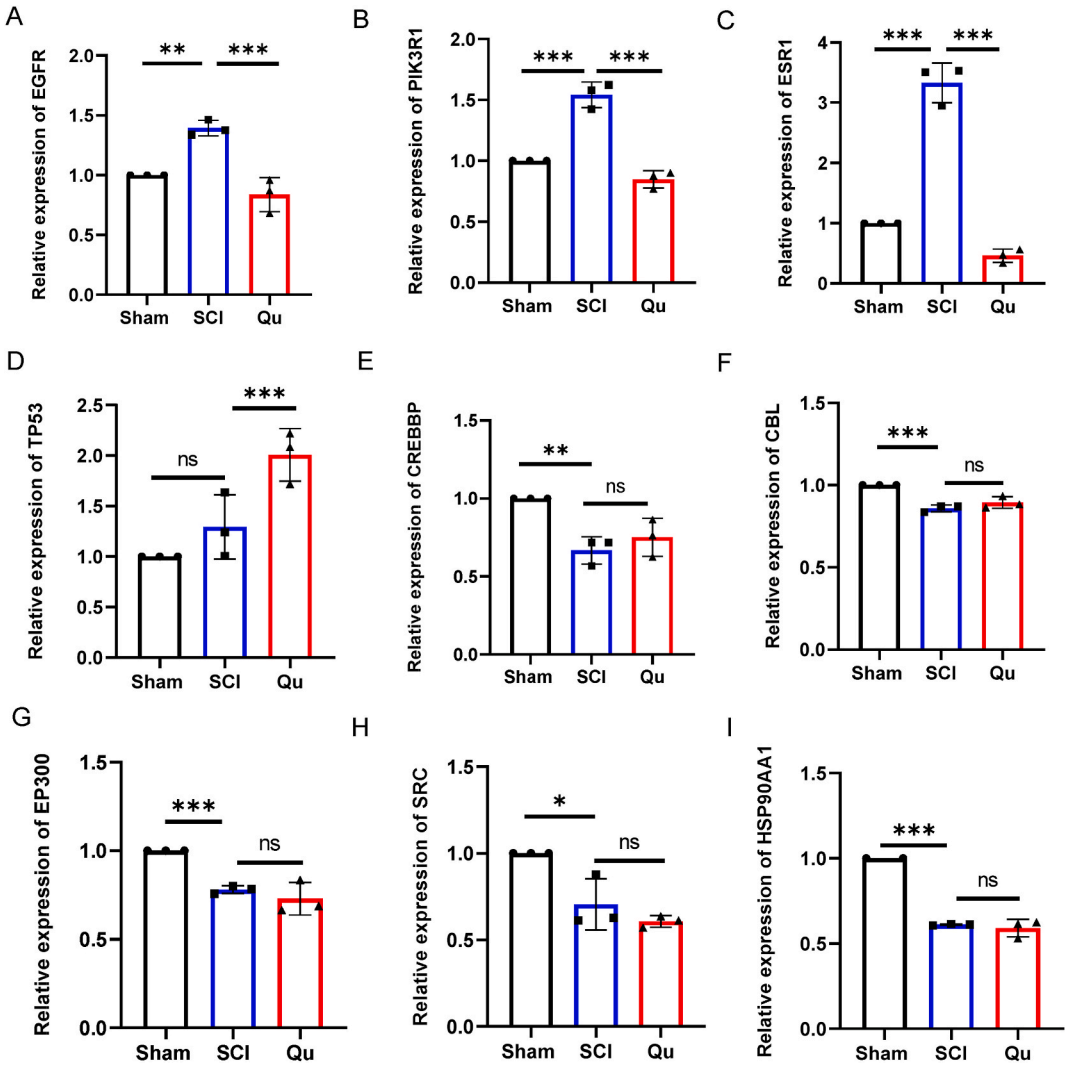

Fig. 7. The mRNA expression levels of target genes. (A–I) One-way ANOVA and Tukey's multiple comparisons test were used to analyze differences among groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, each experiment was repeated three times.

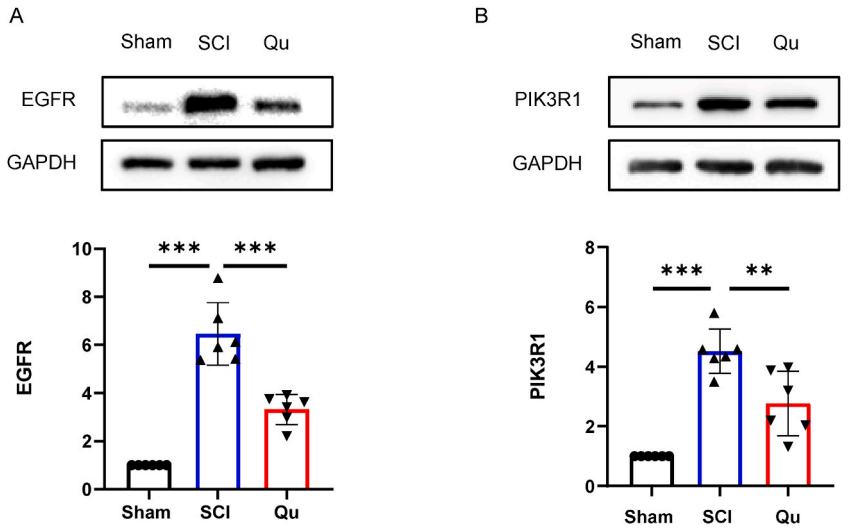

Fig. 8. Protein expression levels of EGFR and PIK3R1 (A) Immunoblot and quantitative analysis plots of EGFR (3 days after SCI). (B) Immunoblot and quantitative analysis plots of PIK3R1. In quantitative analysis, the expression level of each protein was normalized by GAPDH. One-way ANOVA and Tukey's multiple comparisons test were used to analyze differences among groups. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n = 6 in each group. The uncropped images are in Supplementary Figure 1.

approach to natural quercetin compounds treating SCI.

Author contribution statement

Wenyuan Shen: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Quan Liu; Chuanhao Li: Performed the experiments; Wrote the paper.
Chuanhao Li; Muhetidier Abula; Zibo Yang; Zhishuo Wang: Analyzed and interpreted the data.
Jun Cai: Conceived and designed the experiments; Wrote the paper.
Xiaohong Kong: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20024.

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