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To Explore the Core Mechanism of Spinal Cord Injury and the Intervention Effect of Electroacupuncture based on GEO Database

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Abstract

The most serious complication of spinal injury is spinal cord injury, which frequently results in severe limb dysfunction below the injured segment. The GEOquery package was used to download it from the GEO database, and one probe had the probes for multiple molecules removed from it.Only the probe with the highest signal value was retained when multiple probes for the same molecule were found. The LimMA package was used to conduct the difference analysis between the two groups using the PCA and UMAP diagrams, and the sample standardization was checked using the box diagram. Finally, the STRING database is used for both the core process and the signal pathway screening. The effect of electroacupuncture as an intervention on spinal cord injury was then investigated. The primary genes for spinal cord injury are TLR4 and FN1. At various points in time, the malaria signaling pathway is always involved in the mechanism of spinal cord injury. After spinal cord injury, Ea can boost the inflammatory and oxidative stress responses in mice, helping to repair the structure and function of the spinal cord.It is connected to the inhibition of NF-B activation and the enhancement of the Nrf2/HO-1 antioxidant pathway. In mice with acute spinal cord injury, electroacupuncture can effectively promote motor function repair, and its mechanism may be related to the downregulation of HMGB1, TLR4, and Iba1 expression levels.

Keywords

Whole Genome; Spinal Cord Injury; Electric Acupuncture (EA).

1. Introduction

The most serious complication of spinal injury is spinal cord injury, which frequently results in severe limb dysfunction below the injured segment. Not only will spinal cord injuries cause severe physical and mental harm to the patients themselves, but they will also have a significant financial impact on society as a whole. The prevention, treatment, and rehabilitation of spinal cord injuries have emerged as a major medical topic due to the social and economic costs associated with them. By inserting an acupuncture needle into an acupoint to obtain qi and passing a trace current on the needle that is close to human bioelectricity, electric acupuncture, also known as Electmacupuncture, can be used to prevent and treat diseases. Electroacupuncture can, among other things, alter the physiological functions of the human body, act as an analgesic and sedative, increase blood flow, alleviate muscle tension, and so on. Electroacupuncture has a wide treatment range because its range is almost identical to that of filiform needle acupuncture. It is regularly utilized in different agony condition, arTHRALgia disorder, brokenness of heart, stomach, digestive tract, bladder, uterus and different organs, as well as franticness and muscle, tendon, joint injury illnesses, and so forth., and can be used to anesthetize acupuncture. After EA stimulation, rats with spinal cord injuries underwent changes in glial fibrillary acidic protein expression, as Tang Fuyu [1] observed.I n order to

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prevent the formation of a glial scar, ea stimulation can reduce the expression of astrocytes and glial fibrillary acidic protein in the injured area.In addition, it was discovered that electroacupuncture stimulation of the gastric meridian of the foot Yangming can promote the regeneration and repair of nerve cells, promote motor function recovery, and reduce the expression of TNF- and MIP-2 in rats with spinal cord injuries [2].To investigate the core mechanism of spinal cord injury and the intervention effect of EA in rats, this study primarily relied on the GEO database.

2. Methods

1) Software:R (Statistical Analysis and Visualization, version 3.6.3).

2) R package:GEOquery's package 2.54.1 is used to download data; Limma's package 3.42.2 is used for difference analysis; UMAP's package 0.2.7.0 is used for UMAP analysis; GGploT2's package 3.3.3 is used for visualization; and ComplexHeatmap's package 2.2.0 is used to analyze heat maps in their visualization [3-5].

3) methods: The GEOquery package was used to download it from the GEO database, and one probe had the probes for multiple molecules removed. Only the probe with the highest signal value was retained when multiple probes for the same molecule were found. The LimMA package was used to conduct the difference analysis between the two groups using the PCA and UMAP diagrams, and the sample standardization was checked using the box diagram. Finally, the STRING database is used for both the core process and the signal pathway screening.

3. The Results

3.1. Difference Analysis

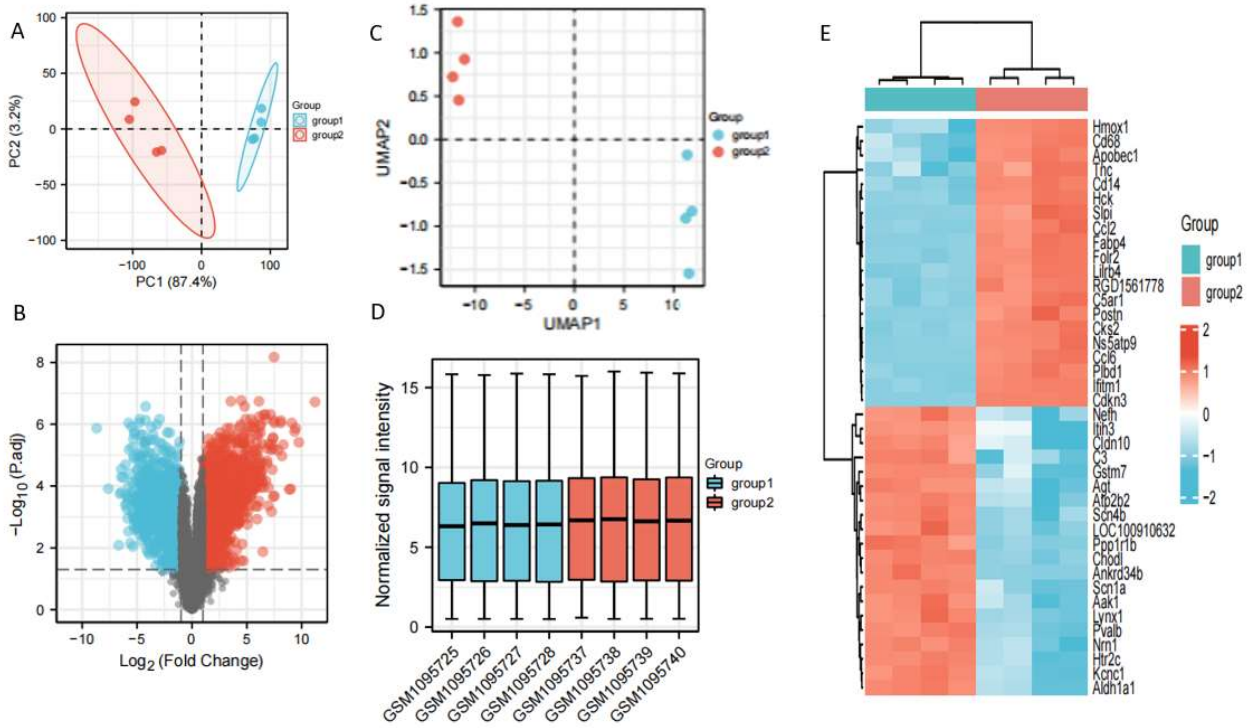

Figure 1. Primary outcome of spinal cord injury at 1 day

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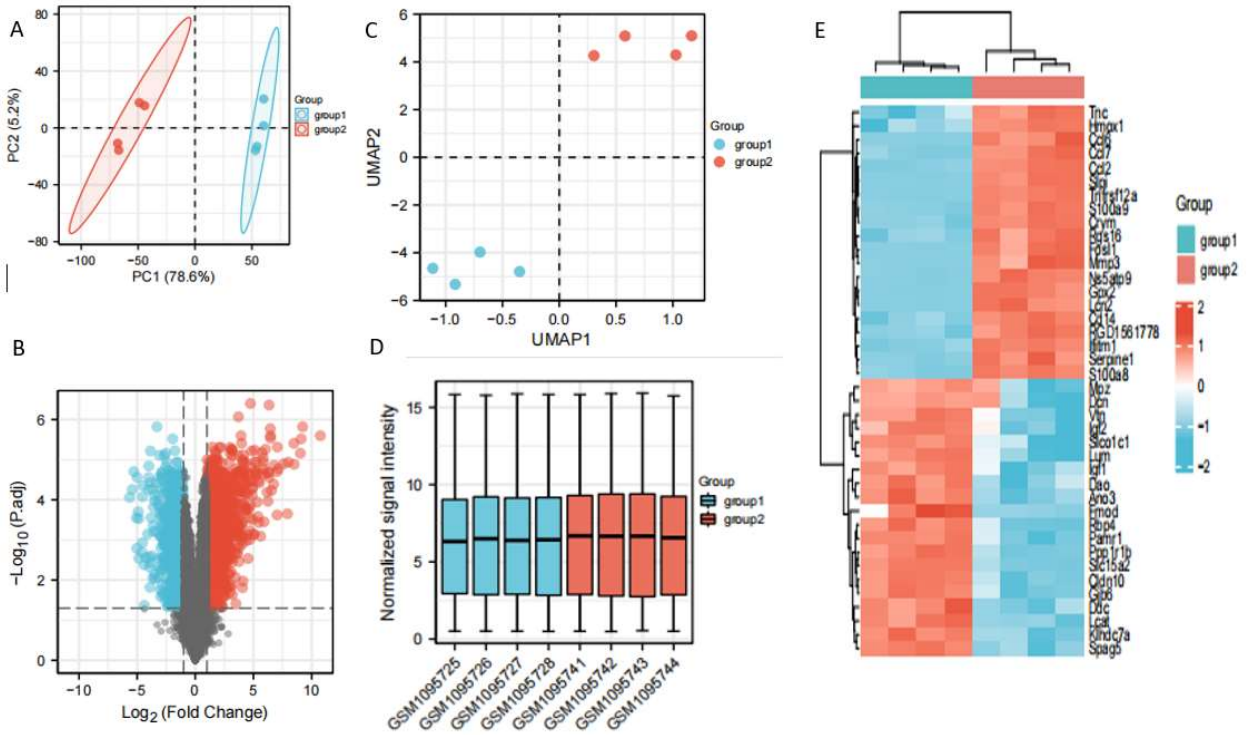

Figure 2. Primary outcome of spinal cord injury at 3 days

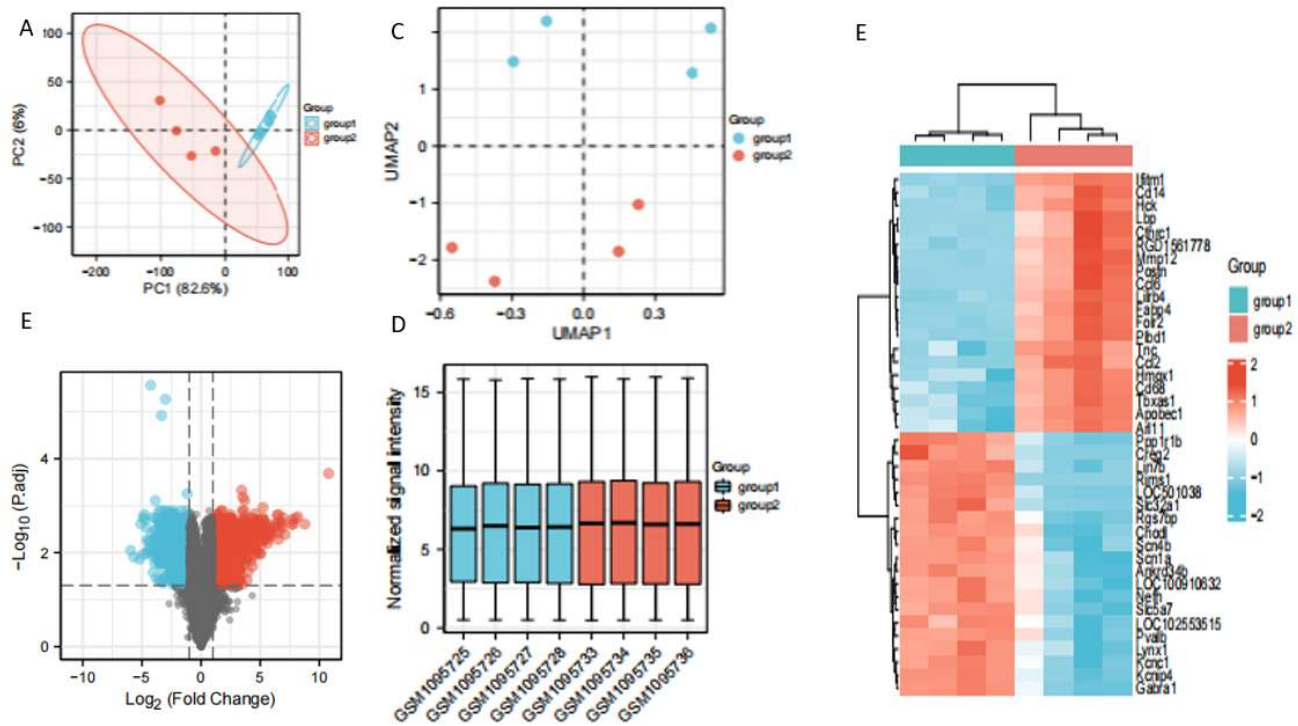

Figure 3. Primary results of spinal cord injury at 1 week

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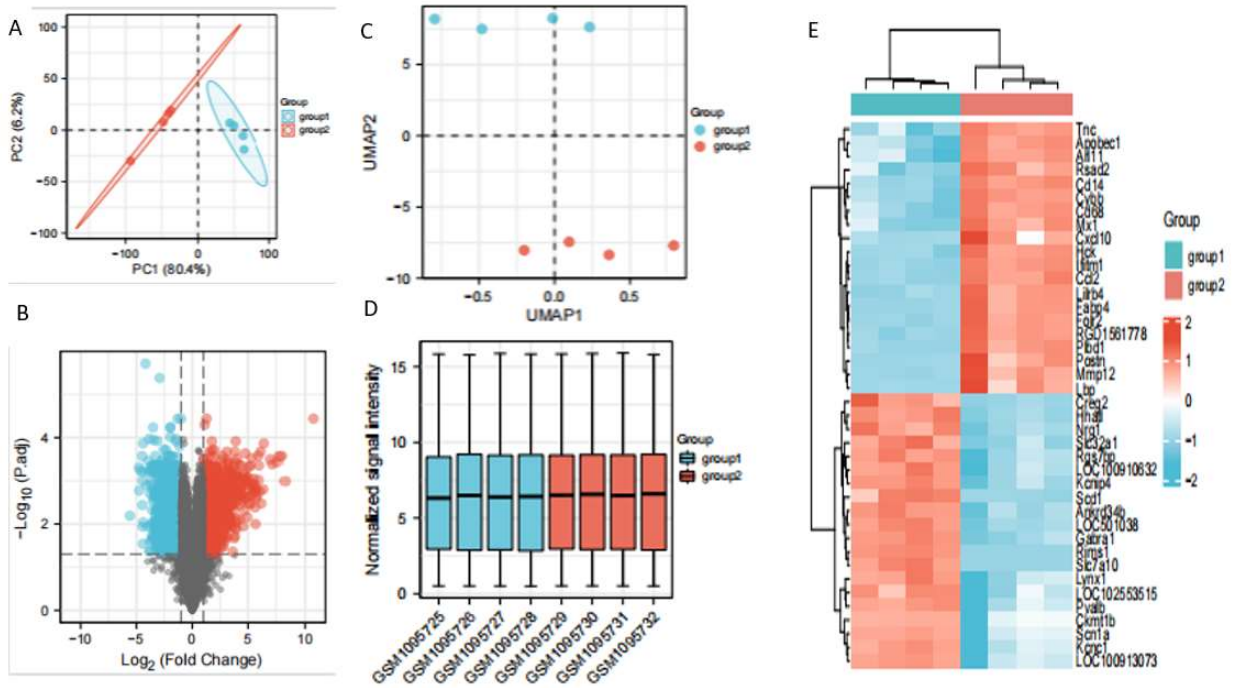

Figure 4. Main results of spinal cord injury at 2 weeks

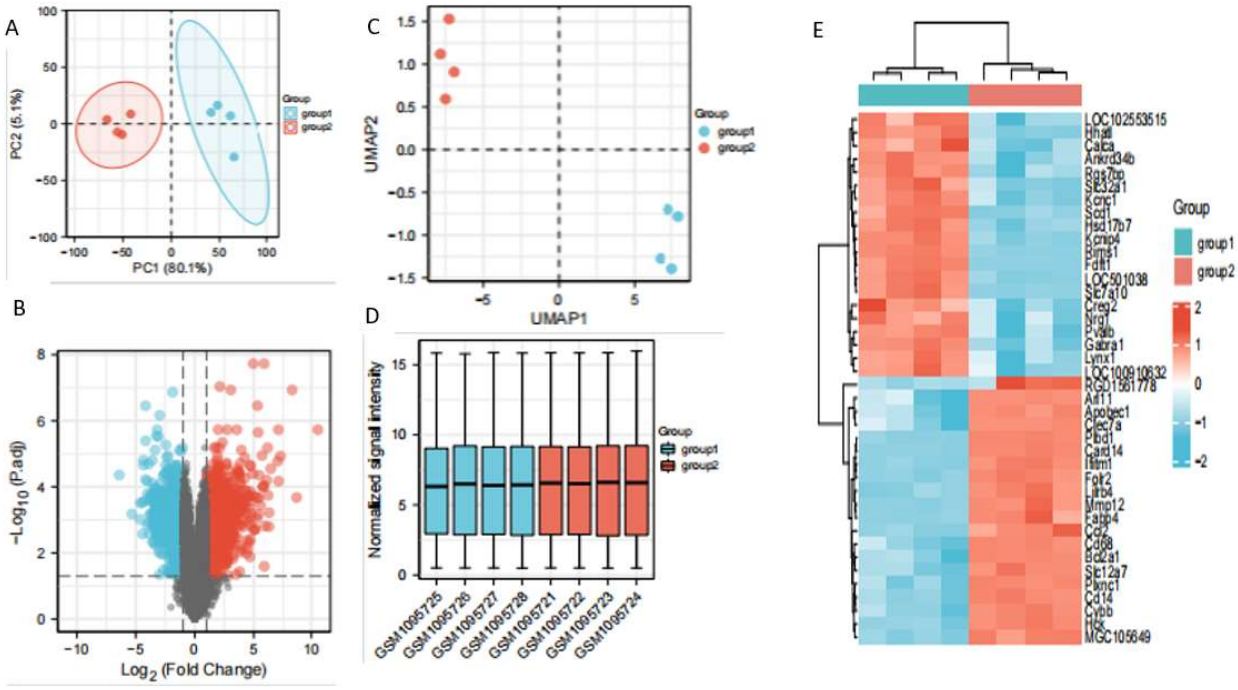

Figure 5. Primary outcome of spinal cord injury at 8 weeks

In this analysis, all selected data sets, corresponding samples and grouping are summarized as group 1 quantity: 4, group 2 quantity: 4. When the median of each sample is basically at a level, it indicates that the normalization degree between samples is good (Figure 1-5D). When the samples of each group are separated and the proportion of PC1 and PC2 is high, it indicates that there is a significant difference between groups, and there may be more meaningful results in subsequent difference analysis (Figure 1-5A). When the samples of each group are separated, it indicates that the differences between groups are obvious, and the subsequent difference

ISSN: 1813-4890

analysis may have more meaningful results (Figure 1-5C). After spinal cord injury time is 1 day difference analysis filtering molecules for a total of 14378, among them, meet | log2 (FC) | > 1 & p.a DJ < 0.05 threshold value ID 3716, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 2178, The number of high expression (negative logFC) in the reference group (group 1) was 1538; Meet | log2 (FC) | > 1.5 & p.a DJ < 0.05 threshold value ID 2388, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 1388, in the reference group (group 1) high expression (logFC is negative), the number of 1000; Meet | log2 (FC) | > 2 & p.a DJ < 0.05 threshold value ID 1592, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 923, in the reference group (group 1) high expression (the number of logFC is negative). There are 669 (FIG. 1 b). The expression of top20 genes with high expression and low expression in the visual expression profile is shown in figure 1E. After spinal cord injury time is 3 days gap analysis filtering molecules for a total of 14378, among them, meet | log2 (FC) | > 1 & p.a DJ < 0.05 threshold value ID 2414, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 1469, The number of high expression (negative logFC) in the reference group (group 1) was 945; Meet | log2 (FC) | > 1.5 & p.a DJ < 0.05 threshold value ID 1217, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 768, in the reference group (group 1) high expression (logFC is negative), the number of 449; Meet | log2 (FC) | > 2 & p.a DJ < 0.05 threshold value ID 661, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 460, in the reference group (group 1) high expression (the number of logFC is negative). There are 201 (FIG. 2 b). The expression of top20 genes with high and low expression in the visual expression profile was shown in FIG. 2E. After spinal cord injury time for 1 week difference analysis filtering molecules for a total of 14378, among them, meet | log2 (FC) | > 1 & p.a DJ < 0.05 threshold value ID 2375, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 1346, The number of high expression (negative logFC) in the reference group (group 1) was 1029; Meet | log2 (FC) | > 1.5 & p.a DJ < 0.05 threshold value ID 1444, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 827, in the reference group (group 1) high expression (logFC is negative), the number of 617; Meet | log2 (FC) | > 2 & p.a DJ < 0.05 threshold value ID 944, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 568, in the reference group (group 1) high expression (logFC is negative), the number of 376. (Figure 3B). The expression of top20 genes with high expression and low expression in the visual expression profile is shown in figure 3E. Spinal cord injury time is 2 weeks after the variance analysis filtering molecules for a total of 14378, among them, meet | log2 (FC) | > 1 & p.a DJ < 0.05 threshold value ID 1972, under the threshold, in the experimental group (group 2) high expression (logFC is) there are 1014 in number, The number of high expression (negative logFC) in reference group (group 1) was 958; Meet | log2 (FC) | > 1.5 & p.a DJ < 0.05 threshold value ID 1204, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 659, in the reference group (group 1) high expression (logFC is negative), the number of 545; Meet | log2 (FC) | > 2 & p.a DJ < 0.05 threshold value ID 749, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 432, in the reference group (group 1) high expression (the number of logFC is negative). There are 317 (FIG. 4 b). The expression of top20 genes with high expression and low expression in the visual expression profile is shown in figure 4E. After spinal cord injury time for eight weeks difference analysis filtering molecules for a total of 14378, among them, meet | log2 (FC) | > 1 & p.a DJ < 0.05 threshold value ID 2044, under the threshold, in the experimental group (group 2) high expression (logFC is) there are 1074 in number, The number of high expression (negative logFC) in the reference group (group 1) was 970; Meet | log2 (FC) | > 1.5 & p.a DJ < 0.05 threshold value ID 1216, under the threshold, in the

ISSN: 1813-4890

experimental group (group 2) high expression (logFC is) as the number of 677, in the reference group (group 1) high expression (logFC is negative), the number of 539;Meet | log2 (FC) | > 2 & p.a DJ < 0.05 threshold value ID 741, under the threshold, in the experimental group (group 2) high expression (logFC is) as the number of 439, in the reference group (group 1) high expression (the number of logFC is negative). There are 302 (FIG. 5 b). The expression of top20 genes with high expression and low expression in the visual expression profile is shown in figure 5E.

3.2. Screening of HUB Genes

FN1, PTPRC, MYC, SNAP25, TLR4 were the core genes of spinal cord injury at 1 day. The core genes of spinal cord injury for 3 days were IL6, FN1, MYC, ITGAM, CCL2; The core genes of spinal cord injury for 1 week were PTPRC, FN1, CD4, TLR4, ITGAM. The core genes of spinal cord injury time of 2 weeks were PTPRC, FN1, TLR4, ITGAM, SNAP25. The core genes of spinal cord injury at 8 weeks were CD4, PTPRC, TLR4, TYROBP and CCL2. Finally, TLR4 and FN1 are the core genes of spinal cord injury.

3.3. KEGG Analysis

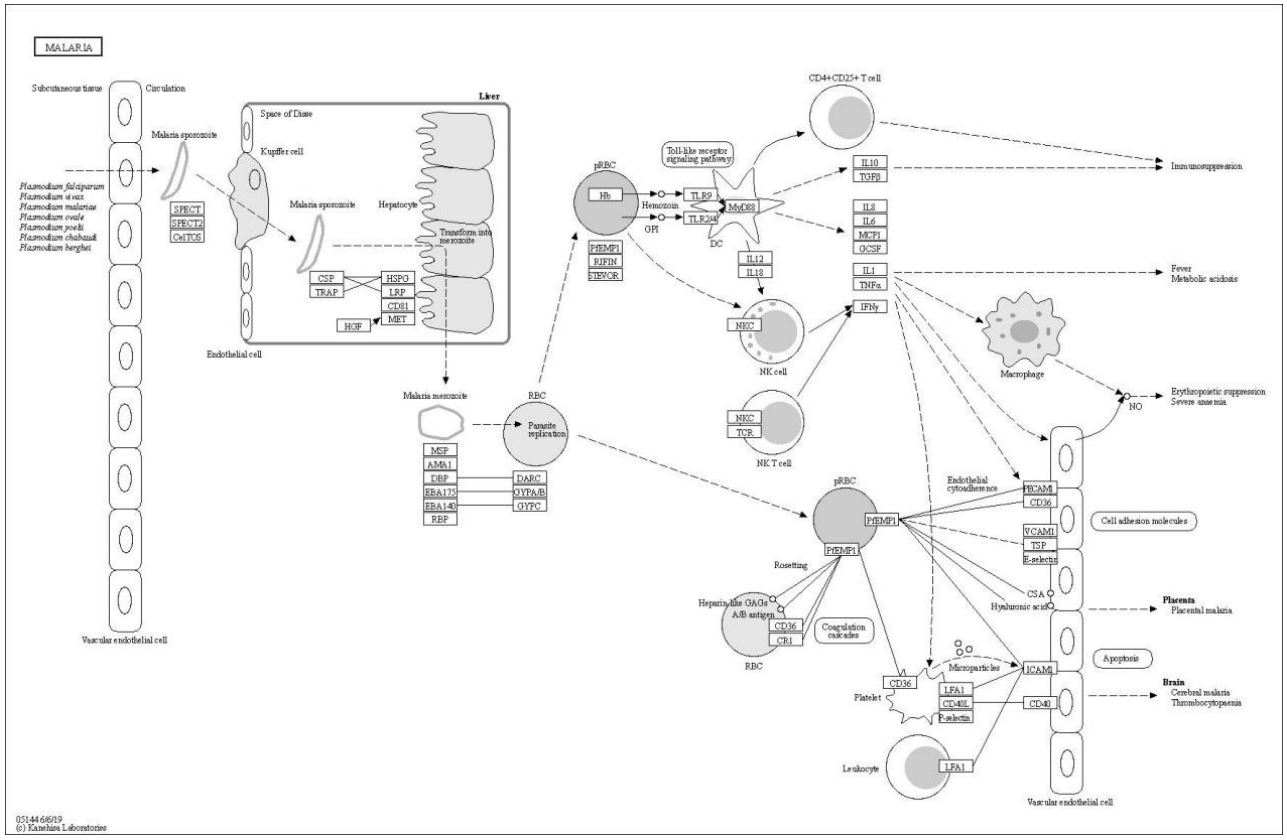

Figure 6. Malaria signaling pathway

The signal pathways of spinal cord injury that took 1 day were DNA replication, Nicotine addiction, Malaria, Mismatch repair, Steroid biosynthesis, Leishmaniasis, Signaling pathway in diabetic complications, GABAergic synapse, Complement and coagulation Cascades Alanine, Aspartate and glutamate metabolism, the signaling pathway of spinal cord injury with a duration of 3 days was Malaria, DNA replication, Signaling pathway in diabetic complications, Legionellosis, Amoebiasis, ECM-receptor interaction, Complement and coagulation Cascades, Cholesterol metabolism, TGF-beta signaling pathway, IL-17 signaling pathway; The signaling pathways of spinal cord injury with a duration of 1 week were Malaria, Leishmaniasis Age-rage signaling pathway in diabetic complications, Alanine, ASpartate and glutamate metabolism,

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Rheumatoid arthritis, Cholesterol metabolism, Nicotine addiction, Legionellosis, NF-Kappa B signaling pathway, Osteoclast differentiation; Steroid biosynthesis, Alanine, Aspartate and glutamate metabolism, Pertussis, Malaria, spinal cord injury time was 2 weeks. Synaptic Vesicle Cycle, Leishmaniasis, Nicotine Addiction, Butanoate Metabolism, Osteoclast Differentiation, Protein wet and absorption. The spinal cord injury took eight weeks and the signal pathway was Steroid biosynthesis,Glycosaminoglycan degradation, Terpenoid backbone biosynthesis, Cholesterol metabolism, Pertussis, Butanoate metabolism, By Malaria, Complement and coagulation Cascades, Alanine, Aspartate and glutamate metabolism, Staphylococcus aureus infection. Malaria signaling pathways are consistently involved in the mechanism of spinal cord injury at different time periods (FIG. 6).

4. Discussion

The Malaria signaling pathway is described on the KEGG website as Plasmodium protozoa, the parasite that causes Malaria infections. The sporozoite forms of the parasite are injected subcutaneously through mosquito bites and taken to the liver, where they develop into merozoite forms. Sporozoon invasion of hepatocytes is mediated by parasite surface proteins such as CSP. It is subsequently infected by merozoites to red blood cells (RBCS), which cause malaria disease through aberrant cytokine production and sequestration of parasite-infected red blood cells (PRBCS) into the host endothelium. Microvascular sequestration in the brain causes cerebral malaria, which can lead to death or sustained neurological damage. PfEMP1 is considered is the key of PRBC adhesion molecules (https://www.kegg.jp/entry/map05144).

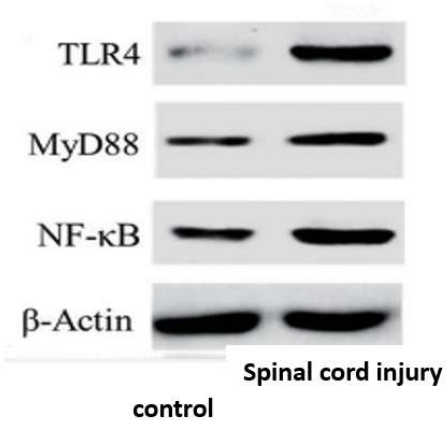

Figure 7. Expression of TLR4/NF-KB related genes by Western-blot analysis

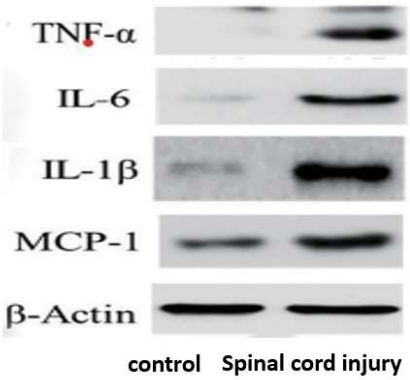

Figure 8. Protein expression of related inflammatory factors detected by Western-blot analysis

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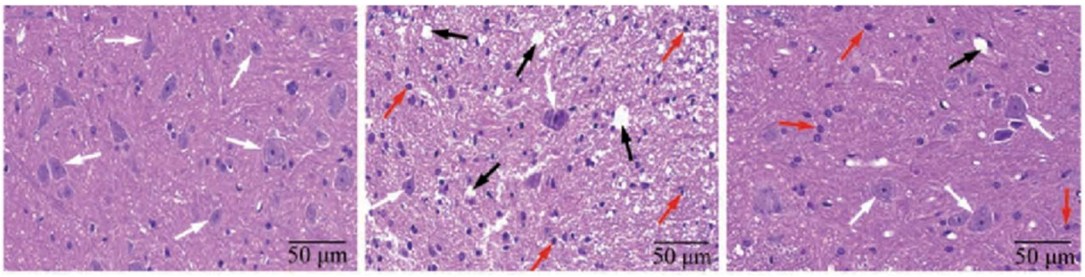
The core genes of spinal cord injury are TLR4 and FN1. TLR4 This gene encodes a protein that is a member of the Toll-like receptor (TLR) family, which plays a fundamental role in pathogen recognition and innate immune activation. After spinal cord injury, TLR4 expression was increased in Mou Leming rats, and TLR4 expression was decreased after BMSCs transplantation. BMSCs in rats may inhibit the inflammatory response after spinal cord injury and treat spinal cord injury by reducing the expression of TLR4 at the injury site [6]. The mRNA expressions of TLR4, MyD88, NF-KB and AP-1 in the spinal cord of rats in the Zhang Lige SCI group were significantly higher than those in the sham-operation group, while the mRNA expressions of TLR4, MyD88, NF-KB and AP-1 in the paeoniflorin group were significantly lower than those in the spinal cord injury group, and the differences were statistically significant ($P<0.05$). The serum levels of IL-6, TNF-α and IL-12 in SCI group were significantly higher than those in sham group. The mRNA expressions of NRF-2 and ARE in the spinal cord of rats in SCI group were significantly higher than those in sham operation group, and the contents of HO-1, SOD and GSH-Px were significantly lower than those in sham operation group [7]. After spinal cord injury, the expression of TLR4, My D88 and NF-κB M RNA was significantly increased, and the expression of TNF-α, IL-6, IL-1β and McP-1 protein in monocytes was significantly increased in the injured group (FIG. 7-8) Reference [8]. Electroacupuncture can effectively promote the repair of motor function in mice with spinal cord injury in the acute stage, and its mechanism may be related to the down-regulation of the expression levels of inflammatory factors HMGB1, TLR4 and Iba1 [9].

Fibronectin 1(FN1) is a high molecular glycoprotein widely present in various tissues and body fluids, which can participate in biological processes such as cell proliferation, adhesion, migration and tissue repair. Recent studies have reported that FN1 is closely related to the proliferation, invasion, migration and chemotherapy resistance of hepatocellular carcinoma, gastric cancer and other malignant tumors, but it has not been reported in spinal cord injury. Wang Xiaoliang found that NRF2-ARE pathway had neuroprotective effect after spinal cord injury in rats [10]. In the Dayni EA group, bilateral "Zusanli" and "Sanyinjiao" were given EA 3 hours after modeling, once a day, for 7 consecutive days. Compared with sham operation group, the expression of ApoE in spinal cord of model group was significantly increased ($P<0.05$). Compared with the model group, the expression of ApoE in the spinal cord of mice in the EA group was significantly increased (FIG. 9, [9]). On day 7 after modeling, the expressions of p-ERK1/2, Nrf2 and HO-1 in the spinal cord of mice in the model group were significantly increased compared with those in the sham operation group ($P<0.05$), but there was no significant difference in the expression of ERK1/2 ($P>0.05$). Compared with the model group, the expressions of p-ERK1/2, Nrf2 and HO-1 in the spinal cord of mice in the EA group were significantly increased ($P<0.05$), but there was no significant difference in the expression of ERK1/2 ($P>0.05$) (FIG. 10, [11]).



control Model group Acupuncture group

Figure 9. Histopathological comparison of spinal cord tissue in each group (HE staining)

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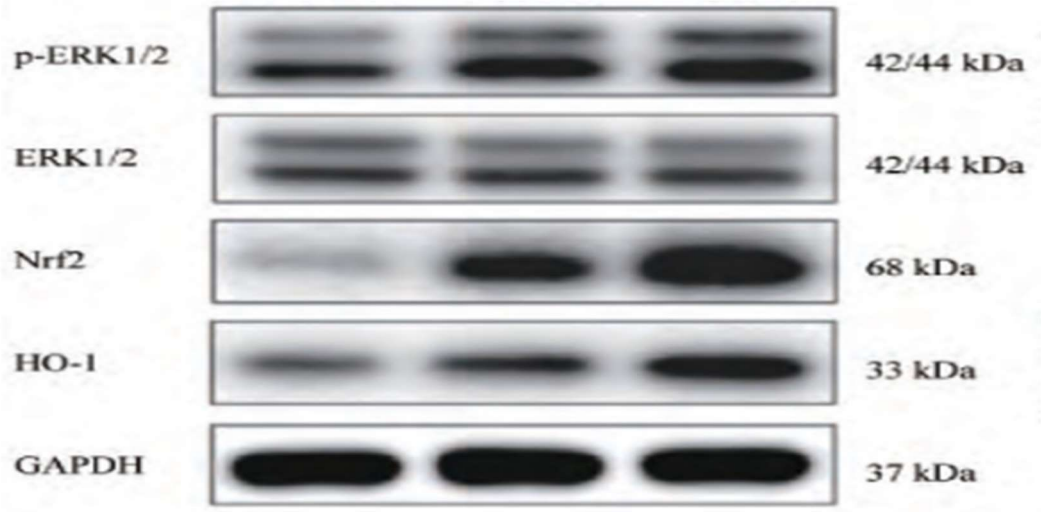

Figure 10. Comparison of the expressions of P-ERK1/2, ERK1/2, Nrf2 and HO-1 in the spinal cord of mice in each group

5. Conclusion

TLR4 and FN1 are the core genes of spinal cord injury and participate in the whole cycle of spinal cord injury. Malaria signaling pathway is always involved in the mechanism of spinal cord injury at different time periods. Ea can improve the inflammatory response and oxidative stress response in mice after SCI, thus promoting the repair of spinal cord structure and function. It is related to the enhancement of Nrf2/HO-1 antioxidant pathway and the inhibition of NF- κ B activation. Electroacupuncture can effectively promote the repair of motor function in mice with spinal cord injury in the acute stage, and its mechanism may be related to the down-regulation of the expression levels of inflammatory factors HMGB1, TLR4 and Iba1.

Acknowledgments

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2) Project of Gansu Research Center of Traditional Chinese Medicine, Study on the effect and regulatory mechanism of different frequencies of electricity on KEAP1-NRF2 /ARE signaling pathway in rat SCI, No. Zyzx-2020-zx10.

3) Gansu Provincial Educational Science and Technology Innovation Project, Study on analgesic mechanism of central sensitization mediated by "copper death" mediated by acupuncture "neural axis" regulating pain rat model, No. 2022A-067.

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