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Multi-omics in Spinal Cord Injury: Diagnosis, Prognosis, and Treatment

Hanxun Xu[1], Wenhai Zhao[2], Yimin Zhou[1], Li Zhang[3*]

[1] College of Traditional Chinese Medicine, Changchun University of Chinese Medicine, Changchun 130021, China
[2] Orthopedic Center, Affiliated Hospital of Changchun University of Chinese Medicine, Changchun 130021, China
[3] Principal's Office, Fujian Vocational College of Bioengineering, Fuzhou 350007, China

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ABSTRACT

A spinal cord injury (SCI) can result in varying degrees of limb movement impairment and sensory impairment, affecting the quality of life severely. The study of the molecular mechanisms underlying SCI disease has advanced greatly. However, there is still room for improvement in terms of the cognitive and systematic approaches used for disease diagnosis, progression, treatment, and prognosis. This situation might change as multi-omics technology advances. Single omics technology has some limitations when it comes to fully comprehending the disease progression pattern and directing SCI treatment. Therefore, a thorough understanding of the state of the art in omics research on SCI can explain the pathogenesis and mechanism of the disease and offer new, multifaceted treatments for SCI. This article reviews recent developments in the application of various omics techniques to diseases associated with SCI and discusses the advantages and disadvantages of using these techniques for disease diagnosis, prognosis, and treatment.

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Introduction

A severe condition that damages the CNS, SCI frequently results in motor and sensory dysfunction, impairs mobility, and has a detrimental impact on quality of life. The prevalence of spinal cord injuries is increasing as the population ages and the number of traffic accidents rises. More than 27 million people worldwide have spinal cord injuries that have left them disabled(1). In China, there are 66.5 spinal cord injuries for every million people(2). The high SCI disability rate presents a challenge for clinical and basic research, and as the patient population grows, so does the financial burden on families and society. Currently, early surgical decompression followed by anti-inflammatory drugs and the stimulation of nerve regeneration is the main treatments for spinal cord injuries(3). Exosomes, recombinant adenovirus, stem cells, and other novel treatments are currently being researched and developed(4,5). The development of Omics techniques may open up new avenues for the treatment of SCI. Omics-related detection and analysis techniques are improving as a result of advancements in modern science and technology. Omics techniques can offer more thorough and quick diagnostic indicators for SCI during the preliminary diagnosis process, shorten the time it takes to confirm the diagnosis, increase the accuracy of the diagnosis, offer patients an earlier and better course of treatment, and offer a prognosis for SCI. Omics techniques can offer new therapeutic targets and concepts for SCI, which researchers can investigate in the future in terms of treatment (6). In order to provide new references for the diagnosis and treatment of SCI, we review the research progress of various Omics techniques in the diagnosis, prognosis, and treatment of SCI and highlight the viability of Omics techniques in the diagnosis and treatment process of SCI.

Genomics

The study of all genes in an organism is possible through genomics, and choosing an appropriate animal model can help to more accurately simulate SCI and serve as a guide for future neurological repair. Due to their capacity to completely repair spinal cord tissue that has been transected, adult zebrafish are frequently used in research on spinal cord injuries(7). Additionally, Shen's team(8) collected spinal cord tissue from zebrafish two weeks after SCI, identified the genome and compared it with the gene pool of mature spinal cord-injured zebrafish. An important area of genomics called epigenomics focuses on reversible, inheritable, and genomic changes that do not involve DNA sequences(9). DNA methylation,

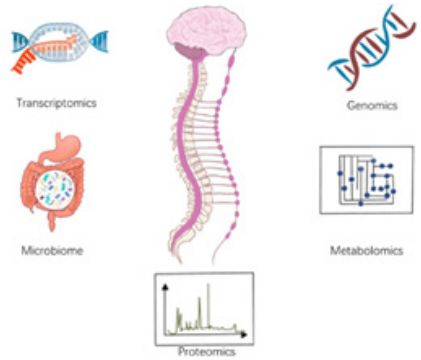

Figure 1. A multi-layer omics approach to study SCI.

* Corresponding author. Email: zhanglil62@163.com

Histone modification, and other common epigenetic modifications are examples. According to research on spinal cord injuries, genes' capacity for transcription and expression heavily influences how quickly the nervous system can heal(10). Researchers focus on the epigenome, which has the ability to change the chromatin state, which has grown recently. According to earlier research, histone acetylation is a potential therapeutic approach to improve axonal regeneration(11). DNA methylation has also been suggested to play a significant role in both proliferation and differentiation of neural stem cells, and neuronal cells(12). Shi(13) used whole genome bisulfite sequencing and methylated DNA immunoprecipitation sequencing to screen and compare the two groups of differentially methylated genes, and then performed biological analysis. Core genes were discovered, including Dnm3, Ntrk3, Smurf1, Dpysl2, Kalrn, and Shank1, as well as signaling pathways like the Hippo signaling pathway and the Endocytosis pathway. Axon guidance, endocytosis, T cell receptor signaling, and Hippo signaling were the four pathways where DNA methylation was most concentrated, according to research done by some teams on rats with SCI (14). These recently discovered target genes might represent a fresh avenue for treating and diagnosing SCI. Another team also concluded that 5-methylcytosine (5mC) and 5-hydroxymethylcytosine(5hmC) could not be distinguished using conventional DNA methylation detection methods (15). The genome-wide analysis of DNA at the 5hmC level revealed that 5mC and 5hmC have distinct functions in the axonal regeneration of spinal cord injured neurons. It also revealed that genes with a significant change in DNA methylation are new therapeutic targets and potential injury mechanisms in SCI. Exercise rehabilitation is a common form of treatment for people with spinal cord injuries. With the help of epigenomic theories, exercise training can enhance the functional recovery of SCI by raising the levels of 5mC and 5hmC(16). UTX/Mir-24 has been demonstrated to inhibit NeuroD1 expression in the form of epigenetic complexes in response to known epigenomic studies of SCI(17). This limits the capacity of SCI neurons to regenerate, and UTX may emerge as a key target for the treatment of SCI.

Transcriptomics

A cell, tissue, or group of organisms' entire RNA assembly is known as its transcriptome. The importance of transcriptome analysis is to quantify the expression of genes in cells, tissues, organs, etc. while capturing the specificity of gene expression in coding and non-coding regions of RNA. This analysis establishes a framework for reconstructing genetic interaction networks(18,19) and offers the first step in functional characterization and annotation of genes or genomes previously revealed by DNA sequencing. Coding region RNA and non-coding region RNA are the two primary types of RNA, and non-coding region RNA are further divided into long-stranded non-coding RNA and microRNA, both of which have recently been proposed to play a crucial role in the development of SCI disease(20,21).

Through the use of transcriptomics techniques, it was discovered that a number of RNAs showed abnormalities in SCI(22) and that these differentially expressed genes were primarily enriched in the neurotrophin TRK receptor signaling pathway, which indirectly suggested that the neurotrophin TRK receptor signaling pathway may be involved in the start of neurological regeneration. LncRNA and miRNA can be used to diagnose SCI, just like other RNAs. Ding et alstudy(23) revealed the expression profile of lncRNAs in the spinal cord of a complete transection model rat and proposed that lncRNAs may be crucial in the early immune-inflammatory response after SCI. Additionally, the ceRNA hypothesis's formulation has opened up new opportunities for the study of transcriptomics. Eight mRNAs associated with SCI were found by Gong et al.(24) in the ceRNA network, and RGD1564534 may function as a ceRNA to control the expression of these eight SCI-associated miRNAs by competitively interacting with miR-29b-5p. Additionally, lncRNAXR 350851 may regulate SCI autophagy and may be a potential new marker and therapeutic target for SCI, according to the ceRNA hypothesis.

The use of peripheral blood cells in the diagnosis of patients with metal injuries for which MRI is contraindicated has a special advantage in the case of hyperacute spinal cord injuries. This is so because it is simple to collect peripheral blood cells, which express about 80% of the human genome's genes. According to a related study, mice's peripheral blood contained altered levels of certain miRNAs, including miR-30b-5p, miR-152-3p, miR-200c-3p, miR-125b, and miR-124-3p, both before and after injury(25). This information is extremely helpful in the diagnosis of hyperacute SCI. After 2 hours of injury, the analysis showed that differentially expressed genes were enriched in the toll-like receptor signaling pathway, p53 signaling pathway, MAPK signaling pathway, and Jak-STAT signaling pathway. LncRNA expression was highly dynamic at various stages of SCI. In the event of an immediate SCI, IL6, MBOAT4, FOS, TNF, JUN, STAT3, CSF2, MYC, CCL2, and FGF2 may be crucial targets(26). Similar to this, two days after SCI, differential transcriptome assays were carried out in mice, and the top 10 core genes identified by the PPI network were IL6, TNF, and MYC(27). The results obtained two hours after the injury are both the same and different from this, and comparing the differences between the two can aid in the identification of an early SCI. By comparing differentially expressed genes at three different time points after SCI, at 1, 4, and 7 days, Gong et al.'s research(28) demonstrated that SCI is a process of continuous transformation of an inflammatory transcriptome into an apoptotic, self-healing transcriptome. Interestingly, pathways and genes essential to the pathology of spinal cord injuries were discovered by sequencing tissue samples from acute and subacute spinal cord injuries(29). The most enriched functional categories were "inflammatory response," "neurological disease," "cell death and survival," and "neurological development," which also support one another.

Spinal cord regeneration studies frequently involve the neurorenewable zebrafish. Specialized glial cells are present in adult zebrafish that spontaneously restore spinal cord damage by growing a regenerative bridge over the damaged tissue. Mammals are incapable of having this. The epithelial-mesenchymal transition and E2F4 gene programs are activated by SCI in zebrafish, according to transcriptomic studies. The results of additional research on bridging glial cells will then serve as a platform for translational applications to enhance bridging and CNS

regeneration in mammals(30,31). Successful regeneration in rodents depends on the regenerative transcriptome profile remaining active continuously throughout the regeneration process. For the first two weeks, the transplantation group's transcriptomic response was nearly identical to that of the SCI-only group in terms of corticospinal axon regeneration. The transcriptome in the injury-only group, however, is downregulated after two weeks, whereas it is maintained in the transplantation group(32). The central hub of the regenerative transcriptome is the huntingtin gene, a transcriptional regulator linked to corticospinal regeneration, and mutations in Htt can impair neuronal repair mechanisms(33). A favorable microenvironment for neurogenesis and the restoration of fully transected spinal cord function is provided by the neurotrophin-3 (NT3)-loaded chitosan, which has been shown through transcriptome analysis to be essential for the spinal cord's regenerative effects(34). After SCI, apoptosis is also unavoidable, and ZBTB38 overexpression significantly increases RB1CC1, a crucial element of the spinal cord's autophagy initiation mechanism, promoting autophagy and partially reversing secondary damage(35). Similar to this, increased miRNA-21 expression levels following SCI may aid in spinal cord repair by inhibiting the expression of its target gene, PDCD4(36). Montazeri et al.(37) 's discovery that neurotrophic factor was highly expressed after SCI in terms of the beginning of neurological repair suggests that the neurotrophin TRK receptor signaling pathway may be involved at the beginning of neurological regeneration and that the function of the p75 neurotrophin receptors to kill injured cells by inducing apoptotic mechanisms. These findings all point to new directions for SCI treatment research.

Proteomics

Because it can thoroughly examine all the protein characteristics of a specific biological sample over a specific period and allow monitoring changes in protein expression under various circumstances, proteomics is frequently used in the search for disease biomarkers, new therapeutic targets, and treatment plan optimization. It is challenging to diagnose, predict, and treat diseases solely based on genetic information in practical genetic information technology applications because proteins are frequently impacted at the transcriptional and translational levels by post-translational modifications, protein heterodimerization, etc. Because proteins are more closely related to biological functions than genetic information, proteomics is a potent complement to genomics and transcriptomics and has been shown to be effective(38–45).

Studies on SCI have frequently used proteomics(46). Biomarkers for diseases related to SCI can be discovered by comparing samples with normal samples. Ding et al(47). conducted proteomic analysis on rats 5 days after SCI and identified 30 upregulation proteins represented by 11-zinc finger protein and Glypic. While Liu and his team subjected 9 female Wistar rats to SCI strikes and 7 days later compared them with the sham-operated group using iTRAQ labeling, and LC-MS/MS techniques for proteome comparison identified 10 significantly different biomarkers represented by complement c(48), compared to these findings, 220 differential proteins, including 3 heat shock proteins, were found in the spinal cord tissue proteome of rats after the 8-hour acute phase of injury in comparison to the normal group of rats(49). During the 24-hour acute phase, a total of 108 differential proteins—including 16 significant differential proteins—were discovered(50). These distinctive proteins could serve as key indicators of diseases related to spinal cord injuries. Proteins in the human body exhibit partial differences at various injury times, and the appearance of these differential proteins can serve as a guideline for determining how SCI disease progresses. After performing a proteomic analysis on mouse spinal cord tissues at weeks 1, 2, 3, and 8 following SCI, Ding et al(51). came to the conclusion that 9 out of 29 significantly different proteins were involved in the disease process. They also noted that the proteomic differences between the spinal cord-injured mice and the sham-operated group shrank as the disease improved, as previously reported in studies(52), in order to be able to regenerate after an SCI, the CNS exhibits strong proteomic differences. By 5 days or later, the damaged spinal cord tissue starts to adjust to this pathological environment and reaches homeostasis. By extending the time for SCI testing to 8 weeks(53), the number of different proteins that are consistently increased or decreased can reach 40 and 48, respectively. Analysis of these differential proteins can identify proteins with the potential to be drug targets in SCI, which opens up new avenues for future drug research for the treatment of SCI. Could a more accessible and safe sample source be used, even though proteomic analysis of cerebrospinal fluid from patients at various stages of injury may produce noticeably different proteins that offer intervention pathways or small molecules for later treatment of SCI(54). This was confirmed by Zhang's team(55), whose proteomic analysis of the erythrocytes from beagle dogs suffering from the acute and subacute phases of SCI and validation of the mass spectrometry results not only revealed 26 differential proteins but also suggested that erythrocytes might be a source of samples for biological markers of oxidative stress in SCI. The team of Moghieb and Lubieniecka(56,57) collected spinal cord tissues and cerebrospinal fluid from moderate and severe SCI rat models for differential proteomic analysis. Proteomics technology will also help establish quantitative and objective biomarkers to gauge SCI severity, which will lead to more precise SCI treatment options. While using SCI patients as the sample source(58,59). They identified TF, FASN, NME1, and various biomarkers that can determine the severity of SCI represented These protein markers that indicate the severity of SCI may also suggestively contribute to the prediction of disease prognosis. Using this feature in conjunction with a machine learning strategy, a prognostic model was created that can predict an improvement in AIS grading after six months.

Post-translational modifications play a significant role in the expression of protein functions in addition to normal protein expression. In addition to identifying early post-translational modifications of proteins after SCI in rats(60), blood-related proteins, heat-shock proteins, glycolytic enzymes, etc., have been shown to be significantly affected by protein phosphorylation(61). Researchers may be able to gain insight into disease mechanisms by studying the changes in these proteins following SCI. A common secondary condition of SCI is neurological inflammation. Identifying the pathological mechanism of neuroinflammation and developing effective

interventions are key steps in improving SCI. Proteomic and transcriptomic data suggest that the lysosome pathway, cholesterol metabolism, and the complement and coagulation cascades are critical pathways for SCI progression. It has also been shown that sequential upregulation of protein clusters at acute and subacute time points after SCI is associated with myeloid cells and immune responses, lipid regulation, and lysosome pathways that may contribute to the neuroinflammatory response following SCI (62). Didangelos(63) also demonstrated that persistent overexpression of extracellular alarmins could trigger inflammation through pattern recognition receptors by targeting a combination of abundant extracellular proteome and transcriptomics after SCI.

The use of proteomics in SCI is not just restricted to diagnosis and prognosis; it can also be very helpful in guiding treatment decisions and fostering the investigation of novel therapeutic approaches for prediction. Mesenchymal stem cells have been demonstrated to be effective in treating SCI(64). Extracellular vesicles, one of the most significant MSC mediators, have been demonstrated to reduce apoptosis, reduce the inflammatory response, and promote angiogenesis, all of which have been shown to improve SCI(65). Specifically, through the regulation of apoptosis and inflammation, MSCs-EVs can be directed to the injured spinal cord tissue when used for SCI treatment, and 883 differential proteins can be identified when proteomics is applied(66). MSCs-Evs pre-treated with melatonin was found to promote better recovery after SCI(67), and after proteomic analysis was conducted, Ubiquitin-specific protease 29 expression was significantly elevated, allowing further research to establish its probable mechanism of action, in order to formulate more valuable methods for the treatment of SCI. Unlike mammals, some animals have the ability to repair and regenerate nerves in close association with age(68-72) and this can provide insight into ways to improve recovery from SCI. When Xenopus regenerative and non-regenerative SCI models of laevis were quantitatively detected by proteome(73), 172 were in the regenerative phase and 240 in the non-regenerative phase. As with Xenopus laevis, Monodelphis Domestica also exhibits age-related CNS repair functions and has significant effects on a postnatal day 5 and postnatal day 18 (Proteomic analysis of spinal cord tissues of Monodelphis Domestica from P18)(74) showed significant differences in proteins associated with neurodegenerative diseases. According to the six differential proteins related to CNS development, the Glial fibrillary acidic protein, Paired box protein Pax-6, Growth associated protein 43, Myelin basic protein and Brain lipid-binding protein content of P18 is greater than that of P5, Paired box protein Pax-2 is the opposite. Injury treatment in the Monodelphis Domestica T10 spinal cord at P7 and P28. A total of 56 differentially regulated proteins associated with SCI were identified at 1 and 7 days after injury and compared with the corresponding age control group(75). Interestingly, 14-3-3 epsilon, 14-3-3 gamma, cofilin, alpha-enolase, heart fatty acid binding protein, brain fatty acid binding protein, and ubiquitin were differentially expressed with age. At the same time, P7 appears to have fewer protein and transcriptional changes in SCI(76), perhaps because the injury occurs during the regeneration of spinal cord tissue. The findings from regenerative animals have the potential to provide us with crucial information regarding spinal cord regeneration and help us understand the mechanism of SCI repair. These differential proteins also provide us with effective therapeutic targets for future research. A spinal cord transection injury is more severe than a normal injury. In a proteomic analysis of SCT rats, α--synuclein was significantly down-regulated (77) and the expression of Endoplasmic Reticulum Protein29 was significantly increased(78). As a result of the later verification of SNCA and ERp29, it was also discovered that inhibiting SNCA reduced the apoptosis of neuronal cells and promoted the recovery of motor function. In addition, ERp29 promotes motor neuron survival and axon regeneration, two differential proteins that may be therapeutic targets for SCI. In addition, eukaryotic translation initiation factor 5A1 was also highly expressed in the SCT model(79). In addition, inhibition of elf-5A1 expression slows functional recovery in rats with SCI. elf-5A1 overexpression increased the expression level of RhoGDIα as well. As a result, certain experiments have also demonstrated(80) that eIF5A1 upregulates RhoGDIα in neurons, thereby promoting neuronal regeneration in vitro. The eIF5A1/RhoGDIα pathway could be used as a new target for the treatment of spinal cord injuries by means of proteomics.

Metabolomics

Since it was first proposed in 1998, metabolomics has developed into a well-established field of study that uses both qualitative and quantitative methods to examine metabolites with molecular weights less than 1000 that are present in cells, tissues, or biological samples at a specific point in time(81). Metabolomics, as opposed to proteomics and genomics, considers omics metabolites as end products and connects downstream phenotypes to upstream biological processes in transcriptomics, genomics, and proteomics(82). Because it can precisely and thoroughly describe the dynamic responses of biological organisms to environmental and genetic influences, metabolomics is frequently used to identify disease biomarkers, disease progression, and new therapeutic targets(83–87).

There are some challenges in the early diagnosis and treatment of SCI because there is no established biological decision-making mechanism. But the development of 1HNMR, mass spectrometry, and other metabolomics technologies offers a fresh perspective on the identification and management of SCI. YANG and his team(88) built four rat models of SCI with varying degrees of injury, and they collected samples of cerebrospinal fluid, plasma, and rat spinal cord tissue 72 hours after the onset of the disease for parallel studies. Their goal was to identify metabolic biomarkers of SCI in the subacute phase. These samples contained 14 metabolites, including uric acid, phosphorylcholine, guanidoacetic acid, and pyridoxine. The pathways phosphorylcholine, pyridoxine, and guanidoacetic acid displayed were strongly linked to SCI. Accordingly, a group of researchers(89) collected cerebrospinal fluid and serum samples from patients with various grades of SCI from ASIA at 24, 48, and 72 hours after injury for detection and analysis. They used differential chemical isotope labeling liquid chromatography-mass spectrometry with a Universal metabolome standard. The content of differential metabolites was positively correlated with the severity of SCI, meaning that the more serious

the disease injury, the higher the content of differential metabolites, which may be a potential marker of SCI severity. Six typical cerebrospinal fluid metabolites and one serum metabolite were determined after comparison between groups. Through metabolic analysis of plasma from rats with various levels of SCI(89,90), fatty acids, VLDL, LDL, triglyceride, 3-methyl-histamine, myc-inositoland arginine, as well as other metabolic markers represented by sp The metabolomic profile of SCI at the initial and long-term stages was determined and provided a potential biomarker profile for the prognosis of individual clinical treatment through the metabolome detection and statistics of human urine at different times(91).

Similar to the non-targeted metabolomics detection results mentioned above, changes in the lipidome in the body after SCI were particularly pronounced(92), the presence of the disease results in an apparent disorder of the body's metabolism of polyunsaturated fatty acids represented by DHA, and the expression of fatty acid-binding protein 5 was also impacted(93). In addition to the ability of FABPs to control the transport metabolism of polyunsaturated fatty acids, the intervention with FABP5 siRNA also hindered the restoration of motor function in SCI and the positive regulation of disease by DHA. Therefore, by regulating amino acid metabolism and associated free radical biochemical characteristics, prophylactically feeding a diet high in omega-3 polyunsaturated fatty acids can improve the recovery of autonomic nerves, sensation, and movement(94). Additionally, DHA deficiency after SCI is improved(95). In addition to a diet high in PUFAs, the ketogenic diet has gained popularity since metabolomics demonstrated that the lipidome of patients with spinal cord injuries was significantly affected. According to preliminary studies on neurological disorders, the ketogenic diet is thought to have a therapeutic effect on promoting nerve fiber repair, nerve protection, and reducing neuroinflammation(96). In rats with spinal cord injuries, the ketogenic diet has also been proposed to quickly raise ketone levels and enhance motor recovery(97). Intriguingly, Mayr and his team's experimental findings(98) revealed that while other monoamine metabolites did not change significantly compared to the control group after SCI, the levels of metabolites such as Tyrosine and Tryptophan decreased in rats given a ketogenic diet. More importantly, the ketogenic diet did not have a significant protective effect on SCI. Traditional Chinese medicine has emerged as a new field of study in modern medicine that is worth exploring. The study found that Miltiorrhiza Salvia Bunge, a widely used herbal remedy in clinical practice, had 51 metabolites and 6 major metabolic pathways identified after metabolomic assay(99), including arachidonic acid metabolism, which can be further investigated for the role of fatty acid/ arachidonic acid metabolism in SCI. Additionally, the metabolome was examined and Licofelone was used to inhibit COX/ 5-Lox. In SCI, lipid metabolites were also noticeably decreased(100). This demonstrates that lipid metabolism plays a crucial role in the histological analysis of the metabolism following SCI. It is possible to gain a new understanding of the pathological mechanism underlying SCI through the study of metabolomics and the integration of related data. It is also possible to find new diagnostic markers and potential treatment targets.

Microbiomics

The host immune system, a source of genetic diversity, and a crucial element affecting drug metabolism are all attributes of the microbiome(101). At the same time, the microbiome is gaining recognition for its capacity to control the physiological relationships and functions of almost all body organs and to help the host coordinate essential survival processes(102). Given the abundance of microbes in the gastrointestinal tract, it has emerged as a significant area of study and interaction between the microbiota and the host(103). The concepts of the CNS-gut axis, brain-gut axis, and spinal cord-gut-immune axis have also been proposed(101, 102, 104, 105). These concepts define the gut, spinal cord, and CNS as a bidirectional communication pathway, in which various signals related to SCI are interspersed. The sympathetic nerve is thought to be the primary mode of CNS innervation that controls the gastrointestinal nervous system. The regulation of intestinal sympathetic nerves, gut-associated lymphoid tissue, intestinal movement, intestinal epithelial permeability, etc., affects the normal function of the gut microbiota either directly or indirectly. Sympathetic nerve damage after SCI causes a number of terrible effects on the gastrointestinal system. Studying the changes in microbiomics after SCI and its reverse effects on the human body has also become a new focus as the significance of the gut microbiome on host physiological function and overall health is recognized, such as dysregulation of the gut microbiota, which can greatly affect the neurological function, host immune function, overall health, and quality of daily life of SCI patients(106,107).

Due to the highly conserved nature of the 16S

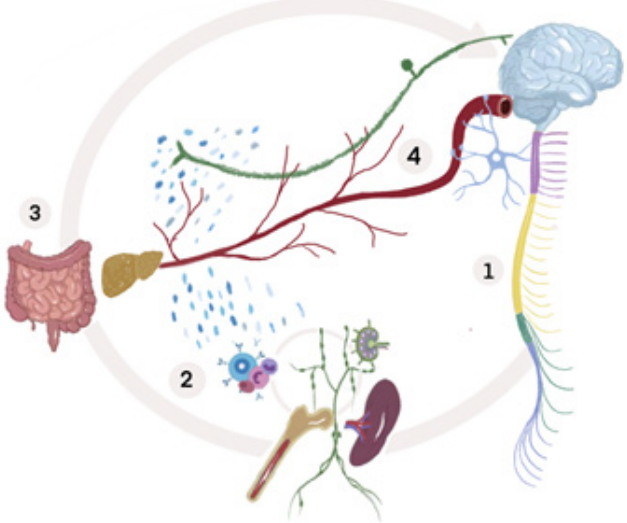

Figure 1. CNS-gut axis (105): The connections between the nervous system, immune system, and gastrointestinal system are abnormally affected by SCI (SCI)1. SCI results in a decrease in the sympathetic nervous system's ability to control the body. As a result, there is a reduction in the physiological regulation of hematopoiesis (bone marrow and spleen) and immune surveillance (bone marrow, spleen, lymph nodes, and gastrointestinal tract-associated lymphoid tissue/ GALT)2. Immune dysfunction and dysregulation of the gut ecosystem are brought on by this endostasis disruption3. The vagus nerve is activated or metabolites and cytokines produced by gut microbes and cells enter the bloodstream, affecting the structure and operation of the CNS through the CNS-gut axis4.

ribosomal RNA gene and its biological uniqueness, 16S rRNA sequencing has become a popular method for identifying the variety of bacterial communities present in the human gut(108). The 30S subunit of the ribosome in prokaryotes contains the 16S rRNA(109). The 16S rRNA gene sequence is roughly 1550 bp long and is made up of variable and conserved regions. Its high conservation status is thought to be one of the primary justifications for the use of 16S rRNA as a target for the detection of the human microbiome(110). A similar effect was observed when gut microbiota was studied in SCI patients. SCI has been shown to affect the composition of the gut microbiota in experimental animals(111), and many bacteria can only return to normal levels after SCI fluctuations in the acute phase(112). It has been demonstrated that the gut microbiota of SCI patients differed from the healthy population's in terms of microbial community structure and quantity(113), Additionally, there may be a connection between serum markers, neurogenic bowel dysfunction, cardiovascular disease, and metabolic disorders and dysregulation of the gut microbiota(114,115). Likewise, the gut microbiota of patients with various types of SCI bowel dysfunction, such as upper motor neuron bowel syndrome and lower motor neuron bowel syndrome, differs(116). These differences can be used as a guide for the early diagnosis of SCI. Patients with different degrees of injury and patients at different stages of SCI(117) also have different bacterial community characteristics, on the assumption that SCI patients show significant dysregulation of gut microbiota. Patients with complete SCI exhibit more severe gut microbiota dysbiosis than patients with incomplete SCI (ITSCI)(118). This difference is thought to play a role in the development of other diseases, such as inflammatory responses, and is closely related to the integrity and severity of the disease(119). These studies offer a strong foundation for the use of microbiomics in clinical practice.

When treating SCI, it was discovered in a therapeutic study that some therapeutic techniques, including melatonin, electroacupuncture, and minocycline, also regulate the microbiota(120,121). This might offer a fresh concept and treatment objective for SCI. Can the control of the gut microbiota's health status slow the progression of SCI and serve as a therapeutic tool? Fecal transplantation(122) is the most direct method of enhancing the gut microbiota because it not only enhances the gastrointestinal tract's motor function and barrier integrity but also encourages the regeneration of neuronal axons and the restoration of motor function in SCI rats(123). Additionally, a more tolerable probiotic therapy can reduce urinary tract infections and urinary tract flora in patients(124), as well as positively regulate gut microbiota by stimulating immune-related cells in the gut-associated lymphoid tissue, which in turn protects the nervous system and aids in associated motor recovery(125). All of these studies demonstrate that improving gut microbiota and gut function health status as adjunctive therapy(126) can effectively halt the progression of SCI disease and aid in the recovery of related functions.

To characterize or quantify various molecules, such as DNA, RNA, microbiota composition, protein types, etc., as well as to categorize SCI into various subtypes and disease stages, various high-throughput sequencing technologies can be used. As a result, it is now possible to not only better understand SCI and pinpoint the disease's stage of development, but also to provide patients with more targeted interventions and treatments for various disease types. There are still some restrictions despite the excellent clinical prediction ability to discover new biomarkers using omics techniques. The outcomes of the current experiments cannot be directly translated to the clinic. Therefore, we must continue to conduct multicenter, standardized, and larger-scale clinical trials to improve the applicability of omics techniques to the diagnosis and course assessment of SCI in order to improve the clinical diagnosis of SCI by biomarkers. Although omics techniques have made great strides to date and there is a lot of data available, there is still a lot of information to learn. The way to translate omics techniques from laboratory studies to real-world clinical applications is through the validation of the results and a more standardized and rigorous scientific experimental method. Databases and multi-omics may work well together as a solution. The joint use of multi-omics is a trend in the advancement of omics techniques, even though the integration of multi-omics and datasets is still a challenge. Diversified data integration can improve the quality and quantity of big data, remove the constraints imposed by a single technology in the diagnosis and treatment of disease, aid in the investigation of the etiology of SCI, and create personalized disease treatment approaches. As a result, we will be able to better understand the disease process and choose the best treatment option for patients to aid SCI recovery as omics techniques continue to advance.

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Table 1. Typical biomarkers of SCI found by multi-omics.

References	Subjects	Sample/Methods	Significant Biomarkers	
			Up	Down
Shi,et al.(13) (Gene)	Wistar rats with SCI (n=6) and Laminectomy group (n=6)	Spinal Ccord tissue/ WGBS	Smurf1,Shank1,Arsb, Dpysl2,Map6,Htr7	Kalrn,Dnm3,Dlg2, Tenm2,Numbl,Prickle2, Bmp5,Lmx1a, Ntrk3,Rein
Yang,et al(127) (Gene)	Gene Expression Omnibus	Bioinformatics		Sdha,Uqcrc2, Ndufa5, Atp5b, Atp5a1 and Cox5a
Wang,et al(22) (Gene)	Healthy controls(n=5) and patients with ISCI (n=10) and CSCI(n=10)	Peripheral blood/ The Illumina HiSeq high-throughput sequencing platform	CDK16,BAD,MAP2K2,EGR	EPHA4,RHOB
Ding,et al(23) (Gene)	SD rats healthy controls (n=12) and SCI group (n=12)	Spinal Ccord tissue/ The Illumina HiSeq	H19,Vof16,Hmox2-ps1,LOC100910973,Ybx1-ps3,Nnat,Gcgr,LOC680254	Rmrp,Terc,Ngrn,Ppp 2r2b,Cox6a2,Rpl37a-ps1,LOC360231, Rpph1
Zhou,et al(26) (Gene)	SD rats control group(n=9) and SCI group(n=9)	Spinal Ccord tissue/ Microarray analysis	nonratt009530, 002354, 027672, 006315, 030938, 018585, 001250, 006614, 008294, 022586	nonratt027883, 023477, 029520, 020419, 013439, 015190, 020882, 020279, 009500, 011215
Liu,et al(48) (Protein)	Female Wistar rats control group (n=9) and SCI group (n=9)	Spinal Ccord tissue/ iTRAQ labeling, and LC-MS/MS	Lyz1, Kng1, Orm1, Apoe, Fabp4, Lgals3, Kng2, Vim, Ctsb, Hexb	Gat3, Atp1a2, Atp1b2, Ina, Sncb, Slc6a1, Tst, Map2, Syt2, Slc4a4
Chen,et al(50) (Protein)	Long-Evans rats control group (n=9) and SCI group (n=9)	Spinal cord tissue/ iTRAQ labeling, and LC-MS/MS	p01946, p02091, p11517, p02770, p10860, p62989, p63012, p27139, p48199, p12346	p47819, q4qrb4, p02688, p09117, p11442, q6ay84,
Ding,et al (51)* (Protein)	Female mice control group (n=15) and SCI group (n=15)	Spinal cord tissue/ iTRAQ labeling	Hist1h1c, S100a10, S100a6.	Hist1h1e, Hbb-b1, Hbb-bs, Hba, Ca1, Apoa4
Moghieb,et al (56)* (Protein)	Fischer rats Naive, sham(4 h, 24 h, 7 day n=15), moderate SCI(4 h, 24 h, 7 day n=15), severe SCI(4 h, 24h, 7d n=15)	Spinal Ccord tissue, Cerebrospinal fluid/ CAX-PAGE and LCMS/MS	TF, FASN, NME1, CTSD, ANXA1, ANXA2, TPI-1	STMN1, PGM1, PEA15.
Lubieniecka,et al(57)** (Protein)	Sprague Dawley rats sham control (n=12), moderate injury (n=12), severe (n=3)	Cerebrospinal fluid/ LCMS/MS	Itih4, Gpx3, Pzp, Car1, MGC72973, Orm1, S100a8, Prdx2, Car2, RGD1564861	
TOMLJANOVIĆ I,et al (74) (Protein)	Monodelphis domestica P5 and P18	Spinal Ccord tissue / Nano-HPLC-MS/MS	GFAP,PAX6,GAP43,MBP,BLBP	PAX2
Yang,et al(85)** (Metabolites)	Female SD rats sham, light injury, moderate injury, and heavy injury groups (n=6)	CSF, plasma, and spinal cord tissue / UHPLC-HRMS	Citric acid, Glyceric acid, Creatine, 5-aminopentanoic acid, 3-hydroxyanthranilic acid, Stearic acid, Linoleic acid, 1-methylnicotinamide, 3,4-dihydroxyphenylacetaldehyde, N-acetylaspartylglutamic acid, Guanidoacetic acid, Phosphorylcholine, Uric acid, Pyridoxine dihydroxyphenylacetaldehyde, N-acetylaspartylglutamic acid, Guanidoacetic acid, Phosphorylcholine, Uric acid, Pyridoxine	
Wu,et al (86)** (Metabolites)	Acute SCI patients with baseline ASIA Impairment Scale (AIS) grades of A (n = 10), B (n= 12) and C (n= 8)	CSF, Serum samples/ LC-MS	uridine, imidazoleacetic acid, methionine sulfoxide, arginine, cystathionine and homocarnosine, uridine, 4-hydroxyproline, N1,N12- diacetylspermine and glycylproline	
Peng,et al (87)** (Metabolites)	Male SD rats control (n=7), mild SCI (n=10), and severe SCI group (n =25)	Plasma samples/^{1}H-NMR	4-Hydroxybutyric acid, Choline, L-Arginine, L-Histidine, L-Methionine, Tyramine, L-Serine, L-Lactic acid, Malonic acid, Phosphocreatine, 3- Indolebutyric acid, 3-Methylhistamine, Putrescine, Urea, Inosine	
Doelman,et al (108)(Microbial)	Female Yucatan pigs Control (n = 9), SCI (n = 8), Diet (n = 3) and Antibiotics (n = 3)	Fecal sample/ 16sRNA sequence	Proteobacteria, Tenericutes, Epsilonbacteraeota, Cyanobacteria	Firmicutes, Bacteroidetes, Spirochaetes

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