RESEARCH ARTICLE

Bioinformatic identification of key candidate genes and pathways in axon regeneration after spinal cord injury in zebrafish

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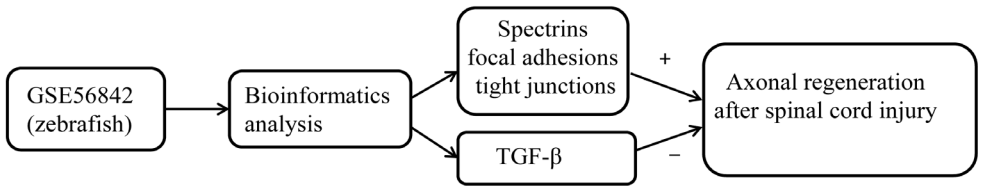
Graphical Abstract

Spectrins, focal adhesions and tight junctions may promote axonal regeneration after spinal cord injury, while transforming growth factor beta (TGF-β) does the opposite



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Abstract

Zebrafish and human genomes are highly homologous; however, despite this genomic similarity, adult zebrafish can achieve neuronal proliferation, regeneration and functional restoration within 6–8 weeks after spinal cord injury, whereas humans cannot. To analyze differentially expressed zebrafish genes between axon-regenerated neurons and axon-non-regenerated neurons after spinal cord injury, and to explore the key genes and pathways of axonal regeneration after spinal cord injury, microarray GSE56842 was analyzed using the online tool, GEO2R, in the Gene Expression Omnibus database. Gene ontology and protein-protein interaction networks were used to analyze the identified differentially expressed genes. Finally, we screened for genes and pathways that may play a role in spinal cord injury repair in zebrafish and mammals. A total of 636 differentially expressed genes were obtained, including 255 up-regulated and 381 down-regulated differentially expressed genes in axon-regenerated neurons. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment results were also obtained. A protein-protein interaction network contained 480 node genes and 1976 node connections. We also obtained the 10 hub genes with the highest correlation and the two modules with the highest score. The results showed that spectrin may promote axonal regeneration after spinal cord injury in zebrafish. Transforming growth factor beta signaling may inhibit repair after spinal cord injury in zebrafish. Focal adhesion or tight junctions may play an important role in the migration and proliferation of some cells, such as Schwann cells or neural progenitor cells, after spinal cord injury in zebrafish. Bioinformatic analysis identified key candidate genes and pathways in axonal regeneration after spinal cord injury in zebrafish, providing targets for treatment of spinal cord injury in mammals.

Key Words: *axonal regeneration; differentially expressed genes; focal adhesions; Gene Ontology; Kyoto Encyclopedia of Genes and Genomes; neural regeneration; protein-protein interaction network; signaling pathway; spectrin; tight junctions; transforming growth factor beta; Wnt signaling pathway*

Chinese Library Classification No. *R446; R364; R741*

Introduction

In mammals, spinal cord injury (SCI) is a destructive neurological disorder that often results in the loss of sensory and motor functions (Yu et al., 2016; Dyck and Karimi-Abdolrezaee, 2018; Sharma et al., 2019). After SCI, the non-regenerative characteristics of the central nervous system lead to neuronal death in the spinal cord (Pinto and Gotz, 2007; Wei et al., 2019). In contrast to mammals, adult zebrafish can achieve neuronal proliferation, regeneration and functional restoration within 6–8 weeks after SCI via several regenerative processes that evade cell death (Becker et al., 1998; Briona et al., 2015). Zebrafish and human genomes are highly homologous; therefore, zebrafish is used as a regeneration model (Howe et al., 2013). However, not all axons regenerate after SCI in adult zebrafish (Vajn et al., 2013) and the mechanisms of recovery after SCI in zebrafish and the significant differences between zebrafish axons are not fully understood.

In the past decade, there have been many studies on SCI in zebrafish that have identified SCI regeneration mechanisms that are similar between zebrafish and mammals. For example, fibroblast growth factor (FGF) signaling in zebrafish can promote regeneration after SCI by means of multiple mechanisms (Goldshmit et al., 2012). Similarly, many studies have demonstrated that FGF1 and FGF2 can protect neurons and promote axonal regeneration and recovery of movement function in mammalian SCI models (Kuo et al., 2011; Zhang et al., 2013). Other similar mechanisms involve bcl-2, phospho-Akt and miR-133b (Seki et al., 2003; Yu et al., 2011; Ogai et al., 2012; Zhang et al., 2016; Theis et al., 2017). Study of zebrafish regeneration mechanisms after SCI leads to understanding the zebrafish regeneration process and provides new research targets for SCI regeneration in mammals.

In recent years, microarray technology has been extensively used to study various biological mechanisms and a growing number of studies have used microarray technology to study the pathophysiology of diseases (Vogelstein et al., 2013; Hao et al., 2018). The purpose of this study was to identify the key genes and pathways involved in zebrafish axonal regeneration after SCI using bioinformatic methods and to provide new ideas and therapeutic targets for the treatment of mammalian SCI.

Materials and Methods

Identification of differentially expressed genes (DEGs)

The original GSE56842 datasets were downloaded from NCBI GEO (available online: http://www.ncbi.nlm.nih.gov/geo/). These datasets were submitted by Vajn et al. (2013) and are based on the GPL1319 Platform (Affymetrix Zebrafish Genome Array, Affymetrix Technologies, Santa Clara, CA, USA). The data were derived from a complete spinal cord transection with fluoro-ruby retrograde tracing performed at the 8th vertebral level in adult zebrafish. The fluoro-ruby dye labeled all the neurons in the brain that projected their axons to the 8th vertebra. Three weeks later, fluoro-emerald was injected 4 mm distal to the spinal cord transection site, thereby labeling all neurons that regenerated their axons to this level. The zebrafish were sacrificed 1 week after fluoro-emerald tracing and the brains were enzymatically dissociated. Cells were sorted using fluorescence-activated cell sorting. The GSE56842 dataset contains nine samples, including three non-regenerated neuron samples, three regenerated neuron samples, and three non-lesion samples. In the present study, the regenerated neuron samples and non-regenerated neuron samples were selected for analysis.

The original gene expression profile data were analyzed on the Morpheus website (available online: https://software.broadinstitu te.org/morpheus/) to obtain a heat map of the most significant DEGs. GEO2R was used to obtain the DEGs (available online: https://www.ncb i.nlm.nih.gov/geo/geo2r/). We considered $P < 0.05$ to show statistical significance and [logFC] > 1 as the cut-off value.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

GO analysis is a common method of gene function annotation that can be used for the analysis of transcriptome data (Ashburner et al., 2000, 2006). KEGG is a database resource for studying advanced functions and biological systems from the molecular level, and is especially useful for genomic sequencing data (Ogata et al., 1999). DAVID (available online: https://david.ncifcrf.gov/) was used to analyze candidate DEG functions and pathway enrichment. $P < 0.05$ was considered statistically significant.

Module screening from integration of protein-protein interaction (PPI) networks

STRING (available online: http://string-db.org) (Franceschini et al., 2013) was employed to evaluate the interaction among DEGs. Cytoscape software (Shannon et al., 2003) was used to analyze the PPI networks. To calculate the number of interconnections, the network analyzer plug-in was used to filter PPI hub genes. The modules of PPI networks were obtained by using Molecular Complex Detection in Cytoscape, and Molecular Complex Detection scores > 5 were used. Finally, DAVID was used to analyze module pathway enrichment. $P < 0.05$ was considered statistically significant.

Results

Identification of DEGs

Taking $P < 0.05$ and [logFC] > 1 as inclusion conditions, we identified 636 DEGs, including 255 upregulated and 381 downregulated DEGs, in the regenerated neuron samples compared with the non-regenerated neuron samples from the GSE56842 expression profile dataset. Using the Morpheus website, we obtained a heat map of the top 50 upregulated and downregulated DEGs, revealing the top 100 significantly DEGs (**Figure 1**).

GO analysis of DEGs

All DEGs were uploaded to DAVID and the overrepresented GO categories and KEGG pathways were identified. The top 30 enriched GO terms are shown in **Figure 2**; they were mainly in the biological process group, such as multicellular organism development, cell differentiation, and negative

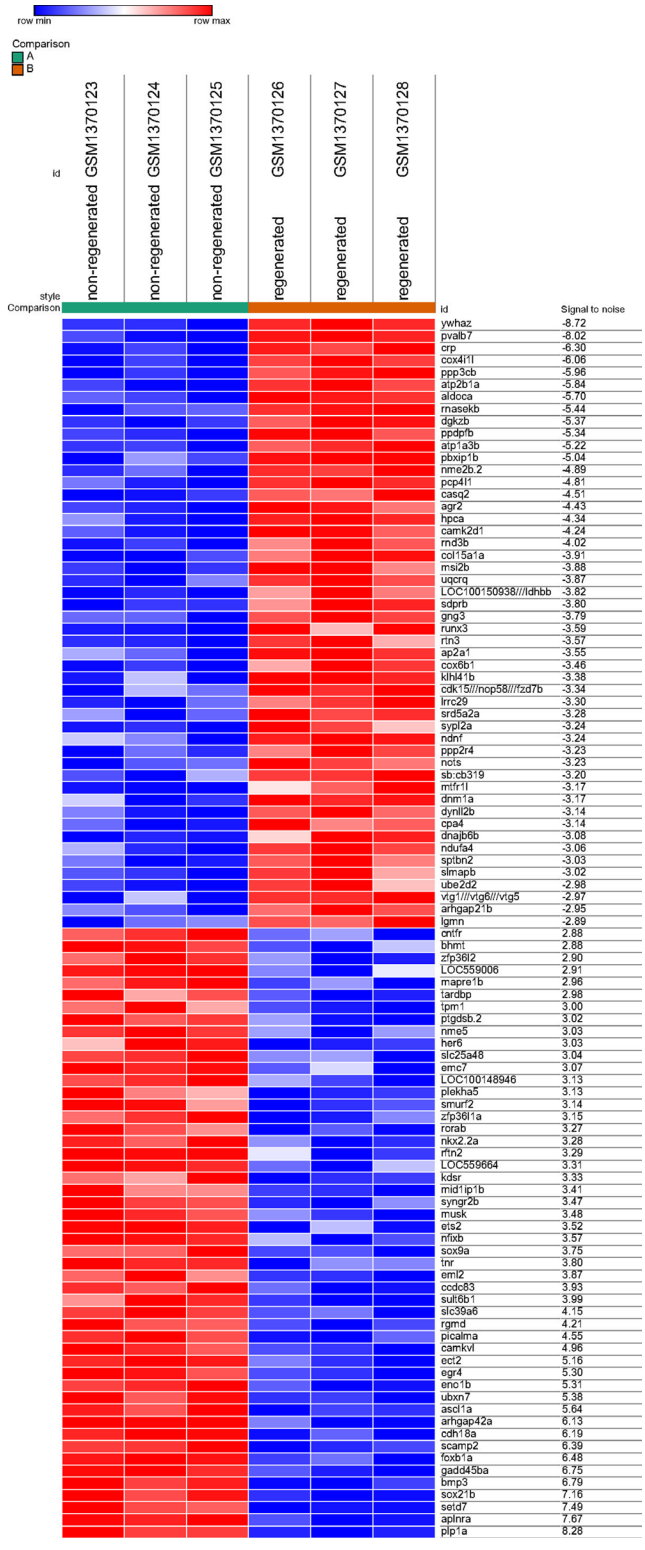

Figure 1 Heat map of the top 50 up-regulated and down-regulated differentially expressed genes.
The three columns on the left are the axon-non-regenerated neuron samples. The three columns on the right are the axon-regenerated neuron samples. Blue indicates down-regulation and red indicates up-regulation.

regulation of neurogenesis. In the molecular function group, DEGs were mainly enriched in structural constituent of cytoskeleton, cytochrome-c oxidase activity, and protein dimerization activity. There were only two GO terms in the cellular component group, mitochondrial respiratory chain complex IV, and spectrin. The upregulated DEGs were mainly enriched in cytochrome-c oxidase activity, structural constituent of cytoskeleton, mitochondrial respiratory chain complex IV, PDZ domain binding, and spectrin (**Table 1**). The downregulated DEGs were mainly enriched in multicellular organism development, negative regulation of neurogenesis, regulation of transcription, positive regulation of sequence-specific DNA binding transcription, and regulation of transcription DNA-templated (**Table 1**).

Signaling pathway enrichment analysis

As shown in **Table 2**, the upregulated genes were mainly enriched in cardiac muscle contraction, oxidative phosphoryla-

Table 1 Top 10 significant enriched gene Gene Ontology (GO) terms of up-regulated and down-regulated differentially expressed genes (DEGs) in regenerated neurons

Term	Description	Count	*P*-value
Up-regulated			
GO: 0004129	Cytochrome-c oxidase activity	6	1.68E-05
GO: 0005200	Structural constituent of cytoskeleton	6	2.98E-04
GO: 0005751	Mitochondrial respiratory chain complex IV	4	5.75E-04
GO: 0030165	PDZ domain binding	4	5.89E-04
GO: 0008091	Spectrin	3	7.43E-04
GO: 0006814	Sodium ion transport	6	1.54E-03
GO: 0006811	Ion transport	15	2.44E-03
GO: 0005890	Sodium:potassium-exchanging ATPase complex	3	2.54E-03
GO: 0005543	Phospholipid binding	4	5.09E-03
GO: 0005388	Calcium-transporting ATPase activity	3	9.17E-03
Down-regulated			
GO: 0007275	Multicellular organism development	31	1.76E-07
GO: 0050768	Negative regulation of neurogenesis	5	3.42E-05
GO: 0006355	Regulation of transcription, DNA-templated	46	3.60E-05
GO: 0051091	Positive regulation of sequence-specific DNA binding transcription Factor activity	5	7.20E-05
GO: 0006351	Transcription, DNA-templated	30	1.34E-04
GO: 0045666	Positive regulation of neuron differentiation	5	2.26E-04
GO: 0007219	Notch signaling pathway	7	2.73E-04
GO: 0046983	Protein dimerization activity	12	3.39E-04
GO: 0030154	Cell differentiation	14	3.63E-04
GO: 0042803	Protein homodimerization activity	10	4.14E-04

PDZ: Post-synaptic density protein 95, Drosophila disc large tumor suppressor and zona occludens 1.

Table 2 Enrichment KEGG pathways of up-regulated and down-regulated DEGs in regenerated neurons

Pathway	Name	Gene count	*P* value	Genes
Up-regulated DEGs				
dre04260	Cardiac muscle contraction	9	1.54E-04	*COX6B1*, *ATP1B1B*, *ATP1B2A*, *COX6A1*, *COX5AB*, *ATP1A3B*, *ATP1B2B*, *COX4I1L*, *COX7A2A*
dre00190	Oxidative phosphorylation	10	4.27E-04	*NDUFA4*, *NDUFB5*, *ATPV0E2*, *SDHC*, *COX6B1*, *COX6A1*, *COX5AB*, *COX4I1L*, *COX7A2A*, *ATP6V1F*
dre04261	Adrenergic signaling in cardiomyocytes	10	4.66E-03	*CAMK2D1*, *ATP2B2*, *PLCB4*, *CAMK2D2*, *ATP1B1B*, *ATP1B2A*, *ATP1A3B*, *ATP1B2B*, *PPP1CB*, *ATP2B1A*
dre04310	Wnt signaling pathway	8	1.96E-02	*CAMK2D1*, *PRICKLE1A*, *PLCB4*, *CAMK2D2*, *PPP3R1B*, *PPP3CB*, *RHOCB*, *RHOAB*
dre04114	Oocyte meiosis	7	2.27E-02	*CAMK2D1*, *YWHAZ*, *ANAPC13*, *CAMK2D2*, *PPP3R1B*, *PPP3CB*, *PPP1CB*
dre00071	Fatty acid degradation	4	3.27E-02	*GCDHB*, *CPT1AB*, *ACAT1*, *ACSL6*
dre04145	Phagosome	7	4.08E-02	*MARCO*, *ATPV0E2*, *TUBA2*, *MRC1A*, *TUBB2*, *TUBA8L2*, *ATP6V1F*
Down-regulated DEGs				
dre01130	Biosynthesis of antibiotics	13	1.28E-03	*HADHAA*, *CYP51*, *SHMT2*, *AK3*, *ADH5*, *HADHB*, *FDFT1*, *ENO1B*, *NME5*, *PAPSS2B*, *IDH1*, *PSAT1*, *PAICS*
dre00260	Glycine, serine and threonine metabolism	5	9.67E-03	*SHMT2*, *TDH*, *BHMT*, *MAO*, *PSAT1*
dre04350	TGF-beta signaling pathway	7	1.04E-02	*INHBB*, *MYCB*, *BMPR1AA*, *SMURF2*, *DCN*, *BMPR1AB*, *ID2B*
dre01230	Biosynthesis of amino acids	6	2.12E-02	*GLULB*, *SHMT2*, *GPT2L*, *IDH1*, *PSAT1*, *ENO1B*
dre04330	Notch signaling pathway	5	2.29E-02	*NOTCH3*, *JAG1B*, *NOTCH1A*, *HER6*, *LFNG*
dre00062	Fatty acid elongation	4	2.72E-02	*HADHAA*, *HACD2*, *ELOVL7A*, *HADHB*
dre01200	Carbon metabolism	7	2.94E-02	*HADHAA*, *SHMT2*, *GPT2L*, *ADH5*, *IDH1*, *PSAT1*, *ENO1B*

DEGs: Differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; TGF: transforming growth factor. The full names of the genes are shown in Additional Table 1.

tion, adrenergic signaling in cardiomyocytes, Wnt signaling pathway, and oocyte meiosis. The downregulated genes were mainly enriched in biosynthesis of antibiotics, glycine, serine and threonine metabolism, transforming growth factor beta (TGF)-β signaling pathway, and biosynthesis of amino acids. As shown in **Figure 3**, the top 30 enriched pathways were obtained. The most significantly enriched pathways of all DEGs were biosynthesis of antibiotics, cardiac muscle contraction, fatty acid degradation, carbon metabolism, and Wnt signaling pathway.

PPI network and module analysis

The PPI network contained 480 nodes and 1976 edges. The top 10 hub nodes were obtained and included ras homolog gene family, member Ab (*RHOAB*); catenin (cadherin-associated protein), beta 1 (*CTNNB1*); YES proto-oncogene 1 (*YES1*); phosphoribosylaminoimidazole carboxylase (*PAICS*); mitogen-activated protein kinase 4 (*MAPK4*); Rho family GTPase 3a (*RND3A*); hydroxyacyl-CoA dehydrogenase, alpha subunit a (*HADHAA*); Rho family GTPase 3b (*RND3B*); fibroblast growth factor receptor 2 (*FGFR2*); and v-myc avian myelocytomatosis viral oncogene homolog b (*MYCB*). The top 10 hub genes and their corresponding degrees are shown in **Table 3**. Two significant modules with high corresponding degrees were obtained using Molecular Complex Detection in Cytoscape. As shown in **Figure 4**, module 1 consisted of nine nodes and 28 edges, and module 2 consisted of 56 nodes and 173 edges. Through pathway enrichment analysis, we conclude that nodes of module 1 were mainly enriched in focal adhesion, tight junctions, and regulation of actin cytoskeleton (**Table 4**), and nodes of module 2 were mainly enriched in cardiac muscle contraction, cytokine-cytokine receptor interaction, and TGF-β signaling pathway (**Table 5**).

Table 3 Top 10 core genes and their corresponding degree (score), and expression in neurons with axonal regeneration

Gene	Full name of gene	Degree	Up or Down
RHOAB	Ras homolog gene family, member Ab	71	Up
CTNNB1	Catenin (cadherin-associated protein), beta 1	69	Down
YES1	YES proto-oncogene 1	60	Down
PAICS	Phosphoribosylaminoimidazole carboxylase	54	Down
MAPK4	Mitogen-activated protein kinase 4	49	Down
RND3A	Rho family GTPase 3a	43	Down
HADHAA	Hydroxyacyl-CoA dehydrogenase, alpha subunit a	42	Down
RND3B	Rho family GTPase 3b	39	Up
FGFR2	Fibroblast growth factor receptor 2	35	Down
MYCB	Myelocytomatosis viral oncogene homolog b	35	Down

CoA: Coenzyme A; GTP: guanosine triphosphate.

Discussion

In this study, 636 DEGs were obtained from analysis of cohort profile datasets of GSE56842. GO and KEGG pathway enrichment analyses were then performed. From the GO analysis results, the up-regulated DEGs were involved in cytochrome-c oxidase activity, spectrin, and phospholipid binding, and down-regulated DEGs were involved in negative regulation of neurogenesis, positive regulation of neuron differentiation, Notch signaling and cell differentiation. Spectrins consist of two α and two β subunits and are widely expressed. They have the ability to assemble neuronal excitable regions (Berghs et al., 2000; Yang et al., 2007). Many studies indicate that breakdown of spectrins is an important mechanism in neurodegenerative diseases and injuries. For example, the lack of αII spectrin in mice and zebrafish leads to the death of embryos and larvae because of a stunted nervous system (Stankewich et al., 2011). In fact, spectrins are potent substrates for calpain, and their degradation has been related to brain injury (Schafer et al., 2009). A recent study showed that αII spectrin is critical for the development and synaptogenesis of dendrites and axons (Wang et al., 2018). In the peripheral nervous system of zebrafish, spectrins in Schwann cells can promote the myelination of axons (Susuki et al., 2011). Moreover, Schwann cells are extensively distributed after SCI in zebrafish (Hui et al., 2010). We suggest that spectrins may synergize with Schwann cells to promote nerve regeneration after SCI in zebrafish, and may also promote repair after SCI in mammals.

In most multicellular organisms, the Notch signaling pathway is a highly conserved cellular signaling system that contains four receptors, Notch1–4 (Artavanis-Tsakonas et al., 1999; Kumar et al., 2016). In mammals, Notch1 expression is enhanced in response to injury, and activation of Notch signaling prevents spinal cord progenitor cells from producing neurons. This inhibitory effect can be inhibited *in vitro* by attenuating the Notch signaling pathway (Yamamoto et al., 2001). In addition, Notch signaling inhibits motor neuron generation and progenitor proliferation in the injured ventromedial spinal cord. In zebrafish at 14 days post SCI, Notch1a and Notch1b are significantly upregulated in the spinal cord. Altogether, Notch signaling is a negative signal for nerve regrowth, and inhibiting Notch signaling can enhance the regeneration of spinal motor neurons in adult vertebrates (Dias et al., 2012).

From the KEGG analysis results, the upregulated DEGs were mainly enriched in cardiac muscle contraction, oxidative phosphorylation, adrenergic signaling in cardiomyocytes and Wnt signaling, while downregulated DEGs were mainly enriched in the biosynthesis of antibiotics, glycine, serine and threonine metabolism, TGF-β, biosynthesis of amino acids, Notch signaling pathways and fatty acid elongation. Upregulated DEGs were enriched in cardiac muscle contraction. Adrenergic signaling in cardiomyocytes is mainly because upregulated DEGs contain some genes for cytochrome oxidase, sodium-potassium ATPase and calcium channel. These illustrate that the basal metabolism of neurons is active after axonal regeneration. The Wnt signaling pathway is crucial in development and growth and is associated with cell differentiation, polarization, and migration during development. Wnt signaling is based on one canonical β-catenin-dependent and two β-catenin-independent non-canonical Wnt pathways: the planar cell polarity signaling pathway and the Ca^{2+} Wnt pathway (Taciak et al., 2018). After damage in animals with the ability to regenerate, the activation of Wnt/ß-catenin signaling is enhanced, and attenuating activation of the signaling can inhibit regeneration (Yokoyama et al., 2007). A previous study in zebrafish showed that Wnt/ß-catenin signaling is activated in glia after SCI, and when the signaling pathway is suppressed, neurogenesis is reduced and axon regrowth fails (Briona et al., 2015).

The TGF-β family, which works mainly through the SMAD pathway is composed of evolutionarily conserved polypeptides that have essential functions in homeostasis, growth and development of cells and tissues. However, they also function in the pathogenesis of diseases, including neuronal degeneration and tumor formation (Yamagata et al., 2005; Ueberham et al., 2006; Chalmers and Love, 2007; Jaskova et al., 2014). TGF-β is also an anti-inflammatory cytokine

Table 4 The enriched pathways in module 1

Term	*P*-value	Count	%	Genes
Focal adhesion	8.77E-04	4	50.0	*CRKL*, *PTENB*, *BCAR1*, *RHOAB*
Tight junction	9.89E-03	3	37.5	*PTENB*, *RHOAB*, *YES1*
Regulation of actin cytoskeleton	2.24E-02	3	37.5	*CRKL*, *BCAR1*, *RHOAB*

The full names of the genes are shown in Additional Table 3.

Table 5 The enriched pathways in module 2

Term	*P*-value	Count	%	Genes
Cardiac muscle contraction	2.00E-03	5	9.80	*COX6B1*, *COX6A1*, *COX5AB*, *TPM1*, *ATP1A3A*
Cytokine-cytokine receptor interaction	2.12E-03	6	11.76	*KITA*, *FLT4*, *VEGFAA*, *BMPR1AA*, *BMPR1AB*, *CXCL12A*
TGF-beta signaling pathway	2.24E-03	5	9.80	*INHBB*, *MYCB*, *BMPR1AA*, *SMURF2*, *BMPR1AB*
Melanogenesis	7.17E-03	5	9.80	*KITA*, *FZD7B*, *CALM1A*, *FZD7A*, *CTNNB1*
Oxidative phosphorylation	7.74E-03	5	9.80	*NDUFA4*, *NDUFB5*, *COX6B1*, *COX6A1*, *COX5AB*
Biosynthesis of antibiotics	4.18E-02	5	9.80	*GOT1*, *PAPSS2B*, *OGDHL*, *IDH1*, *LDHBB*

TGF: Transforming growth factor. The full names of the genes are shown in Additional Table 4.

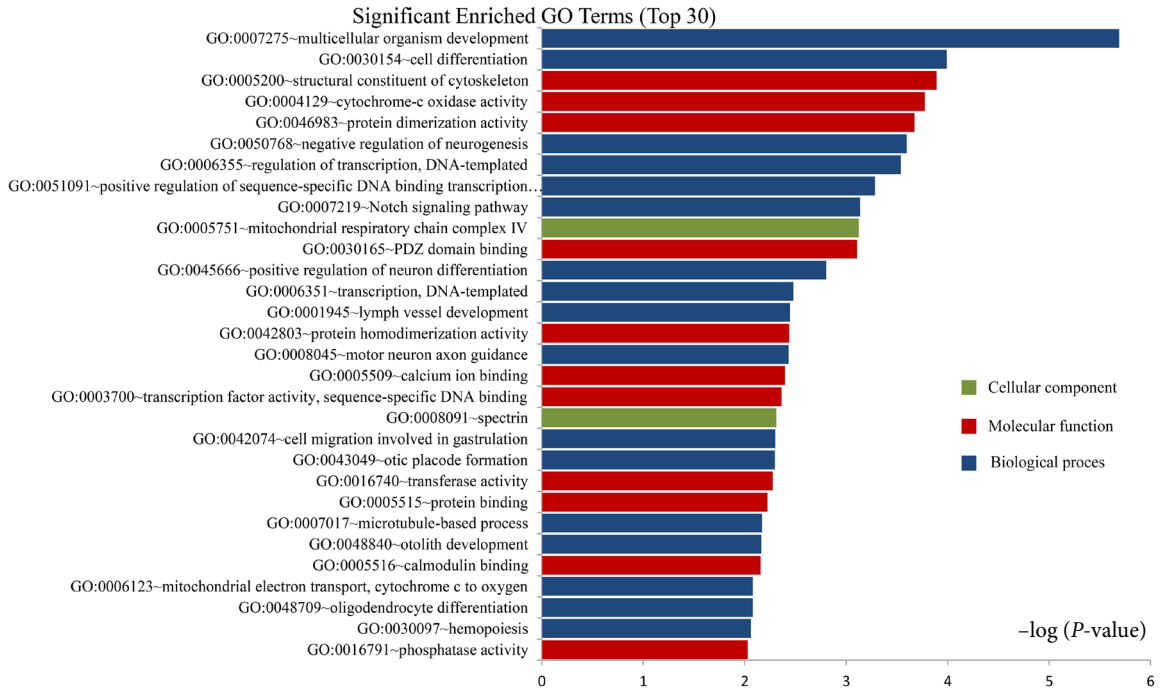

Figure 2 Top 30 significantly enriched gene ontology (GO) terms of differentially expressed genes in regenerated neurons.
The green bars represent cellular component. The red bars represent molecular function. The blue bars represent biological process. The length of the bar represents the –log (*P*-value); credibility increases with bar length. The ordinate is the GO terms, and the abscissa is the –log (*P*-value) of GO terms.

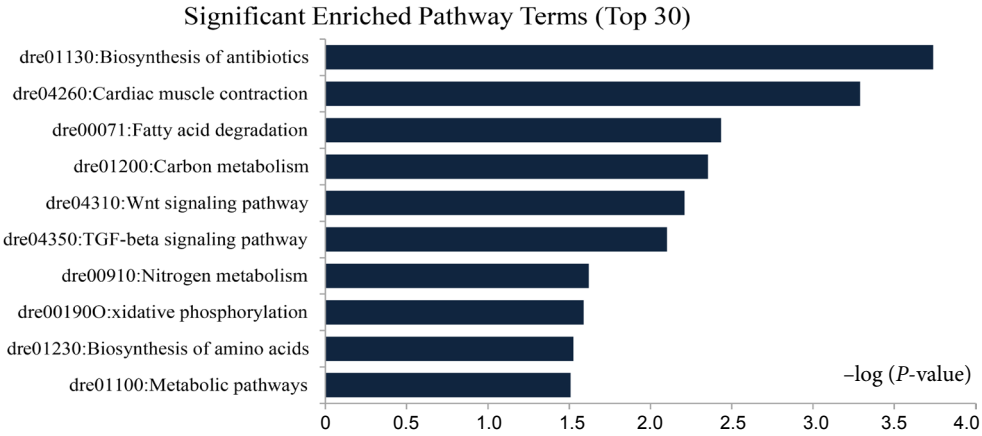

Figure 3 Top 30 significantly enriched pathways of DEGs in regenerated neurons.
The ordinate is KEGG signaling pathways, and the abscissa is the –log (*P*-value) of pathways. DEGs: Differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes.

in the injured spinal cord after SCI (Semple-Rowland et al., 1995; Nakamura et al., 2003). A previous study suggested that SCI can lead to elevation of TGF-β, which might lead to the degeneration of neurons and the formation of glial scar after SCI (Joko et al., 2013). Both neuronal degeneration and glial scar are factors that hinder repair after SCI (Yuan and He, 2013; Sofroniew, 2018). Another study showed that inhibition of TGF-β1 in rats can promote axonal regeneration and preservation, and also reduce the formation of glial scar after SCI (Kohta et al., 2009). In mammals, TGF-β hinders nerve regeneration after SCI, and the down-regulated DEGs in the present study are enriched in the TGF-β pathway, indicating that TGF-β may also play a role in inhibiting nerve regeneration in zebrafish SCI.

We identified the top 10 degree hub genes: *RHOAB*, *CTNNB1*, *YES1*, *PAICS*, *MAPK4*, *RND3A*, *HADHAA*, *RND3B*, *FGFR2*, and *MYCB*. Catenin β-1 is encoded by the *CTNNB1* gene. Catenin β-1 participates in the formation of a cadherin protein complex and is also a signal converter of the Wnt signaling pathway, plays an important role in cell adhesion and gene transcription (Peifer et al., 1991, 1994; Noordermeer et al., 1994). In some cell types, an elevated level of β-catenin helps maintain pluripotency, and it may also promote cell differentiation at the developmental stage (Sokol, 2011). Consistent with the GO term results revealing that neuron differentiation and cell differentiation were downregulated, *CTNNB1* was also downregulated in the axon-regenerated neurons. However, previous studies only focused on the promotion of differentiation to facilitate SCI repair (Xu et al., 2017a; Geissler et al., 2018; Li et al., 2018). No studies have

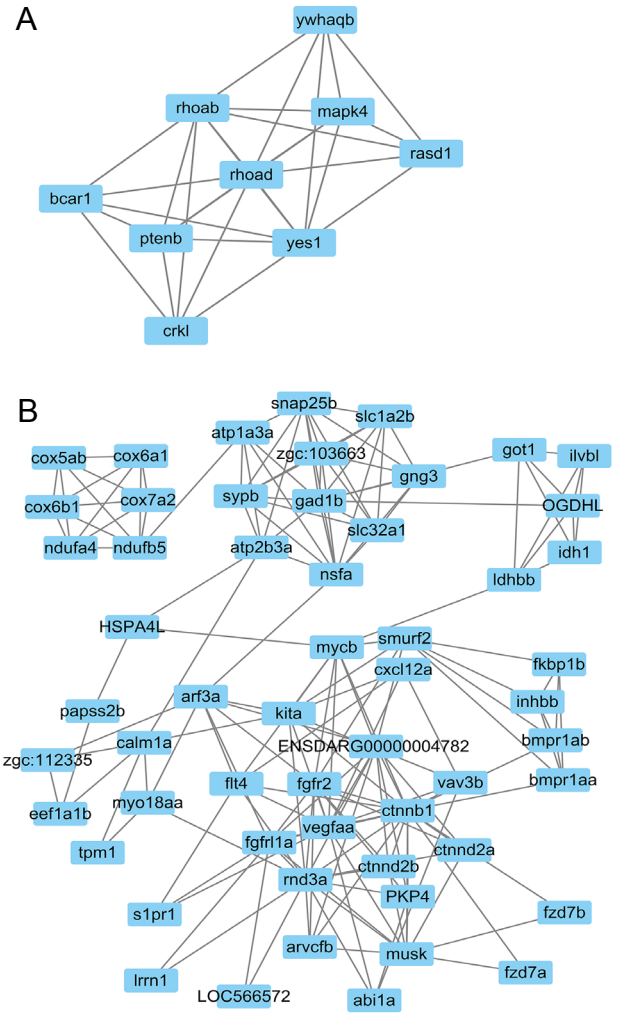

Figure 4 The top two modules with high corresponding degrees from the protein-protein interaction network.
(A) The first module and nodes. (B) The second module and nodes. The full names of the proteins are shown in Additional Table 2.

described inhibition of the *CTNNB1* gene to promote the repair of SCI.

The proto-oncogene tyrosine-protein kinase, YES, is an enzyme encoded by the *YES1* gene (Semba et al., 1985), which belongs to the Src protein family. Secondary damage after SCI includes edema formation. Vascular endothelial growth factor, an endothelial mitogen as well as a potent mediator of vascular permeability, contributes to brain formation by binding of the vascular endothelial growth factor receptor, leading to recruitment and stimulation of the catalytic activity of Src (Senger et al., 1983; Cobbs et al., 1998; Schlessinger, 2000). An inhibitor of Src family tyrosine kinases reduces edema and the inflammatory response to SCI, subsequently improving motor function (Akiyama et al., 2003, 2004). Fibroblast growth factor receptor 2 (FGFR2), also known as cluster of differentiation 332 (CD332), plays vital roles in embryonic development and tissue repair (Xu et al., 2017b; Ishiwata, 2018). FGFR2 is involved in anti-apoptosis and nerve repair activated by FGF10 (Chen et al., 2017). At present, the role of FGFR2 in the central nervous system is poorly understood. These genes are involved in inflammation, cell proliferation and apoptosis, but their role in SCI is unclear; therefore, the role of these genes is worth studying.

The pathway analysis of modules showed that they were mainly associated with focal adhesion, tight junctions, regulation of actin cytoskeleton, cytokine-cytokine receptor interaction, TGF-β signaling, melanogenesis, and oxidative phosphorylation. In cell biology, focal adhesions are associated with mechanical force and signal transduction between cells or between cells and the extracellular matrix. In addition to anchoring the cell, they act as signal carriers, informing the cell about the condition of the extracellular matrix and thus influence their behavior (Riveline et al., 2001). In addition, focal adhesions are involved in cell motility, proliferation, migration and survival (Zaidel-Bar et al., 2004; Fu et al., 2010; Li et al., 2012; Liu et al., 2018; Yan et al., 2018). Tight junctions, also known as occluding junctions or zonulae occludentes, are multiprotein junctional complexes whose general function is to prevent leakage of transported solutes and water and to seal the paracellular pathway (Anderson and Van Itallie, 2009). Tight junctions also play a critical role in cell migration, proliferation, and differentiation (Kurasawa et al., 2011; Wu et al., 2011; Gonzalez-Mariscal et al., 2017). Although, tight junctions are mainly associated with epithelial and endothelial cells, no study has elucidated the relationship between focal adhesions or tight junctions and SCI. Nevertheless, the migration, proliferation and differentiation of Schwann cells and neural progenitor cells play an important role in repair after SCI in zebrafish (Reimer et al., 2008; Hui et al., 2010; Briona and Dorsky, 2014). However, based on the current findings, we believe that focal adhesion or tight junctions may play an important role in the repair of SCI in zebrafish.

In the present study, through functional enrichment analysis for DEGs, we have highlighted many known pathways involved in the repair of SCI, including Wnt and Notch signaling pathways. By analyzing the top 10 hub genes, we identified several genes worth studying, including *CTNNB1*, *YES1* and *FGFR2*. In addition, through the enrichment of DEGs and functional analysis, we found that spectrins may promote the regeneration of SCI axons in zebrafish and mammals, while TGF-β signals may inhibit repair after SCI in zebrafish. In addition, focal adhesion and tight junctions might promote the migration and proliferation of some zebrafish cells after SCI, such as Schwann cells or neural progenitor cells, and promote repair after SCI.

In summary, this study helps us better understand the repair mechanism of SCI in zebrafish and provides new research targets for mammalian SCI. However, pathway or gene functions were not verified in this study, and more in-depth studies are needed to explore the role of identified genes and pathways in the repair of SCI in zebrafish and mammalian species.

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Additional file 1: *Open peer review report 1.*
Additional Table 1: *The full names of the genes in Table 2.*
Additional Table 2: *The full names of the proteins in Figure 4.*
Additional Table 3: *The full names of the genes in Table 4.*
Additional Table 4: *The full names of the genes in Table 5.*

References

Akiyama C, Yuguchi T, Nishio M, Fujinaka T, Taniguchi M, Nakajima Y, Yoshimine T (2003) Src family kinase inhibitor PP1 improves motor function by reducing edema after spinal cord contusion in rats. Acta Neurochir Suppl 86:421-423.

Akiyama C, Yuguchi T, Nishio M, Tomishima T, Fujinaka T, Taniguchi M, Nakajima Y, Kohmura E, Yoshimine T (2004) Src family kinase inhibitor PP1 reduces secondary damage after spinal cord compression in rats. J Neurotrauma 21:923-931.

Anderson JM, Van Itallie CM (2009) Physiology and function of the tight junction. Cold Spring Harb Perspect Biol 1:a002584.

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284:770-776.

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The gene ontology consortium. Nat Genet 25:25-29.

Becker T, Bernhardt RR, Reinhard E, Wullimann MF, Tongiorgi E, Schachner M (1998) Readiness of zebrafish brain neurons to regenerate a spinal axon correlates with differential expression of specific cell recognition molecules. J Neurosci 18:5789-5803.

Berghs S, Aggujaro D, Dirkx R Jr., Maksimova E, Stabach P, Hermel JM, Zhang JP, Philbrick W, Slepnev V, Ort T, Solimena M (2000) betaIV spectrin, a new spectrin localized at axon initial segments and nodes of ranvier in the central and peripheral nervous system. J Cell Biol 151:985-1002.

Briona LK, Dorsky RI (2014) Radial glial progenitors repair the zebrafish spinal cord following transection. Exp Neurol 256:81-92.

Briona LK, Poulain FE, Mosimann C, Dorsky RI (2015) Wnt/ss-catenin signaling is required for radial glial neurogenesis following spinal cord injury. Dev Biol 403:15-21.

Chalmers KA, Love S (2007) Neurofibrillary tangles may interfere with Smad 2/3 signaling in neurons. J Neuropathol Exp Neurol 66:158-167.

Chen J, Wang Z, Zheng Z, Chen Y, Khor S, Shi K, He Z, Wang Q, Zhao Y, Zhang H, Li X, Li J, Yin J, Wang X, Xiao J (2017) Neuron and microglia/macrophage-derived FGF10 activate neuronal FGFR2/PI3K/Akt signaling and inhibit microglia/macrophages TLR4/NF-kappaB-dependent neuroinflammation to improve functional recovery after spinal cord injury. Cell Death Dis 8:e3090.

Cobbs CS, Chen J, Greenberg DA, Graham SH (1998) Vascular endothelial growth factor expression in transient focal cerebral ischemia in the rat. Neurosci Lett 249:79-82.

Dias TB, Yang YJ, Ogai K, Becker T, Becker CG (2012) Notch signaling controls generation of motor neurons in the lesioned spinal cord of adult zebrafish. J Neurosci 32:3245-3252.

Dyck SM, Karimi-Abdolrezaee S (2018) Role of chondroitin sulfate proteoglycan signaling in regulating neuroinflammation following spinal cord injury. Neural Regen Res 13:2080-2082.

Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C, Jensen LJ (2013) STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 41:D808-D815.

Fu XD, Goglia L, Sanchez AM, Flamini M, Giretti MS, Tosi V, Genazzani AR, Simoncini T (2010) Progesterone receptor enhances breast cancer cell motility and invasion via extranuclear activation of focal adhesion kinase. Endocr Relat Cancer 17:431-443.

Geissler SA, Sabin AL, Besser RR, Gooden OM, Shirk BD, Nguyen QM, Khaing ZZ, Schmidt CE (2018) Biomimetic hydrogels direct spinal progenitor cell differentiation and promote functional recovery after spinal cord injury. J Neural Eng 15:025004.

Goldshmit Y, Sztal TE, Jusuf PR, Hall TE, Nguyen-Chi M, Currie PD (2012) Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. J Neurosci 32:7477-7492.

Gonzalez-Mariscal L, Miranda J, Raya-Sandino A, Dominguez-Calderon A, Cuellar-Perez F (2017) ZO-2, a tight junction protein involved in gene expression, proliferation, apoptosis, and cell size regulation. Ann N Y Acad Sci 1397:35-53.

Hao M, Ji XR, Chen H, Zhang W, Zhang LC, Zhang LH, Tang PF, Lu N (2018) Cell cycle and complement inhibitors may be specific for treatment of spinal cord injury in aged and young mice: transcriptomic analyses. Neural Regen Res 13:518-527.

Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L, McLaren S, Sealy I, Caccamo M, Churcher C, Scott C, Barrett JC, Koch R, Rauch GJ, White S, Chow W, et al. (2013) The zebrafish reference genome sequence and its relationship to the human genome. Nature 496:498-503.

Hui SP, Dutta A, Ghosh S (2010) Cellular response after crush injury in adult zebrafish spinal cord. Dev Dyn 239:2962-2979.

Ishiwata T (2018) Role of fibroblast growth factor receptor-2 splicing in normal and cancer cells. Front Biosci (Landmark Ed) 23:626-639.

Jaskova K, Pavlovicova M, Cagalinec M, Lacinova L, Jurkovicova D (2014) TGFbeta1 downregulates neurite outgrowth, expression of Ca^{2+} transporters, and mitochondrial dynamics of in vitro cerebellar granule cells. Neuroreport 25:340-346.

Joko M, Osuka K, Usuda N, Atsuzawa K, Aoyama M, Takayasu M (2013) Different modifications of phosphorylated Smad3C and Smad3L through TGF-beta after spinal cord injury in mice. Neurosci Lett 549:168-172.

Kohta M, Kohmura E, Yamashita T (2009) Inhibition of TGF-beta1 promotes functional recovery after spinal cord injury. Neurosci Res 65:393-401.

Kumar R, Juillerat-Jeanneret L, Golshayan D (2016) Notch antagonists: potential modulators of cancer and inflammatory diseases. J Med Chem 59:7719-7737.

Kuo HS, Tsai MJ, Huang MC, Chiu CW, Tsai CY, Lee MJ, Huang WC, Lin YL, Kuo WC, Cheng H (2011) Acid fibroblast growth factor and peripheral nerve grafts regulate Th2 cytokine expression, macrophage activation, polyamine synthesis, and neurotrophin expression in transected rat spinal cords. J Neurosci 31:4137-4147.

Kurasawa M, Maeda T, Oba A, Yamamoto T, Sasaki H (2011) Tight junction regulates epidermal calcium ion gradient and differentiation. Biochem Biophys Res Commun 406:506-511.

Li JX, Fu LJ, Yang XG, Wang K (2012) Integrin-mediated signaling contributes to gadolinium-containing-particle-promoted cell survival and G(1) to S phase cell cycle transition by enhancing focal adhesion formation. J Biol Inorg Chem 17:375-385.

Li X, Fan C, Xiao Z, Zhao Y, Zhang H, Sun J, Zhuang Y, Wu X, Shi J, Chen Y, Dai J (2018) A collagen microchannel scaffold carrying paclitaxel-liposomes induces neuronal differentiation of neural stem cells through Wnt/beta-catenin signaling for spinal cord injury repair. Biomaterials 183:114-127.

Liu C, Qu L, Zhao C, Shou C (2018) Extracellular gamma-synuclein promotes tumor cell motility by activating beta1 integrin-focal adhesion kinase signaling pathway and increasing matrix metalloproteinase-24, -2 protein secretion. J Exp Clin Cancer Res 37:117.

Nakamura M, Houghtling RA, MacArthur L, Bayer BM, Bregman BS (2003) Differences in cytokine gene expression profile between acute and secondary injury in adult rat spinal cord. Exp Neurol 184:313-325.

Noordermeer J, Klingensmith J, Perrimon N, Nusse R (1994) dishevelled and armadillo act in the wingless signalling pathway in Drosophila. Nature 367:80-83.

Ogai K, Hisano S, Mawatari K, Sugitani K, Koriyama Y, Nakashima H, Kato S (2012) Upregulation of anti-apoptotic factors in upper motor neurons after spinal cord injury in adult zebrafish. Neurochem Int 61:1202-1211.

Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M (1999) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 27:29-34.

Peifer M, Berg S, Reynolds AB (1994) A repeating amino acid motif shared by proteins with diverse cellular roles. Cell 76:789-791.

Peifer M, Rauskolb C, Williams M, Riggleman B, Wieschaus E (1991) The segment polarity gene armadillo interacts with the wingless signaling pathway in both embryonic and adult pattern formation. Development 111:1029-1043.

Pinto L, Gotz M (2007) Radial glial cell heterogeneity-the source of diverse progeny in the CNS. Prog Neurobiol 83:2-23.

Reimer MM, Sorensen I, Kuscha V, Frank RE, Liu C, Becker CG, Becker T (2008) Motor neuron regeneration in adult zebrafish. J Neurosci 28:8510-8516.

Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, Narumiya S, Kam Z, Geiger B, Bershadsky AD (2001) Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J Cell Biol 153:1175-1186.

Schafer DP, Jha S, Liu F, Akella T, McCullough LD, Rasband MN (2009) Disruption of the axon initial segment cytoskeleton is a new mechanism for neuronal injury. J Neurosci 29:13242-13254.

Schlessinger J (2000) New roles for Src kinases in control of cell survival and angiogenesis. Cell 100:293-296.

Seki T, Hida K, Tada M, Koyanagi I, Iwasaki Y (2003) Role of the bcl-2 gene after contusive spinal cord injury in mice. Neurosurgery 53:192-198.

Semba K, Yamanashi Y, Nishizawa M, Sukegawa J, Yoshida M, Sasaki M, Yamamoto T, Toyoshima K (1985) Location of the c-yes gene on the human chromosome and its expression in various tissues. Science 227:1038-1040.

Semple-Rowland SL, Mahatme A, Popovich PG, Green DA, Hassler G Jr., Stokes BT, Streit WJ (1995) Analysis of TGF-beta 1 gene expression in contused rat spinal cord using quantitative RT-PCR. J Neurotrauma 12:1003-1014.

Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF (1983) Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219:983-985.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498-2504.

Sharma S, Goel SA, Sharma S, Chhabra HS (2019) Polyetheretherketone cages used in anterior cervical discectomy and fusion surgery: a meta-analysis. Clin Trials Orthop Disord 4:29-33.

Sofroniew MV (2018) Dissecting spinal cord regeneration. Nature 557:343-350.

Sokol SY (2011) Maintaining embryonic stem cell pluripotency with Wnt signaling. Development 138:4341-4350.

Stankewich MC, Cianci CD, Stabach PR, Ji L, Nath A, Morrow JS (2011) Cell organization, growth, and neural and cardiac development require alphaII-spectrin. J Cell Sci 124:3956-3966.

Susuki K, Raphael AR, Ogawa Y, Stankewich MC, Peles E, Talbot WS, Rasband MN (2011) Schwann cell spectrins modulate peripheral nerve myelination. Proc Natl Acad Sci U S A 108:8009-8014.

Taciak B, Pruszynska I, Kiraga L, Bialasek M, Krol M (2018) Wnt signaling pathway in development and cancer. J Physiol Pharmacol doi: 10.26402/jpp.2018.2.07.

Theis T, Yoo M, Park CS, Chen J, Kugler S, Gibbs KM, Schachner M (2017) Lentiviral delivery of mir-133b improves functional recovery after spinal cord injury in mice. Mol Neurobiol 54:4659-4671.

Ueberham U, Ueberham E, Gruschka H, Arendt T (2006) Altered subcellular location of phosphorylated Smads in Alzheimer's disease. Eur J Neurosci 24:2327-2334.

Vajn K, Plunkett JA, Tapanes-Castillo A, Oudega M (2013) Axonal regeneration after spinal cord injury in zebrafish and mammals: differences, similarities, translation. Neurosci Bull 29:402-410.

Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr., Kinzler KW (2013) Cancer genome landscapes. Science 339:1546-1558.

Wang Y, Ji T, Nelson AD, Glanowska K, Murphy GG, Jenkins PM, Parent JM (2018) Critical roles of alphaII spectrin in brain development and epileptic encephalopathy. J Clin Invest 128:760-773.

Wei WB, Zou ZL, Zhou BB, Li BL, Qin JY, Feng ZF, Li JN, Ye LY, Wu RM (2019) Selection of injury segments in a rat model of spinal cord injury: network meta-analysis. Zhongguo Zuzhi Gongcheng Yanjiu 23:650-656.

Wu CY, Jhingory S, Taneyhill LA (2011) The tight junction scaffolding protein cingulin regulates neural crest cell migration. Dev Dyn 240:2309-2323.

Xu B, Zhao Y, Xiao Z, Wang B, Liang H, Li X, Fang Y, Han S, Li X, Fan C, Dai J (2017a) A dual functional scaffold tethered with egfr antibody promotes neural stem cell retention and neuronal differentiation for spinal cord injury repair. Adv Healthc Mater doi: 10.1002/adhm.201601279.

Xu W, Luo F, Wang Q, Tan Q, Huang J, Zhou S, Wang Z, Sun X, Kuang L, Jin M, Su N, Jiang W, Chen L, Qi H, Zhu Y, Chen B, Chen H, Chen S, Gao Y, Xu X, et al. (2017b) Inducible Activation of FGFR2 in adult mice promotes bone formation after bone marrow ablation. J Bone Miner Res 32:2194-2206.

Yamagata H, Matsuzaki K, Mori S, Yoshida K, Tahashi Y, Furukawa F, Sekimoto G, Watanabe T, Uemura Y, Sakaida N, Yoshioka K, Kamiyama Y, Seki T, Okazaki K (2005) Acceleration of Smad2 and Smad3 phosphorylation via c-Jun NH(2)-terminal kinase during human colorectal carcinogenesis. Cancer Res 65:157-165.

Yamamoto S, Nagao M, Sugimori M, Kosako H, Nakatomi H, Yamamoto N, Takebayashi H, Nabeshima Y, Kitamura T, Weinmaster G, Nakamura K, Nakafuku M (2001) Transcription factor expression and Notch-dependent regulation of neural progenitors in the adult rat spinal cord. J Neurosci 21:9814-9823.

Yan Y, Qin Q, Wu L, Jing X, Deng S, She Q (2018) Insulin-like growth factor 1 receptor signaling regulates embryonic epicardial cell proliferation through focal adhesion kinase pathway. Acta Biochim Biophys Sin (Shanghai) 50:976-983.

Yang Y, Ogawa Y, Hedstrom KL, Rasband MN (2007) betaIV spectrin is recruited to axon initial segments and nodes of Ranvier by ankyrinG. J Cell Biol 176:509-519.

Yokoyama H, Ogino H, Stoick-Cooper CL, Grainger RM, Moon RT (2007) Wnt/beta-catenin signaling has an essential role in the initiation of limb regeneration. Dev Biol 306:170-178.

Yu YM, Gibbs KM, Davila J, Campbell N, Sung S, Todorova TI, Otsuka S, Sabaawy HE, Hart RP, Schachner M (2011) MicroRNA miR-133b is essential for functional recovery after spinal cord injury in adult zebrafish. Eur J Neurosci 33:1587-1597.

Yu YQ, Hu NC, Duan JA, Li DP, Liu C (2016) Neuroprotective effects of sufentanil preconditioning on spinal cord injury in mouse models. Zhongguo Zuzhi Gongcheng Yanjiu 20:5966-5972.

Yuan YM, He C (2013) The glial scar in spinal cord injury and repair. Neurosci Bull 29:421-435.

Zaidel-Bar R, Cohen M, Addadi L, Geiger B (2004) Hierarchical assembly of cell-matrix adhesion complexes. Biochem Soc Trans 32:416-420.

Zhang D, Ma G, Hou M, Zhang T, Chen L, Zhao C (2016) The neuroprotective effect of puerarin in acute spinal cord injury rats. Cell Physiol Biochem 39:1152-1164.

Zhang HY, Zhang X, Wang ZG, Shi HX, Wu FZ, Lin BB, Xu XL, Wang XJ, Fu XB, Li ZY, Shen CJ, Li XK, Xiao J (2013) Exogenous basic fibroblast growth factor inhibits ER stress-induced apoptosis and improves recovery from spinal cord injury. CNS Neurosci Ther 19:20-29.

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