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ORIGINAL PAPER

Identification of Key Genes and Pathways Involved in the Heterogeneity of Intrinsic Growth Ability Between Neurons After Spinal Cord Injury in Adult Zebrafish

Haitao Fu[1,2] · Gonghai Han[3] · Haojiang Li[4] · Xuezhen Liang[5] · Die Hu[6] · Licheng Zhang[2] · Peifu Tang[2]

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Abstract

In the adult central nervous system (CNS), axon regeneration is a major hurdle for functional recovery after trauma. The intrinsic growth potential of an injured axon varies widely between neurons. The underlying molecular mechanisms of such heterogeneity are largely unclear. In the present study, the adult zebrafish dataset GSE56842 were downloaded. Differentially expressed genes (DEGs) were sorted and deeply analyzed by bioinformatics methods. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs were performed with the DAVID. A DEGs-associated protein–protein interaction network was constructed from the STRING database and visualized with Cytoscape software. In total, 621 DEGs were identified. GO analysis showed that the biological processes of DEGs focused mainly on the Notch signaling pathway, cell differentiation and positive regulation of neuron differentiation. The molecular functions mainly included calcium-transporting ATPase activity and calcium ion binding and structural constituents of the cytoskeleton. The cellular components included the plasma membrane, spectrin, and cytoplasmic and membrane-bound vesicles. KEGG pathway analysis showed that these DEGs were mainly involved in the metabolic pathway and Notch signaling pathway, and subnetworks revealed that genes within modules were involved in the metabolic pathway, Wnt signaling pathway, and calcium signaling pathway. This study identified DEG candidate genes and pathways involved in the heterogeneity of the intrinsic growth ability between neurons after spinal cord injury in adult zebrafish, which could facilitate our understanding of the molecular mechanisms underlying axon regeneration, and these candidate genes and pathways could be therapeutic targets for the treatment of CNS injury.

Keywords Axon regeneration · Bioinformatical analysis · Differentially expressed genes · Intrinsic growth ability · Spinal cord injury

✉ Licheng Zhang
zhanglcheng218@126.com

✉ Peifu Tang
pftang301@163.com

[1] School of Medicine, Nankai University, Tianjin 300110, China

[2] Department of Orthopedics, the General Hospital of Chinese People's Liberation Army, Beijing 100853, China

[3] Kunming Medical University, Kunming 650500, China

[4] Department of Microbiology and Immunology, Shanxi Medical University, Taiyuan 030000, China

[5] The First Clinical Medical School, Shandong University of Traditional Chinese Medicine, Shandong 250355, China

[6] Qingdao Eye Hospital, Shandong Eye Institute, Shandong Academy of Medical Sciences, Qingdao 266071, China

Introduction

For more than a century, numerous efforts have been made to investigate the mechanisms of regeneration failure in the adult mammalian central nervous system (CNS), which have been accompanied by substantial progress in the use of cell transplantation and molecular or bioengineering strategies for repairing the injured CNS and improving functional recovery in animal models [1–5]. Enhanced regrowth of some types of axons (e.g., brainstem, propriospinal and sensory ascending axons) are observed in most cases; however, the regrowth of corticospinal axons is relatively refractory to most therapeutic manipulations [6–8]. Thus, understanding the molecular differences underlying the heterogeneity of axon growth will be important to restore cortical-dependent function after injury in adults.

In cases of injury to the nervous system, a key pathological event is the axonal severing induced by the initial trauma, leading to neurological deficits and impairment [6, 9]. A logical repair strategy would be to first promote the regeneration of injured axons. For regeneration to occur, a prerequisite is that these injured axons must have the intrinsic ability to regrow. Compared with the peripheral nervous system (PNS), neurons in the CNS are generally thought to have less intrinsic ability to regenerate [10], especially corticospinal neurons [7]. A major challenge involved in functional recovery after CNS injury is to clarify the underlying molecular mechanisms that control the growth ability of adult neurons. Deciphering the different gene expression profiles in various neurons with different intrinsic growth capacities after CNS injury will facilitate our understanding of the molecular mechanisms involved in axon regeneration failure. Therefore, we aim to explore the key genes and pathways in CNS neurons after injury through bioinformatics analysis.

Gene expression microarrays, as a large-scale gene detection technique, can quickly detect all genes within the same sample time-point expression information, which makes this method a good choice for differentially expressed genes (DEGs) screening in human diseases [11, 12]. With the extensive application of high-throughput sequencing technology, large amounts of data have been created, published, and deposited in public databases. Reanalyzing these data can allow deeper research of molecular mechanisms. To identify the changes of gene expression profiling in spinal cord neurons after axotomy and during axon regeneration, to subsequently analyze the interactions among DEGs and the pathways in the interaction network, and, finally, to improve our understanding of growth heterogeneity between different axons in the CNS, we downloaded the original dataset GSE56842 from the Gene Expression Omnibus (GEO) database, which contained a total of 9 samples, with 3 regenerating, 3 nonregenerating and 3 traced, nonaxotomized samples of spinal neurons. These neuron cells were sorted using a fluorescence-activated cell sorter. We chose only the regenerating and nonregenerating neuron samples to identify regeneration-related genes between heterogeneous spinal neurons and analyze their biological functions and pathways.

Materials and Methods

Microarray Data and Quality Assessment

The gene expression profile dataset GSE56842 (Vajn et al. unpublished data 2018) was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The platform for GSE56842 is GPL1319, [Zebrafish] Affymetrix Zebrafish Genome Array. The platform files and raw data were downloaded as TXT files and CEL files, respectively. Only data from regenerating and nonregenerating neuron samples were extracted and further analyzed. Relative log expression (RLE), normalized unscaled standard errors (NUSE) and RNA degradation curves were performed with the R software (version 3.5.0) package affyPLM to assess the sample quality.

Data Preprocessing and DEG Screening

The R software package affy was used to preprocess the downloaded original CEL data. This process included background adjustment, normalization and expression calculation with the use of RMA. Probes not matching any known genes were removed, and the mean was calculated when multiple probes were matched to the same gene. The probe ID was converted into an international standard name for genes (gene symbol) and saved in a TXT file. The K-nearest neighbor (KNN) test was used to supplement missing values (using the R package impute). The DEGs of regenerating and nonregenerating neuron samples were identified using the R package limma. Genes with an adjusted p value < 0.05 and $|\log FC| > 2$ were considered DEGs.

Function and Pathway Enrichment Analysis of DEGs

To analyze the identified DEGs at the functional level, gene ontology analysis (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using DAVID (https://david.ncifcrf.gov/) [13]. DAVID 6.8 is an updated web server which provides a comprehensive set of functional annotation tools for investigators to understand biological meanings behind a large list of genes. In this study, we analyzed the candidate DEGs that were significantly up- and downregulated, and $P < 0.05$ was set as the threshold value.

Protein–Protein Interaction Network (PPI) and Modular Analysis

To study the interactive relationships among the DEGs, a PPI network of DEGs was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/cgi/input.pl). The combined score of > 0.4 was set, and the isolated nodes were removed. Then, the PPI network was visualized using the Cytoscape software. In this network, each node is a DEG, and the connections between nodes represent the interactions between these genes. Modular analysis was conducted with the Molecular Complex Detection (MCODE) app of Cytoscape software, and an MCODE score > 2 was set as the cut-off criterion. Pathway enrichment analysis was performed for DEGs within the modules. $P < 0.05$ was considered as a significant difference.

Results

Microarray Data Information and Quality

The microarray dataset GSE56842 about the identification of axonal regeneration-associated genes after spinal cord injury was chosen and downloaded, which includes 3 regenerating and 3 nonregenerating samples of spinal neurons. In the aspect of overall design of GSE56842, a complete spinal cord transection with Fluororuby retrograde tracing was performed at the 8th vertebra level in adult zebrafish. Fluororuby labeled all the neurons in the brain that project their axons to the 8th vertebra. Three weeks later Fluoroemerald was injected 4 mm distally from the spinal cord transection site, thereby labeling all neurons that regenerated their axons to this level. The zebrafish were sacrificed 1 week after Fluoroemerald tracing, brain enzymatically dissociated and cells sorted using fluorescence-activated cell sorter. Therefore, the non-and regeneration neurons could be successfully identified. Given that sample quality was essential for subsequent analysis, the CEL files were used to assess the microarray quality, and the results are shown in Fig. 1. In the RLE and NUSE plot, the center of each sample was very close to the position of y-coordinates 0 and 1, respectively. In the RNA degradation plot, the slope of the curve was moderate. Overall, the quality of the chip was considered reliable.

DEGs Identified Between Regenerating and Nonregenerating Samples of Spinal Neurons

Using an adjusted P-value < 0.05 and |logFC| > 2 as the threshold, 621 DEGs were extracted from the microarray datasets GSE56842, including 41 upregulated genes and 580 downregulated genes in regeneration samples compared to nonregenerating samples. A cluster heatmap and volcano plot developed with R software showed the distribution of all DEGs (Fig. 2).

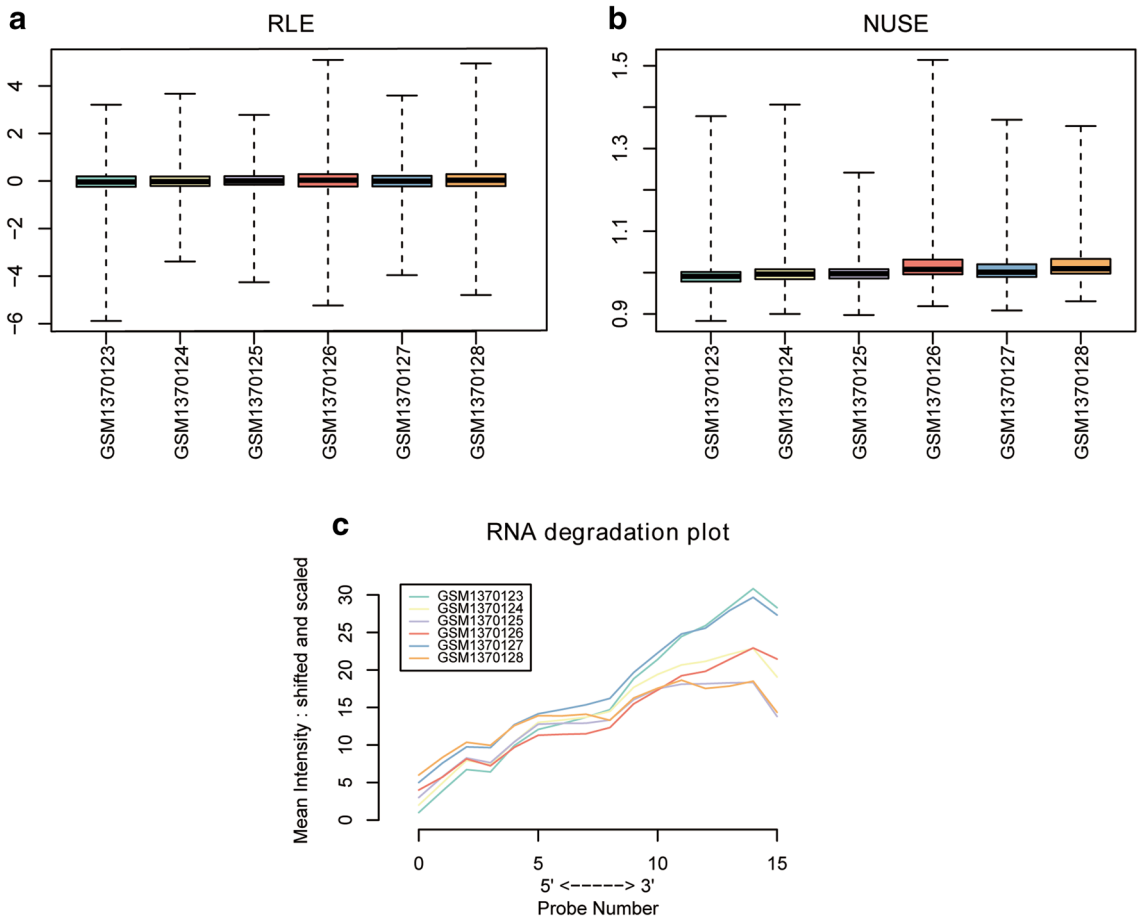

Fig. 1 Quality control of the microarray based on RLE, NUSE, and RNA degradation plots

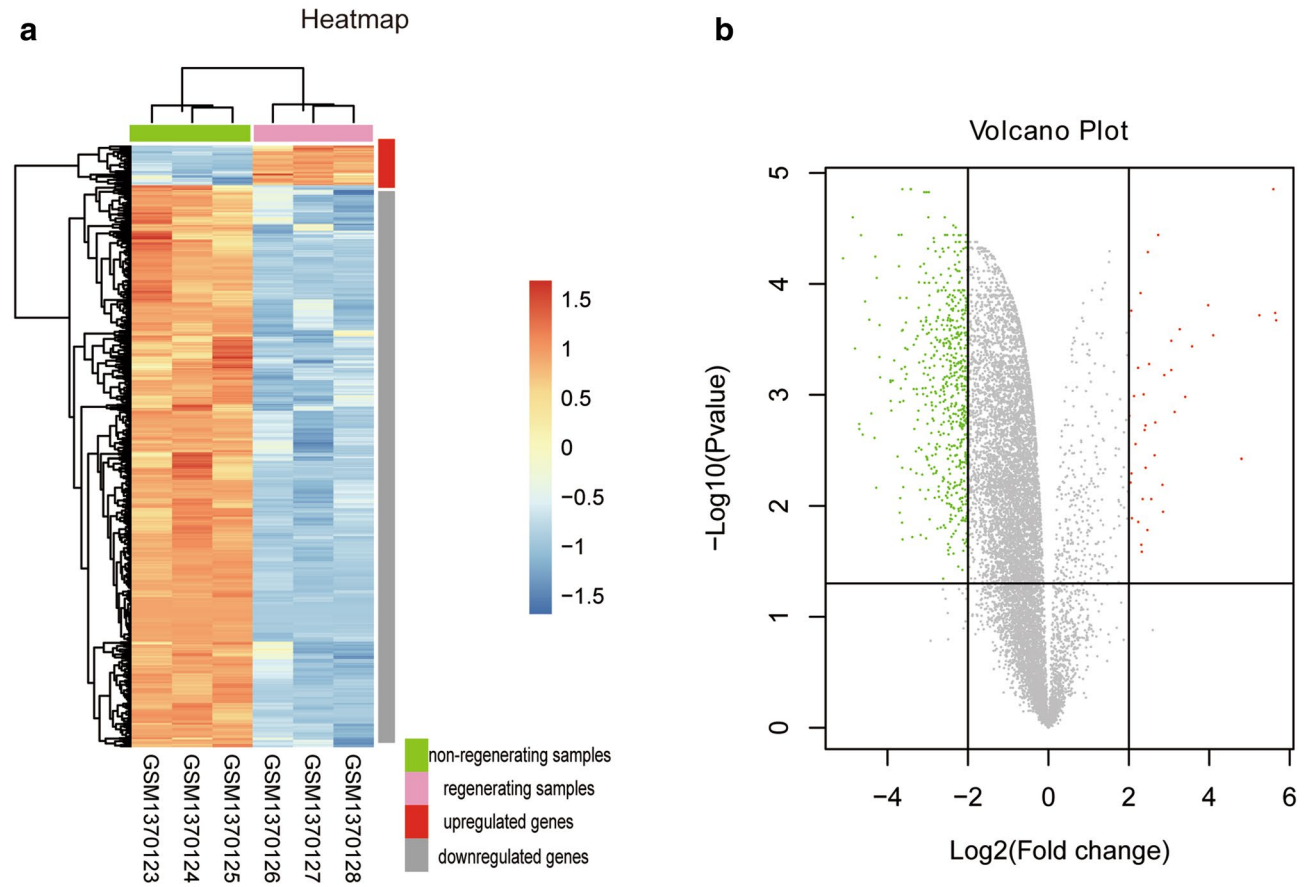

Fig. 2 Hierarchical clustering heatmap and volcano plot of DEGs between two populations of neurons. **a** Hierarchical clustering heatmap of DEGs screened on the basis of |logFC| > 2 and adjusted p value < 0.05. Total 621 DEGs were screened in 3 non-regenerating samples and 3 regenerating samples. Green and pink represent non- and regenerating samples, respectively. Color gradient from red to green indicates expression values change from high to low. Red indicates that the expression of genes is relatively upregulated, blue indicates that the expression of genes is relatively downregulated, and white indicates no significant change in gene expression. **b** Volcano plot of DEGs screened on the basis of |logFC| > 2 and adjusted p value < 0.05. The red points represent upregulated genes, the green points represent downregulated genes, and the gray points represent genes with no significant difference. FC is the fold change (Color figure online)

GO Enrichment Analysis of DEGs

GO analysis of DEGs was performed with DAVID, and genes were classified into three functional groups, including biological processes (BP), molecular functions (MF) and cell compositions (CC). The results of GO analysis are shown in Figs. 3a–c, 4 and Table 1. In the BP group, the upregulated genes were mainly enriched in locomotory behavior, the protein kinase C-activating G-protein coupled receptor signaling pathway, phosphorylation, transport and ion transport, and the downregulated genes were mainly enriched in the Notch signaling pathway, multicellular organism development, regulation of transcription, cell differentiation and positive regulation of neuron differentiation. In the MF group, the upregulated genes were mainly enriched in calcium-transporting ATPase activity, PDZ domain binding, calmodulin-dependent protein kinase activity, ATP binding, calcium ion binding and structural constituents of the cytoskeleton, and the downregulated genes were mainly enriched in protein binding, protein dimerization activity, protein tyrosine kinase activity and DNA binding. In the CC group, the upregulated genes were mainly enriched in integral components of the plasma membrane, spectrin, intracellular membrane-bounded organelles and intermediate filaments, and the downregulated genes were mainly enriched in the condensed chromosome kinetochore, the transcription factor complex, and cytoplasmic and membrane-bound vesicles.

KEGG Pathway Analysis of DEGs

KEGG pathway analysis of DEGs was conducted with DAVID, and the results are shown in Fig. 3d and Table 2. The signaling pathways of DEGs were mainly enriched in

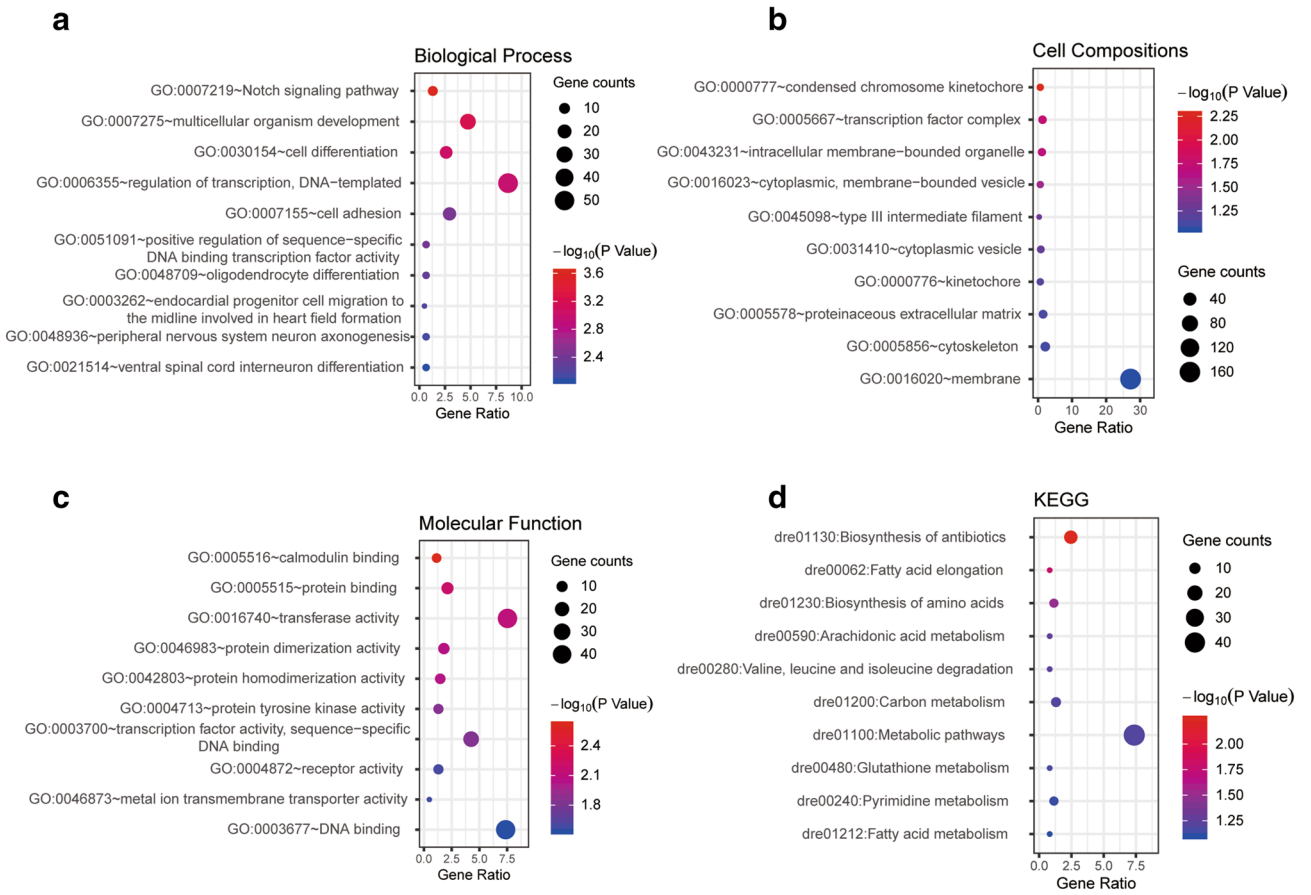

Fig. 3 GO and KEGG pathway enrichment analysis of DEGs in the heterogeneity of intrinsic growth ability between neurons. **a** GO enrichment items of DEGs based on their biological processes. **b** GO enrichment items of DEGs based on their molecular functions. **c** GO enrichment items of DEGs based on their cell compositions. **d** KEGG pathway enrichment items of DEGs. The horizontal and vertical axis represents the name of pathways and gene ratio, respectively. Bubble size represents the number of genes. Color gradient from red to blue indicates enrichment significance from high to low (Color figure online)

the biosynthesis of antibiotics; fatty acid elongation; biosynthesis of amino acids; arachidonic acid metabolism; valine, leucine and isoleucine degradation; glutathione metabolism; pyrimidine metabolism; and the Notch signaling pathway.

PPI Network Construction and Analysis of Modules

The PPI network was constructed with STRING and visualized with Cytoscape software (version 3.6.1). The combined score > 0.4 and degree ≥ 6 were set as the cut-off criteria. After removing isolated nodes, a total of 120 DEGs (13 upregulated and 107 downregulated genes) out of all the altered DEGs were filtered into the DEG PPI network complex, containing 120 nodes and 587 edges (Fig. 5). Among the 120 nodes, 34 central node genes were identified with the filtering criterion of degree ≥ 15, and the top 10 hub nodes with higher degrees were uckl1b, ctnnb1, cad, paics, mapk4, hadhaa, rnd3a, notch3, fgfr2, and ephb2b. In addition, four modules were selected after MCODE analysis of the whole network. The results of pathway enrichment analysis of DEGs within the modules revealed that the DEGs in module 1 were mainly associated with metabolic pathways, those in module 2 with the Wnt signaling pathway and glutathione metabolism, those in module 3 with the calcium signaling pathway, and those in module 4 with metabolic pathways and ECM-receptor interaction.

Discussion

There is significant heterogeneity in the regenerative ability of different types of axons. In the present study, we Identification of several key genes and pathways involved in the heterogeneity of intrinsic growth ability between neurons after spinal cord injury in adult zebrafish. Although regeneration and/or sprouting of brainstem, propriospinal and sensory ascending axons is observed after CNS injury in most studies, there are limited cases

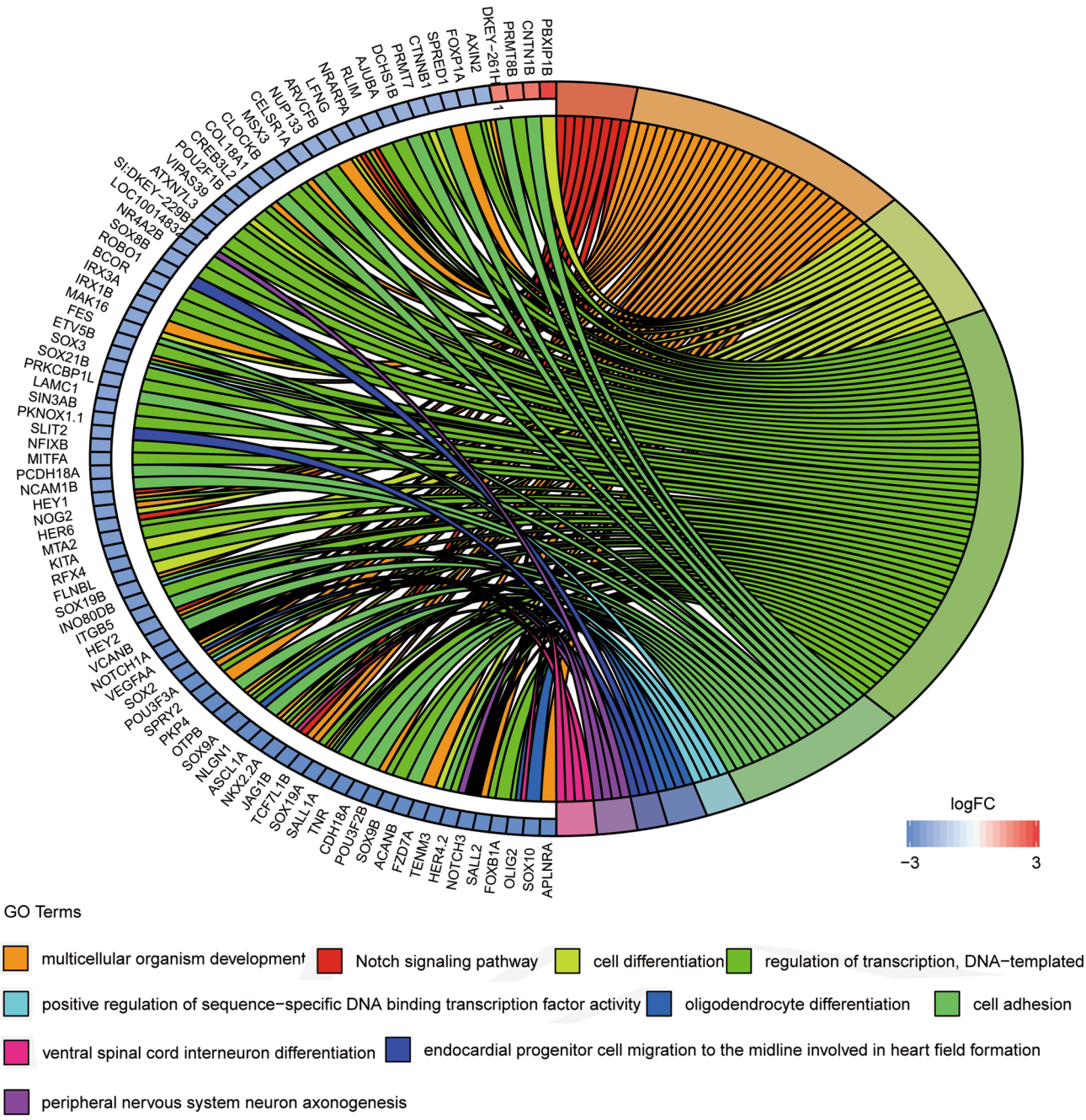

Fig. 4 Distribution of DEGs in spinal cord neurons for different GO-enriched functions. Left side of the plot represents the DEGs. Red represents upregulated genes, and blue represents downregulated genes. Right side represents different GO terms. The connected band represents a gene in a corresponding GO term (Color figure online)

of corticospinal regeneration [2, 14]. Abolishing or neutralizing extracellular inhibitory factors alone is insufficient to allow the majority of injured axons to regenerate, suggesting that a diminished intrinsic regenerative ability of mature neurons critically underlies regeneration failure [15]. Thus, for axon regeneration to occur, one of the determinants is that these injured neurons must possess intrinsic growth ability. Sensory neurons have the highest and corticospinal neurons have the lowest intrinsic regenerative potentials [7]. However, little is known about the mechanism that accounts for such differential regenerative abilities in distinct types of neurons. Deciphering the DEGs or molecular differences underlying the mechanisms that govern intrinsic regenerative ability may open the possibility of inducing regrowth of regeneration-incompetent axons.

Table 1 GO analysis of DEGs in samples of spinal neurons

Term	Description	Count	P-value
Upregulated			
GO:0005887	Integral component of plasma membrane	7	0.002775013
GO:0007626	Locomotory behavior	3	0.003023792
GO:0008091	Spectrin	2	0.006298159
GO:0043231	Intracellular membrane-bounded organelle	3	0.008411331
GO:0007205	Protein kinase C-activating G-protein coupled receptor signaling pathway	2	0.019940843
GO:0016310	Phosphorylation	5	0.022267749
GO:0005388	Calcium-transporting ATPase activity	2	0.025222414
GO:0030165	PDZ domain binding	2	0.029047729
GO:0004683	Calmodulin-dependent protein kinase activity	2	0.029047729
GO:0006810	Transport	6	0.035654887
Downregulated			
GO:0007219	Notch signaling pathway	8	0.000153614
GO:0007275	Multicellular organism development	29	0.000197720
GO:0006355	Regulation of transcription, DNA-templated	52	0.000358116
GO:0030154	Cell differentiation	15	0.001325362
GO:0005515	Protein binding	13	0.003151303
GO:0051091	Positive regulation of sequence-specific DNA binding transcription factor activity	4	0.003255144
GO:0048709	Oligodendrocyte differentiation	4	0.004070003
GO:0000777	Condensed chromosome kinetochore	4	0.004369863
GO:0046983	Protein dimerization activity	11	0.004767922
GO:0003700	Transcription factor activity, sequence-specific DNA binding	26	0.005007191

GO gene ontology, *DEGs* differentially expressed genes

Table 2 KEGG pathway analysis of DEGs in samples of spinal neurons

ID	Pathway	Count	P-value	Genes
dre01130	Biosynthesis of antibiotics	15	0.005656753	CYP51, HADHAA, ALDOCA, OGDHL, ALDH9A1B, ENO1B, HADHB, FDFT1, NME5, PAPSS2B, PHGDH, IDH1, NME2B.2, PSAT1, PAICS
dre00062	Fatty acid elongation	5	0.016052607	HADHAA, ZGC:55,621, HACD2, ELOVL7A, HADHB
dre01230	Biosynthesis of amino acids	7	0.031876662	GLULB, ALDOCA, GPT2L, PHGDH, IDH1, PSAT1, ENO1B
dre00590	Arachidonic acid metabolism	5	0.054373268	PLA2G4AA, PTGDSB.1, PTGDSB.2, GGT5A, CYP2P6
dre00280	Valine, leucine and isoleucine degradation	5	0.057666429	HADHAA, ABAT, ALDH9A1B, HMGCL, HADHB
dre01200	Carbon metabolism	8	0.061804635	HADHAA, ALDOCA, GPT2L, OGDHL, PHGDH, IDH1, PSAT1, ENO1B
dre01100	Metabolic pathways	45	0.063332667	HADHAA, CYP51, PLA2G4AA, ALDOCA, PTGDSB.1, PTGDSB.2, GPT2L, GGT5A, OGDHL, POLA1, CAD, HPRT1, ACSS3, GMP-PAB, HADHB, FDFT1, ALDH1A2, PLCB4, IDH1, NME2B.2, HMGCL, NDUFA4, MTMR1B, GLULB, B3GALT2, PPAP2D, COX4I2, UCKL1B, CDO1, ALDH9A1B, ENO1B, ZGC:55,621, NME5, SI:CH211-15E22.3, RRM2, CYP2P6, PAPSS2B, MBOAT1,
dre00480	Glutathione metabolism	5	0.068172121	GSTT1A, RRM2, GGT5A, IDH1
dre00240	Pyrimidine metabolism	7	0.074773347	NME5, RRM2, POLA1, UCKL1B, CAD, NME2B.2
dre01212	Fatty acid metabolism	5	0.079598577	HADHAA, ZGC:55,621, HACD2, SCD, HADHB
dre04330	Notch signaling pathway	5	0.079598577	NOTCH3, JAG1B, NOTCH1A, HER6, LFNG
dre00910	Nitrogen metabolism	3	0.089808591	GLULB, CA9, CA8
dre04916	Melanogenesis	8	0.090727741	KITA, CAMK2D1, PLCB4, CAMK2D2, MITFA, CREB3L2, FZD7A, CTNNB1
dre00830	Retinol metabolism	4	0.095904508	SDR16C5A, ALDH1A2, SDR16C5B, RETSAT

KEGG Kyoto Encyclopedia of Genes and Genomes, *DEGs* differentially expressed genes

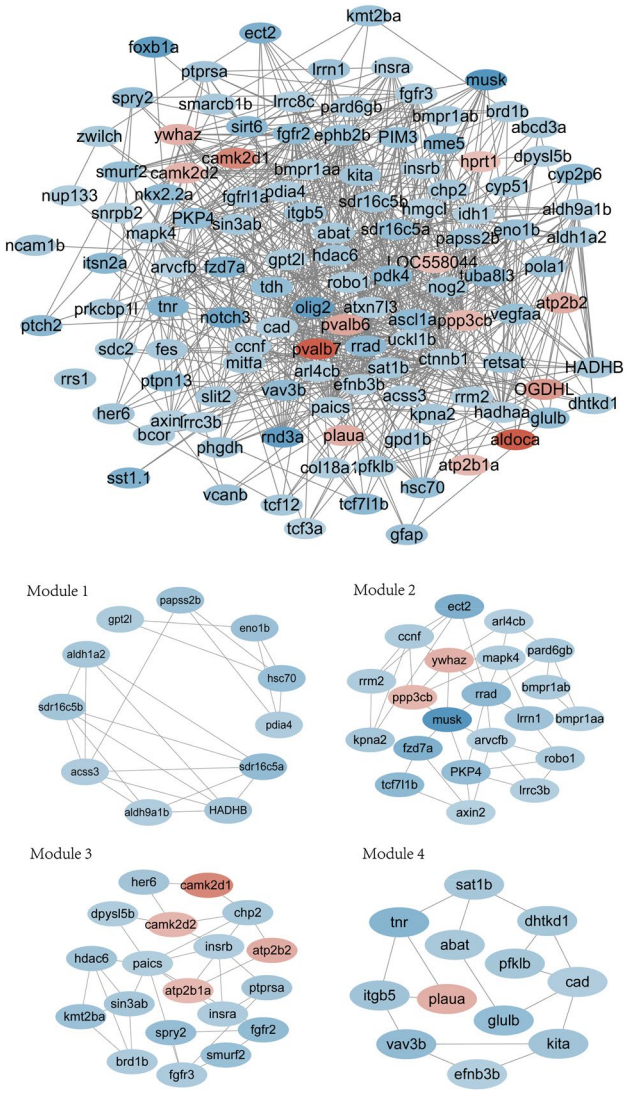

Fig. 5 PPI network and module analysis. A total of 120 DEGs (13 upregulated and 107 downregulated genes) out of all the altered DEGs were filtered into the DEG PPI network complex. Red represents upregulated genes, and blue represents downregulated genes. Lines represent the interaction between proteins produced from the genes. Module 1 consists of 11 nodes and 22 edges, which are mainly associated with metabolic pathways; Module 2 consists of 21 nodes and 42 edges, which are mainly associated with the Wnt signaling pathway and glutathione metabolism; Module 3 consists of 19 nodes and 31 edges, which are mainly associated with the calcium signaling pathway; Module 4 consists of 12 nodes and 16 edges, which are mainly associated with metabolic pathways and ECM-receptor interaction (Color figure online)

Recently, microarray sequencing technologies have been widely used to detect the expression levels of tens of millions of genes. In the present study, we used bioinformatics methods and R language to deeply analyze microarray dataset GSE56842 to identify potential targets for axon regeneration following CNS injury. The results identified 621 DEGs, including 41 upregulated genes and 580 downregulated genes. The top 20 most significantly upregulated genes were casq2, wu:fj45a02, ywhaz, ppp3cb, dgkzb, nme2b.2, pvalb7, pcp4l1, hprt1, aldoca, atp1a3b, camk2d1, si:dkey-35i13.1, pbxip1b, ndufa4, atp2b1a, wu:fc46b01, hpca, s100a1, and atl2. The top 20 most significantly downregulated genes were zgc:92287, cdh18a, tnr, ascl1a, si:ch211-237l4.6, bmp3, egr4, aplnra, kita, sox21b, sept8b, rgmd, sox3, LOC100148946, tpm1, picalma, nudt3a, cln6a, pot1, and foxb1a. We then performed functional enrichment analysis on the DEGs. The up- and downregulated DEGs were divided into three groups (BP, CC, and MF) by GO terms and further clustered based on functions and signaling pathways enrichment analysis. In addition, we constructed the PPI network and analyzed the four modules in the whole network. KEGG pathway analysis revealed that the DEGs in these modules were enriched in metabolic pathways, the Wnt signaling pathway, the calcium signaling pathway, focal adhesion, and ECM-receptor interaction.

Growth cone formation is an important step during the initiation of axon regeneration following CNS injury [16]. One of several steps involved in the growth cone formation of regeneration-competent axons is resealing of the ruptured membrane, which is critically dependent on calcium influx [17]. The increased intracellular calcium activates calpains, and subsequently, activated calpains cleave submembranous spectrin, which allows efficient vesicle fusion with the plasma membrane [18]. In addition to promoting growth cone formation, the elevated intracellular calcium triggers various cell autonomous mechanisms necessary for axon regeneration [19]. A higher amplitude of calcium changes were detected in the cell body of the rat dorsal root ganglion when compared to cortical neurons [20]. In the sensory neurons of C. elegans, the amplitude of axonal calcium waves correlates with the extent of axonal regeneration [21]. However, high calcium levels are deleterious for CNS neurons [22]. These observations suggest that calcium transients are tightly associated with axonal regeneration. In this study, GO analysis of DEGs revealed that the upregulated genes were mainly enriched in calcium-transporting ATPase activity, calmodulin-dependent protein kinase activity, ATP binding, calcium ion binding, and structural constituent of the cytoskeleton at the MF level; in locomotory behavior, the protein kinase C-activating G-protein coupled receptor signaling pathway, phosphorylation, and ion transport at the BP level; and in integral components of the plasma membrane, spectrin, intracellular membrane-bound organelles and intermediate filaments at the CC level. This result is consistent with the knowledge that calcium-mediated signaling is associated with the intrinsic growth capacity of axons [19, 23].

A characteristic postinjury response of neuronal cell bodies during axon regeneration is the change of metabolic status. Most mature neurons are sustained in a metabolically inert state. After injury, regenerating neurons show increased

(anabolic) metabolism, while nonregenerating neurons display catabolic metabolism [24, 23]. For regeneration to occur, injured neurons need to adjust toward anabolic metabolism from inert metabolism so that macromolecules can be continuously synthesized to support axon extension [23]. Consistent with these observations, in the present study, we found a large number of DEGs in metabolism-related signal pathways through KEGG pathway analysis, including fatty acid elongation; amino acids biosynthesis; arachidonic acid, carbon, glutathione, pyrimidine and retinol metabolism; and valine, leucine and isoleucine degradation. MCODE analysis of DEGs within the four modules indicated that they were mainly associated with these metabolic pathways. The mammalian target of rapamycin (mTOR) is important for both anabolic metabolism and axon regeneration [23, 25]. Forced upregulation of mTOR activity in corticospinal neurons by conditional deletion of phosphatase and tensin homolog (PTEN), a negative regulator of mTOR, enhances the regenerative ability of injured neurons [26, 27]. Direct manipulation of genes enriched in these pathways may switch the metabolic status of injured neurons from inert metabolism to anabolic metabolism for axon regeneration.

The intrinsic capacity of axon regeneration varies between different neurons and is regulated by both negative and positive signaling pathways [28, 29]. It is known that Notch and Wnt signaling are closely related to axonal regeneration of neurons in the adult CNS [30, 31]. Using single-neuron analysis of regeneration in vivo, El Bejjani and Hammarlund [32] showed that activating Notch/lin-12 signaling inhibited the regeneration of mature C. elegans neurons. Conversely, blocking Notch activation enhanced axon regeneration following injury. Recently, numerous studies in the adult CNS have suggested that activating Wnt signaling promotes axonal regeneration and neurite growth [31]. Using an optic nerve crush injury murine model, Patel et al. [33] demonstrated that intravitreal injections of Wnt3a, a canonical Wnt signaling activator, resulted in significant axonal growth. Using a spinal cord injury model in adult zebrafish, Strand et al. [34] also showed that Wnt signaling was correlated with axonal regeneration and neuronal function recovery. Through KEGG pathway analysis, we identified 6 DEGs enriched in the Notch pathway. In addition, 3 DEGs were enriched in the Wnt pathway in module 2. Identification of these DEGs and pathways will significantly expand our understanding of the genetic basis of axonal injury responses and repair.

Strengths and Limitations

Nerve regeneration and functional recovery after CNS injury are difficult problems worldwide in regenerative medicine. Neurons in the adult CNS have limited capacity to regenerate their axons after injury [35]. We conducted this study to strengthen our understanding of the molecular mechanisms involved in the heterogeneity of intrinsic growth ability between neurons. To our knowledge, this is the first analysis to explore the key genes and pathways in CNS neurons after injury through a bioinformatics method. We identified several significant genes and pathways associated with axon regeneration including several well-proven genes and pathways (Notch signaling pathway, Wnt signaling pathway, metabolic pathway and calcium signaling pathway). Ueda et al. [36] demonstrated the decrease in expression level of her4 and her6, two target genes of Notch signaling after CNS injury in adult Zebrafish. This substantiate our findings that both of two genes were downregulated in our identified DEGs. In addition, several identified genes were proved to be associated with CNS regeneration in Zebrafish [37–39]. Besides these pathways and their genes, some others may be also likely involved in intrinsic growth ability. For example, the genes enriched in cell differentiation or positive regulation of neuron differentiation were downregulated. It might be helpful for axonal regeneration after spinal cord injury if knocking out these genes to negatively regulate cell differentiation could induce the nonregenerating neurons into a relative immature state from a mature phenotype. However, a limitation of this study is the small sample size. More samples are necessary to further improve the reliability of the analysis, and further molecular biological experiments are required to verify the functions of the identified genes associated with axon regeneration.

Conclusion

We downloaded Zebrafish microarray dataset GSE56842 and used R software and bioinformatics analysis to further investigate this dataset. We identified 621 DEGs, which may be involved in the intrinsic control of axon regeneration. By performing GO and KEGG pathways analysis, we showed that the DEGs were mainly enriched in metabolic pathways, the calcium signaling pathway and the Notch signaling pathway, which provides a theoretical basis for studying the heterogeneity of axon regeneration. We successfully constructed a PPI network of DEGs and performed MCODE module analysis. The genes within modules encoded proteins involved in the Wnt signaling pathway and metabolic pathways. Further study of the PPI network would be beneficial for understanding the interaction between DEGs. These results improve our understanding of the molecular mechanisms underlying the heterogeneity of intrinsic growth ability between neurons, and the key genes and pathways could be used as therapeutic targets in the treatment of CNS regeneration failure. Although these results came from the Zebrafish dataset, the heterogeneity of intrinsic growth

ability between neurons also existed in mammalian SCI models [6]. Therefore, our study also provides a reliable approach for future mammalian SCI experiments to investigate molecular differences underlying the mechanisms that govern intrinsic regenerative ability.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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