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Mechanisms of spinal cord injury regeneration in zebrafish: a systematic review

Zeynab Noorimotlagh [1], Mahla Babaie [1], Mahdi Safdarian [2], Tahereh Ghadiri [3], Vafa Rahimi-Movaghar [4*]

[1] Medical Student, Iran University of Medical Sciences, Sina Trauma and Surgery Research Center, Tehran, Iran
[2] Iran University of Medical Sciences, Sina Trauma and Surgery Research Center, Tehran, Iran
[3] Tabriz University of Medical Sciences, Tabriz, Iran
[4] Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, 11365-3876, Iran

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ABSTRACT

Objective(s): To determine the molecular and cellular mechanisms of spinal cord regeneration in zebrafish.

Materials and Methods: Medical databases of PubMed and Scopus were searched with following key words: Zebrafish; spinal cord injuries; regeneration; recovery of function. The map of mechanisms was performed using Xmind software.

Results: Wnt/ß-catenin signaling, L1.1, L1.2, Major vault protein (MVP), contactin-2 and High mobility group box1 (HMGB1) had positive promoting effects on axonal re-growth while Ptena had an inhibitory effect. Neurogenesis is stimulated by Wnt/ß-catenin signaling as well as HMGB1, but inhibited by Notch signaling. Glial cells proliferate in response to fibroblast growth factor (FGF) signaling and Lysophosphatidic acid (LPA). Furthermore, fgf signaling pathway causes glia bridge formation in favor of axonal regeneration. LPA and HMGB1 in acute phase stimulate inflammatory responses around injury and suppress regeneration. LPA also induces microglia activation and neuronal death in addition to glia cell proliferation, but prevents neurite sprouting.

Conclusion: This study provides a comprehensive review of the known molecules and mechanisms in the current literature involved in the spinal cord injury (SCI) regeneration in zebrafish, in a time course manner. A better understanding of the whole determining mechanisms for the SCI regeneration should be considered as a main goal for future studies.

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Introduction

Spinal cord injury (SCI) in mammals leads to the loss of sensory and motor functions within and below the lesion site due to the non-regenerating nature of the central nervous system, which leads to neuronal cell death in the primary motor cortex (1). It is one of the most damaging conditions among injuries with poor prognosis (2-4). The incidence of SCI has been reported to be 25.5 case per million per year in developing countries (5). Functional recovery after adult mammalian SCI is limited in part by myelin inhibitors of axonal re-growth, in addition to a weak intrinsic neuronal growth response (6).

In contrast to mammals, adult zebrafish is capable of neuronal proliferation, regeneration and functional restoration within 6–8 weeks after complete spinal cord transection via several regenerative processes in addition to surviving upper motor neurons in the brainstem against cell death (7, 8). Adult zebrafish has evolved into a paradigmatic vertebrate system to identify novel genes vital for successful regeneration after SCI. However, exact molecular and cellular mechanisms of recovery in zebrafish CNS are not fully understood. Radial glia, such as resident neural progenitors, plays a key role in remarkable regenerative capacity of zebrafish spinal cord (9). According to previous studies, the nuclei of medial longitudinal fascicle (NMLF) and the intermediate reticular formation (IMRF) in the brain stem of zebrafish are the most potent regions for re-growing of descending axons toward spinal cord (8).

Due to profound re-growing ability combined with genetic tractability, zebrafish has been considered as a useful model to understand the molecular mechanisms of spinal cord regeneration (10). Much of the work on this model has been done over the past decade. Furthermore, embryonic neurons of both peripheral and central nervous systems respond to axonal injury by

*Corresponding author: Vafa Rahimi-Movaghar. Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, Iran. Fax : +98-21-6670 5140; email: V_rahimi@sina.tums.ac.ir

initiating pro-regenerative transcriptional changes that enable axons to extend, and adhere to appropriate targets, in order to retrieve sensorimotor function (11). Behavioral recovery after spinal cord injury is clearly quantifiable using different tests (6). Since most genes in zebrafish genomes have been highly conserved phylogenetically, the discovery of genes or proteins related to regenerative capacity could address new therapeutic strategies on how to deal with functional recovery after SCI in humans (12). Here, we designed a systematic review in order to provide a framework of all the yet-known mechanisms of spinal cord regeneration in zebrafish after injury. Our goal is to understand the nature of the conditioning response in terms of its underlying cellular and molecular mechanisms.

Materials and Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement (13). On January 3, 2016, we searched PubMed using "(spinal cord inj*) AND zebrafish" and Scopus using "TITLE-ABS-KEY (spinal cord inj*) AND TITLE-ABS-KEY (zebrafish)" in order to compile related papers on the spinal cord injury in zebrafish. Due to the lack of a massive literature related to the topic, we chose our search strategies more specifically in order to include all the possible related data. After removing the duplicates, 130 different records were entered for screening by three independent reviewers (Zeynab Noorimotlagh, Mahla Babaie and Mahdi Safdarian). Reviews and non-original articles were excluded at this stage. Full-texts of the remaining manuscripts were retrieved and further screened by reviewers. Fifty three papers were known as eligible for full text review, from which 32 were considered as final papers for full text data extraction (PRISMA diagram). We used the 15-item checklist developed by the Delphi study of Hassannejad *et al.* (14) for potential variables affecting animal studies on SCI. We prepared the related extraction data sheet with a few modifications according to our study (Supplementary file 1). The characteristics of the studies including the subject, definition and function of the molecule as well as the site of its expression were extracted from the text of the papers. Study methods used to investigate the results of studies were shown in an acronym form, in addition to the most critical times of the study following injury described as 'hours post-lesion (hpl)', 'days post-lesion (dpl)' and 'weeks post-lesion (wpl)'.

This information besides fish strain/species, gender, age, weight or length, sham and control group data, method and level of injury, methods and drugs used for anesthesia as well as housing conditions were all extracted from the papers to provide comprehensive information on the quality and methodology of the studies.

Then, the pooled data was used to draw a concept map of mechanisms involved in spinal cord injury regeneration after SCI in zebrafish. To fulfil this aim, Xmind 7.5 update (XMind Ltd, a Hong Kong registered business) drawing map software was utilized. In order to categorize the data into a better classification, a results table was drawn (Supplementary file 2). Consequently, the results table was converted into two maps in order to indicate time course changes (Time course map) and relationships between cells and molecules (Cellular map). A guide to read these maps is constructed below every map.

Results

Characteristics of studies

From the total 32 included studies, 24 used wild type transgenic adult zebrafish (Danio rerio) as the most frequent animal model for the study. Three studies used both adult and embryonic (larva) zebrafish and 3 used embryonic zebrafish. Characteristics of the 32 studies are illustrated in Table 1. In the aspect of age and length of the fishes, four studies used 3-6 month-old fishes, two used 4 months (2-cm-legnth), eight used 6 months, three older than 6 months (> 2.5 cm) fishes, five 3-4 centimeters length and three larger than 2-centimeter length fishes. Immersion in 0.033% phosphate-buffered saline (PBS, pH 7.4) containing 0.033 aminobenzoic acid ethylmethylester or 0.02% tricaine methanesulfonate were the most common anesthesia methods used in the studies. Sixteen studies reported the direction of recovery from caudal to the injury site. Three studies reported that they worked on only male fishes; six used both male and female and others did not mention the sex of the animal. Maintaining the fishes at 28 °C on 14 hr light and 10 hr dark cycles was the most frequently used housing condition in the studies.

Description of the statistical analysis and ethics were reported by all studies. A complete transection (n=27) between the eighth and ninth vertebrae (about 4 mm caudal to the brainstem-spinal cord transitional junction) following a longitudinal incision to the vertebral column were the most common injury method and level (n=26) used for inflating SCI. Thirteen studies used the sham-lesioned control with identical surgical procedures without spinal cord cut versus 15 studies that used un-lesioned control fishes. Immunohistochemistry (n=29) and *in situ* hybridi- zation (n=23) were the most frequent cellular, molecular and histological methods applied to investigate the regeneration followed by quantitative real-time polymerase chain eaction (RT-PCR) (n=15) and locomotor analysis (n=16). The frequency of study methods is shown in Table 2.

Concept map

The time course map (Figure 1) has been drawn to show the main phases of regeneration processes including immediately after the injury (less than 24 hr), defined as acute phase and one to 20 dpl as chronic phase after spinal cord injury in the zebrafish. In each phase of regeneration, we highlighted some days based on the occurrence of specific events or up-regulation of mRNAs and proteins on those days. Considerable events of each phase are described in a time course manner.

Table 1. Characteristics of studies
Abbreviations: hpl: hours post-lesion, dpl: days post-lesion, wpl: weeks post-lesion, NMLF: Nucleus of the medial longitudinal fascicle, IMRF: Intermediate reticular formation, NCAM: Neural cell adhesion molecule, MLF: Medial longitudinal fascicle, IRF: Inferior reticular formation, MON: Medial octavolateralis nucleus
#the acronyms of the study methods and their frequency are shown in Table 2

Ref No.	First author	Year	Subject	Definition of molecule or cell	Function of molecule	Site of expression	Time of study	Study methods#
15	Barreiro-Iglesias	2015	Serotonin	Neurotransmitter	Permissive	Midthoracic spine	24-26, 33 hpl; 10, 14 dpl	ACX
16	Becker	2004	L1.1	Recognition molecule	Permissive (Prompting regeneration)	Lesion site (NMLF)	10 dpl; 6 wpl	ADIJLX
17	Becker	2001	Neuron macrophages/microglial cells	NA	NA	Axons from all descending tracts including lateral funiculus and MLF	2, 14 dpl; 6 wpl	AEFGJL
8	Becker	1998	-L1.1 and L1.2 -GAP-43 -NCAM -Reactive Macrophage/microglia	-Recognition molecules -Marker of axonal growth -Neural cell adhesion molecule	Permissive (Promote regeneration Except to NACM)	NMLF, IMRF	7, 14 dpl; 2, 3, 56, 84 dpl	ABFJX
18	Bormann	1999	zfNLRR	Neuronal-specific adhesion molecule	Permissive (Growth promoting agent)	MLF and IRF in the medulla oblongata, MON, IMRF neurons and radial glial cells	5 dpl	ABEGUX
7	Briona	2015	Wnt/ß- catenin	Signaling pathway	Permissive (Prompting regeneration) Neurogenesis, radial glia differentiation, axon regrowth.	Blastoma and around, the level of anal pore in the spinal cord	1, 3, 5, 7 dpl	AV
19	Briona	2014	Radial glial progenitors (Cells)	NA	NA	Blastema and proximal site of injury, at the level of the anus in the spinal cord	1, 5, 9 dpl	ABFVX
20	Chen	2016	L1.2	L1.1 paralog and ortholog of mammalian L1CAM	Permissive (functional recovery)	Neurons and GFAP-immunoreactive glia (Islet-1, HuC/D and GFAP immunopositive cells)	6, 11 dpl	ABCDEGHIMT
21	Dias	2012	Notch	Notch signaling	Negative regulator of regeneration	Progenitor (radial glial progenitor) cells of specific regions of the ventricular zone and dorsal midline of spine	14 dpl	ABDF
22	Fang	2014	HMGB1 (amphoterin)	Nuclear protein Or extracellular cytokine	Permissive (Prompting neurite outgrowth regeneration)	Along the central canal and in motoneurons	4, 12, 24 hpl; 6, 7, 11, 21 dpl	ABCDGHO
23	Goldshmit	2012	Lysophosphatidic Acid Signaling	inflammatory and wound-healing mediator (phospholipid)	Negative regulator	Brain and spinal cord	3 hpl; 3, 5, 10, 21 dpl	ABCDRX
24	Goldshmit	2012	fgf	Growth factor	Permissive (Prompting regeneration) Glial cell morphogenesis	In glial cell and injury site	3, 5, 10 dpi; 2, 3 wpl	ABCDEGHKX
25	Guo	2011	Sox11b	transcription factor	Permissive (Promotes proliferation of ependymal cells and migration of newly generated neurons)	Ependymal cells lining the central canal and in newly differentiating neuronal precursors or immature neurons	4, 12 hpl; 11 dpl; 6 wpl	ABCDF
26	Hui	2015	Newly generated Cellular profile (Sox2, OCT4+/HuC/D, A 25+ cells progenitors)	NA	NA	Ependyma around the central canal	1, 3, 7, 10; 15 dpl	ABCFKLST

Continued Table 1

27	Hui	2010	Cellular profile	RBC, macrophage, Schwan, neuron	NA	Injury epicenter and the adjacent part, white matter and neurons in the ependyma, subependyma at the level of dorsal fin in the spinal cord	6 hpl; 1, 3, 5, 7, 10, 15 dpl; 4 wpl	AKLMR
28	Kuscha	2012	neurons	Tyrosine Hydroxylase and Serotonergic neurons	Permissive (promotes regeneration)	In the spinal cord	1, 2, 6, 13 wpl	ABEDFQ
29	Kuscha	2012	Cells	interneuron cell type	NA	Around central canal of spinal cord	2, 6 wpl	ABF
30	Lin	2012	Contactin-2 (TAG-1)	Cell Neural Adhesion Molecule	Permissive (locomotor recovery and regrowth of axons)	NMLF, along the central canal and in motoneurons	4, 12 hpl; 6, 11 dpl; 6 wpl	BCDEGHIO
31	Liu	2014	Ptena	Tumor suppressor gene homologs of mammalian PTEN (phosphatase and tensin homolog)	Permissive (locomotor recovery)	Neurons in NMLF in the brainstem, spinal motoneurons and immature neurons lining the central canal	12 hpl; 6, 11 dpl; 4-6 wpl	ABCDEGHI
32	Ma	2014	Legumain (The Asparaginyl Endopeptidase)	Protease (Enzyme)	Permissive (functional recovery)	NMLF, the caudal spinal cord	1, 3, 11 dpl	ABCDIJN
33	Ma	2012	cysteine- and glycine-rich protein (CRP)1a	Growth-associated protein	Permissive (functional recovery)	NMLF, and other nuclei such as the IMRF and superior reticular formation capable of regeneration	3, 11-21 dpl	ABCDHIJN
34	Ogai	2014	The sex-determining region Y-box 2 (Sox2)	Transcription factor	Permissive (proliferation initiator)	Ependymal cells	1 ,3, 5, 20 dpl	ABC
35	Ogai	2012	Bcl-2 and phospho-Akt	Anti-apoptotic factors	NA	Brainstem neurons of the NMLF and IMRF	1–15 dpl	AEFHKMP
36	Pan	2013	MVP (Major vault protein)	Multifunctional protein	Permissive (functional recovery and axonal regrowth)	Ependymal cells (brainstem)	4, 12 hpl; 6, 11 dpl; 4–6 wpl	ABCDEGHIX
37	Reimer	2009	sonic hedgehog a (shha)	Ventral morphogen	Permissive (Neurogenesis)	Ependymoradial glial cells lining the central canal in ventrodorsal positions	1, 2, 6 wpl	ABCEP
			olig2	Ependymoradial glial cells		Ventricular zone		
			nkx6.1	Transcription factors		-		
			pax6	Transcription factors		-		
			Hedgehog)hh(receptors: *patched1* and *smoothened*	Receptor		Ependymoradial glial cells including those of the pMN-like zone		
38	Reimer	2008	olig2-positive (olig2+)	Ependymo-radial glial progenitor cells	Permissive (Motor Neuron Regeneration)	The ventricular zone	1,2, 6–8 wpl	AEFKLX
39	Schweitzer	2007	Contactin1a(Cntn1a)	Homolog of contactin1 (F3/F11/ contactin) in mammals; an immunoglobulin superfamily recognition molecule of neurons and oligodendrocytes	Permissive (oligodendrocyte differentiation and axonal regeneration in the central nervous system)	Brainstem neurons and white matter glial cells	14 dpl	ABEX

Continued Table 1

40	Schweitzer	2003	Protein Zero(P0)	Immunoglobulin superfamily molecule	Permissive	Spinal cord 0 to 1 mm caudal to the lesion (peripheral white matter)	14 dpl	ABUX
41	Vajn	2014	Swimming distance	-	-	Reticular formation, MON, NMLF (at least four millimeters beyond the lesion)	2, 4, 8 wpl	ADEGMP
42	Yu	2013	Syntenin-a	Scaffolding protein involved in mammalian cell adhesion and movement, axonal outgrowth, establishment of cell polarity, and protein trafficking	Permissive (locomotor recovery and axonal regrowth-synapse formation)	Neurons, glia and newly generated neural cells	4, 12 hpl; 6, 11 dpl	ABCDEGHIQ
43	Yu	2011	miR-133b	MicroRNAs (miRNAs)	Permissive (functional recovery)	Regenerating neurons of the brainstem, supraspinal Neurons and NMLF	6, 24 hpl; 7 dpl; 6 wpl	BCDEFHOQ
12	Yu	2011	Tenascin-C	Extracellular matrix glycoprotein	Permissive (locomotor recovery)	Gray matter, in motoneurons	4, 48 hpl; 11 dpl; 6 wpl	ABIDEFG

Table 2. Frequency of study methods used to investigate outcomes

Acronym	Study methods	Frequency of studies
A	Immunohistochemistry	29
B	In situ hybridization	23
C	Quantitative real-time polymerase chain reaction	15
D	Locomotor analysis (swim tracking test)	16
E	Retrograde tracing	14
F	Cell quantification (counting)	13
G	Anterograde tracing	10
H	Western blot analysis	8
I	Anti-sense Morpholino (MO) application	7
J	BrdU(5-bromo-20-deoxy- uridine)labeling assay (application)	5
K	Biocytin Application	5
L	Electron microscopy	5
M	Histology	4
N	Microarray data	2
O	Immunofluorescence analyses	3
P	Behavioral assays	3
Q	Antibody characterization	3
R	Enzyme linked immune- sorbant assay (ELISA)	2
S	TUNEL staining (TdT-Mediated Deoxy-UTPNick End Labeling Assay)	1
T	Northern Blot Analysis	2
U	Confocal microscopy	2
V	Immunoblotting	2

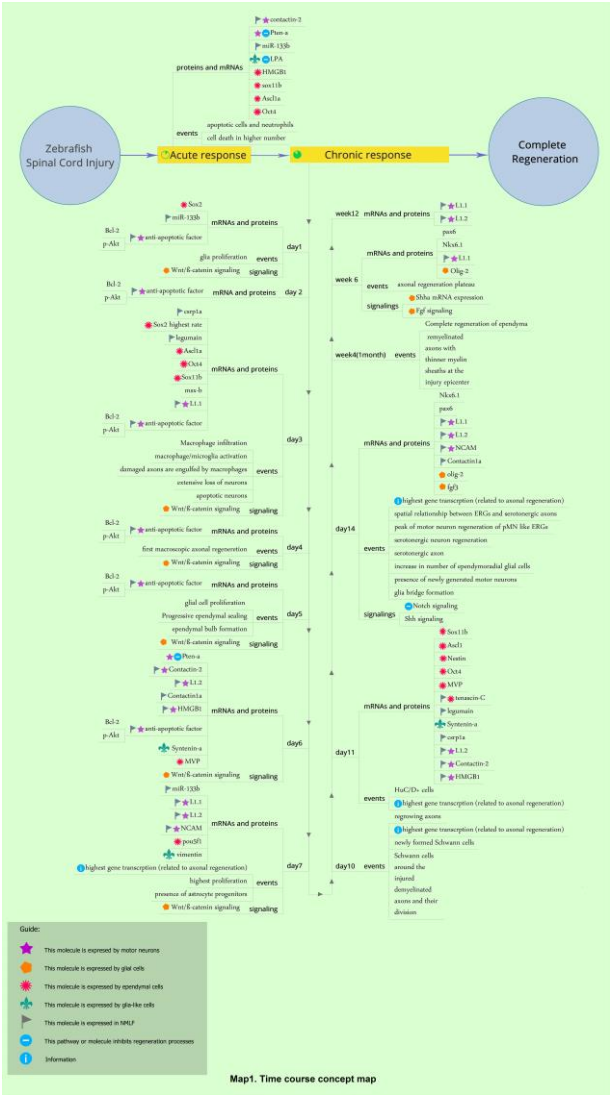

Figure 1. Time course concept map

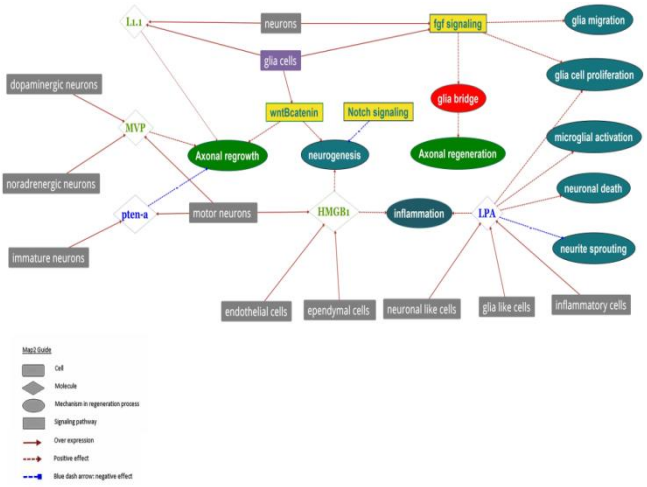

Figure 2. Cellular concept map

The cellular map (Figure 2) was drawn to display two features of each molecule including the origin of secretion or expression and regeneration-related function of each molecule (for example, proteins and mRNAs or other substances such as serotonin).

Discussion

The zebrafish is an excellent model to study the mechanisms underlying axonal regeneration after SCI. Due to embryo transparency, the zebrafish has been established as a model of vertebrate development for several decades and by sequencing its genome, many molecular tools have been developed for detecting neuronal re-growth (41). After SCI, acute and chronic regenerative responses are seen and then functional recovery is achieved in about 6 wpl. No additional improvement has been observed at 10 wpl (16). Based on previous researches, we considered less than 24 hpl as the acute response phase after injury and later than 1 dpl as the chronic phase (30, 25). Here, we discuss the most critical cellular and molecular mechanisms, engaged in successful regeneration of zebrafish spinal cord in a time course manner.

Acute response phase

Immediately after the injury, infiltration of blood cells including RBC and macrophage to the injury site (27). Among all molecular and cellular modifications, some are in favor of regeneration and others could be inhibitory factors. In the acute phase, motor neurons of NMLF express contactin-2, miR-133b, Sox11b and Ascl1 within 6 to 12 hpl (30) play positive roles in axonal regeneration (43). Sox11b (a regulator of nestin), miR-133b and Ascl1 are also upregulated in ependymal cells as well as newly generated neurons (25). Motor and immature neurons around the central canal and also in the white matter express phosphatase and tensin homolog A (Ptena) at 12 hpl to act as an inhibitory factor on re-growth of fibers and regenerating axons from brain stems nuclei such as NMLF (31). Also, at 6 hpl, measurements showed miR-133b upregulation in ependymal cells located around the ventricle as well as in neurons of NMLF, which play a major role in axonal regeneration (43). Large neuronal-like and glia-like cells along the midline and central canal upregulated lysophosphatidic Acid (LPA) in the large neuronal-like and glia-like cells along the midline and central canal also inhibits acute regeneration response (23). In addition, motor neurons and endothelial cells in NMLF release HMGB1, mediate inflammation at the acute phase (4 and 12 hpl) (22) but induce regeneration during chronic phase after injury (27). Sox11b regulates nestin and also Ascl1 expression after the lesion. These molecules in ependymal cells as well as the newly generated neurons are upregulated (25).

Chronic response phase

Apoptotic cell death was detected at 1 and 3 dpl around the injury site and engulfment of damaged axons by macrophages (27) along with upregulation of Msx-b in the gray matter observed at 3 dpl (26). A pathway switch of descending axons from the white matter to the gray matter was observed during the regeneration processes (17).

In order to promote the regeneration of axons in NMLF, IMRF & superior reticular formation (SRF), neurons started to overexpress Csrp1a gene to upregulate cysteine- and glycine-rich protein1a at 3 dpl, continued to 21 dpl (33). Following apoptotic cell loss, anti-apoptotic factors such as Bcl-2 and p-Akt in upper motor neurons of NMLF & IMRF activates at 1-6 dpl (35). As we described earlier in the acute phase, it has been found that upregulation increases in the level of sox-2 starts as soon as 1 dpl by ependymal cells around the central canal and continued to 20 dpl with a peak level at 3 dpl, related to ependymal cell proliferation after SCI (26, 34). Highly expressed Sox11b and Ascl1 expression in ependymal cells as well as newly generated neurons continued in the chronic phase after lesion (25). The increased expression of contactin-2 by motor neurons of NMLF at the early phase, sustained 6 and 11 dpl (30). Additionally, contactin1a was also upregulated in peripheral white matter and in periventricular cell layer of NMLF and IMRF at 6 and 11 dpl (39).

Expression of HMGB1 despite short-term sharp increase at the beginning of the acute phase (4 hpl) shows a significant decrease at the end of the acute phase and remains low until 11 dpl but returns to the control level in 21 dpl (22). Motor neurons, endothelial cells and microglia cells of NMLF produce HMGB1 to promote regeneration and angiogenesis (22). Furthermore, high levels of miR-133b in NMLF continued to 7 dpl (reported at 1 and 7 dpl) (43). Upregulation of legumain expression after spinal cord injury in adult zebrafish, as an essential component of the capacity of injured neurons to re-grow their axons, was also confirmed at 3 and 11 dpl in NMLF, IMRF and the caudal spinal cord. (32). Also, highly expressed pou5f1, a positively mediator of re-growth, by ependymal, neuron-like and glia cells as well as vimentin overexpression in glia like and mesenchymal cells at 7 dpl has been detected (26). At 1-7 dpl, Wnt/ß-catenin signaling was detected in radial glia cells in blastema to induce progenitor differentiation into neurons during the process of neurogenesis and axonal re-growth (7). Overexpression of inhibitory LPA by neuron-like and glia-like cells has been continued in the chronic phase after injury to prevent neural sprouting (23). L1.2, a cell recognition molecule, and major vault protein (MVP) as well as other re-growing permissive proteins were detected in NMLF at 6 and 11 dpl (20). While, L1.2 was preferentially expressed by motor neurons and immature neurons around the central canal, MVP is detected in ependymal cells, motor neurons, noradrenergic and dopaminergic neurons around the central canal in the gray matter and in spinal cord parenchyma (36). In addition to neurons, the upregulation of L1.2 in putative glial cells (most likely astrocytes or oligodendrocytes) caudal to the lesion site at 7 and 14 dpl has also been reported (8).

Another isoform of cell recognition molecules (L1); L1.1, significantly increased after several weeks delay to L1.2, in the projection neurons of NMLF, IMRF, the magno cellular octaval nucleus, the nucleus ruber, nucleus of the lateral lemniscus, and the tangential nucleus of the brain stem at 1-6 wpl (8, 16). L1.1 also upregulated in Mauthner cells, a bilateral pair of giant projection neurons after distal lesion (8).

Syntenin-a, involved in synapse formation, was highly expressed by neuron- and glia-like cells around the central canal and in the white matter 6 and 11 dpl (42). At 11 dpl, neurons of NMLF and ependymal cells overexpressed Tenascin-C for promoting axonal regeneration from brain stem (12).

Production of protein zero (P0) mRNA in the peripheral white matter enhances to caudally regenerate descending axons of brain stem to the lesion site 14 dpl (40). Upregulation of inhibitory Ptena, in motor neurons and immature neurons around the central canal and in the white matter continued to 6 dpl and returned to normal levels at 11dpl (31).

Regenerating neurons as well as neuron-associated glial cells express zfNLRR (zebrafish neuronal leucine-rich repeat) in different parts of zebrafish CNS including the somatosensory medial funicular nucleus (MFN), the reticular formation, vagal motor nucleus (NXm), medial longitudinal fascicle (MLF), inferior reticular formation (IRF) in the medulla oblongata, medial octavolateralis nucleus (MON) and IMRF in the metencephalon. The adhesive strength of neurons is controlled by zFNLRR during neuronal growth in response to extracellular environment (18). Retinoic acid signaling pathway was also detected during the motor neuron regeneration process (37). Glial bridge, as a mechanical facility of zebrafish spinal cord, supports the regenerating axons. Overexpression of fgf3 and its target gene in fgf signaling, spry4, in glia cells and neurons is involved in glia bridge formation and was detected at 2-3 wpl (37, 24).

Cellular changes

Parallel to molecular alterations, various kinds of glial and neural progenitor cells also undergo changes after spinal cord injury; the motor neuron progenitor-like, ependymoradial glial cells proliferated and over expressed olig-2 at 14 dpl to facilitate proliferation and differentiation of motor neurons and continued to 6 wpl (37, 38). Neurogenic spinal radial glia progenitors are able to differentiate neurons and interneurons after injury (19).

Ependymoradial glial cells (lining the central canal) were proliferated and overexpressed patched1

receptor, known as a target gene of the Hedgehog (hh) signaling at 14 dpl, which has a major effect on the regeneration of motor neurons. These cells also upregulated other transcription factors such as Pax6 and Nkx6 at 2-6 wpl (37). One study reported that Pax6 and Nkx6.1 were detected in V2 (ventral domain two) interneurons (differentiated from the V2 interneuron progenitor) after injury (29). At 14 dpl, notch1a and notch1b, receptors of notch signaling pathway were upregulated from undetectable levels around the central canal. In addition to receptors, other ligands of notch signaling pathway including Her9, deltaC, Her4, 5 and Jagged1b were upregulated in the chronic phase after injury. Altogether, notch signaling inhibits motor neuron generation and progenitor proliferation in the ventromedial injured spinal cord (21).

Throughout all mature neurons, dopaminergic and serotonergic neurons were particularly evaluated and showed massive innervation in order to promote motor neuron regeneration by secretion of serotonin and dopamine during regeneration. Serotonin promotes proliferation of these cells after injury (15, 28). Dopaminergic axons are originated from diencephalon, and serotonergic axons are derived from descending axons of the brain stem. PMN-like ependymoradial glial cells, radial glial cells and oligodendrocytes express serotonin receptors; thus, these cells are affected by serotonergic axons after injury.

In summary, as principal mechanisms of regeneration after SCI in zebrafish, Wnt/ß-catenin signaling, L1.1, L1.2, MVP, contactin-2 and HMGB1 had positive effects on axonal re-growth, while Ptena has an inhibitory effect (7, 8, 16, 36). Neurogenesis is stimulated by Wnt/ß-catenin signaling as well as HMGB1, but inhibited by Notch signaling (7, 22, 21). Glial cells proliferate in response to fgf signaling and LPA (23, 24, 37). Furthermore, fgf signaling pathway causes glia bridge formation in favor of axonal regeneration (24,37). In the acute phase, LPA and HMGB1 stimulate inflammatory responses around injury and suppress regeneration (22, 23). LPA also induces microglia activation and neuronal death in addition to glia cell proliferation, but prevents neurite sprouting (23, 24, 37).

Limitations

Since specific search decreases the number of papers in the screening phase, we did not design our search strategies very specifically in order to include all the related papers for screening. In general, a systematic review requires a comprehensive literature involving at least two databases. However, PubMed is the most complete bibliographic database of biomedicine; Mesh-based queries return more relevant articles compared to keyword searching and generally are recommended by librarians. While it is desirable to include the greatest possible number of applicable articles in the first screening level of a systematic review due to lack of relevant literature in the scope of our study, only 130 results were gathered after the duplication removal. Data quality assessment for each article has not been considered and discussed separately. Also, there is no comparison with other systematic reviews.

Conclusion

The zebrafish is an excellent model to study the mechanisms underlying successful and failed axonal regeneration after SCI. However, molecular and cellular mechanisms involved in this phenomenon are not fully understood. Uncovering the molecular mechanism for endogenous regeneration of adult zebrafish spinal cord would give us more clues on important targets for future therapeutic approach in mammalian spinal cord repair and regeneration. This study provides a systematic review of the known molecules and mechanisms in the current literature involved in the SCI regeneration in zebrafish, in a time course manner. A better understanding of the whole determining mechanisms in this process should be considered as the main goal for future studies.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Pinto L, Gotz M. Radial glial cell heterogeneity-the source of diverse progeny in the CNS. Prog Neurobiol 2007; 83:2-23.
2. Rahimi-Movaghar V, Yazdi A, Karimi M, Mohammadi M, Firouzi M, Zanjani LO*, et al.* Effect of decompression on complete spinal cord injury in rats. International J Neurosci 2008; 118:1359-1373.
3. Rahimi-Movaghar V, Vaccaro AR, Mohammadi M. Efficacy of surgical decompression in regard to motor recovery in the setting of conus medullaris injury. J Spinal Cord Med 2006; 29:32-38.
4. Rahimi-Movaghar V, Saadat S, Vaccaro AR, Ghodsi SM, Samadian M, Sheykhmozaffari A*, et al.* The efficacy of surgical decompression before 24 hours versus 24 to 72 hours in patients with spinal cord injury from T1 to L1–with specific consideration on ethics: a randomized controlled trial. Trials 2009; 10:77.
5. Rahimi-Movaghar V, Sayyah MK, Akbari H, Khorramirouz R, Rasouli MR, Moradi-Lakeh M*, et al.* Epidemiology of traumatic spinal cord injury in developing countries: a systematic review. Neuroepidemiology 2013; 41:65-85.
6. Becker CG, Becker T. Zebrafish as a model system for successful spinal cord regeneration. Model organisms in spinal cord regeneration: John Wiley and Sons; 2007. 289-319.

7. Briona LK, Poulain FE, Mosimann C, Dorsky RI. Wnt/ß-catenin signaling is required for radial glial neurogenesis following spinal cord injury. Dev Biol 2015; 403:15-21.
8. Becker T, Bernhardt RR, Reinhard E, Wullimann MF, Tongiorgi E, Schachner M. Readiness of zebrafish brain neurons to regenerate a spinal axon correlates with differential expression of specific cell recognition molecules. J Neurosci 1998; 18:5789-5803.
9. Briona LK, Poulain FE, Mosimann C, Dorsky RI. Wnt/ss-catenin signaling is required for radial glial neurogenesis following spinal cord injury. Dev Biol 2015; 403:15-21.
10. Fleisch VC, Fraser B, Allison WT. Investigating regeneration and functional integration of CNS neurons: Lessons from zebrafish genetics and other fish species. Biochim Biophys Acta 2011; 1812:364-380.
11. Wang Z, Reynolds A, Kirry A, Nienhaus C, Blackmore MG. Overexpression of Sox11 promotes corticospinal tract regeneration after spinal injury while interfering with functional recovery. J Neurosci 2015; 35:3139-3145.
12. Yu YM, Cristofanilli M, Valiveti A, Ma L, Yoo M, Morellini F*, et al.* The extracellular matrix glycoprotein tenascin-C promotes locomotor recovery after spinal cord injury in adult zebrafish. Neuroscience 2011; 183:238-250.
13. Panic N, Leoncini E, de Belvis G, Ricciardi W, Boccia S. Evaluation of the endorsement of the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement on the quality of published systematic review and meta-analyses. PLoS One 2013; 8:e83138.
14. Hassannejad Z, Sharif-Alhoseini M, Shakouri-Motlagh A, Vahedi F, Zadegan SA, Mokhatab M*, et al.* Potential variables affecting the quality of animal studies regarding pathophysiology of traumatic spinal cord injuries. Spinal Cord 2015.
15. Barreiro-Iglesias A, Mysiak KS, Scott AL, Reimer MM, Yang Y, Becker CG*, et al.* Serotonin promotes development and regeneration of spinal motor neurons in zebrafish. Cell Rep. 2015; 13:924-932.
16. Becker CG, Lieberoth BC, Morellini F, Feldner J, Becker T, Schachner M. L1.1 is involved in spinal cord regeneration in adult zebrafish. J Neurosci 2004; 24:7837-7842.
17. Becker T, Becker CG. Regenerating descending axons preferentially reroute to the gray matter in the presence of a general macrophage/microglial reaction caudal to a spinal transection in adult zebrafish. J Comp Neurol 2001; 433:131-147.
18. Bormann P, Roth LWA, Andel D, Ackermann M, Reinhard E. zfNLRR, a novel leucine-rich repeat protein is preferentially expressed during regeneration in zebrafish. Mol Cell Neurosci 1999; 13:167-179.
19. Briona LK, Dorsky RI. Radial glial progenitors repair the zebrafish spinal cord following transection. Exp Neurol 2014; 256:81-92.
20. Chen T, Yu Y, Hu C, Schachner M. L1.2, the zebrafish paralog of L1.1 and ortholog of the mammalian cell adhesion molecule L1 contributes to spinal cord regeneration in adult zebrafish. Restor Neurol Neurosci 2016; 34:325-335.
21. Dias TB, Yang YJ, Ogai K, Becker T, Becker CG. Notch signaling controls generation of motor neurons in the lesioned spinal cord of adult zebrafish. J Neurosci 2012; 32:3245-3252.
22. Fang P, Pan HC, Lin SL, Zhang WQ, Rauvala H, Schachner M*, et al.* HMGB1 contributes to regeneration after spinal cord injury in adult zebrafish. Mol Neurobiol 2014; 49:472-483.
23. Goldshmit Y, Matteo R, Sztal T, Ellett F, Frisca F, Moreno K*, et al.* Blockage of lysophosphatidic acid signaling improves spinal cord injury outcomes. Am J Pathol 2012; 181:978-992.
24. Goldshmit Y, Sztal TE, Jusuf PR, Hall TE, Nguyen-Chi M, Currie PD. Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. J Neurosci 2012; 32:7477-7492.
25. Guo Y, Ma L, Cristofanilli M, Hart RP, Hao A, Schachner M. Transcription factor Sox11b is involved in spinal cord regeneration in adult zebrafish. Neuroscience 2011; 172:329-341.
26. Hui SP, Nag TC, Ghosh S. Characterization of proliferating neural progenitors after spinal cord injury in adult zebrafish. PLoS One 2015; 10:e0143595.
27. Hui SP, Dutta A, Ghosh S. Cellular response after crush injury in adult zebrafish spinal cord. Dev Dyn 2010; 239:2962-2979.
28. Kuscha V, Barreiro-Iglesias A, Becker CG, Becker T. Plasticity of tyrosine hydroxylase and serotonergic systems in the regenerating spinal cord of adult zebrafish. J Comp Neurol 2012; 520:933-951.
29. Kuscha V, Frazer SL, Dias TB, Hibi M, Becker T, Becker CG. Lesion-induced generation of interneuron cell types in specific dorsoventral domains in the spinal cord of adult zebrafish. J Comp Neurol 2012; 520:3604-3616.
30. Lin JF, Pan HC, Ma LP, Shen YQ, Schachner M. The cell neural adhesion molecule contactin-2 (TAG-1) is beneficial for functional recovery after spinal cord injury in adult zebrafish. PLoS One 2012; 7:e52376.
31. Liu D, Yu Y, Schachner M. Ptena, but not Ptenb, reduces regeneration after spinal cord injury in adult zebrafish. Exp Neurol 2014; 261:196-205.
32. Ma L, Shen YQ, Khatri HP, Schachner M. The asparaginyl endopeptidase legumain is essential for functional recovery after spinal cord injury in adult zebrafish. PLoS One 2014; 9:e95098.
33. Ma L, Yu YM, Guo Y, Hart RP, Schachner M. Cysteine- and glycine-rich protein 1a is involved in spinal cord regeneration in adult zebrafish. Eur J Neurosci 2012; 35:353-365.
34. Ogai K, Nakatani K, Hisano S, Sugitani K, Koriyama Y, Kato S. Function of Sox2 in ependymal cells of lesioned spinal cords in adult zebrafish. Neurosci Res 2014; 88:84-87.
35. Ogai K, Hisano S, Mawatari K, Sugitani K, Koriyama Y, Nakashima H*, et al.* Upregulation of anti-apoptotic factors in upper motor neurons after spinal cord injury in adult zebrafish. Neurochem Int 2012; 61:1202-1211.
36. Pan HC, Lin JF, Ma LP, Shen YQ, Schachner M. Major vault protein promotes locomotor recovery and regeneration after spinal cord injury in adult zebrafish. Eur J Neurosci 2013; 37:203-211.
37. Reimer MM, Kuscha V, Wyatt C, Sorensen I, Frank RE, Knuwer M*, et al.* Sonic hedgehog is a polarized signal for motor neuron regeneration in adult zebrafish. J Neurosci 2009; 29:15073-15082.
38. Reimer MM, Sorensen I, Kuscha V, Frank RE, Liu C, Becker CG*, et al.* Motor neuron regeneration in adult zebrafish. J Neurosci 2008; 28:8510-8516.
39. Schweitzer J, Gimnopoulos D, Lieberoth BC, Pogoda HM, Feldner J, Ebert A*, et al.* Contactin1a expression is associated with oligodendrocyte differentiation and axonal regeneration in the central nervous system of zebrafish. Mol Cell Neurosci 2007; 35:194-207.

40. Schweitzer J, Becker T, Becker CG, Schachner M. Expression of protein zero is increased in lesioned axon pathways in the central nervous system of adult zebrafish. GLIA 2003; 41:301-317.
41. Vajn K, Plunkett JA, Tapanes-Castillo A, Oudega M. Axonal regeneration after spinal cord injury in zebrafish and mammals: differences, similarities, translation. Neurosci Bull 2013; 29:402-410.
42. Yu Y, Schachner M. Syntenin-a promotes spinal cord regeneration following injury in adult zebrafish. Eur J Neurosci 2013; 38:2280-2289.
43. Yu YM, Gibbs KM, Davila J, Campbell N, Sung S, Todorova TI, *et al.* MicroRNA miR-133b is essential for functional recovery after spinal cord injury in adult zebrafish. Eur J Neurosci 2011; 33:1587-1597.