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

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**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 keywords: Zebrafish; spinal cord injuries; regeneration; recovery of function. The map of mechanisms was performed using Xmind software.

**Results:** Wnt/β-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 Pten had an inhibitory effect. Neurogenesis is stimulated by Wnt/β-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, IGF 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.

**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 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-length), 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 ethylmethyl ester 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 hybridization (n=23) were the most frequent cellular, molecular and histological methods applied to investigate the regeneration followed by quantitative real-time polymerase chain reaction (RT-PCR) (n=15) and locomotor analysis (n=16). The frequency of study methods is shown in Table 2.
Table 1. Characteristics of studies

| 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 | Mid thoracic spine | 24-26, 33 hpl; 10, 14 dpl | ACX |
| 16      | Becker        | 2004 | L1.1     | Recognition molecule | Permissive | Lesion site (NMLF) | 10 dpl; 6 wpl | ADIJLX |
| 17      | Becker        | 2001 | Neuron | Recognition molecule | Permissive | Axons from all descending tracts including lateral funiculus and MLF | 2, 14 dpl; 6 wpl | AEFGJL |
| 8       | Becker        | 1998 | zFNLRR   | Neuronal specific adhesion molecule | Permissive | MLF and IRF in the medulla oblongata, MON, IMRF neurons and radial glial cells | 5 dpl | ABEGUX |
| 7       | Briona        | 2015 | Wnt/β-catenin | Signaling pathway | Permissive | Blastema and around, the level of anal pore in the spinal cord | 1, 3, 5, 7 dpl | AV |
| 19      | Briona        | 2014 | Radial glial progenitor | (Cells) | NA | Blastema and proximal site of injury at the level of the anus in the spinal cord | 1, 5, 9 dpl | ABFX |
| 20      | Chen          | 2016 | L1.2      | L1.1 paralog and ortholog of mammalian L1CAM | Permissive | Neurons and GFAP-immunoreactive glia (labeled 1, HuC/D and GFAP immunopositive cells) | 6, 11 dpl | ABCDEGHMT |
| 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 | HMG1      | Nuclear protein | Permissive | Along the central canal and in motoneurons | 4, 12, 24 hpl; 6, 7, 11, 21 dpl | ABCDGHO |
| 23      | Goldshmidt    | 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      | Goldshmidt    | 2012 | fgf        | Growth factor | (Promoting regeneration) | Glial cell morphogenesis | In glial cell and injury site | 3, 5, 10 dpl; 2, 3 wpl | ABCDEGHXX |
| 25      | Guo           | 2011 | Sox11b     | transcription factor | (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 d pl; 6 wpl | ABCDF |
| 26      | Hui           | 2015 | Newly generated Cellular profile (Sox2, OCT4/HuC/D, A25+ cells progenitors) | NA | NA | Ependyma around the central canal | 1, 3, 7, 10, 15 dpl | ABCFKLST |
## Continued Table 1

| No. | Author Year | Treatment | Cellular Profile | Molecule | Function | Tissues | Phenotype |
|-----|-------------|-----------|------------------|----------|---------|---------|-----------|
| 27  | Hui 2010    |          | RBC, macrophase, Schwann, neuron | NA       | Injury epicenter and the adjacent part | 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 cental 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 (CGRP)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 | ABCDHJN |
| 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 | Permissive | Brainstem neurons of the NMLF and IMRF | 1–15 dpl | AEFHKMP |
| 36  | Pan 2013    | MVP (Major vault protein) | Multifunctional protein | Functional recovery and axonal regrowth | Ependymal cells (brainstem) | 4, 12 hpl; 6, 11 dpl; 4-6 wpl | ABCDEGHIX |
| 37  | Reimer 2009 | sonic hedgehog (shh) | Ventral morphogen | Permissive (Neurogenesis) | Ependymonal glial cells lining the central canal in ventrodorsal positions | 1, 2, 6 wpl | ABCEP |
|     |             |                  | olig2  | Ependymonal glial cells | Ventricular zone |          |          |
|     |             |                  | nle6.1 | Transcription factors | - |          |          |
|     |             |                  | pax6  | Transcription factors | - |          |          |
| 38  | Reimer 2008 | olig2-positive (olig2+) | Ependymonal glial progenitor cells | Permissive (Motor Neuron Regeneration) | The ventricular zone | 1, 2, 6-8wpl | AEFKLX |
|     |             |                  | Homolog of contactin1 (F3/F11/contacin) in mammals; an immunoglobulin superfamily recognition molecule of neurons and oligodendrocytes | | | |
|     |             |                  | Brainstem neurons and white matter glial cells | 14 dpl | ABEX |
| 39  | Schweitzer 2007 | Contactin1a (Cntn 1a) | Brainstem neurons and white matter glial cells | 14 dpl | ABEX |
Spinal cord injury regeneration in zebrafish
Noorimotlagh et al.

Iran J Basic Med Sci, Vol. 20, No. 12, Dec 2017

Continued Table 1

|   | Study methods                                                                 | Frequency of studies |
|---|------------------------------------------------------------------------------|----------------------|
| 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                                                  | N                   |
|   |                                      |                      |
|   |                                      |                      |
|   |                                      |                      |
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|   |                                      |                      |
|   |                                      |                      |
|   |                                      |                      |
| 42 | Yu 2013 Syntenin-a Scaffold protein involved in mammalian cell adhesion and movement, axonal outgrowth, establishment of cell polarity, and protein trafficking | Permissive           |
|   | Reticular formation, MON, NMLF (at least four millimeters beyond the lesion) | 2, 4, 8 wpl          |
|   | ABCDEGMP                                                                     |                      |
| 43 | Yu 2011 miR-133b MicroRNAs(miRNAs)                                           | Permissive           |
|   | Regenerating neurons of the brainstem, supraspinal neurons and NMLF          | 6, 24 hpl; 7 dp16 wpl|
|   | ABCDEFGHIOQ                                                                 |                      |
| 12 | Yu 2011 Tenascin-C Extracellular matrix glycoprotein                          | Permissive           |
|   | Gray matter, innervation of motoneurons                                       | 4, 48 hpl; 11 dp16 wpl|
|   | ABIDFEG                                                                     |                      |

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                    |
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.

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 with in 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 Mssx-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 contactin2 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/β-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) causal 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 magnocellular oval 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 at 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 11 dpl (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). Gliarial 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 gliarial 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 serotonin axons after injury.

In summary, as principal mechanisms of regeneration after SCI in zebrafish, Wnt/β-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/β-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.

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Noorimotlagh et al.

Spinal cord injury regeneration in zebrafish

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