Non-coding RNAs as key player in stem cell proliferation and differentiation; Potential therapeutic strategies in spinal cord injuries

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ABSTRACT

Spinal cord injury (SCI) is a devastating condition which often results in the loss of sensory and motor function below the lesion site. The consequences of SCI constitute substantial burden to both the patient and society. Existing treatments for SCI injuries have proved inadequate, partly owing to an incomplete understanding of post-injury cellular and molecular changes. SCI triggers a multitude of pathophysiological events that are tightly regulated by the expression levels of specific genes. It has been shown that numerous non coding RNAs (ncRNAs), especially microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are differentially expressed following SCI. MiRNA and lncRNA could, as regulators of gene expression at the post-transcriptional level, affect the pathophysiology of SCI. In addition, these ncRNAs are emerging as key regulators of stem cell differentiation and proliferation, thus, manipulating their levels could improve future therapeutic neural stem cell transplantation strategies. This review will discuss the most common and well-established therapeutic strategies (specially focusing on stem cell and non-coding RNAs studies) in SCI management.

Key Words: Spinal cord injury; Stem cells; miRNA; lncRNA; Therapeutics

Spinal cord injury (SCI) is a devastating condition resulting in severe and permanent neurologic deficits (either sensory, motor, or autonomic function) (1). Spinal cord injuries have become an emerging threat in the last 40 years and understanding their epidemiologyis required to implement the appropriate measures (2). The global incidence of SCI has been reported to vary from 8 to 246 cases per million inhabitants a year with prevalence ranging from 236 to 1,298 per million inhabitants. The annual incidence of SCI per million among geographic region is as follows: USA 20.7 to 83.0, Europe, 8.0 to 130.6, Asia and the Middle East, 14.6 to 246 and Oceania 10.0 to 77.0 (3).

In the United States, the annual incidence of spinal cord injury (SCI) is 54 cases per million population or approximately 17,000 new SCI cases each year (4).

As for Norway the annual incidence rate of SCI increased over time from 6.2 per million in 1952–1956 to 26.3 per million in 1997–2001 (3).

On the other hand, considerable differences are present among the studies from distinct countries in Asia. The annual incidence of SCI ranged from 12.06 to 61.6 per million (5).

No literature has been found regarding the incidence or prevalence of SCI in Africa (2). When it comes to age at which SCI occurs, the highest incidences in all countries were reported between 20 and 50 years old. As for gender, men exhibit higher risk than women (5).

The primary causes of traumatic spinal cord injury (TSCI) are motor vehicle collisions (MVCs) and falls. However, several countries reported war wounds as the major cause. As for severity of SCI, most patients are categorized as AIS/Frankel grades A (5).

Concerning SCI complications, spinal cord lesion can cause physical disability as well as a wide range of secondary complications. Yet, every injury is unique and these complications will not affect everyone (4).

The economic burden associated with traumatic spinal cord injury from a societal perspective includes both direct and indirect costs. A Canadian study using an incidence-based approach estimated the lifetime economic burden per individual with TSCI to range from $1.5 million for incomplete paraplegia to $3.0 million for complete tetraplegia. The annual economic burden associated with 1389 new persons with TSCI surviving their initial hospitalization is estimated at $2.67 billion (6).

Spinal cord injury causes significant morbidity and mortality. Even if the individual survives, it's challenging for them to go through life due to the severe damage that the injury causes. Spinal cord injury requires life-long treatment. And eventually, most of the injured cases don't experience normal life again (7).

This review will discuss the most common and well established therapeutic strategies specially focusing on stem cell and non-coding RNAs studies in SCI management.

Stem cells

Stem cells are unique type of cells derived from an embryo, foetus, or an adult. They have the ability to regenerate and renew themselves, differentiate and develop into many different specialized cells such as: liver cells, muscle cells, blood cells, etc. (8,9).

There are different types of stem cells such as: embryonic stem cells (ESCs), tissue specific stem cell (adult stem cell), and induced pluripotent stem cells (iPSCs) (8).

The first kind of stem cells is embryonic stem cells; they are pluripotent and can be harvested from the inner cell mass of blastocyst. ESCs have the ability to differentiate into every cell type in the body except the placenta and umbilical cord. These cells are of great value since they can be used in studying normal development of body and for testing of drugs and other therapies (10).

The second type of stem cells is tissue-specific stem cells (known as adultor somatic stem cells) which are more differentiated than embryonic stem cells. They can only be used to heal and generate the cell types of the tissue in which they reside. They are harder to find in the body compared to embryonic stem cells. Studying these cells can help understand what happens in aging, injury and pathogenesis (11).

A specific cell type of adult stem cells is mesenchymal stem cells; they are multipotent stromal cells which are derived from stroma surrounding the tissues and organs. Various MSCs are questioned for having

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immunomodulatory properties but this questioning is still being tested. Not all MSCs are the same; they have different characteristics based on the organ from which the cells are obtained (12).

The last kind of stem cells is induced pluripotent stem cells which are converted from tissue-specific cells into cells that act as embryonic stem cells. Although induced pluripotent stem cells share many of embryonic stem cells' properties, they aren't the same (13).

ROLE OF STEM CELLS IN SCI

Many ways for recovery from spinal cord injury have been suggested including oligodendrocytes and neurons replacement, remaining axons re-myelination, neuronal circuitry recovery, conservation of host neuronal and glial cells, neurotrophins/cytokines enhanced manifestation by transplanted or host cells, angiogenesis elevation, cysts or cavities joining, abridged inflammation or gliosis, endogenous precursor cells stimulation, and providing favorable environment for plasticity and axonal renewal (14,15).

Embryonic stem cells can differentiate into all derivatives of the three primary germ layers. Undifferentiated transplanted ES cells can create teratomas, thus, ES cells must be differentiated before transplantation (16). Procedures have been established to differentiate ES cells into neural precursors and specific neuronal and glial ancestries (17). Pre-differentiated mouse ES cells transferred into the spinal cord of an incapacitated rat specialized into neurons and glia and displayed partial functional reclamation (18). SCI causes broad demyelination and oligodendrocytes become mainly susceptible to apoptosis (19,20). ES cells differentiated into oligodendrocyte progenitor cells (OPCs) remyelinated saved axons and enhanced recovery when relocated sub-acutely into the damaged rat spinal cord (21). The benefit of ES cells is that they can be propagated in culture almost indefinitely because of their ability to maintain elevated telomerase activity. However, it has been problematic to spawn high-purity lineage-specific cell lines deprived of karyotypic abnormalities (22).

Neural stem/progenitor cells (NSPCs) are cells dedicated to generating the neural lineage that can self-renovate and expand *in vitro*. The neural stem cells react to the growth factors in the culture environment and selectively multiply in interruption to form neurospheres. When these cells are coated in growth factor–free medium comprehending serum, they specialize into neurons, oligodendrocytes, and astrocytes (23). NSPCs are found in both fetal and adult CNS (24). They exist within niches in the adult CNS. Multipotential, self-renewing NSPCs can be insulated and cultivated from the mature rodent spinal cord when the cultivated tissue embraces regions of the central canal (25). NSPCs mainly discriminate into oligodendrocytes *in vitro* and *in vivo* (26). Their transplantation into SCI rats helped functional recovery with neuroprotective and neuroregenerative effects. Adult mouse brain–derived NSPCs displaced into the injured rat spinal cord with associated infusion of growth factors endorsed oligodendrocyte specialization of the grafted NSPCs, remyelination, and enhanced locomotor function (27) NSPCs taken from fetal rat spinal cord distinguished into neurons that assimilated into the injured cord and enhanced recovery (28) and transplanted NSPCs combined with valproic acid management endorsed neuronal differentiation, resulting in renewal of disrupted neuronal circuitry and boosted recovery (29). In addition, NSPCs have shown some immunomodulatory and pathotropic capability by homing towards injured tissues as well as secreting various neurotrophic factors and cytokines (30,31). Most experimental SCI studies with NSPC transplants have induced rodent cells because human stem cells were either not accessible or hard to cultivate. Human NSPCs have been secluded from fetal brain and spinal cord from terminated fetuses and from adult brain through surgical biopsy specimens and postmortem tissue (32-34). Recently, self-renewing multipotent NSPCs have demonstrated the ability to be channeled from the adult human spinal cord of organ transplant benefactors and these cells specialized into both neurons and glia after being transplanted into rats with SCI (35) Stem cells derived from the human fetal brain were transferred into NOD/SCID mice with SCI, and the embedded cells expressed neural differentiation markers and progressive recovery (36). Broad neuronal specialization of human fetal NSPC grafts was testified after transfer into the adult rat spinal cord (37). Furthermore, human fetal brain NSPCs (adjusted to convey galectin-1) relocated sub-acutely into the contused cervical spinal cord of adult common marmosets created considerably better grip power than controls (38).

NON-CODING RNA IN SPINAL CORD INJURY

Although non-coding RNAs (ncRNAs) almost have no function in protein structural coding, these nucleic acids play a massive role inside the cell. Though, most of it is not discovered yet. Non-coding RNAs as ribosomal RNA and transfer RNA aid in translation process, small nuclear RNA involved in splicing and small nucleolar RNA, which guide modification of rRNA. Newly discovered ncRNAs including short regulatory RNA (microRNA, piwi-associated RNA, endogenous short-interfering RNA) and long non-coding RNA (lncRNA) act as regulatory molecules for gene expression through different levels and mechanisms (39).

ROLE OF miRNA IN SCI

miRNAs are short RNA molecules (around 22 nucleotides long) that prevent the translation of mRNA via their complementary binding at the 3' untranslated region of mRNA (40). This interaction shows cross-reactivity; which means that not only one miRNA can affect several mRNA but mRNA can also be inhibited by more than one miRNA (12). Liu et al were the first to test the changes in miRNA expression following SCI in rats showing that 300 miRNA levels were altered after 4 hours, 1 and 7 days following the injury with 60 showing significant changes. They were then classified into 3 groups: 1- up regulated 2- down regulated 3-up regulated after 4 h then down regulated (41). A study was performed with a moderate SCI revealed that there was a massive decrease in miRNA expression associated with increased mRNA expression. A bioinformatics analysis showed the role of miRNAs in pathophysiological events following SCI (42).

According to their effect on SCI, some miRNAs are considered protective such as miR-146a and miR-138 with their anti-apoptotic effect and miR-181a reducing the inflammatory process, while others like miR-30b, miR-30c, miR-223, miR-15b, miR-1, and miR-145 are considered detrimental as they induce SCI progression (43).

Regarding the events following traumatic SCI, miRNAs have remarkable action on inflammatory response, immune cells infiltration, cell death, astrogliosis and axonal regeneration. This information constitutes a great step for using these molecules as therapeutic targets (44). MiR-133b is correlated with the ability of zebra fish to regenerate their CNS (45). This miRNA shows up-regulation shortly after SCI then is down-regulated 1 day later (14). MiR-124 also shows a similar pattern which reduces axonal regeneration following SCI (46). Astrogliosis is a significant consequence of SCI that stands as an obstacle against neural regeneration (47). A group of miRNAs have shown a role in this process. Mir-21 expression is increased following SCI associated with decreased astrocytic hypertrophy (48). Moreover, exercise-induced recovery is accompanied by increased mir-21 expression providing an evidence of its possible usage as a therapeutic target (49). Mir-81, mir-146 and mir-125b also affect astrogliosis either by enhancement or suppression (17).

MiRNAs strongly affect the inflammatory response following SCI. miRNAs like mir-126, mir-223, and mir-142 affect immune cell infiltration following SCI. mir-124 down regulation cause microglial activation. Overexpression of mir-17-92, mir-98, mir-106a, mir-323, and mir-221 has a significant anti-inflammatory effect. In addition, inflammatory mediators like (TNF-, IL-6, IL-1) and the complement cascade are also targets of post SCI miRNA changes (17).

THERAPEUTIC IMPLICATIONS OF miRNA IN SCI

miRNAs are very promising in therapeutic applications due to their small size which allows them to diffuse easily to cells and their targeting of multiple mRNAs which magnifies their effectiveness and their specificity to cells (50). MiRNA-based therapy for hepatitis c is now in phase 2clinical trials giving hope for their further use in more applications including SCI (51). The difficulty in designing such therapeutic options is in selecting a delivery method and making the proper modifications in the chosen RNA molecules to reduce the dosing and avoid toxicity (22). The designed therapy can be either a mimetic or anti-miRNA depending on the targeted miRNA. For example, animal models neuropathic pain was improved following miR23b infusion compensating for its down-regulation in SCI (52). As miRNAs are modulators of gene expression, so it is easy to speculate that they could become novel therapeutics for promoting neurogenesis after SCI.

Neurogenesis is the process of generation of newborn neuronal cells from NSCs and progenitor cells in the mammalian brain through steps that include NSC self-renewal, proliferation, neuronal differentiation, migration, maturation, and integration (53). It continues in the adulthood at 2 separate sites: a) the hippocampal dentate gyrus b) the sub-ventricular zone (54).

Many miRNAs control the process of neurogenesis as miR-124, miR-9, miR-137and let-7 in NSC proliferation and differentiation, miR-125b in neuronal proliferation and maturation and miR-132, in integration (55).

miR-124

miR-124 is the most abundant miRNA in both the embryonic and adult

CNS (56). Ectopic expression of miR-124 in cultured non-neuronal cells increased the expression of neuronal genes and inhibited non-neuronal gene expression (57,58). miR-124 also decreases the expression of Ezh2 which is a polycomb group protein,Ezh2 is a H3 Lys-27 histone methyltransferase. It decreases neural progenitor cells through repressing the expression of several neurogenesis-promoting genes as master regulator for neuronal lineage commitment and differentiation Ascl1 (Achaete-scute complex homolog 1 (Drosophila). Thus, it promotes neuronal and counters astrocyte-specific differentiation route (59). Moreover, overexpression of miR-124 was associated with increased expression of the protein-III tubulin after 6 days of *in vitro* culture; tubulin is an important structural protein in neurons and is a marker of differentiated neurons. miR-124 not only promotes differentiation in BMSCs " bone marrow stem cells " into neurons, but it also directs them to develop synapses, which is important in nerve function`s recovery (60). miR-124 also regulates neuronal maturation by targeting the expression of cAMP response element-binding protein (CREB) as it represses the expression of fork-head box protein G1(61).

miR-124a and miR-9 overexpression decreased signal transduction and activation of transcription 3 (STAT3), whose phosphorylation leads to the inhibition of the differentiation of neuro-progenitor stem cells into neurons and induction of glia differentiation (29).

There are other miRNAs that control neurogenesis such as miRNA lethal-7b (let-7b). Overexpression of let-7b inhibits neural stem cell proliferation and induces neural differentiation while knockdown of let-7b resulted in increased proliferation (31,62).

Another example of miRNA involvement with neurogenesis is miRNA-125a-3p. It was demonstrated that neural differentiation of cultured adipocyte-derived stem cells was accompanied by a decrease in miRNA-125a-3p expression and an increase in insulin-like growth factor expression. Thus, targeting miRNA 125a- 3p or enhancing insulin-like growth factor expression could improve neural differentiation (63).

miRNA miR-137 also affects mice neural stem cell as increasing its expression leads to decreasing NSCs proliferation and increasing the differentiation of the latter through A regulatory loop of TLX-miR-137-lysine-specific histone demethylase 1(LSD1) (64).

miR-219 is essential to promote oligodendrocyte differentiation in mouse and zebra fish by inhibiting negative regulators of oligodendrocyte differentiation (65).

miR-132 has 2 important functions, (a) it mediates the Integration of newborn neurons into the Adult Dentate Gyrus (66). (b) it affects neuronal plasticity and memory as its over expression in hippocampal dentate gyrus induces neurite outgrowth and increases dendritic length and complexity (67).

ROLE OF LONG NON-CODING RNAs (LncRNAs) IN SCI

In addition to miRNAs, lncRNAs play a regulatory role on mRNA and the translation process. They also regulate signal transduction and miRNA. LncRNAs also play a role in neural development and disease (68,69). The first study of lncRNA expression profile following SCI in mice was performed by Ding et al showing that lncRNA expression was altered with slight changes 1 day post injury, markedly changed within 1 week then declined after 3 weeks providing an evidence of their possible role in SCI (70). While thousands of lncRNAs have been identified, few lncRNAs that control neural stem cell (NSC) behavior are known. Ramos et al, identified Pinky (Pnky) as a neural-specific lncRNA that regulates neurogenesis from NSCs in the embryonic and postnatal brain. Another example oflnc RNAs found to be necessary for neuronal differentiation is the rhabdomyosarcoma 2-associated transcript (RMST), which directly interacts with Sox2 and tethers it to the promoter regions of some neurogenic transcription factors to increase their expression and in turn induce neuronal differentiation of NSCs (71,72).

CONCLUSION

SCI is a complex pathology that induces strong molecular changes. NcRNAs are known as modulators of gene expression and several studies demonstrated that many ncRNAs are differentially expressed after SCI. In addition, they are evolving as regulators of stem cell differentiation and proliferation. Thus, altering ncRNA expressions in NSCs will provide a new means for stem cell based therapies for SCI.

FUTURE PERSPECTIVES

In the coming future, there might be new pharmaceutical approaches as using miRNA mimics or inhibitors, RNAi or emerging gene editing tools such asCRISPR-Cas9, these approaches could improve future therapeutic neural stem cell transplantation strategies through introducing a variety of genetic alterations on stem cells to control their proliferation and differentiation (73,74). This expected progress will bring considerable benefits to the patients suffering from SCI.

ETHICAL STATEMENT

The authors declare that they have no conflict of interest. The manuscript does not contain clinical studies or patient data. This study has not been funded. This article does not contain any studies with human participants or animals performed by any of the authors.

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