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Markers of Peripheral Neuronal Regeneration: A Bioinformatic Study.

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ABSTRACT

After an an injury to a nerve in the peripheral nervous system, there is partial or full loss of function in the innervated organ. This may present as paralysis, in motor neuron injury, or loss of sensation in sensory neurons lesions. Due to this, the body triggers a regenerative process through intrinsic and extrinsic pathways to enable the regrowth of the affected site or discontinuity on the axon. The proximal segment of the injured nerve develops growth cones that grow, with guidance from molecular signaling pathways, towards the ischemic distal stump. The triggering off of this process is dependent on neurotrophic factors (NTFs) and neuropoietic cytokines that are produced during the inflammatory response. This, therefore, creates a growth-friendly milieu around the axon. In conjunction with intrinsic signaling pathways, the regrowth process is coordinated and modulated to enable the axon to resume its functionality. The article used under this review were accessed primarily from Pubmed search engine and NCBI Bioinformatics search engine. Some material were added from Google Scholar. All these articles were relevant to the topic under study and were in Standard English. PRISMA guide was used as the criteria of inclusion and elimination. Twenty out of the initial one hundred and twenty-four made it through the elimination criteria. These sources had spelled out the physiological processes of the relevant molecules and pathways. The research experiments shown in the different resources all added substantial value to the topic of study. These, therefore, enabled simple analysis and comparison of facts and coming up with the conclusion. There are numerous markers of peripheral neuronal regeneration. These include simple molecules, structural proteins or even complex signaling pathways. None of these indicators proves superior to the other. Their functions are either additive or inhibitory to the others within a complex system where the molecules communicate with others in different pathways. Other markers are considered redundant since other markers serve an overlapping purpose.

Keywords: regeneration markers, peripheral neuronal regeneration, bioinformatic

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INTRODUCTION

These signaling pathways and molecules involved have stirred great interest in the minds of many neurologists and neurosurgeons, as they strive to find the primary target for medical practice in line with neuronal regeneration, to trigger off, enhance or modulate the whole process. Since after injury, very many signaling pathways are activated, no specific route has been singled out to be the main one involved in the modulation of the regenerative process. These signaling pathways show dense interconnections and linkages, both inhibitory and stimulatory. Therefore, understanding and singling out the most central pathway in this physiologic process proves to be an endlessly debatable topic (Kaplan, 2015).

Considering the complex interplay of pathways like phosphatidyl inositol 3-kinase (PI3K), Janus kinase/ signal transducers and activators of transcription (JAK/STAT) and cyclic adenosine monophosphate signaling pathway (cAMP) among others, intracellular physiologic processes are activated which is mainly transcription. Transcription of regeneration-associated genes (RAGs) in response to the activation of these pathways, therefore, signifies that the regeneration process involves protein synthesis (Willis, 2015). Activation of these signaling pathways does not necessarily mean that regeneration will take place or that it will progress at any given pace. Therefore, with this, scientists have tried to figure what molecules act as the markers of regeneration to monitor its progress. In this article review, we seek to delve into this issue and try to find out the important molecules that act as beacons or indicators of neuronal regeneration.

Due to the incapacitation or loss of function that follows neuronal loss due to injury, scientists have delved into this issue to elucidate the some of the ways that the problem can be reversed. Since the body has a natural means of neuronal regeneration, the scientists have targeted these pathways Involved in order to find a way to manipulate artificially and enhance the healing and regeneration of the injured neurons. Therefore, the choice to review articles on markers is entirely in tandem with this drive.

In this section, the detailed picture of the relevant neuronal regeneration markers and their physiological roles is explained

Research question

The purpose of this review was to find out the molecules that serve as the indicators of regeneration after injury to neurons I the peripheral nervous system. To act as a guide for this article review, I defined my research question to be:

- What is the main molecule that signifies active neuronal regeneration process and what is its physiological profile?

Response

Many molecules and signaling pathways have been associated with this process of neuronal regeneration. Most of them have additive physiologic activities whereas others have inhibitory effects on others. Moreover, other molecules have overlapping physiological activity, thereby, rendering others redundant. Therefore, no single molecule or marker has the overall role as a marker. The complex interplay of these molecules, thus, serves to be the most significant marker of neuronal regeneration.

METHODS

With a lot of dedication to the success of this work, much effort was put from the start to the end of the review. The conduction and reporting of this work were done on the basis of Preferred Reporting Items for Review and Meta-Analysis. To start with, I formulated the research question regarding the main objectives that drove the systemic review. This was later followed by a research strategy that was designed to streamline, and increase the efficacy of the referencing material. Pubmed (NCBI) and NCBI Bioinformatics search engine were the primary search engines for the review articles. Additional materials were retrieved from Google Scholar. The materials then went through rigorous vetting according to relevance and time of publishing as the main filters.

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SEARCH STRATEGY

The materials used in this review were retrieved from Pubmed (NCBI) search engine and NCBI Bioinformatics search engine as the primary sources. Additional materials were accessed from Google Scholar. Key words guided choice of the articles as a check for relevance. Cross-checking of different articles and reports and consideration of various scientific facts helped in generating the conclusion of the whole review. The time interval between the studies was between the year 2007 to the year 2015. The key words used in this article review research were microtubules, tubulin, S100, Actin, cAMP, neuronal regeneration and Sciatic nerve regeneration.

Study selection

In order to increase the efficacy and efficiency of the search process, the sources accessed underwent strict criteria of inclusion and inclusion. These criteria were crafted in a way that would enable the achievement of the research objectives with ease and sufficiency of research material. Randomized control trial was used as the method of study. This research was mainly centered invertebrates since humans are vertebrates. Therefore, any research that involved invertebrates was excluded at this point. Rats were the principal research animals. The neuronal injury was inflicted upon them by the scientists and monitored under modified conditions to get the results. Controls were also used in these experiments to keep the results of all the tests in check.

A total of one hundred and twenty-six sources were found. All these articles were in Standard English. Therefore, no item was eliminated by language. At the beginning of the elimination process, the year of publishing of the articles was used as a filter. The final articles were published between 2007 and 2015. This was reached after exclusion of twenty-one articles. Analyzing the source titles for relevance to the topic of study led to the elimination of fifty-seven articles. The next step was checking for relevance by reading the abstracts of the remaining articles. Here nineteen articles were excluded. Finally after reading through the articles, seven pieces were excluded. Thus, twenty articles remained.

Peripheral Nerve Injury

After an injury to neurons in the central nervous system, there is the formation of retraction bulbs. These are globular and larger that the growth cones formed by injured neurons in the peripheral nervous system. Structurally, these two features are different. The microtubules in the in the growth cone are well arranged and have 99% of the fibers oriented to the axonal axis. On the contrary, the fibers in the retraction bulbs are disorganized, and only 51 % of them are oriented to the axonal axis (Sainath, 2015). This, therefore, gives the growth cone the upper hand in the ability to grow or regenerate.

Actin is a molecule that forms part of the cytoskeleton and aids in locomotion. It exhibits a treadmill fashion of motion. This type of motion enhances the process of neurite outgrowth. Cross-linkage with other structural proteins forms complex structures that serve to enhance the overall process of regeneration. There are two isoforms of these molecules that play the role of neuronal regeneration. These are Actin β and γ. B-actin is mainly concentrated in the growth cone (Defato, 2011). Therefore, this shows that it plays a supportive role in neuronal growth and regeneration. However, it has been demonstrated that other isoforms can compensate deficiency in the isoforms without significance alteration of the overall physiologic function. Also, the absence of β-actin is well offset by other more potent molecules with overlapping function. Therefore, this makes Actin a weak marker of neuronal regeneration.

S100 proteins have a site for the attachment of calcium ions. They function both in and out of the cell and; thus, this forms the basis for their differentiation. They activate their intended intracellular physiologic activities via the stimulation of surface receptors through autocrine or paracrine activity (Fujiwara, 2014). S100B mainly modulates the function of neuronal regeneration. This protein is primarily found in Schwann cells, and it serves to inhibit their proliferation but increase myelination. Myelination being part of the neuronal regeneration continuum, thus, groups the proteins as markers of neuronal regeneration. Also, S100B through linkage with other structural proteins enhances neuronal regeneration and growth (Donato, 2013).

The main signaling pathways that have been associated with neuronal regeneration and growth are cAMP, PI3K,, and JAK/STAT. As stated above, they are vital in this process,, and they involve activation of different molecules that interact across the pathways in a way that is synergistic. The regulation and fine-tuning of the activities of these pathways leads to a steady, tightly regulated growth of the injured axon. PI3K leads to the activation of transcription factors. The primary factor activated is Smad1 (Jiao, 2013). This, therefore, leads to the generation of proteins that act towards enhancing healing and regeneration of the neuron. cAMP, as a separate signaling pathway, is modulated by G-proteins. Increased intracellular calcium ions activate this pathway. This increase is linked to increased influx. Activation cAMP leads to the formation of transcription of regeneration-associated genes. These genes, therefore, serve to enhance the translation of proteins associated with neuronal growth and regeneration. Finally, JAK/STAT pathway depends on the activation of the JAK molecules in response to ligand-dependent surface receptor activation. The STAT3 is the particular molecule associated with neuronal regeneration and is stimulated by the activated JAK molecules (Lang, 2013). STAT3, thus, through different mechanisms, enhances the process of neuronal regeneration.
From the above pathways, it can be seen that the relevant molecules associated with the pathways can be used as markers of regeneration.

Microtubules

These proteins in are encoded by gene 7846 called tubulin A 1a. This gene is found on chromosome 12q. The proteins are made of dimers and mainly perform the supportive function of the cytoskeleton. These microtubules fall under the superfamily of b tubulin which is made up of 6 different protein families that show high similarity. Only three of these families, alpha, beta and gamma, are found in eukaryotes. Primarily, alpha and beta are the ones found that make up the microtubules Gamma tubulin, on the other hand serves an integral role in microtubule structure nucleation. Nucleation is seen to be an essential step in the process of neuronal growth and regeneration and thus our focus will mostly be narrowed down to this. Therefore, this can serve as a therapeutic target in relation to neuronal regeneration. For proper function of the cytoskeleton, the proteins involved, mainly tubulin and actin, have connections within themselves or with the other proteins. This is the function of microtubule-actin cross-linking factor 1 (MACF 1). The main purpose of these is to add strength to the structure to thus be able to support the cellular functions.

After a neuronal injury, there is the formation of retraction bulbs mainly in the central nervous system. On the contrary, in the peripheral nervous system, there is the formation of the growth cone. Their main difference physiologically is that the growth cone can regenerate, whereas, in the retraction bulb, there is no ability to grow or regenerate. Structurally, the microtubules in growth cones are organized, whereas in the retraction bulbs, they are disorganized, thus slowing down the regeneration process (Kleele, 2014). This property can be seen to have a temporal variation in the size of these structures. With time, the retraction bulb grows, while growth cones remain constant. Also, it has been observed that any disturbance to the microtubules in injured sensory neurons causes the development of retraction bulbs. This feature has been explained to be one of the main reasons why the central nervous system does not regenerate (Bradke, 20070. The growth cone has diverse mechanisms that interplay to maximize the axonal ability to regenerate. One of them is the high concentration of mitochondria on the growing tip, to act as a steady and ample source of energy to drive the process of regeneration. Also, the cone regularly supplies the growing tip with cell membrane that will enable the covering of the elongated axon. Strikingly, it is noted that the microtubules in this pole are rearranged into a well-organized pattern. This serves to enhance the process of regeneration (Sainath, 2015). To find out, conclusively, the role of the microtubules as an indicator of regeneration, different experiments were done on mice. These experiments were directed towards observing the intracellular structure of the growth cone and retraction bulb. Also, induction of axon stalling on the growth cone was done, and the consequences of the regeneration process observed (Bradke, 2007).

In these experiments, green and yellow transgenic protein M and H mice, respectively, were used. Different procedures were performed on these specimen. These were, transecting of the dorsal column, sciatic nerve, treatment of the peripheral axons with nocodazole and also a treatment of central axons with taxol. From the above experiments, it was realized that the peripheral axons of dorsal root ganglions (DRGs) neurons would regenerate after injury, whereas the axonal branches of the DRGs in the dorsal columns could not. It was observed that the peripheral branches, which form part of the PNS, developed growth cones in response to the lesion. Growth cones were seen on the transected sciatic nerve. On the contrary, the branches of the DRGs in the dorsal column formed retraction bulbs on the proximal axon stump.

From the experiments, many deductions were made. One of them is that the microtubules in the retraction bulbs were disorganized (Chen, 2015). Microtubules serve an important role in neuronal growth and regeneration. Specifically, they are involved with the guidance of the shafts and also act as the backbone for the shaft and the neuron at large (Kleele, 2014). This, therefore, means that they are a source of morphological stability to the neuron. Moreover, dynamic microtubules are seen protruding from the growth cone ends to facilitate neural growth. To check for the difference in the arrangement of these microtubules, tubulin was detyrosinated by anti-tyrosine antibodies. These antibodies were directed towards growth cones and retraction bulbs to act as markers. The tyrosinated tubulin (microtubules) in the growth cones were found to be arranged out in a parallel fashion (Marcos, 2009). On the contrary, it was observed the tubulin (microtubules) remained stable and disorganized in the retraction bulbs. Dynamic microtubules were barely noted in the retraction bulb tips. Also, it was found that 99% the microtubules in the growth cones deviated from the axonal axis by <300. This was contrary to what was observed in the retraction bulbs in which on 51% of the microtubules deviated by <300 while 35% deviated 300-600. This showed that in retraction bulbs, the microtubules were more disorganized than seen in the growth cones and unlesioned neurons (control experiment).

Treatment with nocodazole of the growth cones in the injured terminals of the sciatic nerve, disorganized the whole arrangement of the microtubules thus turning the growth cone to a retraction bulb. To check out the growth potential of the newly formed retraction bulb, compared to typical growth bulb, imaging was done. Extension of dynamic microtubules was observed in the growth cones but not in the retraction bulb. This, thus, shows that disorganized microtubules inhibit neuronal growth (Sainath 2015). To further prove this phenomenon, inverse of the first reaction was done. Here, taxol, a compound that stabilizes microtubules, was applied to the injured nerve ending in the dorsal column where retraction bulbs had formed. Repetitive application of taxol leads to the alignment of the microtubules to the axonal axis like the cytoskeleton. This, in return, increased the growth potential of this lesioned neurons (Bradke, 2007).

From the above findings, it can be noted that the presence of microtubules aligned to the axonal axis is an indicator of neuronal regeneration. Transformation of retraction bulbs to growth cones by application of taxol has shown that there can be pharmacological interventions that can be applied to enhance neuronal regeneration. Therefore, with this in mind, it can be safely concluded that formation of well-organized microtubules that are aligned to the axonal axis, is a firm indicator of regeneration process that is underway.

Actin

Actin filaments, precisely F-actin present a treadmill fashion of motion in its activity. In this movement, it dissociates proximally and assembles and engages it the leading end. Tread milling serves to enhance the process of neurite outgrowth. In growing neurons, the actin fibers interact with other filaments, actin binding proteins, and myosin. These leads to the formation of other more complex structure whose coordinated, and modulated functions allow for directionality of neuronal growth. Actin, as a protein, has different isoforms encoded by gene HGNC 132 on locus 7p22. There are six isoforms, but the main ones involved in neuritogenesis and neurogenesis are β and γ. These isoforms show different patterns of distribution within the neuron proper. There is a higher distribution of γ-actin in the soma, and lower levels are found in the growth cone terminal. On the contrary, β-actin is mainly concentrated in the growth cone. This, therefore, highlights the importance of β-actin in the process of neuronal regeneration (Pak, 2009). It has also been shown that these two isoforms can undergo co-polymerization within a single actin filament suggesting an overlap in activity and a possibility of resultant redundancy. It has also been demonstrated that the two isoforms can also compensate for each other's deficiency. This means that in low levels of one, or complete knock-out, for example through gene knock-out, leads to a resultant compensation in activity by the other isoform. Through experiments, it was discovered that a total γ-actin ablation, does not result in any phenotypic change in the physiologic function of the neurons (Flynn, 2013).

The growth cone, which is the main hub of neuronal regeneration and growth is divided into three regions morphologically. These are three concentric layers made of different structures. On the outermost layer lies the peripheral domain (P). This part is made of actin filaments and lacks organelles. Centrally, there is the central domain (C). This is made of organelles, mainly mitochondria, and microtubules that form the core. The transitional zone (T) is the region between P and C. This region serves to compress the contractile actin filaments and prevent further growth of the microtubules peripherally. As said above, actin filaments form

different complex structures like arcs, filopodia, and lamellipodia. Nucleators, which are proteins that are bound to the plasma membrane, serve to activate the actin fibers. The activated filaments extend into filopodia or lamellipodia depending on the nucleator. Arp2/3, leads to the development of lamellipodia, while, formins lead to the development of filopodia. Transverse actin bundles form arcs. These complexes are mainly found in the transitional zone of the growth cone. They are situated behind the filopodia and lamellipodia located at the leading pole(Pak, 2009).

The growth or regeneration of neurons depends on neurite formation as one of the pivotal primary steps. Formation of neuritis is initiated, controlled and guided by an interplay of intracellular and extracellular molecules and signaling pathways (Yishi, 2015). Structurally, the whole process depends on the presence and response of the cytoskeleton that form the supporting structure and shell upon which the neurite forms. Actin is the main protein that serves an integral role in the formation of the cytoskeleton. This, therefore, means that actin plays a significant role in the process of neuronal regeneration. The morphological change of the cytoskeleton, due to the restructuring in the actin filaments leads to the formation of filopodia. Filopodia thus act as the basis for the formation of the neurite. The whole process the triggers off this changes in the actin fibers in preparation for the formation of neuritis and, thus, neuron growth or regeneration is not well understood (Flynn, 2013). Two models have been proposed. One model is de novo. In the de novo model, it has been suggested that there are factors that play a role in nucleation, unidirectional elongation, and formation of filopodial actin bundles via cross-linkage. On the other hand, convergent elongation model explains that elongation and bundling, into filopodia, of the branching actin fibers depend on different proteins. It has been discovered that the two models work hand in hand in to lead finally to neurite formation.
Arp2/3 is a complex of actin binding proteins that help in the nucleation of actin filaments. Binding of the complex to actin forms a nidus unto which other actin filaments attach, thus, leading to branch and development of filopodia. The barbed end of the actin fibers allows for the attachment of G-actin which thus leads to polymerization. Therefore, capping off this capped end inhibits the polymerization of the actin fibers and, thus, preventing neurite formation. Lose of Arp2/3 complex, for example through induced gene knock-out, however, does not inhibit neurite formation. Instead, it leads to increased formation of shorter and irregular neuritis. It has proposed that when the actin filaments are capped, there is increased G-actin levels that can interact with Arp2/3 complex. This, therefore, means that there is a subsequent preferential development of branching as compared to elongation of the fibers which occurs due to polymerization. A membrane protein, Ena/VASP, encourages neurite formation by promoting branching of the actin fibers and their elongation as they invaginate the plasma membrane. Lose of this protein by gene knock-out, however, prevents the formation of filopodia (Pak, 2009).

In other researches, it has been shown that neurons undergoing regeneration present with a phenomenon called waves. These are structures, which resemble growth cones, which move or propagate along the injured axon. It has also been shown that with the arrival of the waves, the growth cone enlarges. These waves, thus act as an alternative mode of transport for actin. Increase in frequency of the waves led to a proportionate increase in the neurite growth (Flynn, 2009).

MAP2, a protein that plays a role in microtubules same as that of Arp2/3 to actin, has been found to have a site that binds to actin. This, therefore, shows that MAP2 serves to link actin to microtubules (Bastiani, 2009). It is proposed that the microtubules attach to the actin fibers and push against the plasma membrane thus allowing for growth and elongation of the neuron. Also, in some researches, it was discovered that in the presence of actin polymerization inhibitors, microtubules were able to push against the plasma membrane and elongate. From this observation, it can be seen that there is redundancy regarding function. In another research, where the scientists tried to figure out the significance and role of ß-actin in neuronal regeneration, it was concluded that ß-actin was not essential or integral in the whole process. This conclusion was reached when the scientists used mice that had their ß-actin knocked out did not show any significant change in phenotype nor physiologic function (Cheever, 2011).

In conclusion, it can be said that the overall role of actin in neuronal growth is not well understood. But as from the documented researches, most of its functions have been overlapped by other more integral molecules like microtubules, thus, making it redundant. Therefore, actin is not a major molecule in neuronal regeneration.

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S100

This is a CA2+-binding family of regulatory proteins that are grouped according to their functionality and distribution in the body. "S100" was coined from the ability of these proteins to dissolve in saturated solution of 100% Ammonium sulfate. The genes to these proteins are mainly found on chromosome 1q21 (Wright, 2009). The other proteins in this family are found on chromosomes 21q22, Xp22, 5d14 and 4p16 (Lesniak, 2011). They contain the EF-hand as seen in other Ca^{2+}-binding proteins like calmodulin. Therefore, due to this feature, glutathionation, phosphorylation or any other form of post-translational modification tightens the affinity to Ca^{2+} (Wright, 2009). There are three main groups. The first group acts intracellularly; the second one acts both intracellular and extracellular; while the final one functions mainly in the extracellular compartment. Some of the functions of S100 in the intracellular compartment are regulation of proliferation, migration and even interaction with various cytoskeletal structures. They depend on surface receptors and multiple signaling pathways, like the G-protein coupled receptor pathways, to initiate its function within cells. Activation of these molecules depend on Ca^{2+} that leads to a conformational change that exposes a hydrophobic pocket that is normally hidden in the apo state. This region enables for the attachment of trigger ligands and the diversity of the chemical structures in this region determines the functional target-specificity. The activity of the S100 occurs in either autocrine or paracrine fashion. On the other hand, extracellular S100 proteins, S100A4, and s100B, work in conjunction with other extracellular molecular factor to mediate their functions. S100A4 works with epidermal growth factor (EGF) whereas S100B links with basic fibroblast growth factor (bFGF). S100B in this family of proteins is the one mainly connected with the function of neuronal regeneration (Fujiwara, 2014).

S100B acts as an initiator of cellular proliferation and enhances migration. On the other hand, its inhibitory effects serve to prevent apoptosis and differentiation. All these features are required for neuronal growth and regeneration. In the intracellular compartment, these S100B molecules bind to molecules that form the cytoskeleton. These molecules include tubulin, caldesmon, and calponin among others. In the central nervous system, this molecule is found mainly in the astroglial cells. Peripherally, S100B is found in the Schwann cells. S100 being a neurotrophic factor, its levels are often elevated during neuronal growth and differentiation (Donato, 2013).

The direct role of S100B in neurogenesis is not well understood. But its role in myelination has been tested and documented. Activation of S100 by SOX10, from Schwann cells in experimental rats with injured sciatic nerve, lead to suppression of Schwann cell proliferation, but stimulated myelination process (Donato, 2013). On the contrary to this great function, s100 has been implicated in many neurological diseases including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Multiple sclerosis among others. This, therefore, stirs a twist on its general importance in neuronal growth and as a pharmacological target as a marker of neurogenesis. The knock down of the SOX10-S100B lead to suppressed myelination in various experiments. This showed the critical role of the molecules in the myelination process (Sorci, 2013).

As a pharmacological target, it has been discovered that loss of function of S100 as a molecule can occur due to methylation of CpG pairs at the cytosine nucleoside. These pairs are few in humans and are found in islands in 5' regulatory regions of S100 genes. Use of DNA methylotransferase inhibitors can be useful to reactivate these proteins to, thus, enhance neuronal regeneration (Lesniak, 2011).

Myelination being part of the final process in the neuronal regeneration chronology of events means that its rise after neuronal injury is a positive marker of neuronal regeneration. Though this may serve as a late marker of regeneration, it surely plays a role in the whole process. But due to the scarcity of knowledge on its direct role in regeneration and growth of neurons, and its involvement in many neuronal pathologies, it, therefore, means that S100 is a poor marker of neuronal regeneration (Donato, 2013).

Signaling pathways

The human body is set in a way that it tries to maintain a certain equilibrium of functionality at anatomical, (Both gross and histological), biochemical and physiological. This steady balance means that there is a linkage between them or a means of communication. Here, the hormonal and nervous system kick in. The neurons being the conduits for molecules (neurotransmitters) that serve as the medium of communication in the body, are, therefore, integral in the body's functionality. Thus, the body has devised a mechanism of

protection and even regeneration of the neurons to avoid disruption of communication within the body. At the molecular level, in response to neuronal injury, many molecules are released. The release of the molecules is either from within the same neuron (intrinsic) or from without (extrinsic) (Kaplan, 2015). Whichever source is recruited, the common goal is the regeneration of the injured neuron. Due to intricate processes involved in the physiological activity, a means of communication that will stimulate, guide and fine-tune the whole process of neuronal regeneration is required. This is where signaling pathways are recruited. Many signaling pathways have been studied in this line, and one striking discovery is that they have a synergistic mode of function in their mode of function. The different pathways are triggered off by different molecules and have unique molecules within their cycles. The interplay of the molecules and their biological activity within a cell may be either inhibitory or stimulatory. The overlap of their biological activity to other signaling pathways, therefore, creates a powerful machinery that drives and coordinates the process of neuronal regeneration. Therefore, with considered, the levels of the main molecules in the major pathways involved in this process can be considered as markers of neuronal regeneration.

The main signaling pathways that have been studied in line with neuron regeneration are phosphatidyl inositol 3-kinase (PI3K), Janus kinase/ signal transducers and activators of transcription (JAK/STAT) and cyclic adenosine monophosphate signaling pathway (cAMP). Below is a brief description of the signaling pathways and their role in neuronal regeneration.

CAMP

Cyclic adenosine monophosphate is a G-protein mediated signaling pathway. The stimulation of this pathway is dependent on calcium ions (Ca2+). After a neuronal injury, there is an increase in intracellular calcium ions due to a breach of the plasma membrane and increased influx. This there activates the dephosphorylating of membrane-bound adenosine triphosphate to form cAMP. This pathway leads to transcription of genes called regeneration-associated genes (RAGs). These genes are proposed to be used in the translation of proteins that are used in the healing and regeneration process (Willis, 2015). Therefore, the main molecules associated with this pathway are the RAGs. They are directly linked to regeneration and thus the detection of increased levels of these genes, therefore, serves as an indicator or marker of regeneration that is underway.

PI3K

Phosphatidylinositol 3-kinase is a pathway that requires the usage of membrane lipids. This signaling pathway has a complex interplay of various molecules that cross-react with molecules in different signaling pathways like the cAMP. This sort of interaction leads to modulation of the levels and functional activity of the affected molecules. This is either through inhibition or stimulation. PI3K leads to the activation of a molecule whose noble function in the neuron is activating and modulating transcription. This transcription factor is called Smad1. Smad1 is an integral protein in the regeneration process. This has been shown through several experiments where the molecule was knocked out, and the regeneration process monitored. These injured axons showed stagnated regeneration (Zou, 2009). This molecule still serves as an activator of the PI3K. This, therefore, shows that once started off, the cycle self-propagates. The features of the PI3K cycle that make it important in the regeneration process are the many mini-pathways triggered off by the activation of this signaling pathway. Also the auto-inhibition of Inositol phosphatases. Smad1 and Phosphatidylinositol kinase levels, therefore, serve as the main molecules in the PI3K signaling pathway which act as markers of regeneration.

JAK/STAT

Janus kinase/ signal transducers and activators of transcription (JAK/STAT) signalling pathway is a system that depends on activation of surface receptors by ligands released in extrinsically in response to the injury. The ligands include cytokines released by inflammatory cells or growth factors released centrally or peripherally. Activated membrane bound JAK molecules lead to the stimulation of the STAT molecules. The main STAT molecule involved in the process of regeneration is STAT3 (Jiao, 2013). Dimerization of these STATs stimulates transcription, therefore, making them transcription factors. These molecules work by recruitment of new fibers to the injured site and enhancing the generation of new fibers (Lang, 2013). The elevation of the levels of STAT3, thus, serves as an indicator of neuronal regeneration.

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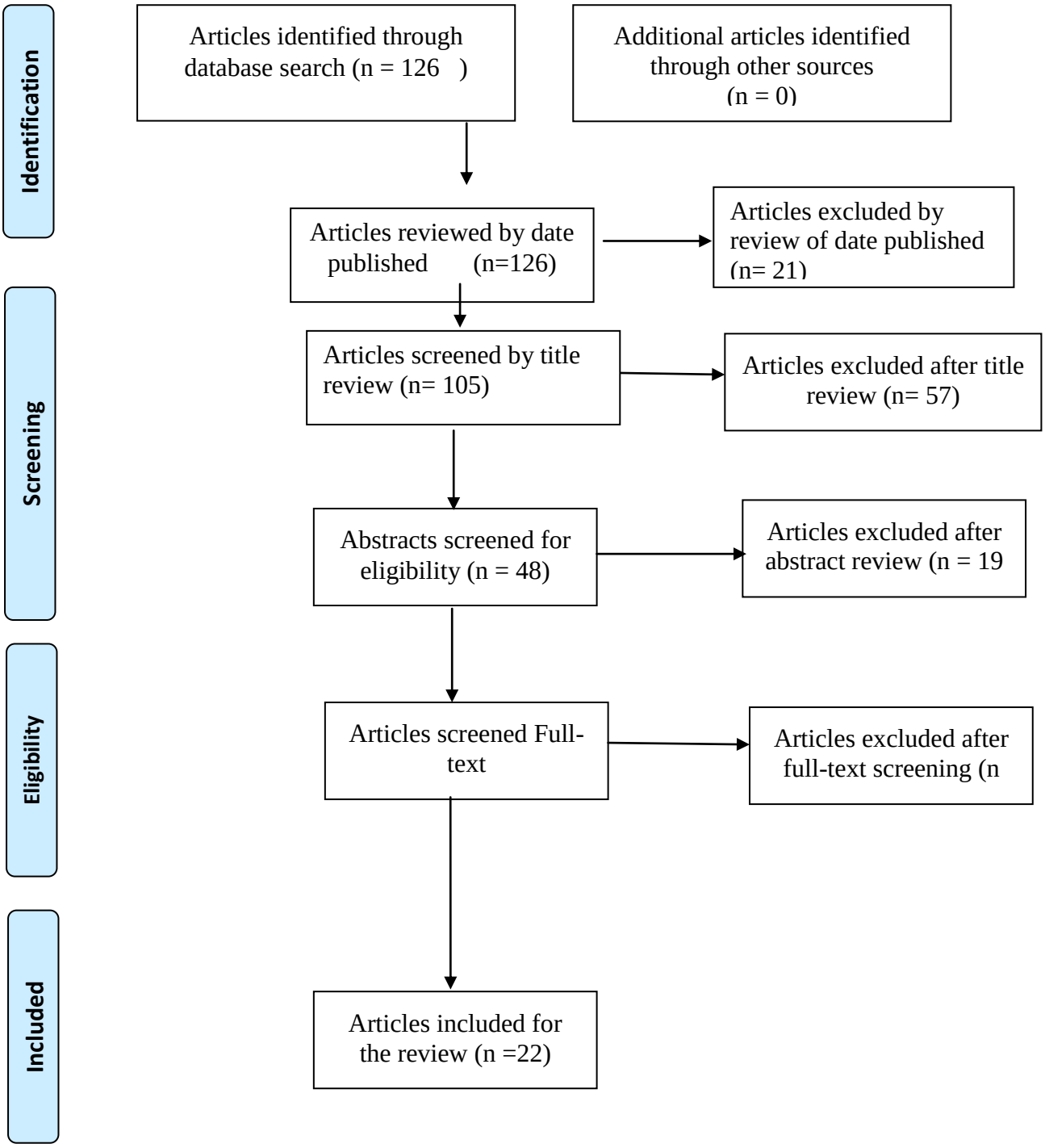
CONCLUSION

A thorough analysis of these markers of regeneration, in their respective pathways and associated physiological roles, has shown that they have both strengths and weaknesses in line with regeneration. The pathways and molecules involved work together to form machinery that drives the whole process of neuronal growth and regeneration. Not a single marker stands out to be the most critical but actin,, and S100 have redundancy in functionality as their major drawback. This means that all the molecules are designed to work in a coordinated fashion that covers for deficiencies, thus, making the whole process efficient. Thus, increased levels of these molecules in the neuron proper after injury indicates that neuronal regeneration is underway.

Appendix 1: Prism Approach



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Appendix 2: Table for analysis of the signaling pathways as markers of neuronal regeneration

Pathway	Major molecules involved (markers)	Mode of activation	Mode of action	Strengths	Weaknesses
Phosphatidylinositol 3-kinase pathway (PI3K)	PI3K, GSK3 and Smad1	Ligand-dependent phosphorylation of the kinase. Ligands include cytokines and neurotrophins	Smad1, a strong transcription factor leads to the generation of genes associated with neuronal growth and regeneration	It has many interconnections with other pathways and some of the molecules involved moderate the activity of the other pathways	Due to the many inter-connections involved with other signaling pathways, it makes it a difficult pharmacological target.
Cyclic monophosphate pathway (cAMP)	Calcium ions (Ca^{2+}), Regeneration Associated Genes (RAGs)	Activated by influx of calcium ions after neuronal injury	Increased generation of RAGs leads to translation of proteins associated with neuronal growth and regeneration	It is triggered off almost immediately after injury since it depends on Ca^{2+} influx and not on protein ligands that have to be synthesized before release	Pharmacological levels alone cannot trigger off regeneration. Meaning it depends on other molecules released after injury, to reach optimum activity. Also, its function is concentration-dependent. Thus pharmacological targets of cAMP require to find out the optimum levels required.
Janus kinases/signal Transducers and Activators of Transcription (JAK/STAT)	STAT3 and Smad1	Ligand-dependent membrane receptor activation	Transcription of regeneration-related protein genes	STAT3 recruits new connections with unlesioned tracts, thus is helpful in hemi-section of the spinal cord	Less is known about the biological activity of STAT3 as an activator of regeneration since it has also been found to be an inhibitor at some levels.

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Author's Contributions

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Ethics

There is no ethical issues that might arises from this article review.

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