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RESEARCH ARTICLE

Review on Cyclic Adenosine Monophosphate signaling pathway (cAMP), DLK Signaling Pathway, RAS/RAF Signaling, Retinoic Acid Signaling, Phosphatidylinositol 3-kinase (PI3K) as the Signaling Pathways Involved in Peripheral Neuronal Generation

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Abstract

Neuronal regeneration is the physiological process wear, after neuronal injury, the body stimulates mechanisms of self-healing. Thus, through coordinated different processes and signaling that involves diverse molecules in the body, the nerves are able to regain, to some extent, their initial capability to transduce and convey signals. After, neuronal injury, there is a defect in the normal signal transduction. This presents with different symptoms depending on the system in which the nerve belongs. This may be mortar or sensory. Injury to mortar neurons means the supply of the muscle innervated is cut off. This, therefore, implies that the muscle will not have enough power or will become spastic. It is in response to this injury, that the nerve, through some intricate intrinsic signaling pathways, is able to regenerate in order to return the normal physiologic functioning of the body. Methode of this paper is summed up with systemic reviews of articles in the same topic under study. The articles were searched using the Pubmed search engine and were all in English. None of the articles was translated. The inclusion and exclusion criteria was guided by PRISMA guide, as a predetermined outline. The result of this article is out of forty-three, eight articles were selected as eligible, this is because they met the PRISMA criteria and had adequate relevant and supporting experiments to back the signaling pathway under study. These articles had a clear and elaborate outline of the whole signaling pathway, the associated molecules, the interconnections between the pathways, supporting experiments and even recommended research in the same field or topic. The comparison of the strengths of facts backing the individual pathways was used to determine the pathway that is most involved, or that can be presumes the "mother" signaling pathway in the process of neuronal regeneration.

Keywords: *Neuronal regeneration, Neuronal injury, Signaling pathway.*

Introduction

Loss of function due to neuronal injury has proved to be a headache in the society today. With the rampant increase of the causes of physical trauma that increase the vulnerability human being as a species has led to increased study on how to reverse this process. Many handicapped people in the society have had minor trauma, to their spinal cord for example, that has proved to be a turning point in their life and has spelled doom in their activities. This coupled with other occupational hazards and even the final threat of being incapacitated and losing jobs has, thereby, attracted so much attention by researchers. With the evidence that sometime after neuronal injury, there is recorded partial or full recovery without any pharmacologic interventions, researchers hypothesized that, there was an intrinsic self-healing pathway that controlled the regeneration processes. With this, many molecules and signaling pathways have been uncovered in relation to regeneration.

However, researchers are still studying and investing to find out the most pivotal pathway in the whole regeneration process. With the discovery of very many molecules and signaling pathways, researchers have tried to narrow down on the single most important and significant pathway that can be targeted pharmacologically to enhance the process of regeneration. Confusion arises when these pathways are interconnected and thus one has to determine which one is superior to the other. Pathways like cyclic adenosine monophosphate (cAMP) and PI3K have very many molecules involved. Some of these molecules serve as activators and promoters, whereas, others act as inhibitors.

The coordinated and controlled functioning of these molecules in their respective pathways is therefore what determines the superior pathway. A pathway like PI3K has a both direct and indirect ways of regulation of the regeneration process. One of it including down-regulation of Inositol phosphatases immediately after neuronal injury [1].

This therefore serve to increase the levels of the PI3K thereby self-propagating. Therefore, this synergistic activity within the signaling pathways plays an integral role in the choice of the most significant pathway. Due to increased susceptibility of the human being to trauma in this millennium, there is a proportionate increase in the risk of acquisition of neuronal injury.

Nerve grafting as solution to this problem has been the main stay. However, due to the costs, failure rates and the invasiveness of the procedure, there has been a campaign to find more less-invasive management procedures. Pharmacological treatment was the best option and therefore choosing the target signaling pathway that can be targeted in order to trigger, enhance and hasten the process of regeneration has been the big question to neurologists and other scientists in this field. This part gives an elaborate description of the different pathways involved in the process of neuronal regeneration.

Literature Review

JAK/STAT is a pathway that involves activation of the JAK molecule after the attachment of a stimulatory ligand on membrane receptors. One of the main ligands is NGF and this leads to a cycle of phosphorylation reactions in the neuron.

This finally stimulates the transcription of genes that will be used in the guidance and modulation of the neuronal regeneration. This pathway has a unique feature in that it has been found to lead to decussation of fibers during the regeneration. Therefore, it causes remodeling of the nerves and tracts mainly in the spinal code. Besides this, there is no other striking thing about this signaling pathway. This therefore calls for more research to be done to determine its linkage with regeneration in a more concrete way. The DLK signaling pathway is a protein kinase dependent pathway responsible for regeneration of neurons in case of injury.

It is unique in that it acts in a contradictory manner depending on the situation. In an axonal injury, it works to activate JNK and MAPK pathways. It has two homologues that respectively regulate re-growth of axons and also another homologue for regeneration purpose. Since it is a protein kinase dependant pathway, it can be modulated through the manipulation of enzyme activity.

A major disadvantage in DLK signaling is that when it has activated MAP2K, it constrains JNK activity on neuritis [2]. Retinoic acid signaling pathway and its effect on neural regeneration is manifested through it receptor called beta Retinoic Acid Receptor (RARß). Retinoic acid induces neurite outgrowths in the injured neurons.

Retinoic acid signaling has a number of effects in the nervous system. It will suppress RhoA activation; it will inhibit Lingo-1 and will promote induction of outgrowths. Through all these three effects, it improves its probability to cause change in an injured neuron. Lingo-1 is exclusively in myelin sheath. Therefore, its inhibition by Retinoic

Acid also leads to a rise in re-myelination. This is important in the neuronal cells that may have lost the myelin sheath during injury. Another mechanism is through the RAS/RAf signaling pathway. Ras is normally used as a molecule by many signaling pathways acting in the upward stream.

A disadvantage with the Ras/Raf mechanism is the availability of numerous mutations that may give rise to a neoplasm even in the

cases of regeneration. Conditional activation of B-RAF kinase has the potential to sufficiently drive the growth of long range peripheral sensory axons in the absence of neurotrophin signaling.

Another disadvantage is that the RAF mechanism is not sufficient to enable in vivo growth of neurons; it has to be complemented with other signaling pathways in order to realize long-range axonal projections. The Kruppel-like factor (KLF) is families of genes each working independently to either suppress of induce the growth of axons [3]. KLF4 forms the focus point in discussing neuronal regeneration because it is an active induce of the same. It is important to note that for normal working of this family of genes, there has to be a mechanism to suppress some growth inducers and also a mechanism to promote some growth-inhibitors.

The balance of all this is what leads to a normal regeneration without mutations. Smad1 is also a mediator in nerve regeneration. It is naturally activated by the presence of a lesion. Axiotomy promotes its induction and it then proceeds to mediates BMP signaling from axonal transport. Cyclic adenosine monophosphate pathway (cAMP) is strongly linked with neuronal regeneration.

It depends on the metabolism of adenosine triphosphate, which is a membrane molecule that is used to provide energy for the cells physiological activities. From the research and experiments, it was seen that cAMP by itself could not trigger off regeneration of the neurons. It had to work hand in hand with other molecules that were released in the body like PI3K in order to sufficiently start the process.

This, therefore, makes this molecule, and pathway as a whole, to be weak in relation to the process of peripheral neuronal regeneration. Phosphatidyl inositol 3-Kinase (PI3K) is the novel signaling pathway. This is because it has proved its superiority by triggering off and linking up with many other signaling pathways in order to propagate the process of neuronal healing and regeneration. Its ability to stimulate Smad 1, which is a strong initiator of regeneration by itself, and also the activation of GAP43 in conjunction with the down regulation of the inositol phosphatases, boosts its capability to enhance neuronal regeneration.

Moreover, LY294002, GSK3 and pten, which are all inhibitors of the pathway, undergo down-regulation. This, thereby, leads to accelerated increase in axonal growth. From all these intertwined meshwork of molecules and signaling pathways, which are triggered off by the PI3K pathway, it shows how strong and invincible it is over the rest of the signaling molecules and pathways.

Objectives

The aim of this paper is to review the main signaling pathway that is responsible for neuronal regeneration in the Peripheral Nervous System, find the physiological process of its function and how it affects the growth of neurons. In order to achieve this objective, we defined the research question as follows; which is the main signaling pathway in human beings that enables neuronal regeneration of the peripheral nervous system and how does it work.

Methods

A lot of both mental and physical input was used in order to come up with this systematic review. The review was conducted and reported using the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines (PRISMA) guidelines. In the development of this review, we first defined the research question basing it on the objective of the systematic review. We later designed a search strategy which would help ease up the workload of looking for materials to be used in order to meet the objective.

The articles used were retrieved through searching for relevant database that had the relevant information. These articles were then assessed using a predefined inclusion and exclusion criteria. After finding the data sources, they had to be analyzed critically in order to meet the cream of the crop that was relevant and could help meet the object of study.

Search Strategy

This review has only used articles retrieved from the Pubmed electronic database. In order to narrow down to the specific articles with relevant information towards my research question, we had to use specific key words to narrow down the results. We

considered various reports on the nerve regeneration since it was the main objective of study.

The conclusion was met from well analyzed results from scientific experiments. The literature search covered some of the well-researched signaling pathways that take part in peripheral nervous system regeneration and their mechanism of action. The time interval between the studies was from June 3, 2009 to August 7, 2015. The key words used in the literature search included: axonal regeneration, peripheral nervous system, jak-raf system, ras-raf system, cAMP, axonal regrowths, morphology, mechanism of action.

Study Selection

In order to improve improved the process of identifying the necessary literature sources, the systematic review employed the use of inclusion and exclusion criteria. This therefore enabled me to separate the necessary sources of study while getting rid of the unnecessary sources that would not help me to meet the research objective.

This systematic review only involved studies conducted on animals with a neural lesion. Some of the animals used included mice and rats that had been artificially had a nervous impairment. Because this systematic review only involved animals with peripheral nerve lesion, normal healthy animals with a fully functional nervous system could not be of any help to the study.

The outcomes from the research could only be validated from the success of in vitro treatment of the nervous impaired animals over a standard period of time and also under a standard environment. The studies had to also include a control experiment. Studies that failed to show the expected effect and desired results were excluded from the study.

The study design used was a randomized control trial. A total of Forty-three articles were derived from the search engine. None of the articles was excluded due to language barrier since all of them were in Standard English. Firstly, these articles were screened by year. All articles were supposed to have been published between 3rd June 2009 to 7th August 2015.With this; we were able to exclude seven articles.

In the next elimination criteria, the titles of the articles were analyzed to figure out the relevant articles for the topic. Seventeen articles were eliminated from here. The next stage was elimination after reading the abstracts. Here, eight articles were eliminated. Finally, after reading through the remaining articles, two articles were eliminated, thereby, leaving us with eight articles.

Discussion

Cyclic Adenosine Monophosphate Signaling Pathway (cAMP)

After neuronal injury, the body switches into a healing and reconstruction state. This concept was adopted by physiologists when it was noted that after neural injury, the cell body of the nerve triggered of a cycle of events like generation of mRNA and synthesis of proteins that were presumed to be in preparation for self-healing. After further analysis and research some of the modulators associated with this process were discovered to be the reconstructive or regenerative associated genes abbreviated as RAGs. In response to injury, these genes are are switched on.

The switching on, in this state, is dependent on the cyclic adenosine monophosphate signaling pathway (cAMP). cAMP pathway is triggered off due to injury to the nerve that leads to increased influx of calcium ions (Ca^{+}) into the neurons [4]. The influx leads to an elevation of cAMP which jam-starts release of transcription factors that are directed towards neuronal regeneration.

This phenomenon was tested by grafting of neurons with rat grafts. The neurons exhibited an intrinsic ability to regrow. The RAGs were detected and on manipulation to increase their intra-neuronal levels, the rate of nervous regeneration was accelerated. This therefore showed that RAGs have a driving effect towards regeneration.

The role of cAMP in neuronal regeneration was seen in young nerves that exhibited high growth rates and regenerative capabilities. In these young neurons, there were very high levels of cAMP that helped the nerve overcome the external nerve regeneration inhibitory factors. In a control experiment where there was administration of pharmacological inhibitors directed towards PKA, a molecule that activates cAMP.

This inhibiting effect, therefore, lead to a resultant reduction and stagnation in the rate of neuronal growth and regeneration. A research at the Filbin lab showed that, secondary to neuronal injury, a PKA-dependent reaction of the neuron lead to increased levels of cAMP. In further investigations and experiments, they decided to find out and ascertain the effect of cAMP levels in the injured nurons.

An analogue of cAMP called db-cAMP was intra-ganglionically to the lesions both in vitro and in-vivo. There were spectacular results in that, the was an increased rate of recapitulation of the affected nerves [4].

Effect of Camp on Regeneration

Cyclic adenosine monophosphate is a molecule synthesized from adenosine triphosphate (ATP), the sole source of intracellular energy. This synthesis process is dependent on the enzyme Adenylate cyclase (AC). AC exists as either membrane-bound or a soluble intracellular molecule. The soluble form of adenylate cylase and so of the isoforms of the AC that is membrane-bound are activated by calcium ions (Ca^+).

Due to neuronal injury, there is depolarization of the neuron and, therefore, increased Ca^+ influx into the cell body. This can also be replicated by the normal neuronal firing that usually leads to Ca^+ influx and thus this leads to an increase in the levels of intracellular cAMP.

During these experiments, it was discovered that inhibition of AC, mainly the soluble form, inhibited the PKA-dependent growth of neurons and prevented the priming of axon growth that is neurotrophin-dependent. This therefore revealed that the soluble AC was the sole source of cAMP. cAMP is likely to be exerting its effects locally to be exerting local effects by affecting the locally accessible proteins that are associated with neural healing and regeneration.

This characteristic, therefore, enables the cAMP to exert its functional effect on the cell bodies and axons. At the axon, through the PKA-guided inactivation of RhoA signaling, cAMP causes axonal guidance. This enables the rearrangement of the cytoskeleton thus enhancing motility of the growth cone. This axon guidance can also be mediated by the modulation of EPAC by the cAMP. In the cell body, the function of cAMP is different from that seen in the axons. Here it is the modulator of axonal growth, not guidance.

This function is dependent on RAG. The link between cAMP and RAG is CREB, whose main function is the activation of gene transcription directly or through other signaling pathways. Both cAMP and CREB have also been linked with a role in synaptic plasticity. This, therefore, highlights the fact that the synaptic plasticity associated with neuronal regeneration also utilizes the same transcription factors.

On the contrary, there was an interesting discovery when the CREB was inhibited by expression of a dominant-negative species of it, there was no change in the axon growth induced by cAMP. Also, the overall rate of regeneration was higher when the two worked together. This, therefore, showed that the two molecules, CREB and cAMP work synergistically in nerve regeneration. Meaning that the individual sum potential to stimulate nerve regeneration was less than the actual resultant potential when working together.

How Sufficient is cAMP in the Regeneration Process

It has been proven than an increase in the level of cAMP alone in the nerves may not be sufficient to start and propagate the whole process of neural regeneration. This was determined when two condition that both lead to an increase in cAMP levels were examined and analyzed in the light of which one would recruit more transcription of injury-induced genes that mediate the healing and regenerating process. One was by pharmacologically inhibiting the breakdown of cAMP, while the other was by use of an injured nerve. The injured nerve showed higher potnial to regenerate than the former.

This, therefore, showed that, injury itself triggered other pathways and release of other molecules that worked synergistically with cAMP the increase the regeneration process. Moreover, it showed that the cAMP increase due to inhibition of its metabolism or, due to electrical stimulation, in the absence of nerve injury, was not sufficient to trigger the transcription of genes that reach the threshold of neural regeneration.

Experiments

Due to the independent, though synergistic, activity of CREB and cAMP, research was done to determine the final genes that drove the whole process of regeneration. The experiment involved using Arginase 1 (Arg1) to model for RAG. With this, on the proximal promoter region, an AP1 site was discovered. The inhibition of this site by the use of dominant-negative Fos protein there was a resultant blockade of the CREB-CA and cAMP activity on axonal regeneration. This was reflected by the levels of expressed Arg1.

It was also discovered that the levels of transcription genes stimulated by the AP1 were more than those detected during normal neuronal injury. This was seen to be due to the AP1 being a heterodimer of c-Fos and Jun.

The combination of AP1 and cAMP was seen to be of lower productive potential without the effect of CREB. With the introduction of CREB to this combination, there was increased productivity. This was reflected by the increase in the transcription genes and appearance of some transcription genes that were not there in the former combination. This, therefore, showed that for optimum activity in the regeneration process, all the molecules had to work synergistically.

In conclusion, in this pathway we can see that many questions have not been elucidated in order to figure out the specific molecule that can be targeted to increase its performance. The synergistic activity and at times the independence in their levels of activity raises many queries and, thus, meaning more research should be done to bring more clarity to this pathway and how it can be optimally managed pharmacologically to help in neuronal regeneration.

DLK Signaling Pathway

Regenerative Response

When an adult mammalian neural system is injured, there is a widespread negative effect on a number of body systems and thus the quality of life is affected. The sprouting ability in the adult Neural System is normally reduced significantly with increase in age. This means that the functional recovery in such instances of injury is severely limited. Spontaneous regeneration may occur but it depends on a number of molecular pathways. The Dual Leucine zipper Kinase pathway is responsible for the regeneration of the axons and also their apoptosis in the development of the neural system. In cases of neuronal injury, the DLK pathway is also key in the neuro-degeneration of the same system. The DLK pathway is a protein kinase that is mitogen activated.

This therefore means that the Dual Leucine Zipper Kinase pathway will act as a contradictory response depending on the situation that is critical. This can be whether it is a neuronal development or an axonal injury. The Dual Leucine zipper Kinase localises in several areas of the nervous system. These areas include the brain, the spinal cord and the sensory ganglia. The DLK normally works as an MAP3K [5]. It is essential in this case to note that the MAPK pathways ahs three modular cascades that are sequential in their organisation. In order of sequence, they are MAP3K, MAP2K and the other one being MAPK. The Dual Leucine Kinase pathway will work to activate JNKs and p38α-δ MAPK pathways.

The DLK has homologues that regulate the regrowth of axons and also regeneration of axons in times of injury. DLK-1 also called *Caenorhabditis elegans* regulates axonal regrowth after having an injury while the other homologue Wallenda also known as *Drosophilla* is responsible for axonal regeneration. In the early regeneration of the axons, the first step will be the migration of the neurons. This is then followed closely by a very extensive growth of dendrites and axons. The growth is further enhanced by synapse formation where the functional connections are refined within a neuronal network.

The Process of Growth and Neuronal Migration

The crucial steps that occur in neural development are axon formation and neural polarisation. This will allow for a direct transmission of information within the nervous system. The induction of the JNK activity by the DLK pathway will always occur in vitro. It is reported that in the absence of DLK, the JNK activity will normally decrease variably in the brain. An activated JNK normally ohosphorylates a couple of both nuclear and non-nuclear

substrates that participate in signal transduction and axon motility. There are three JNK genes in mammals (*jnk1, jnk2, jnk3*). To note is that the deletion of a single family of the *jnk* family is enough to alter the neuronal cytoskeleton integrity. This therefore has a disruption effect on the formation of the axonal tract. JNK activity to neurites is constrained in cases of DLK-mediated activation of MAP2Ks.

DLK-mediated activation of MAP2K7 normally positions signaling modules that are in the neurite shaft thus controlling the microtubule bundling in found in the hippocampal neuron in the brain. It is therefore crucial to mention that activation of the DLK-JNK signaling pathway plays a major role in axogenesis and even in the neuronal polarity maintenance.

Neuronal polarization is influenced by microtubule stability changes. Axon formation is influenced by microtubule regulators. These regulators include tau, MAP1B, CLIPs, DCX, SCG10, MAP2. These modulators serve as substrates in the DLK-JNK pathway. The DLK-JNK pathway plays a major role in the regulation of radial migration in corticogenesis. Radial and tangential formation has a related association with axon formation in the newly generated neurons.

A pharmacological inhibition of the JNK will therefore will alter the migration of the cortical neurons. In a case where there is active inhibition of the JNK with the complete absence of the DLK, the result is noticeable delay in radial migration of the neurons in the cortex. When there is an over expression of the DLK in the neural precursor cells, it leads to an accumulation of neurons in the subventricular zone. DLK kinase activity is very essential in the DLK pathway because in a case where this kinase is absent, there is no notable increase of the neurons. This means that the DLK expression in the precursor cells is dependent on the kinase enzyme.

MAPK Signaling

The DLK-1 pathway has a regulatory effect on *cebp-1* mRNA stability which happens through the MAPKAP kinase MAK-2 in *C. Elegans*. Axon regrowth post-injury is promoted by stability and translation of the mRNA. Within some hours after injury, there develops a filopodia from the axonal stump. In successful axonal regeneration, the filopodia are replaced by the growth cone. The filopodia will act as the points of anchor to support growth. The DLK-1 signaling pathway plays a role in the transition of the filopodia to growth cones. A coordinated activation of both the JNK and the MAPK pathways is also a major requirement in axonal regeneration.

It is also crucial to note that the regeneration of the neurons is dependent on the quick activity of the DLK-1 after injury. In case of a delayed activity of DLK-1, there results a limited regeneration activity. This therefore means that it has to be active immediately after an injury has occurred to the neurons. In this scenario, multiple events have to be activated for the regenerative response to be successful. Calcium ion influx in axonal injury has a certain effect in the DLK-1 function.

Depending on the concentration of the calcium ions, this will result in a switch between an active DLK-1 complex or an inactive DLK-1 complex. It can, therefore, be demonstrated that for DLK-1 to initiate an active neuronal degeneration, it first has to activate JNK and p38 MAPK pathways. A regulatory effect exists in the actin and microtubule cytoskeleton network in the neuronal regeneration after its injury.

For the microtubule growth tobe activated, it has to follow a very distinct phase in *C. elegans* mechanosensory axons. This starts with an increase of dynamic microtubules at the injury site. It is then followed by a down-regulation of KLP-7, which is a depolymerizing kinesin that controls steady state conditions in regeneration. The DLK-1 signaling pathway has an active control over the aspects of microtubule growth found in *C. elegans* mechano-sensory axons.

In the experiments done on mice, it has shown that DLK–MAP2K7–JNK1 signaling pathway has a mechanism through which it regulates the bundling of microtubules in the neurite elongation of the hippocampal neurons. The experiments have also proven that a down-regulated or an inhibited DLK-1 leads to an inhibited accumulation of neuronal cells.

DLK can also not work effectively without the presence of the enzyme kinase.

It has been experimented in mice that in instances of optic nerve injury (Tedeschi, 2013), DLK-dependent signaling will trigger quick transcriptional responses in RGCs. DLK will therefore activate a regenerative process in the optic nerve cells. It was also noted that the same DLK produced pro-apoptotic process thus leading to the conclusion that the DLK responses can be contradictory. In this scenario, the dominant response will therefore depend on the nature of the neurons.

If the neurons are incompetent, the resulting dominant response will be apoptotic thus leading to cell death. In case of normal cells, the dominant response will be regeneration thus causing formation of new neuron cells. The first priority for a neuron after sensing damage should be the process of making a decision whether it should regenerate or undergo natural apoptosis.

The axon terminal has an activation mechanism using several factors e.g. JNK which is phosphorylated and via retrograde motor complexes, it is transported to the nucleus. From such a result, we can conclude that the ability to activate fully the nuclear responses in a backward transport of the signals related to injury might form the major mechanism through which the DLK function is dependent on. In conclusion, of the Dual Leucine Zipper kinase pathway, it has not been very clear whether there is a conservation of the upstream or downstream mechanism of its action in other non-model organisms.

For example, there is a known fact that there is a limited ability for mammalian neurons to regenerate. Re-establishment of functional connections after neurodegenerative diseases and PNS trauma is not very promising. It is a major cause of permanent neuronal deficits. A future understanding of how to modulate the activity of DLK will be crucial in managing cases requiring neuronal repair and regeneration.

RAS/RAF Signaling

In order to establish a functional nervous system and adequately restore then proper neuronal connectivity after injury or disease, axonal growth is very essential. Cell intrinsic RAF signaling forms a crucial pathway that promotes development and regeneration of the axons in both the peripheral nervous system and the central nervous system. If we can reactivate the development growth mechanism, it can help us achieve an axonal regeneration in adults with nervous system injury. The RAS/RAF signaling cascade is used by so many growth factors which transmit signals useful for regulating gene expression and also preventing apoptosis.

In a situation of PTEN mutation, there may be suppression of the RAF-MEK cascade because activated AKt phosphorylates and inactivates different Rafs. RAS is a GTP-binding protein. Several signaling pathways use RAS as a molecule in the upstream pathway. RKIP is a scaffolding protein. It inhibits Raf-1 activation and the downstream signaling. The activation of Raf happens in a complex series of events:

- An interaction with Ras which mediates its recruitment to the plasma membrane
- Dimeralization of the proteins found in Raf
- Different domains undergo either phosphorization or dephosphorization.
- It then disassociates from the Raf kinase inhibitory protein
- It then associates with the scaffolding complexes.

Human beings have intrinsic growth programs which have the duty to promote developmental axon growth. Research shows that activation of these intrinsic growth programs in the site of injured neurons may promote regeneration of the injured adult neurons. Conditional activation of B-RAF kinase has the potential to sufficiently drive the growth of long range peripheral sensory axons in the absence of neurotrophin signaling.

Sensory axons in the spinal cord can be regenerated through B-RAF signaling after a dorsal root crash. It can also cause substantial regrowth of the axon in a nerve in a case of crush injury. Experiments conducted in vitro, show that RAF-MEK signaling promotes axonal growth in primary sensory neurons.

It is however not very clear as to whether the RAF signaling mechanism is sufficient to enable in vivo growth of neurons or whether it has to be complemented with other signaling pathways in order to realize long-range axonal projections.

The experiments have also not put it very clear as to whether the RAF signaling mechanism can promote regeneration beyond the sensory neurons and therefore harnessed for other uses. When B-RAF signaling is activated in its own, it is sufficiently enough to promote sensory axonal regeneration during growth. The neurotrophin growth factor signals the developmental extension DRG peripheral axons through its receptor kinase Trka. B-RAF activation does not affect mTOR phophorylation.

There is also minimal interaction between the MAP-kinase and the PI3-kinase–AKT pathways. The reason why the expression of B-RAF is never changed in the spinal cord is because the kaB-RAF is under the control of endogenous Braf promoter. The growth of Trka positive afferent projections in the dorsal root ganglion is dependent on the Trka signaling. If intrinsic B-RAF signaling is elevated to sufficient levels whether it be in vitro or in vivo, it can induce robust adult re-growth of the axons in the DRG neurons.

It also has the effect of making the axons capable of overcoming signals that inhibit growth which tend to be abundant in the spinal cord. The most crucial driver of cell growth happens to be the classical RAF-MEK signaling pathway that is always cell autonomous. The process that occurs in the neurons is that B-RAF is activated, this then becomes sufficient enough enabling axons in the adults to regenerate across the DREZ and further into the peripheral nerves.

As a result, there is robust regeneration of adult axons. A very important thing to note is that, this signaling pathway supports axonal growth signaling but there is no sufficient evidence to support a theory of whether it supports the survival of neurons. Some research reports indicate that when MEK-ERK signaling was pharmaceutically inhibited, it resulted to abrogation of nerve regeneration.

The activity of ERK in the process of axonal regeneration seems to be dependent on glial cells. This shows an indirect mode of action. The peripheral nervous system provides an environment where the actions of this signaling mechanism can work directly. This is unlike the central nervous system which contains inhibitory environment therefore making the signaling mechanism to work indirectly in order to achieve its purpose.

The approach by RAF-MEK signaling system is a direct effect on the axons to enhance their growth. The direct effect is also positioned towards the regeneration of RGCs as well as DRG neurons. RAF-MEK also has action on the microtubules. When RAF-MEK signaling is activated, it affects the microtubule regulating enzymes.

The result is maintaining the stability of the microtubules in the injured axons. This process is essential in the regeneration of injured axons. A good example of the microtubule-regulating enzyme is the HDAC6. This enzyme takes an active role in the process of axon stability. Experiments show that B-RAF interacts directly with tubulin. When B-RAF signaling is activated, it triggers the expression of axon growth–enhancing gene-sets found in injured neurons.

For the nociceptor's peripheral projections to develop, they have to be dependent on NGF/TrkA signaling. Neurotrophic factors that act through the enzyme tyrosine kinase show an enhanced regeneration mechanism on the receptors. This intracellular signaling pathways- PI3-kinase–mTOR and the JAK–STAT pathways- work in hand with the engagement by tyrosine kinase, a growth factor, its receptors and cytokines to promote regeneration.

In an instance where there is a deletion of PTEN, PI3-kinase-mTOR is strongly activated and their combinations with RAF have a tremendous result of neuronal regeneration. We conclude by saying that a direct genetic activation of the BRAF signaling pathway in terms of the extent of regeneration achieved can be compared favourably with reports that show the resulting activity of growth factors like NGF, GDNF and BDNF. The downside to growth factors as regeneration promoters in the neurons is that there is no sufficient level of expression of the signaling machinery in adults to enable growth factors to elucidate an axon growth in the developing nervous system.

When a person matures enough, his or her growth inhibitory signaling molecules e.g. the phosphatases are up-regulated. To therefore come up with well and workable theories, studies conducted using growth factors have to be combined with an intervention to down-regulate their intrinsic inhibitor.

The genetic intervention can also be a up-regulator of growth factor receptors. It is probable that in future that we combine the activation of several growth signaling pathways while pharmacologically blocking growth inhibitory pathways may so as to develop treatment options for patients with loss of locomotion and even sensation.

Retinoic Acid Signalling

There is a marked difference in terms of the regenerative capacity between the Central nervous system and the Peripheral Nervous System (PNS). The capacity of regeneration in the CNS is limited due to a number of factors. These factors include the existence of an extrinsic environment that is inhibitory. The CNS also lacks intrinsic growth factors. Schwann cells found in the peripheral nervous system will always infiltrate an injured are in the Peripheral nervous system in case of trauma to that part particular place.

The debris in that area is quickly removed, there is release of neurotrophic factors and nervous regeneration quickly sets itself in motion. This is followed by sheathing of the growing axons. Regeneration Associated Genes widely termed as RAGs have been found present in the peripheral nervous system following an injury. This is quite the opposite of the Central nervous system which lacks these RAGs in cases of injury.

The growth inhibitory environment found within the central nervous system is provided by the astrocytes, the glial cells in the CNS and the oligodendrocytes. In case of a CNS lesion, the myelin sheath is fragmented, extrinsic inhibitory molecules are released which inhibit axonal outgrowth. Following a neural injury, they will hamper its functional recovery. Another receptor with high affinity for myelin inhibition molecules is the Paired immunoglobulin-like receptor B (PirB).

This receptor has the capacity to mediate outgrowth inhibition through enzyme dephosphorylation of tropomyosin receptor kinase receptor. The CNS has a repair mechanism that follows a lesion. A glial scar forms at the site of the blood brain barrier to prevent further inflammation. However, this scar disrupts axonal formation while limiting inflammation. For this regeneration to be fully functional just like in the peripheral nervous system, there are a number of limitations that have to be met:

- Managing the inhibitory glial environment. The PNS does not have this inhibitory environment.
- Tackling the issue of lack of neuronal extrinsic capacity for the outgrowths.
- Tackling the axonal demyelination.
- Managing the excessive inflammation in the CNS.

Axonal regeneration in both the PNS and the CNS has been shown to be induced by signaling pathways that involve protein translation. Phosphatase and Tensin homologue counteracts the effect of phosphoinositide-3 conversion PIP2 into PIP3. Inhibition or deletion of Phosphatase and Tensin homologue leads to an increase in axonal regeneration. This then leads to protein synthesis and later cell growth. When retinol has been degraded following its ingestion, the end product formed is retinoic acid.

Retinoic acid readily crosses the blood-brain barrier easily. Research has shown that retinoic acid an active role of inducing neurite outgrowths in the neurons. Results of in vitro experiments done have shown that exogenous Retinoic acid induces the formation of neurite outgrowths in the Dorsal root ganglion. Many neurotrophins have the capacity to up-regulate the expression retinaldehyde dehydrogenase-2 (RALDH-2) thus causing neurite outgrowth of the dorsal root ganglion.

Retinoic acid receptor beta agonists also have the capacity to induce the growth of the neurite outgrowths. The effect of retinoic acid on the neurite outgrowths is manifested exclusively through RARß only. In an experiment done using adult spinal cord explants, the effect of retinoic acid was negative because the explants lacked the required RARß that is the only mechanism that Retinoic acid expresses itself in neuritic outgrowths.

RARß2 is the only receptor in the peripheral nervous system that can be used by retinoic acid to cause neurite outgrowth. Over expression of this receptor in vivo has the ability to induce regeneration of the peripheral nerves.

An experiment done to mice showed that there was an axonal and functional regeneration of the spinal tracts when a lentiviral RARß2 was injected to the mice some weeks prior to an injury to the spinal cord. This therefore proved without doubt that the RA-RARß2 was involved in the neuronal regeneration of the peripheral nervous system. Lentiviral RARß2 expression causes an increase in the levels of cAMP with and induction of neurite outgrowth. An adenylate cyclase inhibitor and a cAMP-dependent Protein Kinase inhibitor has an antagonistic effect on the induction of neurite outgrowths as a result of RARß2 being inhibited.

This therefore draws to conclusion that cAMP plays a crucial role in the neuronal regeneration. PI3K inhibitor, however, has a stronger attenuation effect on the beta retinoic acid receptor. The signaling of Retinoic Acid has various effects in the nervous system. It increases the number of neurite outgrowth, it decreases the RhoA activation and lastly has an inhibitory effect on Lingo-1 gene and protein expression.

This is done specifically through the beta retinoic acid receptor. Lingo-1 inhibitors have an effect of promoting the sprout of axons while the functional recovery is tremendously improved. The Retinoic Acid –dependent suppression of Lingo-1 is exclusively found in neurons that make up the myelin sheath. Therefore, RA-signaling due to inhibition of Lingo-1 causes a rise in remyelination [6].

In spinal neuron development, Retinoic acid recruits CBP and is then activated by Retinoic Acid Receptors to be the promoters for histone acetylation. This increases the accessibility of the promoter to the factors of transcription. It induces the expression of genes belonging to the spinal motor neuron. Experimental results have shown that acetylation of histone at the Retinoic Acid Receptor of the Lingo-1 promoter decreases greatly upon Retinoic acid treatment. This results can however not be taken as very conclusive evidence, more has to be done to prove that there exists complete activator and repressor complexes that work in the Retinoic Acid-dependent regulation of Lingo-1.

It is evident that when PTEN is inhibited PI3 levels are also increased via PI3K. Through phosphorylation, ATK is in turn activated to pAKT which has a number of functions like activating Raf. In the process of axonal regeneration, active pATK leads to activation of mTOR pathway. When the mTOR pathway is activated, it results in high protein synthesis, ribosome biogenesis and cell growth. In order for the results of regeneration to be fully felt, there are a number of things that need to be overcome. Excessive inflammation in case of injury forms one of the obstacles. It is however, possible to manage this excessive inflammation by the use of retinoic acid. Retinoic acid has the inhibitory capability over interferon gamma-induced inflammatory response.

It inhibits JAK and STAT3 activation in the neurons. Another obstacle that needs to be overcome is the low rate of intrinsic neuronal axonal outgrowth capacity. Retinoic acid seems to, however, have the capacity to handle most of these limitations. The RA-RARß2 can overcome the inhibitory myelin environment comfortably. Enhancing it leads to a strong inhibitory signal following an injury.

This thus promotes axonal regeneration. Important to note is that there is a possible transcription role of RA-RARß signaling is actively involved in the cAMP-dependent retrograde signal that causes RAGs to be produced. STAT3 happens to be a retrograde signal. Following a PNS lesion, its inhibition has the effect of hampering axonal regeneration. The Retinoic acid signaling mechanism is capable of taking any pathway whether it is paracrine or autocrine.

All cells in the Peripheral nervous system including fibroblasts and macrophages will differentially express retinoic acid after an injury to the PNS. It is therefore a prudent idea to conduct further investigation over the mechanism of action between the two modes during the time of axonal regeneration. To enhance specific axonal regeneration with limited side effects, more clarification has to be made on the transcriptional targets of RA-RARß signaling following a peripheral lesion.

Phosphatidylinositol 3-kinase (PI3K)

In the peripheral nervous system, in contrast to the central nervous system, injured nerves can regenerate. This has been the focus of many scientists as they partake to find out the uniqueness in the peripheral nervous

system that gives it this precious characteristic. The study of the Phosphatidylinositol 3-Kinase pathway has revealed one of the few secrets behind this phenomenon. It has been revealed that PI3K signaling pathway is activated and modulated in after peripheral neuronal injury or axotomy and that it serves the purpose of enhancing neuronal regeneration [1]. The main system of the nervous peripheral n nervous system affected by this signaling pathway is the sensory neural system. Studies on mice have revealed the molecules that work together with the PI3K in the neuronal regeneration process.

Glycogen synthase kinase 3 (GSK3) has been shown to link with PI3K to induce the synthesis of Smad 1, a transcription factor. Also, it has been shown in vivo that acute depletion of Smad 1 in the mice blocked the processes of neural regeneration. Therefore, this shows how integral the interplay, PI3K-GSK3-Smad 1, is integral in enhancing the regrowth of sensory axons in mammals. After peripheral nerve injury, a radical change occurs within the nerve cells as it triggers pff its intrinsic ability to self-heal and regenerate.

All these activities that sum up as regeneration depend on the activities of mRNA and resultant proteins. This process requires a complex of molecules and pathways to be a success, but here we are narrowing down to the PI3K signaling pathway in particular.

Firstly, in the distal axon, GSK3 helps in the coordination and modulation of the cytoskeletal rearrangement that occurs with the axon. This is a very integral part in neuronal regeneration.GSK3 has also been implicated in the modulation of transcription genes like n-Jun and CREB, which was also seen in the cAMP pathway, and also Smad. These transcription factors mediate the process of neuronal regeneration and healing. However, whether GSK3 has a direct role in the activation of these transcription factors is yet to be uncovered. The study of signaling pathway has mainly been done on the dorsal root ganglion of mice.

This is because it provides a very conducive milieu that is almost of the replica of the human being. The regenerative response seen to be triggered after neuronal injury is said to be strong enough that it can lead to the healing of an injury on another part of the same axon. In all the studies on peripheral neuronal regeneration, it has been seen that there is interplay between molecules and other pathways that thence, lead to augmented healing and regrowth. On this study, PI3K is shown to work by inhibiting GSK3. This leads to the induction of expression of the transcription factor called Smad 1. This pathway and interplay is quite integral in the regeneration of the sensory neurons since blockade of either PI3k or Smad 1, shuts off the process of regeneration.

In Vitro Experiments of Regeneration

A model was set up in a way that would recapitulate the in vivo neural regeneration when induced by axotomy. From these experiments, several proteins, as seen in axotomy, were detected to be up-regulated. These proteins are same as those encoded by the regeneration-associated genes (RAGs). Replating of these cultured neurons revealed a unique characteristic similar to that of conditioning lesioned neurons. This was seen as long extending axons, with sparse branching.

This growth was seen to be different from that triggered off by NGF, which normally leads to extensive branching but average lengthening of the axons. This therefore shows the similarity of the biochemical and morphological changes that occur to the dorsal root ganglion, to those of the neurons in response to the pre-conditional injury. This similarity is seen after the dorsal root ganglion has undergone culture and re-plating.

Procedures: Does Regeneration Require Gene Transcription?

This was tested by use of culture-and-replating method. Here, a reversible polymerase II inhibitor adopted. 5, 6 dichlorobenzimidazole (DBR) was used for this purpose. Its function was to halt all axonal growth which it did almost fully by day 3 of the experiment [1]. This, therefore, strongly proved that neuronal growth or regeneration cannot occur without gene transcription. This experiment therefore tried to narrow down to the target pathway that is involved in neuronal regeneration.

PI3K Signaling in Modulating in Intrinsic Axon Growth

In this experiment, different molecules in the signaling pathways that led to neuronal regeneration were targeted. This was directed towards deciding on the integral molecules in the pathways known beforehand. Targeting PI3K, as one the main molecules in the regeneration pathway, LY294002 served as an inhibitor. Application of this inhibitor before replating led to diminished growth in the neural axons. On the contrary, application of U0126, which inhibits ERK pathway proved futile in the results since it exhibited little effect. LY294002 instead led to increased frequency of axonal branching.

In confirmation to this phenomenon, using pharmacological data, an experiment was set in which the nerves expressed a dominant-negative PI3K (dnPI3K). This ended up blocking the PI3K signaling pathway. With time-intensive analysis, it was discovered that the phosphorylation of AKT peaked by day 3 of the experiment. De-phosphorylation then took over and was down to baseline levels by day 7. This therefore showed that PI3K signaling pathway activation was pivotal in the whole process.

On another experiment where PI3K was acutely blocked prior to sciatic nerve injury, by electroporationally transfecting (in vivo) dnPI3K into adult DRGs. This largely impaired the axonal regeneration of sensory neurons. This acts as proof enough of the importance of this signaling pathway in the regeneration of neurons in mature nervous systems.

To determine the role of PI3K in the neuronal regeneration, there was interference on the PI3K pathway, before and after the beginning of the pro-regenerative responses. The effects of these actions on the growth of the neurons were monitored. When the in vivo electroporation was done prior axotomy, it was realized that the neurons grew shorter than normally under optimal conditions.

On the other hand, it was discovered that there was no inhibition on nerve grown when the electroporation was done 7 days after axotomy. These results, therefore, show that activation and optimum functioning of PI3K signaling pathway has temporal dependence. This is to say, for optimum regeneration of the axons, activation of the PI3k should occur before any additional stimulation or alteration on the normal regeneration. Phosphatase and tensin homolog (pten) encodes for a direct antagonist of PI3K.

Thereby its depletion leads to increased axonal growth. Contrary to its regenerative effect in the central nervous system, mTOR does not effect the axonal regeneration. Also GSK3 is a potent inhibitor of the PI3K signaling pathway. This therefore means that for there to be continued neuronal elongation, this molecule has to be inhibited.

More over the local changes in the axon in relation to the neural injury are not the main controllers of the PI3K-GSK pathway. Rather, it is the intra-somal changes. This, therefore, means that the signaling pathway fully depends on transcription for modulation. This was proved in an experiment where LY294002 was treated to either the axon or the cell body. Inhibition of axonal regeneration was noted in the one treated to the cell body, and not the axonal one.

Smad 1 as an Activator of the PI3K-GSK Pathway

Nerve damage leads to the phosphorylation of Smad 1 in the nucleus. This means that it gets activated in response to the injury. The effect of this activation is augmented regeneration of the injured nerves. LY294002 is a potent inhibitor of Smad 1 and thus a blocker of neural regeneration. GAP43, also associated with the PI3K-GSK pathway, has the same reaction and effects as Smad 1. Experiments revealed that Smad 1 could not stimulate the regeneration of the axon by itself in a dnPI3K-induced inhibition of neuronal regeneration. For this effect to happen, it meant that the inhibition had to be reversed first.

Therefore, it can be easily concluded that the Smad 1 is a downstream regulator of the PI3K-GSK pathway. Smad 1 is regulated in relation to its mRNA level by PI3K-GSK3 pathway. This, therefore, suggests that the regulation is dependent on transcription. The role of Smad 1 as an activator or promoter of axonal regeneration was examined by transfection of the dorsal root ganglia with siRNA against the Smad 1. It showed stagnated growth of the axons, showing the

integral role of Smad 1 in neuronal regeneration and growth.

Down-regulation of Inositol Phosphatases in Response to Axotomy

There is a downward shift in the level of Inositol phosphates that are involved with the breakdown of phosphatidyl inositol. This therefore means more life to the lipid that is integral in the PI3K-GSK3 signaling pathway. In summary, we can see that the PI3K signaling pathway is very rich, which very many interconnected pathways and associated molecules as compared to the other signaling pathways. Smad 1, GAP43 and the down-regulation of the Inositol phosphatases are activators of the pathway.

On the other hand, LY294002, GSK3 and pten were all inhibitors of the pathway. The downregulation of this inhibitor led to increased axonal growth. Due to this meshwork of connections, the PI3K thus serves to be one of the main pathways in the regeneration of injured neurons.

The KLF Members in Regeneration

There exists Kruppel-like factor 4 gene that enhances regeneration of neurons once an injury happens [3]. This gene was identified in early lives of test animals as a cause of neuronal growth in young and newborn animals. This therefore necessitated its test to check whether it can be used to initiate regeneration of neurons in adult animals that had suffered neuronal injury. In natal life, KLF4 works in the Retinal Ganglion Cells to promote its growth.

It works as a transcription repressor of the growth of axons in the neurons. There exist a number of KLF family members each working to either suppress or promote the growth of axons. In experiments done to check the functionality of these genes, the family members can either be down-regulated or up-regulated depending on their mechanism of work.

When the functionality of these KLF families is well regulated, they then lead to the generation of neurons in the peripheral nervous system and also the central nervous system. From all the other pathways that have been explained to cause neuron regeneration, one of the problems that have been greatly featured in the central nervous system is the inhibitory environment.

This disability gives the peripheral nervous system an edge over the CNS in terms of regeneration because the PNS environment is very conducive for regeneration.

For regeneration to work properly in the central nervous system just like in the peripheral nervous system, the limitations have to shake off by any of the signaling pathways participating in regeneration. Regeneration failure in the CNS is also thought to be caused by the deficiency of intrinsic factors within the adult CNS that lead to regeneration.

That is the main reason as to why, at birth there is rapid axonal outgrowth even after injury or lesion to the area in the CNS. The KLf system does not work alone. There exist various cell-autonomous factors for example the cyclic AMP and phosphatise and tensin homologue (PTEN) that are very crucial in the regeneration process. When these regulators of axon growth are manipulated in any manner, there occurs some inhibition on the process. This, however, is not a fully realizable effect. It therefore brings into light the availability of other intrinsic factors of regeneration that are yet unknown at the present.

Kruppel-like factor 4 which is a zinc finger transcription factor tends to be a good suppressor of neurite growth in its inhibition. In a case of KLF4 over-expression at the region of hippocampal neuron, it will result to enlargement of the neurons. In vitro studies and experiments have shown that KLF4 can work independently to cause suppression of axon and dendrite initiation and elongation. KLF4-transfected cells tend to elongate at a slower rate meaning that the over-expression of KLF4 decreases rate of elongation. A KLF4 gene which lacks a C-terminal DNA has completely no effect on the axons.

It happens to be its binding domain and therefore it is the one used in suppression. There is a developmental regulation of the KLF families which means that each one will differentially affect neurite growth and regeneration. It is therefore a possibility to say that in cases of growth, there are those growth-enhancing KLFs that are down-regulated while those that are growth-suppressing are up-regulated. In such a scenario, the balance of this will result in a regulated growth of neuronal axons.

The targets for KLF which are relevant for regeneration have to be those selectively expressed genes in neurons that are very useful in growth cone function. It therefore brings us to the conclusion that if we can manipulate the KLF genes, it may be a useful strategy to increase the regenerative capacity of neural cells in cases of injury.

SMAD1 in Regeneration

When adult neurons in the mammalian peripheral system are injured, they are capable of regenerating if we can reverse the age-dependent loss of intrinsic growth of axons. Smad1 is a neuronal growth promoter which is naturally triggered by axotomy. If we can analyse its working mechanism in regeneration, we can therefore harness it in the promotion of neuronal growth. Smads are a family of intracellular mediators of TGFß/BMP forming signaling pathways in the body.

Transcription factor SMAD1 is required in triggering an axonal growth once an injury to a neuron occurs. In this scenario, the neuronal smad1 will be up-regulated. Once it has been up-regulated, it is then phosphorylated and thus accumulates in the nucleus. This is then followed by an onset of axonal extension.

If by any chance the Smad1 is reduced via RNA manipulation, the growth of the axons will be impaired. It therefore means that for continued uninterrupted growth, smad1 will always be required. Axonal growth capacity can be enhanced marked if one was to inject a smad1 activator e.g. BMP2 into the ganglion. This however, does not mean that it depends on extracellular BMP for activation. The sole activator for smad1 is only axotomy. There has not been any reported effect of blocking extracellular BMP on Smad1 activation. There is, however, a report that indicates smad1 mediates BMP signaling from axonal transport.

This works in regulating neuronal differentiation and synaptic maturation. Naturally, smad1 is activated in cases of axotomy which forms an integral part in the transcription switch promoting growth of neurons. In addition to smad1, there is also an immediate early gene called ATF3 which is also induced in cases of axotomy. When the level of ATF3 is increased, the rate of peripheral regeneration is also substantially increased. The key trigger to nuclear translocation of pSmads is their phosphorylation. These then work to activate downstream target genes. All the signals from members of the BMP family are mediated by Smad1, Smad5 and Smad8. In the discussion on adult peripheral neuronal regeneration, we will focus mainly on Smad1gene.

Induction of Smad1 is always neuronal following an injury to an axon. It peaks sequentially from day of lesion to optimise at around day 7 and will remain above baseline for a few days. It has to be phosphorylated at the C-terminus for it to be active. Once Smad1 undergoes phosphorylation, it can then undergo nuclear translocation. If Smad1 is knocked down, the axonal growth in adult neurons is reduced. The reprogramming process in the Smad1 sequence has been shown to be directly linked to injury [7]. It has been shown to be largely neuro-autonomus.

It has a retrograde transport that is shared by other signaling transport factors like JNK, MAP kinase and pSTAT3. It is possible for these factors to accentuate responses due to peripheral axotomy from the neurons. Not only does Smad1 regulate growth of the axons, it also seems to control expression of other injury-induced genes. BMPs also work at the tips of axons independent of any nuclear signaling.

JAK/STAT Signaling Pathway

After peripheral neural injury, the neuron starts undergoing ischemia. At this stage, the body chooses on whether to lose the nerve or regain its function. Often, the body sets itself towards a self-healing process. Many molecules and pathways are recruited in order to make this a reality. These molecules may be derived or functioning in the extra-cellular or intracellular compartment. Among these many pathways jumpstarted by the body's healing process, is the Janus kinase pathway (JAK/STAT) [8].

This pathway involves the use of two molecules whose linked functions end up in the modulation of signals that lead to in transcription. Transcription is the process behind the development of genes that encode the necessary proteins needed in the regeneration process. Therefore, the process of healing is slow since it involves protein

synthesis under modulated and coordinated milieu and activity in order to fine tune their synthesis and finally regeneration of the injured nerve. Janus kinases are a group of molecules that are activated by the activation of various surface receptors diverse ligands. These include growth factors, interferon and cytokines. This activation leads to phosphorylation.

The phosphorylated and, thus, activated janus kinases recruit the signal transducers and activators of transcription (STAT). Activated STATs dimerise into homodimers and heterodimers and move to the nucleus where they activate transcription of the target genes.

Therefore, the whole signaling pathway depends solely on the activation of the various associated molecules by phosphorylation. In relation to neuronal regeneration, JAK as a molecule acts as a promoter. On the other hand, STAT has mixed functions to an extent that the direct role of either promoting or inhibiting is elusive. But, generally, it is assumed to be an activator of neuronal regeneration. The main STAT involved in neuronal regeneration is STAT3 [9].

Role of STAT3 in Remodeling After Spinal Injury

STAT3 is a transcription factor that serves as an initiator of growth in the body. This function are quite useful when it comes to spinal injury since any lesion to the spinal cord damages the neurons thus causing deficits in motors supply and sensory functions. When there is partial injury to the spinal cord or nerves tracts in particular, the neurons preserve the potential too self-regenerate.

Many studies and investigations have revealed this potential to regenerate and remodel way after an injury. The intrinsic capability of the neurons to start the process of regeneration has been investigated and the various molecules involved studied. One of the most pivotal of them is STAT3. This has been backed by the following supporting findings.

- After neuronal injury, there is increased intra-nuclear STAT3, which coincides with the process of regeneration.
- During experimentation, deletion of the molecule slowed down the peripheral neuronal regeneration process (WANG, 2025).

Here are some of the major findings in relation to this signaling pathway:

Stat3 Augments Sprouting Of Fibers

After the discovery that the levels of endogenously produced STAT3 could not sustain the function of remodeling and regeneration, there was a resolution to test for exogenously augmented STAT3. This was done by the administration and induction of recombinant STAT3 using adenovirus into the hind limb motor cortex.

The detour circuits were assessed in order to test for the duration of time the remodeling could be sustained. From the results, it was noted that there an increase in the formation of the cervical corticospinal tract collaterals by the third week. These, therefore, revealed that sustained expression of STAT3 could jumpstart and propagate the regeneration and remodeling process.

STAT3 Recruits New Connections with Unlesioned Tracts

STA3 leads to recruitment of nerve fibers that are not affected by the lesion in order to hasten the process of neuronal regeneration. This was discovered after there was a unilateral pyramidectomy. The contralateral fibers were monitored to observe their response to the lesion and, thus, to show the relationship with the availability of STAT3 [9].

After 3 weeks of observation, it was discovered that the fibers from the unlesioned side had crossed over to the lesioned side. This meant that the nerves had crossed the midline in order to join the contralateral side.

This can also be termed as decussating. It was thereby noted that the endogenous STAT3 had the potential to cause the nerve fibers to cross the midline as part of the healing process. This had initially been known to be the function of neuronal calcium sensor1 and neurotrophin3. These discoveries, therefore, bring more light and triggers more room for experiments and discovery.

The Decussating Fibers Improve Functional Recovery

Immediately after injury to the spinal cord, there is diminished or complete loss of function in the area supplied by the lesioned fibers. With time, and with the healing from the STAT3 that leads to crossing of fibers to the contralateral side, the affected area starts regaining functional capability. This has been seen in a number of studies and investigations that have been done so far.

Therefore, from the above findings, we can see that STAT3 plays a big role in the regeneration of neurons. It also has the extra capability of reorganizing and remodeling as seen above. The role of stimulating decussation in the verves makes it unique as a molecule involved in the regeneration of injured neurons. Otherwise, STAT3 is not a main player in this field since less of its facts are stated. This cycle is triggered off intrinsically after neuron injury due to influx of Ca^{+} into the injured nerve. This finally leads to generation of RAGs. These are genes involved with the function of neuronal regeneration.

Conclusion

- All the pathways involved have been put through analysis and vetting to measure their strengths and weaknesses. From all of them, as shown above, PI3K has come out on top due to its efficiency and efficacy in the controlling of the whole process of neuronal regeneration. It can be easily targeted pharmacologically and its efficacy easily monitored and modulated. Since the main reason for the study is to decide on the most significant and most efficacious signaling pathway, it can, therefore, be confidently stated that PI3K is the main signaling pathway that modulates and propagates peripheral neuronal regeneration in response to injury.
- Most of the signaling pathways studied under this review had adequate supporting evidence. Some of them though, like Smad 1 and KLF pathways had loopholes that required more investigation and experiments to reach concrete conclusion. On the other hand, some of the pathways like the cyclic adenosine monophosphate and phosphatidyl inositol 3-kinase, had very explicitly laid out cycles and interconnections within their system that they proved to be superior to the rest. All in all, the PI3K finally had the most supporting evidence and proved to be more independent, compared to the other signaling pathways.

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