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SPECIAL COMMUNICATION

The Role Of Cells, Neurotrophins, Extracellular Matrix And Cell Surface Molecules In Peripheral Nerve Regeneration

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Abstract

Wallerian degeneration is a complicated process whereby axons and myelin sheaths undergo degeneration, and eventually are phagocytosed by macrophages and Schwann cells following nerve damage. Schwann cells proliferate and the endoneural tubes persist. In addition, neurotrophins, neural cell adhesion molecules, cytokines and other soluble factors are upregulated to facilitate regeneration. The important role of cellular components, neurotrophins, and extracellular matrix components, including cell surface molecules involved in this regenerative process, is highlighted and discussed in this review.

Keywords: *cell surface molecules extracellular matrix, nerve growth factors, nerve regeneration, Schwann cells, neurosciences*

Introduction

Various cellular components such as Schwann cells, macrophages, fibroblasts, and mast cells are recruited during peripheral nerve regeneration. In addition, research has shown that neurotrophins, extracellular matrix components and cell surface molecules are also vital for successful nerve regeneration. Available research data on these components are discussed below.

Schwann cells

Schwann cells, macrophages and mast cells are the three main cell types that are involved in peripheral nerve regeneration. The importance of Schwann cells in peripheral nerve regeneration was demonstrated in a study carried out by Hall (1). The investigator compared the regeneration that occurred in the cellular and acellular autografts using electron microscopy. The study showed that neurites grew into fresh autografts and rapidly re-establish functional relationships with the Schwann cells lying in bands of Büngner within the graft, however penetration of acellular grafts was less efficient. In another study carried out by the same author (2), following mitomycin C treatment, an anti-mitotic agent known to arrest Schwann cell division after nerve injury, very few neurites grew into a cellular nerve autografts, suggesting that neurite outgrowth from the proximal stump is dependent upon active Schwann cell participation.

There are a variety of mitogenic factors that can stimulate Schwann cell proliferation. These factors include the extracellular matrix components, myelin debris, macrophage-derived cytokines and Schwann cell-Schwann cell signalling (3). In addition, axon-derived signals have also been shown to cause Schwann cell proliferation. One component of the axon-derived signal that has been well characterised is the neuregulin family (4). One of the family members, neuregulin-1 (NGR1) is a survival factor for immature Schwann cells in the developing peripheral nerve. NGR1 mRNA is reported to be present at high levels in the cell bodies of the adult sensory and motoneurons (5).

Based on in vitro studies carried out by Mahanthappa et al. (6) on the effects of recombinant human glial growth factor 2 (rhGGF2), the investigators proposed that the secretion of neuregulin from the growth cones of the regenerating axons may set up a concentration gradient within a local environment. According to their hypothesis, the neuregulin serves as a chemoattractant, so that Schwann cells migrate towards the site of injury where the neuregulin

concentration is high and this stimulates the Schwann cells to proliferate and also secrete trophic factors to support axon regeneration.

Apart from axon-derived signals to regulate Schwann cell survival, investigations carried out by Jessen and Mirsky (7) suggest the presence of autocrine survival loops which provide an axon-independent way of surviving. Their in vitro study has shown that Schwann cells grown at a sufficient density to achieve a concentration of secreted factors are capable of surviving in the absence of neuronal signals. In addition, it has been reported that the Schwann cells in the distal stump also upregulate the expression of both neuregulins and the erbB2 neuregulin receptor (8). These observations indicate that Schwann cells might be able to survive even when the axons are damaged.

Additionally, mitogenic signals can also be released by damaged Schwann cells. Nerve injury that leads to Schwann cell damage releases intracellular ciliary neurotrophic factors (CNTF), which then signals via axonal leukaemia inhibitory factor (LIF) receptors to induce Reg-2 which is a Schwann cell mitogen (9). Additional mitogenic signals for Schwann cells can also come from macrophages which secrete platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß) and fibroblast growth factors (FGF-ß) which are all mitogenic for Schwann cells (10). On the other hand, axotomised Schwann cells can downregulate expression of myelin protein genes (such as myelin-associated glycoprotein, myelin basic protein and PO) and upregulate gene expression of low affinity neurotrophin receptor p75, GAP43, GFAP, neurotrophins like NGF and BDNF, the neu receptor c-erbB2, cell-cell adhesion molecules Neural-Cell Adhesion Molecule (N-CAM) and L1, extracellular matrix molecules like laminin, J1/tenascin and fibronectin. Following establishment of functional contact with axons, Schwann cells also upregulate expression of ß4 integrin, laminin B1 and B2 chains (11).

Clearly, from the studies reviewed above, the Schwann cells appear to be essential for peripheral nerve regeneration. However, macrophages and mast cells are also important cellular components, and will be discussed next.

Macrophages

Major macrophage invasion usually occurs around the third day following nerve injury. The mechanism by which this invasion occurs is not clearly understood, but it is thought that chemokines such as macrophage chemotactic protein-1 (MCP-1) and macrophage inhibitory protein-1a (MIP-1a), are produced by Schwann cells or resident macrophages in response to nerve injury (3). Macrophages deliver a variety of important molecules including basic fibroblast growth factor, granulocycte-macrophage colony stimulating factor, transforming growth factor alpha (TGF-a), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and interleukin-8 (12).

Mast cells

Mast cells are important cells involved in the inflammatory response. Mast cell degranulation and the release of histamine may be important in opening the blood nerve barrier after nerve injury. Mast cells also contain growth factors, proteolytic enzymes, tumour necrosis factor alpha (TNF-a), interleukins and cytokines which may be released following nerve injury (12).

Neurotrophins

The five known neurotrophins are named nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrphin-4/5 (NT-4/5) and neurotrophin-6 (NT-6), and some of them may have important functions in peripheral nerve regeneration. These neurotrophins act via receptor tyrosine kinases (RTK): NGF interacts with trkA; BDNF and NT-4/5 interact with trkB; NT-3 interacts with trkA, trkB and trkC; and all of these neurotrophins (except NT-6 which is not fully characterized yet) interacts with receptor p75 (13). Of these, NGF, NT-3 and BDNF show beneficial effects on the survival and regeneration of primary sensory neurons in the dorsal root ganglion (DRG) and motoneurons in the spinal cord (5).

The roles of neurotrophins were confirmed in knockouts. Studies by Snider and Silos-Santiago (14), showed that both the trkA-/- and trkC-/- receptor mutant mice have striking neurological deficits which can be ascribed to neurons in the DRG. The trkA-/- animals showed massive DRG neuron loss in the lumbar ganglia, and almost all unmyelinated and 50% of myelinated axons were lost. The trkC-/- animals showed writhing movements of the extremities that were noticeable within the first few days of life, reduced size of the DRG, a modest reduction in the DRG neuron number, depletion of large calibre axons in the dorsal roots, and reduction in area of the dorsal column. On the other hand, trkB-/- animals had less profound effects in the DRGs observed. Most of these neurotrophins are target-derived, however,

the localization of BDNF in a population of DRG neurons suggests the additional possibility that BDNF could act in a paracrine or autocrine manner to mediate neuronal survival (15).

In relation to nerve injury, the importance of these neurotrophins can be seen in studies carried out by Verge et al. (16). To assess the role of neurotrophins in vivo, the authors investigated the effects of intrathecal administration of NGF and NT-3 on DRG following unilateral sciatic transection in the adult rats. Their result showed both NGF and NT-3 were able to counteract axotomy induced changes in the DRG neurons. In another study, Sterne et al. (17) reported that NT-3 delivered locally via fibronectin mats enhances peripheral nerve regeneration. Similar enhancement of peripheral nerve regeneration was also reported following delivery of CNTF following sciatic nerve transection in rats (18).

Following nerve injury, the Schwann cells in the distal stump upregulate the synthesis of NGF, BDNF, NT-4 and p75 to promote the proliferation and migration of Schwann cells. BDNF also supports motoneuron survival and its level rises along with NT-4 in the denervated distal sciatic nerve stumps. It was also shown that NT-3 supports large myelinating sensory neurons (12). However, the exact roles of these neurotrophins in peripheral nerve regeneration is not clearly defined yet.

Extracellular matrix

The extracellular matrix (ECM) is a naturally occurring substrate, which cells use to migrate, proliferate and differentiate. Four main ECM molecules that are found in the peripheral nervous system and synthesized by Schwann cells are laminin, fibronectin, type IV collagen and various proteoglycans.

Laminin, which is a glycoprotein of cruciform structure comprising three polypeptide chains (19), is found in the Schwann cell basal lamina (20). Laminin, which is known to be the most effective promoter of neurite outgrowth in vitro (19), is also commonly used in tissue culture studies as a substrate to grow explants such as DRGs. Fibronectin is a large highly asymmetrical glycoprotein which is thought to promote adhesion and spreading of cells by linking them to collagen substrates. On the other hand, type IV collagen, which is also a glycoprotein, is found exclusively in the basement membrane of the Schwann cell (21).

Heparan sulphate proteoglycans (HSPG) and chondroitin sulphate proteoglycans (CSPG) and are also found in the basal lamina. HSPGs consist of a polypeptide backbone (core protein) and linear chains of polysaccharides characterized by repeating disaccharides of hexuronate and N-substituted glucosamine residues. The activity of HSPGs as promoters of neurite outgrowth was observed in in vitro studies in the mid eighties. HSPG complexes greatly enhance the neurite outgrowth promoting abilities of laminin or N-CAM (22,23,24). One of the CSPGs found in the peripheral nerve is NG2. NG2 consists of a core protein bearing GAG chains composed entirely of chondroitin sulfate. NG2 levels are upregulated following central nervous system (CNS) injury and the molecule inhibits axon outgrowth (25). Similarly, in vitro studies carried out by me and my colleagues on damaged rodent peripheral nerves showed that NG2 is a significant component of fibroblastic scar tissue and it appears to contribute to the failure of axonal growth through the scar (26). Generally, CSPGs are thought to inhibit axon regeneration and, in contrast, HSPGs promote neurite outgrowth (27).

Other general observations on the neurite promoting activities of heparan sulphate moieties are supported by studies on specific molecules. Perlecan and agrin ECM associated HSPGs, have been associated with the establishment of neuromuscular junctions and CNS axon pathways, respectively (28,29). Cerebroglycan, which is a member of glypican family, is expressed on the surface of cultured neurons and their growth cones, presumably mediating neuronal migration and axon growth (30). Members of the glypican family have also been detected in the developing nervous system and are generally associated with axons rather than glia (31).

Cell surface molecules

Cell surface molecules mediate axonal adhesion and outgrowth, and Schwann cells have been reported to express a variety of these glycoproteins including N-CAM, L1 and myelin-associated glycoprotein.

The distribution of these molecules was described by Martini and Schachner (32) in the regenerating adult mouse sciatic nerve. The authors reported that during the first 26 days post lesion, L1 and N-CAM were detectable at cell contacts between non-myelinating Schwann cells and degenerating axons, and myelin-associated glycoprotein was detectable in the periaxonal area of the degenerating myelinated axons. These authors also observed that growth cones and regenerating axons expressed N-CAM and L1 at contact sites with fibroblast-like cells. Two weeks after nerve transection, when the regenerating axons were

seen in the distal part of the transected nerve, the authors also reported seeing L1- and N-CAM-positive contacts with Schwann cells. Their study clearly shows that L1 and N-CAM are involved in supporting axon regeneration on the surface of Schwann cells and fibroblast-like cells. In addition, studies by Bixby et al. (33) demonstrated 13that other surface molecules such as cadherin, NgCAM (a homophilically binding immunoglobulin-like adhesion molecule) and integrins (which bind laminin, fibronectin and other ECM molecules) are also important in facilitating axon growth on Schwann cells.

Conclusion

Previous and current research data have shown that during peripheral nerve regeneration, Schwann cells, macrophages, mast cells, extracellular matrix molecules and neurotrophins are found to be highly expressed and indeed vital. However, our current understanding of peripheral nerve regeneration is incomplete. Research is still needed to further understand this process, especially in the area of molecular neurobiology, including genomic and proteomic studies. Nevertheless, the available data provide a step towards a better understanding of this complex regenerative process which is useful for better management of nerve injuries.

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