

Proteomics in Neuropathic Pain Research

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Neuropathic pain is often caused by nerve injury or dysfunction in the peripheral and central nervous system and is frequently associated with allodynia and hyperalgesia. The underlying molecular mechanisms of neuropathic pain are largely unknown, and therefore, pharmacologic treatment is insufficient in many cases. To elucidate translational and posttranslational modifications in the nervous system that arise after nerve injury, a number of proteomic studies have been performed using different animal neuropathy models. The results of these proteomic approaches are summarized in this review to provide a better overview of proteins that are involved into the pathogenesis of nerve injury and neuropathic pain. This might allow a better understanding of the pathophysiologic signaling pathways in this impairment, facilitate the discovery of specific biomarkers, and thus promote the development of novel pain therapies.

NEUROPATHIC pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system. It can arise from a wide variety of injuries to peripheral or central nerves, including metabolic disorders, traumatic injury, inflammation, and neurotoxicity, and is characterized by spontaneous pain, hyperalgesia (increased pain response to a noxious stimulus), and allodynia (pain elicited by a nonnoxious stimulus), which can persist long after the initial injury is resolved.¹ Common causes of neuropathy are diabetes, herpes zoster infections, chronic or acute trauma, and neurotoxins. Furthermore, neuropathic pain occurs frequently in cancer as a direct result of peripheral nerve damage (e.g., compression by a tumor) or as a side effect of many chemotherapeutic drugs.

The underlying molecular mechanisms are still not completely understood, and as a consequence, treatment is unsatisfactory in many cases.²⁻⁴ Despite the large number of approved analgesics such as opioids or nonsteroidal antiinflammatory drugs, treatment of chronic pain is still often aggravated by poor activity of available

drugs and the occurrence of adverse drug reactions.^{5,6} The current pharmacologic treatment of neuropathic pain includes tricyclic antidepressants such as amitriptyline, anticonvulsants such as gabapentin and pregabalin, serotonin-norepinephrine reuptake inhibitors such as duloxetine, and opioids. However, all of these drugs have limited efficacy combined with a number of side effects, and the mechanism of how they relieve pain is not completely understood. Therefore, there is an urgent need to develop novel therapeutics for an effective treatment of neuropathic pain.

The discovery, design, and evaluation of new drugs are critically dependent on the elucidation of protein mechanisms involved in the respective diseases. Neuropathic pain reflects both peripheral and central sensitization mechanisms which involve transcriptional and posttranscriptional modifications in sensory nerves.^{1,7} Therefore, proteome analysis in animal models of neuropathy can help to identify pain-related proteins (biomarkers) which may serve as diagnostic markers or drug targets and therefore ameliorate the treatment conditions for patients with neuropathic pain. This review is intended to summarize recent proteomic approaches in animal models of neuropathic pain, which provide several hypotheses about proteins involved into the pathogenesis of this impairment.

Proteomics

Most physiologic body functions are based on the integrity of proteins. Pharmacologically active drugs often target proteins because of their pathophysiologic relevance. However, among more than 3,000 proteins that are suggested as “drugable,” only approximately 500 are indeed targets for pharmacologic therapy so far.⁸

Proteomics is a term that describes the science and methodology of the investigation of the proteome, which quantitatively acquires the composition of proteins in a cell, a tissue, or an organism. Proteomics is complementary to genomic approaches, which investigate DNA and RNA. Unlike genomics, proteomics delivers information about protein isoforms, posttranslational protein modifications such as glycosylations and phosphorylations, protein-protein interactions, and protein stability and degradation. Whereas the genome of an organism is rather constant, the proteome differs

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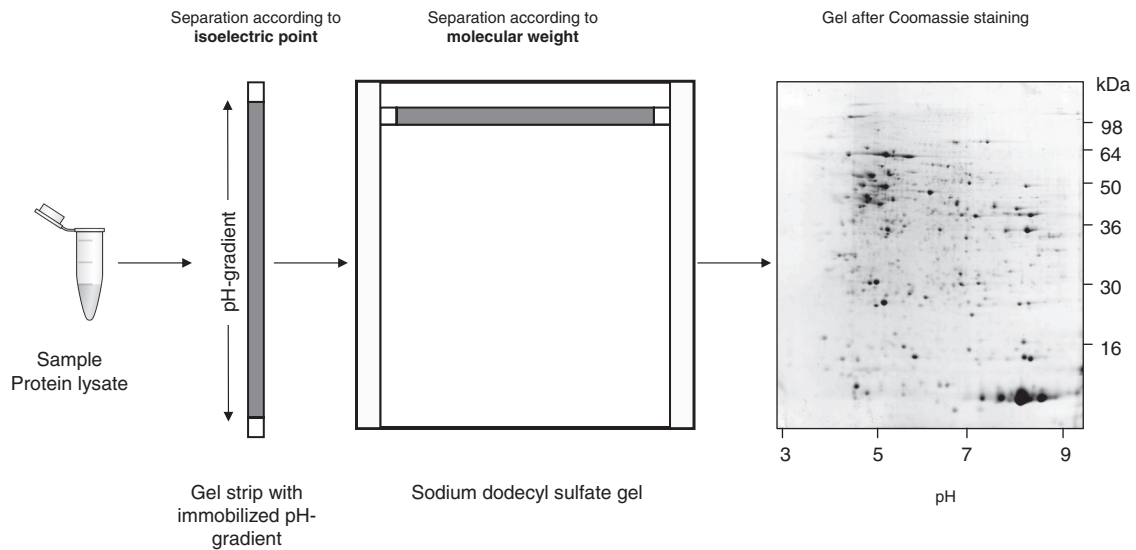


Fig. 1. Schematic illustration of two-dimensional polyacrylamide gel electrophoresis technique. In the first dimension, a protein sample is loaded onto a gel strip with immobilized pH gradient, and the proteins are separated by high voltage according to their isoelectric point. Subsequently, the strip is loaded on the top of a sodium dodecyl sulfate gel, and the proteins are separated according to their molecular weight. Thereafter, proteins are visualized by staining.

strongly from cell to cell and is constantly modulated through biochemical interactions with the genome and the environment. The protein expression is variable in different parts of a body and depends on a number of parameters, such as age, different environmental conditions, or diseases.

Proteomics can be used for the generation of protein maps of certain tissues (profiling or expression proteomics), for studying pathophysiology by investigation of aberrant proteins (functional proteomics), and for correlating nucleic acid levels with proteins (reviewed by Choudhary and Grant⁹ or Gorg *et al.*¹⁰).

2D-PAGE

The main technology in proteomics is two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), which couples isoelectric focusing in the first dimension and sodium dodecyl sulfate polyacrylamide gel electrophoresis in the second dimension and allows the separation and visualization of complex protein mixtures according to their isoelectric point, molecular weight, solubility, and relative abundance.¹¹ Depending on the pH gradient and the gel size, 2D-PAGE makes it possible to separate thousands of proteins. Protein spots in a gel can be visualized using a variety of chemical stains or fluorescent markers. The intensity of the protein stain is used to quantify protein levels. Subsequent protein analysis and identification are generally performed by mass spectrometry (matrix-assisted laser desorption ionization time-of-flight or surface-enhanced laser desorption ionization time-of-flight mass spectrometry).¹²

The first dimension of 2D-PAGE is an isoelectric focusing. Proteins are loaded onto gel strips with an immobilized pH gradient and then separated by high-voltage

isoelectric focusing according to their net charge (isoelectric point).¹³

After isoelectric focusing, the immobilized pH gradient strips are placed onto the top of a sodium dodecyl sulfate polyacrylamide gel, and a separation of the proteins according to their molecular weight is performed. Gels are stained with Coomassie brilliant blue, silver, or fluorescence dyes and analyzed for differences in protein expression between two different samples using appropriate image analysis. 2D-PAGE typically allows for the separation of hundreds to thousands of protein spots onto one gel (fig. 1).

Identification of 2D-separated Proteins

Protein spots of interest are cut out of the gels and digested to peptides by trypsin. The fragmented peptides are prepared for analysis by extraction with appropriate organic solvents. The proteins are identified by matrix-assisted laser desorption ionization time-of-flight or surface-enhanced laser desorption ionization time-of-flight mass spectrometry on the basis of peptide mass matching.^{14,15}

Clinical Applications of Proteomics

The study of clinical proteomics is suggested as a promising field with potential for many applications, such as identification of biomarkers and monitoring of diseases. *Expression proteomics* can be applied for the discovery and validation of diagnostic, prognostic, and predictive biomarkers of diseases, whereas *functional proteomics* is intended to identify new targets as well as clarify known drugs. The potential of proteomics has been discovered in various medical areas, in particular cardiovascular diseases, neurologic disorders, infectious

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diseases, and especially cancer.¹⁶ A number of biomarker proteins in several different cancer types have been identified by surface-enhanced laser desorption ionization time-of-flight mass spectrometry and have been reviewed by Engwegen *et al.*¹⁷ By screening already known drugs with proteomic techniques, it is possible to identify proteins that interact with these drugs. These approaches can provide hints about novel targets of the drugs, *e.g.*, a proteomic analysis of the protein kinase C inhibitor GF109203X revealed other potential targets, such as cyclin-dependent kinase 2.¹⁸

Therefore, in several fields, proteomics will have clinical utility in the future, but to date, the development of proteomics as a technique for high-scale analysis is still in progress. Most approaches are related to cancer diagnosis and treatment. In the context of new drug development, it has to be taken into account that proteomics is mostly the starting point for further investigations which then focus on the most interesting results gained from proteomic studies.

Animal Models of Neuropathic Pain

During the past 10–20 yr, a range of animal models has been established that reflect a variety of different causes for neuropathic pain. They simulate specific human painful conditions, mostly by producing traumatic injuries to the spinal cord or peripheral nerves that result in chronic pain.¹⁹

Central Pain Models

Most of the pain models in the central nervous system are based on spinal cord injury (SCI). In patients, this kind of neuropathic pain can be initialized after traumatic or ischemic injury of the spinal cord. Dysesthesia as well as spontaneous and evoked pain are frequently coming along with this neuropathy.

In animals, SCI can be performed by weight drop,^{20,21} spinal cord compression,²² crushing,²³ photochemically induced injury,^{24,25} excitatory neurotoxin methods,^{26,27} and spinal hemisection^{28–30} as well as complete transection.³¹ These models lead to spontaneous and evoked pain as well as allodynia and hyperalgesia.

Weight Drop Model. In this quite old model, SCI is produced by dropping a weight on the exposed spinal cord surface at the thoracic–lumbar level, which simulates the human clinical condition of traumatic injury of the spinal cord. This treatment leads to paraplegia and complete segmental necrosis.^{20,21}

Excitotoxic SCI. Injection of several neurotoxins into the spinal cord can lead to the development of abnormal pain, which is a correlate to pain induced by SCI in humans, including long-lasting spontaneous pain and mechanical and thermal hyperalgesia.^{26,27}

Photochemically Induced Injury. In this model, spinal cord vessels are damaged by intravenous injection of

a photosensitizing dye, which is excited by an argon ion laser, subsequently leading to parenchymal tissue damage.²⁴ In animals, this treatment leads to autotomy (self-mutilation of the injured paw) and mechanical and cold allodynia as well as hyperalgesia.²⁵

Spinal Cord Hemisection and Complete Transection. In case of spinal cord hemisection, the spinal cord is only partially dissected at the T13 level, thus leading to an immediate flaccid paralysis of the ipsilateral hind paw that is recovered 15 days after injury. Concurrently, noxious stimulation of this paw induces signs of hyperalgesia and allodynia.^{28–30} Complete transection of the spinal cord at thoracic level T9–T10 leads to complete paralysis of the hind legs that cannot be regenerated. This model is suitable to investigate the mechanism of the adult inability to regenerate neurons in the central nervous system.³¹

Peripheral Models

Neuropathic pain in the periphery can be induced in humans by a variety of events, such as trauma, compression, infections, metabolic diseases, neurotoxins, tumors, and others. Common animal models of peripheral neuropathic pain that simulate human neuropathic conditions include spinal nerve ligation (SNL), partial nerve injury, chronic constriction injury (CCI), and spared nerve injury. Key considerations for the use of these animal models should be that most patients with neuropathic pain resulting from nerve trauma have partial lesion of a nerve and that complete nerve lesions are less frequent.

Spinal Nerve Ligation. Kim and Chung^{32,33} reported an experimental model of mononeuropathy with L5 and L6 unilateral (left or right side, respectively) SNL leading to a quick development of hyperalgesia and allodynia (within 24 h) lasting for at least 4 months without autotomy. Deafferentation is moderate in this model. A variant of this model is the ligation of the L5 spinal nerve only,³² which is easier to perform in comparison with combined L5–L6 ligation and also evokes long-lasting allodynia and hyperalgesia. These models simulate the clinical conditions of injury to the nerve plexus or the dorsal root.

Partial Sciatic Nerve Ligation. To achieve partial nerve injury, 33–50% of the sciatic nerve high in the thigh is unilaterally ligated with silk sutures. Within a few hours after the operation and for several months thereafter, rats develop spontaneous pain characterized by guarding behavior of the ipsilateral hind paw and licking. Furthermore, animals exhibit signs of allodynia to mechanical stimulation and of hyperalgesia to thermal and mechanical noxious stimuli. Autotomy is absent in most cases, and deafferentation is moderate. Behavioral changes and sensory disorders in the partial sciatic nerve ligation model correlate with symptoms of complex re-

gional pain syndrome in humans after peripheral nerve injury.^{7,34,35}

Chronic Constriction Injury of the Sciatic Nerve. The CCI model has been reported as painful peripheral mononeuropathy where the sciatic nerve is constricted on the left or right side, respectively, through loose ligatures at the mid-thigh level with chromic gut sutures.^{36,37} This treatment results in nerve inflammation and subsequently leads to a substantial loss of both myelinated and unmyelinated fibers distal to the placement of the ligatures. Therefore, deafferentation is extensive in this model. Compared with human correlates, the CCI model simulates clinical conditions of chronic nerve compression, which can occur after lumbar disk herniation or nerve entrapment, heavy metal poisoning, anoxia, and metabolic disorders.^{38,39}

Chronic constriction injury rats show behavioral signs of spontaneous pain as well as hyperalgesia due to noxious thermal and mechanical stimuli within the first 24 h after surgery. Furthermore, they develop cold and tactile allodynia. All pain signs last over a period of at least 2 months. Because antiinflammatory treatment of CCI rats results in decreased hyperalgesia,⁴⁰ it is suggested that this model also comprises an inflammatory component in the development of neuropathic pain.

Experimental Nerve Crush. In this model, the sciatic nerve is exposed at the mid-thigh level and crushed by hemostatic forceps with grooved jaws. This treatment produces tactile and thermal hyperalgesia and allodynia that manifest first after 3 weeks and last for at least 52 weeks. The nerve crush (NC) leads to wallerian degeneration with subsequent regeneration processes.^{41,42}

Spared Nerve Injury. The spared nerve injury model of Decosterd and Woolf⁴³ is based on section and ligation of two of the three peripheral branches of the sciatic nerve: The tibial and common peroneal nerves are ligated, and the sural nerve remains intact. It differs from the Chung spinal segmental nerve and the Bennett CCI in that the comingling of distal intact axons with degenerating axons is restricted, and it permits behavioral testing of the noninjured skin territories adjacent to the denervated areas. The spared nerve injury model results in behavioral modifications (sensory hypersensitivity) after less than 24 h that last for at least 6 months. The mechanical and thermal hyperalgesia is increased in the ipsilateral sural and, to a lesser extent, saphenous territories, without any change in heat thermal thresholds.⁴³

Protein Regulations Associated with Neuropathic Pain

The regulation of the protein expression pattern in several tissues of the nervous system has been investigated in a number of aforementioned animal models of neuropathy. Protein modifications differed strongly

among the respective models and different tissues, which is in accord with reports showing that most utilized animal neuropathy models show apparent behavioral and morphologic differences.^{44,45} Furthermore, neuropathic pain in humans also displays different pain syndromes and a number of different causes, thus indicating that a specific nerve injury might have a specific underlying mechanism. An overview of the proteomic studies in different models of nerve injury is given in table 1.

Spinal Cord Injury

Differential regulation of proteins in the injured spinal cord of rats has been studied after traumatic injury.⁴⁶ Among 947 protein spots on the 2D-gel, the authors found more than 39 up-regulated and 29 down-regulated proteins (≥ 2 -fold regulation) 24 h after SCI. Protein alterations at this time point may be related to the onset and early development of neuropathic pain. A number of these protein regulations included neural lineage proteins as well as apoptotic signaling proteins after damage of the spinal cord, which could be confirmed by additional immunohistochemical analysis. Based on the results, it has been concluded that secondary events after SCI include apoptotic cell death as well as regeneration processes by restoring chronic function through increased local levels of growth factors that stimulate the migration, proliferation, gliogenesis, and neurogenesis of endogenous neural progenitor cells in the spinal cord.

Another group performed a model of complete spinal cord transection. This study was mainly focused on investigation of proteins associated with the inability of the adult central nervous system to regenerate. Although axonal regeneration is not suggested to restore the original neuronal anatomy before SCI, it is important for functional recovery by enhancing rewiring of the neuronal network^{47,48} and will therefore improve symptoms of neuropathy. Five days after injury, an up-regulation of more than 30 proteins (≥ 1.5 -fold regulation) in the spinal cord has been observed. It has been suggested that these proteins are playing various roles in injury and regeneration processes. In particular, two up-regulated proteins (11-zinc-finger protein and glypican) were estimated as inhibitors for axonal growth and regeneration.³¹ Glypican is a proteoglycan that is expressed in developing immature neurons and prevents axon regeneration as a receptor of axonal growth-inhibiting proteins.⁴⁹ 11-Zinc-finger protein is involved in cell cycle regulation and inhibits cell growth and proliferation in cancer cells.⁵⁰

Spinal Nerve Ligation

In a model of L5-L6 nerve ligation, five proteins with different expression levels (no minimum regulation threshold indicated) in the spinal cord after nerve injury were identified.⁵¹ Among these proteins, the authors

Table 1. Overview of Proteomic Studies in Different Models of Nerve Injury

Study	Animal Model	Dissected Tissue	Time Point	Average of Protein Spots on 2D-gel	Number of Regulated Proteins	Required Change in Protein Expression	Major Findings
Kang <i>et al.</i> ⁴⁶	Spinal cord injury (traumatic)	Spinal cord of the lesion center (T9–T10) (6 mm)	24 h	947	68	≥2-fold	39 up-regulated and 21 down-regulated proteins have been found after nerve injury which can be separated into different functional categories. Some of these proteins are suggested to participate in wound healing responses during neurogenesis and gliogenesis.
Ding <i>et al.</i> ³¹	Spinal cord injury (complete transection)	Whole spinal cord	Day 5	1,400	>30	≥1.5-fold	The study focused on proteins responsible for the inability of the central nervous system to regenerate in adult mammals. A number of regulated spots have been found after complete spinal cord transection comprising two structurally and functionally novel proteins.
Lee <i>et al.</i> ⁵¹	L5–L6 spinal nerve ligation	Spinal cord (L5–L6 level)	Days 4, 7, and 14	1,750	5	NI	Creatine kinase B was down-regulated after nerve ligation, indicating its potential role in the development and maintenance of neuropathic pain.
Alzate <i>et al.</i> ⁵⁵	L5–L6 spinal nerve ligation	Brainstem	Day 7	220	21	≥30%	Differential regulation of several proteins after nerve ligation is suggested to reflect their role in progression and pathogenesis of neuropathic pain.
Komori <i>et al.</i> ⁵⁶	L5 spinal nerve ligation	L4 and L5 dorsal root ganglia (ipsilateral and contralateral)	Day 7	1,300	67	NI*	Comparison of neuropathic (L5) and nonneuropathic (L4) DRG revealed that injury of primary sensory neurons leads to regulation of proteins responsible for multiple cellular mechanisms such as structural and functional integrity of neurons.
Jimenez <i>et al.</i> ⁴¹	Peripheral nerve crush	Sciatic nerve proximal and distal of the crush site	Days 5, 10, and 35	1,500	121	NI*	A wide variety of novel regulated proteins involved in different cellular functions related to nerve injury and recovery have been identified.
Katano <i>et al.</i> ⁵⁸	Partial nerve injury	Lumbar spinal nerve, peripheral and central to the DRG	Days 1, 3, and 7	800	12 (P), 3 (C)	NI	Levels of periCRMP-2 decreased after nerve injury, indicating that periCRMP-2 might be related to pathophysiologic changes in the spinal nerve and regeneration processes in the periphery.
Kunz <i>et al.</i> ⁵⁹	Chronic constriction injury	Lumbar spinal cord	Day 14	500	5	≥40%	1 up-regulated and 4 down-regulated protein spots were identified after chronic constriction injury. The mechanisms were distinct from inflammatory pain.

* Proteins are considered as regulated if levels of certain proteins are significantly different from the standard levels ($P < 0.05$).

2D = two-dimensional; (C) = unique in the central fraction; DRG = dorsal root ganglion; NI = not indicated; (P) = unique in the peripheral fraction; periCRMP-2 = peripheral collapsin response mediator protein 2.

focused on creatine kinase B, which was decreased after nerve injury. Because creatine has been found to reduce glutamate levels and to exhibit neuroprotective properties,^{52–54} the authors concluded that the down-regulation of creatine kinase B might be particularly important for the development and maintenance of neuropathic pain and therefore a valuable therapeutic target for neuropathic pain.

After applying the same neuropathy model, another group investigated the differential protein expression in the brainstem.⁵⁵ They found 14 up-regulated and 7 down-regulated proteins (≥30% regulation) 7 days after nerve ligation in comparison with sham-operated rats. Interestingly, none of the proteins that have been found regulated in the spinal cord were also regulated in the brainstem and *vice versa*, indicating that the nociceptive

transmission involves different proteins in the different tissues.

A third study analyzed protein changes in the L4 and L5 dorsal root ganglia after L5 SNL and found 67 regulations (no minimum regulation threshold indicated; proteins are considered as regulated if levels of certain proteins are significantly different from the standard levels [$P < 0.05$]) among approximately 1,300 separated proteins.⁵⁶ Consistent with Lee *et al.*,⁵¹ a down-regulation of creatine kinase B has been observed; however, this was the only conformity between the different SNL studies.

Sciatic Nerve Crush

The protein expression profile of the rat sciatic nerve has been investigated in a model of experimental NC. The analysis involved immediate responses to injury as well as regeneration processes because tissue was dissected at 5, 10, and 35 days after injury. Among approximately 1,500 spots on each gel, at least 121 regulated proteins (no minimum regulation threshold indicated; proteins are considered as regulated if levels of certain proteins are significantly different from the standard levels [$P < 0.05$]) have been found with respect to different time points investigated. A number of these proteins have not been implicated in nerve regeneration previously. Taken together, it was concluded that the detected proteins might reflect the complexity and the temporal aspects of nerve regeneration and, in particular, pronounce the value of glial and inflammatory determinants.⁴¹

Partial Nerve Injury

Katano *et al.*⁵⁸ investigated the polarity of primary afferent fibers in rats with and without partial nerve injury. They reported the unique expression of 12 proteins in the spinal nerves peripheral to the dorsal root ganglia and 3 in the central region. The proteins in the central region included tubulin $\beta 3$ and $\beta 15$, the peripheral proteins collagen $\alpha 1$, α -tubulin, and an isoform of collapsin response mediator 2 (periCRMP-2). The succeeding study was concentrated on characterization of CRMP-2, which was already well known as a regulator of neuronal polarity, axonal growth, and regeneration after nerve injury.^{34,57} Because total CRMP-2 levels remained unchanged after nerve injury and only peripheral CRMP-2 levels decreased, it has been suggested that periCRMP-2 might be related to pathophysiologic changes in the spinal nerves and regeneration processes in the periphery.⁵⁸

Chronic Constriction Injury

Our group⁵⁹ analyzed the protein expression pattern in the lumbar spinal cord of rats after applying the CCI model. Among an average of 500 protein spots on the 2D-gels, we found 5 significantly regulated protein spots ($\geq 40\%$ regulation) 14 days after induction of CCI. The

regulations of proteins in the spinal cord after CCI have been compared with regulations after inflammatory stimulation (zymosan-induced paw inflammation). Only one overlapping regulation could be observed, indicating that inflammatory and neuropathic pain do have distinct regulatory mechanisms.⁵⁹

Functional Classes of the Identified Proteins

Taken together, all these proteomic studies of neuropathic pain delivered a huge number of proteins that might be involved in the pathogenesis of neuropathy. Although the regulation of the bulk of proteins seems to be unique for the respective neuropathy model and tissue, a number of overlapping protein regulations can be observed by direct comparison of the aforementioned neuropathy studies (table 2). Interestingly, some proteins are regulated in more than one model—however, not in the same direction. Based on their physiologic function, the regulated proteins can be roughly subdivided into fundamental categories, such as proteins related to cellular homeostasis and metabolism; neuronal function proteins; heat shock proteins, chaperones, and antioxidants; proteins related to cell cycle, apoptosis, and neurodegeneration; signaling proteins; proteins related to the immune system; and proteins related to protein synthesis and processing. These proteins are mostly involved in neuronal degeneration, regeneration, and inflammatory processes. Some of the proteins have already been related to pain, but a number of regulated proteins have not been previously implicated in this context and may therefore provide interesting new fields of pain research.

Proteins Related to Cellular Homeostasis and Metabolism

A great number of proteins involved in cellular metabolism and homeostasis has been regulated after nerve injury. Because these proteins are expressed in almost every cell and play essential roles for the cell functions, it is not likely that these proteins themselves can be used as drug targets. However, it might be of interest that changes in albumin protein levels have been found in three neuropathy models (SCI, NC, and L5 nerve ligation). Altered albumin expressions in tissues of the central nervous system indicate a dysfunction of the blood-brain or blood-spinal cord barrier, respectively. It has been shown that the integrity of this barrier is disturbed after nerve injury resulting in an increased immunoreactivity of albumin in spinal cord tissue.⁶⁰ Therefore, albumin could be used as a biomarker for certain disturbances of the nervous system.

Neuronal Function Proteins

Most neuronal function proteins are cytoskeleton proteins. The neuronal cytoskeleton is involved in axonal

Table 2. Concurrent Protein Regulations in Different Animal Models of Neuropathy

Animal Model	Regulated Protein	Protein ID No.	pI	MW	Change in Expression	Physiologic Function	References
Proteins related to cellular homeostasis and metabolism							
NC/SCI	Aconitase 2, mitochondrial	40538860	7.9	85.4	↑	Cellular metabolic enzyme	41,46
NC/SCI/SNL	Albumin	19705431	6.1	68.7	↑	Circulating protein; marker of vascular integrity	41,46,56
NC/SNL	Adenine phosphoribosyltransferase	114075	6.3	19.7	↑	Cellular metabolic enzyme	41,55
NC/SNL	Aldolase C	1334163	6.8	39.7	↓	Cellular metabolic enzyme	41,56
NC/SNL	α-1 Macroglobulin	202857	6.5	167.1	↑	Proteinase inhibitor	41,56
NC/SCI	Annexin V	1421099	4.9	35.8	↓/↑	Membrane organization; Ca ²⁺ signaling; antiinflammatory responses	41,46
NC/SCI	Mitochondrial H1-ATP synthase α	40538742	9.2	59.8	↓/only SCI	Cellular metabolic enzyme	41,46
NC/SNL	ATP synthase β subunit	92350	4.9	50.9	↓/NI	Cellular metabolic enzyme	41,56
SNL	Creatine kinase B	417208	5.4	42.9	↓	Cellular metabolic enzyme	51,56
SNL/CCI	Creatine kinase MM (muscle form)	6978661	6.6	43.2	↑/↓	Cellular metabolic enzyme	56,59
NC/SNL	Fibrinogen α chain	71824	6.6	60.6	↑	Cellular homeostasis	41,56
NC/SNL	Lactate dehydrogenase B	6981146	5.7	37	↓	Cellular metabolic enzyme	41,56
NC/SNL	Phosphoglycerate kinase	16757986	7.5	45.1	↓/↑	Cellular metabolic enzyme	41,56
SCI (TS)/NC	Ubiquitin C-terminal hydrolase	92934	5.1	24.7	↓/↑	Cellular metabolic enzyme	31,41
NC/SNL	UDP glucose dehydrogenase	13786146	7.5	54.9	↑	Cellular metabolic enzyme	41,56
Neuronal function proteins							
SCI (TS)/SNL	Fatty acid-binding protein, brain (FABP-B)	P55051	5.4	15.0	↑	Transport of hydrophobic ligands; axonal growth; neuronal differentiation	31,55
SCI (TS)/SCI	Glial fibrillary acidic protein δ	5030428	5.8	48.8	↑	Neuronal cytoskeleton protein	31,46
SCI/SNL	Neurofilament 3, medium	8393823	4.8	95.7	↑/↓	Neuronal cytoskeleton protein	46,56
NC/SNL	Tropomyosin 4, α	6981672	4.7	28.7	↑	Cytoskeleton protein	41,56
SCI (TS)/NC/SNL	Vimentin	860908	4.8	44.7	↑	Cytoskeleton protein	31,41,56
Heat shock proteins, chaperones, and antioxidant proteins							
SCI (TS)/NC/SCI/SNL	Apolipoprotein A-I	113997	5.5	30.1	↑	Lipid recycling (axon regeneration); antioxidant	31,41,46,56
NC/SNL	Apolipoprotein A-IV	114008	5.1	44.4	↑	Lipid recycling (axon regeneration); antioxidant	41,56
NC/SNL	Apolipoprotein E	114041	5.2	35.8	↑	Lipid recycling (axon regeneration); antioxidant	41,56
NC/CCI	α-B-crystallin	117388	6.8	20.1	↓	Small heat shock protein; molecular chaperone; protein stabilization	41,59
SCI (TS)/NC	Endoplasmic reticulum protein 29	16758848	6.2	28.6	↑	Molecular chaperone	31,41
SCI (TS)/NC/SCI/SNL	Heat shock 27 kd protein 1	14010865	6.1	22.8	↑/↓/↓/↑	Protein stabilization	31,41,46,56
Proteins related to cell cycle, apoptosis, and neurodegeneration							
NC/PNI	Collapsin response mediator 2	135126	5.9	62.8	↓	Regulator of neuronal polarity; axonal growth; nerve regeneration	41,58
NC/SNL	Hemopexin	16758014	7.6	51.3	↑	Heme transport; iron recovery; axonal degeneration	41,56
SCI(TS)/NC/CCI	Protein disulfide isomerase	1352384	5.9	57.0	↑/NI/↑	Redox catalyst; molecular chaperone; neuroprotection	31,41,59
SCI (TS)/NC/SCI	Voltage dependent anion channel	13786200M	8.6	30.8	↑	Initiation and propagation of action potentials	31,41,46
Signaling proteins							
NC/SNL	Calmodulin (CaM)	P02593	4.1	16.7	↓	Ca ²⁺ signaling	41,55
Proteins related to the immune system							
SCI (TS)/NC/SNL	Galectin 3	1346429	8.9	25.6	↑	Immunomodulation; phagocytosis	31,41,56
Proteins related to protein synthesis and processing							
NC/SNL	Heterogenous nuclear ribonucleoprotein L	20824058	6.7	60.9	↑	Control of mRNA stability and splicing	41,56

ATP = adenosine triphosphate; CCI = chronic constriction injury; ID = identification; mRNA = messenger RNA; MW = molecular weight; NC = nerve crush; NI = not indicated; pI = isoelectric point; PNI = partial nerve injury; SCI = spinal cord injury; SNL = spinal nerve ligation; TS = complete transection; UDP = uridine diphosphate.

outgrowth and conductivity. Moreover, it plays a pivotal role in signaling from the axon terminals to the cell bodies. This function modulates the intrinsic neuronal capacity for regeneration and repair after nerve injury. The intermediate filament vimentin was up-regulated in

three models of neuropathic pain, indicating its importance in neuropathy. It is described that vimentin is generated in the injured nerve axoplasm by local translation and calpain-mediated cleavage and thus allows for the retrograde transport of the phosphorylated mitogen-

activated protein kinase Erk. The vimentin–Erk complex is suggested to protect Erk from dephosphorylation, and because the interaction is calcium dependent, the signal generated may provide information about both the injury and the degree of damage as reflected by sustained calcium elevation.⁶¹

Heat Shock Proteins, Chaperones, and Antioxidants

The small heat shock protein HSP 27 was regulated after complete spinal cord transection, SCI, NC, and SNL. The heat shock proteins are stress proteins that mediate protein stabilization in various tissues and protect cells from environmental stress. A number of heat shock proteins are up-regulated in the nervous system in response to stress or injury.^{62,63} Novel evidence suggests that overexpression of the small heat shock protein 27 (Hsp27) in neurons protects against neurotoxic stimuli and may act as an inhibitor of neurodegeneration.^{64–66} Surprisingly, two of four proteomic studies in neuropathy revealed a down-regulation of Hsp27 after NC and SCI, respectively. This might suggest that the nerves are irreversibly damaged in these models.

Up-regulation of apolipoproteins in response to nerve injury has already been described.^{67,68} It is suggested that these proteins serve as vehicles to transport lipids between cells during regeneration and degeneration of neurons. A number of apolipoprotein isoforms are regulated in different neuropathic pain models. The isoform A-I was up-regulated in four of the models, indicating a potential role as a biomarker for neuropathic pain.

Proteins Related to Cell Cycle, Apoptosis, and Neurodegeneration

A number of proteins are involved in apoptotic responses which occur frequently after nerve injury in neurons of the peripheral and central nervous system. Apoptosis seems to induce neuronal sensitization and loss of inhibitory systems, and these irreversible processes might be related to the development of neuropathic pain. Prevention of apoptosis might thus suggest future strategies against neuropathic pain.³⁸ Protein disulfide isomerase, a protein that has been related to neuronal apoptosis, was increased after CCI, NC, and SCI. In the nervous system, up-regulation of protein disulfide isomerase has been described as a result of hypoxia and brain ischemia, thus protecting cells from apoptosis.⁶⁹ The protein acts either as a redox catalyst or as a molecular chaperone that prevents protein aggregation and degradation.⁷⁰ Neurodegeneration is often accompanied by the formation of toxic protein aggregates, which subsequently induce apoptosis and neuronal loss.⁷¹ Therefore, up-regulation of protein disulfide isomerase might constitute a protective mechanism against apoptotic cell death induction in neuropathic pain.

An up-regulation of voltage-dependent anion channels has been found by proteomic analysis in three neuropathy models.^{31,41,46} These channels are major constituents of the outer mitochondrial membrane, where they control membrane permeability and the subsequent release of apoptosis promoting factors.⁷² Therefore, in the context of nerve injury, they might be involved in the degradation of neurons.

Proteins Related to the Immune System

Galactin 3 is a galactose-specific lectin that was regulated in three proteomic studies. The protein is involved in neuronal cell adhesion and neurite outgrowth.⁷³ After peripheral nerve injury in rats, an *N*-methyl-D-aspartate-mediated up-regulation of galactin 3 has been observed in subpopulations of dorsal horn neurons.⁷⁴ Because galactin is also involved in myelin phagocytosis and therefore in wallerian degeneration of neurons, it might serve as a trigger for neuronal apoptosis induction after nerve injury.⁷⁵

Current Limitations of Proteomic Research

At the moment, a number of limitations occurring with proteomics in neuroscience still hinder the analysis of the complete protein spectrum. At first, sample preparation of brain or spinal cord delivers a complex and heterogeneous mix of cells, which cannot be distinguished on 2D-gels. Single, defined cells can only be investigated from neuronal cell cultures. Laser microdissection of single cells might yield a new method to analyze small groups of cells from neuronal tissues. Second, low-level regulatory proteins cannot be detected; protein analysis is substrate limited because no amplification methods are available as yet. Similarly, high- and low-molecular-weight proteins as well as hydrophobic membrane proteins are difficult to separate by 2D-gel electrophoresis. Hence, the great advantage of 2D-PAGE is the evaluation of limited protein patterns under physiologic and pathophysiologic conditions, respectively. This functional proteomic analysis can already deliver a substantial volume of data, which might serve as a basis for the development of further research approaches.^{9,76}

Conclusion

The treatment of pathologic pain coming along with neuropathy requires efficient and highly specific drugs. However, patients are often not adequately treated by the currently available drugs. Therefore, it is necessary to gain more insights into the molecular mechanisms of neuropathy and to find proteins as drug targets that are specifically regulated in neuropathic pain.⁷⁷ A number of animal models of neuropathy have been developed for research of neuropathic pain which result in a highly

reproducible and frequent development of allodynia and hyperalgesia. These models differ strongly among each other by reflecting central or peripheral nerve injury as well as different approaches to produce nerve damage. Therefore, it is difficult to judge which model is the best reflection of the "normal" human response to nerve injury, and data of the different models must be interpreted in the context of the specific pain model.

Despite the importance of collecting new data about signaling pathways in pain, only a few studies have been designed to investigate differences in protein patterns in the nervous system after neuropathic pain. However, these studies revealed a large amount of regulated proteins. Depending on the experimental setting, there were huge differences among the protein regulations, which might be due to different neuropathy models or different neuronal tissues. Furthermore, it should be taken into account that the different approaches to investigate the protein pattern are very heterogeneous. The differential sample preparations, various time points for tissue dissection, differing conditions for isoelectric focusing (pH range, separation protocol, and so on) and sodium dodecyl sulfate polyacrylamide gel electrophoresis might also contribute to the wide variety of regulated proteins.

In principle, proteomics can deliver useful information regarding "pain-associated" proteins and may therefore provide a reasonable technique for the identification of regulated proteins in the nervous system after nerve injury.

Therefore, the studies summarized here, together with previous studies that identified single molecular participants in neuropathic pain, may lead to a better understanding of the molecular mechanisms that are involved in these processes. This might help to control the pathophysiological signaling pathways in pain and thus promote the development of new pain therapeutics by using subsequent systematic investigations.

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