axonal regenerative capacity through the manipulation of intrinsic signaling cascades; and better control of axonal guidance molecules.

Further research is required to fully identify the gene expression profile of all the cells involved in the regeneration process following SCI in a time-dependent manner; such knowledge would enable down- or up-regulation of genes or growth substrates in response to the specific needs of the axons undergoing regeneration [151], which would open the possibility of starting a 'regeneration-associated gene program' where these newly discovered genes and their profile expressions can be studied and shared in the scientific community.

Great attention must be given to finding ways to control and regulate gene delivery where single or multiple neurotrophic factors can be administered to the injured axons in time-dependent gradients or alternating cascades in response to the specific needs for axon regeneration. This will eventually lead to resolution of one of the most complex clinical issues still faced by mankind: the implementation of human trials for SCI where multiple ethical concerns, as well as safety considerations, cannot be overlooked. Even after overcoming implementation issues, including which gene therapy to administrate, the optimal location of gene therapy vehicle administration, the correct use of immuno-suppression regimens if required, definition of target patient populations, and sensitivity and accuracy of outcome measures, the difficulty of properly designing significant randomized clinical trials remains hugely challenging. This enormous difficulty is due to the complex nature of SCI in humans with multiple causes and correlated grades of severity, where clinical trials with small number of patients and the lack of appropriate control groups impose severe limitations. Nonetheless, despite the foreseeable challenges faced, most researchers and clinicians in this area will agree that combinatorial gene therapies are the most promising pathway to achieve neuronal regeneration after SCI. The constant advances in the generation of gene-vectors, as well as new advances in protein engineering, scaffold development and cell technologies, will keep providing us with more effective therapeutic strategies in the coming future.

Executive summary

Background

- Gene-based therapies for spinal cord injury (SCI) are aimed at enhancing the repair process through introduction or inhibition of a particular gene or genes, using a vector as transporter. Delivery to the injured spinal cord can be achieved through gene-activated scaffolds or matrices, plasmid-transfection systems, gene-encoded viral vectors and cell-based therapies.

Viral vectors

- Viral vectors are currently the most widely used system for fine-tuned gene delivery to the CNS. They must be modified for safe clinical use and capable of generating minimal host responses, including neoplasia induction due to immunogenicity. The most important viral vectors are currently adenovirus (Ad5), adeno-associated virus 2, lentivirus and herpes simplex virus.

Non-viral vectors

- Non-viral gene vectors provide a safer alternative, improved neuronal targeting, and a higher DNA carrying capacity than viral vectors. Two methods commonly used are direct injection of plasmids containing the transgene (termed 'naked DNA') into a tissue or using cationic lipids to surround the plasmid DNA facilitating its entrance into the cell (so-called lipofection). Nanocarriers improve gene transfection efficiency with lower cytotoxicity; the most promising agents are polyethylenimine and dendrimers.

Cell-based vectors

- Genetically manipulated cells behave as biological mini-pumps, providing both substrates (cell bridges) for axonal regeneration through the targeted delivery of growth/neurotrophic factors into the lesion. Cells commonly used include fibroblasts, stem cells, olfactory ensheathing cells, bone marrow stromal cells, neural stem/progenitor cells and induced pluripotent stem cells cells.

Combinatorial strategies using gene therapy approaches

- Combinatorial strategies are intended to synergize the effects of different therapies in order to overcome the limitations of a single intervention; these have been adopted to overcome physical barriers and axonal growth inhibitors, which are the main obstacles to regeneration after SCI. The silencing of axonal growth-inhibiting molecules through the administration of vector-encoding shRNAs, the promotion of neurogenesis/oligodendrogenesis, and the administration of transcription factor-encoded vectors are promising approaches.

Future perspective

- Combinatorial gene therapies are the most promising pathway to achieve neuronal regeneration after SCI, but the necessity of developing a fully comprehensive medical trial may impose multiple limitations. Ethical as well as host rejection concerns may ultimately be solved through the use of induced pluripotent stem cell-mediated gene delivery.

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• of interest; •• of considerable interest

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MRI 画像における髄内輝度変化経過と臨床成績

中嶋秀明[1)]，内田研造[1)]，竹浦直人[1)]，本定和也[1)]，坂本拓己[1)]，馬場久敏[1)]

MRI は脊髄の形態的変化を捉え，圧迫性頸髄症の評価には必須の検査であるが，MRI T2 強調画像（WI）における髄内輝度変化の臨床的意義や，除圧手術後の変化，治療成績との関連性については未だ一定の見解が得られていない．本研究では，圧迫性頸髄症に対し手術を行った患者で，術前および術後 6 ヵ月以上経過した時点での MRI follow が可能であった症例を対象とし，輝度変化部位局所の signal intensity を定量的に評価し，手術前後の MRI 輝度変化の経過や治療成績との関連性を調査した．

方　　法

1.5T MRI T2-WI 上，髄内輝度変化を認め，手術を行い術後 6 ヵ月以上経過後に MRI を撮影した 30 症例（頸椎症性脊髄症 20 例，頸椎後縦靭帯骨化症 10 例）を対象とした．MRI 輝度変化の定量的評価として，signal intensity ratio（SIR）（輝度変化部位 intensity（0.05cm^2）/C7-T1 高位 intensity（0.3cm^2））を算出した[1)2)]．評価項目として，MRI では術前・術後 T1-WI および T2-WI における SIR とその変化量，臨床症状として罹病期間，術前・術後 JOA スコア，JOA 改善率を調べ，SIR との関連性を統計学的に調査した．また，単因子解析で JOA 改善率と相関のみられた因子を用いて，Stepwise multivariate regression analysis を行った．

結　　果

術前 T1-WI における SIR は JOA 改善率と正の相関を認めたが，術前 T2-WI における SIR とは相関がみられなかった（図 1）．罹病期間が長い症例ほど，T1-WI における SIR や JOA 改善率は低下する傾向がみられたが，統計学的には有意ではなかった（図 2）．Follow up 時の T1-WI における SIR とその変化量は，JOA 改善率と正の相関を示し，特

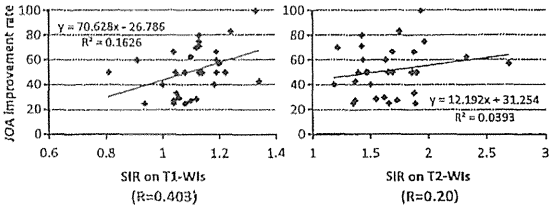

図 1　術前 SIR と JOA 改善率との関連

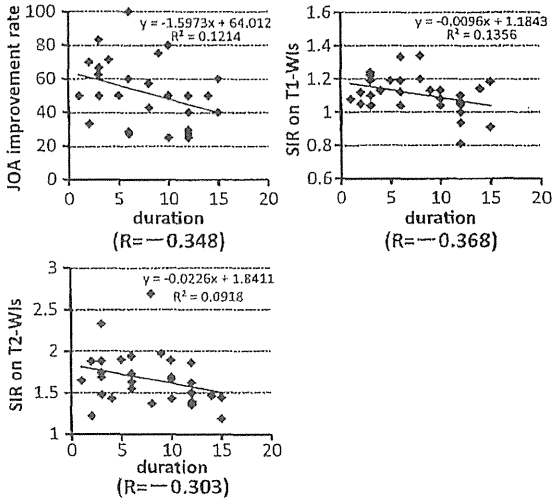

図 2　罹病期間と JOA 改善率・SIR との関連

に術前 1 以上から術後 1 未満に低下した症例は改善率が低かった（図 3，4）．このような症例は 6 例（20%）あったが，これらの平均 JOA 改善率は 37.1% であり，全体の平均 JOA 改善率 51.5% よりも有意に低かった．また，術後 T2-WI における SIR が上昇した症例は改善が低かった．JOA 改善率と相関のみられた因子（術前 T1-WI における SIR，術後 T1-WI における SIR，T1- および T2-WI における手術前後の SIR 変化量）を用いて，Stepwise

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Spinal cord signal intensity changes on MRI images and clinical outcome in patients with compression of cervical myelopathy before and after surgery : Hideaki NAKAJIMA et al. (Department of Orthopaedic and Rehabilitation Medicine, University of Fukui Faculty of Medical Sciences)

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Key words : MRI, Signal change, Compressive cervical myelopathy

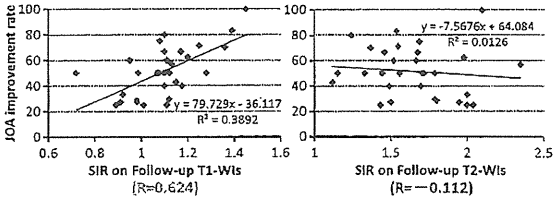

図 3　Follow-up 時の SIR と JOA 改善率との関連

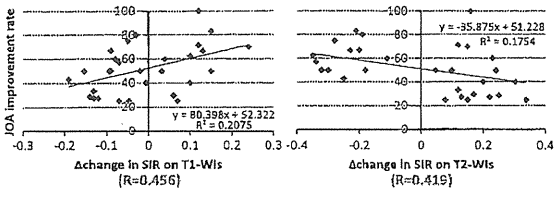

図 4　SIR 変化量と JOA 改善率との関連

表 1　Stepwise multivariate regression analysis

	JOA改善率
	model: (R^2=0.553, p<0.01)
	p value
SIR on T1-WI	0.559
SIR on Follow-up T1-WI	0.0175
Change of SIR on T1-WI	0.00295
Change of SIR on T2-WI	0.00219

multivariate regression analysis を行ったところ，術後 T1-WI における SIR，T1-WI および T2-WI の SIR 変化量は特に相関の高い因子であった（R^2=0.553, p<0.01）（表 1）．

考　　察

MRI の輝度変化の定量的評価を用いて，術前後の変化と治療成績についての関連性を調べた．これまで報告されている MRI での髄内輝度変化の分類は，視覚的情報による分類が多いことに加え，MRI の撮像条件は個々によって異なるという問題点がある．MRI の輝度変化の意義については未だ contraversial な部分が多いが，病理学的には，T2-WI での高輝度変化は，浮腫，虚血，炎症，グリオーシスなどの可逆的な変化を示し，T1-WI での低輝度変化は，壊死や軟化などの不可逆的な変化を示すとの報告がある[3]．本研究結果からは，術前の指標としては T1-WI での低輝度変化が神経学的重篤度と相関する治療上重要な所見であり，術前 T2-WI における高輝度変化の程度は関連性が低いと考えられた．一方で，術後 T1-WI における低輝度変化および T2-WI における高輝度変化が進行する症例，特に術後 T1-WI で低輝度変化がみられる症例では，より改善率が低いと考えられた[1]．

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Chapter 11
Microarray Analysis of Expression of Cell Death-Associated Genes in Spinal Cord Cells with Cyclic Tensile Strain

Kenzo Uchida, Hideaki Nakajima, Takayuki Hirai, Sally Roberts, William E.B. Johnson, and Hisatoshi Baba

Abstract Previous studies have described alterations in gene expression following spinal cord injury, but this response to mechanical stimuli is difficult to investigate in vivo. Therefore, we have investigated the effect of cyclic tensile strain on cultured spinal cord cells from E15 Sprague–Dawley rats. Microarray analysis of gene expression and categorization of identified genes were performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) systems.

The application of cyclic tensile strain reduced the viability of cultured spinal cord cells significantly in a dose- and time-dependent manner. GO analysis identified candidate genes related to "apoptosis" (44) and to "response to stimulus" (17). KEGG analysis identified changes in the expression levels of 12 genes of the mitogen-activated protein kinase (MAPK) signaling pathway, which were confirmed to be upregulated and validated by RT-PCR analysis. Spinal cord cells undergo cell death in response to cyclic tensile strain, which were dose- and time-dependent, with upregulation of various genes, in particular of the MAPK pathway.

Keywords Cultured cell • Cyclic tensile strain • Microarray • Mitogen-activated protein kinase (MAPK) signaling pathway • Spinal cord

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11.1 Introduction

Mechanical stresses applied to the spinal cord can potentially induce profound and irreversible paresis, secondary to induced neuronal cell necrosis and apoptosis [1, 2]. Examples of such mechanically induced spinal cord damage include not only spinal cord compression but also distraction insult [3, 4], where tensile strain forms an important part of the injury mechanism. The primary mechanical event can initiate a cascade of molecular and cellular events such as changes in gene expression levels that are necessary for cell recovery or cell death [5–8].

Such changes have been documented in various in vivo models of spinal cord injury using microarray analysis [9–11], in an attempt to stabilize, both biologically and functionally, the spinal cord once injured [12, 13]. However, these in vivo experimental settings suffer from several disadvantages over in vitro experimentation, such as activation of resident inflammatory cells or invasion of foreign cells from the periphery [14]. The complexity of the in vivo situation limits the access to specific areas of tissue or cell type of interest, preventing real-time and spatial measurement of biological or mechanical parameters [15]. Thus, the importance of in vitro models becomes evident.

The use of neuronal cell culture models allows for better control of the extracellular environment, cell specificity while being relatively easy to manipulate. The Flexercell Strain Unit (FX3000®, Flexercell International, Hillsborough, NC) is a cell-stretching apparatus that allows application of cyclic tensile force to cultured cells [16–18]. In our previous study using this equipment [19], cyclic tensile strain on cultured spinal cord cells increased the expression levels of nerve growth factor, brain-derived neurotrophic factor, trkB, p75 neurotrophin receptor (p75NTR), glial cell line-derived neurotrophic factor, and caspase-9 mRNAs in the acute phase, followed by increased lactate dehydrogenase release and induction of necrotic cell death.

The present study was thus designed to examine further the molecular changes and gene expression profiles in cultured spinal cord cells using the above cell-stretching apparatus and DNA microarray technology.

11.1.1 Cyclic Tensile Stress-Induced Spinal Cord Cell Death

Under the condition of 10 % tensile strain (typically found in spinal cord injuries [19]) at a frequency of 0.5 Hz (Fig. 11.1a, b), the proportion of living green-stained spinal cord cells decreased in a time-dependent manner, whereas that of dead red-stained cells increased simultaneously. Transmission electron microscopy (TEM) examination showed that all cells at 0 h appeared viable, with large nuclei, and dotted with chromatin. When subjected to tensile strain, some cells showed deformity of the nuclei and cytoplasm at 6 h, and chromatin condensation and fragmentation were observed at 24 h; morphological changes indicative of early apoptosis (Fig. 11.1c).

The cell survival rate of cultures under cyclic strain decreased from 83 ± 24 % at 2 h to 40 ± 11 % at 72 h (Fig. 11.2a), becoming significant after 6 h. Figure 11.2b shows that increasing the strain rate independently of the strain level was associated

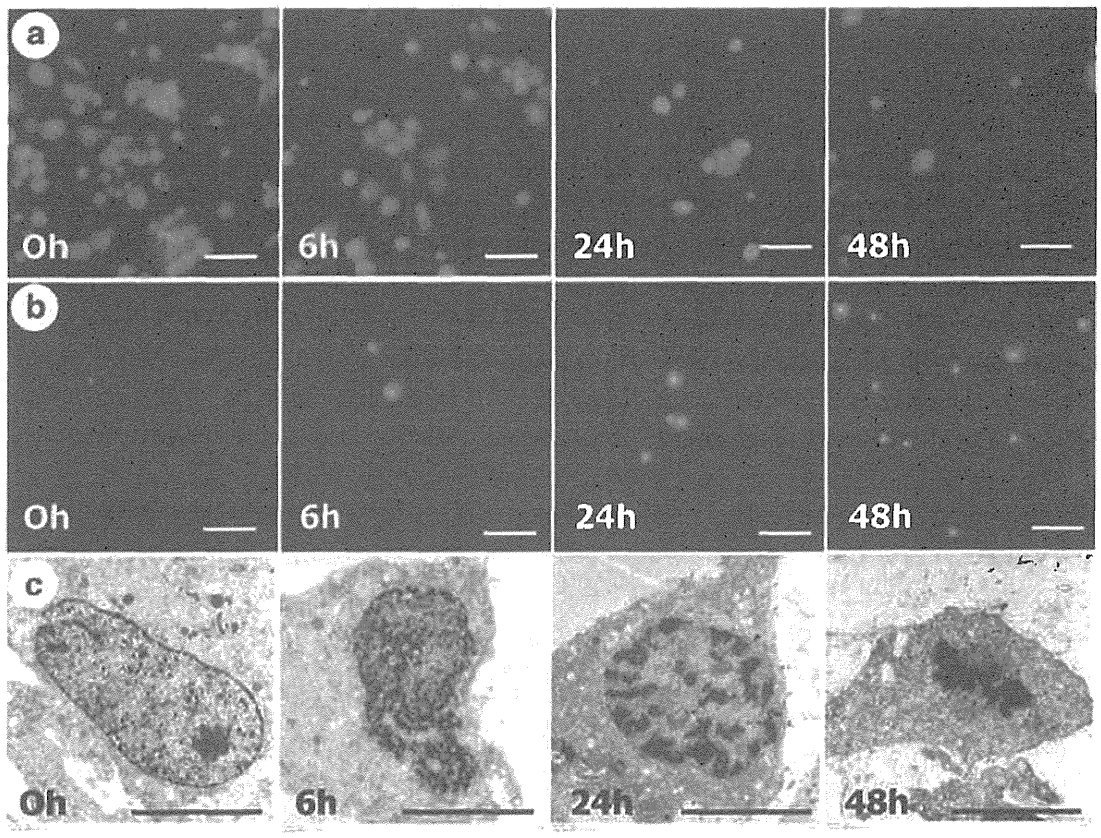

Fig. 11.1 The application of cyclic tensile strain induced apoptotic cell death in spinal cord cells. Representative serial photomicrographs are shown of cells exposed to a tensile strain of 10 % strain at 0.5 Hz at 0, 6, 24, and 48 h (**a**, **b**). The number of *green-stained living cells* decreased (**a**: *top row*) while the number of *red-strained dead cells* increased (**b**: *middle row*) in a time-dependent manner during cyclic tensile strain application. Transmission electron microscopy (TEM) examination (**c**: *bottom row*): all cells at 0 h appeared viable, with large nuclei, and *dotted* with chromatin and abundant rough endoplasmic reticulum; some cells at 6 h showed deformity of nuclei and cytoplasm. TEM at 24 h showed some cells with condensed and fragmented nuclei and condensed chromatin, and the change progressed at 48 h (**c**: *bottom row*). Bar=100 μm (**a**, **b**), 50 μm (**c**) (Reprinted, with permission, from [20])

with increased spinal cord cell death. Conversely, the cell survival rate in cultures subjected to a 15 % strain level at a frequency of 0.5 Hz was also significantly lower than that seen at the standard level. Therefore, increasing the strain level independently of the strain rate was also associated with increased spinal cord cell death.

11.1.2 Cluster Analysis of Gene Expression Profiles

There was altered expression of 3,412 genes after the application of a cyclic tensile strain of 10 % at 0.5 Hz during 72 h, which were profiled using hierarchical cluster analysis in a time course manner and divided into six clusters (Fig. 11.3). Genes of clusters 1, 2, and 3 were upregulated in a time-dependent manner; those of cluster 4 were upregulated in a time-independent manner. Genes of clusters 5 and 6 were downregulated in a time-dependent manner.

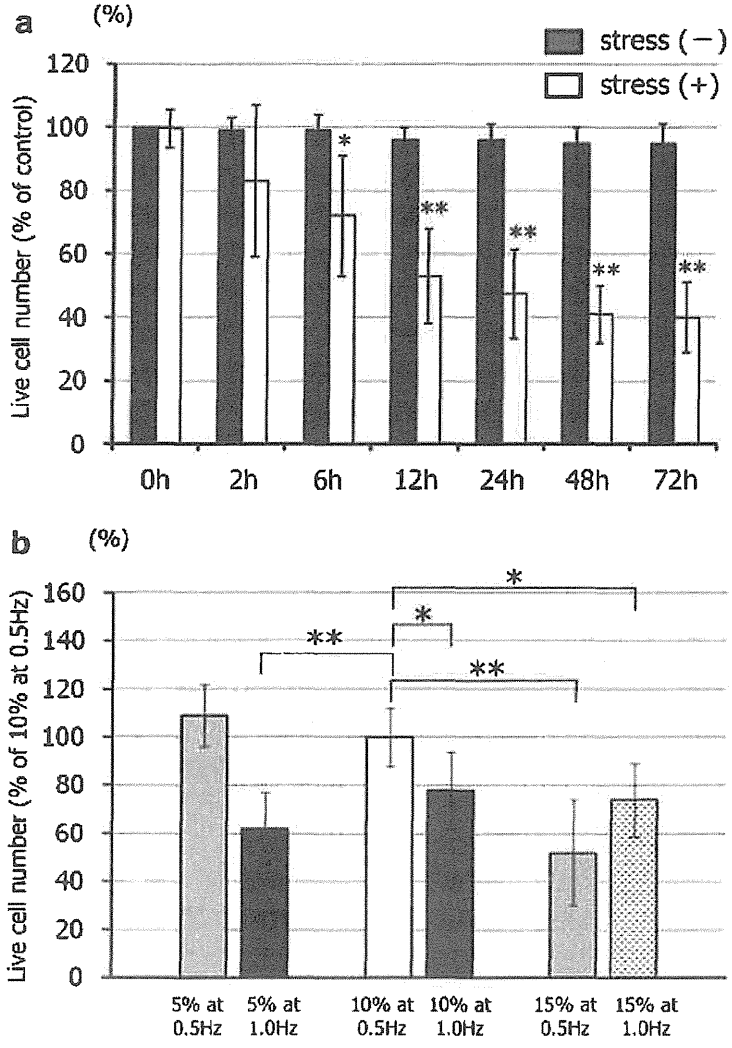

Fig. 11.2 The survival rate of spinal cord cells was dependent on the level, frequency, and duration of the applied tensile strain. The survival rate (%) of living cells during the application of a standardized cyclic tensile strain (10 %, 0.5 Hz) compared with stress-free baseline (**a**). *Gray bar*, stress-free condition; *white bar*, cyclic tensile strain condition. The survival rate (%) of living cells after 6 h at three different strain levels of 5 %, 10 %, and 15 % and two different strain frequencies of 0.5 and 1 Hz compared with that at the standardized strain level of 10 % at 0.5 Hz frequency (**b**). Data are expressed as mean ± SEM of six experiments. $*P<0.05$, $**P<0.01$ (Reprinted, with permission, from [20])

11.1.3 Identification of Upregulated Genes Through Gene Ontology Analysis

Based on the results of clustering analysis, we tested the genes by all GO terms and identified the upregulated genes related to cyclic tensile strain among clusters 1, 2, and 3. Forty-four genes related to "apoptosis" were expressed in the early phase (cluster 3), whereas 17 genes related to "response to stimulus" were expressed in the late phase (cluster 1).

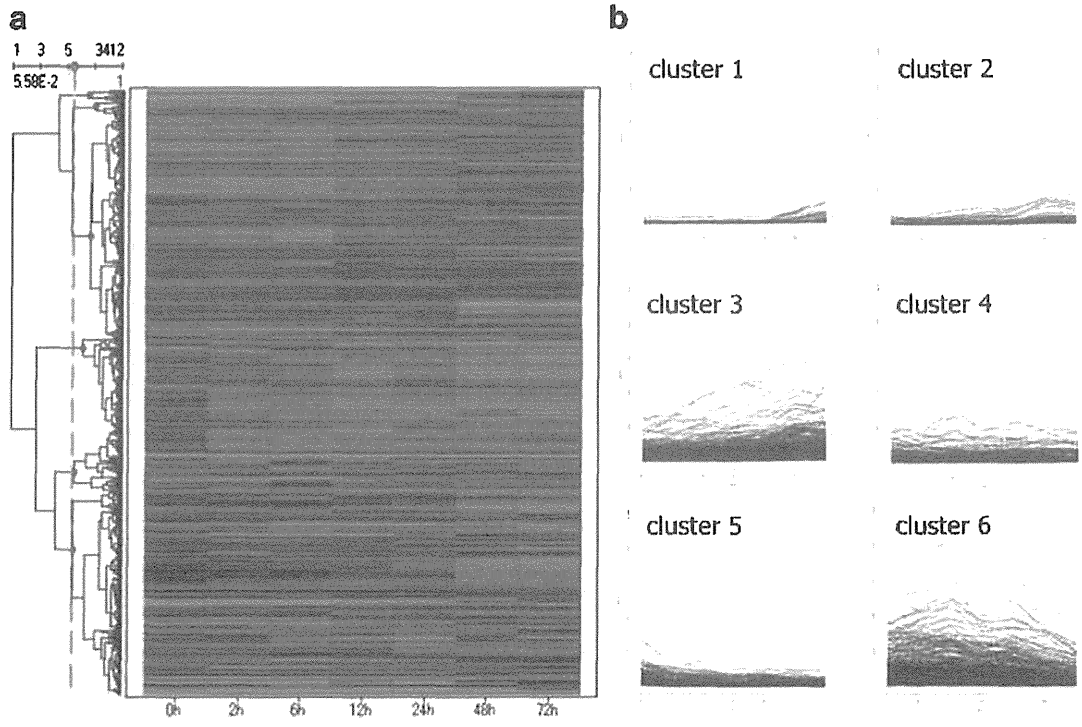

Fig. 11.3 Gene expression profiles in spinal cord cells after the application of cyclic tensile stress. The hierarchical clustering data are presented graphically as a heatmap (**a**). These genes were subsequently grouped into six subclusters (clusters 1–6) according to the time course of gene expression pattern (**b**) (Reprinted, with permission, from [20])

11.1.4 Identification of Upregulated Genes Using Kyoto Encyclopedia of Genes and Genomes Analysis

We tested the genes by all Kyoto Encyclopedia of Genes and Genomes (KEGG) terms in clusters 1, 2, and 3 including those upregulated in a time-dependent manner. Four pathways were significantly included in cluster 1, 3 pathways in cluster 2, and 24 pathways in cluster 3.

11.1.5 Gene-Specific Real-Time Reverse Transcription Polymerase Chain Reaction (Rt-Pcr) in Mapk Signaling Pathway

In further examination of KEGG analysis of cluster 3, which included many of the upregulated genes, we found that the MAPK signaling pathway contained 12 candidate genes among the 15 genes that were significantly upregulated. These genes were calcium channel voltage-dependent L-type alpha-1F subunit (Cacnalf or CACN), neurotrophic tyrosine kinase receptor type 2 (Ntrk2 or trkA/B), fibroblast

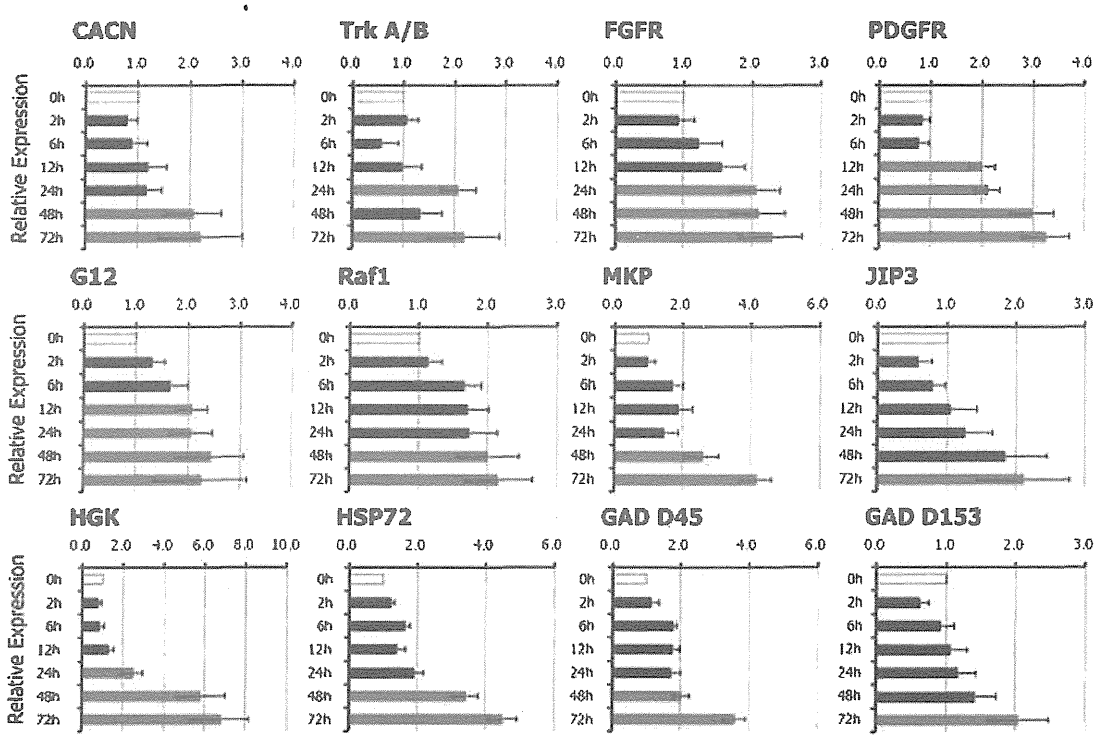

Fig. 11.4 The effects of cyclic tensile stress on gene expression levels analyzed by real-time RT-PCR. Application of cyclic tensile stress resulted in significant increases in mRNA expression levels of platelet-derived growth factor receptor (PDGFR), guanine nucleotide binding protein gamma 12 (G12) at 12 h, neurotrophic tyrosine kinase receptor type 2 (trkA/B), fibroblast growth factor receptor (FGFR), mitogen-activated protein kinase kinase kinase kinase 4 (HGK) at 24 h, and calcium channel voltage-dependent L-type alpha-1F subunit (CACN), v-raf-leukemia viral oncogene 1 (Raf1), dual-specificity phosphatase 1 (MKP), mitogen-activated protein kinase 8 interacting protein 3 (JIP3), heat shock protein 72 (HSP72), DNA-damage-inducible alpha (GAD D45), and DNA-damage-inducible transcript 3 (GAD D153) at 48–72 h. *Red bars* indicate significant differences ($P<0.05$) in upregulation at least twofold in comparison to controls. Data are mean ± SEM of three experiments (Reprinted, with permission, from [20])

growth factor receptor 2 (Fgfr2 or FGFR), platelet-derived growth factor receptor, beta polypeptide (Pdgfrb or PDGFR), v-raf-leukemia viral oncogene 1 (Raf1 or Raf), guanine nucleotide binding protein (G protein) gamma 12 (Gng12 or G12), dual-specificity phosphatase 1 (Dusp1 or MKP), mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4 or HGK), mitogen-activated protein kinase 8 interacting protein 3 (Mapk8ip3 or JIP3), heat shock protein 72 (Hspa 72 or HSP72), growth arrest and DNA-damage-inducible alpha (Gadd45a or GAD D45), and DNA-damage-inducible transcript 3 (Ddit3 or GAD D153). These 12 genes were identified to test differential expression using real-time RT-PCR analysis. The mRNA expression levels of PDGFR and G12 significantly increased from the mid period of application of cyclic tensile stress (12-h stress duration), while CACN, trkA/B, FGFR, Raf1, MKP, HGK, JIP3, HSP72, GAD D45, and GAD D153 mRNA expression levels increased during the late phase of cyclic tensile strain (24–72 h duration) (Fig. 11.4).

11.2 Discussion

In vitro systems offer several advantages over in vivo systems in the analysis of cellular responses to their mechanical environment. By precisely controlling the loading conditions, the quantitative relationship between the severity of mechanical injury and response to the injury can be evaluated [21]. The physiological strain conditions of neuronal cells are not as high as other cells [22], showing ischemic changes at 11 % strain [23], but not at a 6 % strain level [24]. PC12 cells were subjected to cyclic tensile strain levels ranging from 4 to 16 % at strain frequencies of 1–2 Hz as physiological mechanical conditions [22]. Based on these reports and considering primary cultured spinal cord cells [19], we selected the range of cyclic tensile strain most appropriate to our culture system in the present study. Our results suggest that both the level of strain applied and frequency of its application influence cell viability. Furthermore, the results demonstrate that a higher strain level at a lower strain rate can have a similar effect as a lower strain level at a higher strain rate in neuron-rich spinal cord cells.

DNA microarray was used to understand the molecular mechanism of neuronal responses to cyclic tensile strain. Forty-four progressively upregulated genes related to "apoptosis" among cluster 3 and 17 late phase upregulated genes related to "response to stimulus" among cluster 1 were identified using the GO system. Furthermore, the KEGG/pathway analysis identified different pathways in the upregulated genes in a time-dependent manner in the clusters 1, 2, and 3. Indeed, the MAPK kinase pathway was identified by KEGG analysis of the alterations in gene expression seen in the mechanically loaded spinal cord cells. MAPKs are a family of related serine/threonine protein kinases that transduce several signals responsible for cell proliferation or cellular stress and are also intracellular signaling systems that induce optimal stress responses. In KEGG analysis, the MAPK signaling pathway can be classified into three main groups [25]: the classical MAP pathway, the c-Jun N-terminal kinase or stress-activated protein kinase (JNK), the p38 MAPK signaling pathway, and the ERK pathway. In the present study, the mRNA expression levels of PDGFR and G12 significantly increased during the mid period of cyclic tensile strain application, while CACN, Raf1, MKP, JIP3, HSP72, GAD D45, and GAD D153 mRNA levels increased during the late phase of the cyclic tensile strain application. These genes, which were further identified by real-time RT-PCR analysis, may play an important role in the response of spinal cord cells to neuronal injury. Gene expression of trkA/B, FGFR, PDGFR, and Raf1 could facilitate neuronal survival, while gene expression of G12, HSP72, GAD D45, and GAD D153 could be involved in DNA damage.

Considered together with our previous findings [19], we can conclude that certain apoptosis-specific genes are activated in neuronal cell-rich cultures during the application of cyclic tensile stress [20]. The clinical relevance of tensile strain may specifically include the tethering effect with the developmental ascensus medullaris [19], cervical myelopathy in association with kyphotic deformity [4], and complicated spinal cord distraction injury.

11.3 Conclusion

Cultured spinal cord cell death was induced depending on the level and duration of cyclic tensile strain applied with alterations in gene expression and particular upregulation of members of the MAPK pathway.

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Conflict of Interest All authors declare that they have no conflict of interest.

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Chapter 9
Morphological Changes in Anterior Horn Cells, Immunoreactivity to Neurotrophic Factors, and Neuronal Cell Death of Spinal Cord Lesions in the Spinal Hyperostotic Mouse (*twy/twy*) with Chronic Mechanical Cord Compression

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Abstract We examined the morphology of spinal accessory motoneurons, immunoreactivity to brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-3, and reactive astrocytosis in 70 tiptoe-walking Yoshimura (*twy/twy*) mice that develop calcification at C1–C2 vertebral level compressing the spinal cord. At the level of compression, the area of neuronal soma, total length of dendrites, and numbers of wheat germ agglutinin-horseradish peroxidase (WGA-HRP)-labeled accessory motoneurons decreased significantly. Rostral to the compressive lesion, opposite findings were evidenced; enhanced BDNF and NT-3 immunoreactivities were evident in the anterior horn cells, increasing in response to a more severe degree of compression, with larger population of BDNF-positive astrocyte-like cells.

Our results suggest increased functional activity of anterior horn cells at levels rostral to the site of compression. We speculate that the presence of BDNF and NT-3 in neurons and astrocyte-like cells is proportionate to the severity of chronic mechanical compression and may contribute to the heterotropic neuronal reserve and survival. The numbers of TUNEL-positive neurons in the gray matter and oligodendrocytes in the white matter of the spinal cord of the *twy/twy* mouse increased with progressive mechanical compression.

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9.1 Introduction

We have previously documented that a 40–50 % reduction in the transverse area of the cervical cord represents the critical threshold for favorable postoperative neurological recovery [1, 2]. Chronic compression of the spinal cord results in atrophy and loss of anterior horn cells associated with irreversible spinal cord damage [3–5]. Clinically, however, even in cases with severe compression detected on radiological examination, decompression may result in favorable recovery from a profound paresis. This unexpected outcome may be explained by the survival of a group of motoneurons even in the presence of considerable mechanical compression [1, 2].

In order to elucidate the mechanisms of neuronal reserve and survival of anterior horn cells, we used the tiptoe-walking Yoshimura (*twy/twy*) mouse, a model of spinal cord compression [6]. We examined morphological changes in spinal accessory motoneurons, expression of brain-derived neurotropic factor (BDNF) and neurotrophin (NT)-3 [7], and neuronal cell death [8], which have been used as markers of motoneuron survival and neuronal plasticity [9–11].

9.1.1 Topographic Morphology of Wheat Germ Agglutinin-Horseradish Peroxidase-Labeled Spinal Accessory Motoneurons

Representative photomicrographs showing WGA-HRP-labeled accessory motoneurons in the medial cell pool of the ICR and *twy* mice are shown in Fig. 9.1, and results of morphological analysis of these motoneurons are summarized in Table 9.1. At sites A and B of the *twy* mice, the mean area of the neuron soma was significantly larger than in ICR mice ($P<0.05$), where the proportion of the number of WGA-HRP-labeled motoneurons at site C, the compression level, was significantly lower. The total length of dendrites at sites A and B was significantly longer than in the corresponding levels of ICR mice ($P<0.05$).

9.1.2 Three-Dimensional Computerized Display of Spinal Accessory Motoneurons

In the *twy* mice, at site B, the level immediately rostral to the compressive lesion, the elongated dendrites extended rostrally and ventrally as if the motoneuron avoided posterior compression (*arrow*, Fig. 9.2c). At site C dendrites were shorter (Fig. 9.2d)