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Establishment of a mouse model for injury-induced scar formation and the accompanying chronic pain: Comprehensive microarray analysis of molecular expressions in fibrosis and hyperalgesia

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Abstract

Background: Surgery is often accompanied by scar formation, which results in a pathological state called fibrosis. Fibrosis is characterized by the excess deposition of extracellular matrix molecules in the connective tissue, leading to tissue contracture and chronic pain. To understand the molecular mechanisms underlying these processes and their causative relationships, we performed comprehensive analyses of gene expression changes in the hind paw tissue of a mouse model established by generating a scar in the sole.

Results: Subcutaneous tissue was extensively stripped from the sole of the operation group mice, while a needle was inserted in the sole of the sham group mice. Pain threshold, as evaluated by mechanical stimulation with von Frey fiber, decreased rapidly in the operated (ipsilateral) paw and a day later in the nonoperated (contralateral) paw. The reductions were maintained for more than three weeks, suggesting that chronic pain spread to the other tissues via the central nervous system. RNA from the paw and the dorsal root ganglion (L3–L5) tissues were subjected to microarray analyses one and two weeks following the operation. The expressions of a number of genes, especially those coding for extracellular matrix molecules and peripheral perceptive nerve receptors, were altered in the operation group mice paw tissues. The expression of few genes was altered in the dorsal root ganglion tissues; distinct upregulation of some nociceptive genes such as cholecystokinin B receptor was observed. Results of real-time polymerase chain reaction and immune and histochemical staining of some of the gene products confirmed the results of the microarray analysis.

Conclusion: Analyses using a novel mouse model revealed the extensive involvement of extracellular matrix-related genes and peripheral perceptive nerve receptor genes resulting in scar formation with chronic pain. Future bioinformatics analyses will explore the association between these relationships.

Keywords

Mouse model, scar formation, fibrosis, long-lasting pain, gene expression

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Introduction

Epidemiological surveys indicate that most patients suffer from chronic pain, mainly in the lower back, shoulder, and lower extremities.[1] Several etiologies underlie the development and continuation of these chronic pain conditions and surgery (operative intervention) chronic postoperative pain (CPOP) is one of most common complications of surgery. According to previous reports, the incidence of CPOP is anywhere between 5% and 85%, and this kind of chronic pain strongly diminishes the patient's activities of daily life and quality of life.[2] Currently, however, there is no effective cure, reflecting the poor understanding of the pathology of the symptoms.[3]

Surgery in muscles, fingers, shoulders, elbow joints, and peripheral tissues often results in scar formation.[4,5] In this process, a reparative or reactive accumulation of fibrous connective tissue results in the pathological accumulation of extracellular matrix (ECM) proteins. The accumulation is termed fibrosis.[6] A case study highlighted the persistence of fibrosis in the muscle of operated lumbar vertebra (25 years in the cited case).[7] We have observed that in surgery involving locomotive tissues such as fingers, shoulders, and elbows, the occurrence of postoperative scar constraint is often associated with motion pain. Therefore, we hypothesized that postoperative fibrosis can obliterate the architecture and function of the underlying organ and the regeneration of nervous and circulatory systems in them,[8] which contributes to the pathogenesis of chronic pain.

In normal tissues, fibrous connective tissue is defined on a tissue-dependent basis by the characteristic tissue architecture and functions. The tissue consists of a variety of ECM proteins that are characteristically different depending on the tissue type and function. Fibrosis, in a reactive, benign, or pathological state of connective tissue,[6] is described as the process of excess deposition of fibrous connective tissue components[9] and results in scarring and thickening of the affected tissue accompanied by tissue contracture, which interferes with normal organ function.[8] Therefore, it is important to define the pathological accumulation of ECM proteins at the molecular and gene expression levels to understand the mechanisms underlying the pathogenesis of chronic pain in fibrotic tissues and explore potential cures.

Painful scar tissue formations in patients involve neuropeptide-containing nerve fibers. Similarly, regenerated nerve fibers in postoperative scars were reported to be positive for calcitonin gene-related peptide (Cgrp) and substance P in rats.[10] These observations suggest that scar formation and accumulation of neuropeptide-containing nerve fibers are parts of the mechanism of chronic pain.[11]

We previously developed a rat model of chronic pain and scarring by stripping the subcutaneous tissue of the plantar in the hind paw, which caused hypersensitivity to mechanical stimuli that persisted for over 12 weeks.[12] Histologically, the dermis and the epidermis were thickened and contained a large number of collagen fibers with migrating cells having round- or oval-shaped nuclei. In addition, the boundary between the dermis and the subcutaneous area was less defined. In this study, we used the mouse as the animal model of post-surgical scarring because many gene knockout mice models have already been established. The model was used to assess the gene expression changes during injury and scar formation from multidisciplinary viewpoints, which we believe might be important to understand "pain." In particular, comprehensive microarray analyses of genes involved in the ECM and pain-associated molecules were performed.

Materials and methods

Mouse model

Ten-week-old male C57BL/6NCrSlc (C57b) mice were purchased from Japan SLC Co. (Shizuoka, Japan). The mice were given water and food ad libitum and were housed in conditions of constant temperature ($23^{\circ}C \pm 1^{\circ}C$) and humidity ($50\% \pm 15\%$) with a 12-h light and dark cycle. Experimental procedures were approved by the Experimental Animal Committee of Aichi Medical University.

Surgical operation

The surgical operation closely followed the protocol previously described.[12] The sole of the left hind paw of mice was punctured using a 19 G needle, followed by insertion of a steel rod through the hole to reach the toe. The subcutaneous tissue of the entire left sole was stripped to generate an adhesive scar (operation group, $n = 7$). The sole of the left hind paw of mice was punctured without stripping any subcutaneous tissue to generate the control (sham group, $n = 7$). In all mice, the right hind paw was not altered. An outline of this procedure is depicted in Figure 1.

Measurement of changes in body weight and appearance

Body weight changes were measured to ensure that the surgery had no significant effect on the general health of the mice. The visual appearance of the body, such as the condition of the fur, was examined at the same time for the same purpose. All mice were weighed one and two days before the operation, and 2 h; one, two, and three

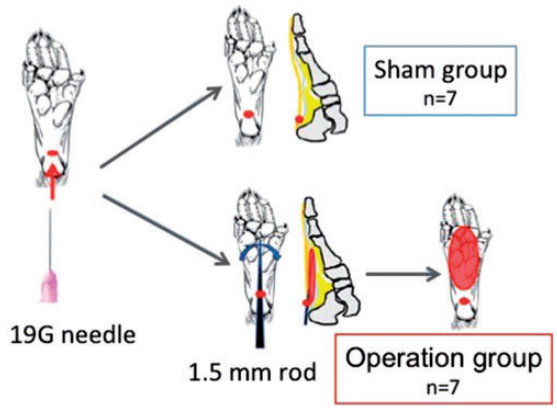

Figure 1. Surgery protocol. Mice were divided into two groups (n = 7 each). In the operation group mice, a pinhole was made in the sole of the left hind paw using a 19G needle and the subcutaneous tissue was stripped through the hole using a steel rod ($\Phi = 3$ mm). In the sham group mice, the pinhole was made in the left heel using a 19G needle. In all mice, the right hind paw was not manipulated.

days; and once a week until 10 weeks after the operation. No significant statistical differences in body weight were observed between the operation and sham group mice. We examined the body appearances at the same time but only in a descriptive manner.

Measurement of hind paw response to mechanical stimuli

Hind paw pain was evaluated by measuring the threshold of mechanical stimuli that induced withdrawal of hind limbs as previously described.[12,13] This method has been frequently implemented and is known as the "up-down method."[14] Mice were allowed to acclimatize on the metal mesh for at least 15 min prior to testing. If necessary, they were gently stroked with a writing brush as a calming action. Mechanical stimuli were then applied to the proximal end of the toes of hind paws using von Frey filaments (0.008, 0.4, 0.6, 1, 1.35, 2, 2.12, 3.14, 4, 5.25, and 8.19 g; Bioseb-In Vivo Research Instruments, Vitrolles, France) from beneath the metal mesh; the lowest stimulus strength was used first and the strength was gradually increased. Enough force was applied in each case to generate a slight bend in the filament. When a filament was able to consecutively induce foot withdrawal or rapid kicking twice, its strength was recorded as the threshold of mechanical stimulus. The test was performed between 10:00 and 13:00 h one and two days before the operation and 2 h, one day, two days, three days, and once a week until 10 weeks after the operation. At each time point, the body weight of each mouse was measured,[14] and any visible difference in body appearance was recorded. Recently, a new mechanosensitivity testing method, termed the simplified up-down (SUDO) method,[15] is being used commonly. In this method, the values of the von Frey filaments are supposed to represent the number of the filament within a complete set of 20 von Frey filaments that span a range of force from 0.008 g to 300 g (Stoelting, Dale Wood, IL, USA). In the original mouse tests, filaments 2 to 9 were used. The test always starts with filament 5 for mice, and the sequence progresses following an up-down sequence where a positive response to a particular filament indicates that the next lower value filament be used in the subsequent test, while a negative response indicates the next higher value filament be used, as in the old method.[14] The test is stopped if a positive response to the lowest possible filament or a negative response to the highest possible filament is observed. For the SUDO method, the paw withdrawal threshold (PWT) estimate was calculated by taking the value of the fifth filament used in each test and adding an adjustment value of ± 0.5 stimulus intervals. The adjustment factor was positive if there was no response to the fifth filament of the sequence to generate a PWT slightly higher than the fifth filament value or negative if there was a withdrawal to generate a PWT slightly lower than the fifth filament value. In some cases, the PWT was converted from filament number to force and was expressed in grams using equation (1)

$$\text{PWT force} = 10^{(X \times F + B)} \tag{1}$$

where F is the calculated PWT value in terms of filament number using either SUDO or the method described by Chaplan et al.[14] X and B are determined from a linear regression of the logarithm of the empirically measured filament bending force plotted against the filament number using equation (2)

$$\text{Log (bending force)} = X \times \text{filament number} + B (X = 0.240\ B = -2.00) \tag{2}$$

Statistical analyses

Statistical analysis of the difference of PWTs within the groups was performed by the nonparametric Freedman's test. When the intergroup difference was determined to be significant, post hoc tests were conducted. The difference of PWTs between the operation and sham groups was determined by the Mann–Whitney test.

Tissue sampling for microarray analysis

Mice were euthanized with an overdose of isoflurane anesthesia (Forane; Abbott, Tokyo, Japan). The dorsal

skin was incised, the muscles surrounding the spine were removed, and the spine was opened with Luer Bone Rongeurs. The dorsal root ganglion (DRG, L2–L5) was promptly excised under a microscope, immediately frozen in liquid nitrogen, and stored at −80°C until use. The scar tissues of the operated left hind paw were also excised, frozen, and preserved in the same manner. Frozen DRG and scar tissues were ground using the Beads Clasher μT-12 (Taitec, Saitama, Japan). The powders were mixed with 1 ml TRIzol (Invitrogen, Carlsbad, CA, USA) to isolate total RNA in separate 1.5 ml microcentrifuge tubes and homogenized using a hand homogenizer. Total RNA was isolated following the manufacturer's instruction and quantified using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All the samples contained >120 ng/μl RNA for the DRG samples and >250 ng/μl for the hind paw tissue samples. RNA purity was evaluated by the ratio of the optical density at 260/280 nm and 260/230 nm, which were >1.8 and >1.9, respectively, for all samples. The values completely satisfied the conditions required for the microarray analysis of gene expression. In certain samples, changes in gene expression were further confirmed by quantitative real-time polymerase chain reaction (PCR) as described below.

Microarray analysis

Microarray analysis was performed by Oncomics Co. (Hangzhou, Japan) using RNA samples from the hind paw and DRG tissues collected from both operation and sham group mice one and two weeks after the operation (Ope-1w, Ope-2w, Sham-1w, and Sham-2w, respectively, for both hind paw and DRG tissues; a total of eight samples were analyzed). The purity and integrity of the samples were first checked using a model 2100 Bioanalyzer (Agilent Technologies, Tokyo, Japan). The complementary DNA (cDNA) was prepared from 100 ng of total RNA using the Low Input Quick Amp Labeling kit and the One Color and RNA Spike-in kit (Agilent Technologies) in which the poly dT primer was conjugated to a T7 promoter. The cDNA was then amplified with T7 RNA polymerase to generate Cy3-labeled RNA, which was purified using the RNeasy mini kit (Qiagen, Germantown, MD, USA) and used as the probe. The SurePrint G3 Mouse GE microarray kit 8 × 60k (Agilent Technologies), which contains approximately 50,000 sequences derived from roughly 25,000 genes, was hybridized with the RNA probes, and the results were analyzed using GeneSpring GX analyzing software (Agilent Technologies). The microarray slide was washed and scanned using a DNA microarray scanner (Agilent Technologies). The scanned data were quantified using the Feature Extraction software (version 11.0.1.1, Agilent Technologies), and the signal intensities were considered to be raw values. Significant gene expression was defined as a raw signal intensity value >50; genes with signal intensities <50 were generally excluded from the analysis, with some exceptions where the gene expressions increased significantly in tissue samples collected after the operation. Signal intensities were normalized as previously described,[16] along with the background signals. The normalized data in samples of the sham and operation groups were compared at the same time points (one and two weeks). The comparison of expression levels among the four different samples (Ope-1w, Ope-2w, Sham-1w, and Sham-2w) was validated after normalizing the expression level of the representative constitutive gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in each sample (Table 1). Upregulated and downregulated genes were defined as an increase of >2-fold (or 1.5-fold in some cases) and a decrease of <0.5-fold in the operation samples as compared with the sham samples, respectively, although some exceptions were considered when the changes in values were not so high but significant in some tissues and genes (e.g., <0.66 or <0.75).

Real-time PCR

Forty-nine genes that displayed a significant change in their expression levels according to the microarray assay, especially those associated with the ECM and pain, were further analyzed by real-time PCR. The primers for real-time PCR were purchased from Takara Bio (Shiga, Japan). Primer sequences used are shown in Supplemental Materials, Table S1. The remaining portions of total RNA (2 μg) were used to generate cDNA with the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Tokyo, Japan). The real-time PCR assay was carried out using SYBR Premix Ex Tag II (Takara Bio) using an ABI Prism 7000 apparatus (Applied Biosystems). The cycling conditions were as follows: denaturation at 95°C for 30 s, 40 cycles of denaturation at 95°C for 5 s, annealing and elongation at 55°C for 30 s, and a final extension at 72°C for 34 s. The mean expression level values determined by comparison to the GAPDH expression level were obtained from three independent PCR runs for each RNA sample (n = 3). The relative expression levels were compared with the expression level of the RNA collected from Sham-1w being designated as 1.

Preparation of tissues for histological survey and immunohistological analysis

One and two weeks after the surgery, three mice from both operation and sham groups were euthanized with

an overdose of isoflurane anesthesia. The left hind paw entire tissues including bones, skin, and tendon, and the ipsilateral (operated) and contralateral (nonoperated) sides of L5 DRG were harvested under microscopic guidance as described above. The tissues were immediately dipped and fixed overnight in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.2. Tissue samples obtained from three different mice in each group were embedded in paraffin (Sakura Finetek Japan, Tokyo, Japan). At least two 5 to 10 µm thick sections from each tissue paraffin block were obtained (n = 6 in each group). Following deparaffinization with alcohol, the sections were incubated in blocking solution (4% Block Ace; DS Pharma Biomedical, Osaka, Japan) for 2 h at room temperature. They were then washed three times in washing buffer (0.4% Block Ace containing 0.1% Tween 20), followed by incubation with rabbit antibody against collagen I, collagen III, substance P, tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), or c-Fos (1:1000 dilution in phosphate-buffered saline (PBS)) for 24 h at 4°C. After washing the sections three times in washing buffer, they were incubated with goat antirabbit IgG antibody conjugated with horseradish peroxidase (1:500; Invitrogen) at room temperature for 2 h. The samples were then washed with PBS three times and were incubated with 0.05% 3′,3-diaminobenzidine and 0.00006% hydrogen peroxide for color development. Finally, they were washed five times in washing buffer and mounted on coverslips. The sections were observed to examine the stained areas under light microscope (Olympus BX51/52, Tokyo, Japan) for the assessment of target molecule staining. When the statistical comparison of stained areas was needed, the immunoreactive areas on the section were examined using Image J software (National Institutes of Health, Bethesda, MD, USA), and the significance was evaluated using the Mann–Whitney test.

Histochemical analysis

Paraffin sections with a thickness of 5 to 10 µm (n = 6 in each group) prepared as described above were deparaffinized and washed with PBS. All the sections were stained with hematoxylin–eosin (HE; Wako Laboratory Chemicals, Wako, Japan) and Sirius-red (Sigma-Aldrich, St. Louis, MO, USA) for histopathological examination of the collagenous scar formation in the left sole. Staining with Safranin O was also performed for the detection of cartilage, mucin, and mast cell granules on formalin-fixed, paraffin-embedded tissue sections, and Toluidine blue staining was used to identify nucleic acids (blue) and polysaccharides (purple).

Results and discussion

Effects of hind paw surgery on mouse appearance and body weight

Mice in both sham and operation groups demonstrate normal growth after the operation, with no significant change in body weight observed between the two groups (Figure 2). The only apparent visual effect is swelling of the left (operated side) hind paws of mice in the operation group, which continues for at least four weeks. As discussed below in the "Histochemical Analysis" section, this is probably due to inflammation and scar formation.

Pain-associated responses to mechanical stimulation following surgery, and comparison between ipsilateral and contralateral hind paws

Pain is evaluated by measuring the PWT of mechanical stimulation with von Frey fibers until 10 weeks after the operation (Figure 3). The mean PWT values were observed to be 4 g before the operation was performed in the ipsilateral paws of both the operation and sham group mice. The values are compared before and after the operation within the same groups (Figure 3(a)) as well as between the two groups (Figure 3(b)) and are found to decrease rapidly after the operation. The PWT values in the operated group mice are maintained at significantly lower levels (1 g) compared with the values in the sham group mice for three weeks ($P < 0.05$). The PWT values in the operation group mice increase and return to a comparable level with the withdrawal threshold values in the sham group mice seven weeks after the operation. Interestingly, in the operation group mice, sensitivity to mechanical stimulation is also detected in the contralateral hind paw one day after sensitivity is detected in the ipsilateral hind paw; three days later, the PWT values of

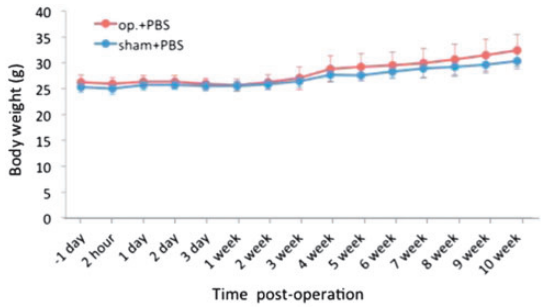

Figure 2. Body weight changes in the mice. Mice in the sham (n = 7) and operation (n = 7) groups were weighed one and two days before the operation, as well as 2 h, one, two, and three days after the treatments; they were also weighed at weeks 1 to 10 after the treatments. No significant differences in weight between the two groups are detected by the Mann–Whitney Test. PBS: phosphate-buffered saline.

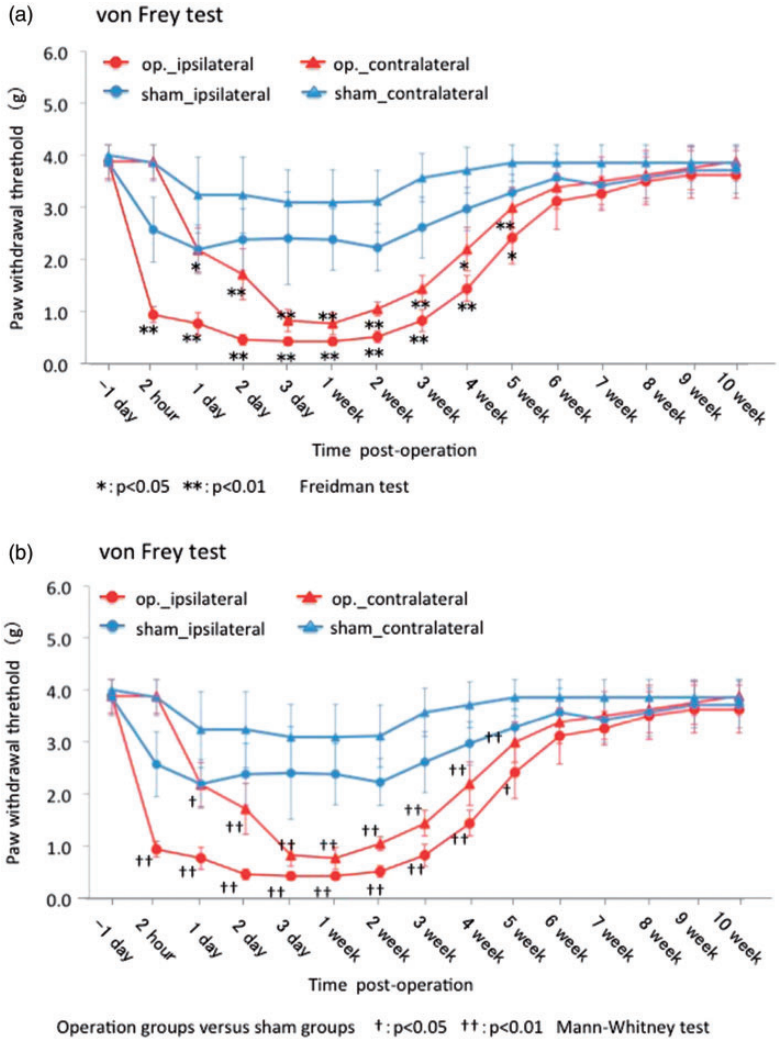

Figure 3. Changes in response to mechanical stimulation. (a) Withdrawal threshold of the hind paw in response to mechanical stimuli was measured by the von Frey test at the time points indicated before and after the operation. Data are presented as mean values. Preoperation and postoperation values in each hind paw are compared between the operation (op) and sham groups at each indicated time point ($^{*}P < 0.05$, $^{**}P < 0.01$). (b) Withdrawal threshold of the treated hind paw is compared with the contralateral side at same time points in both the groups ($^{\dagger}P \leq 0.05$, $^{\dagger\dagger}P < 0.01$).

the contralateral hind paw decrease to values similar to the values of the ipsilateral hind paw and remain comparable for a period of time (Figure 3). These data suggest a possible transmission of pain-associated response signals from the injured left paw to other tissues, possibly through the nervous system of the DRG. The same phenomenon has previously been reported in a rat pain model.[12] Therefore, the DRGs, especially those in L3 to L5 areas, were analyzed for genes involved in the transmission of pain-related response signals.

Histopathological and immunohistological analyses of the injured tissue architecture

Histological analysis shows that the muscles and tendons of the left hind paw in the operation group mice are disordered with a lack of thick bundles and recruitment of fibroblasts and inflammatory cells among the collagen fibers (Figure 4). In addition, the collagen bundles vary in size; fibrous tissues are present under the dermis and the subcutaneous area, and numerous migrating cells with round- or oval-shaped nuclei are observed between the collagen fibers in HE-stained sections (Figure 4(a) to (c)). Furthermore, the tissue sections from the operation group mice exhibit a large number of collagen fibers that ran in random directions in Sirius-red-stained sections (Figure 4(d) to (f)) and show significantly more glycosaminoglycan depositions around fibroblasts in sections stained with Safranin O and Toluidine blue (data not shown) compared with the tissue sections from the sham group mice, suggesting the occurrence of fibrosis in the operation group mice. Immunohistological

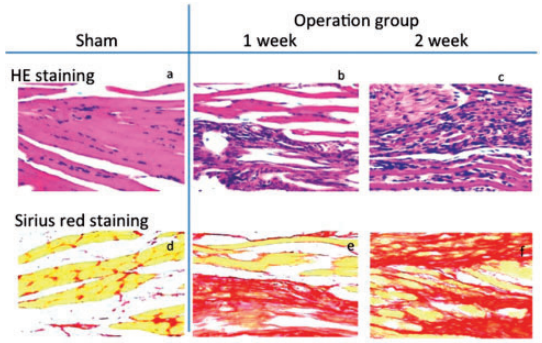

Figure 4. Histopathological comparisons of paw tissues in the operation and sham groups. Paraffin sections (5–10 μm thick) were deparaffinized and washed with phosphate-buffered saline. All serial sections are stained with hematoxylin–eosin and Sirius-red for the histopathological examination of collagenous scars (n = 6 in each group). Staining with Safranin O was also performed for the detection of cartilage, mucin, and mast cell granules in formalin-fixed paraffin-embedded tissue sections. Nucleic acids and polysaccharides are stained blue and purple, respectively, with Toluidine blue. A representative image is shown.

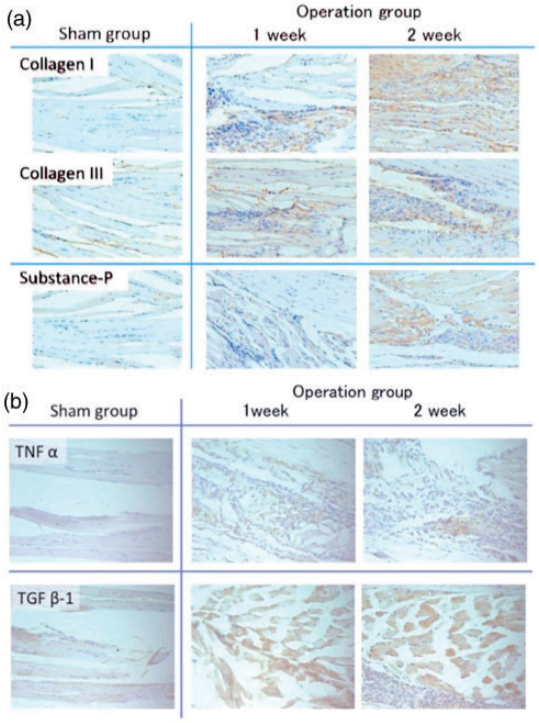

Figure 5. Immunohistological staining characteristics of paw tissues in the operation and sham groups. Two 5 to 10 μm thick tissue sections were obtained from three individuals of each group (n = 6). The sections were stained for (a) fibrotic and pain-associated molecules, which included collagen I, collagen III, and substance P (1:1000 diluted with phosphate-buffered saline (PBS)), and (b) growth and translational factors, which included TNF-α, TGF-β, and c-Fos (1:500 diluted with PBS). The staining patterns and their intensities in the sections from each group are very similar. As the sections consist of a variety of tissues (muscle, bone, tendon, connective tissue, skin, and other components), it is difficult to quantify staining intensities because the intensities are different in different tissues. Therefore, representative images for each group are shown here to illustrate the staining characteristics of the tissue architectures. TGF-β: transforming growth factor-beta; TNF-α: tumor necrosis factor-alpha.

analysis also reveals the characteristics of fibrosis: the abundant accumulation of collagen type I and III fibers in the extracellular spaces in the operation group mice (Figure 5(a)). Furthermore, significant distribution of substance P (Figure 5(a)) and inflammation-stimulating factors (TNF-α and TGF-β1) are also found in samples from the operation group mice (Figure 5(b)). The paw tissue samples were also stained with anti-c-Fos antibody; no statistical difference was detected in the c-Fos immunoreactive neurons between the operation and sham groups (data not shown). Similar histologically different characteristics were observed in all six sections from three mice in each of the one-week and two-week operation and sham groups.

DRG tissues were also analyzed by histopathological and immunohistological staining. No statistical difference was detected in the staining patterns in the ipsilateral and contralateral sides of DRG L5 level in either group (data not shown).

Microarray analyses of gene expression in hind paws and DRGs of sham and operation group mice after surgery

Samples that were extracted from the DRG and paw samples one and two weeks after surgery were examined by microarray analysis. To validate the microarray analysis, we examined the consistency of gene expressions obtained using microarray analysis by comparing the results with the results from real-time PCR examinations. We chose 49 molecules related with ECM, pain, and signaling for the analysis (Supplemental Materials, Table S1) and synthesized PCR primers for real-time PCR (the forward and backward sequences are shown in Supplemental Materials, Table S1). Using the RNA left after microarray analysis, three independent real-time PCR runs (n = 3) were performed for each RNA sample to determine the gene expression levels. The relative expression levels were compared with the expression level of the RNA collected from Sham-1w being designated as 1. Although only 49 genes were analyzed by real-time PCR, the changes in gene expression were similar between the two quantitative methods. Representative results for six genes are shown in Supplemental Materials, Figure S1. Therefore, only results from the microarray analyses are shown and discussed hereafter.

Genes with raw signal intensity values >50 are evaluated and genes with raw signal intensity values <50 are generally excluded, with the exception of the genes whose expression values increase significantly in tissue samples after the operation. Postoperative gene expressions (upregulation or downregulation) are normalized to the expressions in Sham-1w, the raw value of which is designated as 1.000. As summarized in Table 2, 1419 genes and 407 genes in paw samples are upregulated (fold change ≥2.0) one week and two weeks after the operation, respectively. We also found that 386 genes are continuously upregulated (fold change ≥2.0) for two weeks after the operation. In addition, 1299 genes and 461 genes are downregulated (fold change ≤0.5) one week and two weeks after the operation, respectively, and 219 genes are continuously downregulated (fold change ≤0.5) for two weeks after the operation. In contrast, in the DRG samples, the number of upregulated or downregulated genes is much smaller compared with the number of upregulated or downregulated genes in the paw samples; 104 and 94 genes are upregulated (fold change ≥2.0) one week and two weeks after the operation, respectively, and only 6 genes are continuously upregulated for two weeks after the operation. In addition, 99 genes and 213 genes are downregulated (fold change ≤0.5) one week and two weeks after the operation, respectively, and 82 genes are continuously downregulated for two weeks after the operation. Heatmaps of the upregulated and downregulated genes are constructed to obtain an overall view of gene expression changes using normalized values of each sample with Treeview software (Figure 6). The involved genes in DRG samples were distinct from the involved genes in paw samples.

We investigated details of the expression level changes in genes involved in scar formation and the accompanying chronic pain. Extensive literature searches were done to identify molecules directly related to tissue fibrosis and chronic pain and potential molecules involved in signaling pathways and regulation of their gene expressions. Based on this information, we chose a number of upregulated and downregulated genes for detailed discussion (see Tables 3 to 11).

Table 1. Comparison of expression level of the glyceraldehyde-3-phosphate dehydrogenase constitutive gene among the four samples.

Used primer sequence: GAGCCTAGGGAGCCCTACCTACTC TCTTGAATACCATCAATAAAGTTCGCTGCACCCACA	
Paw samples	
Ope-1w versus Sham-1w: 1.098[a]	
Sham-2w versus Sham-1w: 1.114	
Ope-2w versus Sham-2w: 1.046	
DRG samples	
Ope-1w versus Sham-1w: 1.089	
Sham-2w versus Sham-1w: 1.022	
Ope-2w versus Sham-2w: 1.007	

Note: w: week; Ope: operation; DRG: dorsal root ganglion.
[a]Fold change of expression level in each sample was calculated relative to the sham expression level at the same time of 1.000.

Expression of genes encoding ECM and other related proteins

The expression of genes encoding ECM and other associated proteins differ significantly between the paw (Table 3) and DRG samples (Table 6). Molecules expressed at significant levels (>50 in raw values) in both paw (30%, 14/47) and DRG (48%, 32/66) samples are specific to each tissue. Interestingly, molecules commonly expressed in both tissues, such as decorin, lumican, collagen type I alpha 1, and fibronectin, show high expressions with raw values >5000, which may correspond to the level of intrinsic and predominant ECM proteins in both types of tissues. In the paw, upregulation (fold change ≥2.0) of 23 ECM-related genes is detected continuously for two weeks, that of 18 genes is detected one week, and that of 1 gene is detected two weeks after the operation. On the other hand, none of the genes show reduced expressions (fold change <0.66) one week and two weeks after the operation. Notably, expression of *Tnn*, which encodes Tenascin N protein and is weakly expressed only in the paw samples, is increased significantly continuously for two weeks after the operation. The elevated expression of genes for ECM proteins and the enhanced staining and irregular arrangements of ECM components (Figure 5, Table 3) support the suggestion that molecular processes underlying tissue fibrosis initiate at an early time point within one week after the operation. In the DRG samples, genes encoding many neuronal ECM proteins, such as brevican, neurocan, agrin, haplans, laminin, and fibulin, are significantly expressed (raw values >50), while expression of these genes is very low in the paw samples. In the DRG samples, genes encoding some ECM-modified proteins (enzymes), including genes for matrix metalloproteinase 2, 8, 15, and 17 (*Mmp2*, *8*, *15*, and *17*), and the tissue inhibitor of metalloproteinase 3 gene (*Timp3*) are significantly expressed (raw values >50). Interestingly, paw samples exhibit specific expression of *Mmp3* and *14*. In addition, both *Timp1* and *2* genes are highly expressed in both paw and DRG samples, and their expressions are significantly increased in the paw samples one week after the operation. In the DRG samples, only the expression of thrombospondin 2 gene (*Tsp2*) increases more than 1.5-fold continuously for two weeks after the operation. In addition, expression of three genes encoding aggrecan (*Acan*), syndecan 1

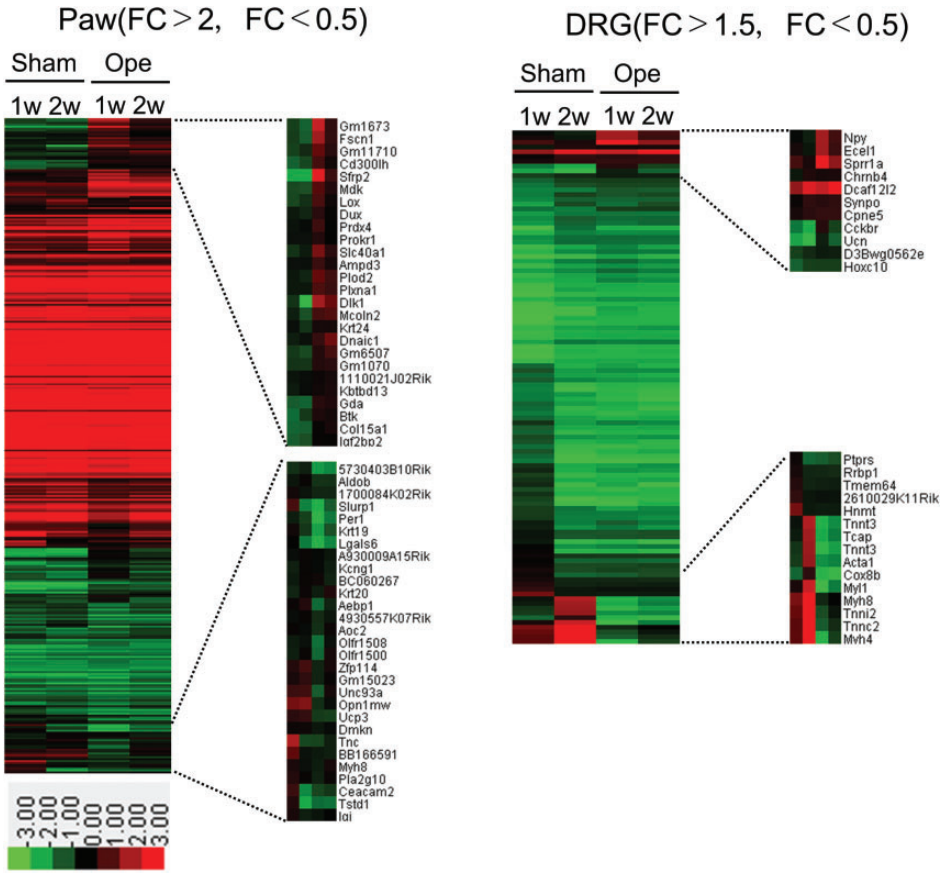

Figure 6. Gene expression profiles in the paw and/or DRG after the operation. Heatmaps of the relative expression of upregulated and downregulated genes in the paw (left) and the DRG (right) in the operation and sham groups. Red indicates upregulated gene expression, and green indicates downregulated expression. The corresponding gene names are mentioned in the right side of the heatmaps. DRG: dorsal root ganglion; FC: fold change.

Table 2. Number of genes that the expression levels were changed one week, two weeks, and one to two weeks after the operation, respectively, in the foot paw samples and in the DRG samples.

Changes of expression level[a]	Weeks after operation	Number of genes detected	
		Foot	DRG
≥2-fold	1 week	1419	104
	2 weeks	407	94
	1 week and 2 weeks	386	6
≤0.5-fold	1 week	1299	99
	2 weeks	461	213
	1 week and 2 weeks	219	82

Note: DRG: dorsal root ganglion.
[a]Fold change of the expression level in each sample was calculated relative to a sham expression level at the same time of 1.000.

(*Sdc1*), and tissue inhibitor of metalloproteinase 1 (*Timp1*) increases one week after the operation. The increased expressions are not evident two weeks after the operation. On the other hand, expression of four genes encoding inter-α-trypsin inhibitor heavy chain 2 (*Itih2*), *Mmp8*, *Mmp9*, and *Mmp13* decreases <0.75-fold continuously for two weeks after the operation. There is no decrease in the expression of any of the genes encoding cell surface receptors for ECM molecules one week after the operation; however, the expression of one gene encoding integrin *β*3 decreases after two weeks (Table 6). Expression of *Timp1* and *2* gene is higher than the expression of other Timp family genes in both paw and DRG samples, and their upregulation is detected only in the paw samples one week after the operation (Tables 3 and 6).

We also examined the expressions of genes encoding growth factors, cytokines, and their receptors (Tables 4 and 7) because these molecules are closely associated with the upregulated and downregulated expressions of the genes encoding the aforementioned ECM molecules. Upregulated expression of inflammation-related genes including bone morphogenetic protein 1 (*Bmp1*), TNF (ligand) superfamily member 15 (*Tnfsf15*), TNF-α-induced protein 6 (*Tnfaip6*), insulin-like growth factor 1 (*Igf1*), platelet-derived growth factor

Table 3. Selected ECM-related genes in the paw-ECM molecules.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.257557	Adamts9	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 9	1.000	1.957	0.913	2.024	96.519
Mm.71963	Adamts19	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 19	1.000	2.762	0.930	2.530	44.570
Mm.65867	Adamts15	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 15	1.000	2.817	0.700	2.974	370.324
Mm.358571	Acan	Aggrecan	1.000	6.102	1.347	3.293	18.358
Mm.423621	Cd44	CD44 antigen	1.000	4.233	0.568	1.680	1390.600
Mm.1571	Cdh11	Cadherin 11	1.000	3.417	1.623	2.117	74.528
Mm.277735	Col1a1	Collagen, type I, alpha 1	1.000	2.856	0.663	1.748	32,888.895
Mm.7281	Col5a1	Collagen, type V, alpha 1	1.000	4.280	0.681	1.737	2729.255
Mm.249555	Col3a1	Collagen, type III, alpha 1	1.000	2.513	0.703	2.362	57,473.367
Mm.233547	Col15a1	Collagen, type XV, alpha 1	1.000	2.416	1.058	2.825	126.905
Mm.56769	Dcn	Decorin	1.000	0.922	1.331	1.449	141,758.830
Mm.2608	Bgn	Biglycan	1.000	2.622	0.902	1.167	974.425
Mm.18888	Lum		1.000	2.268	1.101	2.096	12,542.905
Mm.338790	Srgn	Serglycin	1.000	2.121	1.142	1.523	1186.320
Mm.2580	Sdc1	Syndecan 1	1.000	3.899	0.820	1.403	181.435
Mm.158700	Vcan	Versican	1.000	2.219	0.777	1.595	461.186
Mm.255701	Has1	Hyaluronan synthase1	1.000	2.190	0.856	1.416	125.691
Mm.5148	Has2	Hyaluronan synthase 2	1.000	1.133	1.279	1.482	198.298
Mm.87150	Itgb3	Integrin beta 3	1.000	2.970	0.735	1.416	654.601
Mm.482186	Itga1	Integrin alpha 1	1.000	2.632	0.654	4.163	424.992
Mm.217000	Itgb8	Integrin beta 8	1.000	3.437	1.164	9.239	50.116
Mm.172	Lox	Lysyl oxidase	1.000	3.448	1.189	2.032	162.738
Mm.172674	Lamb1	Laminin B1	1.000	1.819	0.749	1.533	5641.006
Mm.3900	Ltbp2	Latent transforming growth factor-beta binding protein 2	1.000	6.487	0.567	1.577	917.148
Mm.193099	Fn1	Fibronectin 1	1.000	3.222	0.725	1.896	34,658.168
Mm.249146	Fbln2	Fibulin 2	1.000	1.871	1.006	1.527	10,101.423
Mm.4993	Mmp3	Matrix metallopeptidase 3	1.000	1.669	2.057	3.880	374.823
Mm.4561	Mmp11	Matrix metallopeptidase 11	1.000	1.738	1.177	1.626	722.731
Mm.280175	Mmp14	Matrix metallopeptidase 14 (membrane-inserted)	1.000	3.419	0.657	1.842	356.224
Mm.8245	Timp1	Tissue inhibitor of metalloproteinase 1	1.000	4.368	0.471	1.038	4433.954
Mm.206505	Timp2	Tissue inhibitor of metalloproteinase 2	1.000	1.949	1.261	1.196	2497.030
Mm.46221	Tnmd	Tenomodulin	1.000	1.715	1.220	1.538	531.432
Mm.26688	Thbs2	Thrombospondin 2	1.000	3.498	1.031	1.826	1090.658
Mm.2114	Thbs3	Thrombospondin 3	1.000	1.995	0.918	1.601	7962.698
Mm.20865	Thbs4	Thrombospondin 4	1.000	1.536	1.130	1.337	29,962.049
Mm.90140	Tnn	Tenascin N	1.000	34.325	0.910	3.045	12.036
Mm.454219	Tnc	Tenascin C	1.000	11.232	0.811	2.423	31.712
Mm.454219	Tnc	Tenascin C	1.000	7.765	0.622	2.176	127.965

Note: Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased more than 1.50-fold only two weeks after the operation: . Expression increased less than 0.66-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . w: week; Ope: operation.

Table 4. Selected ECM-related genes in the paw—growth factors, chemokines, other molecules in ECM, and their receptors and signaling molecules.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (sham 1w)
Mm.27757	Bmp1	Bone morphogenetic protein 1	1.000	4.375	0.791	1.969	767.535
Mm.271745	Nrp1	Neuropilin 1	1.000	1.118	0.790	0.544	160.368
Mm.248380	Tgfb1	Transforming growth factor, beta 1	1.000	1.623	1.528	0.671	235.068
Mm.18213	Tgfb2	Transforming growth factor, beta 2	1.000	0.485	0.888	0.948	2429.841
Mm.3992	Tgfb3	Transforming growth factor, beta 3	1.000	1.772	1.237	1.068	3597.482
Mm.172346	Tgfbr2	Transforming growth factor, beta receptor II	1.000	1.540	1.043	1.247	3279.329
Mm.14455	Tgfbi	Transforming growth factor, beta induced	1.000	1.552	1.048	1.182	11,147.733
Mm.3248	Tgfb1i1	Transforming growth factor-beta 1-induced transcript 1	1.000	2.645	1.082	1.491	651.339
Mm.7320	Smad3	MAD homolog 3 (*Drosophila*)	1.000	0.550	0.547	0.568	1329.652
Mm.100399	Smad4	MAD homolog 4 (*Drosophila*)	1.000	0.904	1.207	1.040	3044.519
Mm.208152	Tnfsf15	Tumor necrosis factor (ligand) superfamily, member 15	1.000	1.916	1.335	2.693	32.910
Mm.41171	Tnfsf9	Tumor necrosis factor (ligand) superfamily, member 9	1.000	1.491	1.212	1.712	103.330
Mm.1258	Tnfrsf1a	Tumor necrosis factor receptor superfamily, member 1a	1.000	1.717	1.206	1.173	3218.103
Mm.235328	Tnfrsf1b	Tumor necrosis factor receptor superfamily, member 1b	1.000	1.955	0.521	1.175	184.165
Mm.28518	Tnfrsf12a	Tumor necrosis factor receptor superfamily, member 12a	1.000	1.066	1.553	2.055	6715.547
Mm.265915	Tnfrsf13b	Tumor necrosis factor receptor superfamily, member 13b	1.000	2.284	0.787	1.385	212.595
Mm.281356	Tnfrsf19	Tumor necrosis factor receptor superfamily, member 19	1.000	0.369	0.754	0.663	1816.735
Mm.290780	Tnfrsf23	Tumor necrosis factor receptor superfamily, member 23	1.000	3.464	0.449	0.735	361.337
Mm.386774	Tnfaip1	Tumor necrosis factor, alpha-induced protein 1 (endothelial)	1.000	1.069	1.089	1.081	5930.855
Mm.255332	Tnfaip2	Tumor necrosis factor, alpha-induced protein 2	1.000	1.800	1.010	1.430	598.973
Mm.3509	Tnfaip6	Tumor necrosis factor-alpha-induced protein 6 (TSG-6)	1.000	4.506	0.951	2.971	347.194
Mm.268521	Igf1	Insulin-like growth factor 1	1.000	2.802	0.988	1.742	1442.476
Mm.275742	Igf1r	Insulin-like growth factor 1 receptor	1.000	0.874	0.838	0.988	1098.206
Mm.2675	Pdgfa	Platelet-derived growth factor, alpha	1.000	0.714	1.003	0.845	7725.119
Mm.4146	Pdgfrb	Platelet-derived growth factor receptor, beta polypeptide	1.000	1.757	1.081	1.771	1541.038
Mm.877	Cxcl10	Chemokine (C–X–C motif) ligand 10	1.000	0.901	0.267	0.404	282.380
Mm.131723	Cxcl11	Chemokine (C–X–C motif) ligand 11	1.000	1.039	0.996	1.228	219,852.690
Mm.303231	Cxcl12	Chemokine (C–X–C motif) ligand 12	1.000	2.912	1.166	2.302	1035.064
Mm.30211	Cxcl14	Chemokine (C–X–C motif) ligand 14	1.000	0.796	1.539	1.215	6983.855
Mm.425692	Cxcl16	Chemokine (C–X–C motif) ligand 16	1.000	1.500	0.347	0.778	331.724
Mm.337035	Cxcr1	Chemokine (C–X–C motif) receptor 1	1.000	1.013	1.024	1.295	353.875
Mm.12876	Cxcr3	Chemokine (C–X–C motif) receptor 3	1.000	3.162	0.584	1.404	38.475
Mm.1401	Cxcr4	Chemokine (C–X–C motif) receptor 4	1.000	1.535	0.421	0.755	38.702
Mm.6522	Cxcr7	Chemokine (C–X–C motif) receptor 7	1.000	1.185	1.013	1.111	2275.335
Mm.290320	Ccl2	Chemokine (C–C motif) ligand 2	1.000	4.718	1.491	2.004	282.129
Mm.1282	Ccl3	Chemokine (C–C motif) ligand 3	1.000	5.460	0.316	1.744	48.880
Mm.244263	Ccl4	Chemokine (C–C motif) ligand 4	1.000	10.210	1.202	4.799	19.851
Mm.284248	Ccl5	Chemokine (C–C motif) ligand 5	1.000	1.910	0.227	0.645	309.851
Mm.137	Ccl6	Chemokine (C–C motif) ligand 6	1.000	3.776	0.941	2.103	2549.211
Mm.341574	Ccl7	Chemokine (C–C motif) ligand 7	1.000	5.718	0.918	3.053	148.973
Mm.42029	Ccl8	Chemokine (C–C motif) ligand 8	1.000	7.473	0.929	4.375	687.353
Mm.416125	Ccl9	Chemokine (C–C motif) ligand 9	1.000	2.421	1.003	1.497	1884.263
Mm.4686	Ccl11	Chemokine (C–C motif) ligand 11	1.000	0.499	1.866	1.141	1918.918
Mm.41988	Ccl17	Chemokine (C–C motif) ligand 17	1.000	0.634	1.138	1.013	205.774
Mm.31505	Ccl24	Chemokine (C–C motif) ligand 24	1.000	1.330	1.050	1.529	341.442
Mm.425176	Ccl27a	Chemokine (C–C motif) ligand 27A	1.000	0.650	1.368	1.059	1266.784
Mm.274927	Ccr1	Chemokine (C–C motif) receptor 1	1.000	0.565	0.579	0.717	14,643.228
Mm.6272	Ccr2	Chemokine (C–C motif) receptor 2	1.000	1.261	0.541	0.842	371.863
Mm.8007	Ccr6	Chemokine (C–C motif) receptor 6	1.000	1.306	1.677	1.926	15,443.569
Mm.222830	Il1b	Interleukin 1 beta	1.000	1.542	0.296	0.592	18.554
Mm.1019	Il6	Interleukin 6	1.000	1.077	0.610	0.561	41.772
Mm.35814	Il11	Interleukin 11	1.000	2.373	0.819	0.870	508.559
Mm.10137	Il16	Interleukin 16	1.000	1.130	0.959	0.953	328.685
Mm.222808	Il17c	Interleukin 17C	1.000	1.068	1.099	1.350	43,705.300
Mm.390726	Il17d	Interleukin 17D	1.000	0.953	1.338	1.204	2996.427
Mm.131480	Il19	Interleukin 19	1.000	0.952	1.957	1.212	1625.052
Mm.103794	Il20	Interleukin 20	1.000	0.499	0.715	0.640	89.625
Mm.77697	Il34	Interleukin 34	1.000	1.694	0.823	1.330	4521.061
Mm.2565	Il4i1	Interleukin 4-induced 1	1.000	2.416	0.873	1.225	103.005
Mm.896	Il1r1	Interleukin 1 receptor, type I	1.000	1.858	1.177	1.376	363.214
Mm.289824	Il1rl1	Interleukin 1 receptor-like 1	1.000	1.347	1.085	1.441	195.029

(continued)

Table 4. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (sham 1w)
Mm.253424	Il1rap	Interleukin 1 receptor accessory protein	1.000	0.491	0.498	1.518	40.000
Mm.426143	Il1rapl1	Interleukin 1 receptor accessory protein-like 1	1.000	1.051	1.046	1.285	618.773
Mm.915	Il2ra	Interleukin 2 receptor, alpha chain	1.000	0.756	0.777	0.676	2086.175
Mm.35287	Il2rb	Interleukin 2 receptor, beta chain	1.000	3.135	0.455	0.852	188.629
Mm.2923	Il2rg	Interleukin 2 receptor, gamma chain	1.000	4.880	0.293	0.465	157.499
Mm.425857	Il3ra	Interleukin 3 receptor, alpha chain	1.000	2.234	0.789	1.060	1443.059
Mm.2856	Il6ra	Interleukin 6 receptor, alpha	1.000	1.183	0.779	1.087	2950.859
Mm.4364	Il6st	Interleukin 6 signal transducer	1.000	0.894	1.099	0.815	2719.444
Mm.389	Il7r	Interleukin 7 receptor	1.000	2.111	0.105	0.971	1352.508
Mm.379327	Il10ra	Interleukin 10 receptor, alpha	1.000	1.604	0.516	0.923	122.370
Mm.4154	Il10rb	Interleukin 10 receptor, beta	1.000	1.912	0.822	1.216	302.925
Mm.368330	Il13ra2	Interleukin 13 receptor, alpha 2	1.000	1.259	1.609	1.596	20,876.816
Mm.253664	Il18r1	Interleukin 18 receptor 1	1.000	1.294	0.941	2.088	577.273
Mm.155643	Il21r	Interleukin 21 receptor	1.000	2.252	0.411	0.928	1570.504
Mm.38386	Il27ra	Interleukin 27 receptor, alpha	1.000	1.793	1.264	1.294	340.354

Note: Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased more than 1.50-fold only two weeks after the operation: . Expression increased less than 0.66-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . w: week; Ope: operation; MAD: mothers against decapentaplegic; TSG-6, TNF-stimulated gene 6.

Table 5. Selected ECM-related genes in the paw—changes in glycosyltransferases and sulfotransferases for the synthesis of glycosaminoglycans and sugar chains.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.100638	Xylt2	Xylosyltransferase II	1.000	1.710	0.941	1.330	1053.633
Mm.139825	B4galt7	Xylosylprotein beta1,4-galactosyltransferase, polypeptide 7 (galactosyltransferase I)	1.000	1.512	0.904	1.050	1047.007
Mm.393827	B4galt6	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6	1.000	1.548	0.682	1.353	135.098
Mm.11132	B3galt4	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 4	1.000	1.701	1.315	0.939	196.260
Mm.334569	Csgalnact1	Chondroitin sulfate N-acetylgalactosaminyltransferase 1	1.000	4.262	0.860	1.984	393.823
Mm.300317	Csgalnact2	Chondroitin sulfate N-acetylgalactosaminyltransferase 2	1.000	0.562	1.087	0.898	2526.246
Mm.333916	Chsy1	Chondroitin sulfate synthase 1	1.000	2.292	0.909	1.327	705.601
Mm.482404	Chst14	Carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 14	1.000	2.025	0.975	1.160	220.386
Mm.34557	Dse	Dermatan sulfate epimerase	1.000	1.820	0.881	1.533	160.641
Mm.103468	Dsel	Dermatan sulfate epimerase-like	1.000	1.780	1.418	1.527	662.678
Mm.360747	Chst11	Carbohydrate sulfotransferase 11	1.000	2.606	1.026	0.974	25.935
Mm.28934	Chst12	Carbohydrate sulfotransferase 12	1.000	1.631	1.205	1.056	217.488
Mm.213582	Chst15	Carbohydrate (N-acetylgalactosamine 4-sulfate 6-O) sulfotransferase 15	1.000	1.460	0.887	0.828	78.566
Mm.12866	Chst3	Carbohydrate (chondroitin 6/keratan) sulfotransferase 3	1.000	0.691	0.989	0.841	215.931
Mm.44827	Chst7	Carbohydrate (N-acetylglucosamino) sulfotransferase 7	1.000	2.111	1.294	1.290	167.148
Mm.309395	Ext1	Exostoses (multiple) 1	1.000	1.749	1.237	1.221	430.741
Mm.4336	Ext2	Exostoses (multiple) 2	1.000	2.490	0.992	1.228	32.854
Mm.30978	Extl1	Exostoses (multiple)-like 1	1.000	0.660	1.109	0.977	528.590
Mm.103748	Extl3	Exostoses (multiple)-like 3	1.000	1.026	0.962	0.914	4554.838
Mm.12863	Hs2st1	Heparan sulfate 2-O-sulfotransferase 1	1.000	0.629	0.892	0.767	543.519
Mm.24411	Glce	Glucuronyl C5-epimerase	1.000	0.832	0.771	0.796	1153.899
Mm.213566	Hs6st1	Heparan sulfate 6-O-sulfotransferase 1	1.000	0.664	1.433	0.707	159.164
Mm.252561	Hs6st2	Heparan sulfate 6-O-sulfotransferase 2	1.000	0.543	0.995	0.713	398.640
Mm.328848	Hs3st6	Heparan sulfate (glucosamine) 3-O-sulfotransferase 6	1.000	1.112	1.055	1.591	59.114
Mm.181862	Ndst1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	1.000	0.711	0.947	0.696	730.496
Mm.485574	B3galt1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 1	1.000	0.472	1.042	0.752	168.534
Mm.285580	B3galt2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	1.000	0.255	0.995	0.907	349.793
Mm.15622	B4galt1	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	1.000	1.550	0.873	1.278	3906.908
Mm.182377	B4galt4	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 4	1.000	1.510	1.033	1.183	341.531
Mm.21686	B3galnt2	UDP-GalNAc:betaGlcNAc beta 1,3-galactosaminyltransferase, polypeptide 2	1.000	0.758	1.302	1.017	956.080
Mm.153710	B3galnt1	UDP-GalNAc:betaGlcNAc beta 1,3-galactosaminyltransferase, polypeptide 1	1.000	3.028	1.113	1.495	363.838
Mm.386762	B4galnt1	Beta-1,4-N-acetyl-galactosaminyl transferase 1	1.000	2.597	0.588	0.898	750.477
Mm.334477	Fut10	Fucosyltransferase 10	1.000	1.173	0.834	0.804	399.024
Mm.440185	B3galt6	UDP-Gal:betaGal beta 1,3-galactosyltransferase, polypeptide 6	1.000	1.608	1.279	1.002	70.286

(continued)

Table 5. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.154783	B3galt5	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5	1.000	0.602	0.132	0.193	40.415
Mm.285580	B3galt2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	1.000	0.255	0.995	0.907	349.793
Mm.1203	Fut7	Fucosyltransferase 7	1.000	2.002	0.794	1.491	52.599
Mm.306228	St8sia4	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4	1.000	1.668	0.931	1.324	547.713
Mm.4954	St8sia2	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2	1.000	1.093	0.971	0.561	38.444
Mm.393827	B4galt6	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6 (should be B3galt6)	1.000	2.121	0.861	0.838	73.732

Note: Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased more than 1.50-fold only two weeks after the operation: . Expression increased less than 0.66-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . w: week; Ope: operation.

receptor-beta protein (*Pdgfrb*), chemokine (C–X–C motif) ligand 12 (*Cxcl12*), and chemokine (C–C motif) ligands 2, 3, 4, 6, 7, and 8 (*Ccl2*, *Ccl3*, *Ccl4*, *Ccl6*, *Ccl7*, and *Ccl8*) is evident. Genes including mothers against decapentaplegic homolog 3 (*Smad3*) and TNF (ligand) superfamily member 9 (*Tnfsf19*) are downregulated in the paw samples continuously for two weeks after the operation (Table 4). In addition, 29 and 6 genes are upregulated one week and two weeks after the operation, respectively, and 6 and 5 genes are downregulated one week and two weeks after the operation, respectively (Table 4). In contrast, none of the genes upregulated in the paw samples are upregulated in the DRG samples one week and two weeks after the operation; however, two genes, *Tnfaip2* and *Tnfsf25*, are continuously downregulated for two weeks after the operation (Table 7). We also found that six genes encoding ECM-related molecules including Src homology 2 domain-containing transforming protein C3 (Shc3), TNF receptor superfamily member 12a (*Tnfrsf12a*), chemokine (C–C motif) ligands 8 and 17 (*Ccl8* and *Ccl17*), interleulin 6 (Il6), and interleukin 7 (*Il7*) are upregulated one week after the operation but not after two weeks. Four genes (*Cxcl14*, *Il1b*, *Il1rl1,* and *Il6ra*) are downregulated two weeks after the operation (Table 7).

Since the paw was the site of the surgery, operation-derived necrosis may have caused the secretion of a high concentration of proteases from the necrotic cells, inducing higher expression of inflammation-related genes than that in the DRG samples. It would be of interest to investigate whether prevention of necrosis-induced inflammation-related gene expression in the paw is related to chronic pain in the mouse model.

Enzymes implicated in ECM saccharide chain synthesis

All ECM molecules bear saccharide chains. Some of the chains are long saccharide chains recognized as glycosaminoglycans, such as chondroitin sulfate (CS). The synthesis of glycosaminoglycans, especially CS, negatively or positively controls plasticity and regeneration of the neuronal network after injury and neurodegenerative diseases.[17–19] Therefore, it was of interest to investigate the changes in the expression of genes encoding sugar chain synthases after the injury. In the paw samples, the expression of genes involved in CS synthesis increases by >1.5-fold one week after the operation, which was similar to previous reports. In contrast, the expression of most genes involved in heparan sulfate (HS) synthesis decreases by <0.75-fold, although the expression of two genes involved in HS synthesis, *Ext1* and *Ext2*, increases (Table 5). Changes in the expression of genes involved in other sugar chain synthases are also observed in the paw samples. Interestingly, the expression of genes involved in sugar chain synthases in the DRG samples is very low (raw values <50) and also shows no significant changes after the operation (data not shown), which suggests that changes in the expression of these genes occur only in injured nerve tissues at least during the study period.

Expression of genes for pain-associated molecules; neuropeptides, neurotransmitters, and receptors

Details of molecules involved in nociceptive and neurogenic pain, and effects of the operation on their gene expression levels are described and discussed below.

Genes expressing pain-related neuropeptides, including *Tac1* (which encodes tachynin 1, a precursor protein of substance P and neurokinin A) are upregulated in the DRG samples (Table 9). It should be noted that *Tac1* expression in the DRG samples is >700-fold higher than that in the paw. However, the operation group mice display a nearly two-fold increase in *Tac1* expression in the paw. *Cck*, a gene encoding a peptide hormone cholecystokinin, is found both in the brain and in the intestine where it acts as a neurotransmitter and a regulator of

Table 6. Selected ECM-related genes in the DRG-ECM molecules.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.358571	Acan	Aggrecan	1.000	1.875	1.059	1.022	59.171
Mm.4598	Bcan	Brevican	1.000	1.255	1.373	1.353	14,562.961
Mm.423621	Cd44	CD44 antigen	1.000	1.194	1.248	1.118	5197.634
Mm.277735	Col1a1	Collagen, type I, alpha 1	1.000	1.162	0.906	0.854	11,919.386
Mm.249555	Col3a1	Collagen, type III, alpha 1	1.000	0.768	0.779	0.761	9851.486
Mm.7281	Col5a1	Collagen, type V, alpha 1	1.000	0.858	0.956	1.002	852.757
Mm.233547	Col15a1	Collagen, type XV, alpha 1	1.000	0.810	0.755	0.650	138.265
Mm.56769	Dcn	Decorin	1.000	0.831	0.830	0.810	172,673.360
Mm.2608	Bgn	Biglycan	1.000	1.049	0.938	1.158	680.136
Mm.18888	Lum	Lumican	1.000	0.956	0.790	0.768	13,765.940
Mm.268079	Ncan	Neurocan	1.000	1.193	1.242	1.255	331.374
Mm.338790	Srgn	Serglycin	1.000	0.889	0.800	0.822	4331.386
Mm.273098	Agrn	Agrin	1.000	0.886	0.818	0.918	8834.358
Mm.2580	Sdc1	Syndecan 1	1.000	1.911	1.140	1.266	1152.383
Mm.234266	Sdc2	Syndecan 2	1.000	0.951	0.986	1.064	2480.722
Mm.206536	Sdc3	Syndecan 3	1.000	1.144	1.142	1.149	3853.221
Mm.3815	Sdc4	Syndecan 4	1.000	1.068	1.151	1.230	233.354
Mm.158700	Vcan	Versican	1.000	0.977	0.913	1.000	285.012
Mm.182043	Itih2	Interalpha trypsin inhibitor, heavy chain 2	1.000	0.736	0.629	0.742	517.973
Mm.4517	Itih3	Interalpha trypsin inhibitor, heavy chain 3	1.000	0.912	0.849	0.940	171.878
Mm.313876	Itih5	Interalpha (globulin) inhibitor H5	1.000	1.035	1.062	1.056	2302.226
Mm.266790	Hapln1	Hyaluronan and proteoglycan link protein 1	1.000	0.789	0.842	0.903	1387.906
Mm.178759	Hapln3	Hyaluronan and proteoglycan link protein 3	1.000	1.207	1.139	1.330	714.373
Mm.152048	Hapln4	Hyaluronan and proteoglycan link protein 4	1.000	0.940	1.234	1.007	440.505
Mm.87150	Itgb3	Integrin beta 3	1.000	1.045	0.952	0.740	2803.320
Mm.482186	Itga1	Integrin alpha 1	1.000	0.995	0.982	1.020	162.884
Mm.217000	Itgb8	Integrin beta 8	1.000	1.020	0.859	0.805	249.617
Mm.303386	Lama1	Laminin, alpha 1	1.000	1.374	1.304	1.432	511.484
Mm.172674	Lamb1	Laminin B1	1.000	0.978	1.000	1.012	12,456.387
Mm.1249	Lamc1	Laminin, gamma 1	1.000	0.949	0.958	0.982	5748.124
Mm.193099	Fn1	Fibronectin 1	1.000	0.856	0.879	0.992	9890.401
Mm.297992	Fbln1	Fibulin 1	1.000	0.852	0.739	0.877	855.236
Mm.249146	Fbln2	Fibulin 2	1.000	1.206	1.230	1.226	5243.587
Mm.288381	Fbln5	Fibulin 5	1.000	1.013	1.044	1.039	9095.349
Mm.5107	Fbln7	Fibulin 7	1.000	0.765	0.888	0.848	1080.418
Mm.29564	Mmp2	Matrix metallopeptidase 2	1.000	0.877	0.781	0.796	1145.888
Mm.16415	Mmp8	Matrix metallopeptidase 8	1.000	0.521	0.351	0.321	778.120
Mm.4406	Mmp9	Matrix metallopeptidase 9	1.000	0.702	0.429	0.346	208.902
Mm.4561	Mmp11	Matrix metallopeptidase 11	1.000	1.040	1.144	1.225	212.527
Mm.5022	Mmp13	Matrix metallopeptidase 13	1.000	0.653	0.148	0.308	192.473
Mm.217116	Mmp15	Matrix metallopeptidase 15	1.000	1.287	1.267	1.256	1312.885
Mm.42047	Mmp17	Matrix metallopeptidase 17	1.000	1.014	1.091	1.120	738.841
Mm.8245	Timp1	Tissue inhibitor of metalloproteinase 1	1.000	1.507	0.681	0.783	980.763
Mm.206505	Timp2	Tissue inhibitor of metalloproteinase 2	1.000	1.119	1.344	1.261	145,578.690
Mm.4871	Timp3	Tissue inhibitor of metalloproteinase 3	1.000	0.979	0.912	0.991	16,308.405
Mm.4159	Thbs1	Thrombospondin 1	1.000	1.038	1.128	1.123	821.780
Mm.26688	Thbs2	Thrombospondin 2	1.000	1.522	1.603	1.565	154.773
Mm.2114	Thbs3	Thrombospondin 3	1.000	0.909	0.806	0.889	1222.697
Mm.20865	Thbs4	Thrombospondin 4	1.000	0.862	0.829	0.845	2849.937
Mm.290527	Tnxb	Tenascin XB	1.000	0.852	0.870	0.912	2335.082

Note: No apparent changes in glycosyltransferases and sulfotransferases for the syntheses of glycosaminoglycans and sugar chains although their expressions were significant. Although there are high expressions (>500 raw values) in Adamts1, 2, 4, 5, 8, 14, and 15 (especially high in Adamts2 and 15), no significant change was observed among Sham-1w, Ope-1w, Sham 2-w, and Ope-2w groups. Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . w: week; Ope: operation.

Table 7. Selected ECM-related genes in the DRG—growth factors, chemokines, other molecules in ECM, and their receptors and signaling molecules.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.27757	Bmp1	Bone morphogenetic protein 1	1.000	1.353	1.101	1.161	638.950
Mm.1442	Bdnf	Brain-derived neurotrophic factor	1.000	1.279	1.033	0.961	1299.840
Mm.906	Mdk	Midkine	1.000	1.126	1.048	0.929	1428.512
Mm.248380	Tgfb1	Transforming growth factor, beta 1	1.000	1.344	0.899	0.928	286.958
Mm.18213	Tgfb2	Transforming growth factor, beta 2	1.000	1.199	1.169	1.262	848.305
Mm.3992	Tgfb3	Transforming growth factor, beta 3	1.000	1.092	1.211	1.212	2943.462
Mm.197552	Tgfbr1	Transforming growth factor, beta receptor I	1.000	1.052	0.946	0.984	886.366
Mm.172346	Tgfbr2	Transforming growth factor, beta receptor II	1.000	0.980	0.993	1.003	3636.591
Mm.200775	Tgfbr3	Transforming growth factor, beta receptor III	1.000	1.067	1.142	1.182	2556.895
Mm.271745	Nrp1	Neuropilin 1	1.000	1.070	0.907	0.951	547.147
Mm.246069	Tgfbrap1	Transforming growth factor, beta receptor associated protein 1	1.000	1.128	1.313	1.234	286.382
Mm.14455	Tgfbi	Transforming growth factor, beta induced	1.000	0.877	0.878	0.935	21,443.662
Mm.223717	Smad1	MAD homolog 1 (*Drosophila*)	1.000	1.362	1.306	1.221	608.409
Mm.391091	Smad2	MAD homolog 2 (*Drosophila*)	1.000	1.035	0.982	1.001	1306.672
Mm.7320	Smad3	MAD homolog 3 (*Drosophila*)	1.000	0.831	1.114	0.899	708.494
Mm.100399	Smad4	MAD homolog 4 (*Drosophila*)	1.000	1.172	1.263	1.247	4616.912
Mm.272920	Smad5	MAD homolog 5 (*Drosophila*)	1.000	1.020	1.063	0.994	3949.937
Mm.325757	Smad6	MAD homolog 6 (*Drosophila*)	1.000	0.923	1.145	1.207	174.597
Mm.34407	Smad7	MAD homolog 7 (*Drosophila*)	1.000	1.068	1.088	1.302	198.854
	Smad9	MAD homolog 9 (*Drosophila*)	1.000	1.091	1.042	1.294	1288.767
Mm.37801	Shcbp1	Shc SH2-domain binding protein 1	1.000	0.805	0.667	0.873	211.611
Mm.480460	Shc1	Src homology 2 domain-containing transforming protein C1	1.000	1.135	1.045	1.147	6871.065
Mm.39424	Shc2	SHC (Src homology 2 domain containing) transforming protein 2	1.000	1.175	1.281	1.218	5061.852
Mm.131870	Shc3	Src homology 2 domain-containing transforming protein C3	1.000	1.504	1.109	0.979	80.982
Mm.472964	Shc4	SHC (Src homology 2 domain containing) family, member 4	1.000	1.373	0.976	1.064	6915.565
Mm.27735	Smurf1	SMAD-specific E3 ubiquitin protein ligase 1	1.000	0.985	1.109	1.046	510.965
Mm.340955	Smurf2	SMAD-specific E3 ubiquitin protein ligase 2	1.000	0.779	0.843	0.742	1056.188
Mm.1062	Tnfsf10	Tumor necrosis factor (ligand) superfamily, member 10	1.000	0.756	0.985	1.133	188.933
Mm.8983	Tnfsf12	Tumor necrosis factor (ligand) superfamily, member 12	1.000	1.145	1.057	1.064	922.328
Mm.386774	Tnfaip1	Tumor necrosis factor, alpha-induced protein 1 (endothelial)	1.000	1.173	1.191	1.206	9439.455
Mm.255332	Tnfaip2	Tumor necrosis factor, alpha-induced protein 2	1.000	0.643	0.707	0.653	277.883
Mm.3509	Tnfaip6	Tumor necrosis factor-alpha-induced protein 6 (TSG-6)	1.000	1.000	0.877	0.919	241.719
Mm.1258	Tnfrsf1a	Tumor necrosis factor receptor superfamily, member 1a	1.000	1.044	0.989	1.021	1992.850
Mm.6251	Tnfrsf11a	Tumor necrosis factor receptor superfamily, member 11a	1.000	1.096	1.292	1.404	2352.915
Mm.28518	Tnfrsf12a	Tumor necrosis factor receptor superfamily, member 12a	1.000	1.521	0.678	0.766	2161.853
Mm.290780	Tnfrsf23	Tumor necrosis factor receptor superfamily, member 23	1.000	0.960	0.933	0.843	196.858
Mm.101198	Tnfrsf25	Tumor necrosis factor receptor superfamily, member 25	1.000	0.624	0.417	0.520	709.815
Mm.766	Cxcl9	Chemokine (C–X–C motif) ligand 9	1.000	1.175	0.831	0.937	97.213
Mm.131723	Cxcl11	Chemokine (C–X–C motif) ligand 11	1.000	1.050	0.919	1.019	243,571.170
Mm.30211	Cxcl14	Chemokine (C–X–C motif) ligand 14	1.000	1.111	0.788	0.606	209.169

(continued)

Table 7. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.1401	Cxcr4	Chemokine (C–X–C motif) receptor 4	1.000	0.890	0.831	1.097	315.310
Mm.124289	Cxcr6	Chemokine (C–X–C motif) receptor 6	1.000	1.290	0.851	0.930	124.976
Mm.6522	Cxcr7	Chemokine (C–X–C motif) receptor 7	1.000	0.769	0.676	0.677	1358.595
Mm.1283	Ccl1	Chemokine (C–C motif) ligand 1	1.000	0.818	0.919	0.970	473.782
Mm.290320	Ccl2	Chemokine (C–C motif) ligand 2	1.000	1.182	0.806	0.876	758.840
Mm.137	Ccl6	Chemokine (C–C motif) ligand 6	1.000	0.627	0.526	0.518	1653.252
Mm.42029	Ccl8	Chemokine (C–C motif) ligand 8	1.000	1.997	0.716	0.714	391.056
Mm.41988	Ccl17	Chemokine (C–C motif) ligand 17	1.000	2.474	1.283	1.451	58.584
Mm.425176	Ccl27a	Chemokine (C–C motif) ligand 27A	1.000	0.773	0.865	0.903	6250.515
Mm.6272	Ccr2	Chemokine (C–C motif) receptor 2	1.000	0.742	0.767	0.813	261.003
Mm.8021	Ccr10	Chemokine (C–C motif) receptor 10	1.000	0.867	1.093	1.187	253.073
Mm.222830	Il1b	Interleukin 1 beta	1.000	1.179	0.623	0.532	43.916
Mm.1019	Il6	Interleukin 6	1.000	2.176	0.761	0.841	88.520
Mm.3825	Il7	Interleukin 7	1.000	1.540	0.870	0.963	92.573
Mm.35814	Il11	Interleukin 11	1.000	0.902	0.877	0.769	71.788
Mm.10137	Il16	Interleukin 16	1.000	0.744	0.738	0.768	1393.593
Mm.390726	Il17d	Interleukin 17D	1.000	0.916	0.970	0.917	2316.509
Mm.1410	Il18	Interleukin 18	1.000	0.964	0.892	1.095	921.322
Mm.90154	Il25	Interleukin 25	1.000	1.057	1.042	1.163	137.154
Mm.222632	Il27	Interleukin 27	1.000	1.099	1.067	1.147	262.909
Mm.182359	Il33	Interleukin 33	1.000	0.742	0.663	0.722	985.900
Mm.77697	Il34	Interleukin 34	1.000	0.907	0.891	0.839	4333.002
Mm.896	Il1r1	Interleukin 1 receptor, type 1	1.000	1.183	0.961	1.052	503.944
Mm.289824	Il1rl1	Interleukin 1 receptor-like 1	1.000	0.748	0.738	0.667	315.875
Mm.2856	Il6ra	Interleukin 6 receptor, alpha	1.000	0.727	0.586	0.550	2044.217
Mm.379327	Il10ra	Interleukin 10 receptor, alpha	1.000	0.885	0.888	0.907	449.424
Mm.4154	Il10rb	Interleukin 10 receptor, beta	1.000	1.139	0.908	0.956	2480.624
Mm.193451	Il11ra1	Interleukin 11 receptor, alpha chain 1	1.000	0.861	0.917	0.887	14,409.509
Mm.213397	Il17rc	Interleukin 17 receptor C	1.000	0.920	1.087	1.081	2449.070
Mm.269363	Il17rb	Interleukin 17 receptor B	1.000	0.676	0.710	0.816	155.249
Mm.206726	Il17rd	Interleukin 17 receptor D	1.000	0.983	1.024	1.129	529.300
Mm.380801	Il31ra	Interleukin 31 receptor A	1.000	1.202	1.490	1.342	981.582
Mm.439649	Grb2	Growth factor receptor bound protein 2	1.000	1.168	1.318	1.337	10,112.787
Mm.464229	Grb10	Growth factor receptor bound protein 10	1.000	1.106	0.968	1.000	263.521
Mm.214554	Grb14	Growth factor receptor bound protein 14	1.000	1.101	1.116	1.152	2708.602

Note: No apparent changes in glycosyltransferases and sulfotransferases for the syntheses of glycosaminoglycans and sugar chains although their expressions were significant. Although there are high expressions (>500 raw values) in Adamts1, 2, 4, 5, 8, 14, and 15 (especially high in Adamts2 and 15), no significant change was observed among Sham-1w, Ope-1w, Sham-2w, and Ope-2w groups. Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . w: week; Ope: operation; MAD: mothers against decapentaplegic; SMAD: the fusion of Caenorhabditis elegans Sma genes and the Drosophila Mad, Mothers against decapentaplegic.

gastrointestinal functions, respectively.[20] This hormone induces various biological effects such as pathological thermal hyperalgesia, which is mediated by cholecystokinin A and B receptors encoded by *Cckbr* and *Cckar* genes, respectively. Mostly, cholecystokinin B receptors are found in the brain and the spinal cord,[20] and cholecystokinin A receptors are found in the peripheral nervous systems.[20–23] *Cckar* is highly expressed in the DRG which is the center of the peripheral neurons; interestingly, *Cckbr* is also highly expressed in the paw tissues having the peripheral nervous systems (Tables 8 and 9). This observation suggests that *Cckbr* may also be expressed to function in the peripheral tissues. *Cckbr* expression increases one week after the operation in the DRG samples, which is almost comparable to that in the paw samples; this change appears to be associated with the operation-induced increase in *Cck*.

Expression of *Npy* (which encodes neuropeptide Y) is significantly increased one week after the operation in both paw and DRG samples (Tables 8 and 9). This peptide acts as a neurotransmitter in the brain and the autonomic nervous system[24] and is thought to have several functions, including reduction of pain perception.[25]

Table 8. Selected genes of pain-related molecules in the paw (≥1.50).

UniGeneID	Gene symbol	Gene name	Sham 1w	Ope 1w	Sham 2w	Ope 2w	Raw (Sham 1w)
Neuropeptides and receptors							
Mm.1440	Tac1	Tachykinin 1 substance P is one of the proteolytic products	1.000	2.097	1.419	1.823	144.417
Mm.8054	Tacr2	Tachykinin receptor 2	1.000	1.206	1.180	1.456	5510.782
Mm.2619	Cck	Cholecystokinin	1.000	1.883	0.513	1.525	147.387
Mm.3521	Cckar	Cholecystokinin A receptor	1.000	1.013	1.022	1.265	3.593
Mm.44513	Cckbr	Cholecystokinin B receptor	1.000	1.889	1.053	2.459	1018.039
Mm.154796	Npy	Neuropeptide Y 36AA peptide acts as neurotransmitter in brain and in autonomic nerve system	1.000	5.612	0.549	1.219	555.866
Mm.64201	Nts	Neurotensin 13AA neuropeptide implictd in reg. of LH and PL release and intact with dopaminergic system. Analgesia. Increase of locomotor activity.	1.000	9.109	2.416	6.677	67.692
Mm.281715	Ntsr2	Neurotensin receptor 2	1.000	0.760	1.292	0.743	154.835
Mm.57149	Galr2	Galanin receptor 2	1.000	1.561	0.815	1.348	24.054
Mm.6219	Galr1	Galanin receptor 1	1.000	1.065	1.090	1.343	8.771
Mm.4655	Gal	Galanin	1.000	0.951	0.638	0.674	699.462
Membrane protein, a cell adhesion protein or ligand-receptor, enzyme							
Mm.19283	Cd163l1	CD163 molecule-like 1	1.000	1.622	1.454	2.233	45.136
Mm.167781	Dbh	Dopamine beta-hydroxylase	1.000	0.425	0.090	0.098	190.104
Mm.244393	Tank	TRAF family member-associated Nf-kappa B activator	1.000	1.010	1.129	1.270	164.076
Mm.222329	Camk4	Calcium/calmodulin-dependent protein kinase IV	1.000	4.865	1.110	2.324	31.646
Receptors on cell surfaces, channels, peptides, neurotransmitters							
Mm.4679	Gdnf	Glial cell line-derived neurotrophic factor	1.000	1.276	0.767	0.740	282.092
Mm.159842	Cacna2d1	Calcium channel, voltage-dependent, alpha2/delta subunit 1	1.000	0.422	0.931	0.896	12,415.046
Mm.439747	Htr2b	5-Hydroxytryptamine (serotonin) receptor 2B	1.000	2.131	0.616	0.616	79.605
Mm.254266	Htr7	5-Hydroxytryptamine (serotonin) receptor 7	1.000	1.132	1.533	1.660	52.034
Mm.214351	Htr2a	5-Hydroxytryptamine (serotonin) receptor 2A	1.000	1.124	0.428	1.393	58.023
Mm.151293	Nlgn2	Neuroligin 2	1.000	1.686	0.888	1.282	1830.992
Mm.250418	Ogfr	Opioid growth factor receptor	1.000	0.951	1.054	1.071	1499.651
Mm.250418	Ogfr	Opioid growth factor receptor	1.000	1.038	1.061	1.098	10,795.503
Mm.28013	Ogfrl1	Opioid growth factor receptor-like 1	1.000	1.134	1.023	1.143	178.986
Mm.365444	Olfr1500	Olfactory receptor 1500	1.000	0.266	0.798	0.369	252.488
Mm.37324	Piezo1, Fam38a	Family with sequence similarity 38, member A	1.000	1.973	1.042	1.613	3446.311
Mm.158720	Piezo2, Fam38b	Family with sequence similarity 38, member B	1.000	2.372	1.004	2.283	67.722
Mm.1418	Scn1b	Sodium channel, voltage-gated, type I, beta	1.000	0.580	1.431	1.033	5115.720
Mm.477575	Scn2b	Sodium channel, voltage-gated, type II, beta	1.000	1.316	1.475	0.939	60.461
Mm.330256	Scn3a	Sodium channel, voltage-gated, type III, alpha	1.000	0.827	0.722	0.707	81.520
Mm.290083	Scn3b	Sodium channel, voltage-gated, type III, beta	1.000	2.074	0.736	3.338	4424.105

(continued)

Table 8. Continued.

UniGeneID	Gene symbol	Gene name	Sham 1w	Ope 1w	Sham 2w	Ope 2w	Raw (Sham 1w)
Mm.432528	Scn4a	Sodium channel, voltage-gated, type IV, alpha	1.000	0.556	1.106	0.689	289.822
Mm.335112	Scn4b	Sodium channel, type IV, beta	1.000	0.258	1.031	0.790	14,749.829
Mm.103584	Scn5a	Sodium channel, voltage-gated, type V, alpha	1.000	1.212	0.915	1.234	238.286
Mm.38127	Scn7a	Sodium channel, voltage-gated, type VII, alpha	1.000	1.839	0.950	1.462	68.586
Mm.385012	Scn8a	Sodium channel, voltage-gated, type VIII, alpha	1.000	0.844	0.903	0.948	2484.323
Mm.35247	Scnn1g	Sodium channel, nonvoltage-gated 1 gamma	1.000	1.053	1.073	1.336	238.286
Mm.217171	Slc24a3	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	1.000	1.823	1.055	1.504	426.994
Mm.291070	Slc24a6	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 6	1.000	1.288	1.132	1.875	129.100
Mm.288064	Trpv2	Transient receptor potential cation channel, subfamily V, member 2	1.000	2.157	0.724	1.260	569.669
Mm.266450	Trpv4	Transient receptor potential cation channel, subfamily V, member 4	1.000	1.735	0.869	1.751	495.302
Mm.38875	Trpm1	Transient receptor potential cation channel, subfamily M, member 1	1.000	1.055	0.649	1.540	746.703
Mm.215171	Trpm6	Transient receptor potential cation channel, subfamily M, member 6	1.000	2.474	0.808	1.734	69.567
Mm.244705	Trpm7	Transient receptor potential cation channel, subfamily M, member 7	1.000	0.816	0.962	1.046	1776.056
Mm.333327	Hrh1	Histamine receptor H1	1.000	0.993	1.179	1.176	80.855
Mm.207073	Hrh4	Histamine receptor H4	1.000	1.139	1.155	1.037	51.096
Mm.41665	Grina	Glutamate receptor, ionotropic, NMDA-associated protein 1 (glutamate binding)	1.000	0.817	1.353	0.671	1832.325
Mm.21094	Grinl1a	Glutamate receptor, ionotropic, NMDA-like 1A	1.000	0.737	1.328	1.064	12,537.344
Mm.209263	Gria4	Glutamate receptor, ionotropic, AMPA4 (alpha 4)	1.000	1.107	1.048	1.427	995.962
Mm.18072	Crcp	Calcitonin gene-related peptide-receptor component protein	1.000	1.002	1.138	0.746	1363.275
Mm.209312	Rxfp3	Relaxin family peptide receptor 3	1.000	2.260	1.144	3.915	32.794
Mm.3770	Sos2	Son of sevenless homolog 2 (*Drosophila*)	1.000	0.587	1.051	0.754	1126.500
Mm.464229	Grb10	Growth factor receptor bound protein 10	1.000	2.501	0.501	1.353	839.430
Mm.439649	Grb2	Growth factor receptor bound protein 2	1.000	0.816	1.366	0.724	153.220
Mm.214554	Grb14	Growth factor receptor bound protein 14	1.000	0.281	1.380	1.066	3399.423
Mm.3272	Ramp1	Receptor (calcitonin) activity modifying protein 1	1.000	0.352	1.460	1.250	8204.198
Mm.260698	Ramp2	Receptor (calcitonin) activity modifying protein 2	1.000	1.397	0.884	1.105	6642.900
Mm.39884	Ramp3	Receptor (calcitonin) activity modifying protein 3	1.000	0.712	1.339	1.548	161.895

Note: Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased more than 1.50-fold only two weeks after the operation: . Expression increased less than 0.66-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . w: week; Ope: operation; TRAF: TNF receptor (TNFR) associated factor.

Neurotensin is a peptide distributed throughout the central nervous system (CNS). It is involved in the regulation of dopamine pathways and induces various effects, including analgesia, hypothermia, and increased locomotor activity.[26] Interestingly, the *Nts* gene encoding this peptide is also upregulated in both paw and DRG samples after the operation (Tables 8 and 9).

Gal encodes galanin, which is also a neuropeptide widely expressed in the brain, spinal cord, and gut, and signals through three G protein-coupled receptors (*Galr1*, *2*, and *3*). It is predominantly involved in the modulation and inhibition of action potentials in neurons and has been implicated in many biologically diverse functions including nociception, cognition, and blood pressure regulation.[27] In addition, it has been reported that the biosynthesis of galanin is increased upon axotomy in the peripheral nervous system.[28] Consistent with these findings, the expression of *Gal* increases three-fold in the DRG one week after the operation, which seems to correspond to its neuroprotective activity during the acute phase, as reported previously.[28] However, *Galr* which encodes the Gal receptor is not significantly expressed before and after the operation in both DRG and paw samples (raw values <50), with the exception of *Galr*1 in the DRG (Tables 8 and 9).

Cd163 encodes the macrophage scavenger receptor, which is a marker of the monocyte/macrophage lineage.[29] Macrophages are critical to injury and repair. Two types of macrophages are found in cells: M1, which secretes inflammatory cytokines that activate nociceptors and promote pain, and M2, which secretes anti-inflammatory cytokines that inhibit nociceptors and promote analgesia. Thus, they may regulate both chronic musculoskeletal pain and analgesia during regular physical activity.[30,31] The expression of *Cd163* increases approximately two-fold on week 1 and continues two weeks after the operation in both paw and DRG samples. Thus, the elevated expression might play some roles to suppress the scar formation in the paw and the associated pain.

Dbh encodes dopamine beta-hydroxylase, an enzyme involved in the synthesis of norepinephrine. Its expression is significantly increased in the DRG samples but reduced in the paw samples, which may be a neuronal response to the shock of the operation.

The expressions of *Ogfr* gene encoding the opioid growth factor receptor and *Htr* gene encoding the serotonin receptor do not show a marked change as a result of the operation in both paw and DRG samples, although their expressions in the DRG samples are higher than the expressions in the paw samples.

Piezo1 and its close homolog *piezo2* are genes encoding mechanosensitive ion channel proteins. These genes are expressed in the lungs, bladder, and skin, where mechanosensation has important biological roles, and *piezo2* is highly expressed in the sensory neurons.[32] In our study, *piezo1* is highly expressed in the paw samples and it increases two-fold continuously for two weeks after the operation, which is similar to previous reports. In contrast, *piezo2* is highly expressed in the DRG samples but weakly in the paw samples, although the expression increases after the operation.

Scns encode integral membrane proteins that form ion channels, which facilitate the transport of sodium ions (Na^+) through the cell membrane. In excitable cells such as neurons, myocytes, and certain types of glia, the channels are responsible for the rising phase in the action potentials.[33] *Scns* are highly expressed in the DRG samples but mostly unaffected by the operation. In contrast, their expressions in the paw samples change after the operation albeit their expression levels being less than those in the DRG samples (Tables 8 and 9).

TRP (transient receptor potential) channels are ion channels comprising six protein families. Based on their structural similarities, they are grouped into two broad groups. Group 1 includes TRPC ("C" for canonical), TRPV ("V" for vanilloid), TRPM ("M" for melastatin), TRPN ("N" for no mechanoreceptor potential C), and TRPA ("A" for ankyrin), and group 2 includes TRPP ("P" for polycystic) and TRPML ("ML" for mucolipin). TRPV1/TRPV2/TRPV3 and TRPV4 have recently been found to be clinically significant in their roles as thermoreceptors and mechanoreceptors, respectively. Reduction in chronic pain might be achieved by targeting ion channels involved in thermal, chemical, and mechanical sensations to reduce their sensitivity to stimuli.[34] The present microarray analysis shows that almost all genes for TRPVs and TRPMs are highly expressed in the DRG samples compared with the expressions in the paw samples. Interestingly, expressions of *TrpV6* and *Trpm1* in the DRG are increased by approximately two-fold one week after the operation. In the paw samples, expressions of *Trpv2*, *Trpv4,* and *Trmp6* are increased two-fold one week after the operation, and the expressions of *Trpv4* and *Trpm6* remain elevated two weeks after the operation (although they decrease from the levels at one week). Considering these results and their known functions described above, TRPV4 may be an important factor involved in algesia after the operation.

Grin and Gria, genes for glutamate receptors of ionotropic NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methoxazole-4-propionate), respectively, are, as expected, highly expressed in the DRG samples compared with the expressions in the paw samples. However, only *Gria1* expression in the DRG and *Grina* expression in the paw samples change significantly after the operation (Tables 8 and 9), suggesting that these receptors do not play significant roles in pain perception. Glutamate receptors are present in CNS glial

Table 9. Selected genes of pain-related molecules in the DRG (≥1.50).

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Neuropeptides and receptors							
Mm.1440	Tac1	Tachykinin 1 substance P is one of the proteolytic products	1.000	0.973	0.980	1.009	111,882.410
Mm.8054	Tacr2	Tachykinin receptor 2	1.000	1.149	1.107	1.285	9114.199
Mm.2619	Cck	Cholecystokinin	1.000	1.445	1.043	1.082	327.288
Mm.3521	Cckar	Cholecystokinin A receptor	1.000	1.396	1.141	1.150	1270.071
Mm.44513	Cckbr	Cholecystokinin B receptor	1.000	7.198	0.531	1.914	184.889
Mm.154796	Npy	Neuropeptide Y 36 AA peptide acts as neurotransmitter in brain and in autonomic nerve system	1.000	8.080	0.735	2.635	747.400
Mm.64201	Nts	Neurotensin 13AA neuropeptide Reg. of LH and PL release and intact with dopaminergic system. Increase of locomotive activity	1.000	2.572	0.628	1.034	3897.031
Mm.281715	Ntsr2	Neurotensin receptor 2	1.000	1.078	1.146	1.240	419.464
Mm.57149	Galr2	Galanin receptor 2	1.000	2.542	0.687	0.488	13.785
Mm.6219	Galr1	Galanin receptor 1	1.000	1.194	1.135	1.031	150.854
Mm.4655	Gal	Galanin	1.000	3.166	0.889	1.073	13,176.159
Membrane protein, a cell adhesion protein or ligand-receptor, enzyme							
Mm.19283	Cd163l1	CD163 molecule-like 1	1.000	2.366	1.222	1.920	30.448
Mm.167781	Dbh	Dopamine beta-hydroxylase	1.000	5.898	2.457	2.495	48.550
Mm.4926	Pacsin1	Protein kinase C and casein kinase substrate in neurons 1	1.000	1.169	1.428	1.372	9746.880
Mm.244393	Tank	TRAF family member-associated Nf-kappa B activator	1.000	0.953	0.891	0.933	282.948
Mm.222329	Camk4	Calcium/calmodulin-dependent protein kinase IV	1.000	1.318	1.080	0.877	136.030
Receptors on cell surfaces, channels, peptides, neurotransmitters							
Mm.4679	Gdnf	Glial cell line-derived neurotrophic factor	Not significant				32.218
Mm.159842	Cacna2d1	Calcium channel, voltage-dependent, alpha2/delta subunit 1	1.000	1.591	1.250	1.192	5412.147
Mm.217000	Itgb8	Integrin beta 8	1.000	1.020	0.859	0.805	249.617
Mm.5040	Htr1f	5-Hydroxytryptamine (serotonin) receptor 1F	1.000	1.101	1.188	1.079	347.892
Mm.40573	Htr1d	5-Hydroxytryptamine (serotonin) receptor 1D	1.000	1.019	1.228	1.336	380.441
Mm.214351	Htr2a	5-Hydroxytryptamine (serotonin) receptor 2A	1.000	0.915	0.768	0.830	347.106
Mm.4831	Htr3a	5-Hydroxytryptamine (serotonin) receptor 3A	1.000	0.811	1.076	0.975	7244.666
Mm.20440	Htr4	5-Hydroxytryptamine (serotonin) receptor 4	1.000	0.804	0.927	0.920	1107.711
Mm.4833	Htr5b	5-Hydroxytryptamine (serotonin) receptor 5B	1.000	0.973	1.360	1.171	439.514
Mm.254266	Htr7	5-Hydroxytryptamine (serotonin) receptor 7	1.000	0.842	0.962	0.958	2502.003
Mm.248684	Tph1	Tryptophan hydroxylase 1(serotonin synthetic enzyme)	1.000	0.857	0.784	0.785	53.494
Mm.316080	Nlgn1	Neuroligin 1	1.000	1.014	0.957	1.040	282.700
Mm.151293	Nlgn2	Neuroligin 2	1.000	1.365	1.309	1.348	18,788.574
Mm.121508	Nlgn3	Neuroligin 3	1.000	0.994	1.447	1.118	577.787
Mm.457998	Oprm1	Opioid receptor, mu 1	1.000	1.124	1.073	1.230	18,262.445
Mm.250418	Ogfr	Opioid growth factor receptor	1.000	0.985	0.980	0.994	13,026.637

(continued)

Table 9. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.28013	Ogfrl1	Opioid growth factor receptor-like 1	1.000	0.831	0.987	0.872	3097.117
Mm.377575	Olfr1026	Olfactory receptor 1026	1.000	29.141	0.847	0.812	7.808
Mm.329753	Olfr1463	Olfactory receptor 1463	1.000	15.826	0.850	0.814	7.688
Mm.377652	Olfr429	Olfactory receptor 429	1.000	5.471	0.117	0.113	55.140
Mm.37324	Piezo1,Fam38a	Family with sequence similarity 38, member A	1.000	0.981	0.933	0.979	1780.688
Mm.158720	Piezo2,Fam38b	Family with sequence similarity 38, member B	1.000	1.010	1.372	1.369	7164.400
Mm.439704	Scn1a	Sodium channel, voltage-gated, type I, alpha	1.000	0.876	0.947	1.071	7637.490
Mm.1418	Scn1b	Sodium channel, voltage-gated, type I, beta	1.000	1.023	1.140	1.050	7432.533
Mm.477575	Scn2b	Sodium channel, voltage-gated, type II, beta	1.000	1.074	1.449	1.330	4954.048
Mm.290083	Scn3b	Sodium channel, voltage-gated, type III, beta	1.000	1.070	1.119	1.137	3625.366
Mm.335112	Scn4b	Sodium channel, type IV, beta	1.000	1.049	1.232	1.154	25,317.773
Mm.38127	Scn7a	Sodium channel, voltage-gated, type VII, alpha	1.000	0.865	0.902	1.022	1468.625
Mm.385012	Scn8a	Sodium channel, voltage-gated, type VIII, alpha	1.000	1.030	1.105	1.098	3056.059
Mm.440889	Scn9a	Sodium channel, voltage-gated, type IX, alpha	1.000	1.138	1.025	1.226	795.757
Mm.247042	Scn10a	Sodium channel, voltage-gated, type X, alpha	1.000	1.116	1.240	1.248	75,487.940
Mm.89981	Scn11a	Sodium channel, voltage-gated, type XI, alpha	1.000	1.078	1.392	1.231	59,342.344
Mm.281691	Sfrp1	Secreted frizzled-related protein 1	1.000	1.045	1.172	1.129	695.010
Mm.42095	Sfrp4	Secreted frizzled-related protein 4	1.000	0.964	0.857	0.890	9175.229
Mm.470071	Sfrp5	Secreted frizzled-related sequence protein 5	1.000	0.925	0.990	0.994	26,854.572
Mm.217171	Slc24a3	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	1.000	1.176	1.271	1.358	5476.262
Mm.485915	Slc24a2	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 2	1.000	1.143	1.257	1.423	18,983.328
Mm.330538	Slc24a5	Solute carrier family 24, member 5	1.000	0.925	0.843	0.943	387.274
Mm.447485	Trpv1	Transient receptor potential cation channel, subfamily V, member 1	1.000	1.218	1.344	1.292	5064.208
Mm.288064	Trpv2	Transient receptor potential cation channel, subfamily V, member 2	1.000	1.139	1.157	1.168	10,565.155
Mm.266450	Trpv4	Transient receptor potential cation channel, subfamily V, member 4	1.000	0.894	0.974	1.069	481.735
Mm.296889	Trpv6	Transient receptor potential cation channel, subfamily V, member 6	1.000	1.898	1.154	1.244	108.708
Mm.186329	Trpa1	Transient receptor potential cation channel, subfamily A, member 1	1.000	1.042	1.066	1.308	5115.754
Mm.38875	Trpm1	Transient receptor potential cation channel, subfamily M, member 1	1.000	1.689	1.014	1.146	553.997
Mm.440339	Trpm3	Transient receptor potential cation channel, subfamily M, member 3	1.000	0.867	0.795	0.877	169.541

(continued)

Table 9. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.439890	Trpm4	Transient receptor potential cation channel, subfamily M, member 4	1.000	1.012	1.286	1.174	488.978
Mm.244705	Trpm7	Transient receptor potential cation channel, subfamily M, member 7	1.000	0.860	0.889	0.854	2461.384
Mm.218753	Trpm8	Transient receptor potential cation channel, subfamily M, member 8	1.000	0.799	1.046	0.976	216.932
Mm.333327	Hrh1	Histamine receptor H1	1.000	0.993	1.014	1.059	435.679
Mm.285360	Hrh3	Histamine receptor H3	1.000	1.009	1.402	1.144	696.883
Mm.207073	Hrh4	Histamine receptor H4	1.000	1.202	1.281	1.291	126.832
Mm.41665	Grina	Glutamate receptor, ionotropic, NMDA-associated protein 1 (glutamate binding)	1.000	1.168	1.252	1.254	20,735.664
Mm.278672	Grin1	Glutamate receptor, ionotropic, NMDA1 (zeta 1)	1.000	1.161	1.424	1.369	1540.792
Mm.21094	Grin1la	Glutamate receptor, ionotropic, NMDA-like 1A	1.000	1.059	1.213	1.168	19,679.764
Mm.440095	Grin3a	Glutamate receptor ionotropic, NMDA3A	1.000	1.092	1.214	1.235	958.367
Mm.4920	Gria1	Glutamate receptor, ionotropic, AMPA1 (alpha 1)	1.000	1.469	1.616	2.002	248.100
Mm.327681	Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)	1.000	0.905	0.924	0.941	416.481
Mm.209263	Gria4	Glutamate receptor, ionotropic, AMPA4 (alpha 4)	1.000	1.003	0.949	1.012	3849.313
Mm.322667	Gabrg3	Gamma-aminobutyric acid A receptor, subunit gamma 3	1.000	1.496	1.707	1.632	171.226
Mm.275639	Glrb	Glycine receptor, beta subunit	1.000	0.804	0.806	0.748	4215.388
Mm.18072	Crcp	Calcitonin gene-related peptide-receptor component protein	1.000	1.100	0.960	1.060	7440.255
Mm.4361	Calca	Calcitonin/calcitonin-related polypeptide, alpha	1.000	1.026	0.866	0.909	301,215.720
Mm.3272	Ramp1	Receptor (calcitonin) activity modifying protein 1	1.000	0.837	0.966	0.990	411.499
Mm.260698	Ramp2	Receptor (calcitonin) activity modifying protein 2	1.000	0.769	0.796	0.779	11,089.589
Mm.39884	Ramp3	Receptor (calcitonin) activity modifying protein 3	1.000	1.174	1.118	1.162	671.492

Note: Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . w: week; Ope: operation; TRAF: TNF receptor (TNFR) associated factor; AMPA: α-amino-3-hydroxy-5-methoxazole-4-propionate; NMDA: N-methyl-D-aspartate.

cells as well as neurons and play a role in modulating the expression of genes involved in glial cell differentiation and brain development.[35] Spinal NMDA receptors are reportedly involved in hyperalgesia and link the pain sensory region to the thalamus, the pain-processing center of the brain.[35] However, we observed the unaltered expression of *Grin* after the operation and the upregulated expression of *Gria1* at one week and two weeks after the operation in the DRG samples (Table 9).

GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter of the mammalian CNS. It is synthesized from glutamate in the brain. GABA regulates neuronal excitability throughout the nervous system and is directly responsible for regulation of muscle tone via the GABA receptor, which is encoded by *Gabr*.[36] The *Gabr* expression is significantly increased in the DRG samples after the operation, as expected from its function (Table 9).

The expression of genes for other neurotransmitter receptors is not significantly changed in the DRG samples by the operation. Altered expressions are observed for only a few neurotransmitter receptor genes (e.g., *Ramp1* and *Ramp3*) in the paw samples.

Expression of genes associated with signaling pathways and molecules involved in muscle fibrosis and associated hyperalgesia

It is important to investigate how the changes in gene expression result in muscle fibrosis and associated hyperalgesia. We first examined changes in the gene expressions of muscle constituents (Table 10). As expected, higher expression of genes involved in muscle components is observed in the paw samples, but expressions of myosin heavy polypeptides (*Myhs*) are reduced after the operation, which may reflect the loss of muscle differentiation by the operation. Contrary to this, as also expected, there are almost no gene expression changes in the DRG samples (Table 11). Interestingly, the gene encoding myosin 1H (*Myo1h*, nonskeletal muscle type), which functions in vesicle transport, is highly expressed in the DRG samples.

Similarly, the expression of genes related to myogenesis is highly expressed in the paw samples, and a few of these genes are expressed in the DRG samples (Tables 10 and 11). For example, gene expression of *Pax7*, a transcription factor that plays a role in myogenesis through regulation of muscle precursor cell proliferation,[37] is detected in the paw samples. Interestingly, the expression decreases continuously following the operation.

Wnt and Shh (Sonic hedgehog) signaling pathways function during embryogenesis in processes that include not only cell fate specification, cell proliferation, and cell migration but also control tissue regeneration.[38,39] Thus, we expected some involvement of these signaling molecules in fibrosis and associated hyperalgesia. Many genes associated with the Wnt and Shh pathways are expressed in both paw and DRG samples but at distinct expression levels. *Wnt1* and *Shh* expressions significantly increase in the paw samples, after the operation, although their expressions are mostly unaffected by the operation in the DRG samples (Tables 10 and 11). The expressions of *Sfrp2* and *Sfrp1*, which encode secreted frizzled-related protein 2 and protein 1, respectively, increase significantly in the paw samples after the operation. Thus, the Wnt and Shh signaling pathways may play important roles in fibrosis and associated hyperalgesia.

The AKT/mTOR pathway regulates the cell cycle.[39] In many cancers, this pathway is overactive, which reduces apoptosis and allows cell proliferation.[40] However, this pathway is important to promote growth and proliferation over differentiation of adult stem cells, specifically neural stem cells.[41] Consistent with these observations, we observed that the genes encoding the molecules involved in this pathway are significantly expressed in both paw and DRG samples but are largely unaltered by the operation (Tables 10 and 11).

The Notch signaling network regulates interactions between physically adjacent cells and development of neurons and somites.[42] Genes encoding the molecules involved in this pathway are highly expressed in both paw and DRG samples, but there are no significant changes in their expressions after the operation (Tables 10 and 11), suggesting no significant involvement in the observed events.

Expression of genes encoding neuronal signaling molecules, protein kinases, and transcription factors

Activating transcription factor 3, encoded by *Atf3*, is a member of the mammalian activation transcription factor/cAMP responsive element-binding protein family of transcription factors that are specifically expressed in a variety of stressed tissues.[43] The observed four-fold increase in *Atf3* expression one week after the operation is consistent with the above report, and this expression in the DRG samples provides confirmation of regenerative response being one of neuronal signaling molecules following injury in DRG neurons.[44] Since the Atf3 protein interacts with several signaling molecules such as c-Jun and Smad3,[45,46] knowledge of their interactions can provide a better understanding of the present results.

Significantly high expressions of genes for a variety of mitogen-activated protein kinases (*Mapks*), MAPK-activated protein kinases (*Mapkapk*), and Rho GTPase-activating proteins (*Arhgap*) are observed in both DRG and paw tissues. Interestingly, while the expressions of these genes, especially *Arhgap*, are significantly affected even two weeks after the operation in the paw samples, they remain constant in the DRG samples. This suggest the frequent occurrence of membrane-involved biological processes, such as endocytic recycling,[47] and their involvement in the fibrotic events in the paw tissues.

Runx1, the gene for a transcription factor that regulates the differentiation of hematopoietic stem cells into mature blood cells, has recently been demonstrated to play a major role in the development of the neurons that transmit pain.[48] However, we observed that *Runx1* expression increases only in the paw samples after the operation, while *Runx2* and *Runx3* expressions increase in the DRG samples (Tables 10 and 11).

The Fos gene family consisting of four members (*Fos*, *Fosb*, *Fosl1*, and *Fosl2*) that encode leucine zipper proteins can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1 and being implicated in the regulation of cell proliferation, differentiation, and transformation.[49] *Fosl1* expression in the DRG samples and *Fosl1* and *Fosl2* expressions in the paw samples are significantly elevated after the operation. However, their expression values are

Table 10. Selected genes of signaling molecules and differentiation-involved molecules in the paw (≥1.50).

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Molecules for muscle components							
Mm.121878	Tpm1	Tropomyosin 1, alpha	1.000	2.979	1.060	1.102	388.843
Mm.121878	Tpm1	Tropomyosin 1, alpha	1.000	0.900	1.100	1.233	422,258.530
Mm.646	Tpm2	Tropomyosin 2, beta	1.000	0.694	1.535	1.057	51,336.640
Mm.240839	Tpm3	Tropomyosin 3, gamma	1.000	1.351	0.842	0.854	14,139.394
Mm.295124	Tpm4	Tropomyosin 4	1.000	2.252	0.839	1.137	6352.871
Mm.477065	Myh1	Myosin, heavy polypeptide 1, skeletal muscle, adult	1.000	0.611	0.938	1.047	257,901.640
Mm.422801	Myh2	Myosin, heavy polypeptide 2, skeletal muscle, adult	1.000	0.650	1.030	0.898	301,862.340
Mm.340090	Myh3	Myosin, heavy polypeptide 3, skeletal muscle, embryonic	1.000	0.633	0.142	0.177	32,171.072
Mm.297382	Myh4	Myosin, heavy polypeptide 4, skeletal muscle	1.000	0.250	0.390	0.379	54,374.130
Mm.250705	Myh11	Myosin, heavy polypeptide 11, smooth muscle	1.000	0.720	0.669	0.837	223.819
Mm.1000	Myl1	Myosin, light polypeptide 1	1.000	0.963	1.105	1.237	313,707.380
Mm.7353	Myl3	Myosin, light polypeptide 3	1.000	0.430	1.531	1.412	53,880.582
Mm.390355	Myl4	Myosin, light polypeptide 4	1.000	1.470	0.194	0.265	51,167.040
Mm.238285	Myo1h	Myosin 1H	1.000	2.863	2.287	2.068	24.891
Myogenic genes							
Mm.29798	Cd34	CD34 antigen	1.000	1.196	0.988	1.412	30,536.896
Mm.218760	Pax7	Paired box gene 7	1.000	0.616	0.677	0.546	100.252
Mm.4984	Myf5	Myogenic factor 5	1.000	0.787	0.992	0.990	6.244
Mm.1526	Myod1	Myogenic differentiation 1	1.000	1.165	1.334	0.916	381.228
Mm.329100	Mdfi	MyoD family inhibitor	1.000	3.891	1.468	2.010	160.600
Mm.16528	Myog	Myogenin	1.000	1.155	0.276	0.249	3330.099
Mm.253067	Myt1l	Myelin transcription factor 1-like	1.000	1.047	1.065	3.830	15.528
Mm.136217	Ascl1	Achaete-scute complex homolog 1 (*Drosophila*)	1.000	2.207	0.715	2.123	61.411
Mm.389520	Pou3f2	POU domain, class 3, transcription factor 2 (Brn2)	1.000	1.028	1.374	3.594	3.817
Myogenic signalings—Wnt and Hedgehog signaling pathways							
Mm.1123	Wnt1	Wingless-related MMTV integration site 1	1.000	2.310	1.051	3.336	3.876
Mm.57202	Shh	Sonic hedgehog	1.000	1.038	1.052	1.313	4.054
Mm.228798	Ptch1	Patched homolog 1	1.000	0.539	0.993	1.066	86.766
Mm.287037	Ptch2	Patched homolog 2	1.000	0.870	1.155	0.850	6417.877
Mm.29279	Smo	Smoothened homolog (*Drosophila*)	1.000	1.794	1.000	1.433	2134.633
Mm.391450	Gli1	GLI-Kruppel family member GLI1	1.000	0.811	1.113	1.272	735.725
Mm.273292	Gli2	GLI-Kruppel family member GLI2	1.000	1.695	1.126	1.510	20.242
Mm.5098	Gli3	GLI-Kruppel family member GLI3	1.000	1.328	0.658	0.802	469.799
Mm.246003	Fzd1	Frizzled homolog 1 (*Drosophila*)	1.000	1.452	0.917	1.264	655.057
Mm.86755	Fzd4	Frizzled homolog 4 (*Drosophila*)	1.000	0.415	0.780	0.739	7417.048
Mm.4769	Fzd6	Frizzled homolog 6 (*Drosophila*)	1.000	1.920	1.179	1.601	44.355
Mm.297906	Fzd7	Frizzled homolog 7 (*Drosophila*)	1.000	0.506	1.176	0.859	522.855
Mm.281691	Sfrp1	Secreted frizzled-related protein 1	1.000	3.741	0.903	1.868	74.011
Mm.19155	Sfrp2	Secreted frizzled-related protein 2	1.000	25.619	0.979	6.823	73.564
Mm.42095	Sfrp4	Secreted frizzled-related protein 4	1.000	1.261	0.929	2.275	1293.556
Mm.470071	Sfrp5	Secreted frizzled-related sequence protein 5	1.000	0.429	0.977	0.988	4119.221
Mm.294664	Gsk3a	Glycogen synthase kinase 3 alpha	1.000	0.801	1.382	0.924	5055.916
Mm.394930	Gsk3b	Glycogen synthase kinase 3 beta	1.000	0.679	0.757	0.840	6365.856
Mm.3406	Tcf3	Transcription factor 3	1.000	2.013	1.030	0.978	29.362
Mm.4269	Tcf4	Transcription factor 4	1.000	1.078	1.056	1.102	3623.097

(continued)

Table 10. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.31630	Tcf7	Transcription factor 7, T-cell specific	1.000	1.819	0.589	1.063	32.497
Mm.171615	Tcf12	Transcription factor 12	1.000	1.368	0.845	1.303	501.344
Mm.3881	Tcf15	Transcription factor 15	1.000	0.664	1.608	1.806	616.577
Mm.11434	Tcf19	Transcription factor 19	1.000	2.139	0.855	0.917	168.466
Mm.252156	Tcf20	Transcription factor 20	1.000	0.646	0.758	0.818	128.112
Mm.178818	Tcf25	Transcription factor 25 (basic helix-loop-helix)	1.000	1.239	1.290	0.988	3559.424
Myogenic signalings—AKT/mTOR pathway							
Mm.6645	Akt1	Thymoma viral proto-oncogene 1	1.000	1.173	1.391	1.041	7800.907
Mm.177194	Akt2	Thymoma viral proto-oncogene 2	1.000	0.951	1.083	1.063	10,858.169
Mm.235194	Akt3	Thymoma viral proto-oncogene 3	1.000	1.096	1.037	0.886	707.138
Mm.245395	Pten	Phosphatase and tensin homolog	1.000	1.436	1.149	1.414	1191.963
Mm.30435	Tsc2	Tuberous sclerosis 2	1.000	0.845	0.930	0.983	46.584
Mm.319175	Rheb	Ras homolog enriched in brain	1.000	0.794	1.264	1.018	1578.323
Mm.21158	Mtor	Mechanistic target of rapamycin (serine/threonine kinase)	1.000	0.988	1.071	1.488	233.903
Myogenic signalings —Notch pathway							
Mm.290610	Notch1	Notch gene homolog 1 (*Drosophila*) (require for the stem cell commitment to its differentiation)	1.000	1.374	0.700	1.722	224.574
Mm.485843	Notch2	Notch gene homolog 2 (*Drosophila*)	1.000	1.190	0.900	1.148	1208.306
Mm.439741	Notch3	Notch gene homolog 3 (*Drosophila*)	1.000	1.029	0.848	1.016	1578.507
Mm.173813	Notch4	Notch gene homolog 4 (*Drosophila*)	1.000	1.823	1.160	1.120	225.537
Mm.4875	Dll1	Delta-like 1 (*Drosophila*)	1.000	1.060	0.781	0.619	122.125
Mm.1371	Pax3	Paired box gene 3	1.000	0.861	1.013	0.805	37.007
Mm.12926	Med1	Mediator complex subunit 1 (require for the stem cell commitment to its differentiation)	1.000	1.122	0.969	1.044	2164.906
Mm.485382	Med10	Mediator of RNA polymerase II transcription, subunit 10 homolog (NUT2, S. cerevisiae)	1.000	1.425	1.008	0.947	2103.597
Mm.46424	Med11	Mediator of RNA polymerase II transcription, subunit 11 homolog (S. cerevisiae)	1.000	2.271	0.950	1.089	598.854
Mm.20873	Med12	Mediator of RNA polymerase II transcription, subunit 12 homolog (yeast)	1.000	0.692	0.756	1.057	107.439
Mm.260089	Med16	Mediator complex subunit 16	1.000	1.249	1.182	1.348	1956.158
Mm.44151	Med17	Mediator complex subunit 17	1.000	0.936	1.040	1.074	299.416
Mm.219643	Med19	Mediator of RNA polymerase II transcription, subunit 19 homolog (yeast)	1.000	0.976	1.010	1.068	558.516
Mm.246493	Med24	Mediator complex subunit 24	1.000	0.598	1.148	0.683	381.934
Mm.235885	Med26	Mediator complex subunit 26	1.000	0.811	0.972	0.865	899.050
Mm.4645	Six1	Sine oculis-related homeobox 1 homolog (*Drosophila*)	1.000	0.573	1.125	0.979	4749.476
Mm.5039	Six2	Sine oculis-related homeobox 2 homolog (*Drosophila*)	1.000	0.623	1.208	0.721	830.434
Mm.249575	Six4	Sine oculis-related homeobox 4 homolog (*Drosophila*)	1.000	0.612	1.023	0.670	657.696
Mm.3410	Six5	Sine oculis-related homeobox 5 homolog (*Drosophila*)	1.000	1.587	1.108	1.099	212.273
Mm.485537	Nanog	Nanog homeobox	1.000	1.009	1.008	1.330	679.405
Mm.4325	Klf4	Kruppel-like factor 4 (gut)	1.000	0.880	0.890	1.333	2640.308
Mm.65396	Sox2	SRY-box containing gene 2	1.000	3.409	0.904	1.985	3.758

(continued)

Table 10. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.455819	Sox4	SRY-box containing gene 4	1.000	2.849	0.919	2.475	974.457
Mm.355478	Sox5	SRY-box containing gene 5	1.000	0.910	0.773	1.369	42,600.816
Mm.42162	Sox7	SRY-box containing gene 7	1.000	1.402	1.377	1.543	97.708
Mm.276739	Sox10	SRY-box containing gene 10	1.000	1.299	1.156	1.438	565.574
Mm.41702	Sox11	SRY-box containing gene 11	1.000	2.716	0.228	0.813	37.905
Mm.8575	Sox13	SRY-box containing gene 13	1.000	0.905	1.174	0.932	400.782
Mm.279103	Sox17	SRY-box containing gene 17	1.000	1.161	1.139	1.209	327.911
Mm.264904	Sox18	SRY-box containing gene 18	1.000	2.337	1.711	1.757	155.475
Mm.436572	Dock1	Dedicator of cytokinesis 1	1.000	1.410	0.986	1.297	658.575
Mm.380679	Dock2	Dedicator of cytokinesis 2	1.000	2.086	0.291	0.720	45.605
Mm.341423	Dock4	Dedicator of cytokinesis 4	1.000	0.886	0.745	1.064	80.490
Mm.258155	Dock5	Dedicator of cytokinesis 5	1.000	1.793	0.844	1.319	211.272
Mm.133473	Dock10	Dedicator of cytokinesis 10	1.000	1.925	0.647	1.412	195.548
Mm.32873	Dock11	Dedicator of cytokinesis 11	1.000	1.444	1.202	1.339	658.214
Mm.2444	Myc	c-Myc, myelocytomatosis oncogene	1.000	1.099	1.081	1.062	168.038
Mm.446553	Mycbp	c-myc binding protein	1.000	1.009	0.825	1.000	128.580
Mm.6478	Mycbp2	MYC binding protein 2	1.000	0.730	1.039	1.047	4.685
Neuronal signaling molecules, protein kinases, transcription factors							
Mm.2706	Atf3	Activating transcription factor 3	1.000	1.290	1.619	4.815	7651.993
Mm.143737	Ankrd2	Ankyrin repeat domain 2 (stretch responsive muscle)	1.000	1.197	1.662	1.210	19,016.723
Mm.218760	Pax7	Paired box gene 7	1.000	0.616	0.677	0.546	100.252
Mm.5035	Pax9	Paired box gene 9	1.000	1.827	1.330	1.326	34.343
Mm.24614	Prkce	Protein kinase C, epsilon	1.000	1.071	0.651	1.102	62.933
Mm.244393	Tank	TRAF family member-associated Nf-kappa B activator	1.000	1.010	1.129	1.270	164.076
Mm.196581	Mapk1	Mitogen-activated protein kinase 1	1.000	0.972	1.121	1.061	5022.614
Mm.8385	Mapk3	Mitogen-activated protein kinase 3	1.000	1.399	1.101	0.935	1891.409
Mm.480076	Mapk6	Mitogen-activated protein kinase 6	1.000	0.868	0.969	0.968	2009.583
Mm.38172	Mapk7	Mitogen-activated protein kinase 7	1.000	1.790	1.018	1.189	886.235
Mm.68933	Mapk9	Mitogen-activated protein kinase 9	1.000	0.763	1.060	1.081	1315.982
Mm.91969	Mapk11	Mitogen-activated protein kinase 11	1.000	1.033	0.961	0.996	180.155
Mm.38343	Mapk12	Mitogen-activated protein kinase 12	1.000	0.502	1.243	0.867	17,691.670
Mm.311337	Mapk14	Mitogen-activated protein kinase 14	1.000	0.946	1.041	1.128	5010.004
Mm.270866	Mapkap1	Mitogen-activated protein kinase associated protein 1	1.000	0.871	1.132	0.763	480.334
Mm.221235	Mapkapk2	MAP kinase-activated protein kinase 2	1.000	0.579	1.178	0.851	3768.003
Mm.272206	Mapkapk5	MAP kinase-activated protein kinase 5	1.000	0.698	1.297	1.161	8372.942
Mm.22413	Arhgap1	Rho GTPase-activating protein 1	1.000	1.463	1.006	1.186	1481.943
Mm.482337	Arhgap4	Rho GTPase-activating protein 4	1.000	2.000	0.822	1.506	137.316
Mm.128411	Arhgap8	Rho GTPase-activating protein 8	1.000	1.887	1.048	2.166	40.598
Mm.227198	Arhgap9	Rho GTPase-activating protein 9	1.000	2.305	0.507	1.307	674.657
Mm.217350	Arhgap15	Rho GTPase-activating protein 15	1.000	1.626	0.971	1.387	120.174
Mm.443529	Arhgap20	Rho GTPase-activating protein 20	1.000	0.424	1.130	1.059	1712.559
Mm.318350	Arhgap22	Rho GTPase-activating protein 22	1.000	1.687	1.037	1.269	742.576
Mm.119564	Arhgap25	Rho GTPase-activating protein 25	1.000	2.166	0.651	1.473	261.168
Mm.9935	Arhgap28	Rho GTPase-activating protein 28	1.000	0.552	1.465	0.952	189.197
Mm.46683	Arhgap32	Rho GTPase-activating protein 32	1.000	1.555	0.895	2.661	81.867
Mm.4081	Runx1	Runt-related transcription factor 1	1.000	2.163	0.853	1.228	623.365
Mm.322821	Scx	Scleraxis	1.000	0.858	0.936	0.966	6062.514
Mm.6645	Akt1	Thymoma viral proto-oncogene 1	1.000	1.173	1.391	1.041	7800.907
Mm.177194	Akt2	Thymoma viral proto-oncogene 2	1.000	1.082	1.494	1.053	387.451
Mm.235194	Akt3	Thymoma viral proto-oncogene 3	1.000	1.096	1.037	0.886	707.138

(continued)

Table 10. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.294664	Gsk3a	Glycogen synthase kinase 3 alpha	1.000	0.801	1.382	0.924	5055.916
Mm.394930	Gsk3b	Glycogen synthase kinase 3 beta	1.000	0.679	0.757	0.840	6365.856
Mm.21158	Mtor	Mechanistic target of rapamycin (serine/threonine kinase)	1.000	0.913	1.277	0.808	252.065
Mm.30963	Itpkc	Inositol 1,4,5-trisphosphate 3-kinase C	1.000	1.094	1.258	1.234	435.645
Mm.400954	Nras	Neuroblastoma ras oncogene	1.000	1.776	0.899	1.077	492.165
Mm.334313	Hras1	Harvey rat sarcoma virus oncogene 1	1.000	1.052	1.632	1.189	2335.527
Mm.400954	Nras	Neuroblastoma ras oncogene	1.000	0.852	0.766	0.776	688.566
Mm.383182	Kras	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	1.000	0.689	1.229	1.061	396.962
Mm.375031	Sykb	Spleen tyrosine kinase	1.000	1.611	0.597	0.840	491.071
Mm.293120	Stat2	Signal transducer and activator of transcription 2	1.000	1.397	0.717	1.323	692.397
Mm.249934	Stat3	Signal transducer and activator of transcription 3	1.000	1.374	0.843	1.061	5557.969
Mm.121721	Stat6	Signal transducer and activator of transcription 6	1.000	1.186	1.223	0.884	51.195
Mm.277403	Stat5a	Signal transducer and activator of transcription 5A	1.000	1.090	1.059	1.069	806.367
Mm.34064	Stat5b	Signal transducer and activator of transcription 5B	1.000	0.526	1.149	0.823	6529.135
Mm.246513	Fos	FBJ osteosarcoma oncogene	1.000	0.990	0.831	1.167	409.651
Mm.6215	Fosl1	Fos-like antigen 1	1.000	3.227	1.337	2.505	8.218
Mm.24684	Fosl2	Fos-like antigen 2	1.000	1.517	0.895	1.193	50.453
Mm.248335	Fosb	FBJ osteosarcoma oncogene B	1.000	1.159	0.939	1.434	4.432
Apoptosis signaling proteins							
Mm.257460	Bcl2	B-cell leukemia/lymphoma 2	1.000	1.094	1.126	0.839	131.776
Mm.4387	Bad	BCL2-associated agonist of cell death	1.000	1.628	1.074	1.139	4572.613
Mm.1051	Casp1	Caspase 1	1.000	1.615	0.712	1.194	514.638
Mm.3921	Casp2	Caspase 2	1.000	1.245	0.953	1.097	1550.184
Mm.34405	Casp3	Caspase 3	1.000	3.672	0.720	1.508	50.852
Mm.1569	Casp4	Caspase 4, apoptosis-related cysteine peptidase	1.000	1.877	1.197	1.788	832.388
Mm.281379	Casp6	Caspase 6	1.000	2.366	1.088	1.236	286.846
Mm.35687	Casp7	Caspase 7	1.000	1.221	0.932	1.011	182.985
Mm.336851	Casp8	Caspase 8	1.000	2.524	0.947	1.353	280.373
Mm.88829	Casp9	Caspase 9	1.000	1.456	0.819	0.994	756.757
Mm.20940	Casp14	Caspase 14	1.000	0.955	1.129	0.941	84.916
Mm.184163	Raf1	v-raf-leukemia viral oncogene 1	1.000	0.640	1.017	0.970	11,143.813
Mm.292510	Rac1	RAS-related C3 botulinum substrate 1	1.000	1.189	1.081	0.983	6019.936
Mm.1972	Rac2	RAS-related C3 botulinum substrate 2	1.000	2.562	0.413	0.818	238.192
Mm.34008	Rac3	RAS-related C3 botulinum substrate 3	1.000	2.965	1.033	1.487	191.182
Mm.287052	Tbx2	T-box 2	1.000	0.826	0.984	1.288	263.099
Mm.219139	Tbx3	T-box 3	1.000	0.653	1.029	0.982	2303.188
Mm.275336	Tbx4	T-box 4	1.000	0.843	0.901	0.768	588.571
Mm.727	Tbx6	T-box 6	1.000	1.008	1.079	1.188	215.448
Mm.246555	Tbx10	T-box 10	1.000	0.854	1.123	1.023	79.598
Mm.158789	Tbx18	T-box18	1.000	2.952	1.191	2.167	69.861
Mm.137011	Tbx22	T-box 22	1.000	1.670	0.708	2.709	3506.660

(continued)

Table 10. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Gene expressions involved in mirror-image pain and other noted molecules							
Mm.23253	Lpar2	LPA receptor 2	1.000	1.298	0.785	1.161	54.037
Mm.155520	Lpar3	LPA receptor 3	1.000	0.511	0.566	0.259	86.608
Mm.90147	Lpar4	LPA receptor 4	1.000	1.210	0.887	0.974	155.610
Mm.390681	Lpar6	LPA receptor 6	1.000	2.559	1.317	2.106	973.757
Mm.250256	Enpp2	Ectonucleotide pyrophosphatase/ phosphodiesterase 2, LPA synthase (from LPC)	1.000	0.453	1.287	1.129	4.303
Mm.211047	Lrrc16a	Leucine-rich repeat-containing 16A	1.000	1.686	0.765	1.264	362.520
Mm.302602	Gpnmb	Glycoprotein (transmembrane) nmb	1.000	3.180	0.559	1.462	637.196
Mm.220853	Gm1987	Predicted gene 1987	1.000	2.882	0.707	4.081	17.006
Mm.378888	Bcl2a1d	B-cell leukemia/lymphoma 2-related protein A1d	1.000	5.211	0.562	1.608	517.158
Mm.46301	Tyrobp	TYRO protein tyrosine kinase binding protein	1.000	4.840	0.625	1.364	3582.910
Mm.28520	Ski	Ski sarcoma viral oncogene homolog (avian)	1.000	1.139	0.862	0.716	4.305

Note: Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased more than 1.50-fold only two weeks after the operation: . Expression increased less than 0.66-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . Raw data, too low to be trusted: . w: week; Ope: operation; TRAF: TNF receptor (TNFR) associated factor; TYRO: tyrosine kinase; LPA: lysophosphatidic acid; LPC: lysophophatidyl choline; BCL: B-cell lymphoma; MAP: Mitogen-activated protein; MYC: Myelocytomatosis; SRY: sex-determining region Y; Nut 2: negative regulation of upstream regulatory sequence (NRS) 2; AKT: protein kinase B; mTOR: mammalian target of rapamycin; GLI: glioma-associated oncogene; MMTV: mouse mammary tumor virus; POU: the Pituitary-specific Pit-1, the Octamer transcription factor proteins Oct-1 and Oct-2, the neural Unc-86 transcription factor from Caenorhabditis elegans.

low (Tables 10 and 11), suggesting less significant involvement in the observed events.

Expression of genes for apoptosis signaling protein

A process of programmed cell death[50] should be stimulated in our mouse model. Expressions of genes encoding apoptosis signaling molecules are at adequate levels to induce signaling in both DRG and paw samples. However, some tissue-specific differences are evident. Significant changes in the expressions of more genes for apoptosis signaling molecules (*Casp1*, *3*, *4*, *6*, and *8*, and *Bad*, *Raf1*, *Rac2*, *3*, *Tbx3*, *18*, and *22*) are observed in the paw samples, while the expressions of fewer genes (*Casp3*, *Rac2*, *Tbx1*, *Tbx2*, and *Tbx3*) significantly change in the DRG samples after the operation (Tables 10 and 11). The results suggest the close involvement of apoptosis in both injury-induced fibrosis and the associated chronic pain, but the processes differ between paw tissues and the DRG.

Expression of genes involved in mirror-image pain

The data (Figure 3(a) and (b)) suggest that chronic pain could spread from the primary somatosensory cortex to other regions. It has been reported that nerve damage induces the production of lysophosphatidic acid (LPA) via the autotaxin (ATX)-mediated conversion of lysophophatidyl choline and LPA signals through six cognate G protein-coupled receptors (LPAR1-6). These receptors are expressed on most cells within the central and peripheral nervous tissues and are linked to many neural processes and pathways. LPA stimulates LPAR3 on activated microglia, resulting in a feed-forward LPA release that can activate LPAR1 on Schwann cells, which leads to the downregulation of myelin proteins, progressive demyelination, and the initiation of neuropathic pain.[51,52] Consistent with these reports, *Lpar1-6* and *Enpp2* (a gene encoding for ectonucleotide pyrophosphatase/phosphodiesterase family member 2, known as ATX) are highly expressed in the DRG samples and their levels remained constant following the operation. On the other hand, the expressions of these genes are low at onset in the paw samples and significantly change, with the expressions of *Lpar3* and *Enpp2* decreasing, after the operation (Tables 10 and 11).

Ohmichi et al.,[13] using the rat chronic postcast pain models, reported that activation of lumbar cord astrocytes is an important factor in widespread mechanical hyperalgesia and that Atf3 may be involved in this activation. Consistent with this suggestion, a >4-fold increase in Atf3 expression, in addition to its high expression levels, is observed one week and two weeks after the operation in DRG and paw samples, respectively. This suggests that Atf3 may also be an important

Table 11. Selected genes of signaling molecules and differentiation-involved molecules in the DRG (≥1.50).

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Molecules for muscle components							
Mm.121878	Tpm1	Tropomyosin 1, alpha	1.000	1.075	1.107	1.058	4204.390
Mm.646	Tpm2	Tropomyosin 2, beta	1.000	0.933	1.566	1.043	632.308
Mm.240839	Tpm3	Tropomyosin 3, gamma	1.000	1.000	0.978	0.966	27,377.762
Mm.295124	Tpm4	Tropomyosin 4	1.000	1.039	0.776	0.849	14,834.851
Mm.158289	Myh14	Myosin, heavy polypeptide 14	1.000	1.084	1.407	1.303	14,108.714
Mm.7353	Myl3	Myosin, light polypeptide 3	1.000	0.854	3.412	0.946	294.642
Mm.390355	Myl4	Myosin, light polypeptide 4	1.000	0.898	1.047	0.980	1875.792
Mm.238285	Myo1h	Myosin 1H	1.000	1.108	1.028	0.960	5697.960
Myogenic genes							
Mm.4984	Myf5	Myogenic factor 5	1.000	0.845	0.823	0.824	8.129
Mm.329100	Mdfi	MyoD family inhibitor	1.000	1.051	0.754	0.733	310.810
Mm.16528	Myog	Myogenin	1.000	0.878	1.059	0.856	7.578
Myogenic signalings—Wnt and hedgehog signaling pathways							
Mm.1123	Wnt1	Wingless-related MMTV integration site 1	1.000	1.132	1.057	0.968	709.310
Mm.57202	Shh	Sonic hedgehog	1.000	1.060	1.149	1.074	319.358
Mm.228798	Ptch1	Patched homolog 1	1.000	1.075	1.304	1.471	250.940
Mm.287037	Ptch2	Patched homolog 2	1.000	1.523	1.262	1.425	37.753
Mm.29279	Smo	Smoothened homolog (*Drosophila*)	1.000	0.907	0.960	0.918	3966.727
Mm.391450	Gli1	GLI-Kruppel family member GLI1	1.000	0.959	0.805	0.868	1942.966
Mm.273292	Gli2	GLI-Kruppel family member GLI2	1.000	1.275	4.071	1.616	17.227
Mm.5098	Gli3	GLI-Kruppel family member GLI3	1.000	0.856	0.800	1.004	159.735
Mm.246003	Fzd1	Frizzled homolog 1 (*Drosophila*)	1.000	1.082	1.312	1.294	1431.651
Mm.297906	Fzd7	Frizzled homolog 7 (*Drosophila*)	1.000	0.752	1.078	1.237	38.671
Mm.6256	Fzd9	Frizzled homolog 9 (*Drosophila*)	1.000	1.314	1.319	1.214	143.483
Mm.281691	Sfrp1	Secreted frizzled-related protein 1	1.000	1.045	1.172	1.129	695.010
Mm.42095	Sfrp4	Secreted frizzled-related protein 4	1.000	0.964	0.857	0.890	9175.229
Mm.470071	Sfrp5	Secreted frizzled-related sequence protein 5	1.000	0.925	0.990	0.994	26,854.572
Mm.294664	Gsk3a	Glycogen synthase kinase 3 alpha	1.000	1.096	1.248	1.171	14,844.347
Mm.394930	Gsk3b	Glycogen synthase kinase 3 beta	1.000	1.265	1.216	1.333	24,899.932
Mm.3406	Tcf3	Transcription factor 3	1.000	1.156	1.198	1.330	76.983
Mm.4269	Tcf4	Transcription factor 4	1.000	0.964	1.012	1.039	1891.414
Mm.31630	Tcf7	Transcription factor 7, T-cell specific	1.000	1.121	1.118	1.216	32.440
Mm.171615	Tcf12	Transcription factor 12	1.000	1.038	1.020	0.899	757.978
Mm.3881	Tcf15	Transcription factor 15	1.000	1.190	1.178	0.807	45.012
Mm.11434	Tcf19	Transcription factor 19	1.000	1.106	0.971	0.803	513.015
Mm.252156	Tcf20	Transcription factor 20	1.000	1.053	1.173	1.181	4627.177
Mm.178818	Tcf25	Transcription factor 25 (basic helix-loop-helix)	1.000	1.179	1.089	1.130	12,271.728
Myogenic signalings—AKT/mTOR pathway							
Mm.6645	Akt1	Thymoma viral proto-oncogene 1	1.000	1.134	1.222	1.198	26,537.600
Mm.177194	Akt2	Thymoma viral proto-oncogene 2	1.000	1.037	1.134	1.206	7255.185
Mm.235194	Akt3	Thymoma viral proto-oncogene 3	1.000	1.141	0.943	1.061	275.333
Mm.177194	Akt2	Thymoma viral proto-oncogene 2	1.000	0.989	1.067	1.079	6844.500
Mm.245395	Pten	Phosphatase and tensin homolog	1.000	1.072	1.142	1.130	9874.612
Mm.30435	Tsc2	Tuberous sclerosis 2	1.000	1.175	1.553	1.146	64.975
Mm.319175	Rheb	Ras homolog enriched in brain	1.000	0.910	0.900	0.909	5776.891
Mm.21158	Mtor	Mechanistic target of rapamycin (serine/threonine kinase)	1.000	1.103	1.380	1.221	1016.137
Myogenic signalings—Notch pathway							
Mm.290610	Notch1	Notch gene homolog 1 (*Drosophila*) (require for the stem cell commitment to its differentiation)	1.000	1.651	0.792	0.952	239.918

(continued)

Table 11. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.485843	Notch2	Notch gene homolog 2 (*Drosophila*)	1.000	0.907	1.044	1.098	1563.774
Mm.439741	Notch3	Notch gene homolog 3 (*Drosophila*)	1.000	1.113	1.264	1.215	616.700
Mm.173813	Notch4	Notch gene homolog 4 (*Drosophila*)	1.000	0.921	1.003	1.042	348.427
Mm.4875	Dll1	Delta-like 1 (*Drosophila*)	1.000	0.961	0.761	0.856	38.766
Mm.12926	Med1	Mediator complex subunit 1	1.000	1.050	1.021	0.961	2055.559
Mm.24159	Med7	Mediator complex subunit 7	1.000	0.958	0.857	0.903	3072.517
Mm.46424	Med11	Mediator of RNA polymerase II transcription, subunit 11 homolog (S. cerevisiae)	1.000	0.937	0.848	0.808	4209.490
Mm.20873	Med12	Mediator of RNA polymerase II transcription, subunit 12 homolog (yeast)	1.000	1.301	1.174	1.487	144.602
Mm.208970	Med15	Mediator complex subunit 15	1.000	1.307	1.508	1.555	3634.469
Mm.44151	Med17	Mediator complex subunit 17	1.000	0.874	0.967	0.885	779.204
Mm.219643	Med19	Mediator of RNA polymerase II transcription, subunit 19 homolog (yeast)	1.000	1.054	1.012	0.965	2496.371
Mm.283045	Med28	Mediator of RNA polymerase II transcription, subunit 28 homolog (yeast)	1.000	1.096	1.223	1.161	314.683
Mm.4645	Six1	Sine oculis-related homeobox 1 homolog (*Drosophila*)	1.000	1.086	1.247	1.319	4771.977
Mm.249575	Six4	Sine oculis-related homeobox 4 homolog (*Drosophila*)	1.000	0.897	1.203	1.143	2073.919
Mm.3410	Six5	Sine oculis-related homeobox 5 homolog (*Drosophila*)	1.000	0.763	0.770	0.814	443.102
Mm.485537	Nanog	Nanog homeobox	1.000	0.991	1.368	1.395	173.754
Mm.4325	Klf4	Kruppel-like factor 4 (gut)	1.000	0.823	0.805	0.806	859.743
Mm.65396	Sox2	SRY-box containing gene 2	1.000	1.045	1.157	1.020	306.970
Mm.35784	Sox3	SRY-box containing gene 3	1.000	1.441	1.717	1.362	199.971
Mm.455819	Sox4	SRY-box containing gene 4	1.000	1.108	1.250	1.171	2418.596
Mm.355478	Sox5	SRY-box containing gene 5	1.000	1.119	0.971	1.053	43,109.082
Mm.42162	Sox7	SRY-box containing gene 7	1.000	1.970	1.236	1.083	53.506
Mm.258220	Sox8	SRY-box containing gene 8	1.000	0.899	0.961	0.907	172.529
Mm.276739	Sox10	SRY-box containing gene 10	1.000	0.920	1.046	1.024	5970.493
Mm.41702	Sox11	SRY-box containing gene 11	1.000	2.915	1.005	1.407	266.525
Mm.8575	Sox13	SRY-box containing gene 13	1.000	1.192	0.919	0.909	535.987
Mm.279103	Sox17	SRY-box containing gene 17	1.000	0.841	0.828	0.944	423.220
Mm.264904	Sox18	SRY-box containing gene 18	1.000	0.963	0.849	0.931	181.857
Mm.70950	Sox21	SRY-box containing gene 21	1.000	1.407	1.060	1.230	96.658
Mm.436572	Dock1	Dedicator of cytokinesis 1	1.000	0.972	1.102	1.096	1152.460
Mm.258155	Dock5	Dedicator of cytokinesis 5	1.000	1.129	1.115	1.153	3850.394
Mm.128153	Dock6	Dedicator of cytokinesis 6	1.000	0.894	0.959	1.065	2867.670
Mm.260623	Dock7	Dedicator of cytokinesis 7	1.000	1.150	1.175	1.073	208.271
Mm.133473	Dock10	Dedicator of cytokinesis 10	1.000	0.808	0.877	0.859	1910.556
Mm.32873	Dock11	Dedicator of cytokinesis 11	1.000	0.983	1.091	1.044	9900.318
Mm.360004	Sos1	Son of sevenless homolog 1 (*Drosophila*)	1.000	1.346	1.412	0.921	297.030
Mm.3770	Sos2	Son of sevenless homolog 2 (*Drosophila*)	1.000	0.587	1.051	0.754	1545.853
Mm.16469	Mycn	v-myc myelocytomatosis viral-related oncogene, neuroblastoma derived (avian)	1.000	1.312	1.330	1.306	530.555
Mm.2444	Myc	c-Mic. myelocytomatosis oncogene	1.000	1.099	1.081	1.062	508.955
Mm.6478	Mycbp2	MYC binding protein 2	1.000	1.040	1.166	1.256	3799.196

(continued)

Table 11. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.446553	Mycbp	c-myc binding protein	1.000	0.643	0.608	0.647	650.678
Neuronal signaling molecules, protein kinases, transcription factors							
Mm.2706	Atf3	Activating transcription factor 3	1.000	4.188	0.514	1.190	2502.960
Mm.143737	Ankrd2	Ankyrin repeat domain 2 (stretch responsive muscle)	1.000	0.458	0.720	0.385	173.506
Mm.16469	Mycn	v-myc myelocytomatosis viral-related oncogene, neuroblastoma derived (avian)	1.000	1.312	1.330	1.306	530.555
Mm.2444	Myc	Myelocytomatosis oncogene	1.000	1.099	1.081	1.062	508.955
Mm.6478	Mycbp2	MYC binding protein 2	1.000	1.040	1.166	1.256	3799.196
Mm.446553	Mycbp	c-myc binding protein	1.000	0.643	0.608	0.647	650.678
Mm.480076	Mapk6	Mitogen-activated protein kinase 6	1.000	1.339	0.968	1.164	113.328
Mm.480076	Mapk6	Mitogen-activated protein kinase 6	1.000	1.284	1.111	1.198	1847.275
Mm.311337	Mapk14	Mitogen-activated protein kinase 14	1.000	1.197	1.168	1.223	5830.189
Mm.196581	Mapk1	Mitogen-activated protein kinase 1	1.000	1.016	1.039	1.055	19,639.100
Mm.8385	Mapk3	Mitogen-activated protein kinase 3	1.000	1.053	1.190	1.140	73,230.850
Mm.254517	Mapk4	Mitogen-activated protein kinase 4	1.000	0.956	1.217	1.054	377.634
Mm.480076	Mapk6	Mitogen-activated protein kinase 6	1.000	1.284	1.111	1.198	1847.275
Mm.38172	Mapk7	Mitogen-activated protein kinase 7	1.000	1.094	1.071	1.075	4541.513
Mm.21495	Mapk8	Mitogen-activated protein kinase 8	1.000	0.841	0.898	0.977	372.046
Mm.68933	Mapk9	Mitogen-activated protein kinase 9	1.000	1.050	1.143	1.152	12,266.253
Mm.39253	Mapk10	Mitogen-activated protein kinase 10	1.000	0.961	0.992	0.940	50,159.950
Mm.91969	Mapk11	Mitogen-activated protein kinase 11	1.000	1.070	1.268	1.215	5136.187
Mm.38343	Mapk12	Mitogen-activated protein kinase 12	1.000	0.895	0.972	1.019	7628.115
Mm.27970	Mapk13	Mitogen-activated protein kinase 13	1.000	1.104	0.950	0.974	403.822
Mm.311337	Mapk14	Mitogen-activated protein kinase 14	1.000	1.197	1.168	1.223	5830.189
Mm.43081	Mapk8ip3	Mitogen-activated protein kinase 8 interacting protein 3	1.000	1.182	1.282	1.229	85,576.766
Mm.270866	Mapkap1	Mitogen-activated protein kinase associated protein 1	1.000	0.988	1.013	0.965	1321.612
Mm.221235	Mapkapk2	MAP kinase-activated protein kinase 2	1.000	1.109	1.041	1.031	1171.873
Mm.272206	Mapkapk5	MAP kinase-activated protein kinase 5	1.000	0.975	0.958	0.970	4600.907
Mm.22413	Arhgap1	Rho GTPase-activating protein 1	1.000	1.265	1.225	1.194	4766.664
Mm.443529	Arhgap20	Rho GTPase-activating protein 20	1.000	1.052	1.271	1.284	1365.274
Mm.46683	Arhgap32	Rho GTPase-activating protein 32	1.000	1.131	1.218	1.174	368.116
Mm.480450	Arhgap36	Rho GTPase-activating protein 36	1.000	1.147	1.396	1.329	3983.115
Mm.322931	Arhgap39	Rho GTPase-activating protein 39	1.000	1.053	1.150	1.125	14,519.543
Mm.134338	Arhgap44	Rho GTPase-activating protein 44	1.000	1.129	1.293	1.208	8469.994
Mm.322821	Scx	Scleraxis	1.000	0.858	0.936	0.966	4856.714
Mm.391013	Runx2	Runt-related transcription factor 2	1.000	2.078	1.665	1.781	66.117
Mm.378894	Runx3	Runt-related transcription factor 3	1.000	1.474	1.907	1.585	90.270
Mm.6645	Akt1	Thymoma viral proto-oncogene 1	1.000	1.134	1.222	1.198	26,537.600
Mm.177194	Akt2	Thymoma viral proto-oncogene 2	1.000	1.037	1.134	1.206	7255.185
Mm.235194	Akt3	Thymoma viral proto-oncogene 3	1.000	1.141	0.943	1.061	275.333
Mm.294664	Gsk3a	Glycogen synthase kinase 3 alpha	1.000	1.096	1.248	1.171	14,844.347
Mm.394930	Gsk3b	Glycogen synthase kinase 3 beta	1.000	1.265	1.216	1.333	24,899.932
Mm.21158	Mtor	Mechanistic target of rapamycin (serine/threonine kinase)	1.000	1.103	1.380	1.221	1016.137
Mm.30963	Itpkc	Inositol 1,4,5-trisphosphate 3-kinase C	1.000	1.148	1.079	1.014	574.319
Mm.257460	Bcl2	B-cell leukemia/lymphoma 2	1.000	1.081	1.063	0.961	1319.177
Mm.2045	Mras	Muscle and microspikes RAS	1.000	1.123	1.138	1.039	696.835
Mm.291120	Fras1	Fraser syndrome 1 homolog (human)	1.000	1.238	1.281	1.490	116.569

(continued)

Table 11. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.400954	Nras	Neuroblastoma ras oncogene	1.000	1.108	0.987	0.981	1961.214
Mm.334313	Hras1	Harvey rat sarcoma virus oncogene 1	1.000	1.064	1.081	0.979	16,036.868
Mm.389894	Rras	Harvey rat sarcoma oncogene, subgroup R	1.000	1.084	1.024	1.024	8248.441
Mm.383182	Kras	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	1.000	0.930	0.917	0.928	3219.141
Mm.375031	Sykb	Spleen tyrosine kinase	1.000	0.720	0.623	0.581	693.808
Mm.277406	Stat1	Signal transducer and activator of transcription 1	1.000	1.107	1.109	1.013	266.162
Mm.293120	Stat2	Signal transducer and activator of transcription 2	1.000	1.134	1.251	1.315	4399.081
Mm.249934	Stat3	Signal transducer and activator of transcription 3	1.000	1.344	1.347	1.272	19,239.986
Mm.277403	Stat5a	Signal transducer and activator of transcription 5A	1.000	1.316	1.119	1.179	471.345
Mm.34064	Stat5b	Signal transducer and activator of transcription 5B	1.000	1.141	1.254	1.196	3602.220
Mm.121721	Stat6	Signal transducer and activator of transcription 6	1.000	0.986	1.055	0.982	2848.014
Mm.6215	Fosl1	Fos-like antigen 1	1.000	1.927	0.518	0.922	15.472
Mm.24684	Fosl2	Fos-like antigen 2	1.000	1.152	0.861	0.918	924.243
Mm.248335	Fosb	FBJ osteosarcoma oncogene B	1.000	1.088	1.302	1.112	118.685
Apoptosis signaling proteins							
Mm.257460	Bcl2	B-cell leukemia/lymphoma 2	1.000	1.081	1.063	0.961	1319.177
Mm.474472	Bcl2l14	BCL2-like 14 (apoptosis facilitator)	1.000	0.719	0.934	1.084	262.856
Mm.4387	Bad	BCL2-associated agonist of cell death	1.000	0.967	0.941	0.889	15,246.985
Mm.1051	Casp1	Caspase 1	1.000	0.978	0.713	0.733	3164.117
Mm.3921	Casp2	Caspase 2	1.000	0.777	0.772	0.747	2703.238
Mm.34405	Casp3	Caspase 3	1.000	1.691	1.018	1.007	92.956
Mm.1569	Casp4	Caspase 4	1.000	0.892	0.787	0.941	875.289
Mm.35687	Casp7	Caspase 7	1.000	1.083	1.106	1.191	133.239
Mm.336851	Casp8	Caspase 8	1.000	0.810	0.858	0.806	306.155
Mm.88829	Casp9	Caspase 9	1.000	1.053	1.145	1.130	7396.020
Mm.42163	Casp12	Caspase 12	1.000	0.783	0.901	0.768	242.640
Mm.184163	Raf1	v-raf-leukemia viral oncogene 1	1.000	1.029	1.066	1.134	13,304.687
Mm.292510	Rac1	RAS-related C3 botulinum substrate 1	1.000	1.134	1.217	1.202	16,666.912
Mm.1972	Rac2	RAS-related C3 botulinum substrate 2	1.000	0.648	0.525	0.484	793.589
Mm.34008	Rac3	RAS-related C3 botulinum substrate 3	1.000	1.169	1.089	1.026	5387.126
Mm.295194	Tbx1	T-box 1	1.000	0.636	0.764	0.928	195.369
Mm.287052	Tbx2	T-box 2	1.000	1.340	1.366	1.520	1617.514
Mm.219139	Tbx3	T-box 3	1.000	1.181	1.538	1.465	2199.175
Mm.727	Tbx6	Rac2, T-box	1.000	0.776	0.488	0.747	262.328
Mm.88761	Tbx15	T-box 15	1.000	0.753	1.281	1.015	165.204
Mm.137011	Tbx22	T-box 22	1.000	1.331	0.629	1.054	579.177
Gene expressions involved in mirror-image pain and other noted molecules							
Mm.4772	Lpar1	Lysophosphatidic acid receptor 1	1.000	1.036	0.837	0.959	415.523
Mm.155520	Lpar3	Lysophosphatidic acid receptor 3	1.000	0.935	1.253	1.223	2374.570
Mm.90147	Lpar4	Lysophosphatidic acid receptor 4	1.000	1.095	1.032	1.050	210.419
Mm.333386	Lpar5	Lysophosphatidic acid receptor 5	1.000	1.052	1.251	1.379	70.810
Mm.390681	Lpar6	Lysophosphatidic acid receptor 6	1.000	0.837	0.796	0.876	2500.294

(continued)

Table 11. Continued.

UniGeneID	Gene symbol	Gene name	Sham-1w	Ope-1w	Sham-2w	Ope-2w	Raw (Sham-1w)
Mm.250256	Enpp2	Ectonucleotide pyrophosphatase/ phosphodiesterase 2	1.000	0.829	0.877	0.889	4597.964
Mm.290677	Lrrc15	Leucine-rich repeat-containing 15	1.000	2.604	0.879	1.118	53.711
Mm.428639	Lrrc57	Leucine-rich repeat-containing 57	1.000	0.897	0.970	0.891	1492.573
Mm.33498	Lrrc33	Leucine-rich repeat-containing 33	1.000	0.789	0.831	0.815	1226.373
Mm.151577	Lrrc16b	Leucine-rich repeat-containing 16B	1.000	1.035	1.189	1.149	1137.193
Mm.211047	Lrrc16a	Leucine-rich repeat-containing 16A	1.000	0.527	0.425	0.566	169.810
Mm.46301	Tyrobp	TYRO protein tyrosine kinase binding protein	1.000	0.876	0.614	0.615	3863.647
Mm.28520	Ski	Ski sarcoma viral oncogene homolog (avian)	1.000	0.688	0.786	0.653	211.132

Note: Blue number: expression fold more than 1.50. Red number: expression fold less than 0.66. Expression increased more than 1.50-fold only one week after the operation: . Expression increased more than 1.50-fold only two weeks after the operation: . Expression increased less than 0.66-fold only one week after the operation: . Expression increased less than 0.66-fold only two weeks after the operation: . Raw data, too low to be trusted: . w: week; Ope: operation; AKT: protein kinase B; mTOR: mammalian target of rapamycin; TYRO: tyrosine kinase; BCL: B-cell lymphoma; MAP: Mitogen-activated protein; Myc: myelocytomatosis; SRY: sex-determining region Y; RAS: Rat sarcoma; MMTV: mouse mammary tumor virus; GLI: glioma-associated oncogene.

signaling molecule involved in the observed spreading of chronic pain.

Other genes with large changes in expression one and two weeks after the operation

Lrrc16a is a member of the leucine-rich repeat-containing family of proteins that have functions in diverse biological pathways.[53] The expression of *Lrrc16a* is reduced by half in the DRG samples but is increased 1.7-fold in the paw samples after the surgery (Tables 10 and 11), suggesting some involvement in the present events. *Gpnmb* which encodes the non-metastatic gene B (NMB) transmembrane glycoprotein has been characterized as osteoactivin in mice.[54] Significant and increased expression of *Gpnmb* after the operation is detected only in the paw samples (Tables 10 and 11), which is consistent with previous studies demonstrating high *Gpnmb* expression during chondrogenesis and osteogenesis.[55] *Tyrobp* which encodes the TYRO protein tyrosine kinase (SYK)-binding protein is a transmembrane signaling polypeptide that contains an immune-receptor tyrosine-based activation motif in its cytoplasmic domain and may bind zeta-chain-associated protein kinase 70 kDa (ZAP-70) and SYK to facilitate signal transduction in bone modeling, brain myelination, and inflammation.[56] It is notable that the expression of *Tyrobp* is increased almost five-fold one week after the operation in the paw samples (Tables 10 and 11).

General discussion

Our findings using a mouse model confirm that persistent postoperative pain can be established through extensive injury of peripheral tissues. Microarray analyses have revealed that hundreds of genes were involved in the operation-induced fibrosis and the accompanying chronic pain, which lead to muscle fibrosis and associated hyperalgesia. Based on an extensive literature search for molecules implicated in tissue fibrosis and chronic pain, we initially focused on gene expressions related to molecules involved in the syntheses, degradation, and regulation of tissue ECM and pain-associated molecules. Our focus subsequently broadened to include molecules involved in the related signaling pathways and molecules related to transcriptional and translational regulation.

The extent of the gene expression changes and the raw gene expression values can provide an estimate of their involvement in these phenomena. Thus, this report could be the first study to semiquantitatively and comprehensively examine the actual molecules and signal transduction pathways that are implicated in injury of peripheral tissues, subsequently inducing scar formation and chronic pain.

The present study might help elucidate the mechanism of DRG participation in the processes by which the injury of the peripheral tissues sensitizes the CNS to perceive pain. We originally expected that the expression of many genes in the DRG would change, accompanied by a change in the gene expression in the injured tissues. Interestingly, although the expression levels of many genes had not changed, the expression levels of some of the genes in the DRG samples did change (see ECM-related genes in Tables 6 and 7, and see pain-related molecule genes in Table 9). However, it should be noted that there were many cases where significant changes in gene expression occurred in the one-week DRG samples. It should be also noted that higher gene expression levels of pain-related molecules were

detected in DRGs, although there were no remarkable changes in the gene expression levels of DRGs after the injury. These data suggest that DRGs can function to mediate neuronal pulse and signals between the peripheral nerve system in the injured tissues and the brain CNS without long-term gene expression changes.

The results obtained in this study indicate that hundreds of genes are involved in and interacted with to result in chronic pain and hyperalgesia, which are caused by tissue fibrosis. Recently developed computer-based comprehensive analyses, such as bioinformatics,[57,58] may delineate possible signaling pathways and functional molecules implicated in the above phenomena. In addition, the information gathered may be used to conduct further studies using genetically modified mice, including gene-targeting mice.

Before concluding, it should be noted that we observed similar behavioral and molecular changes in some samples after the sham operations. These changes were significant, albeit less than the changes observed in the operation group, and suggest that the insertion of a 19G needle may itself cause scar formation and chronic pain. Thus, the comparison of the expression levels between the sham and operation groups should be made with prudent consideration. In addition, the gene expression data presented here do not necessarily reflect the protein expression levels. Therefore, protein expressions of the potential genes involved should be confirmed prior to final conclusions.

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Authors' Contributions

YL and HI contributed to the experiments equally and are listed as cofirst authors, and YL is taking this study forward. TU and KK conceived and coordinated the study and directed the experiments. KK, AO, and LZ wrote and edited the paper. KK and LZ provided technical support for the immunohistochemical and biochemical experiments. AO provided technical information and support for the microarray and contributed to the analysis of the microarray results. MD, XY, and SK evaluated the experiments and the clinical information. All authors reviewed the results and approved the final version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

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