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Differential Spinal Cord Gene Expression in Rodent Models of Radicular and Neuropathic Pain

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Background: **Neuropathic pain and radicular low back pain both have a major impact on human health worldwide. Microarray gene analysis on central nervous system tissues holds great promise for discovering novel targets for persistent pain modulation.**

Methods: **Rat models of lumbar radiculopathy (L5 nerve root ligation) and neuropathy (L5 spinal nerve transection) were used for these studies. The authors measured mechanical allodynia followed by analysis of global gene expression in the lumbar spinal cord at two time points (7 days and 14 days) after surgery using the Affymetrix RAE230A GeneChip® (Santa Clara, CA). The expression patterns of several genes of interest were subsequently confirmed using real-time reverse transcriptase polymerase chain reaction.**

Results: **The authors observed similarly robust mechanical allodynia in both models. Second, they observed significant differences in lumbar spinal cord gene expression across chronic pain models. There was little overlap between genes altered in each injury model, suggesting that the site and type of injury produce distinct spinal mechanisms mediating the observed mechanical allodynia. The authors further confirmed a subset of the genes using reverse transcriptase polymerase chain reaction and identified several genes as either neuropathy-associated genes or radiculopathy-associated genes.**

Conclusions: **These two models of persistent pain produce similar allodynic outcomes but produce differential gene expression. These results suggest that diverging mechanisms lead to a common behavioral outcome in these pain models. Furthermore, these distinct pathophysiologic mechanisms in neuropathic *versus* radicular pain may implicate unique drug therapies for these types of chronic pain syndromes.**

ONE form of persistent pain, chronic low back, affects more than two thirds of the Western population at any given time,[1] and a recent study[2] suggests that approximately half of these patients experience a neuropathic element to their low back pain. Furthermore, the healthcare cost of painful neuropathic disorders, including diabetic neuropathy, causalgia, and back or neck pain with neuropathic involvement, has been estimated to total threefold more than matched controls.[3] Clinically, patients present with pain of multiple etiologies and varying symptoms; differential responses to treatment are therefore common and not unexpected. The development and introduction of novel analgesics guided by a refined knowledge of the pathogenesis of pain is dire.

Pain researchers have developed several animal models mimicking chronic pain-like behaviors to study the cellular and molecular correlates of aberrant pain transmission. To date, most pain research using animal models has focused on injury to a peripheral nerve, usually the sciatic or spinal nerve [4–9]. In addition, specific animal models of nerve root or radicular pain have also been developed.[10–13] Although Wall and Devor[14] and Kawakami *et al.*[11,13] have postulated the importance of the location of a nerve injury to the severity of clinical symptoms, there have been few studies comparing nerve lesions central to the dorsal root ganglion (DRG) *versus* peripheral. Root lesions central to the DRG lead to a different sequence of events as compared with lesions of peripheral nerves[14]; therefore, it is imperative to investigate the unique pathophysiologic changes that occur after injury to these distinct sites.

In recent years, the development of high-density microarray technology has allowed investigators to determine the expression levels of several thousand messenger RNA (mRNA) transcripts concurrently.[15,16] The utility of these assays in identifying candidate genes involved in the development of chronic pain syndromes has been proposed.[17,18] Specifically, gene expression patterns after peripheral nerve injury in rats has been investigated in the DRG[19,20] and spinal cord,[21–23] as well as in peripheral nerve stumps after sciatic axotomy[24] and spinal cord after spinal cord injury–inducing central pain.[25] Taken together, these studies have identified several hundred candidate genes, many of which have yet to be confirmed by secondary assays, which may help to describe the complex cellular and molecular cascade triggered by nerve injury.

Here we describe the use of two of these models: L5 nerve root ligation (LR), a central radicular injury with an inflammatory component, and L5 spinal nerve transection (L5tx), a peripheral nerve injury. Previously, we have demonstrated that these models differ in their re-

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sponses to drug therapy; specifically, radicular pain has been previously shown to be resistant to a cytokine antagonist cocktail (interleukin-1 receptor antagonist and soluble tumor necrosis factor receptor), whereas in a peripheral neuropathic pain model, this cocktail was effective in reducing tactile allodynia.[26] These findings led us to question whether peripherally induced neuropathic and centrally induced radicular pain produced differential spinal cord gene expression.

Materials and Methods

Animals

Male Sprague-Dawley (Hsd:Sprague-Dawley) rats used in this study were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and were approximately 8 weeks of age at the time of surgery. All animals were housed one per cage under US Department of Agriculture and Association for Assessment and Accreditation of Laboratory Animal Care International–approved conditions with a 12:12-h light–dark cycle and free access to food and tap water. Care was taken to minimize animal discomfort and to limit the number of animals used. All experimental procedures were approved by the Dartmouth College Institutional Animal Care and Use Committee (Lebanon, New Hampshire). All surgical procedures were performed during inhalation anesthesia induced with 4% gaseous halothane in oxygen and maintained at 1.5–2%.

Surgical Procedures

Animals were divided into two groups according to the site of injury used: (1) a group of rats (n = 2/time point) that received a unilateral mononeuropathy by transection of the L5 spinal nerve (L5tx) distal to the L5 dorsal root ganglion,[27–29] or (2) a group of rats (n = 2/time-point) that received an experimental lumbar radiculopathy (LR) with loose ligation of the dorsal and ventral L5 nerve roots with 5-0 chromic gut suture along with placement of 5 pieces (3–5 mm in length) of 4-0 chromic gut on the nerve roots.[10,30] Two sham groups (n = 2/group) were prepared by performing the surgical procedures without either nerve transection or nerve root ligation.

Mechanical Allodynia

The development of mechanical allodynia was assessed using 2-g (20.02 mN) and 12-g (115.26 mN) calibrated von Frey filaments (Stoelting, Wood Dale, IL) by a single investigator blinded to the surgical status of the rats. In each testing session, the animals were subjected to 30 tactile stimulations to the plantar surface of the ipsilateral (nerve-injured) hind paw in the region of the L5 innervation. Mechanical allodynia was assessed by recording the total number of responses elicited during three successive trials (10 stimulations/trial, 30 stimulations total) separated by at least 10 min. The resulting data were then expressed as the percent responses out of total possible responses. Animals were acclimated to the testing procedure twice before the surgical procedure to record baseline values. Any animals displaying abnormal baseline paw withdrawals were excluded from the study.

Tissue Isolation and RNA Preparation

Rats were quickly killed by carbon dioxide asphyxiation followed by decapitation. The spinal cord was then quickly removed, rinsed in phosphate-buffered saline (PBS), and immediately placed on dry ice. Total RNA was prepared from the lumbar region of the spinal cord of the sample using the TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocol. The crude RNA was further purified using RNeasy columns (Qiagen Inc., Valencia, CA) according to manufacturer's RNA cleanup protocol. Spectrophotometric analysis was used to quantify RNA concentration and to assess sample purity.

Affymetrix GeneChip® Microarray Protocol

Affymetrix RAE230A GeneChip® microarrays (Santa Clara, CA) containing 4,699 known rat sequences, 700 nonexpressed sequence tags and 10,467 expressed sequence tags, were used for these experiments. Approximately 5 μg of starting RNA was used for each sample. The double-stranded complementary DNA (cDNA), created using SuperScript II (Invitrogen) reverse transcriptase, was used as a template for an *in vitro* transcription reaction using biotin-labeled nucleotides to synthesize the labeled complementary RNA samples to be hybridized to the microarrays (BioArray HighYield RNA Transcript Labeling Kit [T7], Cambridge BioScience, Cambridge, United Kingdom). A second purification procedure was performed and the labeled RNA was fragmented at 94°C for 35 min. For each sample, hybridization cocktails were assembled, including 20 μg of fragmented biotin-labeled RNA and hybridization controls as established by Affymetrix. Two hundred microliters of hybridization cocktail, containing 15 μg of probe, was loaded onto each GeneChip® and placed in a 45°C rotisserie at 60 rpm for 16 h to hybridize (GeneChip® Hybridization Oven 640). After the hybridization step, the Affymetrix Fluidics Station 400 was used to administer streptavidin-phycoerythrin and antibody stains to the microarrays. The individual GeneChips® were then scanned using the Agilent GeneArray Scanner.

GeneChip® Normalization and Expression Level Analysis

The individual raw image.DAT files from each individual microarray were normalized to the group median intensity using DNA-Chip Analyzer software (dChip ver-

sion 1.3#) as described previously.[31,32] After normalization, the individual expression levels for each gene were calculated using the probe match–mismatch (PM-MM) model–based expression index. The calculated expression levels for each gene were then used in the subsequent statistical analyses to determine which genes from each experimental GeneChip® were differentially expressed as compared with control GeneChips®.

Microarray Data Analysis

In this study, the treatment groups were 7 days postinjury, 14 days postinjury, and 7 days post–sham surgical control, with three gene expression comparisons of interest (7 days–sham, 14 days–sham, and 14 days–7 days). With these three comparisons for each of the two groups defined by the different nerve injury models (L5 nerve root ligation or L5 spinal nerve transection), a total of six separate analyses were performed to identify outliers. Two animals were used for each treatment. The stability of scores between animals in each treatment group was evaluated using the Cronbach α internal consistency reliability index as well as a test–retest reliability estimate. Extremely high reliability estimates supported the decision to average the gene expression scores for each group. For the linear regression model, the target group (*e.g.*, 7 days post–L5 nerve root ligation) was regressed on the reference group (*e.g.*, sham) and a confidence interval around the prediction of Y (7 days post–L5 nerve root ligation) from X (sham) established. The target gene was then defined to be an outlier if the observed score (Y) was outside of the confidence interval. In this study, with 15,866 probe sets being examined, a 99% confidence interval around the regression line was chosen as the basis for defining a discrepancy between the predicted and observed gene expression sufficiently large to suggest differential gene expression. All analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Quantitative Real-time RT-PCR

The purified total RNA from the lumbar spinal cords from a replicate surgical experiment (n = 3/group) Sprague-Dawley rats was reverse transcribed to cDNA using the Superscript III First Strand Synthesis kit (Invitrogen). The resulting cDNA was diluted to 10 ng/μl. Primers for rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH), decorin (DCN), aquaporin (AQP4), insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2), prostaglandin D2 synthase (PTGDS), insulin-like growth factor binding protein 3 (IGFBP3), CD74 antigen (HLADG), glial fibrillary acidic protein (GFAP), and integrin-αM (ITGAM) were designed according to our previously published protocol[33] and are shown in supplementary table 1 (additional information regarding this is available on the ANESTHESIOLOGY Web site at http://www.anesthesiology.org). Primers were synthesized by Applied Biosystems (Foster City, CA). The quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) reactions were performed in a total reaction volume of 25 μl containing a final volume of 12.5 μl of SYBR® Green PCR Master Mix (Applied Biosystems), 900 nM of forward and reverse primers, and 5 μl of diluted cDNA (10 ng/μl). The reactions were performed in 96-well plates using the iCycler IQ Multicolor Real-Time PCR detection system (Bio-Rad, CA). The C_T values for each quantitative real-time RT-PCR reaction were determined using the iCycler software (Bio-Rad). Relative expression of each of the genes was determined using the $2^{-\Delta\Delta C}{}_T$ method[34] using the expression of GAPDH as a control housekeeping gene. All samples gave a single peak upon melt curve analysis, indicating there were no contaminating products or primer dimers for the genes assayed.

Statistical Analyses

Statistical significance for the behavioral experiments was determined by mixed factorial analysis of variance followed by Bonferroni multiple comparison *post hoc* test using GraphPad Prism version 4.02 (GraphPad Software Inc., San Diego, CA). Statistical significance for the RT-PCR experiments was determined by one-way analysis of variance followed by Bonferroni multiple comparison *post hoc* test. A statistical threshold of $\alpha = 0.05$ ($P < 0.05$) was considered significant for all experiments.

Results

Mechanical Allodynia in Rats after L5 Nerve Root Ligation and L5 Spinal Nerve Transection

We have previously demonstrated that both the L5 nerve root ligation model of lumbar radiculopathy (LR) and the L5 spinal nerve transection model (L5tx) of peripheral neuropathy produce reliable, robust, and long-lasting mechanical allodynia.[26,29,35–37] In this study, age-matched male Sprague-Dawley rats exhibited robust mechanical allodynia after LR and L5tx beginning at day 1 after surgery and lasting throughout the testing period. During the course of the study, no significant difference in the magnitude of mechanical allodynia was observed between the LR and L5tx groups in response to the 2-g (fig. 1A) or 12-g (fig. 1B) von Frey filaments. No significant mechanical allodynia was observed in either sham surgery group (fig. 1).

Identification of Differential Gene Expression

In these experiments, gene expression values ranged from 1 to 12,237, with 95% of the scores below 1,000 in

Available at: http://www.dchip.org/. Accessed August 20, 2005.

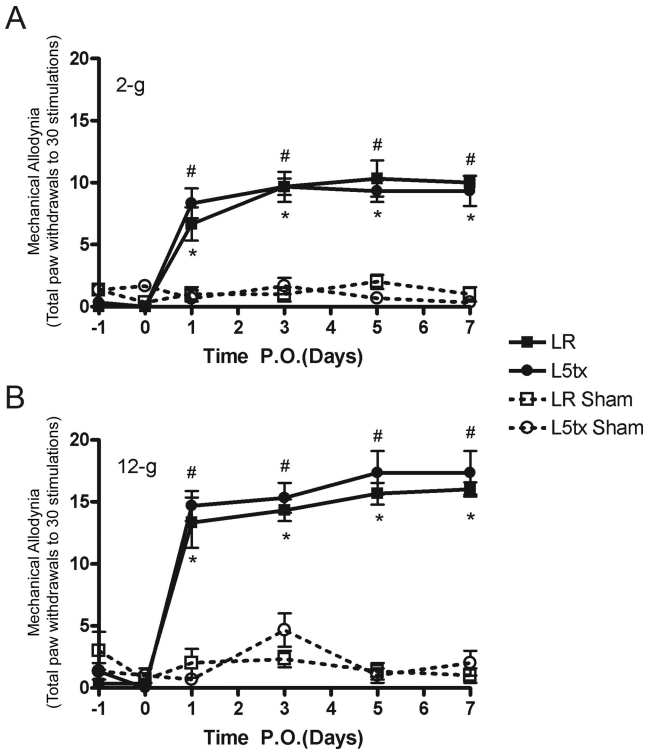

Fig. 1. Mechanical allodynia measured as the paw withdrawal frequency to stimulation with 2-g (*A*) and 12-g (*B*) von Frey filaments in Sprague-Dawley rats. Responses were measured after L5 spinal nerve transection (L5tx, n = 3), sham L5 spinal nerve transection (L5tx sham, n = 3), L5 nerve root ligation (LR, n = 3), or sham L5 nerve root ligation (LR sham, n = 3). Allodynia responses are reported as the number of paw withdrawals out of 30 possible responses ± group SEM. * Significant ($P < 0.01$) difference in LR paw withdrawals compared with LR sham group. # Significant ($P < 0.01$) difference in L5tx paw withdrawals compared with L5tx sham group. P.O. = postoperative.

most cases. The reliability estimates associated with the gene expression scores within experimental groups (*i.e.*, replicates) are summarized in table 1. We observed high reliability estimates for both the internal consistency (coefficient α) and the test–retest reliability estimates, with all indices exceeding 0.97. From this analysis, we determined that replicate consistency was considered adequate grounds for averaging the results from replicate rats from each group before evaluating differences in gene expression across the experimental groups. Differential gene expression across experimental groups was evaluated by using a linear regression method. We followed this analysis with further classification of the genes identified by the linear regression method based on the relative magnitude change to group the genes into subcategories based on expression magnitudes.

Table 1. Summary of Reliability Estimates for the Gene Expression Values from Duplicate Animals in Each Experimental Group

Model	Experimental Group	Coefficient α	Test–Retest
LR	Sham	0.99514	0.99022
LR	7 days after surgery	0.99410	0.98827
LR	14 days after surgery	0.99554	0.99113
L5tx	Sham	0.98820	0.97655
L5tx	7 days after surgery	0.99530	0.99052
L5tx	14 days after surgery	0.99469	0.98926

n = 2 animals/group.

Coefficient α = internal consistency; L5tx = L5 spinal nerve transection; LR = L5 nerve root ligation.

Peripheral Nerve Transection and Nerve Root Ligation Induce Differential Spinal Cord Gene Expression after Injury

Microarray analysis of gene expression in the lumbar spinal cord at two time points (7 days and 14 days) after LR or L5tx was performed. In the 7 days postsurgery groups, we identified 417 genes (2.6% of total probesets) regulated in the L5tx rats (figs. 2A and B) and 386 genes (2.4% of total probesets) in the LR rats (figs. 2C and D). Further subsetting of the L5tx outlier genes by fold change from the L5tx sham-operated group showed 49 genes with a greater than 1.5-fold change, 186 genes with a 1- to 1.5-fold change, 179 genes with a −1- to −1.5-fold change, and 3 genes with a greater than −1.5-fold change (fig. 2B). Similarly, further subsetting of the LR outlier genes by fold change from the LR sham-operated group showed 29 genes with a greater than 1.5-fold change, 176 genes with a 1- to 1.5-fold change, 164 genes with a −1- to −1.5-fold change, and 17 genes with a greater than −1.5-fold change (fig. 2D).

Of these specific L5tx and LR genes, we identified 139 genes (17.3% of L5tx/LR outlier genes) that were coordinately regulated in both models (fig. 2E). These commonly regulated genes were nearly equally distributed between up-regulated genes (7 genes with a greater than 1.5-fold change, 68 genes with a 1- to 1.5-fold change) and down-regulated genes (61 genes with a −1- to −1.5-fold change, 3 genes with a greater than −1.5-fold change). A summary of the outlier genes identified 7 days after surgery can be found in supplementary table 2 (additional information regarding this is available on the ANESTHESIOLOGY Web site at http://www.anesthesiology.org).

In the 14 days postsurgery groups, we identified 367 genes (2.3% of total probesets) regulated in the L5tx rats (figs. 3A and B) and 362 genes (2.3% of total probesets) in the LR rats (figs. 3C and D). Further subsetting of the L5tx outlier genes by fold change from the L5tx sham-operated group showed 7 genes with a greater than 1.5-fold change, 161 genes with a 1- to 1.5-fold change, 188 genes with a −1- to −1.5-fold change, and 11 genes with a greater than −1.5-fold change (fig. 3B). Similarly, further subsetting of the LR outlier genes by fold change from the LR sham-operated group showed 16 genes with a greater than 1.5-fold change, 154 genes with a 1- to 1.5-fold change, 161 genes with a −1- to −1.5-fold change, and 31 genes with a greater than −1.5-fold change (fig. 3D).

Of these specific L5tx and LR genes, we identified 132

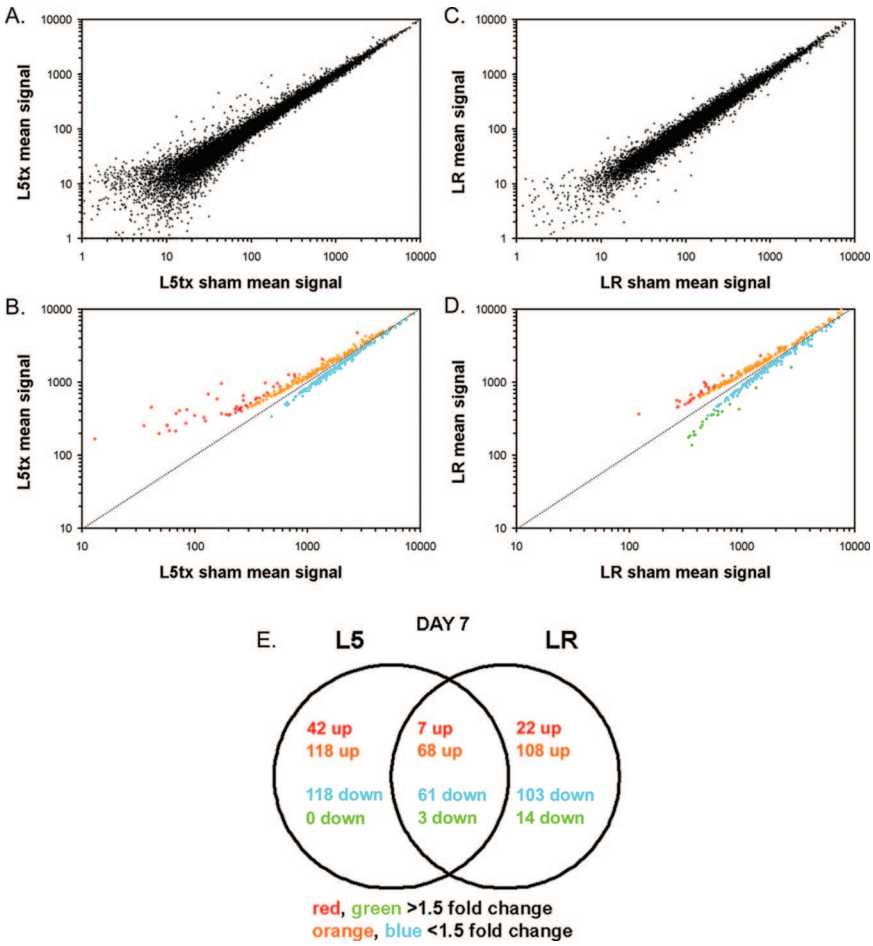

Fig. 2. Scatter plot representation of lumbar spinal cord gene expression comparing L5 spinal nerve–transected (L5tx) rats with sham-operated rats (*A*) and L5 nerve root–ligated (LR) rats with sham operated rats (*C*) at 7 days after surgery. Each *point* represents the mean expression (n = 2/group) of a single gene obtained from Affymetrix RAE230A GeneChip® analysis. Outlier genes identified as having differential expression in L5 spinal nerve–transected (L5tx) rats compared with sham-operated rats (*B*) and in L5 nerve root–ligated (LR) rats compared with sham-operated rats (*D*). L5tx and LR outlier genes are further defined by fold change from their corresponding sham operated controls: greater than 1.5-fold change (*red*), 1.0- to 1.5-fold change (*orange*), −1- to −1.5-fold change (*blue*), greater than −1.5-fold change (*green*). A Venn diagram illustrating the relation of the L5tx and LR outlier genes is shown (*E*).

genes (18.1% of L5tx/LR outlier genes) that were coordinately regulated in both models (fig. 3E). Commonly regulated genes in the 14 days postinjury group were also nearly equal between up-regulated genes (4 genes with a greater than 1.5-fold change, 67 genes with a 1- to 1.5-fold change) and down-regulated genes (56 genes with a −1- to −1.5-fold change, 5 genes with a greater than −1.5-fold change). A summary of the outlier genes identified 14 days after surgery can be found in supplementary table 3 (additional information regarding this is available on the ANESTHESIOLOGY Web site at http://www.anesthesiology.org).

We observed differences in gene expression across the two time points measured as well. In the LR group, we found 62% of the genes to be differentially regulated between the two time points after L5 nerve root ligation. Similarly, in the L5tx group, we found 64% of the genes to be differentially regulated between the two time points after L5 spinal nerve transection.

We further classified the model-specific, nonexpressed sequence tag, outlier genes into functional categories at days 7 and 14 after surgery (fig. 4). Genes that were commonly expressed in both models were not included. This analysis suggests that functional groups of genes that were differentially regulated at day 7 between the two nerve injury models occurred in genes involved in enzyme/metabolism (33% L5tx *vs.* 19% LR). At day 14 after surgery, the functional groups of genes that were differentially regulated were involved in enzyme/metabolism (29% L5tx *vs.* 20% LR), channels/transporters (7% L5tx *vs.* 14% LR), and structural proteins (11% L5tx *vs.* 20% LR).

Confirmation of Microarray Targets via *Real-time RT-PCR*

To confirm the potential genes identified by the microarray analyses, we performed real-time RT-PCR analysis on lumbar spinal cord homogenates from a second group of male Sprague-Dawley rats 7 days after LR or L5tx. We chose PTGDS, IGF1, IGF2, IGFBP3, DCN, HLADG, and AQP4 that were identified as model-specific outlier genes expressed at 7 days after L5tx or LR (fig. 5). In addition, we chose two genes previously shown to be regulated in both models,[26] GFAP and ITGAM. The relative expression of these genes compared with their corresponding sham-operated controls as measured by both Affymetrix microarray analysis and real-time RT-PCR is summarized in table 2. We observed paradoxical differences in the expression of DCN in the LR group and HLADG in the L5tx group between the two assays. These differences were not significantly different ($P >$

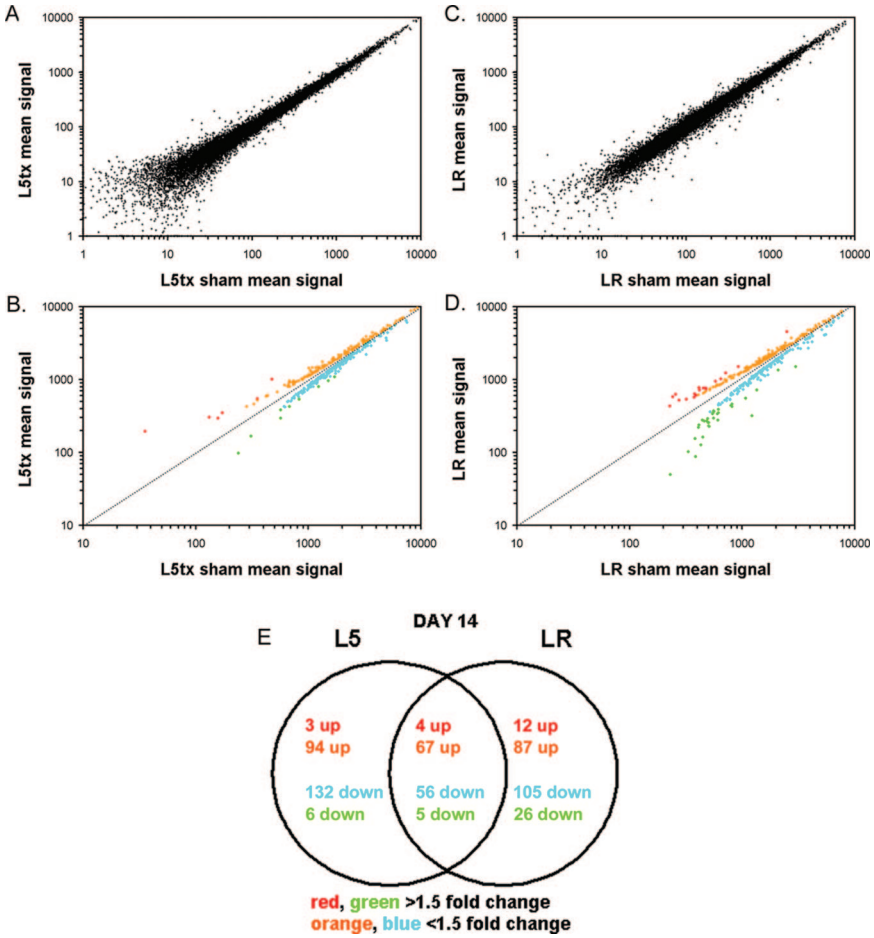

Fig. 3. Scatter plot representation of lumbar spinal cord gene expression comparing L5 spinal nerve–transected (L5tx) rats with sham-operated rats (*A*) and L5 nerve root–ligated (LR) rats with sham-operated rats (*C*) at 14 days after surgery. Each *point* represents the mean expression (n = 2/group) of a single gene obtained from Affymetrix RAE230A GeneChip® analysis. Outlier genes identified as having differential expression in L5 spinal nerve–transected (L5tx) rats compared with sham-operated rats (*B*) and in L5 nerve root–ligated (LR) rats compared with sham-operated rats (*D*). L5tx and LR outlier genes are further defined by fold change from their corresponding sham operated controls: greater than 1.5-fold change (*red*), 1.0- to 1.5-fold change (*orange*), −1- to −1.5-fold change (*blue*), greater than −1.5-fold change (*green*). A Venn diagram illustrating the relation of the L5tx and LR outlier genes is shown (*E*).

0.05) from each other, suggesting some interassay or interanimal variability or both. It is notable, however, that we were able to confirm by a secondary method eight out of nine genes that we selected from the microarray analysis.

As demonstrated previously,[26] the astrocyte marker, GFAP, and the microglial marker, ITGAM, were significantly increased in both models of nerve injury (figs. 5A and B). Similarly, PTGDS, which converts PGH_2 to PGD_2 and is thought to play a role in PGE_2-induced allodynia, was up-regulated approximately 2-fold after both L5tx and LR (fig. 5C). In this study, we have identified several novel genes uniquely regulated after L5tx, including DCN, IGF1, IGF2, and IGFBP3. Several genes in the insulin-like growth factor family (IGF1, IGF2, and IGFBP3) were increased 5- to 10-fold in the L5tx group but not in the LR group (figs. 5D–F). Also notable was DCN, a member of the small, leucine-rich proteoglycan family known to interact with the epidermal growth factor receptor,[38] was significantly increased approximately 6-fold after L5 nerve transection but was unchanged after LR surgery (fig. 5G). In contrast, we found that HLADG (aka CD74), the major histocompatability class II (MHC-II)–associated invariant chain, is increased (approximately 1.4-fold) only after LR surgery and showed no increase in the L5tx animals (fig. 5H). We observed no significant difference in AQP4 expression between any of the treatment groups (fig. 5I).

Discussion

Neuropathic pain is defined as pain caused by a lesion of the peripheral or central nervous system manifesting with sensory signs and symptoms.[39] The DRG is the boundary dividing the central and peripheral nervous systems, and this nomenclature implies that lesions distal to the DRG are peripheral, whereas injuries proximal to the DRG are central. Clinically, a peripheral nerve injury (such as our L5 nerve transection model) presents with a "burning" pain with occasional lancinating pain that is sometimes controlled with opioid analgesics, gabapentin, and tricyclic antidepressants. Radicular pain, however, has a neuropathic (*i.e.*, nerve root injury) component, although there are no accepted diagnostic criteria for defining it.[40] Acute low back pain is often managed with nonsteroidal antiinflammatory drugs and muscle relaxants,[41] whereas chronic radicular pain with obvious disc involvement is often managed surgically with varying degrees of success. For example, the number of spine surgeries with fusion has increased dramatically within recent years without an adequate evidence base

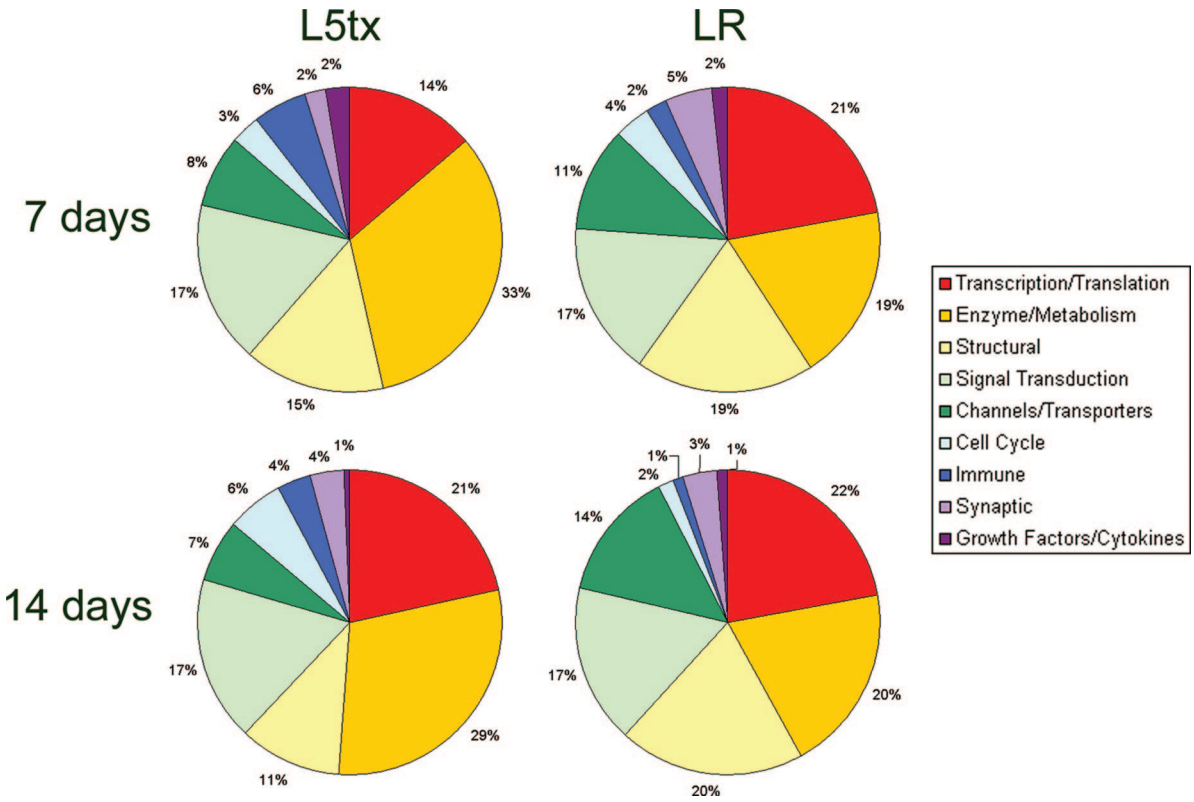

Fig. 4. Functional categorization of genes identified as significant, nonexpressed sequence tags, outlier genes from the GeneChip® microarray analysis at 7 and 14 days after L5 spinal nerve transaction (L5tx) or L5 nerve root ligation (LR). The genes represented here do not included genes commonly expressed in both models.

to justify its use.[42] In a different study, surgical discectomy demonstrated a significantly higher success rate compared with nonsurgical treatment.[43] Clearly, there is significant disconnect in the clinical approaches to treating neuropathic pain and chronic radicular pain. Therefore, in the current study, we sought to determine whether radiculopathy and neuropathy differed in their abilities to alter spinal gene transcription in an effort to identify novel candidate genes that may be pain etiology subtype specific. We observed that these two models display similar magnitudes of mechanical allodynia. Furthermore, gene expression patterns diverged significantly. Many genes were found to be coregulated in the two models and likely represent a basic set of genes transcriptionally altered by any type of injury to the nervous system, whereas other genes diverged significantly between the two models.

A similar magnitude of mechanical allodynia to tactile stimulus was observed after both the L5tx (peripheral) and LR (central) models of chronic pain, in agreement with a previously published study.[26] This finding, in light of the differences in the site and type of nerve injury used in these two models, suggests that allodynia as a symptom or phenomenon is a behavioral outcome mediated by several different pathophysiologic pathways. This concept has been explored in a previous study in which allodynia from the L5tx and LR models was shown to correlate significantly across six strains of mice.[30] These data also suggest that treating neuropathies originating from disparate sites of injury (*e.g.*, central *vs.* peripheral) with pharmacotherapies developed for their ability to treat specific symptoms (*e.g.*, antiallodynic drugs) without regard to the unique pathophysiologic mechanisms that underlie each type injury may not be globally effective.

Our laboratory, among others, has extensively investigated the role of central nervous system neuroimmune activation (glial activation and immune mediator expression) in the development and maintenance of chronic pain states.[28,44,45] As in the current study, previous results have shown that markers for astrocytic and microglial activation (GFAP and OX-42, respectively) were found to be similarly increased in both injury models.[26] These findings suggest that because of their central spinal placement, glial cells may participate in the genesis and maintenance of pain, regardless of the site of the precipitating injury.

A group of genes belonging to the insulin-like growth factor family were confirmed to be up-regulated after injury exclusively in the L5tx group. Insulin-like growth factor-I (IGF-I, somatomedin C) and insulin-like growth factor-II (IGF-2, somatomedin A) are small, mitogenic polypeptides that act as general growth and/or trophic factors for all central nervous system cell types.[46] Previous studies have demonstrated up-regulation of IGF-I after peripheral nerve freeze injury[47] or peripheral nerve crush.[48] Together, these findings suggest that the insulin-

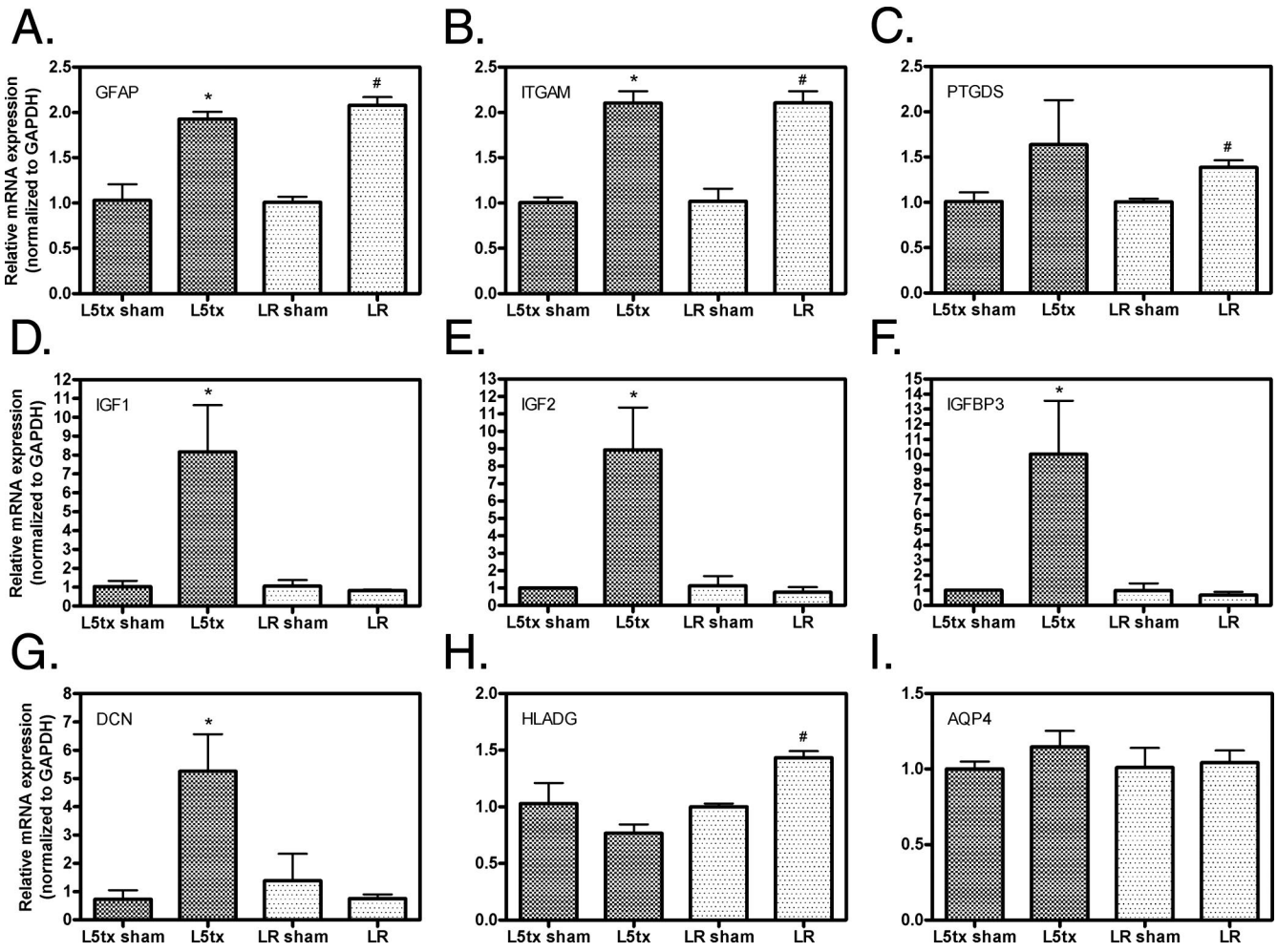

Fig. 5. Reverse transcriptase polymerase chain reaction confirmation of glial fibrillary acidic protein (GFAP, *A*), integrin-αM (ITGAM, *B*), prostaglandin D2 synthase (PTGDS, *C*), insulin-like growth factor 1 (IGF1, *D*), insulin-like growth factor 2 (IGF2, *E*), insulin-like growth factor binding protein 3 (IGFBP3, *F*), decorin (DCN, *G*), CD74 antigen (HLADG, *H*), and aquaporin 4 (AQP4, *I*) messenger RNA (mRNA) expression in Sprague-Dawley rats 7 days after L5 nerve root ligation (LR, n = 3) or L5 spinal nerve transection (L5tx, n = 3). Results are reported as glyceraldehyde 3-phosphate dehydrogenase (GAPDH)–normalized, fold change from corresponding sham surgery. * Significant ($P < 0.05$) change in mRNA expression in L5tx group compared with the L5tx sham. # Significant ($P < 0.05$) change in mRNA expression in LR group compared with the LR sham.

like growth factors may be a peripheral nerve injury–

Table 2. Summary of Results for Eight Genes Identified by GeneChip® Microarray Analysis and Selected for Confirmation by RT-PCR

		Fold Change (LR)		Fold Change (L5tx)		
Gene Name	Gene ID	GeneChip®	RT-PCR	GeneChip®	RT-PCR	*P* Value (RT-PCR), LR *vs.* L5tx
Glial fibrillary acidic protein	GFAP	1.71	2.08	1.40	1.93	0.274 (NS)
Integrin-αM	ITGAM	1.01	2.11	1.57	2.10	0.988 (NS)
Prostagladin D2 synthase	PTGDS	1.11	1.39	1.69	1.64	0.642 (NS)
Insulin-like growth factor 1	IGF1	−1.12	−1.20	5.15	8.17	0.038*
Insulin-like growth factor 2	IGF2	1.11	1.14	5.48	8.94	0.029*
Insulin-like growth factor binding protein 3	IGFBP3	−1.05	−1.01	12.63	10.01	0.040*
Decorin	DCN	1.15	−1.22	3.49	5.27	0.026*
CD74 antigen	HLADG	1.63	1.53	1.17	−1.30	0.002†
Aquaporin 4	AQP4	1.07	1.04	1.36	1.15	0.477 (NS)

* $P < 0.05$. † $P < 0.01$.

L5Tx = L5 spinal nerve transection; LR = L5 nerve root ligation; NS = not significant; RT-PCR = reverse transcriptase polymerase chain reaction.

specific response that may be involved in axonal regeneration, a function not required after LR injury.

Previously, we have observed spinal MHC class II expression after both peripheral and nerve root injury with a qualitatively greater expression after nerve root injury. Interestingly, peripheral inflammation from either zymosan[49] or formalin[50] did not lead to MHC class II expression in the spinal cord. Furthermore, decreased MHC class II expression after administration of the immunosuppressant agents methotrexate or leflunomide was associated with a decrease in existing mechanical allodynia after injury.[51,52] In the current study, we observed an increase in the mRNA for the HLADG gene, which encodes the invariant polypeptide chain of MHC class II, after LR but not L5tx. This suggests a transcriptional regulation of the HLADG gene after root injury proximal to the DRG.

Two previous studies by Wang *et al.*[22] and Yang *et al.*[21] have examined, using microarray technology, gene expression in the spinal cord after spinal nerve ligation or sciatic nerve transection, respectively. Both studies examined gene expression at 14 days after injury and, thus, are comparable to our 14 days post-L5tx group. The experiments by Yang *et al.*[21] show few correlative genes as compared with the current study, most likely because of differences in the microarray platform (cDNA spotted arrays *vs.* Affymetrix GeneChip®). In contrast, comparing the study by Wang *et al.*[22] with the current study demonstrates several genes that are positively identified in both studies (*e.g.*, MHC class II genes, complement protein genes, CD37 leukocyte antigen).

Overall, our results suggest that the characteristic patterns of spinal gene transcription observed after peripheral or nerve root injury may implicate differential responses in neuropathic and radicular pain. We found unique gene expression patterns after LR and L5tx surgery, with surprisingly little overlap between the groups (only 8.7% and 10.9% at days 7 and 14, respectively). We also observed a significant time effect on the gene expression after nerve injury, which suggests different phases of gene expression at different time points after spinal nerve and nerve root injury. All of these differential gene expression profiles may explain divergent behavioral outcomes as well as distinct analgesic responsiveness in peripheral nerve and root injury models[26] and in human pain conditions. This suggests that the site of injury makes a seminal contribution to the ensuing spinal plasticity, which has key implications for therapy. Specifically, although similar pain responses may be observed (patients often exhibit allodynia, dysesthesias, and hyperesthesias) the underlying mechanisms may differ, suggesting that radicular and neuropathic syndromes may respond to unique drug therapies. These findings further suggest that chronic pain may be responsive to specific pharmacotherapy depending on the site of injury and that the current trend toward treating radicular pain principally with surgery may need to be reexamined. Finally, our findings highlight the importance of elucidating basic pain mechanisms to allow for the development of etiology-specific treatments with increased efficacy over currently available therapies.

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