Dissecting the Mechanisms of Chronic Postsurgical Pain

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Neuronal excitability

Remodeling nociceptor

Changes in gene expression, release of pain signaling molecules

Neural plasticity

Central sensitization

Surgical nerve injury
Presynaptic terminal

Cell membrane

CaMKII

New AMPAR

NK1R

mGluR

AMPAR

NMDAR

CGRPR

PKC

P

PKA

P

ERK1/2

CaMKII

CREB

Long-term potentiation

Cell 2009; 139: 267-84

Neurobiol Learn Mem 2013; 105: 133-50
“Have you tried turning it off and on again?
Animal Models of Nerve Injury

- SNI
- SNL
- CCI

Anesthesiol Res Pract 2011; 124898
Incisional Pain Model

Days

0 0.5 1 2 3 4 5 6

Paw withdrawal threshold (g)

0.0

0.5

1.0

1.5

2.0

2.5

Sham

Days

0 0.5 1 2 3 4 5 6

Paw withdrawal time (s)

0

1

2

3

4

5

6

7

8

9

10

Sham
Incisional Pain Model

Incisional Pain Model

Incisional Pain Model

Incisional Pain Model
Spinal Cord Microarray

Agilent 4X44k whole rat genome microarray

Log\textsubscript{10} Raw Intensity value (Control)

Log\textsubscript{10} Raw Intensity value (pain)

Hierarchical cluster analysis

Whole dataset  Differential genes

Ctrl  Pain

Ctrl  Pain

Hierarchical cluster analysis
## Differential Gene Expression

<table>
<thead>
<tr>
<th>Gene Ontology / Pathway</th>
<th>p-value</th>
<th>Genes (observed)</th>
<th>Genes (total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium channel regulator activity</td>
<td>4.93E-04</td>
<td>3</td>
<td>19</td>
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<tr>
<td>Kinase regulator activity</td>
<td>5.68E-04</td>
<td>5</td>
<td>81</td>
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<tr>
<td>Protein kinase regulator activity</td>
<td>2.33E-03</td>
<td>4</td>
<td>67</td>
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<tr>
<td>Transcription factor activity</td>
<td>3.67E-03</td>
<td>14</td>
<td>746</td>
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<tr>
<td><strong>Protein serine/threonine kinase inhibitor activity</strong></td>
<td>5.84E-03</td>
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<td>14</td>
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<tr>
<td>Enzyme inhibitor activity</td>
<td>6.05E-03</td>
<td>7</td>
<td>260</td>
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<tr>
<td><strong>Channel regulator activity</strong></td>
<td>7.81E-03</td>
<td>3</td>
<td>49</td>
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<tr>
<td>Immune effector process</td>
<td>1.09E-04</td>
<td>8</td>
<td>193</td>
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<tr>
<td>Cytokine production</td>
<td>8.89E-04</td>
<td>7</td>
<td>203</td>
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<tr>
<td>Regulation of gene expression</td>
<td>6.64E-03</td>
<td>25</td>
<td>1,989</td>
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<tr>
<td>Adaptive immune response</td>
<td>6.77E-03</td>
<td>4</td>
<td>99</td>
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<tr>
<td>Cellular biogenic amine metabolic process</td>
<td>7.51E-03</td>
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<td>102</td>
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<tr>
<td>Stored secretory granule</td>
<td>1.91E-03</td>
<td>7</td>
<td>212</td>
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<tr>
<td>Membrane-bounded vesicle</td>
<td>4.01E-03</td>
<td>12</td>
<td>599</td>
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<td>Extracellular region</td>
<td>7.30E-03</td>
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<td>1,349</td>
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<td>TGF β</td>
<td>1.63E-03</td>
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<td>1,307</td>
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<td><strong>Dopamine receptor</strong></td>
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<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Protein kinase A</td>
<td>3.44E-03</td>
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<tr>
<td>Nuclear receptor</td>
<td>8.51E-03</td>
<td>6</td>
<td>156</td>
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## Target genes

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Genebank accession</th>
<th>Description</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctsg</td>
<td>NM_001106041</td>
<td>Cathepsin G</td>
<td>6.17</td>
</tr>
<tr>
<td>Ace2</td>
<td>NM_001012006</td>
<td>Angiotensin I converting enzyme 2</td>
<td>1.77</td>
</tr>
<tr>
<td>Usp18</td>
<td>NM_001014058</td>
<td>Ubiquitin specific peptidase 18</td>
<td>-1.74</td>
</tr>
<tr>
<td>Ctsq</td>
<td>BC107913</td>
<td>Cathepsin Q</td>
<td>-1.51</td>
</tr>
<tr>
<td>Serpine1</td>
<td>NM_012620</td>
<td>Serine (or cysteine) peptidase inhibitor</td>
<td>-2.65</td>
</tr>
<tr>
<td>Aif1l</td>
<td>NM_001108578</td>
<td>allograft inflammatory factor 1-like</td>
<td>3.35</td>
</tr>
<tr>
<td>Bdnf</td>
<td>NM_012513</td>
<td>Brain-derived neurotrophic factor</td>
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Cathepsin G Inhibition

![Graph showing Paw withdrawal latency over time post-incision with baseline and different time points indicated. The graph shows a decrease in latency over the first few hours post-incision, followed by stabilization.]
Cathepsin G Inhibition

Intrathecal injection of CatGI

![Graph showing the effect of intrathecal injection of CatGI on paw withdrawal latency over time. The graph compares Incision (dashed line) and CatGI (solid line) treatments.](image)
Cathepsin G Inhibition

Intrathecal injection of CatGI
Cathepsin G Inhibition

Intrathecal injection of CatGI

Paw withdrawal latency (s)
MPO Staining of Spinal Dorsal Horn

Control

Incisional + vehicle

Incisional + CatGI

Cell count (No./field)

Control Pain + Vehicle + Pain + CatGI

*
**IL1β**

**Myeloperoxidase**

**Merge**

**Incision+CatGI**

<table>
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<tr>
<th>Saline</th>
<th>Incision</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
</table>

**pro-IL1β**

**β-actin**
CTSG Reactivates Spinal Astrocytes
CTSG Reactivates Spinal Astrocytes

- Sham + Vehicle
- Sham + CTSG
- Incision + Vehicle
- Incision + CTSG

GFAP Cell count (No./field):

- Sham + Vehicle: 50
- Sham + CTSG: 60
- Incision + Vehicle: 70
- Incision + CTSG: 70

* indicates significant reactivation with CTSG.
CTSG Increases IL-1β Release

Vehicle

CTSG

GFAP
IL1β
Merge

100 µm
Spinal astrocyte after CTSG inhibition

![Western Blot Image]

- CTR, LPS, CG1, CG2 conditions
- Time points: 0.5h, 1h, 6h
- Proteins analyzed:
  - p-IκB
  - p-p38MAPK
  - p38MAPK
  - p-JNK
  - JNK
  - p-Erk1/2
  - Erk1/2
  - β-actin
CTSG and Central Sensitization

Surgical incision → CTSG → transactivate Membrane bounded receptors → Cell recruitment → Immune cells → Astrocytes → p38MAPK → IL6, MMP9 → Central Sensitization

Allodynoia, Hyperalgesia
Persisted Pain After Surgery Study

1,530 patients who fulfilled the eligibility criteria

217 patients were not recruited because:
175 did not consent
42 family or surgeon did not allow patient to participate

1,313 patients recruited to the persistent pain after surgery study (85.8% of all eligible patients)

161 patients (12.3%) were excluded from the genetic substudy because:
107 did not consent to have blood sample collected
31 died before 12-month follow-up
49 unable to complete 12-month due to cognitive or physical disability
4 unable to contact for 12-month follow-up

1,152 patients included in the genetic substudy (75.3% of all eligible patients)

• Adult patients undergoing surgery with an incision
• In-patient or ambulatory surgery
• Provided consent and available for interview
Persistent Pain After Surgery Study

- Primary outcome: Pain over the wound site at 12 months after surgery
- mBPI
- NPQ
- Anxiety/depression scores
- EQ-5D
Persistent Pain After Surgery Study

- 246 (21.4%) patients had pain
- Average pain score was 3.2 ± 2.0
**Persistent Pain After Surgery Study**

- 246 (21.4%) patients had pain
- Average pain score was 3.2 ± 2.0

<table>
<thead>
<tr>
<th>Interference items</th>
<th>Number of patients reporting interference</th>
<th>Interference score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal work</td>
<td>106 (43.2%)</td>
<td>1.87 ± 2.70</td>
</tr>
<tr>
<td>Walk</td>
<td>140 (56.8%)</td>
<td>2.34 ± 2.76</td>
</tr>
<tr>
<td>Relation with others</td>
<td>62 (25.5%)</td>
<td>0.83 ± 1.82</td>
</tr>
<tr>
<td>Enjoyment of life</td>
<td>123 (50.0%)</td>
<td>2.11 ± 2.77</td>
</tr>
<tr>
<td>General activity</td>
<td>131 (53.4%)</td>
<td>2.48 ± 3.00</td>
</tr>
<tr>
<td>Mood</td>
<td>146 (59.3%)</td>
<td>2.46 ± 2.71</td>
</tr>
<tr>
<td>Sleep</td>
<td>117 (47.9%)</td>
<td>2.19 ± 2.86</td>
</tr>
</tbody>
</table>
## Persistent Pain After Surgery Study

<table>
<thead>
<tr>
<th>EQ-5D items</th>
<th>Difficulty in EQ-5D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chronic postsurgical pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pain-free</td>
<td></td>
</tr>
<tr>
<td>Total no. of patient</td>
<td>246</td>
<td>906</td>
</tr>
<tr>
<td>Motion</td>
<td>56 (22.7%)</td>
<td>109 (12.0%)</td>
</tr>
<tr>
<td>Self-care</td>
<td>32 (12.8%)</td>
<td>70 (7.7%)</td>
</tr>
<tr>
<td>Usual activities</td>
<td>46 (18.7%)</td>
<td>92 (10.1%)</td>
</tr>
<tr>
<td>Pain/discomfort</td>
<td>56 (22.9%)</td>
<td>94 (10.4%)</td>
</tr>
<tr>
<td>Anxiety/Depression</td>
<td>71 (28.9%)</td>
<td>186 (20.5%)</td>
</tr>
<tr>
<td>EQ-5D VAS*</td>
<td>69 ± 19</td>
<td>76 ± 47</td>
</tr>
</tbody>
</table>
GoldenGate Genotyping

2-3 µm beads coated with 768 specific oligonucleotides from 65 genes

Oligonucleotides are put in the end of microfibers and then embedded in the sample wells
SNPs in CTSG gene

- >296 SNPs (hapmap.ncbi.nlm.nih.gov)
- Only 4 with minor allele frequency > 5% in Chinese

2.71 kb

Exon 5
rs1885597 (G/T)

4
rs11623400 (A/G)

3
rs2070697 (G/A)

2
rs2236742 (G/A)

1
# Gene Association

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate</th>
<th>p value</th>
<th>Multivariate</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95%CI)</td>
<td></td>
<td>Odds ratio (95%CI)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 65</td>
<td>1.53 (1.09-2.15)</td>
<td>0.015</td>
<td>1.50 (1.36-1.64)</td>
<td>0.041</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>1.56 (1.07-2.27)</td>
<td>0.004</td>
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<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 12 years</td>
<td>0.89 (0.46-1.73)</td>
<td>0.09</td>
<td></td>
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</tr>
<tr>
<td>Prior pain history</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>0.89 (0.24-3.31)</td>
<td>0.16</td>
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<tr>
<td>Employment</td>
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<tr>
<td>Yes</td>
<td>1.34 (0.88-2.05)</td>
<td>0.18</td>
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<tr>
<td>rs2236742</td>
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<tr>
<td>GG</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>0.87 (0.21-3.61)</td>
<td>0.467</td>
<td></td>
<td></td>
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<tr>
<td>AA</td>
<td>0.27 (0.16-0.46)</td>
<td>0.038</td>
<td>0.24 (0.13-0.45)</td>
<td>0.048</td>
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<tr>
<td>rs2070697</td>
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<td></td>
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<tr>
<td>GG</td>
<td>Reference</td>
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<td></td>
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<tr>
<td>GA</td>
<td>1.26 (0.89-1.77)</td>
<td>0.182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>0.64 (0.43-0.96)</td>
<td>0.043</td>
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</tr>
</tbody>
</table>
CTSG and Chronic Postsurgical Pain

- CTSG up-regulation in animal chronic incisional pain model
- Chemotactic effects
- Release of inflammatory mediators
- CTSG inhibition alleviates chronic postsurgical pain
- CTSG polymorphisms reduce risk of chronic postsurgical pain
- Possible target for drug development / biomarker for pain prediction
# Brain-derived neurotrophic factor

<table>
<thead>
<tr>
<th>rs6265</th>
<th>Univariate</th>
<th>Multivariate</th>
<th>PAR (95%CI)</th>
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<tbody>
<tr>
<td>GG</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>0.81 (0.55-1.20)</td>
<td>0.30</td>
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</tr>
<tr>
<td>AA</td>
<td>0.69 (0.51-0.97)</td>
<td>0.032</td>
<td>0.57 (0.31-0.99)</td>
</tr>
</tbody>
</table>

[Image of Sham and Incision with neuronal fluorescence intensity graph]
Epigenetic Regulation?

Transcriptional regulation without changing the underlying DNA sequences
P300-CREB-binding protein Association Factor (PCAF)

IL1β → NFKB p65 → Acetylation → PCAF → Histone acetylation → COX-2 promoter → COX-2 transcription
PCAF inhibition

Thermal hyperalgesia

Time

Baseline 6h Day 1 Day 2 Day 3 Day 4 Day 5

Paw withdrawal latency (s)

Sham, Vehicle

Incision, Vehicle

Paw withdrawal latency (s)

Time
PCAF inhibition

Thermal hyperalgesia

Paw withdrawal latency (s)

- Sham, Vehicle
- Incision, Vehicle
- Incision, PCAF siRNA

Time

Baseline 6h Day 1 Day 2 Day 3 Day 4 Day 5
PCAFC inhibition

Mechanical allodynia

Time
0 6h Day 1 Day 2 Day 3 Day 4 Day 5

Paw withdrawal threshold (g)

Sham, Vehicle
Incision, Vehicle
Incision, PCAF siRNA
PCAF-siRNA reduce COX-2 expression in rat dorsal horn
Conclusions

- Neurobiology of chronic postsurgical pain is complex
- Regulation of transcriptional factors may be the key to prevent central sensitization and chronic postsurgical pain
- Further studies to develop drugs on these targets in the chronic pain pathways
Acknowledgements

General Research Fund, Research Grant Council, Hong Kong (CUHK4612/07M)
Project Grant, ANZCA (09/015)
Academic Enhancement Grant, ANZCA (13/001)