Ueno S et al, “Early gene expression profile in retinal ganglion cell layer after optic nerve crush in mice”

Supplementary materials

Supplementary Figure S1: Representative images showing laser captured microdissection.

Supplementary Figure S2: In situ apoptosis assay of retinas at 0-7 days after ONC.

Supplementary Table S1: Primer sequences using in RT-PCR

Supplementary Table S2: Atf3, Lcn2, Tnfrsf12a upregulation in the rodent model of optic neuropathy reported in the previous studies utilizing whole transcriptome analysis
**Supplementary Figure S1**: Representative images showing laser captured microdissection. Frozen sections of retinas were stained with toluidine blue (upper left) and dissected GCL and IPL (upper middle). After that, residual retinal tissues were also collected (upper right). RT-PCR analysis for *Thy1* indicated that RGC population was almost same in dissected RGC-enriched fractions at 0, 1, 4 days after ONC (lower). GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, ONL: outer nuclear layer. Bar = 100 μm.
**Supplementary Figure S2:** In situ apoptosis assay of retinas at 0-7 days after ONC.

TUNEL-reactivity (upper and middle rows), cleaved caspase-3 immunoreactivity (lower row), and nuclei are shown by green, red, and blue color respectively. TUNEL or cleaved caspase-3 positive cells in GCL are indicated by arrow heads. GCL: ganglion cell layer, INL: inner nuclear layer, ONL: outer nuclear layer. Bar = 20 μm

**TUNEL (fluorescein-dUTP)**

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<th>1d</th>
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**TUNEL (alkyne-dUTP)**

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**Cleaved caspase-3**

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## Supplementary Table S1 Primer sequences using in RT-PCR

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<thead>
<tr>
<th>Gene name (Symbol)</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Size (bp)</th>
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<tr>
<td>Protease, serine 56 (<em>Prss56</em>)</td>
<td>CGGTCACAATCCCCGAGAAC</td>
<td>AGCCGCTAACTTCTGTGTCAG</td>
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<td>Tubulin, beta 5 class I (<em>Tubb5</em>)</td>
<td>GATCGGTCGTAACTTCTGTCAGGA</td>
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<td>Metallothionein 2 (<em>Mt2</em>)</td>
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<td>Endothelin 2 (<em>Edn2</em>)</td>
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<td>Lipocalin 2 (<em>Lcn2</em>)</td>
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<td>Growth arrest and DNA damage-inducible 45 beta (<em>Gadd45b</em>)</td>
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<td>Tumor necrosis factor receptor superfamily, member 12a (<em>Tnfrsf12a</em>)</td>
<td>GTGTTGGGATTCGGCTTGGT</td>
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<td>Activating transcription factor 3 (<em>Atf3</em>)</td>
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<td>DNA damage-inducible transcript 4 (<em>Ddit4</em>)</td>
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<td>RNA polymerase I polypeptide C (<em>Polr1c</em>)</td>
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<td>Mitogen-activated protein kinase kinase kinase 3 (<em>Map4k3</em>)</td>
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**Supplementary Table S2** Atf3, Lcn2, Tnfrsf12a upregulation in the rodent model of optic neuropathy reported in the previous studies utilizing whole transcriptome analysis

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<thead>
<tr>
<th>Technique used in whole transcriptome analysis</th>
<th>Retinal samples for Atf3, Lcn2, Tnfrsf12a expression analysis</th>
<th>Genes defined as upregulated among Atf3, Lcn2, Tnfrsf12a</th>
<th>Applications for validating Atf3, Lcn2, Tnfrsf12a expression</th>
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<tr>
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<td>Current study</td>
<td>Microarray</td>
<td>Retina, GCL (LCM)</td>
<td>Atf3, Lcn2, Tnfrsf12a</td>
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<td>RNA-seq</td>
<td>Retina</td>
<td>Atf3, Tnfrsf12a</td>
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<td>Yasuda M et al (2014)</td>
<td>CAGE</td>
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<td>Atf3, Tnfrsf12a</td>
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<td>Sharma TP et al (2014)</td>
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<td>Retina, optic nerve</td>
<td>Tnfrsf12a (optic nerve)</td>
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<td>Yasuda M et al (2016)</td>
<td>RNA-seq</td>
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<td><strong>Ocular hypertension</strong></td>
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