Neurotrophin and Neurotrophin Receptor Expression by Cells of the Human Lamina Cribrosa

Wendi Lambert,1 Rajnee Agarwal,1 William Howe,2 Abbot F. Clark,2,5 and Robert J. Wordinger1,5

PURPOSE. To determine whether cells and tissue from the human lamina cribrosa (LC) express neurotrophin and tyrosine kinase (trk) receptor mRNA and protein and whether these cells secrete neurotrophins.

METHODS. Synthesis of cDNA and the reverse transcription-polymerase chain reaction (RT-PCR) were conducted using total RNA obtained from well-characterized cell lines from the human LC and human optic nerve head (ONH) tissue. Immunofluorescent localization and Western blot analysis were used to evaluate neurotrophin and trk protein expression in cells and tissue from the human LC. Immunohistochemistry and ELISA systems were used to detect the secretion of neurotrophins.

RESULTS. Two morphologically distinct cell types (LC cells and ONH astrocytes) were isolated and characterized from the human LC. Messenger RNA for each of the neurotrophins, three full-length trk receptors, and two truncated trk receptors were detected in both cell types and in human ONH tissue. Protein for the neurotrophins and trk receptors were detected in LC cells, ONH astrocytes, and ONH tissue. Neither cell type expressed mRNA or protein for the low-affinity neurotrophin receptor p75. The secretion of neurotrophins was observed in both cell types.

CONCLUSIONS. Cells from the human LC express mRNA and protein of neurotrophins and trk receptors. In addition, cells from the LC secrete neurotrophins, which suggests that there is paracrine and/or autocrine signaling within the LC. Neurotrophin signaling within this region of the ONH may play important roles in the maintenance of the normal LC and in such diseases as glaucoma.

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Supported by the Morgan Stanley & Co., Inc. Research Fund of The Glaucoma Foundation and Alcon Laboratories Inc.

Submitted for publication September 19, 2000; revised March 8, 2001; accepted May 17, 2001.

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Neurotrophins (NTs) constitute a family of polypeptide growth factors that have previously been reported to promote the development, survival, and differentiation of neurons. Four members of this family have been identified in humans: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, and NT-4.1 NTs bind with high affinity to a specific class of tyrosine kinase (trk) receptors, as well as to a low-affinity receptor, p75.1 Three trk receptors have been identified, and their interaction with the NTs appears to be specific. The first trk receptor discovered was trkA, the signaling receptor for NGF.2 BDNF and NT-4 signal through trk B, and NT-3 signals through trk C.3 In addition to the full-length trk receptors, truncated isoforms of trk B and trk C, each without the trk domain, have been identified in humans.1,5 The function of the truncated trk receptors is unknown.

The classic NT hypothesis was based on the action of NGF on sympathetic neurons.4 According to this hypothesis, NTs secreted by target cells are taken up by receptors at the axon terminal and carried back to the neuronal cell body by retrograde transport.5 However, recent evidence suggests that this theory accounts for only one aspect of NT action. The expression of NTs by nontarget cells6,7 and the localization of NT receptors to neuronal cell bodies and dendrites and along axons8,9 challenges the claim that NT support is strictly from retrograde sources. Also, the non-neuronal expression of trk receptors suggests that cells other than neurons may be capable of responding to NTs.10,11 Finally, the colocalization of NT and trk receptor mRNAs and proteins within the same cell population12,13 further supports possible paracrine and/or autocrine NT interaction. As a result of this evidence, the classic theory of NT action has been modified to include paracrine and autocrine NT pathways.5

Primary open-angle glaucoma (POAG) is an optic neuropathy that is characterized by the gradual death of retinal ganglion cells and can eventually result in blindness. The exact mechanism of ganglion cell injury is still unknown, although a major site of injury appears to be the lamina cribrosa (LC) within the human optic nerve head (ONH).14 The LC is composed of glial columns and connective tissue plates that align to form channels that guide and support ganglion cell axons as they exit the eye.15 Evidence suggests that retinal ganglion cell axonal transport is blocked at the level of the LC in POAG.16,17 One theory of ganglion cell injury in POAG suggests that an interruption in axonal transport denies the ganglion cells the trophic factors necessary for survival, thus causing them to undergo apoptosis.16,17

Recent studies have examined the ability of exogenous NTs to rescue retinal ganglion cells after injury 19,20 However, endogenous NT sources for ganglion cells within the LC have not been extensively studied. Because of their intimate association, cells within the LC could serve as paracrine NT sources for these neurons. Two major cell types have been reported to be present within the LC: LC cells and ONH astrocytes.21,22 It is unknown at this time which, if any, NTs and/or trk receptors are expressed by LC cells and ONH astrocytes. It is also unknown whether these cells signal each other through NTs. The objectives of this study were to determine whether cells and tissue from the human LC secrete NTs.

MATERIALS AND METHODS

Materials

DMEM and fetal bovine serum were purchased from HyClone Laboratories (Logan, UT). Astrocyte growth media and normal human brain...
astrocytes were purchased from Clonetics (San Diego, CA). The following materials were purchased from Sigma-Aldrich (St. Louis, MO): 0.25% trypsin solution; monoclonal antibodies to elastin, glial fibrillary acidic protein (GFAP), neural cell adhesion molecule (NCAM), and α-smooth muscle actin; polyclonal antibodies to laminin and fibronectin; NP40; sodium deoxycholate; phenylmethylsulfonyl fluoride (PMSF), aprotinin; pepstatin; leupeptin; and sodium orthovanadate. The following materials were purchased from Gibco BRL-Life Technologies (Grand Island, NY): TRIzol reagent, normal rabbit and normal goat serum, and a DNA ladder. Taq Start antibody, human whole brain tissue, and human skeletal muscle tissue were purchased from Clontech Laboratories, Inc. (Palo Alto, CA). Triton X-100, microscope slides (ProbeOn Plus), and 96-well plates (Nunc ELISA/EIA Maxisorp) were purchased from Fisher Scientific (Pittsburgh, PA). Secondary antibodies (Alexa Fluor 488) and 4',6-diamidino-2-phenylindole (DAPI) nuclear stain were purchased from Molecular Probes, Inc. (Eugene, OR). Polyclonal antibodies to collagen types I, III, and IV were purchased from Southern Biotechnology Assoc., Inc. (Birmingham, AL).

LC Dissection and Cell Culture

Human LC explants were obtained and cultured as described by Hernandez et al.21 with some modifications. Human donor eyes from regional eye banks were received within 24 hours of death, and the LC was dissected from the remaining ocular tissue. The LC tissue was cut into three to four explants and placed in culture plates containing DMEM plus 10% fetal bovine serum (FBS).21 The majority of cells that grew out of the explants were LC cells that were then cultured in Ham's F-10 medium with 10% FBS and passaged using a 0.25% trypsin solution. ONH astrocytes were isolated from populations containing both LC cells and ONH astrocytes using a method described by Hernandez (personal communication, 1995). Mixed cell populations were trypsinized and plated in serum-free astrocyte growth medium (AGM). After 24 hours in culture, the medium was changed to AGM containing 5% FBS. LC cells failed to attach in serum-free medium and were removed when the medium was removed. Cultured ONH astrocytes were maintained in AGM and passaged as described. For comparison purposes, normal human brain astrocytes were also cultured. All cultures were maintained in 5% CO2/95% O2 at 37°C, and medium was changed every 2 to 3 days.

RNA Isolation and cDNA Synthesis

Total cellular RNA was prepared using TRIzol reagent (Gibco BRL-Life Technologies). After ethanol precipitation, RNA was resuspended in water and stored at −80°C until needed. First-strand cDNA synthesis was performed as described in Wordinger et al.22 Total RNA from human ONH tissue, skeletal muscle, and whole brain and from PC-12 cells (American Type Culture Collection [ATCC], Rockville, MD) was used to generate cDNA. All cDNA samples were stored at −80°C until used for PCR.

Primer Design

Primers for GFAP, BDNF, NT-3, trk C, truncated trk B, and truncated trk C were designed and verified according to the parameters specified in Table 1.

Table 1. NT and NT Receptor Primer Pairs

<table>
<thead>
<tr>
<th>Factor</th>
<th>Size (bp)</th>
<th>Upstream Primer (5'-3')</th>
<th>Downstream Primer (5'-3')</th>
<th>Optimal Annealing Temperature (°C)</th>
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<tr>
<td>GFAP</td>
<td>285</td>
<td>TCGAGCTTCGCGAGGAGGAGAT</td>
<td>TAGTCGTCCTCGGTGCTG</td>
<td>59.3</td>
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<tr>
<td>BDNF</td>
<td>369</td>
<td>CCTTCTCTCCTCTCTCTCT</td>
<td>AATTCTCTCTTTTGTCTCAT</td>
<td>54.2</td>
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<tr>
<td>NT-3</td>
<td>302</td>
<td>ACAGGAGACACTAAGGAGC</td>
<td>CCACTCCAGCTGCTG</td>
<td>57.0</td>
</tr>
<tr>
<td>Trk B</td>
<td>202</td>
<td>AAGCTCTCTCCTCCAGGAGAC</td>
<td>CCACTCCAGCTGCTG</td>
<td>57.0</td>
</tr>
<tr>
<td>Trk B</td>
<td>266</td>
<td>GCTTCTCTCTCCTCTCTTAG</td>
<td>GGATCAGCTGCTG</td>
<td>57.0</td>
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<tr>
<td>Trk B.T</td>
<td>430</td>
<td>GGGAGGGATAGAGAAAAGAGGATT</td>
<td>GGATCAGCTGCTG</td>
<td>54.0</td>
</tr>
<tr>
<td>Trk C</td>
<td>572</td>
<td>GTGGGTTTGTTTTGAGGAGGCT</td>
<td>CTCACGAGAGGAGGAGGAGG</td>
<td>55.5</td>
</tr>
</tbody>
</table>

Trk B.T, truncated trk B; trk C.T, truncated trk C.
Manufactured Chemists, Inc., Cincinnati, OH) for 5 seconds at room temperature. After a brief wash in PBS, sections were placed in 0.1% Triton followed by 0.02 M glycine for 15 minutes each. Nonspecific binding was blocked by a 30-minute incubation in 10% normal serum. Sections were washed briefly and treated with primary antibodies or nonimmune serum (negative controls) diluted 1:100 in 1.5% normal serum for 1 hour at room temperature. After three washes in PBS, sections were incubated with appropriate secondary antibodies (Alexa Fluor 488; Molecular Probes) for 45 minutes. Sections were treated with DAPI nuclear stain, washed, and mounted as described earlier. Images were captured and underwent deconvolution, as described for LC images.

Protein Extraction and Western Blot Analysis
Total cellular protein was collected from cultured cells in a lysis buffer containing the following: 10 mM Tris-HCl, 0.5% NP40, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 0.2 mM PMSF in ethanol, 1 µg/ml aprotinin, 4 µg/ml pepstatin, 10 µg/ml leupeptin, and 1 mM sodium orthovanadate (10 µM/ml). Protein concentration was measured using a commercial system (Dc Protein Assay System; Bio-Rad Laboratories, Richmond, CA). Cellular lysate was separated on denaturing polyacrylamide gels and then transferred by electrophoresis to nitrocellulose membranes. Blots were processed using primary NT, trk, and p75 antibodies (Santa Cruz Biotechnology, Inc.) and a chemiluminescence immunodetection system (WesternBreeze; Invitrogen, Carlsbad, CA). Blots were then exposed to film (Hyperfilm-ECL; Amersham, Arlington Heights, IL) for various times depending on the amount of target protein present. Human recombinant NGF, BDNF, NT-3, and NT-4 (Sigma-Aldrich) were used as positive controls.

Immunoassays for NTs
Immunoassays (ELISAs) were used to determine the amount of NT secreted by LC cells and ONH astrocytes. Conditioned medium was collected from preconfluent adult LC cells and ONH astrocytes after a 72-hour treatment with serum-free medium containing 0.5 mg/ml BSA. Immunoassays (Emax Immunoassay Systems; Promega), specific for each NT were performed according to the manufacturer’s instructions. Medium was added to 96-well plates (Nunc ELISA/EIA Maxisorp; Fisher Scientific) coated with anti-NT polyclonal antibodies. Secreted NT was detected by treating the plates with the respective NT monoclonal antibody, followed by a horseradish peroxidase–conjugated secondary antibody. Enzyme substrate was added to generate a color product with an absorbance read at 450 nm. An NT standard was included in each assay and was used to generate a standard curve. Samples were assayed in triplicate.

Exogenous NGF and the Cell Proliferation Assays
Confluent LC cells and ONH astrocytes were trypsinized and seeded in triplicate into six-well plates at a density of 10,000 cells per well in 2 ml medium. Cells were allowed to plate down for 24 hours and were then treated with serum-free medium. Twelve hours later, cells were washed twice with serum-free medium and treated with either 50 ng/ml human recombinant NGF (Sigma-Aldrich) in serum-free medium, serum-free medium alone, or medium plus 10% FBS. Cells were maintained in 5% CO₂, 95% O₂ at 37°C and the media were changed every 2 days during the 14-day treatment. Cells were then washed twice with serum-free medium, trypsinized, and counted (Coulter Counter ZM; Technical Communications, Hialeah, FL).

RESULTS
Isolation of Two Distinct Cell Types from Explants of the Human LC
LC cells grew as broad, flat cells and displayed a prominent nucleus containing nucleoli (Fig. 1A). Eleven LC cell lines were obtained from donors that ranged in age from 21 postconception weeks to 90 years. ONH astrocytes grew as large stellate cells with many long, thin processes that extended to make contact with neighboring cells (Fig. 1B). Seven ONH astrocyte cell lines were obtained from donors that ranged in age from 36 postconception weeks to 90 years. Immunofluorescent staining for various LC cell and ONH astrocyte markers was performed to characterize the cell lines used in this study. Both cell types demonstrated positive staining for NCAM, α-smooth muscle actin, and a variety of extracellular matrix proteins, including collagen types I, III, and IV; elastin; laminin; and fibronectin (data not shown). These results are consistent with previous findings.21-22,26

Expression of GFAP in ONH Astrocytes from the Human LC
All cDNA samples from cultured cell lines, positive control cells, and tissues underwent PCR amplification of β-actin to ensure that no genomic DNA contamination was present. The β-actin primer pair was designed to span exons so that the amplification of contaminating genomic DNA sequences would produce a PCR product substantially larger (790 bp) than the expected mRNA product (350 bp). A β-actin amplification product of the expected size was observed in adult LC and ONH astrocyte cell lines (Fig. 2). A similar product was observed in all other cell lines and tissues used (data not shown). Samples of cDNA from LC and ONH astrocyte cell lines were also used in the amplification of GFAP (Fig. 2). No amplification products for GFAP were detected in any of the LC cell lines (Fig. 2, lanes 1–4). An amplification product at the expected size of 285 bp was detected in ONH astrocyte cell lines (lanes 5–8), ONH tissue (lane 9), and brain astrocytes (lane 10). Positive immunofluorescent staining of GFAP was also observed in ONH astrocytes and brain astrocytes (Figs. 3A, 3B). LC cells showed no immunoreactivity to GFAP (Fig. 3C).
Expression of NT and Trk Receptor mRNAs in Human LC Cells

Amplification products of expected size for each NT or trk primer pair in adult cell lines and tissue from the LC are shown in Figure 4. Although only adult cell lines are shown, all LC cell and ONH astrocyte lines expressed message for the NTs, and for both full-length and truncated trk receptors. The low-affinity receptor p75 was not detected in any LC cell or ONH astrocyte cell line, but was detected in the positive control cell line (Fig. 4, lane 8; PC-12 cells). An alternate amplification product (450 bp) was detected in the truncated trk C PCR reaction. This product will be sequenced to determine its identity. Control reactions without cDNA did not result in amplification products (Fig. 4, lane C), indicating that reagents and primers were free of DNA or RNA contamination.

Expression of NT and Trk Receptor Proteins in Human LC Cells

Representative immunofluorescent staining for NTs and trk receptors in adult LC cells and ONH astrocytes is shown in Figure 5. Both LC cells and ONH astrocytes demonstrated positive staining for all the NTs and trk receptors. No significant differences in intensity or staining pattern were observed between the two cell types. A diffuse cytoplasmic staining pattern was observed in cells stained for NGF (Fig. 5A), BDNF (Fig. 5B), and NT-4 (not shown). Cells stained for NGF also demonstrated a punctate nuclear staining pattern. Staining for NT-3 appeared to be concentrated over the nucleus (Fig. 5C). Immunofluorescent staining for full-length trk C and truncated trk B (not shown) were similar to the punctate pattern observed for trk A (Fig. 5D) and trk B (Fig. 5E). No staining was observed when primary antibody was omitted (Fig. 5F) or when appropriate control peptides were added (not shown).

Representative immunofluorescent staining for NTs and trk receptors in ONH tissue from human eyes is shown in Figure 6. GFAP staining (Fig. 6A) was used as a positive control. Positive staining for all the NTs and trk receptors was observed in ONH tissue. NGF appeared to localize to cells on or within the laminar plates (Fig. 6B), as did BDNF and NT-4 (data not shown). Similar to the cell-staining results, NT-3 appeared to be concentrated near the nuclei of cells within the LC (Fig. 6C). A punctate staining pattern along the laminar plates was observed for Trk A and Trk B (Figs. 6D, 6E), as well as for trk C and truncated trk B (data not shown). No staining was observed when nonimmune serum was used in place of primary antibody (Fig. 6F) or when the primary antibody was omitted (not shown).

Figure 7 represents chemiluminescent detection of NT and trk receptor proteins in adult cell lines from the LC. Similar to the immunofluorescent data, all cell lines studied expressed NT and trk receptor protein. Recombinant human NGF, BDNF, NT-3, and NT-4 were used as positive controls (Fig 7, lane 6). The NTs were detected in adult cell lines (lanes 1–5) and...
positive control lanes at molecular weights between 30 and 50 kDa, which suggests NT dimers. Cells from the LC expressed trk receptors that ranged in molecular weight from 120 to 180 kDa. Two bands were detected in the trk A and truncated trk B blots, which most likely represent glycosylated and partially glycosylated forms. Trk B was the only molecular weight exception, with a band at 65 kDa. This band may represent a nonglycosylated form of the trk receptor similar to that previously discovered for trk A.27 No p75 protein was detected in any cell line from the LC, which is consistent with both immunostaining and RT-PCR data.

**Secretion of NTs by Human LC Cells**

The secretion of NTs by cultured cells from the human LC, as detected using immunoasays (ELISAs) for each of the NTs, is shown in Table 2. The results for NGF and NT-3 obtained from LC cells and ONH astrocytes were within the sensitivity range of the NT immunoasays. However, although BDNF was detected in media collected from LC cells and ONH astrocytes, the values were below the sensitivity range of the assay. The secretion of NT-4 was detected in conditioned medium from the 66-year-old POAG LC cell line. No other cell line from the LC (including a POAG ONH astrocyte line) appeared to secrete NT-4 to any detectable level.

**DISCUSSION**

In this study we examined the expression of NT and trk receptors by cells and tissue from the LC region of the human ONH. LC cells, ONH astrocytes, and ONH tissue expressed NTs and trk receptors, and cells from the LC were capable of secreting NTs in culture. To our knowledge, this is the first time that NT and trk receptor expression or NT secretion has been shown in cultured cells and tissue from the LC region of the human ONH.

The cells that were isolated from the human LC were similar to those previously reported by Hernandez et al.,21 and Clark et al.,22 with respect to morphology and immunoreactivity to GFAP and other cell markers (e.g., elastin, collagen, and laminin). Based on positive GFAP and NCAM expression,28,29 we...
believe that the ONH astrocytes used in this study were type 1B astrocytes. LC cells also expressed NCAM, but did not show positive staining for GFAP. To further ensure the specificity of these cell types, we examined the expression of GFAP using the sensitive RT-PCR procedure. Message for GFAP was detected in ONH tissue, ONH astrocytes, and brain astrocytes, but not in any LC cell line. Because astrocytes are defined by GFAP expression, and our results indicate that LC cells do not express mRNA or protein for GFAP, we believe that LC cells are a distinct cell subpopulation within the human LC.

Our results demonstrate that LC cells and ONH astrocytes express mRNA for NGF, BDNF, NT-3, and NT-4. The positive immunofluorescent staining, Western blot results, and NT secretion by these cell types indicate that NT message was translated to protein within cultured LC cells and ONH astrocytes. The apparent nuclear staining observed for NGF and NT-3 within our cells was similar to that reported for NT-3 in human trabecular meshwork cells, for ciliary neurotrophic factor in rat astrocytes, and for FGF-2 in human endothelial cells. This staining pattern was observed in our cells, by using two different NGF and three different NT-3 antibodies (not shown). The concentration of secreted NTs in conditioned medium is similar to concentrations that have been shown to elicit cellular responses in a variety of cells, including neurons. This implies that both LC cells and ONH astrocytes secrete NTs that have biological effects within the LC. Although LC cells and ONH astrocytes expressed NT-4 mRNA and protein, NT-4 secretion was detected in only one cell line from the LC. Other cell lines from the LC may secrete NT-4, but below the sensitivity range of the assay (9.4 pg NT-4/ml). It is also possible that NT-4 acts within the cell in an intracrine fashion, or that because NT-4 and BDNF use the same receptor (e.g., trk B), these cells do not have to secrete both NTs.

In addition to the expression of NTs, we also detected the expression of mRNA and protein for three full-length trk receptors and two truncated trk receptors by cultured LC cells and ONH astrocytes. None of the cell lines expressed mRNA or protein for the low-affinity p75 receptor. Selective cell types have been reported to require p75 as a coreceptor to form high-affinity NGF binding sites with subsequent signaling. However, trk A expression in the absence of p75 is adequate for NT signaling by other cell types. We observed LC cell proliferation in response to exogenous NGF, which suggests that LC cells do not require the expression of p75 to respond to NGF. Whether ONH astrocytes respond to NGF in the absence of p75 will be determined.

Another interesting aspect of this study was the expression of truncated trk receptors by LC cells and ONH astrocytes. Two truncated trk C RT-PCR amplification products were detected in our cell lines. We believe the 450-bp product to be one of the four truncated trk C isoforms previously described. Sequencing of the 450-bp product will determine which of the truncated trk C isoforms is expressed by LC cells and ONH astrocytes. At the present time the function of truncated trk receptors by LC cells and ONH astrocytes in eliciting cellular responses in a variety of cells, including neurons, is unclear.5,6 This implies that both LC cells and ONH astrocytes secrete NTs that have biological effects within the LC. Although LC cells and ONH astrocytes expressed NT-4 mRNA and protein, NT-4 secretion was detected in only one cell line from the LC. Other cell lines from the LC may secrete NT-4, but below the sensitivity range of the assay (9.4 pg NT-4/ml). It is also possible that NT-4 acts within the cell in an intracrine fashion, or that because NT-4 and BDNF use the same receptor (e.g., trk B), these cells do not have to secrete both NTs.

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receptors has not been determined in any cell type. It has been suggested that truncated trk receptors function as naturally occurring dominant negative elements when coexpressed with their full-length isoforms. It also has been suggested that truncated trk receptors serve as coreceptors, similar to p75. They may act to sequester NTs within the extracellular space or signal through a yet undiscovered pathway. It is unknown at this time whether truncated trk receptors effect NT signaling within the LC.

The classic mechanism of NT action states that only neuronal target cells synthesize and secrete NTs. In addition, it was commonly thought that the expression of NTs was primarily restricted to the nervous system. However, both LC cells and ONH astrocytes are not considered to be target cells for neurons. Our results support a growing body of evidence that indicates non-neuronal cells within the central nervous system as well as non-neural cells express and secrete NTs. The expression and secretion of NTs by LC cells and ONH astrocytes may help maintain the normal microenvironment of the LC. Alternatively, because of their close association to retinal ganglion cell axons in the anterior and posterior LC, ONH astrocytes may secrete NTs to act directly on retinal ganglion cell axons, thus maintaining the viability of the ganglion cell. In either case, nonneuronal target cells are synthesizing and secreting NTs, which supports a modified concept of NT action.

NTs secreted by LC cells and ONH astrocytes could serve as an alternative source of NTs for retinal ganglion cells. Although trk receptors have not been examined on unmyelinated retinal ganglion cell axons within the LC, they have been localized on neuronal cell bodies, dendrites, and axons within the CNS. Okazawa et al. localized trk B and trk C immunoreactivity to the cell bodies and dendrites of cerebral cortex neurons, as well as to the axons and dendrites of retinal ganglion cells within the retina. Similar localization was reported by Yan et al. after the staining of adult rat brain tissue with trk B. Positive trk B staining was observed in neuronal cell bodies, axons, and dendrites in many brain regions, including the cerebral cortex, the hippocampus, the thalamus, and the cerebellum.

In regard to retinal ganglion cells and their axons, Jelsma et al. demonstrated trk B and truncated trk B staining in the ganglion cell layer, in retinal ganglion cell axon bundles, and in the optic nerve, suggesting that retinal ganglion cells bind NTs from sources along their axons. One such source may be LC cells and/or ONH astrocytes. For example, under normal circumstances retinal ganglion cells may receive most of their NT support from retrograde NT sources. However, retrograde transport is blocked at the level of the LC in POAG. To remain viable, retinal ganglion cells may require additional NT support from paracrine sources such as LC cells and ONH astrocytes. Decreased NT and/or trk expression by LC cells or ONH astrocytes would ultimately reduce the amount of NT available to retinal ganglion cells. Ganglion cell death would slowly die as the amount of NTs available from retrograde and paracrine sources decreased. Similarly, NTs meant for retinal ganglion cells may instead bind and activate trk receptors on nearby LC cells and ONH astrocytes. The reactive astrocytes associated with glaucomatous damage to the ONH would appear to be consistent with the latter hypothesis.

The expression of NTs and trk receptors by LC cells and ONH astrocytes could be entirely non-neuronal in function. Recent evidence contains many examples of autocrine and paracrine NT signaling between non-neuronal cells. Seilheimer and Schachner demonstrated the expression of NGF, p75, and trk A by Schwann cells and then demonstrated that autocrine NT signaling within this cell population influences the expression of L1, an NCAM. Actively differentiating retinal pigment epithelial (RPE) cells express BDNF and trk B, and after inhibition of BDNF signaling through dominant negative trk B expression, RPE differentiation is arrested. Human keratinocytes have been shown to regulate cell proliferation through an autocrine NGF loop. Similarly, paracrine NGF signaling between NGF-producing stromal cells and trk A-expressing epithelial cells appears to regulate epithelial cell growth within the human prostate. The rate of hair follicle morphogenesis is believed to be controlled by spatiotemporal expression of NTs and their receptors by follicular epithelium and mesenchyme. Maroder et al. examined thymocyte maturation in response to BDNF produced by thymic stromal cells. Signals that influence thymocyte maturation also influenced the expression of trk B by the thymocytes. The function of the LC region of the ONH is to guide and support retinal ganglion cell axons as they exit the eye. Paracrine and/or autocrine NT signaling by LC cells and ONH astrocytes within this region may be important in regulating the normal function of the ONH.

LC cells and ONH astrocytes produce the extracellular matrix (ECM) that gives strength and resiliency to the ONH. Paracrine NT signaling within the LC may regulate ECM metabolism through transforming growth factor (TGF)-isoforms. The expression of TGF- isoforms and their receptors has been shown in LC cells and ONH astrocytes, as well as in human ONH tissue. In addition, NT signaling induces the transcription of TGF- isoforms. In POAG, there are marked changes in the ECM of the LC that are due to the abnormal biosynthesis and/or degradation of ECM proteins by LC cells and ONH astrocytes. Changes in the expression of NTs and/or trk receptors within the LC could alter the expression of TGF-, thus upsetting the normal balance of ECM synthesis and degradation. This scenario would explain some of the ECM changes observed in POAG.

In conclusion, the results of this study indicate that two specific cell populations within the LC express NTs and trk receptors and also secrete NTs. The data presented in this report support a growing body of evidence demonstrating NT and trk expression by non-neuronal cells. Taken as a whole, this evidence suggests NT signaling may regulate more than neuronal development, survival, and differentiation. Further studies demonstrating the activation of trk receptors in LC cells and ONH astrocytes and the cellular processes regulated by NT signaling are required to fully understand NT and trk expression within the LC. The expression of NTs and trk receptors by LC cells and ONH astrocytes in glaucomatous eyes may help to determine the role of these cells in POAG in terms of retinal ganglion cell survival and/or the metabolism of the ECM. Using this knowledge, new therapeutic strategies involving NTs could be developed to better treat patients with POAG.

Acknowledgments

The authors thank The Central Florida Lions Eye and Tissue Bank for ocular tissue and Sherry English-Wright, Paula Billman, Anne-Marie Brun, and Lawrence X. Oakford for technical assistance.

References


