Reverse Transcription-PCR Analysis of Laser-Captured Cells Points to Potential Paracrine and Autocrine Actions of Neurotrophins in Pancreatic Cancer

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Abstract

**Purpose:** Neurotrophins (NTs) can stimulate cell proliferation and differentiation in various cell types, and play a role in certain human cancers. In this study we analyzed the expression and localization of NTs and their receptors in micro-dissected pancreatic cancer tissue samples, and studied their ability to stimulate cell growth.

**Experimental Design:** The expression of nerve growth factor, brain-derived neurotrophic factor, NT-3, and NT-4/5, and the receptors tropomyosin receptor kinase A, B, and C, and P75 was studied in pancreatic cancer cell lines, and normal and pancreatic ductal adenocarcinoma (PDAC) tissue samples by quantitative reverse transcription-PCR. Laser capture microdissection was performed in 21 PDAC samples, and mRNA levels were determined in cancer cells, acinar cells, desmoplastic stroma areas, and nerve fibers. Cell growth assays with NTs and in coculture with dorsal root ganglia were performed.

**Results:** NT receptors were differentially expressed in the cancer cell lines, whereas tropomyosin receptor kinase C was not detectable. NTs modulated pancreatic cancer cell growth. Analysis of nonmicrdissected samples revealed that all of the receptors and tested ligands were over-expressed in PDAC when compared with normal pancreas. Analysis of laser captured samples revealed that NTs and their receptors were expressed in the cancer cells but were especially abundant in the intratumoral nerves. Coculture of dorsal root ganglia with T3M4 cells significantly enhanced the proliferation of this cell line.

**Conclusion:** The abundance of NTs in the intratumoral nerves in PDAC and the presence of NT receptors in the cancer cells, in conjunction with the ability of NTs to modulate pancreatic cancer cell growth, point to potential paracrine and autocrine effects of NTs in PDAC. Thus, our findings provide additional evidence that blocking NT actions may have a therapeutic potential in PDAC.

Introduction

PDAC is the fourth leading cause of cancer death in the Western world (1, 2). Its aggressive growth behavior with early local invasiveness and early metastasis disqualifies a high percentage of patients from having surgical resection at the time of diagnosis. Unfortunately, chemotherapy and radiotherapy have not been effective at prolonging survival beyond a few weeks, resulting in overall 5 year survival rates of <5% (3). Furthermore, PDACs exhibit perineural invasion in 70–98% of the tumor samples, and extratumoral, intrapancreatic nerve invasion is found in >50% of these cancers (4–7). Because perineural invasion in PDAC correlates with a worse prognosis (4), greater pain intensity (8), and advanced tumor stage and grade (7), it is important to determine the mechanisms that contribute to nerve cell-cancer cell interactions in PDAC.

The family of structurally related neurotrophic growth factors comprises the prototypic NGF and the related ligands BDNF, NT-3, NT-4/5, NT-6, and NT-7. These factors are synthesized as precursor proteins that are proteolytically cleaved to the mature peptides. All of the NTs are structurally similar and exhibit ~50% amino acid sequence homology with each other (9). They promote and modulate neuronal development, survival, plasticity, and function (10), and modulate axonal outgrowth during development in both the peripheral nervous system and central nervous system (11). NTs initiate their actions by binding to two distinct groups of cell surface receptors. One class of NT receptors is represented by three distinct transmembrane tyrosine kinase Trk receptors termed TrkA, TrkB, and TrkC (12). NGF and NT-3 preferentially bind TrkA and TrkC, respectively, whereas BDNF and NT-4/5 preferentially bind TrkB (12). Thus, NT-3 also binds to TrkA and TrkB, but with lower affinity than to TrkC (12). The other class of NT receptors

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2 The abbreviations used are: PDAC, pancreatic ductal adenocarcinoma; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT, neurotrophin; Trk, tropomyosin receptor kinase; MAPK, mitogen-activated protein kinase; Erk, extracellular signal-regulated kinase; LCM, laser capture microdissection; RT-PCR, reverse transcription-PCR; MTT, 3-(4,5-methylthiazol-2-yl)-2,5-diaphenylterazolium bromide.

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is represented by p75 (13). It belongs to the tumor necrosis factor receptor/Fas/CD40 superfamily and binds all of the NTs with a low binding affinity (14).

Trk receptors activate multiple signaling pathways that include the Ras/Raf/mitogen-activated protein/Erk kinase/MAPK pathway (15), the insulin receptor substrate/Gab-1/phosphatidylinositol 3'-kinase/protein kinase B/AKT (16, 17) pathway and the phospholipase C-γ/diacylglycerol/protein kinase C/IP3/Ca2+ pathway (18, 19). In the presence of TrkA, p75 can participate in the formation of high-affinity binding sites, resulting in enhanced NGF responsiveness and growth-promoting signals (20). In contrast, in the absence of Trk receptors, p75 is capable of mediating signals that lead to cell death (21, 22).

NGF is overexpressed in PDAC, and this overexpression is associated with increased perineural invasion (8). In vitro, NGF induces the growth of pancreatic cancer cells through phosphorylation of TrkA and subsequently via MAPK, and the effects of NGF on pancreatic cancer cell growth are dependent on the relative expression levels of TrkA and p75 (23). Despite these observations, the specific site of expression of NTs and their receptors in PDAC have not been clearly delineated. Therefore, in the present study we performed LCM and quantitative PCR analysis for NTs and their receptors in PDAC samples. We also studied the effects of NTs on cell growth and MAPK activation in pancreatic cancer cells, in relation to the receptor expression profile, and sought to determine whether NTs released by nerve cells can stimulate pancreatic cancer cell growth in vitro. We now report that the overexpression of NTs and their receptors in PDAC is attributable mainly to their abundance in the intrapancreatic nerves. It is also suggested that nerve cell-derived NTs can signal and stimulate the growth of pancreatic cancer cells.

Materials and Methods

Cell Culture. Human pancreatic cancer cells were routinely cultured in complete medium consisting of DMEM (COLO-357 and PAN-C1) or RPMI 1640 (ASPC-1, CAPAN-1, and T3M4) that was supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (all from Irvine Scientific, Santa Anna, CA) at 37°C in a humidified air atmosphere with 5% CO2. For experiments with NTs (R&D Systems, Minneapolis, MN) cells were incubated for 24 h in serum-free medium (DMEM or RPMI 1640 containing 0.5% BSA, 5 μg/ml apo-transferrin, 5 ng/ml sodium selenite, and antibiotics) and then incubated in serum-free medium in the presence or absence of ligands for the indicated times.

Tissue Samples. Pancreatic cancer tissue samples were obtained from patients with pancreatic cancer undergoing surgery for their disease. Normal pancreatic tissue samples were obtained from organ donors. Tissue samples were frozen in liquid nitrogen immediately after removal and stored at −80°C until use. All of the studies were approved by the Human Subjects Committees at the University of California, Irvine, and the University of Heidelberg.

LCM. For LCM the tissue samples were embedded in OCT (Sakura Finetek, Torrance, CA) by freezing the blocks in an acetone bath within liquid nitrogen. Tissues were then stored at −80°C until use. Sections (6–8 μm) were prepared using a Reichardt-Jung Cryocut 1800 cryostat, attached to glass slides, and immediately frozen on a block of dry ice followed by fixation (75% ethanol) and staining (1.5% Eosin Y; Sigma) for 30 s. Sections were then washed with 95% ethanol and dehydrated in 100% ethanol. The sections were then incubated with xylene, air dried, and subjected to LCM, using the Pix Cell instrument and CapSure LCM Caps (Arcturus Engineering, Mountain View, CA), as reported previously (24).

RNA Extraction. RNA was extracted using the SV Total RNA isolation System (Promega, Madison, WI) according to the manufacturer’s protocol. Cells from 5 to 15 serial sections were pooled in one tube of lysis buffer. For total tissue analysis, sections of OCT-embedded tissue samples (20 μm) were cut, mechanically separated from the embedding medium, and transferred into the lysis buffer. After elution from the column the samples were concentrated by ethanol precipitation in the presence of 100 mM sodium acetate and 5 μg glycogen. The concentration was measured using RiboGreen (Molecular Probes, Eugene, OR) and a CytoFluor fluorescence plate reader (Per-Septive Biosystems, Framingham, MA). To ensure the integrity of the RNA, ~5 ng were analyzed on an Agilent 2100 bioanalyzer using the RNA LabChip. Only RNA exhibiting a ratio of 28S:18S >1 was subjected to RT-PCR analysis. Cell line RNA was extracted by the single-step acid guanidinium isothiocyanate-phenol chloroform method (25) and integrity confirmed by ethidium bromide staining after separation on a 1.2% agarose gel.

Reverse Transcription and TaqMan Quantitative PCR. To analyze expression in the cell lines, RNA was DNase treated and reverse transcribed using the SuperScript kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s recommendations using random hexamer primers. To analyze expression in tissues (50 ng) and LCM (5 ng) samples, RNA was reverse transcribed using random hexamers and the Sensiscript kit (Quiagen, Valencia, CA). One tenth of each reaction was used as template for the PCR reaction. Q-PCR was performed using the 7700 Sequence Detector (Applied Biosystems, Foster City, CA) and TaqMan technology. Reactions (25 μl) were set up using the 2× Universal PCR Master Mix (Applied Biosystems), template cDNA, and adequate concentrations of primers and probes. All of the samples were done in duplicates, data were analyzed using the relative standard curve method with serial dilutions of cDNA made of human fetal brain RNA (Clontech, Palo Alto, CA), and expression levels were normalized to the expression of 18S or β-actin as internal control. To ensure the sensitivity and authenticity of the primers and probes, the PCR-derived products were subcloned into the TA-cloning Vector (Invitrogen), and authenticity confirmed by sequencing. Plasmid concentrations were measured using the PicoGreen dsDNA quantitation kit (Molecular Probes), and the molar concentration was calculated. Serial dilutions of pure plasmid in an artificial DNA carrier solution [1 ng/μl poly(deoxadenylate-deoxythymidylic acid); Amersham Pharmacia Biotech, Piscataway, NJ] were subjected to PCR analysis to determine the sensitivity of the PCR system, which was between 200 and 200 molecules per reaction for each primer pair. The following sequences were used for the primers and probes. NGF forward: AGACCA-AGTGGCGGGACC, NGF reverse: CAGTGGCTTGTAGTCA-ATGCC, NGF Probe: VIC-AATCCCGTTGACACGGGT-
Western Blot analysis of NT receptors. Cell lysates (20 μg/sample) were subjected to immunoblotting with antibodies specific for the indicated target. Erk-2 antibody was used to confirm equal loading of lanes. A, 140 kDa TrkA; B, 110 kDa TrkB; and C, 75 kDa p75.

**Cell Growth Assay.** Cells were seeded in 96-well plates at a density of 4,000–10,000 cells per well, and incubated overnight in complete medium. Cells were then serum starved for 24 h and then incubated in serum-free medium in the presence or absence of ligand. Growth assays were initiated by adding MTT solution at a final concentration of 62.5 μM of MTT per well. After 4-h incubation at 37°C the medium was removed, and the dye crystals were dissolved in acidified isopropanol. The absorbance was measured at 570 nm and 650 nm with an ELISA plate reader (Molecular Devices, Menlo Park, CA; Ref. 27). Data were expressed as percentage of control cell growth. In pancreatic cancer cells, the results of the MTT assay correspond with results obtained by cell counting with a hemocytometer or by monitoring [3H]thymidine incorporation into DNA (28).

**Coculture of Dorsal Root Ganglia and Pancreatic Cancer Cell Lines.** T3M4 and PANC-1 cells were plated in 24-well plates at a density of 20,000 cells per well, allowed to adhere overnight, and then incubated in serum-free medium for 24 h before the application of transwell porous cell culture inserts (0.4 μm pore membrane; Corning Inc., Corning, NY). Dorsal root ganglia were removed from E13 fetal Swiss Webster mice using a dissecting microscope and washed in serum-free medium. Ganglia (3/transwell insert) were then cocultured for 72 h with the pancreatic cancer cells. The inserts were then removed and MTT assays were performed as described above. For this set of experiments, empty inserts served as controls. The experiments with mouse-derived dorsal root ganglia were approved by the...
Results

Expression of NTs and Their Receptors in Pancreatic Cancer Cell Lines. We first analyzed the expression levels of NTs and their receptors in five pancreatic cancer cell lines. NGF, BDNF, NT-3, and NT-4/5 mRNA moieties were detected in all of the cell lines. All four of the NTs were expressed at relatively high levels in ASPC-1 cells. PANC-1 cells expressed relatively high NGF mRNA levels, T3M4 cells expressed relatively high BDNF mRNA levels, whereas CAPAN-1 and COLO-357 cells expressed relatively high NT-4/5 mRNA levels (Fig. 1A). TrkA, TrkB, and p75 mRNA moieties were detected in all five of the cell lines, whereas TrkC was not detected. By contrast, TrkC mRNA was detected in fetal brain RNA, and the sequenced PCR product confirmed its authenticity. TrkA mRNA levels were relatively high in PANC-1, CAPAN-1, and COLO-357 cells, and lower in ASPC-1 and T3M4 cells (Fig. 1A). TrkB mRNA was most abundant in T3M4 cells and expressed at lower levels in the other cell lines. By contrast, p75 mRNA was high in T3M4 and COLO-357 cells, moderate in CAPAN-1 and PANC-1 cells, and low in ASPC-1 cells (Fig. 1B).

Expression of NTs and Their Receptors in Pancreatic Cancer Tissues. Next, total RNA from 14 normal human pancreatic tissues and from 18 PDAC samples was analyzed by quantitative RT-PCR (Fig. 3). Although these samples were not subjected to LCM, the presence of cancer cells in each PDAC sample was verified histologically. There was a 4.1-fold \((P < 0.02)\) increase in NGF mRNA levels in the tumor samples by comparison with normal controls, whereas BDNF and NT-3 mRNA levels were \(\sim 6.5\)-fold greater \((P < 0.01 \text{ and } P < 0.001)\) in the cancer samples. The NT-4/5 mRNA transcript was only detectable in two normal samples (15%) but was present in 11 of 18 cancer samples (61%). Furthermore, there was a 27-fold
overall increase in NT-4/5 mRNA levels in the cancers by comparison with the normal pancreas ($P < 0.03$). PDAC samples also exhibited a 3.1-fold increase ($P < 0.05$) in TrkA mRNA levels by comparison with the normal pancreas, whereas the corresponding increases in TrkB, TrkC, and p75 were not statistically significant (Fig. 3).

**Expression of NTs and Their Receptors in Laser Captured Samples.** To accurately measure NT/receptor expression levels in specific cell types within the tumor mass we performed LCM in 21 PDAC samples, and separately captured cancer cells, nerves, acinar cells, and stromal elements for analysis by Q-PCR. This analysis revealed that NTs and their receptors were always present at markedly higher levels in nerves by comparison with the other cell types (Figs. 4 and 5). BDNF and NT-4/5 mRNA levels were similar in the cancer cells, acinar cells, and stromal areas, whereas NGF and NT-3 mRNA levels were lower in the cancers cells in comparison with the stromal areas. The levels of all four of the NT receptors were generally similar in the cancer cells and in the stromal...
areas, with the exception of TrkC, which was especially abundant in the stromal areas. Although TrkA mRNA levels were relatively high in the acini, the other receptors were expressed at very low levels in this compartment. The expression levels for NTs and their receptors did not correlate significantly with tumor stage or grade. However, the five PDAC samples in which the nerves did not exhibit perineural invasion expressed relatively low levels of p75, whereas all four of the samples in which the nerves exhibited perineural invasion exhibited higher levels of p75 (P < 0.004).

**Effects of NTs and Mouse Dorsal Root Ganglia on Pancreatic Cancer Cell Growth.** NGF (5 nM) stimulated the growth of PANC-1 cells by 28% (P < 0.05) but inhibited the growth of T3M4 cells by 18% (P < 0.004; Fig. 6). By contrast, BDNF and NT-3 increased the growth of T3M4 cells by 31% (P < 0.05) and 43% (P < 0.02), respectively, but did not significantly alter the growth of PANC-1 cells. None of the NTs significantly altered the growth of CAPAN-1 cells (Fig. 7). NT-3 and NT-4/5 enhanced MAPK activation in both T3M4 and PANC-1 cells after 10 min of incubation (Fig. 7). In contrast, NGF and BDNF stimulated MAPK activation in T3M4 cells but not in PANC-1 cells (Fig. 7).

To assess to what extent the activation of the MAPK pathway is responsible for the growth-stimulating effects in PANC-1 and T3M4 we treated the cells with the highly specific MAPK kinase inhibitor UO126. The stimulatory effects of all of the NTs on cell growth were inhibited in both tested cell lines (Fig. 8). Furthermore, simultaneous culture of freshly prepared fetal mouse dorsal root ganglia with T3M4 cells resulted in a 37% increase in cancer cell growth (P < 0.005) compared with controls without the ganglia (Fig. 9). By contrast, coculture of fetal mouse dorsal root ganglia with PANC-1 cells did not alter the growth of the cancer cells (data not shown).

**Discussion**

The pancreas is a complex organ consisting of endocrine islet cells that produce and secrete hormones such as insulin and glucagon, exocrine cells that synthesize and secrete digestive enzymes such as amylase and lipase, and duct cells that generate a bicarbonate-rich fluid. In mammals, an intrapancreatic portal circulation assures that numerous regions within the exocrine pancreas are exposed to high levels of islet-derived endocrine hormones, thereby allowing for functional interactions between the endocrine and exocrine pancreas (29, 30). Similarly, a variety of studies have documented the existence of an enteropancreatic nervous system that consists of intrapancreatic ganglia and a network of nerves that innervate the acini and islets, and that participate in the coordinated regulation of pancreatic endocrine and exocrine functions (31, 32). Whereas it is generally accepted that pancreatic cancer cells can invade blood vessels and lymphatics that are present in the pancreas, it is less well appreciated that these cancer cells also frequently invade the intrapancreatic nerve tracts (33). Indeed, nerve involvement by cancer cells is quite high in PDAC (7) and often extends into extrapancreatic nerve bundles in the retroperitoneal space that is dorsal to the pancreas (4). This site often becomes a nidus for disease recurrence after attempts at curative resection (4, 34), underscoring the need for obtaining a better understanding of the mechanisms that contribute to perineural invasion.

Several studies have focused on the use of immunostaining to assess the potential role of altered expression of NTs and their receptors in PDAC as one mechanism that may contribute to perineural invasion. Such studies have yielded conflicting results, attributable perhaps to the quality of the different antibodies that were used, the different antigen exposure methods, and the different methods of fixing and storing pancreatic...
tissue samples. One study revealed the presence of moderate to strong NGF immunoreactivity in the cancer cells, and also reported that TrkA is absent in these cells both by immunostaining and by in situ hybridization (8). Another study reported the presence of relatively low levels of TrkA immunoreactivity in pancreatic cancer cells (35). By contrast, a third study reported that TrkA, B, and C expression was moderately increased in the cancer cells, as determined both by immunostaining and by in situ hybridization, whereas all four of the NTs were principally present in the stromal compartment (36). In a fourth study, TrkA, B, and C immunoreactivity was reportedly increased in the cancer cells, whereas the nerves exhibited high levels of NT-3 immunoreactivity, but not NGF or BDNF (37). Finally, a fifth study reported that NGF, TrkA, and TrkC, as well as p75 immunoreactivity, was increased in both the cancer cells and nerves, whereas NT-4 was present in the cancer cells but not the nerves, and BDNF and NT-3 was absent in both cell types (38).

In the present study, we performed two types of experiments with PDAC samples. First, we examined expression levels in nonmicrodissected PDAC samples by comparison with nonmicrodissected normal samples. In agreement with our previous findings by Northern blotting (8), Q-PCR of RNA isolated from nonmicrodissected PDAC specimens revealed 3.1- and 4.1-fold increases in TrkA and NGF mRNA levels in the cancers by comparison to the normal tissues. In addition, we determined that BDNF and NT-3 (~6.6-fold), as well as NT-4/5 (~27-fold) were overexpressed in PDAC by comparison to the corresponding levels in the normal pancreas. By contrast, the increases in TrkB, TrkC, and p75 mRNA levels in nonmicrodissected PDAC samples were not statistically significant when compared with normal controls.

In a second set of experiments, we delineated the relative levels of expression of NT and their receptors in different cell types within the pancreatic tumor mass by performing Q-PCR on laser captured cells. This analysis, which has the advantage of examining the expression levels of multiple mRNA moieties in the same defined populations of PDAC-derived cells, revealed the presence of relatively high levels of all four of the NTs and all four of the NT receptors in the nerves within the tumor mass. Moreover, there were significantly higher p75 mRNA levels in nerves in which the cancers exhibited perineuronal invasion compared with unaffected nerves. Because p75 promotes myelinization and is up-regulated in Schwann cells after nerve injury, this expression pattern may reflect attempts at nerve cell repair (39).

NGF, BDNF, and NT-4/5 mRNA moieties were also present in the cancer cells, but at much lower levels than in the nerves. Furthermore, in contrast with the nerves, the cancer cells did not express NT-3. All four of the NT receptor mRNA species were also detected in the cancer cells, but at relatively low levels. NGF, BDNF, NT-3, NT-4, and TrkC mRNA levels were also relatively high in the stromal compartment, whereas TrkA, TrkB, and p75 were similar to the levels observed in the cancer cells. NT-3 was the only NT that was not detected in acinar cells, whereas TrkA was the only receptor that was detectable in these cells. These observations suggest that, with the exception of NT-3, the cancer cells in PDAC have the capacity to synthesize multiple NTs and their receptors, but at lower levels than the nerve cells. Thus, when assessed globally in tissue homogenates, NT and NT receptor overexpression in PDAC is attributable mainly to their abundance in the nerve cells and in the stromal compartment in this malignancy. The differential expression of NTs and their receptors in the stroma and acinar cells also raises the possibility that certain NTs and their receptors may have specific functions in these compartments.

It has been reported previously that intrapancreatic neural cells exhibit strong NGF expression (40), and that both nerve cells and Schwann cells express NTs and their receptors (41). The present findings document, for the first time, that all four of the NTs and their receptors were expressed at higher levels in the nerve bundles in PDAC than in the cancer cells. Because these nerve bundles consist of a mixture of different cell types including perineural, endoneural, and Schwann cells in addition to nerve cells and nerve fibers (42), it is not clear from our analysis which of these components contributes the most to these high expression levels. Furthermore, all five of the pancreatic cancer cell lines that were examined in the present study expressed at least one NT moiety, and, as in the case of ASPC-1 cells, all four of the NTs. With the exception of ASPC-1 cells, which expressed very low levels of NT receptors, these cell lines also expressed variable levels of the various NT receptors. Furthermore, NTs enhanced cell growth and MAPK activation...
in vitro. Both of these effects were reversed by low concentrations of the specific MAPK kinase inhibitor UO126, indicating that the growth stimulatory effects of NTs on some of these pancreatic cancer cell lines is most likely mediated via this pathway. In addition, NGF inhibited the growth of T3M4 cells, yet activated MAPK in these cells, indicating that activation of MAPK cannot block the inhibitory actions of NGF. Growth-inhibitory effects of NGF in cancer cells have been reported previously and have been attributed to the induction of apoptosis by p75 (43). Although T3M4 cells expressed high levels of p75, we did not detect any evidence for apoptosis induction by NGF in T3M4 cells (data not shown).

Several theories have been proposed to explain the phenomenon of perineural invasion in PDAC. First, it has been suggested that cancer cells spread into the perineural space because it represents the path of least resistance (44). Second, the perineural space may provide an environment that is suitable for enhancing tumor cell proliferation and spread. Thus, transforming growth factor-α, the epidermal growth factor receptor, and neural cell adhesion molecules may be especially abundant in the enlarged pancreatic nerves that are present in PDAC, and these molecules may exert chemoattractant and growth-promoting effects on the cancer cells (42, 45). Third, recent findings that Schwann cell derived myelin sheets are rich in NTs (46) raise the possibility that the high expression of multiple NTs in the nerves that was observed in the present study is because of their abundance in myelin. These NTs may then exert chemoattractant effects on the cancer cells (47), as well as enhance their growth (present study and ref (36). Fourth, NGF can increase heparinase expression, an enzyme thought to play a major role in cancer infiltration (48, 49). Thus, the abundance of NTs in the nerves and the expression of NT receptors in the cancer cells may result in abnormal paracrine pathways that enhance cancer cell growth and promote their proclivity to infiltrate the nerve tracts in PDAC.

Our study also documents, for the first time, that T3M4 pancreatic cancer cells exhibit increased proliferation when cocultured with dorsal root ganglia. Because these cells express high levels of TrkB and p75, and are growth inhibited by NGF, it is possible that this stimulatory effect is mediated by BDNF or NT-3, because both ligands bind to TrkB. Several lines of evidence support this hypothesis. First, dorsal root ganglia are known to express high levels of BDNF and other NTs (50). Second, neutralizing antibodies against several NTs decrease pancreatic cancer growth in a xenograft mouse model (51). Third, in the present study BDNF and NT-3 increased the growth of T3M4 cells, which express high levels of TrkB. In contrast, neither factor altered the growth PANC-1 cells, which express very low levels of TrkB. Similarly, T3M4 cells, but not PANC-1 cells, were growth stimulated when cocultured with dorsal root ganglia. Whereas these findings do not exclude the
posibility that dorsal root ganglia produce other factors that are mitogenic toward pancreatic cancer cells, they suggest that the production of NTs by intratumoral nerves can contribute to the growth of some pancreatic cancer cells and may be partly responsible for the perineural invasion that is commonly observed in PDAC. In view of the abundance of NTs in the intrapancreatic nerves and the potential of NTs to stimulate pancreatic cancer cell growth, the development of therapies disrupting this pathway might be of use against cancer progression and may help improve the quality of life of these patients.

References


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