Expression Levels of the Nerve Growth Factor Receptors TrkA and p75 in Effusions and Solid Tumors of Serous Ovarian Carcinoma Patients

Ben Davidson,1 Philip Lazarovici,3 Alexandra Ezersky, Jahn M. Nesland, Aasmund Berner, Bjørn Risberg, Claes G. Tropé, Gunnar B. Kristensen, Mariusz Goscinski, Gregg van de Putte, and Reuven Reich3

Departments of Pathology [B. D., J. M. N., A. B., B. R., M. G.] and Gynecologic Oncology [C. G. T., G. B. K., G. v. d. P.], The Norwegian Radium Hospital, affiliated with the University of Oslo, Montebello N-0310, Norway, and Department of Pharmacology and Experimental Therapeutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel [P. L., A. E., R. R.]

ABSTRACT

Purpose: The purpose of this study was to analyze the expression of the high- and low-affinity nerve growth factor (NGF) receptors TrkA and p75 in effusions and in primary and metastatic tumors of serous ovarian carcinoma patients, as well as to evaluate their association with clinicopathological parameters and disease outcome.

Experimental Design: Sections from 77 malignant effusions and 78 primary and metastatic lesions were evaluated for protein expression of TrkA and p75 using immunohistochemistry (IHC). Expression of the phosphorylated form of TrkA (p-TrkA) was evaluated in 75 effusions using IHC. TrkA and p75 mRNA expression was studied in 44 effusions using reverse transcription-PCR (RT-PCR).

Results: TrkA protein membrane expression was detected in carcinoma cells in 30 of 77 (39%) effusions and 64 of 78 (82%) solid tumors. The decrease in TrkA expression in effusions approached, but did not reach, statistical significance when only corresponding lesions were analyzed (P = 0.06 in the comparison of effusions and primary tumors, P = 0.09 for effusions and metastases). Conversely, p75 protein membrane expression was more common in effusions, which was detected in 16 of 77 (21%) effusions as compared with 6 of 78 (8%) solid tumors (P > 0.05 in analysis of corresponding lesions). Expression of p-TrkA in carcinoma cells was limited to 5 of 75 effusions. Interestingly, 11 of 16 p75-positive effusions were also immunoreactive for the antibody against TrkA (P = 0.001), suggesting NGF activation using two signaling pathways. TrkA and p75 protein expression in tumor cells was similar in pleural and peritoneal effusions (P > 0.05). Using reverse transcription-PCR, TrkA mRNA was detected in 2 of 45 effusions, whereas p75 mRNA was present in 3 of 45 specimens. TrkA and p75 showed no association with tumor grade, Federation Internationale des Gynaecologistes et Obstetricistes stage, chemotherapy status, the extent of residual disease, or survival (P > 0.05).

Conclusions: TrkA and p75 are both expressed in advanced-stage ovarian carcinoma, but whereas p75 expression is elevated in effusions, TrkA shows an opposite trend. The different expression of NGF receptors in effusions may relate to the different microenvironment and growth factor availability in body cavities, as also supported by the infrequent activation of TrkA in effusions. The similar expression of TrkA and p75 in carcinoma cells in pleural and peritoneal effusions provides further evidence for our hypothesis that there are few, if any, phenotypic differences between ovarian carcinoma cells at these two sites. TrkA and p75 expression in effusions does not appear to be a predictor of disease outcome in advanced-stage serous ovarian carcinoma.

INTRODUCTION

Neurotrophins are a family of growth factors, consisting at present of NGF,4 brain-derived neurotrophic factor, and the neurotrophins NT-3, NT-4 and NT-6 (1, 2). Neurotrophins bind to specific high-affinity receptors, the three tyrosine kinase receptors TrkA, TrkB, and TrkC. NGF binds to TrkA, whereas brain-derived neurotrophic factor and NT-4 associate with TrkB. NT-3 can associate with all three receptors, but its biological effects are mediated through TrkC (1). Several isoforms of Trk receptor have been described, all containing an immunoglobulin-rich domain in the extracellular part of the receptor and transmembrane domains (1). Whereas TrkA receptors possess a tyrosine kinase catalytic domain, isoforms lacking this domain have been reported for TrkB and TrkC (1). p75, an additional neurotrophin receptor, belongs to the tumor necrosis receptor family, has a different structure, lacks intrinsic catalytic activity, and is able to bind all neurotrophins (1). NGF ligand binding to TrkA receptors activates several intracellular signal pathways.
transduction pathways and is involved in the differentiation and survival of neuronal cells (2). It is believed that p75-mediated signals are involved in apoptosis (2).

Although originally isolated from neural tissues, Trk and p75 expression has been reported in nonneural cell lines and in both benign and malignant human tissues, many of epithelial origin (3–15). TrkA expression has been shown to be associated with the malignant phenotype in prostate carcinoma (5) and with high histological grade and disease stage in esophageal carcinoma (15). Elevated expression of TrkA, TrkB, and TrkC was detected in pancreatic carcinomas as compared with normal pancreatic tissue (11). Reduced TrkB and elevated TrkA and p75 expression has been reported in nonneural cell lines and in ovarian carcinoma (15). Elevated expression of TrkA, TrkB, and TrkC was expressed in 14 of 16 ovarian carcinomas in a study of carcinomas of different origins (8). p75 expression has not been investigated in ovarian carcinoma. Nor has the expression of TrkA or p75 been studied in malignant effusions to date. Reduced TrkA and elevated TrkC expression showed an association with tumor progression in medullary thyroid carcinoma (10). In in vitro studies, TrkA activation resulted in cell compaction in embryonal carcinoma cells (14) and with cell proliferation in prostate carcinoma cell lines (12). Poor data are available regarding the role of p75 in epithelial malignancy. Two studies of clinical specimens of prostate carcinoma led to inconclusive results, because both elevated (5) and reduced (4) expression was reported in carcinomas, as compared with nonneoplastic tissue.

As opposed to rodent species, human and primate ovaries show evidence of well-developed sympathetic innervation by catecholaminergic fibers (16). Target cells for this pathway include the ovarian vasculature, interstitium, and thecal cells lining follicles (16). Both TrkA (17) and p75 (16) NGF receptors have been localized to cells lining the ovarian follicles. Furthermore, signals mediated through these receptors have an important role in normal ovarian function, as evidenced by a 100-fold increase in TrkA levels at puberty (17). Treatment of neonatal rat ovaries with anti-NGF antibodies results in failure of sympathetic innervation to develop, followed by impaired ovulation and fertility (18).

Ovarian cancer is the leading cause of death from gynecological cancer in women in industrialized countries, accounting for 4.4% of cancer cases and 4.5% of cancer deaths in women (19). Both the incidence and death rate from this tumor appear to be on the rise in western countries, as evidenced by a 30% rise in incidence and an 18% rise in death rate in the United States (19). As opposed to the ample data regarding the expression and activity of NGF and its receptors in endocrine functions of the normal ovary, little is known about neurotrophin receptor expression in ovarian carcinoma to date. NGF receptor expression was detected in 2 of 17 carcinomas while absent from benign ovaries (3). Trk expression was not found in benign ovarian surface epithelium in an additional study (7). TrkA was expressed in 14 of 16 ovarian carcinomas in a study of carcinomas of different origins (8). p75 expression has not been investigated in ovarian carcinoma. Nor has the expression of TrkA or p75 been studied in malignant effusions to date.

The objective of this study was to analyze the expression of TrkA and p75 in effusions and primary and metastatic tumors of serous ovarian carcinoma patients. Activation status of TrkA was studied using an antibody against the phosphorylated form of this receptor. In addition, we attempted to evaluate their association with clinicopathological parameters and disease outcome.

### Materials and Methods

#### Effusion Specimens.

The material consisted of 77 fresh unfixed peritoneal and pleural effusions submitted to the Division of Cytology, Department of Pathology, The Norwegian Radium Hospital, during the period of January 1998–April 2000. Specimens were obtained preoperatively, intraoperatively, or at disease recurrence from 66 patients diagnosed with ovarian carcinoma and 4 patients diagnosed with primary peritoneal carcinoma. Effusion specimens consisted of 51 peritoneal and 26 pleural effusions. All effusion specimens, as well as relevant clinical data, were obtained from the Department of Gynecological Oncology, The Norwegian Radium Hospital.

The distribution of the studied malignant effusions according to histological type, as well as their location, is detailed in Table 1. Handling procedure and morphological evaluation of specimens were undertaken according to established guidelines (20), as detailed previously (21).

#### Tumor Specimens.

Seventy-eight surgical specimens, consisting of primary tumors (=27) and metastatic lesions (=51) of the above patients, were additionally studied. Formalin-fixed, paraffin-embedded tissue blocks were obtained from archival material in the Department of Pathology, The Norwegian Radium Hospital. All tissue specimens underwent microscopic confirmation of diagnosis, tumor type, and histological grade, following established criteria (22). Tumor distribution according to site and histological type is shown in Table 2.

#### Immunohistochemical Analysis.

For TrkA and p75, immunohistochemistry was performed on all specimens (total, 155). Staining for TrkA was performed using the 203 anti-TrkA antibody (23). Pretreatment consisted of microwave oven antigen retrieval for 4 × 5 min in citrate buffer. Staining for p75 was undertaken using an antibody directed against the cytoplasmic domain of the human p75 protein (Promega Corp., Madison, WI). Pretreatment consisted of microwave oven antigen retrieval for 4 × 5 min in citrate buffer. Staining using both antibodies was done using the Envision peroxidase system (Dako, Glostrup, Denmark). Negative controls consisted of sections that underwent a similar staining procedure, with the exclusion of primary antibody application. A schwannoma specimen in which immunoreactivity for the studied antigen was demonstrated previously was used as a positive control.

For p-TrkA, a mouse monoclonal IgG1 antibody was raised against a decapeptide corresponding to an amino acid sequence containing a phosphorylated tyrosine residue (Tyr-490) of human TrkA. The antibody was isolated from a serum-free hybridoma culture medium (24) by sequential affinity chromatography on unphosphorylated and phosphorylated peptide.

#### Table 1

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Peritoneal effusion</th>
<th>Pleural effusion</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>44</td>
<td>25</td>
<td>69</td>
</tr>
<tr>
<td>Combined*</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Primary peritoneal carcinoma</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>26</td>
<td>77</td>
</tr>
</tbody>
</table>

* All containing a serous component.
Sepharose gels. The antibody reacts specifically with phosphorylated-Tyr-490 of human TrkA by Western blotting and immunohistochemistry and is non-cross-reactive to phosphorylated TrkB or TrkC. The antibody was concentrated to 150–200 µg/ml in PBS containing 0.1% sodium azide and 0.1% gelatin and stored at 4°C. Staining procedure was identical to the one described above for TrkA and p75. Positive control consisted of a specimen of immature ovarian teratoma containing primitive neural elements.

**Evaluation of IHC Results.** The presence and intensity of immunoreactivity were scored in tumor cells. Only membrane or combined membrane and cytoplasm immunoreactivity was interpreted as positive. The extent of staining was scored using the following scale: 0, no staining; 1, staining of 0–5% of tumor cells; 2, staining of 6–25% of cells; 3, staining of 26–75% of tumor cells; and 4, staining of 76–100% of tumor cells. A minimum of 500 cells, when present, was evaluated.

**RT-PCR.** Fresh frozen cells from 44 effusion specimens were analyzed for mRNA expression of TrkA and p75 using RT-PCR. These consisted of 19 cases evaluated using IHC and 25 additional specimens. Total RNA was isolated using the Tri Reagent (Sigma Chemical Co., St. Louis, MO). For first-strand cDNA synthesis, 5 µg of total RNA were reverse transcribed using 25 µg/ml oligo(dT)12-18 Primer in a final volume of 20 µl, in the presence or absence of 200 units of Moloney murine leukemia virus reverse transcriptase (Promega). Samples were first heated at 70°C for 15 min. The reaction was then carried out at 42°C for 1 h, followed by 5 min at 95°C. PCR was performed in a total volume of 25 µl containing 1 µl of the cDNA reaction mixture, 5 pmol of each upstream and downstream primer, and 1.2 units of Taq polymerase. The cycle program for each primer set consisted of 40 runs of denaturation at 94°C for 45 s, annealing at 62°C for 1 min, and elongation at 72°C for 1 min. The cycle program was preceded by an initial denaturation at 94°C for 3 min, followed by a final extension at 72°C for 10 min. PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized by ethidium bromide. The following RNA transcripts were detected by amplification of the corresponding cDNA: (a) β-actin, sense primer 5′-GTACCACGTGGCATTGAGGACT-3′ and antisense primer 5′-ACATCGTGAAGAGTGGTCTC-3′ (product size, 410 bp); (b) p75 (25), sense primer 5′-AGCCACCAAGACCGTGTTG-3′ and antisense primer 5′-TTGACGGCTTACCTCCTT-3′ (product size, 663 bp); and (c) TrkA (26), sense primer 5′-CCATCGTGGAAGAGTGTTCTC-3′ and antisense primer 5′-GGTGAACATTGGCCAGGTTCA-3′.

The genomic regions chosen for RT-PCR are highly conserved through primary and metaphases, including the rat and human genes. The PC-12 rat pheochromocytoma cell line was used as a positive control.

**Statistical Analysis.** Statistical analysis was performed applying the SPSS-PC package (version 9.0, 1999; SPSS, Chicago, IL). Probability of <0.05 was considered statistically significant. Comparative analyses of TrkA and p75 results in malignant cytological specimens primary tumors and metastatic lesions were executed using Wilcoxon signed ranks test. In cases for which more than one metastatic lesion was available, the lesion showing the most diffuse staining was included in the statistical evaluation. Full clinical and pathological data were available for all patients. Studies of the association between TrkA and p75 staining results in effusions, and clinicopathological parameters were undertaken using the two-sided χ² test. These consisted of analyses of the association between IHC results and effusion site, FIGO stage, tumor grade, the extent of residual disease, and chemotherapy status (pre- versus posttreatment specimen). Analysis of the association between TrkA, p-TrkA, and p75 expression were similarly performed using the two-sided χ² test. Univariate survival analyses for TrkA and p75 expression in effusion specimens were executed using the Kaplan-Meier method and log-rank test.

**RESULTS**

**TrkA and p75**

**Effusion Specimens.** TrkA protein membrane expression was detected in carcinoma cells in 30 of 77 (39%) effusions, most often with a concomitant cytoplasm immunoreactivity.³ p75 protein membrane expression was observed in 16 of 77 (21%) effusions, similarly accompanied by cytoplasmic staining (Table 3; Fig. 1, C and F). Ovarian carcinoma cells in pleural (n = 26) and peritoneal (n = 51) effusions showed comparable TrkA and p75 expression. Similarly, no association was seen between TrkA and p75 expression in effusions and patient age. FIGO stage, tumor grade, the extent of residual disease, or chemotherapy status. Using RT-PCR, TrkA mRNA was detected in 2 of 45 effusions, whereas p75 mRNA was present in 3 of 45 specimens (Fig. 2). TrkA and p75 mRNA was detected in different specimens. Three of the 5 specimens showing mRNA expression showed membrane expression of TrkA or p75, whereas the remaining two cases showed only cytoplasmic immunoreactivity and were therefore scored as negative. Because the amount of TrkA receptors is very low (1,000–10,000 molecules) in both neuronal and nonneuronal cells (27), the amount of mRNA is also very low. Furthermore, because only

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Ovary</th>
<th>Omentum</th>
<th>Peritoneum</th>
<th>Intestine</th>
<th>Lymph node</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>23</td>
<td>18</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>9</td>
<td>65</td>
</tr>
<tr>
<td>Combined</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Primary peritoneal carcinoma</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>20</td>
<td>8</td>
<td>11</td>
<td>2</td>
<td>10</td>
<td>78</td>
</tr>
</tbody>
</table>

⁴ All containing a serous component.

⁵ Six patients with tumors in both ovaries. The larger lesion was included in comparative studies of effusions and primary and metastatic lesions. Two ovarian lesions in patients diagnosed with primary peritoneal carcinoma were additionally excluded from comparative analysis (see also Table 4).
TrkA and p75 Neurotrophin Receptors in Ovarian Carcinoma

a fraction of the cell population is expressing the receptor, and accordingly the mRNA, an enrichment procedure is required to increase the amount of TrkA mRNA submitted for PCR reaction. In view of these technical problems, which in the future will be resolved, it is not surprising that the PCR evaluation was under the limit of sensitivity required for the detection of TrkA mRNA.

Tissue Sections. The distribution of TrkA and p75 protein expression in primary tumors and metastatic lesions is detailed in Table 3. TrkA protein membrane expression was detected in carcinoma cells in 64 of 78 (82%) solid tumors, whereas that of p75 was observed only in 6 of 78 (8%) lesions. As in effusions, a combined membrane and cytoplasmic localization was most frequently seen (Fig. 1, A, B, and D). Positive controls showed immunoreactivity for both markers in all staining rounds. In addition, staining of benign ganglion cells in the vicinity of metastatic tumors provided an internal control in some cases (Fig. 1E).

p-TrkA

Functional Evaluation of the p-TrkA Antibody. PC-12 cells overexpressing TrkA receptors (28) were treated for 5 min with NGF (100 ng/ml) and lysed. Samples of 5 and 25 μg of protein were immunoblotted. Lysates were separated on 10% SDS-PAGE gels and electrotransferred to Immobilon membranes. Membranes were incubated with either anti-TrkA (203) antibody, to measure receptor level, or p-TrkA, to measure receptor activation (Fig. 3). NGF-induced receptor activation was seen. Both the number of receptors and the number of NGF-induced activated receptors were related to the amount of lysate used (Fig. 3). A double TrkA protein band of Mr 140,000 and Mr 110,000 was seen, representing the mature and nonglycosylated precursor TrkA protein, respectively (Ref. 29; Fig. 3).

Effusion Specimens. Expression of p-TrkA in carcinoma cells was limited to 5 of 75 effusions (Fig. 1, G–I). Membranous staining was limited to 1–5% of the malignant cell population in 4 of these specimens, with an additional case showing expression in 6–25% of cells.

Comparison of Cancer Cells in Effusion Specimens and Tissue Sections

Sixteen patients with effusions had both primary tumors and metastases for comparative evaluation. Three additional patients had primary tumors only, and 6 had metastases only (total = 25 effusions, 19 primary tumors, and 22 metastases). TrkA protein membrane expression was by far more common in carcinoma cells in solid tumors, as compared with effusions (82% versus 39%), when the entire material was analyzed. The decrease in TrkA expression in effusions approached, but did not reach, statistical significance when only corresponding lesions were analyzed (P = 0.06 in the comparison of effusions and primary tumors, P = 0.09 for effusions and metastases; Table 4). Conversely, p75 protein membrane expression was more common in effusions (21%), as compared with solid tumors (8%). This finding failed to reach significance when corresponding lesions exclusively were analyzed (P > 0.05; Table 4).

Coexpression of NGF Receptors in Effusion Specimens

Statistical analysis of staining results of effusions revealed that 11 of 16 p75-positive effusions showed tumor cell populations immunoreactive for the antibody against TrkA (P = 0.001).

Clinicopathological Data and Survival Analysis

For 55 patients with a total of 58 effusions, the association between IHC results and clinical and pathological data were analyzed. Patient ages ranged from 41 to 78 years. One patient was diagnosed with stage IIc disease, 28 with stage III, and 26 with stage IV disease. The extent of residual disease after primary operation ranged from 1 to 5 cm in largest diameter. Two patients had well-differentiated, 23 had moderately differentiated, and 30 had poorly differentiated tumors. The follow-up period ranged from 1 to 64 months (mean, 19 months). Twenty-two patients died of disease, 24 patients were alive with disease, and 12 patients were free of disease at the time of last follow-up. In univariate survival analysis, TrkA and p75 expression in carcinoma cells in effusions did not correlate with survival (P > 0.05).

DISCUSSION

Cancer cells are characterized by a lack of cell growth regulatory control, in part through the use of growth signals generated by a variety of growth factor receptors, such as tyrosine kinase receptors. In many cells, overexpression of these

### Table 3  TrkA and p75 protein expression in the entire study material

<table>
<thead>
<tr>
<th>Site</th>
<th>0%</th>
<th>1–5%</th>
<th>6–25%</th>
<th>26–75%</th>
<th>76–100%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TrkA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effusions</td>
<td>47 (62%)</td>
<td>20 (26%)</td>
<td>5 (6%)</td>
<td>5 (6%)</td>
<td>0 (0%)</td>
<td>77</td>
</tr>
<tr>
<td>Primary tumors</td>
<td>3 (12%)</td>
<td>14 (52%)</td>
<td>5 (18%)</td>
<td>5 (18%)</td>
<td>0 (0%)</td>
<td>27</td>
</tr>
<tr>
<td>Metastases</td>
<td>11 (21%)</td>
<td>23 (46%)</td>
<td>11 (21%)</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td>51</td>
</tr>
<tr>
<td><strong>p75</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effusions</td>
<td>61 (79%)</td>
<td>9 (12%)</td>
<td>7 (9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>77</td>
</tr>
<tr>
<td>Primary tumors</td>
<td>26 (96%)</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>27</td>
</tr>
<tr>
<td>Metastases</td>
<td>46 (90%)</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>51</td>
</tr>
</tbody>
</table>

Downloaded from clincancerres.aacrjournals.org on October 15, 2017. © 2001 American Association for Cancer Research.
receptors or derived signal transduction pathways are involved in the malignant transformation of the cell. In the present study, expression of both TrkA and p75 was detected in ovarian carcinoma cells. The frequency of TrkA expression in solid tumors (82%) observed by us is in agreement with a previous report by Koizumi et al. (8), in which 14 of 16 ovarian carcinomas showed immunoreactivity for this protein. We found only infrequent and focal expression of p75 in solid lesions, previously not investigated in ovarian carcinoma. These findings would be in agreement with the proliferation-promoting effect of TrkA in experimental models (29, 30), as opposed to the proapoptosis effect of p75, documented previously in neurons (2). The general alterations observed in effusion specimens, i.e., the marked overall reduction in TrkA expression and the up-regulated expression of p75, may possibly be attributed to changes in the environment. It is noteworthy, however, that the pattern seen in NGF receptor-expressing cancer cells in effusions was not infrequently that of coexpression of TrkA and p75, suggesting NGF activation using two signaling pathways in some tumors.

We subsequently wished to evaluate whether the altered ratio of p75:TrkA receptors will be reflected in TrkA receptor autophosphorylation activity and thereby on intracellular activity, as documented previously (29). Specifically, since the phosphorylated antibody recognizes Tyr-490, the SHC-binding tyrosine, which is phosphorylated during the autophosphorylation of

---

**Fig. 1** TrkA and p75 protein expression in effusions, primary carcinomas, and metastases. A, TrkA staining in a primary serous ovarian carcinoma. All carcinoma cells show cytoplasmic immunoreactivity, and some cells show additional localization to the cell membrane. B, TrkA immunoreactivity in a colon metastasis that originated from the primary tumor in A. All cells show similar cytoplasmic immunoreactivity, but the number of membrane-positive cells is higher than in the primary tumor. C, TrkA immunoreactivity in a peritoneal effusion obtained from the same patient. Staining pattern resembles that of tumor cells in the solid lesions. D, p75-positive colon metastasis of a primary serous ovarian. Tumor cells show membrane and cytoplasmic immunoreactivity. E, a p75-negative colon metastasis. Ganglion cells (upper right corner) are stained, providing an internal control in this case. F, p75-positive tumor cells in a peritoneal effusion. Tumor cells show weak cytoplasmic and focal (arrow) membrane immunoreactivity. G, p-TrkA staining in a peritoneal effusion. A group of carcinoma cells show clear membrane immunoreactivity. H, p-TrkA staining in another peritoneal effusion. The majority of carcinoma cells show only cytoplasmic immunoreactivity (not scored as positive), whereas only a few cells show additional localization to the cell membrane (arrow). I, a third peritoneal effusion showing absence of any cells immunoreactive for p-TrkA. This was by far the most common pattern observed using this antibody. The specimens shown in D–F were obtained from three different patients, none of which is the patient whose tumor is shown in A–C. The same is true regarding G–I.
TrkA upon binding of NGF (29), immunoblotting results for the antibody used in the current study are suggestive of a functional Ras-Erk pathway initiated by SHC adaptor protein binding (31). We found only infrequent activation of TrkA in serous ovarian carcinoma cells in effusions, suggesting the finding of general down-regulation in TrkA expression at this site.

TrkA protein.

Fig. 2 TrkA and p75 mRNA expression in effusions as detected using RT-PCR. A, TrkA mRNA expression is evident in two effusions. B, mRNA expression of p75 in two specimens. Positive control (the lane nearest to the DNA ladder) consists of the PC-12 rat pheochromocytoma cell line in both figures.

Ovarian carcinoma presents most often with abdominal discomfort, caused by the accumulation of ascites. The presence of ovarian carcinoma cells in peritoneal effusions has been traditionally attributed to direct shedding from the ovarian tumor surface. However, their phenotype and genotype are poorly understood, largely because of a lack of large comparative studies of primary tumors, effusions, and metastatic lesions. Furthermore, the biological differences between ovarian carcinoma cells in the peritoneal and pleural cavity, the latter defining FIGO stage IV disease, are unknown to date. Thus, despite the practically universal presence of malignant effusions in the clinical course of epithelial ovarian cancer, tumor cells at this site are poorly characterized in terms of both effector (invasion- and metastasis-associated) molecules and the transcriptional level. The investigation of cancer-associated molecule expression in these cells is therefore of high significance for the understanding of disease progression.

The comparison between pleural and peritoneal carcinoma cells did not reveal differences in the expression of TrkA and p75 in the present study. The phenotypic similarities between carcinoma cells at these two sites are in agreement with our previous studies of ovarian carcinoma cells in effusions. We recently reported a similar expression of carbohydrate antigens (32), E-cadherin complex proteins (33), matrix metalloproteinases and their inhibitor TIMP-2 (34, 35), the adhesion molecule CD44 (36), and angiogenic genes (37) in pleural and peritoneal effusions. The present findings provide further evidence in support of our hypothesis that ovarian carcinoma cells in peritoneal effusions closely resemble those in pleural effusions and therefore are truly metastatic. Furthermore, carcinoma cells at both sites show altered expression of several key molecules involved in invasion and metastasis (angiogenic genes, proteolytic enzymes, and growth factors) as compared with primary tumors. The findings in the present study further underline the unique phenotypic profile of cancer cells in effusions and the significant biological alterations undergone by these disseminated malignant epithelial cells as compared with cells at the primary tumor site. Collectively, these findings question the validity of the shedding hypothesis and suggest a reevaluation of the biological and prognostic significance of positive ascitic cytology in the metastatic sequence of patients diagnosed with advanced-stage ovarian carcinoma.

In conclusion, the expression of two neurotrophin receptor expression in epithelial malignancies remains largely unknown. Absence of TrkA and p75 expression correlated with advanced disease stage, high histological grade, and poor survival in a recent study of esophageal carcinoma (15). However, this finding was not reproduced in multivariate survival analysis, in which disease stage was the only significant predictor of survival (15). Reduced TrkB and elevated TrkA and TrkC expression showed an association with tumor progression in medullary thyroid carcinoma (10). In the present study, no association was seen between TrkA and p75 expression in effusions and established prognostic parameters, including FIGO stage, histological grade, and the extent of residual disease, and the expression of these markers did not correlate with disease outcome. These findings suggest that neurotrophic receptor expression in ovarian carcinoma cells in effusions may be of biological relevance not yet characterized, rather than of prognostic significance.

In conclusion, the expression of two neurotrophin receptors, TrkA and p75, in ovarian carcinoma in primary and metastatic sites is reported. Altered expression of both proteins was seen in effusion specimens as compared with solid lesions, possibly as a result of changes in microenvironment conditions. Infrequent activation of TrkA in effusions suggests a biological role for this signaling pathway in only a subset of these tumors, a finding that merits further investigation. The consistent similarity between ovarian carcinoma cells in the peritoneal and
pleural cavity is in full agreement with our recent observations, pointing to a largely identical genotypic and phenotypic expression of cancer- and metastasis-associated molecules.

ACKNOWLEDGMENTS

We gratefully acknowledge the competent technical help of Inger-Liv Nordli and Ann Larsen at the Department of Pathology, The Norwegian Radiation Hospital.

REFERENCES

Expression Levels of the Nerve Growth Factor Receptors TrkA and p75 in Effusions and Solid Tumors of Serous Ovarian Carcinoma Patients

Ben Davidson, Philip Lazarovici, Alexandra Ezersky, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/7/11/3457

Cited articles
This article cites 33 articles, 6 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/7/11/3457.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/7/11/3457.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.