Tamoxifen Inhibits Nerve Growth Factor-induced Proliferation of the Human Breast Cancers Cell Line MCF-7

Andrea Chiarenza, Philip Lazarovici, Laurence Lempereur, Giuseppina Cantarella, Alfredo Bianchi, and Renato Bernardini

Department of Experimental and Clinical Pharmacology, University of Catania School of Medicine, I-95125 Catania, Italy. [A. C., L. L., G. C., A. B., R. B.], and Department of Pharmacology and Experimental Therapeutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91420, Israel. [P. L.]

Abstract

An array of polypeptide growth factors contribute to the development of breast cancer, the most common tumor-related cause of death in women of Western countries. Therefore, breast cancer therapy should be aimed at inhibition of growth factor-dependent breast cancerous cell proliferation. However, the relative contribution of each individual factor in the development and maintenance of the transformed phenotype is largely unknown. Here we report for the first time that the proliferative effects of nerve growth factor (NGF), a typical neurotrophin, are similar to those of epidermal growth factor (EGF) and insulin-like growth factor II, and are enhanced by 17β-estradiol in the human breast cancer cell line MCF-7. The effect of NGF appeared to be mediated by its trkA receptors (trkANGFR) as suggested by the potent inhibition of both MCF-7 cell proliferation and trkANGFR phosphorylation occurring upon treatment of cultures with the selective trkANGFR inhibitor K252a. Surprisingly, the antiestrogen drug tamoxifen (TAM) inhibited NGF-induced MCF-7 cell proliferation and trkANGFR phosphorylation in a concentration-related fashion. The effect of TAM seemed to be estrogen receptor-independent, because the pure estrogen receptor antagonistICI 182,780 was unable to block NGF-induced trkANGFR phosphorylation. Our data underline the new emerging role of trkANGFR in breast tumor growth, and suggest a related novel therapeutic use of TAM in breast cancer.

Introduction

Breast cancer is the most common cause of tumor-related death among women in the Western world (1). Transformation of normal breast cells, initiation, and maintenance of breast cancer growth all depend upon the interplay of a number of inhibitory and stimulatory factors, including estrogens, and an array of members of the cytokine/growth factor superfamily. The dedifferentiating and proliferative effects of each single factor are mediated by specific receptors (2). In addition, it is likely that breast cancer development and growth possibly require the concerted intervention of two or more of these growth factors (3). Indeed, it has been reported that estrogens may enhance the effects of EGF (4) or of IGF-I and -II (4, 5).

NGF, a member of the neurotrophin family of growth factors, is a candidate pleiotropic agent that participates in a number of relevant biological processes other than neuronal differentiation and growth (6) including the proliferation of cancerous cells (7, 8).

The major direct evidence that the trkANGFR induces cell proliferation was achieved upon transfection of a variety of neuronal and non-neuronal cells, resulting in strong proliferation upon NGF treatment (9).

The working concept today is that trkANGFR serves as an autocrine loop to regulate cell proliferation (10). For instance, trkANGFR, along with the other NGF receptor, p75NGFR, have been proposed to participate in the paracrine cross-talk between stromal and epithelial cells in the human prostate (11, 12), suggesting that NGF contributes to the genesis of androgen-dependent prostatic cancer. Indeed, it appears that the trkANGFR plays the prominent role in mediating the effects of NGF on prostatic cancerous cell proliferation (13). In this line, the trkANGFR inhibitors of the indol-carbazole series, such as K252a and CEP 7511, inhibit the effects of NGF (14), including NGF-stimulated tyrosine kinase activity (9), as well as estrogen cancer cell growth in vitro (15). Recently, a trkANGFR-mediated mitogenic effect of NGF has also been described on different lines of breast cancer cells (16).

Therefore, we found it of interest to investigate the role of NGF in stimulating the proliferation of the human breast cancer cell line MCF-7, focusing on (a) the compared effects of NGF, EGF, and IGF-II on MCF-7 breast cancer cell proliferation, and the role of trkANGFR, and (b) the possibility of pharmacological inhibition of NGF-induced MCF-7 cell proliferation by antiestrogens.

In fact TAM, an antiestrogen used in the treatment of ER breast cancer (17), has also been successfully used as an agent in various estrogen-independent tumors (18, 19). Clinical data are supported by in vitro studies showing the effectiveness of TAM in inhibiting the proliferation of ER breast cancer cells (20). In addition, TAM has been shown to interfere with tyrosine phosphorylation promoted by IGF-I in breast cancer cell lines (21).

Thus, we finally decided to evaluate further the effects of TAM on NGF-induced MCF-7 human breast cancer cell proliferation and trkANGFR tyrosine residue phosphorylation.

Materials and Methods

Materials. Tissue culture medium, FCS, and other supplies were purchased from Life Technologies Italia (Milan, Italy). E2, IGF-I, EGF, aprotinin, leupeptin, pepstatin, phenylmethylsulfonyl fluoride, glycero, Triton X-100, BSA, and TAM were purchased from Sigma Chemicals (Milan, Italy). NGF and the tyrosine kinase inhibitor K252a were obtained from Calbiochem, Novabiochem (San Diego, CA). The pure ER antagonist ICI 182,780 was a kind gift from Prof. Alvin M. Kaye, Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

Normal rabbit serum, biotinylated goat antirabbit IgG, avidin-biotin complex, and 3,3′-diaminobenzidine kit were obtained from Vector (Burlingame, CA). IgG-agarose beads were purchased from Pharmacia Biotech AB (Uppsala, Sweden); second antibodies conjugated to horseradish peroxidase and ECL reagent were purchased from Amersham Life Science (Buckingham-
TAMOXIFEN INHIBITS NGF EFFECT ON BREAST CANCER

Tench, United Kingdom). The anti-pan-trkANGFR203 antibody was the kind gift of Prof. David Kaplan, McGill University, Montreal Neurological Institute, Montreal, Canada (10). The monoclonal anti-phosphotyrosine antibody 4G10 (UBI, New York) was a kind gift from Dr. Oreste Segato, Istituto Regina Elena, Rome, Italy. Moloney murine leukemia virus-reverse transcriptase and Taq polymerase were purchased from Life Technologies Italia.

**Cell Culture.** PC12 cells, originally from NIH, were cultured as described previously (9) in DMEM without sodium pyruvate and with 7.5% horse serum and 7.5% FCS, penicillin (100 units/ml), and streptomycin (100 µg/ml). The human breast cancer MCF-7 cells were provided by Dr. Daniela Callari, Department of General Pathology, University of Catania, Italy. The cells were cultured in DMEM (with sodium pyruvate), supplemented with 10% FCS, penicillin and streptomycin. MCF-10 noncancerous mammary cells (from Dr. Antonio Uccella, Institute of Internal Medicine and Endocrine and Metabolic Diseases, University of Catania School of Medicine, Catania, Italy), were cultured in DMEM/F12 medium supplemented with 5% horse serum, penicillin (100 units/ml), streptomycin (100 µg/ml), hydrocortisone (0.5 µg/ml), cholera toxin and (0.1 µg/ml) to stimulate cAMP formation, insulin (10 µg/ml), and EGF (0.02 µg/ml).

Cells were grown in 25-cm² or 75-cm² flask with 10% FCS-DMEM, containing antibiotics, in a water-jacketed incubator at 37°C in a 5% CO₂ atmosphere.

**Proliferation Assay.** Cells were plated at a density of 5 × 10⁴ in 35-mm plastic Petri dishes in a volume of 5 ml/dish. After 12 h, the medium was replaced with 3 ml of fresh DMEM (1% FCS), containing graded concentrations of E₂ (10 pm to 100 nM), IGF-II (5, 10, 15, 20, and 25 ng/ml), EGF (5, 10, 15, 20, and 25 ng/ml), NGF (5, 10, 15, 20, and 25 ng/ml), or TAM (60 nM). In other experiments, TAM was used alone or in combination with E₂ (1 nM), IGF-II, EGF, and NGF (25 ng/ml). The pure ER antagonist ICI 182.780 was used at a concentration of 60 nM. In the experiments for trkA NGFR inhibition, the selective trkA NGFR inhibitor K252a was used at a concentration of 200 nM. In the experiments for trkA NGFR inhibition, two aliquots of the same sample were processed as follows. The first aliquot was immunoprecipitated with anti-trkA NGFR rabbit polyclonal antibody (sc118; Santa Cruz Biotechnolog): the second aliquot was immunoprecipitated with the monoclonal anti-phosphotyrosine (PY) antibody. After SDS-PAGE and electroblotting, the membranes were blocked with 1% BSA and incubated with anti-trkANGFR antibody for 2 h at 4°C, rinsed four times, and incubated with horseradish peroxidase-conjugated antirabbit IgG (second antibody) for 45 min at room temperature. The membranes were again washed four times for 15 min and exposed to ECL.

**Reverse Transcriptase-PCR.** Total RNA from cells grown to confluence was isolated after solubilization in guanidinium thiocyanate by phenol-chloroform extraction and precipitation (22). For first-strand cDNA synthesis, 5 µl of total RNA was 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. After first heating at 70°C for 15 min, the reaction mixture was carried out at 42°C for 1 h and subsequently heated for an additional 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 pair of primers 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 before a final extension at 72°C for 10 min. PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized with ethidium bromide. The following mRNA transcripts were detected via amplification of the corresponding cDNAs: (a) glyceraldehyde-3-phosphate dehydrogenase using the primer set composed of the sense 5'-TAGACAAGATGGTGAAAG and the antisense primer 5'-TCCTTGAGGCCTATGAG, yielding an amplicon of 1006 bp; (b) the p75NGFR using a primer pair, already described (23), composed of the sense primer 5'-GGCAGAAGCGCTGGTG and the antisense primer 5'-TTTG-CAGCCTTTCACCTTT, yielding a 663-bp PCR product; and (c) trkA NGFR using a primer pair described previously (24), composed of the sense primer 5'-CAGCCTGGAGGCTGGTG and the antisense primer 5'-AGCCTGGAGGCTGGTG, with an expected amplicon length of 476 bp. Amplification of glyceraldehyde-3-phosphate dehydrogenase was used to judge DNA contamination in the RNA samples examined and as a reference for estimating the level of NGF receptor mRNAs in the different cell lines.

**Statistical Analysis of Results.** Results were analyzed by one-way ANOVA, followed by Fisher’s least significant difference test. *P < 0.05* was considered significant.

**Results**

**NGF Proliferative Effects on MCF-7 and MCF-10 Cells.** NGF stimulated proliferation of MCF-7 cells in a concentration-dependent manner, with an EC₅₀ value of 7.1 ng/ml (Table 1). We also assessed the relative potencies of other growth factors, compared with that of E₂. IGF-II, EGF, and E₃ stimulated MCF-7 cell proliferation in a concentration-dependent fashion, but with different potencies. The relative EC₅₀ values were 5.0, 10.8, and 0.027 ng/ml, respectively, for IGF-II, EGF, and E₃ (Table 1). Coincubation of MCF-7 cells with both EGF and NGF resulted in an additive stimulatory effect on MCF-7 cell proliferation (EC₅₀, 4.4 ng/ml; *P < 0.05*).

NGF-stimulated MCF-7 cell proliferation was inhibited by treatment with the specific trkA NGFR inhibitor K252a (Ref. 14; Fig. 1),
Table 1 Stimulation of MCF-7 human breast cancer cell proliferation by different mitogens

<table>
<thead>
<tr>
<th>Proliferating stimulus</th>
<th>EC\textsubscript{50} (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>10.8</td>
</tr>
<tr>
<td>E\textsubscript{2}</td>
<td>0.027</td>
</tr>
<tr>
<td>IGF-II</td>
<td>5.0</td>
</tr>
<tr>
<td>NGF</td>
<td>7.12</td>
</tr>
<tr>
<td>NGF + EGF</td>
<td>4.4</td>
</tr>
<tr>
<td>NGF + IGF-II</td>
<td>2.84</td>
</tr>
<tr>
<td>NGF + estradiol</td>
<td>3.44</td>
</tr>
</tbody>
</table>

Fig. 1. Effects of NGF on proliferation of noncancerous and cancerous human breast cell lines. Time-related proliferation of MCF-7 human breast cancer cells stimulated by NGF (○), in the presence of the trk\textsuperscript{A NGFR} selective inhibitor K252a (△). ● curve represents the effect of NGF on the human noncancerous breast epithelial cells MCF-10. ■ curve represents proliferation of nonstimulated, untreated MCF-7 human breast cancer cells. Symbols are the means (P < 0.05; ANOVA, and then Fisher’s test); bars, SE.

suggested that the NGF high-affinity receptor trk\textsuperscript{A NGFR} mediates the NGF proliferative effect. On the other hand, treatment with an equal concentration of K252a did not affect both EGF- and E\textsubscript{2}-stimulated MCF-7 cell proliferation (data not shown).

In addition, NGF failed to stimulate proliferation when added to MCF-10 human noncancerous breast cells incubated with low, non-proliferative concentrations of EGF (2 ng/ml; Fig. 1).

Analysis of NGF Receptors Protein and mRNA Expression in MCF-7 and MCF-10 Cells. Western blot analysis showed a band of 75 kDa corresponding to the p75\textsuperscript{NTR} protein in the positive control-transfected PC12 cells (Fig. 2A, Lane 3), a cell line known to express both NGF receptors (10) as well as in MCF-7 cells (Fig. 2A, Lane 2). However, p75\textsuperscript{NTR} protein expression was undetectable in MCF-10 noncancerous human breast cells (Fig. 2A, Lane 1).

A 145 kDa molecular mass protein, corresponding to the trk\textsuperscript{A NGFR} protein was also present both in MCF-7 cells (Fig. 2A, Lane 5) and in the positive control PC12 cells (Fig. 2A, Lane 6). Densitometric analysis revealed a 8- to 9-fold increase in trk\textsuperscript{A NGFR} expression in MCF-7 human breast cancerous cells in comparison with the noncancerous human breast epithelial cell line MCF-10 (Fig. 2A, Lane 4).

Immunohistochemical analysis also revealed the presence of the high-affinity trk\textsuperscript{A NGFR}-like immunoreactivity in MCF-7 cells (Fig. 2B).

Total mRNA was analyzed in PC12, MCF-10, and MCF-7 cells by reverse transcriptase-PCR for the presence of p75\textsuperscript{NTR} and trk\textsuperscript{A NGFR} transcripts. The p75\textsuperscript{NTR} transcript was present in either PC12 (Fig. 2C, Lane 3) or MCF-7 (Fig. 2C, Lane 2) but not in MCF-10 cells (Fig. 2C, Lane 1). In addition, all cell lines contained the trk\textsuperscript{A NGFR} transcript (Fig. 2C, Lanes 4, 5 and 6).

Autophosphorylation of the NGF trk\textsuperscript{A NGFR} receptors in MCF-7 cells is shown in Fig. 2D. Indeed, NGF induced tyrosine phosphorylation of these receptors within 10 min, a selective effect that was completely inhibited by K252a at 200 nM. This concentration has been reported previously to be specific to the trk\textsuperscript{A NGFR} receptor (14, 25), a property shared with other compounds of the same family (15).

TAM Inhibits Estradiol-, EGF-, and NGF-stimulated MCF-7 Cell Proliferation. Treatment with the estrogen receptor antagonist TAM resulted in significant inhibition of MCF-7 cell proliferation stimulated by E\textsubscript{2} (Fig. 3A1), NGF (Fig. 3A2) and EGF (Fig. 3A3). The combination of NGF and E\textsubscript{2} stimulated MCF-7 cell proliferation additively. Proliferation induced by combined treatment with NGF and E\textsubscript{2} was inhibited by pretreatment with TAM (Fig. 3B). The effect of TAM was compared with that of the pure ER antagonist ICI
182.780; both TAM and, to a lesser extent, ICI were able to inhibit NGF-stimulated MCF-7 cell proliferation (Fig. 3). The concentration of TAM and ICI 182.780 used (60 nM) is known not to affect protein synthesis (26).

**Effect of TAM and ICI 182.780 on NGF-stimulated trkANGFR Tyrosine Phosphorylation.** To study the possible mechanism of action of TAM and ICI 182.780 as inhibitors of NGF-stimulated MCF-7 cell proliferation, we assessed their effects upon NGF-induced tyrosine phosphorylation of trkANGFR. MCF-7 cells were incubated respectively for 1 and 48 h with TAM and ICI 182.780 and then stimulated for 10 min with NGF. No effect on trkANGFR phosphorylation was observable after 1 h of treatment with both drugs (Fig. 4A). On the other hand, 48 h treatment with TAM, but not ICI 182.780, resulted in significant inhibition (90%) of tyrosine phosphorylation of trkANGFR (Fig. 4B). The expression of unphosphorylated trkANGFR in MCF-7 cells was comparable in all experiments (Fig. 4; A, top, and B, top), suggesting that TAM is effective on receptor activity rather than on its level of expression.

The expression of the NGF p75NTR receptor was not affected by 48 h of treatment with TAM or ICI 182.780 (Fig. 4C).

In additional experiments, we assessed the concentration-dependence of the effect of TAM on trkANGFR phosphorylation, by performing concentration-response experiments. Indeed, TAM inhibited trkANGFR phosphorylation in a concentration-dependent manner. The inhibitory effect of TAM appeared at 60 nM and reached its maximum at 120 nM (Fig. 5).

**Discussion**

Here we provide the first evidence that the antiestrogen TAM is able to inhibit NGF-induced MCF-7 human breast cancer cell proliferation in a fashion similar to other breast cancer growth factors, such as EGF and IGF-II (27). Our results support the proposal of Descamps et al. (16) that NGF is a promotion mitogen for breast cancer cells. In addition, we found that the proliferative efficacy of NGF on MCF-7 cells was comparable with those of EGF and IGF-II. Moreover, our experiments indicate that the effect of NGF is additive to that of EGF, supporting the prevalent hypothesis that more than one factor is required at the same time for a breast tumor to reach and maintain its ideal rate of growth (2). The enhancing effect of NGF on MCF-7 breast cancer cell proliferation appears to be mediated by its tyrosine kinase trkANGFR (28). These findings are strongly supported by the ability of the specific trkANGFR inhibitor K252a (14, 25) to selectively block the proliferative effect of NGF on these cells at a concentration that completely inhibited its autophosphorylation at tyrosine residues after NGF stimulation.

The trkANGFR, which we have detected by immunohistochemistry on the surface of MCF-7 cells, has also been regarded among medi-
The notion that NGF is a mitogenic factor for cancerous breast cells might find additional support in the increased expression of trkA NGFR that we observed in the cancerous MCF-7 cell line, as compared with the MCF-10 noncancerous human breast cells. On this basis, it seems reasonable that so significantly different expression of the trkA NGFR in MCF-7 and MCF-10 could be one of the reasons underlying the lack of proliferative effects of NGF on the latter cell line, and adds convincing evidence to the important role of a high number of trkA NGFRs in breast cancer growth.

It has been suggested that p75 NTR increases the affinity of the trkA NGFR in the presence of low concentrations of NGF in PC12 cells (32). Considered the lack of p75 NTR expression in MCF-10 cells, the previous finding could well explain the higher activity of trkA NGFR in MCF-7.

We found that the mixed ER agonist/antagonist TAM inhibited estradiol-, EGF-, and NGF-induced MCF-7 human breast cancer cell proliferation.

Estrogens profoundly affect the organization and the activity of the nervous system (33), and tight relationships have been described between the expression of trkA NGFR and ERs (33). In addition, signal transduction cross-talks have been proposed between estrogen and growth factor receptors (34, 35). With this in mind, it was not surprising to observe the inhibitory effect of TAM when used in combination with either estradiol, EGF, or NGF. In fact, clinical work provides evidence that TAM is quite efficient in the control of both estrogen (36), and non-estrogen-dependent breast tumor cell growth (17, 37), a hypothesis that has been verified experimentally in various malignant cell lines (38), as well as clinically, in protocols for treatment of melanoma (39) and leukemias (40).

In fact, tyrosine residue phosphorylation of the IGF-I receptor in MCF-7 cells is inhibited by TAM (21), a result which goes along with the TAM-dependent inhibition of EGF receptor phosphorylation observed in MCF-7 cells, in accordance to Freiss et al. (41), describing that the TAM metabolite 4-hydroxy-TAM decreases autophosphorylation of the EGF receptor in vitro.

Although the bulk of clinical evidence, supported by meta-analysis (42, 43) and chemoprevention (44) data, strongly suggests that TAM is most effective in the treatment of ER+ breast cancer, growing experimental evidence describes an alternate estrogen-independent pharmacological mechanism of action of this antiestrogen. In fact, Kanter-Lewensohn et al. (45) reported that inhibition of proliferation of malignant, ER- human melanoma cells by TAM is probably attributable to a direct effect of the antiestrogen with the IGF-I receptor phosphorylation, a concept affirmed previously by Guvakova and Survacz (21), suggesting inhibition of the IGF-I receptor phosphorylation by TAM in MCF-7 cells. It appears that TAM is also able to inhibit tumor angiogenesis induced by an ER- fibrosarcoma in vivo in the rat (46), and estrogen-independent effects of TAM have been shown in several ER- and ER+ tumor cell lines, in synergism with...
affected by TAM in modulating trkANGFR autophosphorylation re-

tation, it could use a general mechanism not selective for a partic-

Acknowledgments

In conclusion, NGF most probably acts as a progression mitogen in

for Drug Design and Novel Therapeutics at the School of Pharmacy, The

chemotherapy as an adjuvant agent for the growth inhibition of tumors

References

1. Newcombe, P. A., and Lantz, P. M. Recent trends in breast cancer incidence,


growth factors and type I insulin-like growth factor receptor in the estrogen-stimu-
lated proliferation of human breast cancer cells. J. Biol. Chem., 26: 21172–21178,
1990.


7. Marchetti, D., Menter, D., Jin, L., Nakajima, M., and Nicolson, G. L. Nerve growth
factor functions in human and mouse melanoma cell invasion and heparinase pro-

8. Nakagawara, A., and Brodeur, G. M. Role of neurotrophins and their receptors in
1997.

9. Jiang, H., Movsesyan, V., Fink, D. W., Jr., Fasler, M., Whalin, M., Katagiri, Y.,
of human p140NGFR receptors in p140NGFR-deficient, PC12/endothelial cells results in
nerve growth factor-induced signal transduction and DNA synthesis. J. Cell. Bio-

Overexpression of the trk tyrosine kinase rapidly accelerates nerve growth factor-

protein and nerve growth factor receptor in human benign prostatic hyperplasia

12. Sortino, M. A., Condorelli, F., Vancheri, C., Chiarenza, A., Bernardini, R., Consoli,
and, and Canonico, P. L. Mitogenic effect of nerve growth factor (NGF) in LNCap
prostate adenocarcinoma cells: role of the high and low affinity NGF receptors. Mol.

affinity nerve growth factor receptor in benign and malignant human prostate tissue
and loss of expression in four human metastatic prostate tumor cell lines. Cancer Res.,

K252a: a specific inhibitor of the action of nerve growth factor on PC 12 cells.

15. George, D. J., Dionne, C. A., Jani, J., Angeles, T., Murakata, C., Lamb, J., and Isaac,
S. T. Sustained in vivo regression of Dunn H rat prostate cancers treated with com-
binations of androgen ablation and trk tyrosine kinase inhibitors, CEP-751 (KT-6587)

growth factor is mitogenic for cancerous but not normal human breast epithelial cells.

17. Elledge, R. M., Green, S., Pugh, R., Allred, D. C., Clark, G. M., Hill, J., Ravdin, P.,
Minshall, J., and Osborne, C. G. Estrogen receptor (ER) and progesterone receptor
(PgR), by ligand-binding assay compared with ER, PgR and p52, by immuno-
histochemistry in predicting response to tamoxifen in metastatic breast cancer: a

Metcalfe, J. S., and Maize, J. C. The effect of tamoxifen and cisplatin on the
disease-free and overall survival of patients with high risk malignant melanoma. Br.

tamoxifen plus cisplatin and etoposide in the treatment of patients with advanced,

20. Perry, R. R., Kang, Y., and Greaves, B. Effects of tamoxifen on growth and apop-

factor I receptor (IGF-IR) signalling pathway in breast cancer. Cancer Res., 57:


factor and nerve growth factor receptor tyrosine kinase Trk in activated CD4-positive

functional trk protooncogene in human monocytes. Proc. Natl. Acad. Sci. USA, 90:

25. Tapley, P., Lamballe, F., and Barbacid, M. K252a is a selective inhibitor of the
tyrosine protein kinase activity of the trk family of oncogenes and neurotrophin

26. Guille, M. J., and Armstrong H. R. The anti-oestrogen drug tamoxifen is an elongation

cell lines MCF-7 and T47D to growth factors and 17 β-estradiol. Cancer Res., 48:

28. Klein, R., Jing, S., Nanduri, V., O’Rourke, E., and Barbacid, M. The trk proto-

nerve growth factor receptor during malignant transformation of the human prostate.

30. Djakiev, D., Delisle, R., Pflug, B. R., Wrathall, J., Lynch, J., and Onoda, M. Regula-
tion of growth by a nerve growth factor-like protein which modulates paracrine
interactions between a neoplastic epithelial cell line and stromal cells of the human

cycle-specific action of nerve growth factor in PC12 cells: differentiation without

32. Barker, P. A., and Shooter, E. M. Disruption of NGF binding to the low affinity
nerve growth factor receptor p75NGFR reduces NGF binding to TrkA in pcA cells.

33. Miranda, R. C., Sohrabji, F., and Toran Allerand, D. Interactions of estrogens with
the neurotrophins and their receptors during neural development. Horm. Behav., 28:
Tamoxifen Inhibits Nerve Growth Factor-induced Proliferation of the Human Breast Cancerous Cell Line MCF-7

Andrea Chiarenza, Philip Lazarovici, Laurence Lempereur, et al.

*Cancer Res* 2001;61:3002-3008.

**Updated version**  
Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/61/7/3002

**Cited articles**  
This article cites 47 articles, 14 of which you can access for free at:  
http://cancerres.aacrjournals.org/content/61/7/3002.full#ref-list-1

**Citing articles**  
This article has been cited by 9 HighWire-hosted articles. Access the articles at:  
http://cancerres.aacrjournals.org/content/61/7/3002.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.