

The Role of Neurotrophin Receptor p75^{NTR} in Cancer

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No generalizations about the role of this enigmatic receptor in cancer can be made. As in all other aspects of its biology, the effects of p75^{NTR} remain cell type specific, and their pattern has yet to be discovered.

p75 neurotrophin receptor (p75^{NTR}) was the first receptor to be found for the neurotrophins (NT).¹ This important family of growth receptors affect cell survival, differentiation and cell death.² Thus their connection to unchecked cell growth, i.e. cancer, is interesting and important from both a scientific and a therapeutic point of view.

The NTs (NGF, BDNF, NT3 and NT4/5) are a family of small (14 kD) basic proteins containing three cysteine bonds. All NTs are generated as pre-pro-neurotrophin precursors, about 240-260 amino acids long, which are further processed until they are secreted as mature homodimeric proteins. They bind to two types of receptors: each neurotrophin binds to a specific Trk receptor (NGF to TrkA, BDNF and NT4/5 to TrkB and NT3 to TrkC), and all neurotrophins bind to the p75^{NTR} receptor.³ The residues involved in binding of NT to

Trk receptors are different than those involved in binding to p75^{NTR}.

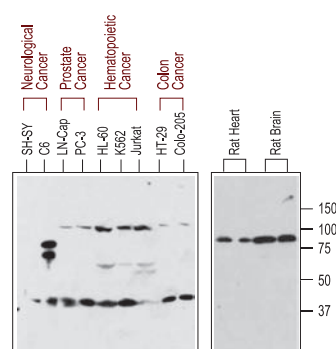
p75^{NTR} is a 399 amino acid Type 1 transmembrane protein.⁴ Its extracellular domain contains four repeated modules of six cysteines, a distinguishing structure of the tumor necrosis factor receptor (TNFR) superfamily members, of which it was the first to be discovered. The most prominent feature of the intracellular section is the death domain, an 80 amino acid module arranged in two bundles each containing three alpha helices.⁵ p75^{NTR} is devoid of intrinsic catalytic activity and signal transduction necessitates the recruitment of cytoplasmic interactors.

The role of p75^{NTR} is contradictory: p75^{NTR} signaling has been found to cause both cell death and cell

survival.⁶ There are many factors that need to be considered: the interaction between p75^{NTR} and Trk receptors, the absence or presence of ligand, and the cytoplasmic interactors involved. Thus it is impossible to generalize when considering the role of p75^{NTR} in any biological situation, and cancer is no exception.

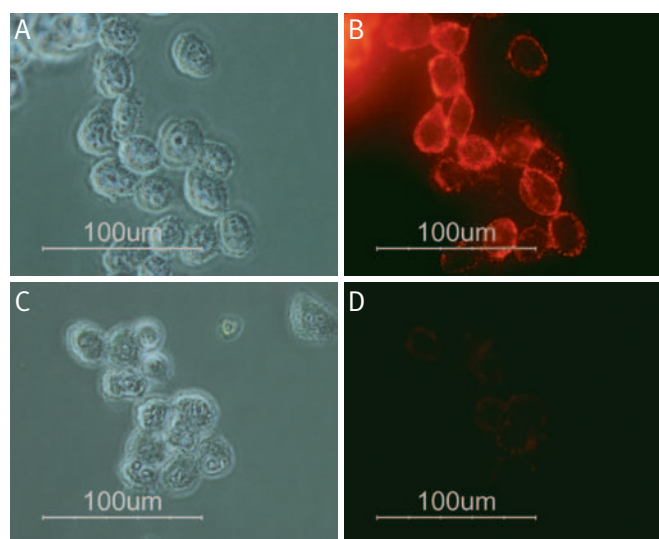
p75^{NTR} expression is mostly correlated with development.⁷ In the adult, it is retained in specific cells of the central nervous system, in sympathetic and sensory neurons of the peripheral nervous system, at a number of mesenchymal/epithelial boundaries, in perivascular fibroblast and dental pulp cells. Normal lung tissue does not express p75^{NTR},⁸ however a line derived from normal breast epithelial cell⁹ and normal prostate¹⁰ do exhibit positive staining for p75^{NTR}. It is also induced by

Expression of p75^{NTR} in Cancer Cell Lines



Expression of p75^{NTR} in normal rat tissue (right blot) and in human cancer cell lines (left blot). p75^{NTR} was visualized with Anti-Human p75^{NTR} extracellular antibody (#ANT-007) diluted 1:200. Note that human cancer cell lines from hemopoietic origin show high p75^{NTR} expression, while cell lines from prostate and colon cancer origin show lower levels. Interestingly, p75^{NTR} from rat (right blot and the C6 cell line) and human (left blot) samples run with a different apparent MW, probably due to species-specific differential glycosylation.

p75^{NTR} Expression in Human Melanoma Cells



Staining of human melanoma cells A875 with Anti-Human p75^{NTR} extracellular antibody (#ANT-007). Cells were incubated with Anti-Human p75^{NTR} extracellular (1:200) (A and B) or with anti-Human p75^{NTR} preincubated with the control antigen (C and D), followed by goat-anti-rabbit-Alexa Fluor 555 (Red). (x100).

injury in a wide variety of cell types,¹¹ and with chronic diseases such as multiple sclerosis,¹² and Alzheimer's.¹³

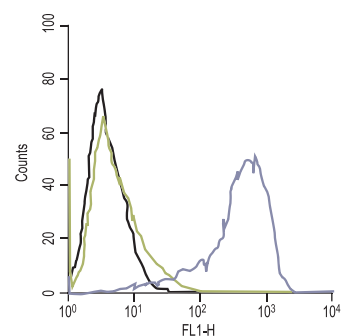
The expression patterns of p75^{NTR} in normal tissues and various types of cancer, and the correlation between p75^{NTR} expression and prognosis have been studied. The largest study done involved 1,150 tumors and fetal and adult normal tissue.¹⁴ A summary of these results can be found in Table 1. The striking observation to be made from these results is that p75^{NTR} seems to be absent in as many cancers as it is present. Thus, the expression of p75^{NTR} is not an indication of the cancerous phenotype. However, p75^{NTR} can be used as a marker in certain situations, such as to distinguish dermatofibrosarcoma protuberans (a soft tissue neoplasm) from benign fibrous histiocytoma.¹⁴ In ovarian cancer, the level of p75^{NTR} was not informative.¹⁵

In acute leukemia it was found that 8% of adult patients with acute leukemia and 26% of patients with acute lymphoblastic leukemia show expression of p75^{NTR}.¹⁶

In prostate carcinoma p75^{NTR} has been found to act as a tumor suppressor.¹⁰ The expression of p75^{NTR} in tissue sections from radical prostatectomies and in the prostate tumor cell lines DU-145, PC-3, LNCaP and TSU-pr1 (established from metastasis) was examined. It was found that there was a progressive loss of p75^{NTR} expression in prostate epithelial cells during the malignant progression of organ confined adenocarcinomas with complete loss of expression in the naturally occurring prostate tumor cell lines.

An additional paper by the same authors examines the pattern of gene expression in PC3 prostate tumor cells overexpressing p75^{NTR}.¹⁷ They found that p75^{NTR} overexpression affected the expression of genes critically involved in regulation of differentiation, cell adhesion, signal transduction, apoptosis, tumor cell invasion and metastasis. The levels of protein expression in genes showing the largest change upon p75^{NTR} overexpression were determined by immunoblot. These results confirmed that the levels of cellular retinoic acid binding protein I (CRAP1), affecting

FACS Analysis of Human Melanoma Cells With Anti-p75^{NTR} extracellular Antibody



— A875 cells alone
 — A875 cells + Goat-anti-Rabbit-FITC
 — A875 cells + Anti-p75^{NTR} extracellular antibody (#ANT-007) + Goat-anti-Rabbit-FITC

Table 1. Expression of p75^{NTR} in Normal and Malignant Tissues

Normal adult tissues positive for p75 ^{NTR}	Normal fetal first trimester tissues positive for p75 ^{NTR}	Normal adult tissues negative for p75 ^{NTR}	Tumors consistently positive for p75 ^{NTR}	Tumors variably (32-69%) positive for p75 ^{NTR}	Tumors negative to p75 ^{NTR}
Pericytes	Sub-epithelial mesenchyme	Sub-epithelial mesenchyme	Dermatofibrosarcoma protuberans	Fibrosarcoma variants	Paragangliomas
Perivascular fibroblasts	Skeletal muscle	Skeletal muscle	Embryonal and alveolar rhabdomyosarcoma	Solitary fibrous tumor	Neuroblastoma
Basal cells of several types of epithelia		GI-smooth muscle	Synovial sarcoma	Hemangiopericytoma	Meningioma
Perineural cells, variable positivity in the nerves		Bile duct mesenchyme	Spindle cell hemangio(endothelial)oma	Spindle cell lipoma	Perineuroma
Dendritic reticulum cells		Adrenals	Schwann cell	Ewings sarcoma	Extraskeletal osteosarcoma
Connective tissue only around vessels and adnexa			Ganglioneuroma	Mesenchymal chondrosarcoma	Benign fibrous histiocytoma
Sympathetic ganglion (weak)			Granular cell tumor	Malignant melanoma	Fibroma
Intestinal nervous plexus			Malignant peripheral nerve sheath tumor		Alveolar soft part sarcoma
Spleen, periarteriolar sheaths			Mixed tumor and adenoid cystic carcinoma		Epithelioid sarcoma
Kidney, perivascular mesenchyme (focally positive)					Smooth muscle and gastrointestinal stoma
Lung subepithelial stroma (narrow positive zone) and bronchial epithelia-basal cells**					Angiosarcoma
					Mesothelioma
Lower, sinusoidal endothelia					Adenocarcinoma
Scattered duct cells*					Squamous cell carcinoma
Breast epithelial cell line ⁹					
Prostate ¹⁰					

* Schneider, M.B. et al. (2001) *J. Histochem. Cytochem.* 49, 1205.

** contradicts with ref. 8

retinoic acid mediated differentiation and proliferation processes, and insulin like growth factor binding protein 5 (IGFBP5), which affects cell growth and maintenance, were increased. On the other hand, the level of plasminogen activator urokinase receptor (PLAUR), which has been shown to be involved in tumor cell invasion and metastasis of prostate cancer, was decreased upon p75^{NTR} overexpression.¹⁷

These researchers also investigated proliferation in the human T24 bladder cell line, (which does not express endogenous p75^{NTR}), transfected with varying levels of p75^{NTR}. They found p75^{NTR} to be an effective tumor suppressor of bladder cancer cell growth *in vivo*.¹⁸ This was attributed to the alteration of cell cycle regulator proteins by p75^{NTR} and was ameliorated by NGF. They also found that p75^{NTR} protein expression induced a dose-dependent increase in pro-apoptotic effectors and a decrease in pro-survival effectors of mitochondrial-mediated apoptosis.¹⁹

The role of p75^{NTR} as a tumor suppressor in pancreatic cancer has been suggested. In a study of 56 human primary pancreatic cancers it was found that the expression of p75^{NTR} was associated with a longer overall survival. In studies of pancreatic cell cancer lines it was found that NGF exhibited both stimulatory and inhibitory effects, depending on the expression levels and ratio of TrkA to p75^{NTR}.²⁰

In one of the first studies on the effects of NT on breast cancer, it was found that NGF could stimulate the proliferation of breast cancer cell lines MCF-7 and MDA-MB231 but was unable to stimulate growth of normal epithelial breast cells in culture.⁹ Both the cancerous and the normal cells expressed p75^{NTR} and TrkA. In addition, breast cancer cells, but not normal breast cells, produce NGF.²¹ Further studies showed that the mitogenic effect of NGF required TrkA, but the anti-apoptotic effect was mediated by p75^{NTR} through the NF- κ B pathway.²² The TNF-receptor associated death domain protein (TRADD) interacts with p75^{NTR} upon NGF stimulation, thus mediating the anti-apoptotic activity of NGF in breast cancer cells.²³ The level of p75^{NTR} has been shown to be related to tumor type, with low levels being more frequently found in ductular type tumors. In an immunohistochemical study of human invasive ductal breast carcinoma it was found that patients who were NGF positive and p75^{NTR} negative had significantly poorer survival rate than those who were NGF negative and p75^{NTR} positive.²⁴ The phenotypic alterations during the progression of breast carcinoma from primary tumor to pleural effusion has shown that p75^{NTR} expression was less frequent in effusions compared to both primary tumor and lymph node metastases.²⁵ The loss of the p75^{NTR} related pro-apoptotic mechanism may provide one biological explanation as to why the presence of malignant cells in effusions is associated with a rapidly

fatal course in both breast cancer and malignant mesothelioma.²⁵

The connection between neurotrophins, neurotrophin receptors and the ability of cancer cells to metastasize and colonize tissues has been investigated. This process is especially important in the case of malignant melanoma, which has one of the highest frequencies of metastasis to the brain.²⁶ It was known that the overexpression of p75^{NTR} is frequently associated with advanced stages of human melanoma progression.²⁷ The human melanoma MeWo cell line and its sublines 70W and 3S5 express varying levels of p75^{NTR}, the highest being found in the 70W subline.²⁸ The expression level of p75^{NTR} was found to correlate with a number of properties:

1. the ability of the cells to metastasize in nude mice. The A875 cell line, which also expresses high levels of p75^{NTR}, is also highly metastatic.
2. the extent of invasion of extracellular matrix stimulated by NGF.
3. the ability of the cells to grow under stress conditions (normal melanocytes did not grow under these conditions).

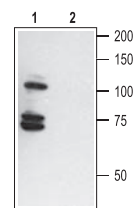
It was hypothesized that brain metastasis essentially represents a traumatic event related to brain injury processes. Thus the same upregulation of NT and NT receptors found in response to chemical or mechanical brain injury is also found when the brain is colonized by metastatic melanoma cells.²⁹

A mechanistic hint to the opposing roles played by p75^{NTR} may arise from work that examines their interaction with the other NT receptors. The effects of p75^{NTR} on neuroblastoma cells were intriguing. SK-N-BE neuroblastoma cell clones transfected with p75^{NTR} and lacking TrkA undergo extensive spontaneous apoptosis from which they are rescued by NGF.³⁰ This process was postulated to involve nitric oxide synthetase.³¹ Another study also confirmed this effect of NGF, but found that it was reversed in another cell line, Neuro-2a, which also expresses p75^{NTR} but not TrkA.³² The authors note that the level of expression of p75^{NTR} was different in the two lines, being higher in Neuro-2a and speculate that this may explain their conflicting results, however they caution that many other factors, most importantly the distribution of the p75^{NTR}, (in domains such as microlipid rafts) need to be determined before reaching any conclusions.

In summary, much work remains to be done in elucidating the role of p75^{NTR} in cancer. Tools such as Anti-rat p75^{NTR} (#AN-170), Anti-rat p75^{NTR}-FITC (#AN-170F), Anti-Human p75^{NTR}-extracellular (#ANT-007), Anti-Human p75^{NTR}-extracellular-FITC (#ANT-007-F), Anti-Human p75^{NTR}-intracellular (#ANT-011), will certainly prove indispensable to this endeavor.

Western Blotting of Human

Melanoma Cells A875



1. Anti-human p75^{NTR}-intracellular (#ANT-011) (1:200).
2. Anti-human p75^{NTR}-intracellular, preincubated with the control peptide antigen.

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Related Products

Compound	Product #
Antibodies to p75^{NTR} Receptor	
Anti-Human p75 ^{NTR} -extracellular	ANT-007
Anti-Human p75 ^{NTR} -extracellular-FITC	ANT-007-F
Anti-Rat p75 ^{NTR}	AN-170
Anti-Rat p75 ^{NTR} -FITC	AN-170-F
Anti-Human p75 ^{NTR} -intracellular	ANT-011