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Review

p75^{NTR} and the concept of cellular dependence: seeing how the other half die

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Abstract

Cells depend on specific stimuli, such as trophic factors, for survival and in the absence of such stimuli, undergo apoptosis. How do cells initiate apoptosis in response to the withdrawal of trophic factors or other dependent stimuli? Recent studies of apoptosis induction by neurotrophin withdrawal argue for a novel form of pro-apoptotic signal transduction – 'negative signal transduction' – in which the absence of ligand-receptor interaction induces cell death. We have found that the prototype for this form of signaling – the common neurotrophin receptor, p75^{NTR} – creates a state of cellular dependence (or addiction) on neurotrophins, and that this effect requires an 'addiction/dependence domain' (ADD) in the intracytoplasmic region of p75^{NTR}. We have recently found other receptors that include dependence domains, arguing that dependence receptors, and their associated dependence domains, may be involved in a rather general mechanism to create cellular states of dependence on trophic factors, cytokines, adhesion, electrical activity and other dependent stimuli.

Keywords: apoptosis; neurotrophin; receptor; cell death

Abbreviations: ADD, addiction/dependence domain; ADD_A, addiction/dependence domain for androgens; ADD_{NT}, addiction/dependence domain for neurotrophins; AR, androgen receptor; BDNF, brain-derived neurotrophic factor; ChAT, choline acetyltransferase; EGFR, epidermal growth factor receptor; IRE, intrinsic receptor effect; NFκB, nuclear factor κB; NGF, nerve growth factor; NT, neurotrophin; NTR, neurotrophin receptor; PC12 cells, pheochromocytoma 12 cells; PTM, post-translational modification

Apoptosis and negative signal transduction

Cells, especially during development, may depend on specific stimuli for their survival, such as trophic factors, cytokines, adhesion, hormones, or electrical activity. Mature cells may in many cases be more resistant to the loss of these stimuli, but often retain at least some degree of dependence on such stimuli. In the absence of the requried stimulus, the dependent cells undergo apoptosis, so the cells are quite literally addicted to the stimulus or stimuli. For example, developing cerebellar granule cells require depolarization for survival, and undergo apoptosis readily in the absence of depolarization (Chang and Wang, 1997; Miller *et al*, 1997).

It has generally been assumed that apoptosis resulting from a lack of such stimuli is simply due to the lack of a positive survival signal, such as that initiated by tyrosine phosphorylation of the Trk receptors and the resultant downstream signaling. While this undoubtedly accounts for part of the effect, results obtained over the past few years argue for a distinct and complementary form of signal transduction (Rabizadeh *et al*, 1993; Bredesen and Rabizadeh, 1997; Rabizadeh *et al*, unpublished observations). Moreover, since apoptosis is an active form of cell death, the signal initiating this active program must be generated or propagated in some way as a result of the lack of binding of growth factor, cytokine, or other dependent stimulus. However, despite the biological rationale for such 'negative signal transduction' – i.e., signaling resulting from the absence of binding of a ligand to its receptor – this form of pro-apoptotic signal transduction has not been appreciated previously.

The recognition of this novel form of signal transduction raises a number of questions: How might an unbound receptor participate in signal transduction? How long might a receptor 'wait' for ligand binding before initiating or mediating a death signal? Might this form of signal transduction utilize similar pathways to those of classical signal transduction? Our work to date argues that 'negative signal transduction' differs from classical signal transduction in critical aspects: for example, in classical signal transduction receptors serve to bind ligand and then initiate a signal; in contrast, in negative signal transduction, molecules that serve as 'receptors' for the classical arm of a negative signal transduction pathway may serve as downstream mediators of the signal propagated along the negative signaling arm of a bifid pathway. Feedback loops create a two-state (or multi-state) system that tends toward one stable state over the other(s) by a combination of feedback inhibition of the alternative arm(s) and feedforward enhancement of the designate arm.

p75NTR, the prototype addiction/dependence receptor

In 1993, we reported that p75NTR, the common neurotrophin receptor, induces apoptosis in the absence of ligand binding, with apoptosis being inhibited by ligand binding. We have reported similar results recently for a related receptor, CD40 (Ruan *et al*, 1997). This novel profile suggested that p75NTR creates a state of cellular dependence, or addiction, on neurotrophins (Figure 1). This concept has been supported by data from p75 null ('knockout') mice (Sauer *et al*, 1996; Yeo *et al*, 1997), as well as by *in vivo* antisense experiments (Cheema *et al*, 1996). Sauer *et al* (1996) found that mice deficient in NGF display atrophy and hypoplasia of medial septal cholinergic neurons, but that crossing those NGF hemizygous mice with p75NTR null mice restored cell size and number to that of the p75NTR null mice, i.e., to supranormal. They concluded that, in the presence of a reduced concentration of NGF, p75NTR mediates a reduction in cholinergic neuronal number and size in the medial septal region.

Recent results from Yeo *et al* (1997) are compatible with those of Sauer *et al* (1996): p75 null mice were found to have a 50% increase in cholinergic neuronal number in the diagonal band and medial septal region, with an increase in somal volume of approximately 30%. Perry Bartlett and Graham Barrett, along with their colleagues, found that the use of antisense oligonucleotides to decrease p75 expression also blocks neuronal apoptosis in another paradigm of neurotrophin-withdrawal-induced death, that of axotomy-induced neuronal cell death (Cheema *et al*, 1996).

Not surprisingly, the dependent state created by p75NTR is relative rather than absolute. For example, the withdrawal of NGF from PC12 cells leads to apoptosis that can be blocked by downregulation of p75NTR (Rabizadeh and Bredesen, 1994; Barrett and Georgiou, 1996) or expression of bcl-2 (Mah *et al*, 1993; Martinou *et al*, 1992). Thus, the net effect is that p75NTR expression in the absence of neurotrophin alters the cellular apostat (Bredesen, 1996; Salvesen and Dixit, 1997), enhancing the likelihood of apoptosis. It is of course possible that the same effect will be associated with binding of an antagonistic ligand, rather than the lack of binding of an agonist, but, in the case of p75NTR, neurotrophin withdrawal is not currently known to be associated with the binding of a neurotrophin-antagonist ligand; nonetheless, this remains a formal possibility.

The effect of p75NTR on cellular neurotrophin dependence may underlie aspects of both neural and extraneural cellular behavior: for example, prostate epithelial cells express p75NTR, which we have argued (Bredesen, 1994) ties them to a source of neurotrophin, supplied by the stromal cells. Pflug *et al* (1992) found a progressive decrease in p75NTR expression associated with the development of prostate neoplasia, with cell lines from metastatic prostate carcinomas failing to express p75NTR. We have found that the re-expression of p75NTR in PC3 prostate carcinoma cells restores a state of neurotrophin dependence, resulting in apoptosis if NGF is not supplied (Rabizadeh *et al*, unpublished observations). Thus, p75NTR may play a role in both neural and extraneural cellular paradigms that share the common thread of neurotrophin dependence.

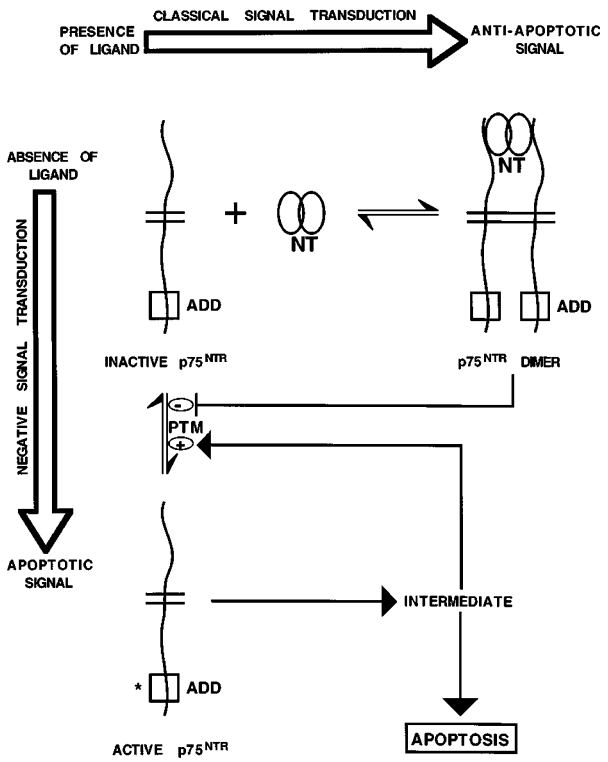

Figure 1 Creation of a neurotrophin-dependent cellular state by expression of p75NTR, the common neurotrophin receptor. The likelihood of undergoing apoptosis (setting of the cellular 'apostat') is increased by p75NTR expression, but this effect can be blocked either by binding of neurotrophins to p75NTR or by downregulation of p75NTR. In the absence of neurotrophin, p75NTR is activated, and, in a process that requires the addiction/dependence domain (ADD), triggers apoptosis. Activation of p75 triggers, through an intermediate, both further activation of p75 and apoptosis, creating a positive feedback loop. NT, neurotrophin; PTM, post-translational modification

An addiction/dependence domain (ADD) in p75NTR

What is the mechanism by which p75NTR expression creates a state of neurotrophin dependence? Site-directed mutagenesis studies of p75NTR have identified a region within the intracellular domain that is required for the creation of cellular neurotrophin dependence (Rabizadeh *et al*, unpublished observations). This region is referred to as an 'addiction domain' or 'dependence domain', since it is required for the induction of apoptosis by p75NTR in the absence of NT binding ('addiction' implies that the cells have been exposed to the NT prior to their requirement for it, whereas 'dependence' does not. We have used these non-equivalent terms interchangeably here because p75NTR can create a state of cellular NT dependence in cultured cells, but *in vivo*, p75NTR expression may follow neurotrophin exposure, and hence in that case the cellular state may more accurately be called an 'addiction' than a 'dependence'). Deletions that include the addiction/

dependence domain (ADD), and point mutations within this region, both destroy the Trk-independent intrinsic receptor effect (IRE (Bredesen and Rabizadeh, 1997)) on apoptosis. Furthermore, the ADD synthesized as a peptide is a relatively potent pro-apoptotic peptide (Hileman *et al*, 1997; Rabizadeh *et al*, unpublished observations). Thus the ADD is both necessary and sufficient for apoptosis induction. Control peptides, including a scrambled $p75^{NTR}$ ADD peptide, mutant p75 peptides, p75-derived peptides from outside the ADD, and control peptides matched for predicted helicity, all failed to induce apoptosis. Mutations that affect the ability of the ADD peptide to induce apoptosis have parallel effects when introduced into the full-length $p75^{NTR}$, further supporting the importance of the ADD in apoptosis induction by $p75^{NTR}$ (Rabizadeh *et al*, unpublished observations).

In a cell-free system of neuronal apoptosis (Ellerby *et al*, 1997), the $p75^{NTR}$ ADD peptide induces apoptosis, as assessed by caspase activation, mitochondrial cytochrome c release, and other apoptosis parameters (Rabizadeh *et al*, unpublished observations). However, control peptides did not induce the neural cell-free system. This system should allow a determination of the organellar and molecular requirements for apoptosis induction by the p75 neurotrophin addiction domain.

Solution structure of fragments of the intracellular region of $p75^{NTR}$ have recently been obtained (Liepinsh *et al*, 1997; Hileman *et al*, 1997). These have disclosed predominantly α-helical structure in the terminal two-thirds of the $p75^{NTR}$ intracellular domain. Liepinsh *et al* (1997) identified six alpha helices, $\alpha1-\alpha6$, in this region of $p75^{NTR}$. They pointed out that this region of $p75^{NTR}$ is reminiscent of the death domain of Fas, but that there are important structural and functional differences. Whereas Fas and TNFR I have death domains of subtype I, the putative $p75^{NTR}$ death domain is of subtype II. Type I death domains display the following characterisics:

1. Binding by ligand induces a pro-apoptotic signal.
2. Self association.
3. Specific arrangement of six alpha helices.

In contrast, the term 'death domain' applied to type II death domains may turn out to be a misnomer. For example, one of the subunits of NFκB displays a type II death domain, but the expression of NFκB inhibits, rather than induces, apoptosis (Van Antwerp *et al*, 1996; Beg and Baltimore, 1996). Furthermore, we have found that mutation of conserved residues in $p75^{NTR}$ whose analogous residues are required for apoptosis induction by Fas and TNFR I – not only point mutations but even large deletions – have no effect on apoptosis induction by $p75^{NTR}$ following ligand withdrawal (Rabizadeh *et al*, unpublished observations).

An additional distinction between apoptosis induction by Fas and $p75^{NTR}$ relates to the requirement for multimerization: for both Fas and TNFR I, multimerization is required for apoptosis induction. However, for $p75^{NTR}$, recent studies utilizing FK binding protein chimeras have shown that the monomeric receptor is required for apoptosis induction, whereas multimerization blocks apoptosis induction (Wang *et al*, unpublished observations; Rabizadeh *et al*, unpublished observations) – just the reverse of the situation for Fas and TNFR I.

Therefore, we have argued that $p75^{NTR}$ has a neurotrophin addiction domain (ADD; Figures 1 and 2) that is distinct from its putative death domain; whether or not it also has a functional death domain remains to be determined, but certainly the studies by Casaccia-Bonnefil *et al* (1996) and Frade *et al* (1996) would argue that it does. However, Liepinsh *et al* (1997) found no tendency toward self-aggregation for the putative death domain, which is further confirmation that the putative $p75^{NTR}$ death domain is unlike the type I death domains of Fas and TNFR I.

Helix formation may be crucial to the pro-apoptotic effect of $p75^{NTR}$, since disruption of helix formation in the $p75^{NTR}$ ADD destroys the pro-apoptotic effect of $p75^{NTR}$. However, additional mutants of the $p75^{NTR}$ ADD must be tested to establish this possibility conclusively.

In theory, it should be possible to create states of cellular dependence on other ligands by fusing the p75 ADD to the extracellular and transmembrane domains of other receptors such as the EGFR; such studies are underway.

Other dependence receptors

$p75^{NTR}$ represents a prototype dependence receptor. Identification and analysis of other functionally similar dependence receptors would be advantageous, in part because this may help to shed light on the $p75^{NTR}$ dependence mechanism, and in part because it may provide an indication of the breadth of cellular utilization of dependence receptors.

Recently, we have found that the androgen receptor (AR) is a dependence receptor similar to $p75^{NTR}$ in functional effect, even though the two receptors are not

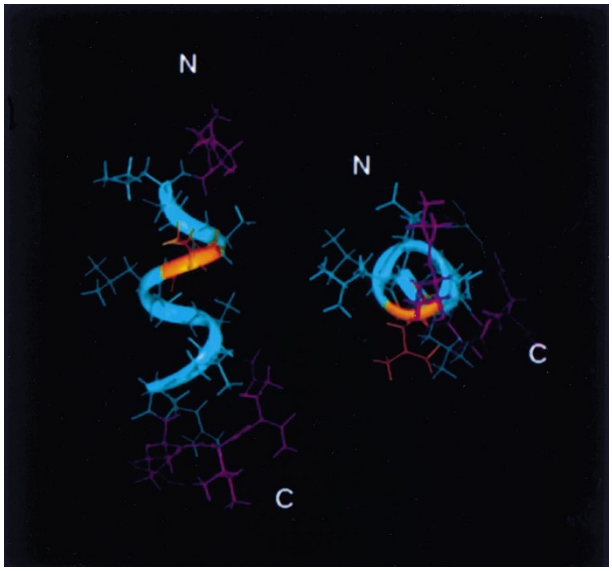

Figure 2 Structure of the $p75^{NTR}$ neurotrophin addiction domain (ADD_{NT}), based on work by Hileman *et al* (1997) and Rabizadeh *et al* (unpublished observations). The orange region highlights a mutation that, in the context of the peptide, destroys both helix formation and pro-apoptotic effect (Hileman *et al*, 1997)

structurally similar (Ellerby *et al*, unpublished observations). Expression of the AR in cells lacking endogenous AR expression induced the cells to become androgen dependent; apoptosis was induced in the absence of testosterone, but inhibited in the presence of testosterone. Furthermore, the AR has an androgen dependence domain (ADD_A, to distinguish it from the $p75^{NTR}$ ADD_{NT}), and, just as for $p75^{NTR}$, a pro-apoptotic peptide sequence is embedded in the AR.

Studies of the AR may shed light on the mechanism by which dependence receptors create cellular states of dependence: we have found that the AR undergoes post-translational modification (PTM), without which apoptotis induction is markedly reduced. Testosterone inhibits both the PTM and apoptosis induction; furthermore, inhibition of AR PTM by mutagenesis of the AR also blocks apoptosis induction (Ellerby *et al*, unpublished observations).

We have identified the same PTM in $p75^{NTR}$, suggesting that the mechanism of apoptosis induction by these two dependence receptors may, at least in principle, be similar.

Subapoptotic dependence effects of $p75^{NTR}$

The initial studies of $p75^{NTR}$ effects on cell death showed that cells overexpressing $p75^{NTR}$ in the absence of trophic factor support undergo neurite retraction and atrophy as well as apoptosis (Rabizadeh *et al*, 1993; Rabizadeh *et al*, unpublished results), but it was assumed that the atrophy and neurite retraction simply occurred as the immediate forerunners of apoptosis. Somewhat surprisingly, however, studies in $p75^{NTR}$ null mice have confirmed that $p75^{NTR}$ expression may indeed lead to neuronal atrophy and synapse loss in surviving, i.e., non-apoptotic (and apparently long-lived) cells, in addition to decreasing neuronal number (Yeo *et al*, 1997). It is tempting to speculate that such changes may represent a stable pre-apoptotic state, i.e., that the cellular biochemical changes resulting from $p75^{NTR}$ expression that lead, in the extreme, to apoptosis, may in some cases have subapoptotic manifestations that include neuronal atrophy and alterations in neurite outgrowth. However, it is not yet clear whether the p75 ADD_{NT} mediates neuronal atrophy and synapse loss, or whether a different $p75^{NTR}$ region is required for those effects.

A role for dependence receptors in input/output matching?

Studies of $p75^{NTR}$ *in vivo*, employing p75 null mice, have produced widely varying results. Lack of $p75^{NTR}$ expression may result in an increase in medial septal neuronal number (Peterson *et al*, 1997; Yeo *et al*, 1997), no increase, or a slight decrease (Peterson *et al*, 1997); it may result in an increase in striatal ChAT activity (Van der Zee *et al*, 1996) or no change in striatal ChAT activity (Yeo *et al*, 1997). In the dorsal root ganglia, lack of $p75^{NTR}$ may lead to fewer neurons (Lee *et al*, 1992), but may also inhibit apoptosis (Greene and Kaplan, 1995). How can such disparate results be reconciled?

Recent work (Sauer *et al*, 1996) may shed some light on these apparent discrepancies. Sauer *et al* (1996) found that NGF hemizygotes, with a reduction in NGF concentration, demonstate both neuronal atrophy and a decrease in medial septal cholinergic neuronal number. This pattern thus represents essentially the inverse of the findings in the $p75^{NTR}$ null mice. However, crossing of the NGF hemizygotes with the $p75^{NTR}$ null mice produced mice with medial septal cholinergic neuronal sizes and numbers indistinguishable from the $p75^{NTR}$ nulls, i.e., with increases in neuronal size and number. The authors concluded that, in the presence of a reduced concentration of NGF, $p75^{NTR}$ mediates neuronal atrophy and loss. Interestingly, their work demonstrates that, in the absence of $p75^{NTR}$, medial septal neuronal size and number are *independent* of NGF concentration over the range of concentrations represented by the NGF hemizygotes and homozygotes. If one constructs an idealized graph of these results (Figure 3), it becomes apparent that $p75^{NTR}$ is likely to serve a role in input/output matching, in which the input is ligand and the output controls neuronal size, survival, and synaptogenesis. In other words, in the presence of $p75^{NTR}$, neuronal size, and probability of undergoing apoptosis, are matched to the NGF availability; but in the absence of $p75^{NTR}$, this input/output matching does not occur.

The curves shown in Figure 3 may help to explain the disparate results obtained by the different groups: in A, an increase is depicted in neuronal size and number in the $p75^{NTR}$ nulls due to the loss of cell number and size mediated by $p75^{NTR}$ in the presence of a reduced NGF concentration. This was the result reported by Sauer *et al* (1996). B depicts an increase in neuronal size and number

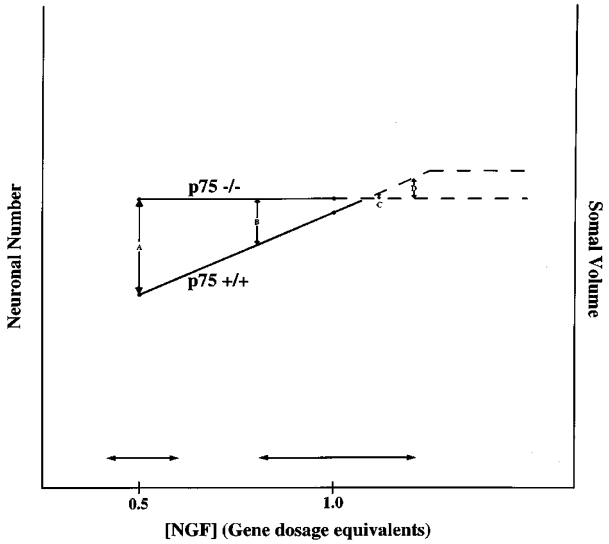

Figure 3 Idealized diagram demonstrating the role of $p75^{NTR}$ in relating neuronal atrophy and loss to NGF concentration. In the absence of $p75^{NTR}$ expression, cholinergic neuronal number and size within the medial septal region are independent of NGF concentration over the range represented by the hemizygotes and homozygotes. In contrast, in $p75^{NTR}$ +/+ animals, these same parameters are NGF dependent. A–D represent differences between the p75 nulls and p75 +/+ animals at various NGF concentrations (discussed in the text). The NGF concentration itself may of course vary as a function of time, site, species, and other parameters, and the cellular response to NGF may vary as a function of $p75^{NTR}$:TrkA ratio and other parameters

in the p75 nulls on a homozygous NGF background in which NGF concentration is still limiting. This result is similar to those reported by van der Zee *et al* (1996) and Yeo *et al* (1997). C depicts a lack of difference (insignificant decrease) for the $p75^{NTR}$ nulls on a homozygous NGF background, in which a higher concentration of NGF than in B is present. Such a minimal decrease in neuronal number for the $p75^{NTR}$ nulls was reported for $TrkA^+$ septal neurons by Peterson *et al* (1997). D depicts a decrease in neuronal size and number for the $p75^{NTR}$ nulls in a setting in which NGF concentration, in a homozygote, is relatively high. This result is reminiscent of that reported by Peterson *et al* (1997) for septal neurons.

Neurotrophin-induced vs neurotrophin withdrawal-induced apoptosis: is there really a controversy?

The initial reports that $p75^{NTR}$ mediates neural apoptosis induced by trophic factor withdrawal appeared in 1993 and 1994 (Rabizadeh *et al*, 1993; Barrett and Bartlett, 1994; Rabizadeh and Bredesen, 1994). Some subsequent reports have described cell death induced by neurotrophins rather than by NT withdrawal (von Bartheld *et al*, 1994; Frade *et al*, 1996; Casaccia-Bonnefil *et al*, 1996). Are these reports really at odds?

It is well established that NGF withdrawal may induce apoptosis in responsive neural populations (Martin *et al*, 1988). This effect is not explained by the finding that NGF may, under certain circumstances, enhance apoptosis. The argument that $p75^{NTR}$ is a mediator of NT withdrawal-induced apoptosis is based on the following observations:

1. Overexpression of $p75^{NTR}$ in neural cells enhances apoptosis induced by serum withdrawal (Rabizadeh *et al*, 1993).
2. PC12 mutants that do not express $p75^{NTR}$ have a reduced apoptotic response to serum withdrawal, whereas those that retain $p75^{NTR}$ expression retain the apoptotic response. Return of $p75^{NTR}$ expression to the p75-negative mutants returns their apoptotic response (Rabizadeh *et al*, 1993).
3. Cell sorting of PC12 cells into those with high and low expression of $p75^{NTR}$ demonstrates a correlation with high and low propensity to undergo apoptosis following serum withdrawal (Rabizadeh and Bredesen, 1994; Barrett and Georgiou, 1996).
4. Antisense inhibition of $p75^{NTR}$ expression is associated with inhibition of apoptosis induced by serum withdrawal, both in cell lines and primary cultures of dorsal root ganglion neurons (Barrett and Bartlett, 1994; Rabizadeh and Bredesen, 1994; Barrett and Georgiou, 1996).
5. Expression of $p75^{NTR}$ in NIH3T3 cells, which do not express Trk family members, enhances apoptosis following serum withdrawal. This effect is inhibited by NGF, BDNF, and NT-3, but affected only minimally by a mutant NGF (Ibanez *et al*, 1992) that binds Trk but binds $p75^{NTR}$ with 100-fold lower affinity than NGF binds $p75^{NTR}$ (Rabizadeh and Bredesen, 1994).
6. A peptide that binds $p75^{NTR}$ inhibits NGF withdrawal-induced death in cultures of dorsal root ganglion cells (Longo *et al*, 1997). This peptide has no effect on similar cultures from p75-null mice.
7. As noted above, mice with reduced NGF expression due to NGF hemizygosity have a reduced number and size of medial septal cholinergic neurons, but this is rescued by crossing with p75-null mice (Sauer *et al*, 1996).

Thus, there is little argument that NT withdrawal can lead to neuronal apoptosis, or that NTs generally inhibit apoptosis, and, based on the results listed above, it is highly likely that $p75^{NTR}$ plays a mediating role. This is not to say that other mediators may not also be discovered.

On the other hand, it is also clear that, under certain circumstances, NGF can enhance cell death by an unknown mechanism in which $p75^{NTR}$ participates. The assumption that $p75^{NTR}$ cannot mediate both NT withdrawal-induced apoptosis and, under different conditions, NGF-induced apoptosis, ignores both a large volume of data and the complexity of the systems involved. Indeed, some other members of the $p75^{NTR}$/TNFR family, such as the TNF receptors, can induce or inhibit apoptosis following ligand binding (Cheng *et al*, 1994).

How might $p75^{NTR}$ mediate both NT withdrawal-induced apoptosis and NGF-induced apoptosis? This is currently a matter of speculation. Based on a comparison of $p75^{NTR}$ effects on cells that also express Trk family members with $p75^{NTR}$ effects on Trk-negative cells, we have previously suggested that $p75^{NTR}$ displays two general types of pro-apoptotic effects:

1. An intrinsic receptor effect (IRE), activated by NT withdrawal, that occurs both in the presence and absence of Trk (although Trk may inhibit this effect, so that it may be less pronounced in the presence of Trk).
2. Trk-dependent effects, which represent the sum of the following:
   (a) High-affinity binding of NTs by the combination of $p75^{NTR}$ and Trk (Hempstead *et al*, 1991). This effect implies that a decrease in $p75^{NTR}$ will decrease high-affinity binding of NTs, enhancing apoptosis induction at limiting NT concentrations.
   (b) Mutual repression of signaling by $p75^{NTR}$ and Trk, such that signaling via either alone is inhibited by the other, unless the mutal repression is relieved by binding of the appropriate NT to both $p75^{NTR}$ and the co-expressed Trk (Bredesen and Rabizadeh, 1996). Competition from a mismatched NT thus leads to a net pro-apoptotic effect.
   (c) A decrease in Trk phosphorylation in the presence of $p75^{NTR}$ (Kaplan and Miller, 1997).

It is noteworthy that the reports of NGF-induced apoptosis have occurred in systems in which mismatched Trk members were expressed (TrkB or TrkC), or in which TrkB and TrkC expression were not evaluated. Thus, it will be of interest to determine whether or not NGF-induced apoptosis requires the expression of a mismatched Trk family member.

Models of apoptosis induction by p75NTR

Two models for NGF-induced apoptosis have been proposed recently. Both have been proven incorrect. The first model (Kaplan and Miller, 1997) featured NGF-induced apoptosis mediated by $p75^{NTR}$ when the $p75^{NTR}$: Trk ratio is high, including the limiting case in which it is infinite (i.e., Trk is absent). Published results have shown just the opposite: Taglialatela *et al* (1996) found that BDNF inhibited, rather than induced, apoptosis in PC12 cells, but that this occurred only with a high $p75^{NTR}$: TrkA ratio (approximately 70 : 1), which in the reported experiments was achieved through the use of antisense oligonucleotides. Similarly, Cortazzo *et al* (1996) found that neuroblastoma cells with a high $p75^{NTR}$: Trk ratio (approximately 100 : 1) were inhibited from undergoing apoptosis, rather than induced to undergo apoptosis, by binding $p75^{NTR}$ to the exclusion of TrkA. In neuroblastoma cells lacking Trk expression, Bunone *et al* (1997) found that $p75^{NTR}$ induced apoptosis, and NGF inhibited apoptosis. Thus Taglialatela *et al* (1996), Cortazzo *et al* (1996), and Bunone *et al* (1997) all reported the inverse of the model's predictions.

The second model (Dechant and Barde, 1997) proposed that signaling through Trk is anti-apoptotic, whereas signaling through $p75^{NTR}$ is pro-apoptotic. Again, published results clearly exclude this model. In addition to the studies of Taglialatela *et al* (1996), Cortazzo *et al* (1996), and Bunone *et al* (1997) quoted above, Longo *et al* (1997) developed a peptide that binds $p75^{NTR}$ but not Trk. This peptide was shown to block the apoptosis of dorsal root ganglion neurons in primary culture, demonstrating that binding to $p75^{NTR}$ may initiate an anti-apoptotic signal. Interestingly, the peptide was only effective as a cyclic dimer, with the monomer being inactive; this finding argues that $p75^{NTR}$ dimerization is crucial to the initiation of an anti-apoptotic signal.

The FK binding protein results quoted above argue that, at least in some paradigms, monomeric $p75^{NTR}$ is required for apoptosis induction, and that dimerization blocks the pro-apoptotic effect. However, whether or not this will be the case in neurons *in vivo* remains to be seen.

Conclusions

$p75^{NTR}$, the common neurotrophin receptor, is a prototypic dependence receptor. The characteristics of receptors with this newly recognized effect include the following:

1. Expression in the absence of the dependent ligand shifts the cellular apostat toward a greater probability of apoptosis.
2. Ligand binding blocks the pro-apoptotic effect.
3. These receptors include novel domains referred to as addiction domains or dependence domains (ADDs), which are required for apoptosis induction by the receptors in the absence of ligand. These ADDs are distinct from death domains.
4. Peptides representing ADDs are pro-apoptotic.

$p75^{NTR}$ may also mediate subapoptotic events such as somal size specification and synaptogenesis (the latter perhaps via an effect on neurite retraction).

In some cases, NGF may induce, rather than inhibit, apoptosis. Whether this effect will turn out to require the coexpression of a mismatched Trk (TrkB or TrkC) with $p75^{NTR}$ remains to be determined.

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References

Barrett GL and Bartlett P (1994) The p75 nerve growth factor receptor mediates survival of death depending on the stage of sensory neuron development. Proc. Natl. Acad. Sci. USA 91: 6501 – 6505

Barrett GL and Georgiou A (1996) The low-affinity nerve growth factor receptor p75NGFR mediates death of PC12 cells after nerve growth factor withdrawal. J. Neurosci. Res. 45: 117 – 128

Beg AA and Baltimore D (1996) An essential role for NF-κB in preventing TNF-α-induced cell death. Science 274: 782 – 789

Bredesen DE (1994) Neuronal Apoptosis: Genetic and biochemical modulation. In: Apoptosis II: The Molecular Basis of Apoptosis in Disease. Cold Spring Harbor Laboratory Press, pp. 397 – 421

Bredesen DE (1996) Keeping neurons alive: the molecular control of apoptosis. The Neuroscientist 2: 181 – 190

Bredesen DE and Rabizadeh S (1997) $p75^{NTR}$ and apoptosis: Trk-dependent and Trk-independent effects. Trends Neurosci. 20: 287 – 290

Bunone G, Mariotti A, Compagni A, Morandi E and Valle GD (1997) Induction of apoptosis by p75 neurotrophin receptor in human neuroblastoma cells. Oncogene 14: 1463 – 1470

Casaccia-Bonnefil P, Carter BD, Dobrowsky RT and Chao MV (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature 383: 716 – 719

Chang Y and Wang JZ (1997) Morphological and biochemical changes during programmed cell death of rat cerebellar granule cells. Neurochem. Res. 22: 43 – 48

Cheema SS, Barrett GL and Bartlett PF (1996) Reducing p75 nerve growth factor receptor levels using antisense oligonucleotides prevents the loss of axotomized sensory neurons in the dorsal root ganglia of newborn rats. J. Neurosci. Res. 46: 239 – 245

Cheng B, Christakos S and Mattson MP (1994) Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. Neuron 12: 139 – 153

Cortazzo MH, Kassis ES, Sproul KA and Schor NF (1996) Nerve growth factor (NGF)-mediated protection of neural crest cells from antimitotic agent-induced apoptosis: the role of the low-affinity NGF receptor. J. Neurosci. 16: 3895 – 3899

Dechant G and Barde Y-A (1997) Signalling through the neurotrophin receptor $p75^{NTR}$. Curr. Opin. Neurobiol. 7: 413 – 418

Ellerby HM, Martin SJ, Ellerby LM, Naiem SS, Rabizadeh S, Salvesen GS, Casciano C, Cashman NR, Green DR and Bredesen DE (1997) Establishment of a cell-free system for neuronal apoptosis: comparison of pre-mitochondrial, mitochondrial, and post-mitochondrial phases. J. Neurosci. 17: 6165 – 6178

Frade JM, Rodriguez-Tebar A and Barde Y-A (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature 383: 166 – 168

Garcia I, Martinou I, Tsujimoto Y and Martinou J-C (1992) Prevention of programmed cell death of sympathetic neurons by the *bcl-2* proto-oncogene. Science 258: 302 – 304

Greene LA and Kaplan DR (1995) Early events in neurotrophin signalling via Trk and p75 receptors. Curr. Opin. Neurobiol. 5: 579 – 587

Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF and Chao MV (1991) High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature 350: 678 – 683

Hileman MR, Chapman B, Rabizadeh S, Krishnan VV, Bredesen D, Assa-Munt N and Plesniak LA (1997) A cytoplasmic peptide of the neurotrophin receptor p75^{NTR}: Induction of apoptosis and NMR determined helical conformation. FEBS Lett. 415: 145 – 154

Ibanez CF, Ebendal J, Barbany G, Murray-Rust J, Blundell TL and Persson H (1992) Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the product of the trk gene product. Cell 69: 329 – 341

Kaplan DR and Miller FD (1997) Signal transduction by the neurotrophin receptors. Curr. Opin. Cell Biol. 9: 213 – 221

Lee K-F, Li E, Huber LJ, Landis SC, Sharpe AH, Chao MV and Jaenisch R (1992) Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell 69: 737 – 749

Liepinsh E, Ilag LL, Otting G and Ibanez CF (1997) NMR structure of the death domain of p75 neurotrophin receptor. EMBO J. 16: 4999 – 5005

Longo FM, Manthorpe M, Xie YM and Varon S (1997) Synthetic NGF peptide derivatives prevent neuronal death via a p75 receptor-dependent mechanism. J. Neurosci. Res. 48: 1 – 17

Mah SP, Zhong LT, Liu Y, Roghani A, Edwards RH and Bredesen DE (1993) The protooncogene bcl-2 inhibits apoptosis in PC12 cells. J. Neurochem. 60: 1183 – 1186

Martin DP, Schmidt RE, deStefano PS, Lowry OH, Carter JG and Johnson EM (1988) Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. J. Cell Biol. 106: 829 – 844

Miller TM, Tansey MG, Johnson Jr EM and Creedon DJ (1997) Inhibition of phosphatidylinositol 3-kinase activity blocks depolarization- and insulin-like growth factor I-mediated survival of cerebellar granule cells. J. Biol. Chem. 272: 9847 – 9853

Peterson DA, Leppert JT, Lee KF and Gage FH (1997) Basal forebrain neuronal loss in mice lacking neurotrophin receptor p75. Science 277: 837 – 839

Pflug BR, Onoda M, Lynch JH and Djakiew D (1992) Reduced expression of the low 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. 52: 5403 – 5406

Rabizadeh S, Oh J, Zhong L, Yang J, Bitler C, Butcher L and Bredesen D (1993) Induction of apoptosis by the low-affinity NGF receptor. Science 261: 345 – 348

Rabizadeh S and Bredesen DE (1994) Is p75^{NGFR} involved in developmental neural cell death? Dev. Neurosci. 16: 207 – 211

Ruan Y, Camerini D, Rabizadeh S and Bredesen DE (1997) Expression of CD40 induces neural apoptosis. J. Neurosci. Res. 50: 383 – 390

Salvesen GS and Dixit VM (1997) Caspases: intracellular signaling by proteolysis. Cell 91: 443 – 446

Sauer H, Nishimura MC and Phillips HS (1996) Deletion of the p75^{NTR} gene attenuates septal cholinergic cell loss in mice heterozygous for a deletion of the NGF gene. Soc. Neurosci. Abs. 22: 513

Taglialatela G, Hibbert CJ, Hutton LA, Werbach-Perez K and Perez-Polo J (1996) Suppression of p140trkA does not abolish nerve growth factor-mediated rescue of serum-free PC12 cells. J. Neurochem. 66: 1826 – 1835

Van Antwerp DJ, Martin SJ, Kafri T, Green DR and Verma IM (1996) Suppression of TNF-alpha-induced apoptosis by NF-κB. Science 274: 787 – 789

Van der Zee CE, Ross GM, Riopelle RJ and Hagg T (1996) Survival of cholinergic forebrain neurons in developing p75NGFR-deficient mice. Science 274: 1729 – 1732

von Bartheld CS, Kinoshita Y, Prevette D, Yin QW, Oppenheim RW and Bothwell M (1994) Positive and negative effects of neurotrophins on the isthmo-optic nucleus in chick embryos. Neuron 12: 639 – 654

Yeo T, Chua-Couzens J, Valletta J, Butcher LL, Bredesen DE, Mobley WC and Longo FM (1997) Absence of p75^{NTR} causes increased basal forebrain cholinergic neuron size, ChAT activity and target innervation. J. Neurosci. 17: 7594 – 7605