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Research Report

Proteomic assessment of sympathetic ganglia from adult mice that possess null mutations of *ExonIII* or *ExonIV* in the p75 neurotrophin receptor gene

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ABSTRACT

Neurotrophins, such as nerve growth factor (NGF), are capable of binding to the transmembrane p75 neurotrophin receptor (p75NTR), which regulates a variety of cellular responses including apoptosis and axonal elongation. While the development of mutant mouse strains that lack functional p75NTR expression has provided further insight into the importance of this neurotrophin receptor, there remains a paucity of information concerning how the loss of p75NTR expression may alter neural phenotypes. To address this issue, we assessed the proteome of the cervical sympathetic ganglia from two mutant lines of mice, which were compared to the ganglionic proteome of age-matched wild type mice. The ganglionic proteome of mice possessing two mutant alleles of either *exonIII* or *exonIV* for the p75NTR gene displayed detectable alterations in levels of Lamin A, tyrosine hydroxylase, and Annexin V, as compared to ganglionic proteome of wild type mice. Decreased expression of the basic isoform of tyrosine hydroxylase may be linked to perturbed NGF signaling in the absence of p75NTR in mutant mice. Stereological measurement showed significant increases in the number of sympathetic neurons in both lines of p75NTR-deficient mice, relative to wild type mice. This enhanced survival of sympathetic neurons coincides with shifts toward the more basic isoforms of Annexin V in mutant mice. This study, in addition to providing the first comparative proteomic assessment of sympathetic ganglia, sheds new light onto the phenotypic changes that occur as a consequence of a loss of p75NTR expression in adult mice.

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Abbreviations: 1-DE, 1-dimensional gel electrophoresis; 2-DE, 2-dimensional gel electrophoresis; HRP, horseradish peroxidase; IgG, immunoglobulin G; IPG, Immobilized pH gradient; kDa, kiloDalton; MALDI-TOF, matrix assisted LASER desorption ionization-time of flight; MS, mass spectrometry; NGF, nerve growth factor; NTR, neurotrophin receptor; p75(III)$^{-/-}$, homozygous mice carrying null mutation in the 3rd exon for p75NTR; p75(IV)$^{-/-}$, homozygous mice carrying null mutation in the 4th exon for p75NTR; PVDF, polyvinylidene fluoride; SCG, superior cervical ganglion; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; trkA, tyrosine kinase receptor A; WT, wild type

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1. Introduction

First described as the low affinity receptor for nerve growth factor (NGF), the transmembrane p75 neurotrophin receptor (p75NTR) is now recognized for its ability to bind all members of the neurotrophin family of molecules (e.g., NGF, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin 4/5) with similar affinity (Chao, 1994; Barker, 1998, 2004). The p75NTR belongs to the superfamily of tumor necrosis factor receptors, which includes tumor necrosis factor receptors I and II and Fas (Dechant and Barde, 1997). In the adult mammalian nervous system, several neuronal populations express robust levels of p75NTR, including ganglionic sympathetic neurons, primary sensory neurons in the trigeminal ganglia and dorsal root ganglia, and basal forebrain cholinergic neurons (Hefti et al., 1986; Johnson et al., 1989). Peripheral glial cells, such as non-myelinating Schwann cells, satellite cells of sensory and autonomic ganglia, and olfactory ensheathing cells also express p75NTR (Taniuchi et al., 1986; Zhou et al., 1996; Ramon-Cueto et al., 1993). While levels of p75NTR often decrease among injured neurons, the opposite appears to be true for glial levels of p75NTR after trauma (Taniuchi et al., 1986; Hagg et al., 1989; Verge et al., 1992; Zhou et al., 1996). Sympathetic, sensory, and basal forebrain cholinergic neurons do, however, display marked increases in p75NTR expression in response to elevated NGF levels, achieved by either infusion of exogenous NGF or the generation of NGF transgenic mice (Hagg et al., 1989; Kawaja et al., 1992; Verge et al., 1992; Miller et al., 1994; Coome and Kawaja, 1999).

The development of mice that lack functional p75NTR, through the targeted mutation of either *exon III* or *exon IV*, has provided valuable evidence concerning the *in vivo* roles of this transmembrane receptor. The third exon of the p75NTR gene encodes for the last three of four cysteine-rich repeats in the extracellular domain (Welcher et al., 1991; Yan and Chao, 1991), whereas the fourth exon encodes for the transmembrane domain (Chapman and Kuntz, 1995). Mice that have two mutated alleles for p75NTR^{exonIII} (herein referred to as p75(III)$^{-/-}$ mice) were first reported by Lee et al. in 1992, and as such there is considerably more information concerning the sympathetic phenotype of these animals, as compared to mice with two mutated alleles for p75NTR^{exonIV} (herein referred to as p75(IV)$^{-/-}$ mice), reported by von Schack et al. (2001). First, p75(III)$^{-/-}$ mice display a substantial increase in the number of post-ganglionic sympathetic neurons, as compared to age-matched wild type mice (Bamji et al., 1998; Majdan et al., 2001). Second, densities of sympathetic axons in target organs of p75(III)$^{-/-}$ mice may be increased (e.g., in the submandibular salivary gland; Jahed and Kawaja, 2005), decreased (e.g., in the pineal gland; Lee et al., 1994), or unchanged (e.g., in the heart and urinary bladder; Jahed and Kawaja, 2005). Third, in the absence of p75NTR expression, the accumulation of retrogradely-derived NGF in trkA-positive somata (as identified by immunostaining) is markedly depleted, as compared to NGF immunostaining seen in sympathetic neurons of wild type mice (Coome et al., 1998; Walsh et al., 1999a,b; Krol et al., 2000). Parenthetically, there is no evidence as to whether the phenotype of ganglionic Schwann cells or satellite cells, which are found in immediate proximity to post-ganglionic sympathetic neurons, is affected in p75(III)$^{-/-}$ mice.

Numerous studies (Lee et al., 1992, 1994; Bamji et al., 1998; Brennan et al., 1999; Kohn et al., 1999; Majdan et al., 2001), including our own (Coome et al., 1998; Kawaja, 1998; Hannila and Kawaja, 1999; Walsh et al., 1999a,b; Krol et al., 2000; Hannila et al., 2004; Jahed and Kawaja, 2005) have used mice with two mutated alleles for p75NTR^{exonIII} to assess sympathetic responses to a loss of receptor function. It is now recognized that these mutant mice express a truncated protein product, resulting in the presence of a cleaved intracellular portion of the receptor (von Schack et al., 2001). In light of this observation, a second line of mice was developed that possess two mutated alleles for p75NTR^{exonIV}. Though no splice variants were initially detected in p75(IV)$^{-/-}$ mice, a study by Paul et al. (2004) raised new questions as to whether these mice may likewise express a hypomorphic form of p75NTR.

Given the availability of the two mutant animal models for p75NTR, we undertook a proteomic comparison of the superior cervical ganglia (SCG) from adult p75(III)$^{-/-}$ and p75(IV)$^{-/-}$ mice, with the expectation of revealing phenotypic similarities and differences between these lines of mice, as compared to age-matched wild type mice. We chose to examine the SCG proteome for two primary reasons. First, as already mentioned, a wealth of information exists regarding the survival and axonal growth of sympathetic neurons in p75(III)$^{-/-}$ mice, thus making the SCG an obvious candidate for proteomic investigation. Second, because the SCG also possess non-myelinating Schwann cells and satellite cells, alterations in ganglionic protein levels might be attributable to these two populations of p75NTR-expressing glia as well. In this study we conducted 2-dimensional gel electrophoresis of solubilized SCG proteins pooled from age-matched p75(III)$^{-/-}$, p75(IV)$^{-/-}$, and wild type mice, and spot differences between the three groups of mice were selected for identification by mass spectrometry. Changes in ganglionic levels of identified proteins were validated by immunoblotting and immunostaining. This first proteomic assessment of the SCG of adult p75(III)$^{-/-}$ and p75(IV)$^{-/-}$ mice reveals several proteins that are differentially expressed between these mutant mice and wild type mice, thus demonstrating similar phenotypic shifts in two independent lines of p75NTR mutant mice. Our finding that shifts in the expression of tyrosine hydroxylase and Annexin V isoforms in mutant mice may be linked to altered NGF signaling events, as a consequence of p75NTR dysfunction, that normally regulate the catecholaminergic phenotype and survival of SCG neurons, respectively.

2. Results

2.1. *Proteomic analyses of the SCG*

Large format (24×20 cm) 2-dimensional gel electrophoresis (2-DE) of whole tissue lysates from mouse SCG resolved approximately 500 distinct spots with isoelectric points between pH 4 and 7, and molecular weight between 10 and 250 kDa for each genotype (Fig. 1). Image analysis revealed a number of ganglionic proteins present in different abundance between adult WT, p75(III)$^{-/-}$, and p75(IV)$^{-/-}$ mice, as observed by variable spot intensities on silver stained gels (Figs. 2A–F). These differently abundant proteins were identified using