Commentary: Impact of a deletion of the full-length and short isoform of p75NTR on cholinergic innervation and the population of postmitotic doublecortin positive cells in the dentate gyrus

Mohamed A. Sabry1*, Mona Fares2, Ronnie Folkesson3, Mariam Al-Ramadan4, Jarrah Alabkal1, Ghada Al-Kafaji5 and Moustapha Hassan2

1 Department of Medical Biochemistry, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain, 2 Experimental Cancer Medicine, Laboratory Medicine, Karolinska Institute, Stockholm, Sweden, 3 Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Stockholm, Sweden, 4 Biotechnology Program, College of Postgraduate Studies, Arabian Gulf University, Manama, Bahrain, 5 Department of Molecular Medicine/Al-Jawhara Centre for Molecular Medicine, Genetics and Inherited Disorders, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain

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A commentary on

Impact of a deletion of the full-length and short isoform of p75NTR on cholinergic innervation and the population of postmitotic doublecortin positive cells in the dentate gyrus

by Poser, R., Dokter, M., von Bohlen und Halbach, V., Berger, S. M., Busch, R., Baldus, M., et al. (2015). Front. Neuroanat. 9:63. doi: 10.3389/fnana.2015.00063

We read with great interest the article by Poser et al. (2015). Full-length human/murine P75NTR (FL-p75NTR; ENST00000172229, ENSMUST00000000122, ENSRNOT00000007268) is encoded by six exons. Four cysteine-rich repeats span P75NTR extracellular domain and exhibit high-pro-neurotrophins-affinity and low-affinity to mature neurotrophins.

Brain P75NTR plays pivotal roles in neurogenesis. Embryonic neurogenesis derives from ventricular zone radial glia (Mochida et al., 2007). Adult neurogenesis is localized to stem cell niches in hippocampal dentate gyrus and the sub-ventricular zone (SVZ). P75NTR is detected in deep layers of developing neocortex (Bishop et al., 2002) and defines adult neurogenesis stem cells in SVZ (Young et al., 2007) and hippocampus (Catts et al., 2008). P75NTR regulates synaptic plasticity through its interactions with pro-BNDF to produce long-term depression (Woo and Lu, 2009). P75NTR interacts with Nogo/Lingo-1 leading to growth cone collapse, neurite retraction, and spine density decrease (Meeker and Williams, 2015).

To verify the existence of the presumed mouse s-p75NTR, we conducted Rapid Amplification of cDNA Ends (RACE) cloning on mouse P75NTR mRNA and ascertained many exon...
III-containing clones without any evidence of continuous exon II/exon IV sequence with exon III-skipping (Figure 1I). Also we conducted RT-PCR using oligos flanking exon III and validated our results by sequencing the identified PCR fragments (Figure 1II). Additionally, we used the same discontinuous, exon III-skipping primers which were used in the original reports as
“specific” oligos to the presumed s-75NTR variant (von-Schack et al., 2001). These specific primers contain exons 2/4 boundary sequences. Also, this approach did not identify any short, exon III-excluding isoform in the mouse (Figure III).

Western blotting (WB) on mouse, rat, and human brain protein extracts using polyclonal antibodies specific to the intracellular region of P75NTR (Figure III) identified the 75 kD FL-p75NTR in the three species while a 62 kD band (corresponding to the claimed s-p75NTR) was not detected in any of the three species investigated. Apart from 75 kD FL-p75NTR, no other bands were detected in mouse and rat brains (Figure III). Of note is the fact that the report by (von-Schack et al., 2001) failed to show the protein band corresponding to the presumed 62 kD s-p75NTR in their WB. They rather showed a WB “smear” around 75 kD and argued that the 62 kD s-p75NTR band has been masked by the high level expression of FL-p75NTR! This is hardly convincing since the issue could have been easily resolved by simple technical improvements. It is noteworthy that our results, verifying only one murine FL-p75NTR band, are compatible with the documentations in major resources like Ensembl, NCBI, and UniProt database.

In addition to the 75 kD FL-p75NTR, our WB results on control and Alzheimer’s disease human brains also detected an additional, previously unreported, human 36 kD isoform, (Figure III). The 36 kD P75NTR isoform does not seem to be a marker for Alzheimer’s disease since we detected the two isoforms (75 and 36 kD), either simultaneously or alternatively, in individual human samples from control or Alzheimer’s brains (Figure III). It is notable that three protein-coding P75NTR human transcripts (ENST00000172229, ENST00000504201, and ENST00000509200) are listed in ENSEMBL and UniProt. We are embarking on further analysis to determine the nature of our 36 kD isoform.

It is seriously alarming that the report by von-Schack et al. (2001) did not show any experimental work to support their claim for the presence of a 62 kD s-p75NTR. In addition to the lack of WB evidence, they did not even sequence their RT-PCR fragments to prove their claims. Such unsubstantiated reporting caused considerable confusion in the neuroscience community since other groups (Fujii and Kunugi, 2009; Poser et al., 2015) innocently reiterated such un-validated reports and, moreover, interpreted their important results based on such unfounded claims (Poser et al., 2015).

AUTHOR CONTRIBUTIONS
All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Sabry et al. P75NTR Isoforms

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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