*Neuroscience* 175 (2011) 49–65

# DIFFERENTIAL EXPRESSION OF RET RECEPTOR ISOFORMS IN THE OLFACTORY SYSTEM

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**Abstract—The glial cell line-derived neurotrophic factor (GDNF) family supports neurons by activating the tyrosine kinase receptor RET. The two main isoforms of RET, RET9 and RET51, differ in their carboxyl termini and have been implicated with distinct functions in the enteric and central nervous systems. Previously we reported the cellular localization of GDNF, neurturin and RET9 in the olfactory system [Maroldt H, Kaplinovsky T, Cunningham AM (2005) J Neurocytol 34:241–255]. In the current study, we examined immunohistochemical expression of RET9 and RET51 in neonatal and adult rat olfactory neuroepithelium (ON) and bulb to explore their potential functional roles. In the ON, RET9 was expressed by olfactory receptor neurons (ORNs) throughout the olfactory neuroepithelial sheet, whereas RET51 was restricted to ORNs situated in ventromedial and ventrolateral regions. Within these regions, RET51 was expressed by a subset of RET9-expressing ORNs. In olfactory bulb, RET9 expression was primarily on cell bodies, including olfactory ensheathing and periglomerular cells, and again, RET51 was expressed by a subset of RET9-expressing cells. RET51 was identified on axons in the olfactory nerve layer and glomerular neuropil, but only in the ventromedial and ventrolateral regions of the bulb. This regionalization correlated with the predicted axonal projection from expressing regions of the ON. RET51 was also expressed on dendrites in the external plexiform layer and glomerular neuropil. These results suggest RET9 may be the predominant functional isoform in the ON while RET51 plays a more selective role in a restricted region of the olfactory neuroepithelial sheet. In the bulb, RET9 is likely the main functional isoform while RET51 may be important in axonal and dendritic function/targeting.**


**Key words: RET isoforms, olfactory system, immunohistochemistry, olfactory ensheathing cells, olfactory receptor neurons, dopaminergic neurons.**

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*Abbreviations:* BDNF, brain derived neurotrophic factor; CNS, central nervous system; EPL, external plexiform layer; GAP-43, growth associated protein-43; GDNF, glial cell line-derived neurotrophic factor; GFR$\alpha$, growth factor receptor alpha; GL, glomerular layer; MAP2, microtubule-associated protein 2; MCL, mitral cell layer; NGF, nerve growth factor; NPY, neuropeptide Y; NS, normal serum; NST, neuron specific tubulin; NTN, neurturin; NT-3, neurotrophin-3; NT-4, neurotrophin-4; OECs, olfactory ensheathing cells; OMP, olfactory marker protein; ON, olfactory neuroepithelium; ONL, olfactory nerve layer; OR, odorant receptor; ORNs, olfactory receptor neurons; pAb, polyclonal antibody; PBS, phosphate buffered saline; PFA, paraformaldehyde; PGs, periglomerular cells; TH, tyrosine hydroxylase.

The *c-ret* proto-oncogene encodes the transmembrane RET receptor tyrosine kinase (Takahashi et al., 1985), which serves as a receptor for the glial cell line-derived neurotrophic factor (GDNF) family of ligands: GDNF, neurturin (NTN), artemin and persephin. Mutations of *ret* lead to a number of human pathological conditions including Hirschsprung's disease and various cancers (Solari et al., 2003; Lodish and Stratakis, 2008). RET signaling is known to be essential for the development of the enteric nervous system as well as kidney organogenesis (Costantini and Shakya, 2006). Sensory neurons in the peripheral nervous system and various neuronal types in the central nervous system (CNS), including motoneurons and dopaminergic neurons, also depend on RET signaling for their maturation and survival (Baloh et al., 2000; Airaksinen and Saarma, 2002).

GDNF family ligands bind to growth factor receptor alpha (GFR$\alpha$1–4) coreceptors located in lipid raft domains in the surface membrane with differing preferences in affinity existing between the ligands and coreceptors (Fig. 1) (Airaksinen et al., 1999; Airaksinen and Saarma, 2002). *In vivo*, GDNF preferentially binds to GFR$\alpha$1, NTN to GFR$\alpha$2, artemin to GFR$\alpha$3 and persephin to GFR$\alpha$4; however, other ligand-coreceptor interactions have been described *in vitro* (Jing et al., 1997). Recently Sidorova et al. (2010) showed persephin can signal via GFR$\alpha$1 in explants of sympathetic ganglia when the usual coreceptor for persephin, GFR$\alpha$4, was not expressed. When ligands bind to the coreceptors, two RET proteins are recruited into the lipid raft and an active signaling complex is formed. Subsequent transphosphorylation of RET tyrosine kinase domains induces intracellular signaling cascades, affecting cell function (Fig. 1A). RET has also been shown to function as a "dependence receptor" (Bordeaux et al., 2000; Porter and Dhakshinamoorthy, 2004). Such receptors promote positive cell functions such as growth and survival in the presence of their ligands but initiate apoptotic mechanisms in the absence of ligands.

Alternative splicing of RET pre-mRNA yields two predominant isoforms, RET9 and RET51, differing at their carboxyl termini, regions involved in intracellular signaling (Tahira et al., 1990; Lorenzo et al., 1995). The long isoform, RET51, is 42 amino acids longer and has two extra tyrosine kinase domains (tyrosines-1090 and 1096). Additionally, the amino acid sequences around tyrosine-1062 vary between the two splice variants (Fig. 1B). These differences potentially allow for activation of different intracellular signaling pathways and thus distinct functions of each isoform (Arighi et al., 2005). Differences in turnover rates and intracellular locations have also been shown

0306-4522/11 $ - see front matter 
doi:10.1016/j.neuroscience.2010.11.047

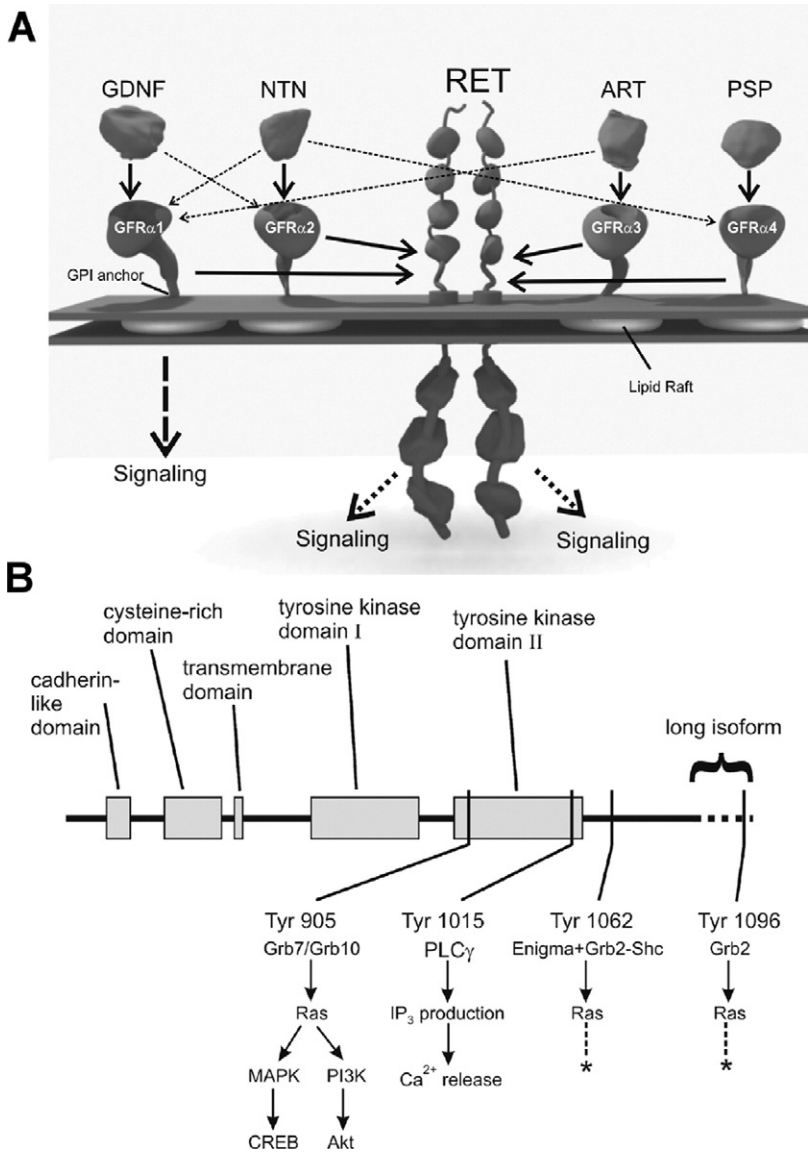


**Fig. 1.** GDNF family of ligands and RET isoforms. (A) The GDNF family of neurotrophic factors (GDNF, NTN, ART, PSP) activate RET receptor tyrosine kinase by first binding their specific GDNF family receptor (GFR) accessory protein (GFRα1, GFRα2, GFRα3, GFRα4). The GFRα proteins are attached to the plasma membrane by a glycosyl phosphatidyl inositol (GPI) anchor situated in lipid rafts. The GFRα-trophic factor complex then recruits RET receptor tyrosine kinase homodimers to merge and cross-phosphorylate, subsequently initiating downstream intracellular signaling cascades. Solid arrows indicate the preferred ligand-receptor interactions that are known to occur *in vivo*, whereas dotted arrows indicate possible crosstalk interactions. For review, see Airaksinen et al., 1999; Airaksinen and Saarma, 2002. (B) The two predominant RET isoforms (9 and 51) differ at their carboxyl termini. The longer isoform (RET51) has two extra tyrosine kinase domains, one of which (tyrosine-1096) is illustrated. The amino acid sequences near tyrosine-1062, a key phosphorylation site, vary between the isoforms.

for the two isoforms (reviewed in Runeberg-Roos and Saarma, 2007; Tsui and Pierchala, 2010), and both isoforms are evolutionarily conserved across many species suggesting each isoform retains a functional role (Carter et al., 2001).

Evidence for distinct functions of RET9 and RET51 has come from work in the renal system. de Graaff et al. (2001) generated monoisoformic mice and demonstrated that RET9-null animals died in the neonatal period from renal dysgenesis, while RET51-null animals appeared to develop normally. These results suggested that RET9 signaling might be essential for kidney development whereas RET51 might be redundant. However, subsequently Jain et al. (2006) reported both strains of monoisoformic mice exhibited normal kidney development and survived to adulthood, suggesting either RET9 or RET51 signaling may be sufficient for renal development. Ivanchuk et al. (1998) provided evidence for both isoforms being functionally important in kidney organogenesis but at different stages of development. They showed RET9 may be necessary throughout embryonic kidney development whereas the importance of RET51 signaling may be at later developmental stages. Furthermore, it is known that a naturally occurring RET51 spe-