The effect of the ret- mutation on the normal development of the central and parasympathetic nervous systems

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The c-ret proto-oncogene, a member of the receptor tyrosine kinase (RTK) superfamily, plays a critical role in tumour formation and embryonic organogenesis. Germline mutations have been identified in patients with multiple endocrine neoplasia types 2A and 2B (characterized by medullary thyroid carcinoma and pheochromocytoma) and familial medullary carcinoma (FMTC), as well as in patients with congenital megacolon (Hirschsprung's disease, characterized by absence of a subset of enteric ganglia). Consistent with these studies in humans, targeted inactivation of the murine c-ret locus (ret-) results in kidney hypoplasia and agenesis of the enteric and superior cervical ganglia (Schuchardt et al., 1994; Dubec et al., 1994a). A number of laboratories have recently established that glial cell line-derived neurotrophic factor (GDNF), a distant member of the TGF-β family of growth factors, is a functional ligand of the Ret receptor. Given the potent neurotrophic activity of GDNF on midbrain dopaminergic and motor neurons of the central nervous system (CNS), it was of interest to examine the potential in vivo role of c-ret in the development of the CNS.

\[\text{c-ret expression in the CNS}\]

To investigate the potential role of c-ret function in the CNS, we first characterized the distribution of c-ret mRNA in this system. Our results revealed that c-ret mRNA in the CNS is expressed predominantly in the developing spinal cord and brain stem (Fig 1).

In the spinal cord, c-ret expression was mainly detected in the lateral and medial somatic motor columns, and in the visceral motor column. Within the brain stem, high levels of c-ret expression were observed in cranial motor and sensory nuclei throughout embryonic, perinatal and adult development. In: cranial motor neurons, c-ret is primarily expressed in branchiomotor and somatic motor nuclei and, to a lesser extent, in visceral motor nuclei. In the cranial sensory system, c-ret expression is restricted to the general somatic nervous system.

\[\text{c-ret transcripts were also observed in the reticular formation and the main monoaminergic systems of the brain stem: serotonergic and catecholaminergic (dopaminergic, noradrenergic and adrenergic). Among these systems, the highest levels of c-ret expression were detected in the developing midbrain dopaminergic neurons, namely the substantia nigra (SN) and ventral tegmental area (VTA).}\]

\[\text{The CNS of ret- homozygous mice}\]

To gain insight into the role of c-ret function in the CNS, we examined the development of c-ret positive cell groups in the CNS of ret- homozygous neonatal mice. For this purpose, we employed in situ hybridization and immunohistochemical methods using a variety of established neuronal markers (Fig 2).

To examine the development of midbrain dopaminergic c-ret neurons in ret-heterozygous and homozygous mice, we used tyrosine hydroxylase (TH) as a specific marker for this group of cells. As revealed by TH immunoreactivity, a normal complement of dopaminergic neurons in these cell groups was observed. Moreover, their axonal projections to the striatum also appeared normal.

To study the effect of the ret- mutation on motor neurons, we examined the expression pattern of Isl-1 mRNA in the CNS by in situ hybridization. Our results indicate similar numbers of differentiated motor neurons are present in the brain stem and spinal cord of ret-heterozygous and homozygous mice.

Overall, our findings revealed that none of the CNS populations analysed are either absent or display any gross morphological...
abnormalities in ret-homozygous mice.

Parasympathetic nervous system

Given the dramatic effect of the ret mutation in the enteric nervous system (ENS), we wished to examine the function of c-ret in other parasympathetic ganglia. High levels of expression were detected normally in the ciliary, pterygopalatine, submandibular, otic, cardiac and pelvic ganglia. Furthermore, our findings revealed c-ret function is required for the development of at least a subset of parasympathetic ganglia. Using SCG10 and Phox-2 as specific cellular markers, we have observed the ciliary and pterygopalatine ganglia do not develop in ret-mutant mice (fig 3).

Conclusions

The spatiotemporal distribution of c-ret transcripts in the developing mouse CNS is complex and comprises a variety of cell populations of distinct embryonic origins. Overall, the highest levels of c-ret mRNA are detected in two cell populations highly responsive to GDNF: midbrain dopaminergic neurons and motor neurons. c-ret expression in GDNF-responsive tissues suggests the GDNF-ret signaling mechanism is involved in the specification and/or maintenance of these neuronal populations.

Analysis of ret-homozygous mice revealed that c-ret function is not required for the development of catecholaminergic, motor or serotonergic neurons during embryonic development. Similarly, GDNF-deficient embryonic mice also show no apparent morphological abnormalities in motor or midbrain dopaminergic neurons (Moore et al., 1996; Pichel et al., 1996; Sanchez et al., 1996).

These findings strongly contradict the effects of exogenously supplied GDNF on midbrain dopaminergic neurons in vivo and in vitro. To reconcile these apparently contradictory findings, two possibilities are being suggested. First, the GDNF-Ret ligand-receptor signalling system is required for the development of specific CNS populations after birth and not during embryogenesis. Given that GDNF and c-ret null mice die in their first day of life, the functional analysis of these genes thus far is limited to embryonic development. To study the role of GDNF and/or c-ret in the postnatal and adult CNS, the c-fos/kap system could be used to disrupt these loci with precise spatiotemporal control. A second alternative possibility for the lack of a CNS phenotype in ret mutants is that compensation occurs by means of other neurotrophin-neurotrophin receptor signalling system(s). It is possible that the GDNF-Ret signalling system acts synergistically with another ligand-receptor complex on neuronal survival in the CNS during normal development. Such complex could compensate for the loss of GDNF and c-ret function. For instance, known neurotrophic factors, such as CNTF, BDNF, NT-3 or NT-4/5, and their receptors, may play a role in such functional redundancy. Also, it is possible that other GDNF-like or Ret-like molecules might compensate for the loss of the GDNF-Ret signalling pathway. However, no such molecules have been reported so far.

We have shown that c-ret function is required for the neuronal development of a subset of parasympathetic neurons, namely the ciliary and pterygopalatine ganglia. These ganglia are derived from cranial neural crest. Interestingly, the other two main groups of autonomic neurons also affected by the ret-mutation, the ENS and superior cervical ganglia (SCG), are also cranial neural crest derivatives (Schuchardt et al., 1994; Durbec et al., 1996a). These findings suggest c-ret function is specifically required for survival and/or differentiation of autonomic neurons of cranial neural crest origin and that the development of autonomic neurons derived from trunk neural crest is independent of c-ret function.

References


