Catecholaminergic neuron survival: getting hooked on GDNF

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Pascual *et al.* conditionally delete glial cell line-derived neurotrophic factor (GDNF) expression in adult mice. They report that GDNF is indispensable for the survival of adult catecholaminergic neurons.

The discovery of GDNF in 1993 by scientists from the now-defunct company Synergen has been hailed as a milestone in Parkinson's disease and neurotrophic factor research[1]. GDNF is a potent survival factor for mesencephalic dopaminergic neurons both *in vitro* and in rodent and primate animal models of Parkinson's disease[2,3] and has shown promise in some clinical trials[4]. Despite more than 500 publications on this topic, however, the physiological function of GDNF in dopaminergic neuron development and maintenance has remained unclear, and previous studies have failed to demonstrate a substantial role for endogenous GDNF signaling in dopaminergic neuron survival. In this issue, Pascual *et al.*[5] found that conditional ablation of GDNF expression in 2-month-old mice resulted in a marked reduction of ventral midbrain dopaminergic neurons and a nearly complete elimination of locus coeruleus noradrenergic neurons. This unexpected result reinstates the importance of GDNF as an endogenous survival factor for adult catecholaminergic neurons.

For most proteins, the first function ascribed to them becomes permanently associated with their identity. GDNF was no exception to this and was associated with the survival of dopaminergic neurons from its discovery. In fact, GDNF is able to rescue nearly 100% of these neurons, both in tissue culture and in various lesion models *in vivo*[6]. However, later work established that GDNF has many other functions, some of which are not at all related to the nervous system, such as branching of ureteric buds in the developing kidney[6]. In fact, mice lacking GDNF die at birth with kidney agenesis[6]. Notably, however, newborn mice lacking GDNF have a full complement of midbrain dopaminergic neurons[7]. Similar to GDNF knockout mice, mouse mutants lacking either the obligatory ligand binding subunit, GDNF family receptor α1 (GFRα1), or the signaling receptor subunit, RET receptor tyrosine kinase, are also born without kidneys, but with a normal complement of midbrain dopaminergic neurons[6,8]. As all of these mutants die at birth and dopaminergic neuron maturation occurs postnatally, the possibility remained that profound deficits might have appeared had the animals survived to later postnatal stages.

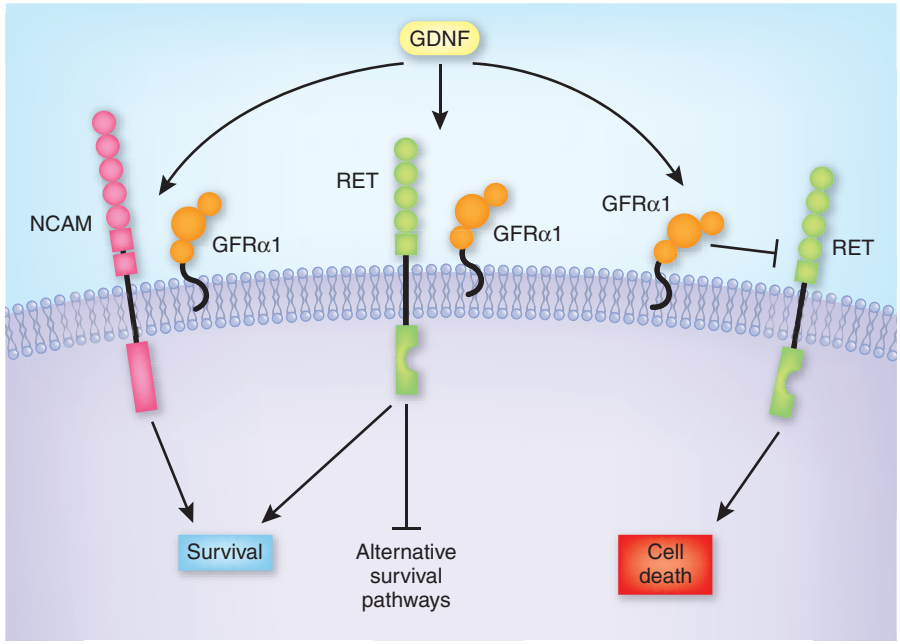

Figure 1 Three speculative scenarios that could explain the discrepancies between the phenotypes of GDNF and RET conditional mutant mice. Left, catecholaminergic neuron survival may be mediated by alternative GDNF signaling receptors that are distinct from RET, such as NCAM. Center, early GDNF signaling may render catecholaminergic neurons dependent on GDNF by suppressing alternative survival pathways. Right, RET may function as a dependence receptor in catecholaminergic neurons.

Pascual *et al.*[5] generated a conditional allele of the *Gdnf* gene that they excised in all cells of the body at 2 months of age using a tamoxifen-inducible CRE recombinase. They estimated that the procedure resulted in the inactivation of over 80% of *Gdnf* alleles in the striatum and a reduction of approximately 60% in the levels of GDNF mRNA and protein in this structure. The fact that less than 20% of *Gdnf* alleles were still able to produce about 40% of the normal amount of GDNF suggested a compensatory upregulation of *Gdnf* gene transcription in the remaining functional alleles. The surprise came, however, when the authors analyzed survival of catecholaminergic neurons 7 months after *Gdnf* deletion and discovered that 60–70% of substantia nigra and ventral tegmental area dopaminergic neurons were missing, whereas noradrenergic neurons in the locus coeruleus were almost completely absent. A proportional decline in the pan-neuronal marker NeuN confirmed that the effects were indeed the result of neuronal loss and not of mere downregulation of the catecholaminergic cell marker tyrosine hydroxylase, the rate-limiting enzyme for dopamine production. Dopaminergic

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cell numbers were also decreased in the carotid body and in the superior cervical ganglion, where RET and GFRα1 are highly expressed, but not in other dopaminergic neurons that are also known to express these receptors, such as those in the arcuate nucleus. Behavioral motor abnormalities consistent with deficits in the dopaminergic nigrostriatal pathway were also observed in these animals.

These results contrast with the view of adult GDNF function offered by the recent studies in conditional inactivation of the *Ret* gene in catecholaminergic neurons carried out by two different groups[9,10]. Notably, neither study found substantial deficits in these neurons in mutant mice younger than 1 year, although one of the studies reported a late-onset decline that resulted in a 38% loss of dopaminergic neurons in the substantia nigra by 2 years of age[10]. However, dopaminergic neurons in the ventral tegmental area and noradrenergic neurons in the locus coeruleus, which are also known to respond to exogenous GDNF[11], were unaffected. Two years is a very long time for a mouse, and the relatively modest deficits that were observed were clearly below everyone's expectations. Throwing their arms in the air, researchers in the field braced for the possibility that, against all odds, endogenous GDNF signaling may not have such an important function in the survival of dopaminergic neurons *in situ* after all.

Pascual *et al.*'s results[5] indicate that endogenous GDNF has an essential role in the survival of catecholaminergic neurons in the adult nervous system and highlight the importance of a continuous supply of neurotrophic factors for the maintenance of adult neuron survival. However, why are these results so different from the relatively mild defects that are observed in conditional RET knockouts? At least three possibilities come to mind (**Fig. 1**). One straightforward explanation would be to invoke the existence of alternative GDNF receptors that are distinct from RET in catecholaminergic neurons. The fact that GFRα1 is expressed in many cells that do not express RET has led to the idea that GDNF may be able to utilize other transmembrane signaling receptors that are distinct from RET[12]. One such receptor is the 140-kDa isoform of the neural cell adhesion molecule NCAM, which GDNF can bind in collaboration with GFRα1 (ref. 13). A previous study has indicated that NCAM-blocking antibodies can antagonize the effects of exogenous GDNF on midbrain dopaminergic neurons[14]. Although NCAM is known to mediate many different functions, including cell migratory responses to GDNF[13], neuronal survival is not prominent among them. Nonetheless, the results of Pascual *et al.*[5] indicate that a closer look at the dopaminergic system of NCAM mutant mice is warranted.

A second possibility, one favored by the authors, is that survival of catecholaminergic neurons in the absence of RET from embryonic stages may have been supported by compensatory mechanisms independent of GDNF, or at the very least through RET-independent pathways. In this scenario, GDNF signaling during embryonic development would suppress such mechanisms, thereby rendering neurons dependent on this factor. This alternative can be tested by inducing a *Ret* gene deletion at later stages of development, as Pascual *et al.*[5] have done with *Gdnf*, and by inactivating *Gdnf* specifically in dopaminergic neurons during embryonic development, as it had previously been done with *Ret*.

Finally, a third option would be to consider the possibility that RET may induce the death of dopaminergic neurons unless engaged by GDNF, in which case the sole function of GDNF would be to prevent constitutive apoptotic signaling by the receptor. Such receptors have been described before and are known as dependence receptors, as they make cells that express them dependent on their ligands. Interestingly, evidence (primarily from transformed cell lines) suggests that RET could indeed function as a dependence receptor[15]. A major problem with this concept has been that elimination of such receptors *in vivo* has, until now, resulted in the same phenotype as elimination of their ligands, which contradicts the predictions made by the theory. In this regard, the discrepancies between the phenotypes of Ret and GDNF conditional mutants would appear to favor this hypothesis. Nonetheless, the lack of effect of *Gdnf* deletion in the arcuate nucleus remains unexplained. Moreover, as the *Ret* and *Gdnf* genes have so far been inactivated at different stages, it would be premature to invoke a dependence-receptor effect in this case. This hypothesis can now be tested, as it predicts that the loss of dopaminergic neurons observed by Pascual *et al.*[5] should be rescued in the absence of RET. Moreover, a loss of dopaminergic neurons after inactivation of *Gdnf* from embryonic stages would also support this hypothesis, whereas a milder, late-onset defect, similar to that observed after inactivation of *Ret*, would favor a compensatory mechanism.

The requirement of GDNF for the survival of adult catecholaminergic neurons brings renewed interest to the field and should serve to rekindle efforts to use this protein in the clinic. Some of the alternatives discussed above would in fact suggest that the development of small molecule RET agonists may not be a viable therapeutic route and refocus attention on GDNF itself. During the course of Parkinson disease, the degeneration of dopaminergic neuron terminals will progressively lower the amount of target-derived GDNF that is available to these neurons. The results of Pascual *et al.*[5] now suggest that this process may also be an important contributor to neurodegeneration in Parkinson disease, reinforcing the rationale for using GDNF therapeutically.

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