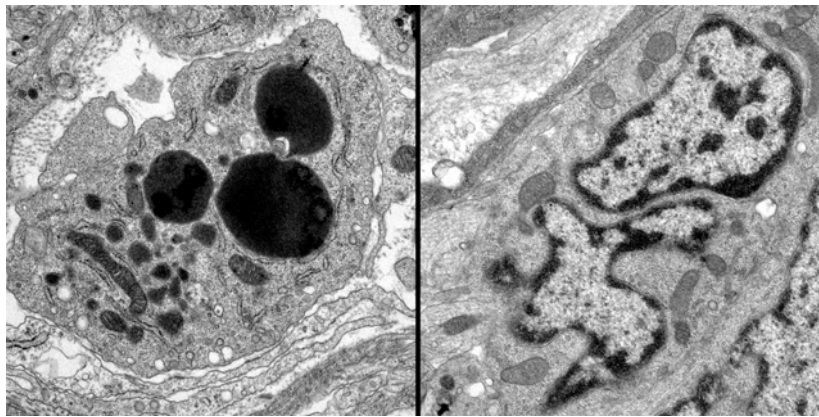


A new route to programmed cell death: Enteric neurons follow non-canonical apoptotic pathway

May 30, 2007 – Neurons rely on molecular cues, known as neurotrophic factors, for their survival, and the culling of cells deprived of such factors plays an important part in the development of most regions of the nervous system. The enteric nervous system (ENS) that innervates the gut, however, appears to be an exception, as it shows no signs of undergoing the form of physiological programmed cell death known as apoptosis. The ENS does depend on signals, nonetheless, as enteric neurons fail to develop in the distal intestine in mice deficient for either the neurotrophic factor GDNF, or either of its receptor components, RET or GFR α 1. Unfortunately, the severity of the phenotype hampers the ability to study the mechanism by which RET/ GFR α 1-mediated GDNF signaling enables ENS development, as the embryos of mice carrying loss-of-function mutations in any of these genes exhibit no neurons whatsoever in the small or large intestine.



GFR α 1 regulates enteric neuron survival in the distal bowel

Distinct morphological characteristics are observed in sympathetic neurons undergoing apoptosis (left) and degenerating enteric neurons in conditional GFR α 1 KO colon (right).

Scientists interested in the function of GDNF and its receptor complex have tried various workarounds to overcome this obstacle to understand, but none has yet conclusively demonstrated the physiological role of this signaling trio in the enteric nervous system. Now, however, by an elaborate feat of genetic engineering, Toshihiro Uesaka and others in the Laboratory for Neuronal Differentiation and Regeneration (Hideki Enomoto; Team Leader) have conditionally abolished GFR α 1 late in the ENS development, and made the unexpected finding that while these neurons do die en masse in the absence of GDNF signaling, they do so by a different process than canonical apoptosis. The findings are described in detail in an article published in the journal *Development*.

“This discovery that an unconventional form of neuronal cell death is triggered by inactivation of GFR α 1 in the distal colon is exciting for us,” says Uesaka, “as it may provide us with valuable insights into the pathogenesis of Hirschsprung’s disease, a disease in which ganglia fail to form in the distal colon in humans.”

Early in ENS development, the GFR α 1 receptor is expressed in the majority of enteric neuronal progenitors differentiating neurons throughout the gut, but its distribution gradually shifts over time until it is predominantly expressed in enteric neurons in the colon. This caught the team’s attention, because GDNF is also detected at particularly high levels in the colon and caecum during later stages of embryogenesis. Uesaka was interested in studying the role of GFR α 1, so to avoid the total loss of enteric neurons characteristic of the unconditional knockout of the gene, he painstakingly generated a line of mice in which the function of *GFR α 1* could be

excised contingently and cells carrying an inactivated allele traced by the expression of green fluorescent protein.

When he looked at embryos in which *GFR α 1* was inactivated at day 15.5 (by which point neuronal *GFR α 1* expression is nearly confined to the colon), he found that the normal pattern of lower gut innervation was completely disrupted, with a massive loss of enteric ganglia. Tests of the conditional knockout at other developmental time points showed that this effect was time-dependent; embryos in which *GFR α 1* was excised earlier in development showed defects in enteric neuronal proliferation, but not survival.

Curiously, however, the conditional E15.5 knockouts showed none of the telltale signs of apoptosis; tests revealed no significant upregulation of typical cell death markers such as activated caspase, nor the DNA fragmentation apoptotic cells undergo (as revealed by TUNEL assay). Following up on this startling observation, the Enomoto lab turned next to the proapoptotic factor, Bax, but found that Bax deficiency had little influence on enteric neuron death induced by GDNF deprivation in culture. And, while a number of abnormal morphological features were seen in the nuclei cells in colonic neurons of conditional *GFR α 1* knockout embryos, they showed none of the nuclear hallmarks of apoptosis, such as globular or crescent-shaped chromatin condensation, nor did dying cells appear to be succumbing to autophagy, as many apoptotic cells do.

In addition to demonstrating that GDNF is a survival factor for enteric neurons, a role it plays in many other arenas of neurodevelopment, Uesaka's study points to an intriguing new form of physiological cell death, which may be important in other developmental contexts and neuronal pathologies as well. Enomoto notes of the study's medical implications, "We think that the possible links to the etiology of Hirschsprung's disease are especially interesting, as we know that RET, the signaling component of the GDNF receptor complex, is most frequently mutated in those patients. It will certainly be worth looking into whether and how this novel cell death mechanism might apply in humans."