Review

Developmental determinants of the independence and complexity of the enteric nervous system

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Enteric nervous system (ENS) development is relevant to Hirschsprung's disease (HSCR; congenital aganglionosis of the terminal bowel), which is still imperfectly treated. Mutations in genes encoding the RET receptor tyrosine kinase and endothelin receptor type B (EDNRB) are involved in HSCR pathogenesis; however, also important in ENS development are molecules that mediate events that are more restricted than those of *RET* and *EDNRB*, act later in development and which might not be HSCR-associated. Examples are molecules that function in the guidance of enteric neural crest-derived cells (ENCDCs) and vagal axons, and in regulating the terminal differentiation of enteric neurons from ENCDCs. It is probable that highly prevalent disorders of gastrointestinal sensation and motility result from subtle defects in ENS development.

Introduction

An intact and functioning enteric nervous system (ENS) is required for life [1,2,4,8]. Smooth muscle activity is not, by itself, sufficient for gastrointestinal transit although propagating "ripples", which are entirely myogenic, develop before the ENS assumes control [9]. The segmental absence of the ENS, which occurs either as a congenital defect (Hirschsprung's disease, HSCR; congenital megacolon; Glossary) [10] or acquired defects (Chagas disease) [11], causes a pseudo-obstruction that impedes gastrointestinal transit. Modern understanding of ENS development dates from 1954, when the neural crest was identified as the source of the ENS [12]. This discovery was refined to the vagal (somites 1–7) and sacral (caudal to somite 28) axial levels in 1973 [13]. Since then, studies of ENS development have focused on HSCR, which is invariably lethal if not treated. Although not rare, HSCR occurs in approximately 1/5000 births and thus is not epidemic [10]. In ~70% of cases, HSCR occurs as an isolated trait [10]. Less commonly (~12%) patients with HSCR have a chromosomal defect, usually trisomy 21 (which occurs in ~90% of this group of patients). In the remaining 18%, HSCR occurs as a component of a syndrome. HSCR can be treated by removing the aganglionic bowel [14,15]; nevertheless, post-treatment gastrointestinal function and quality of life are often disappointing, particularly in older patients [16]. HSCR thus serves to provide "clinical relevance" for

Glossary

DCC: deleted in colorectal cancer. The neuronal receptor through which netrins mediate chemoattraction of ENCDCs, neurons and neurites.
ECE-1: a proteolytic endothelin converting enzyme that cleaves the active endothelin peptides from corresponding "big endothelins" at the bond between W^{21} and V^{22}. Big endothelins are secreted but inactive. They are cleaved intracellularly from 200 amino acid prepropolypeptides, which are encoded by separate genes.
ENCDC: enteric neural crest-derived cell. Cells that have completed their migration to the bowel are different from their predecessors in the premigratory neural crest or those en route to the gut. Although these cells are thus derived from the neural crest, they are not of it. There are no enteric neural crest cells; the neural crest is a transient embryonic structure that disappears as its component cells migrate, lose developmental potential and acquire terminally differentiated characteristics in their final sites of residence.
ET-1, ET-2, ET-3: the endothelins, 21 amino acid active peptide ligands.
ET_A and ET_B: the two G-protein-coupled endothelin receptors that are activated by the endothelin peptides. The affinities of ET_B for each endothelin is approximately the same; however, the affinities of ET_A for the endothelins follow the rank order ET-1>ET-2>>ET-3.
GDNF family: a family of extracellular signaling molecules that includes glial cell-derived neurotrophic factor (GDNF), neurturin, artemin and persephin.
GFRα: GDNF family receptor α. A glycosylphosphatidylinositol-anchored co-receptor that activates Ret in a complex with a preferred member of the GDNF family of ligands. These are, respectively, GFRα1 for GDNF, GFRα2 for neurturin, GFRα3 for artemin and GFRα4 for persephin.
HSCR: Hirschsprung's disease or congenital megacolon. The segmental absence of ganglia in the terminal portion of the bowel; length of the aganglionic region varies and can be present as a long- or short-segment disorder.
Integrins: obligate heterodimers of α and β subunits. Integrins are receptors that are expressed on cell surfaces that function in the attachment of cells to their neighbors or to the extracellular matrix (ECM). Integrins also enable cells to respond to signals from ECM molecules.
Mowat–Wilson syndrome: mental retardation, characteristic facial abnormalities and a plethora of additional birth defects accompanying HSCR.
Neogenin: the receptor through which netrins affect the vasculature and morphogenesis in pancreas and mammary glands.
Netrins: a class of diffusible guidance molecules with a structural resemblance to laminins. Netrins are bifunctional ligands for receptors that can mediate chemoattraction (UNC-40/DCC) or chemorepulsion (UNC5). Netrins also enhance neurite outgrowth and stimulate growth. They are vascular mitogens and promote morphogenesis of the pancreas and mammary glands. Netrin-1 is found in the CNS and gut, netrin-2 is found in avians and netrin-3 is most abundant in the periphery, including the bowel, early in development. Netrin-4 is least like other netrins; it is produced by astrocytes and has been linked to stimulating growth of CNS stem cells.
NT-3: a member of the nerve growth factor (NGF) family of neurotrophins. It supports the differentiation/survival of existing neurons and enhances the growth and development of new neurons and synapses. NT-3 is named as the third of the neurotrophin factors to be characterized. NGF was the first and brain derived neurotrophic factor (BDNF) was the second.
TrkC: this is the high-affinity neurotrophin receptor that mediates the neurotrophic effects of NT-3. It is also called neurotrophic tyrosine kinase receptor type 3 and is encoded in humans by the *NTRK3* gene. TrkC is a member of the family of neurotrophin receptors that also includes TrkA and TrkB.
Waardenburg syndrome type IV: hearing loss and pigmentary abnormalities of skin, hair, iris and inner ear accompanying HSCR.

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investigators who fear appearing excessively basic. This focus has caused a veritable "war" on HSCR. Although this concentration of effort has increased our understanding of critical aspects of ENS development, it might also have obscured abnormalities of the bowel that are not as apparent as the aganglionosis of HSCR. Defects in ENS development that do not cause HSCR, however, undoubtedly occur and can contribute to dysmotility syndromes, including functional bowel disorders.

ENS defects are not easily diagnosed if ganglia are present. The pathology of ENS disorders has not been systematically classified and routine methods of pathological examination mainly determine whether ganglia are present or absent. The presence of ganglia, however, does not preclude abnormalities of gastrointestinal motility, such as chronic intestinal pseudo-obstruction, which can require parenteral nutrition and be life-threatening, and functional bowel disorders, which although not life-threatening can be misery-creating and disabling [17]. Functional bowel disorders, moreover, are prevalent and expensive to individuals and to society (Box 1).

Because functional bowel disorders are compatible with life, they obviously must also be compatible with gangliogenesis and sufficient propulsion of luminal contents to avoid pseudo-obstruction. A developmental defect that gives rise to a functional bowel disorder is likely to affect committed lineages of neural or glial precursors, rather than pluripotent stem cells, and thus occur relatively late in ENS ontogeny (Figure 1). This review will focus not only on the early events that contribute to HSCR, which have been the subject of several outstanding recent reviews [18–20], but on later events that sculpt the complexity of the ENS and can contribute to highly prevalent gastrointestinal disorders.

Box 1. What is the societal cost of functional bowel disorders?

In the United States, the direct cost of the 11,648,000 outpatient visits and 1,241,000 hospitalizations due to functional bowel disorders in 2004 was $3,661,200,000 [1]. Indirect costs due to missed work, non-prescription remedies and alternative therapies were much higher. Because causative lesions in the gut have not been identified, functional bowel disorders are often considered psychogenic [3]; however, their pathogenesis is unknown and the symptom complex implies that the ENS is involved [4–6]. Drugs targeting enteric serotonin receptors, moreover, were effective before their use was curtailed because of unrelated safety concerns [7,8].

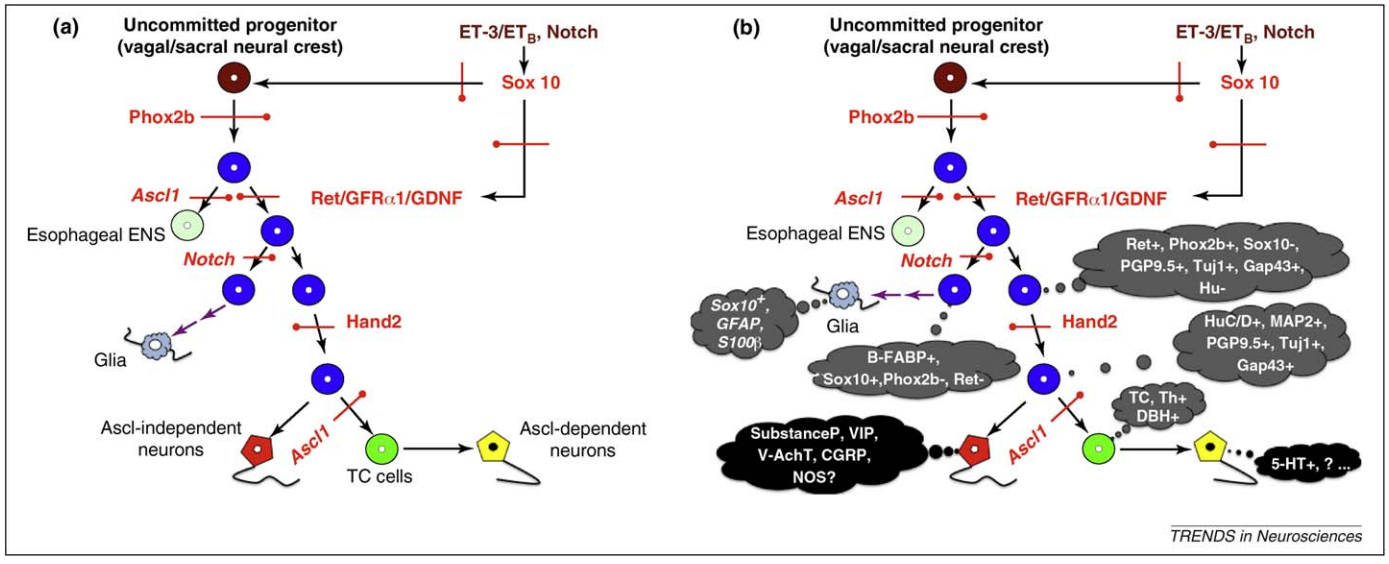

Figure 1. **(a)** The developmental potential of ENCDCs is progressively restricted as development proceeds and occurs in stages that require expression of particular transcription factors and stimulation by specific growth factors. Deletion of these transcription factors (red capped lines) prevents development of all of the cells that arise from the precursors after the expression of these factors is needed. Note that the earlier a factor is needed, the greater the defect caused by its deletion. For example, the uncommitted progenitors that exit from the vagal or sacral neural crest (top) obligatorily express Sox10 and respond to Notch and ET-3/ET$_B$ signaling. These factors tend to maintain the uncommitted state. The cells thus proliferate prior to reaching the gut and continue to proliferate within it. Phox2b is obligatorily expressed within the bowel. Because Sox10 and Phox2b expression are required by early precursors, the entire gut becomes aganglionic (red capped lines) when they are deleted. The activation of Ret by GDNF/GFRα is not required for ganglia to form in the esophagus and adjacent stomach but is essential for gangliogenesis in the remainder of the bowel. Knockout of Ret/GDNF/GFRα, therefore, allows enteric ganglia to form in the esophagus but causes aganglionosis distal to the cardiac stomach. In contrast to Ret, esophageal gangliogenesis is Ascl1-dependent. At a later stage, Hand2 is required for the terminal differentiation of enteric neurons, but Ascl1 is not required for the colonization of the gut by ENCDCs or the early manifestation of some neural markers. Ascl1 is again required for the development of transiently catecholaminergic (TC) cells and the early-born serotonergic neurons that develop from them. Late-born neurons are not derived from TC precursors and are Ascl1-independent. Glia arise relatively late in development from a common glial/neuronal precursor and Notch signaling [108] has been implicated in glial differentiation. **(b)** Genes and/or other markers enable many of the putative progenitor stages of enteric neuronal development express to be recognized. These markers are indicated in callout clouds linked to their associated cells. Note that a common Ret/GDNF/GFRα-dependent progenitor gives rise to committed lineages of enteric neurons and glia, which can be distinguished. Cells in the neuronal lineage express Ret, Phox2b and the neuronal markers, PGP9.5 (protein gene product 9.5, a ubiquitin hydrolase that is present in all neurons), Gap43 and TuJ1, but they downregulate Sox10 and do not yet express Hu. Cells in the glial lineage express Sox10 and the glial marker, B-FABP, but they downregulate Phox2b and Ret. Differentiated glia express Sox10 and the markers glial fibrillary acidic protein (GFAP) and S100β. After a Hand2-dependent step, neuronal progenitors acquire Hu. Expression of the catecholamine biosynthetic enzymes, TH and DBH, allow TC cell precursors to be recognized. Serotonergic neurons, which are derived from them, synthesize serotonin (5-HT). A variety of markers are found in the Ascl1-independent neurons that do not arise from TC cell progenitors. The other line of terminally differentiated neurons is late-born and *Ascl1*-independent and arises from precursors that are never TC. Neurons in this group include those containing calcitonin gene related peptide (CGRP), substance P and vasoactive intestinal peptide (VIP). Some NOS-containing neurons and those expressing the vesicular acetylcholine transporter (V-AchT) are among this group. Cholinergic neurons are a heterogeneous class because >70% of enteric neurons are cholinergic. The glial lineage diverges from the neuronal after E14–E15 in the mouse. Brain fatty acid binding protein (B-FABP) is an early glia marker. Sox10 expression is retained in glia, which lose expression of Phox2b.