Perspective

Neural crest cells and the mesentery

Rishabh Sehgal

Department of Colorectal Surgery, University Hospital Limerick, Limerick, Ireland

Correspondence to: Rishabh Sehgal. Department of Colorectal Surgery, University Hospital Limerick, Limerick, Ireland. Email: rishsehgal@gmail.com.

Abstract: The enteric nervous systems (ENS) in humans contain more neurons than the aggregate of all other peripheral ganglia and are responsible for orchestrating and maintaining all aspects of gastrointestinal (GI) homeostasis. The development of the ENS is a complex process derived from neural crest cells (NCCs) that commences predominantly at the vagal level of the neural tube and ends in the distal hindgut. The traditional model of enteric neural crest cells (ENCCs) migrating in a unidirectional (rostral-to-caudal) fashion within the gut mesenchyme to colonize the entire length of the gut has recently come into question with an alternative trans-mesenteric migratory model. Errors in migrations of ENCCs lead to the development of several congenital and acquired disorders of the digestive tract. Novel stem cell tissue-engineering approaches provide an exciting avenue in future treatments of enteric neuropathies.

Keywords: Enteric nervous systems (ENS); neural crest cells (NCC); trans-mesenteric migration; stem cells; tissue-engineering

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We often are told to follow our "gut feeling or gut instinct" when faced with making a difficult decision. When we become stressed or anxious it is not uncommon to have that "sinking feeling in our guts". It has long been recognized that a person's emotional state can alter the function of the gastrointestinal (GI) tract. This brain-gut axis is linked by an extensive network of neurons, neurotransmitters and hormones that communicate between the two poles providing constant status updates. In fact it is the enteric nervous system (ENS) within the intestinal wall that is responsible for orchestrating and maintaining all aspects of GI homeostasis. The ENS has been described as the second brain due to the fact that although it normally communicates with the central nervous system (CNS) through the parasympathetic (via the vagus nerve) and sympathetic (via the prevertebral ganglia) nervous systems, the ENS also has an innate capability to function autonomously independent of inputs from the brain or spinal cord (1-3).

The human ENS is comprised of more neurons than the entire peripheral nervous system put together (3). These neurons are elegantly organized into microcircuits that encompass four major classes of neurons: motor neurons, sensory intrinsic primary afferent neurons (IPANs), intestinofugal neurons, and interneurons (4). The majority of ganglia in the ENS are located and organized within two plexuses, the myenteric and submucosal plexuses. The myenteric (Auerbach's) plexus is located between the circular and longitudinal muscle layers and is distributed from the level of the upper oesophagus to the internal anal sphincter. It is primarily responsible for the peristaltic movements of the GI tract. In contrast, the submucosal plexus (Meissner's plexus) is responsible mostly for sensing the environment within the gut lumen, regulating GI blood flow and controlling epithelial cell function. In regions where these functions are limited, such as the oesophagus, the submucous plexus is sparse and may be absent (3-5).

Given the pivotal role of the ENS in regulating key functions of the GI tract (secretion, motility, mucosal maintenance and immunological defence) it is not surprising to note that abnormalities of the ENS can lead to serious congenital and acquired digestive disorders. Hirschsprung's disease is one of the most studied congenital neuropathies affecting 1 in 5,000 neonates (6). It is characterized by the absence of enteric ganglia

(cluster of neurons and glia) along variable lengths of the intestine (6). The resulting aganglionosis leads to severe intestinal obstruction necessitating surgical resection of the affected bowel segment. Furthermore these patients often experience recurrent bouts of enterocolitis and dysmotility (1,2,6). Work done in understanding the pathophysiology of Hirschsprung's disease (2,6) and recent clarification in the mesenteric organ (7-10) has provided insights into the migratory ability of neural crest cells and the development of the ENS. These data could lead to the development of stem cell based therapies for the treatment of neurointestinal diseases.

The neural crest was discovered by the Swiss embryologist, Wilhelm His in 1868 while studying neurula-stage chick embryos in 1868 (11). Work since has demonstrated the ENS to be derived from a highly specialised multipotent and migratory cell type known as the neural crest cell (NCC). The ENS predominantly originates from the vagal neural crest, an area between the brain and the spinal cord (postotic hindbrain adjacent to somites 1–7) (1,5,12,13). From here several cell groups follow various paths of migration. One group of NCCs migrate dorsolaterally under the ectoderm to colonize the pharyngeal arches and the cardiac outflow tract. A second group follow a ventral path to form the sympathetic dorsal root ganglia and another group of NCCs enter the proximal foregut to give rise to the ENS (1,5,12,13). Each lineage of NCCs commits to its path influenced by the surrounding mesenchyme. For example, NCCs exposed to retinoic acid (RA) interact via their RA receptors (α and γ) to produce a protein important in ENS development known as receptor tyrosine kinase, RET (14-17). Niederreither *et al.* demonstrated agenesis of the ENS in mice lacking an enzyme involved in RA production, retinaldehyde dehydrogenase (RALDH2) (18).

Once intrinsic ENS NCCs reach the foregut they are referred to as enteric neural crest-derived cells (ENCCs). ENCCs chronologically arrive at various regions of the developing GI tract at specific time intervals: the stomach at week 4, caecal region at week 6 and distal end of the hindgut at week 7 (1). ENCCs are initially located in the outer mesenchyme of the foregut and midgut as the smooth muscle has not yet differentiated. As they migrate towards the cecum smooth muscle begins to differentiate and ENCCs are positioned between the smooth muscle layer and serosa, where they will later lead to the myenteric ganglia. In the midgut, the myenteric ganglia travel inwards towards the epithelium to colonize the submucosal mesenchyme generating the submucosal plexus (Meissner's plexus) (1,2,19).

NCCs derived from the sacral neural crest (located caudal to somite 28 in chick embryos) contribute to the development of the hindgut ENS. Sacral NCCs follow a similar ventral pathway to vagal NCCs as they accumulate on either side of the cloaca and distal hindgut to form a pelvic plexus. Of note sacral NCCs enter the hindgut only after vagal ENCCs have arrived at that level. Vagal derived NCCs are highly migratory compared to sacral counterparts. This may relate to 4-fold over-expression of receptor kinase RET transcripts in vagal NCCs (20).

A substantial proportion of nerves innervating the hindgut originate from a unique NCC population that does not follow the pathways described above but rather utilize the mesentery to access their destination. Nishiyama *et al.* utilized time-lapse imaging analysis to capture an ENCC population migrating from the midgut to the hindgut via a mesenteric route (21). During the E10.5–11.5 period of mouse development, the gut tube coils to form a hairpin bend just rostral to the caecum resulting in midgut and hindgut mesentery being closely apposed. This morphological conformation allows a subset of ENCCs to directly access the hindgut from the midgut by crossing mesenteric regions. Trans-mesenteric migration is only possible during E10.5–11.5 as thereafter mesentery growth increases the distance between the mid- and hindgut. Trans-mesenteric migration of ENCCs is promoted by GDNF signalling which activates RET (22,23). ENCCs at this developmental stage express a RET co-receptor (GFRα1) that binds GDNF and acts as a long range chemoattractant for both emigration from midgut and incorporation into the hindgut (21-23). Once in the hindgut, trans-mesenteric ENCCs bind with circumflex ENCCs that arrive later and colonize the entirety of the hindgut. The authors propose trans-mesenteric ENCCs that cross the mesentery are the principal source of colonic ENS. This challenges the concept that ENCCs undergo a unimodal rostral-to-caudal migration within the gut mesenchyme to colonize the entire length of the gut. It is feasible that error during this process could be associated with the development of aganglionic segments due to failure of trans-mesenteric ENCCs and circumflex ENCCs to merge. This could provide an elegant histopathological basis for Hirschsprung's disease as failure of merging could lead to aganglionic segments (21-23).

Research has demonstrated incorporation of functional enteric neurons from human pluripotent stem cells (PSC) into aganglionic chick and murine models.

A similar cell based therapy could work in the clinical context. Workman *et al.* utilized embryonic and PSCs to generate human intestinal tissue containing a functional ENS (24). Their engineered organoid model contained NCCs forming complex ganglionic structures and interganglionic fibers that in turn resembled myenteric and submucosal neural plexuses (24). The cellular organization observed indicates that engineered tissue may contain the information for coordinated cell migration, proliferation, lineage commitment and plexus assemblage (24).

In addition to mutations in the gene encoding the tyrosine receptor RET, mutations in PHOX2B have been linked with complete aganglionosis of bowel in humans and mice (25-27). Workman *et al.* studied the effect of PHOX2B mutations on ENS development and devised a novel model to study genetic causes of neurointestinal diseases (24). The authors focused on engineering PSC-derived tissue with a view to generating functional intestinal tissue for potential transplantation into patients with short bowel syndrome. This study was the was one of the earliest to generate human-PSC-derived intestinal tissue with a functional ENS. This could provide an excellent model for studying motility disorders of the human GI tract (24).

In summary, development of the ENS is a complex process. The classical theory is that ENCCs undergo unimodal rostral-to-caudal migration within the gut mesenchyme to colonize the entire length of the gut. This theory is now being challenged with alternative models involving a *trans*-mesenteric migration of NCCs. Errors in migration of ENCCs lead to aganglionic gut segments which are the hallmark of Hirschsprung's disease. Novel stem cell tissue-engineering approaches provide an exciting avenue in future treatments of enteric neuropathies.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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