Development of the Enteric Nervous System

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Neural crest arises at the lateral edges of the neural plate

- Migrate either along dorsolateral (melanocytes) or ventral (ganglia, Schwann cells, etc.) pathways
- Developmental potential of crest cells is heterogeneous when they begin to migrate.
  - Some are committed, others are pluripotent

The neural crest form during neurulation but is transient

Migration of crest is timed in a rostro-caudal sequence

- Crest cells undergo an epithelial-mesenchymal transformation to delaminate and become migratory.
  - Lose junctional proteins and adhesion molecules

Crest-derived cells migrate preferentially through the rostral halves of somites

- Segmental pattern is the same as that later followed by spinal nerves.
- Gives rise to the segmentation of DRG.

There are many derivatives of the neural crest
Crest gives rise to DRG and sympathetic chain ganglia at almost all levels

Different Hox genes are expressed by neural crest cells migrating into branchial arches

The connective tissue of the head and neck is largely derived from the neural crest

- These are called "mesectoderm".
- Specific regions of the crest colonize specific regions of the head and neck

Sympathetic ganglia, the ENS, and the adrenal medulla arise from post-otic levels of the crest

- ENS: somites S1-7, 28-
  - Vagal
  - Sacral
- Sympathetic S6-
- Adrenal S18-24

- The cardiac outflow tract is also derived from the neural crest (called the cardiac crest; levels overlap with vagal crest)

Crest-derived ganglia in a chick embryo

- DRG ganglia are crest derivatives
- Some cranial ganglia are crest-derivatives
  - Some are mixed, placodes and crest
- Parasympathetic ganglia are crest-derived
- ENS is crest-derived.

Fates of crest cells varies with their axial level but developmental potential is broader

- Defined migratory pathways lead crest-derived cells to their ultimate destinations.
  - Cells may thus not realize their developmental potential
  - Fates are shaped by cues delivered in the migratory pathway or in the target organ
- All levels from mesencephalon and further caudal can give rise to sensory ganglia, sympathetic ganglia, and ENS, but only S1-7 and caudal to S28 do so in situ.
The ENS is a unique part of the nervous system

- Mediates behavior of gut in absence of input from CNS.
  - Most neurons not connected to CNS
- Lacks internal collagen
- Support from enteric glia
- Many neurons and many types of neuron
  - Every class of neurotransmitter found in CNS is also in ENS
  - More neurons than spinal cord
  - More neurons than remainder of PNS
  - Greatest phenotypic diversity in PNS

Crest cells are traced by using quail-chick interspecies chimeras

- Quail-chick interspecies chimeras reveal the migration pathways of crest-derived cells
  - Chick crest is removed before migration begins.
  - Replaced with a graft of quail crest.
  - Quail crest cells migrate in host.
  - Quail crest cells are stably marked by their distinctive nucleolus-associated heterochromatin.
  - Location of quail cells reveals destinations reached by migrating crest-derived cells.

The gut is colonized by precursors that migrate from the neural crest.

- Vagal level: whole gut. Anterior → posterior
- Truncal level: rostral foregut (Esophagus).
- Sacral level: postumbilical gut. Posterior → anterior

DiI-labeled sacral crest cells colonize the post-umbilical bowel

- DiI was injected into neural crest of a chick embryo caudal to somite 28.

Sacral crest contributes enteric neurons

- Demonstrated with quail chick-chimeras
  - NADPH-d (purple) marks enteric neurons
  - NADPH-d = NOS
  - QCPN (antibody to quail) marks quail cells
Microenvironmental signals determine the fates of crest-derived cells

- Signals from the environment received by crest cells regulate their:
  - migratory paths
  - proliferation
  - restriction of developmental potential
  - survival
  - formation of terminally differentiated derivatives.
- As crest-derived cells migrate they change:
  - cell surface receptors
  - intracellular transduction mechanisms.
- Postmigratory cells in the gut are thus different from their premigratory precursors in the neural crest.

Congenital aganglionosis causes pseudoobstruction

- Hirschspung’s disease results from aganglionosis of the terminal colon.
- Associated with the development of megacolon.
- Relatively common disease
  - 1/5000 births in general population
  - 1/500 births in Mennonites (due to inbreeding)
- Most commonly due to defect in RET > EDNRB.

Enteric neurons are Ret-dependent

- GDNF binds to GFRα1 and stimulates Ret.
- Mice that lack Ret (or GDNF or GFRα1) lack enteric neurons below the level of the esophagus.
- Loss of function mutations in RET, GDNF, or GFRα1 are associated with Hirschsprung’s disease.

Crest-derived cells are isolated by immunoselection.

- Ret is a receptor tyrosine kinase that is expressed in the gut only by crest-derived cells.
- Activated by ligands that bind to co-receptors.
- Ret stimulates proliferation early in development, is a chemottractant for migrating crest-derived cells, and supports survival.

The GDNF family of growth factors activate Ret

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Wild-type

Ret +/-
Neurons develop in cultures of isolated crest-derived cells.

- Precursors express nestin (as in CNS neuroepithelium)
- Neurons express PGP9.5 (a neuronal form of ubiquitin hydrolase).

GDNF is mitogenic and promotes neurogenesis at E12

- GDNF increases precursors (nestin) and neurons (peripherin)
- NT-3 affects neither.

GDNF attracts enteric crest-derived cells

- Cos cells expressing GDNF attract Ret expressing cells from gut explants.
- These cells give rise to neurons (Tuj1).

GDNF is express first in stomach then in cecum

- Ret-expressing crest-derived cells follow GDNF gradient, but how do they get past the cecum?

GDNF/GFRα1/Ret is required to

1. Expand the population of crest-derived émigrés sufficiently to colonize the gut.
   - Stimulates mitosis of early precursors.
2. Provide a chemoattractive force that directs the proximo-distal migration of crest-derived cells
   - But other factor must break in to limit the proliferation of precursor and allow them to escape the trap of the cecum where GDNF expression is highest.
3. If Ret is inadequate: the terminal bowel (last colonized) becomes aganglionic and Hirschsprung’s disease results.

Crest-derived cells require Edn3 (ET-3) and Ednrb (ET_B) to complete their colonization of the gut

- The endothelins are vasoactive peptides
  - edn1 (ET-1), edn2 (ET-2), edn3 (ET-3)
- Big endothelins are secreted and converted in tissues to active peptides by endothelin converting enzymes (1 and 2).
- There are 2 endothelin receptors.
- Edna (ET_A) and Ednrb (ET_B).
  - edn1 and edn2 stimulate both edn3 only activates Ednrb.
  - ENS development requires edn3 and ednrb.
HD10 - Development of the Enteric Nervous System

Ret and Ednrb interact in humans and in mice (mice tested to verify human data)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Mean length of aganglionosis (cm)</th>
<th>Range (cm)</th>
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<tbody>
<tr>
<td>Male</td>
<td>2.6</td>
<td>1.7-4.0</td>
</tr>
<tr>
<td>Female</td>
<td>1.0</td>
<td>0.2-2.0</td>
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</tbody>
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Megacolon occurs in mice that lack edn3 (ET-3)

The terminal colon of ET-3-deficient mice is aganglionic

- The aganglionic bowel is not denervated.
  - It contains large nerve trunks containing extrinsic axons and projections from the proximal hypoganglionic bowel.

Co-cultured sources of crest fail to colonize presumptive end3ls gut

- Donor neurons marked by AChE activity.
- Donor neurons enter wild-type mouse colon but not end3ls colon.

Presumptive aganglionic gut from end3ls mice cannot be entered by quail crest cells

- Mouse colon was grafted into a quail crest migration pathway.
- Crest is immunostained blue (HNK1).
- Mouse nuclei are different from those of quail, enabling a graft of mouse gut to be recognized in a quail host.

The terminal colon is normally colonized in end3ls <> WT chimeric mice

- Cells of WT mice have low and end3ls mice have high levels of β-glucuronidase.
- Crypts are clonal in origin.
- Neurons and connective tissue cells are either WT or end3ls.
- Edn3ls neurons are found in the terminal colon.
Edn3 inhibits the development of neurons from crest-derived precursors

- Edn3 effects are mimicked by the ETB agonist, IRL1620 and blocked by the antagonist BQ788, but neurons develop in the presence of BQ788. Edn3 is not required for neural development.

Exogenous Edn3 enables crest-derived cells to enter the terminal colon of Edn3-deficient mice

Exogenous ET-3 allows crest-derived cells to colonize the entire colon in vitro

Ectopic ganglia develop in the pelvis of endls mice

- Structure is that of peripheral nerve, not ENS.
- Thought to be derived from sacral crest cells that have stopped migrating before reaching the gut.

Specific transcription and growth factors define stages in ENS development

- The earlier a gene acts in development, the more massive the defect that follows its deletion
  - Genes that lead to complete aganglionosis when knocked out
    - Phox2b
    - Sox10
    - Ret/GDNF;GFRa1 (below esophagus)
  - Genes that lead to limited lesions when knocked out
    - Mash-1
    - Edn3/Ednrb
    - NTN/GFRa2
    - NT-3/TrkC
Genes associated with Hirschsprung’s disease

- Phox2b: Transcription factor expressed by the most primitive of the crest-derived cells that colonize the gut.
- Sox10: Transcription factor: required early in development.
- Ret, its co-receptors, and ligands: Receptor tyrosine kinase activated first by GDNF, and then NTN.
- EDN3 and EDNRB: collaborates with Ret and needed by non-crest-derived cells of colon
- SIF1: Encodes Smad protein, involved in BMP signaling

Crest-derived cells colonize the bowel and then migrate from the gut to the pancreas.

- Vagal crest-derived cells remain in the foregut while pancreatic buds form.
- At E13 they enter the pancreas.

Crest-derived cells migrate in the outer gut mesenchyme; the submucosal plexus forms secondarily.

- Vagal crest-derived cells migrate proximo-distally down the bowel in its outer mesenchyme.
- At E13 subsets of these cells migrate into the submucosa. This pattern is true for the entire bowel in the mouse and for the small intestine in the chick.

Netrins and their receptor, DCC are expressed in the developing gut and pancreas

- Netrins are secreted by GI mucosa and pancreatic exocrine cells.
- DCC is expressed by migrating crest-derived cells and by descending vagal sensory axons.

Crest-derived cells migrate from explants of bowel toward transfected cells expressing netrin-1.

- Enteric cells do not migrate toward control, non-transfected cells.
- Netrin-1 has a chemoattractive effect on crest-derived cells in the gut explant.

Crest-derived cells migrate toward cells that secrete netrin-1.

- Immunoselected E6 chick crest-derived cells were plated over a clump of HEK 293 Netrin-1 secreting cells and grow for 2 days
Crest-derived cells isolated from chick gut migrate toward co-cultured netrin-1-expressing cells.

- Stably transfected cells expressing netrin-1 were embedded in 3-D collagen gels. Immunoselected crest-derived cells were plated over the gels.
- Netrin-1 has a chemotactic effect on immunoselected enteric crest-derived cells in vitro.

Antibodies to DCC block the inward migration of crest-derived cells in chick gut explants.

- Crest-derived cells were identified with anti-HNK-1.
- Bar = 100 µm.

The pancreas of DCC -/- mice is aganglionic

- Neurons (PGP9.5- or acetylcholinesterase-labeled) were found in wt but not DCC -/- mice at P0 (g = gut; p = pancreas; bar = 100 µm).
- Netrin/DCC play an important role in directing the migration of crest-derived cells into submucosa and pancreatic bud in vivo.

Netrin/DCC is required to form submucosal and pancreatic plexuses

- DCC-expressing crest-derived cells migrate toward the netrin-secreting mucosa.
  - Stop when they encounter laminin (and other proteins?)
- DCC-expressing crest-derived cells migrate toward the netrin-secreting pancreas when the pass the pancreatic buds.
Summary & Conclusions

- The ENS is derived from a multipotent set of precursors that migrate to the bowel from the neural crest.
- Signals from the migratory and enteric microenvironments determine the fates of the crest-derived ENS precursors.
- Developmental potential is restricted and commitment increases as development proceeds.
  - Stages in development can be recognized by the dependence of cells on a succession of essential transcription factors, growth factors and their receptors.
    - Early factors include Phox2b, Sox10, Ret/GFRα1/GDNF
    - Later factors include Mash-1, EDNRB/EDN3, NT-3/TrkC
- Guidance molecules are needed to colonize the gut and form submucosal and pancreatic plexuses.