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POREKLO ĆELIJA ENTERIČKOG NERVNOG SISTEMA I PUTEVI MIGRACIJE TOKOM EMBRIONALNOG RAZVOJA

Miloš Đuknić[1], Nela Puškaš[2], Milica Labudović Borović[2], Radmila Janković[3*]

[1] Univerzitet u Beogradu, Medicinski fakultet, Republika Srbija
[2] Univerzitet u Beogradu, Medicinski fakultet, Institut za histologiju i embriologiju „Prof. dr Aleksandar Đ. Kostić“, Beograd, Republika Srbija
[3]Univerzitet u Beogradu, Medicinski fakultet, Institut za patologiju „Prof. dr Đorđe Joannović“, Beograd, Republika Srbija

[*] Korespondencija: radmila.jankovic011@gmail.com

SAŽETAK

Enterički nervni sistem (ENS) predstavljen je kompleksnom mrežom neurona, glijalnih i drugih ćelija unutar zida digestivne cevi. ENS ostvaruje brojne, vitalno važne funkcije u našem organizmu. Tako, ENS reguliše motilitet digestivnog trakta, sekreciju u lumen creva, razmenu tečnosti i elektrolita kroz sluznicu, kao i perfuziju sluznice. Da bi pravilno funkcionisao i ostvarivao ove važne funkcije, neophodan je pravilan embrionalni razvoj ENS. Ćelije ENS nastaju od prekursorskih ćelija poreklom od nervnog grebena. Dve populacije koje doprinose najvećem broju budućih ćelija ENS jesu ćelije vagalnog i sakralnog dela nervnog grebena. Prekursorske ćelije vagalnog dela nervnog grebena ulaze u primitivno crevo u regionu budućeg jednjaka (prednje crevo) i započinju svoju migraciju, preko srednjeg, ka zadnjem crevu, odnosno ka budućem analnom otvoru. Ćelije sakralnog dela nervnog grebena ulaze u region zadnjeg creva prateći ekstrinzička nervna vlakna i nastavljaju svoju migraciju rostralno, ka ćelijama poreklom od vagalnog dela nervnog grebena. Uporedo sa procesom migracije, prekursorske ćelije prolaze i kroz druge važne procese, kao što su proliferacija, neuro-glijalna diferencijacija, gangliogeneza i stvaranje aksonskih puteva, kao i sinaptogeneza. Svi ovi procesi strogo su regulisani brojnim signalnim putevima, od kojih se mnogi još uvek aktivno istražuju. Savremena dostignuća u nauci omogućila su praćenja pojedinačnih ćelija na razvojnom putu i druge metode u istraživanju koje će značajno doprineti razumevanju embrionalnog razvoja ENS. Ovo može imati reperkusije u poboljšanju dijagnostike i terapije razvojnih (npr. Hiršprungova bolest) i drugih poremećaja ENS, ali i oboljenja u kojima disfunkcija ENS značajno doprinosi patogenezi.

Ključne reči: enterički nervni sistem, embrionalni razvoj, ćelijska migracija, Hiršprungova bolest

Uvod

Enterički nervni sistem (ENS) predstavljen je kompleksnom mrežom ćelija unutar digestivnog trakta. U njegovoj izgradnji učestvuje oko 400-600 miliona neurona i, pretpostavlja se, još veći broj glijalnih i drugih ćelija, što ga čini najvećim delom perifernog nervnog sistema (1). Neuroni i glija ćelije ENS formiraju sitne ganglije koje su međusobno povezane gradeći nervne pleksuse. Dva glavna nervna pleksusa, submukozni (Majsnerov) i mijenterički (Auerbahov), smešteni su u samom zidu digestivne cevi. Submukozni pleksus, kao što mu ime govori, nalazi se u submukozi, dok se mijenterički pleksus nalazi između longitudinalnog i cirkularnog mišićnog sloja zida digestivne cevi (slika 1) (2). Za razliku od submukoznog pleksusa, koji nedostaje u jednjaku, mijenterički pleksus prisutan je čitavom dužinom digestivnog trakta (2,3). Svi neuroni ENS, tokom međusobnih interakcija i primanja spoljašnjih signala, pre svega od drugih delova nervnog sistema, ostvaruju brojne, vitalno važne funkcije u našem organizmu. Tako ENS reguliše motilitet digestivnog trakta, sekreciju u lumen creva, razmenu tečnosti i elektrolita kroz sluznicu, kao i perfuziju sluznice (1,2,4). ENS je sposoban da neke od ovih funkcija obavlja potpuno autonomno, refleksnim lukovima koji su u celini

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ORIGIN OF ENTERIC NERVOUS SYSTEM CELLS AND MIGRATION PATHWAYS DURING EMBRYONIC DEVELOPMENT

Milos Djuknic[1], Nela Puskas[2], Milica Labudovic Borovic[2], Radmila Jankovic[3*]

[1] University of Belgrade, Faculty of Medicine, Belgrade, Republic of Serbia
[2] University of Belgrade, Faculty of Medicine, Institute of Histology and Embryology „Prof. dr Aleksandar Đ. Kostić", Belgrade, Republic of Serbia
[3] University of Belgrade, Faculty of Medicine, Institute of Pathology „Prof. dr Đordje Joannović", Belgrade, Republic of Serbia

[*] Correspondence: radmila.jankovic011@gmail.com

SUMMARY

The enteric nervous system (ENS) is represented by a complex network of neurons, glial and other cells within the wall of the digestive tract. ENS is responsible for numerous, vital functions in our body. Thus, ENS regulates motility of the digestive tract, secretion into the intestinal lumen, exchange of fluid and electrolytes through the mucosa, as well as mucosal perfusion. In order to perform these important functions, proper embryonic development of ENS is necessary. ENS cells are derived from precursor cells of the neural crest (NCCs – neural crest cells). Two cell populations that contribute to the largest number of future ENS cells are the vagal and sacral NCCs. Vagal NCCs enter the primitive gut tube in the region of the future esophagus (foregut), and begin their migration, through the midgut towards the hindgut and the future anal region. Sacral NCCs enter the hindgut region following the extrinsic nerve fibers and continue their migration rostrally, towards vagal NCCs. Along with the migration process, these cells undergo other important processes, such as proliferation, neuro-glial differentiation, gangliogenesis, axonal pathway formation and synaptogenesis. All these processes are strictly regulated by numerous signaling pathways, which are still being actively researched. Modern lineage tracing and other technologies, that enabledfollowing of individual precursor cells through their development pathways, will significantly contribute to the better understanding of development of ENS. This may have repercussions in improving the diagnosis and treatment of some developmental (Hirschsprung disease) and other ENS disorders.

Keywords: enteric nervous system, embryonic development, cell migration, Hirschsprung disease

Introduction

The enteric nervous system (ENS) is represented by a complex network of cells within the digestive tract. Around 400-600 million neurons take part in its development, and even a greater number of glial and other cells, as it is assumed, which makes it the largest part of the peripheral nervous system (1). Neurons and glial cells of the ENS form tiny ganglia that are interconnected, thus creating nerve plexuses. Two main nerve plexuses, the submucous plexus (Meissner's plexus) and myenteric plexus (Auerbach's), are located within the wall of the digestive tract. The submucous plexus, as its name implies, is located in the submucosa while the myenteric plexus is situated between the longitudinal and circular muscle layer in the digestive tract (Figure 1) (2). In contrast to the submucous plexus, which is not found in the esophagus, the myenteric plexus extends the entire length of the digestive tract (2,3). All neurons of the ENS, with their mutual interactions and extrinsic inputs, primarily from other parts of the nervous system, regulate numerous, vital functions in our body. Thus, the ENS regulates the motility of the digestive tract, the secretion into the intestinal lumen, the exchange of fluids and electrolytes through the mucosa, as well as mucosal perfusion (1,2,4). The ENS is able to perform some of these functions completely independently by reflex arcs,

unutar ENS (5). Zbog svoje kompleksne građe i autonomnog ostvarivanja svojih funkcija, ENS se često naziva i „drugim mozgom“ (6).

Da bi pravilno funkcionisao i ostvarivao životno važne funkcije za naš organizam, neophodan je pravilan embrionalni razvoj ENS. Tokom razvoja, prekursorske ćelije ENS različitog porekla se mobilišu i precizno utvrđenim putanjama migriraju ka ciljnom mestu gde će se diferenovati u neurone ili glijalne ćelije (2). Svi procesi tokom embrionalnog razvoja ENS strogo su kontrolisani brojnim signalnim putevima koji se još uvek aktivno izučavaju, pre svega sa ciljem poboljšanja dijagnostike (7) i eventualne modifikacije njihove aktivnosti kod različitih razvojnih poremećaja ENS, kao što je Hiršprungova bolest (8).

Cilj ovog istraživanja je sveobuhvatni prikaz dosadašnjeg znanja o pravilnom embrionalnom razvoju ENS i potencijalnim kritičnim tačkama za razvoj nekih patoloških stanja. Nekoliko skorašnjih preglednih radova se, takođe, sveobuhvatno i detaljno bavilo ovom tematikom (9-13).

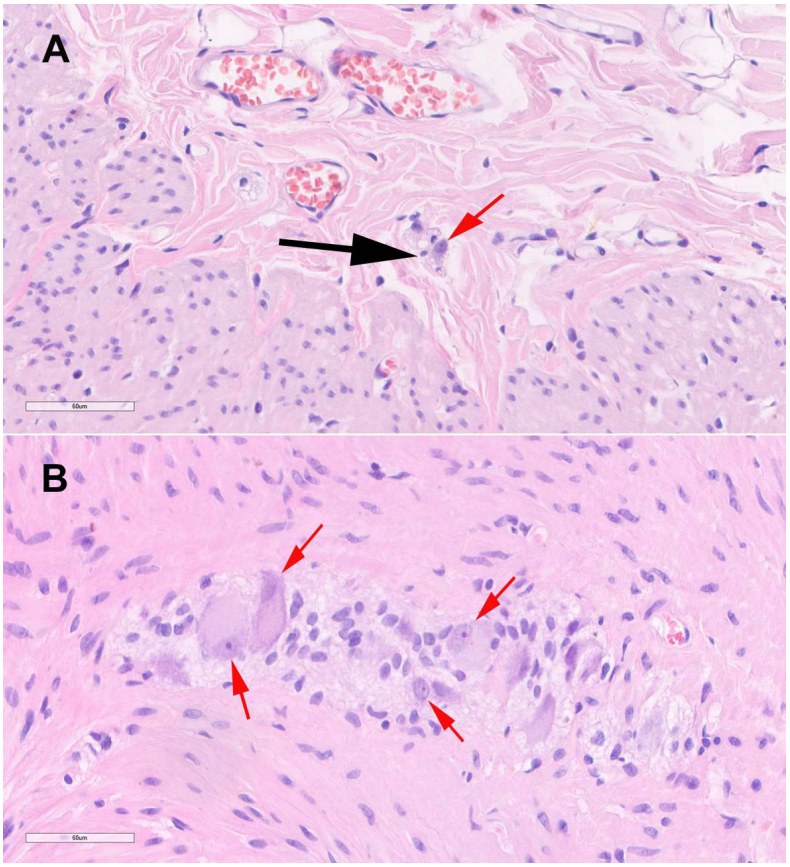
Metode

U ovom preglednom radu, radi što preciznijeg i sveobuhvatnijeg prikaza embrionalnog razvoja ENS, korišćena je literatura dobijena pretraživanjem MEDLINE baze podataka uz pomoć servisa PUBMED. Literatura objavljena na engleskom jeziku, u poslednjih 10 godina, dobijena je pretraživanjem sledećih ključnih reči: enterički nervni sistem, embrionalni razvoj, ćelijska migracija, Hiršprungova bolest.

Poreklo ćelija ENS

Tradicionalno se za proučavanje embrionalnog razvoja ENS koriste animalni modeli, i to mišji model, pileći embrion, zebrice i dr. U poslednje vreme sve više se istražuje i na humanom materijalu, a razvijaju se i različiti *in silico* modeli ENS.

Kao i gotovo svi drugi delovi perifernog nervnog sistema, i ćelije ENS nastaju od prekursorskih ćelija nervnog grebena (NCC – *neural crest cells*). Ovo je potvrđeno studijom Inteme i Hamonda još 1954. godine, i sve buduće studije se oslanjaju



Slika 1. Mikroskopski izgled ganglija enteričkih nervnih pleksusa (submukoznog i mijenteričkog) (HE, x1000).

Submukozne ganglije su sitne (crna strelica), sadrže mali broj ganglijskih ćelija (crvena strelica) (A), dok su mijenteričke ganglije krupnije i sadrže veći broj krupnijih ganglijskih ćelija sa vezikularnim jedrom i uočljivim jedarcetom (crvene strelice) (B).

which are completely within the ENS (5). Due to its complex structure and autonomy of its functions, the ENS is often called the "second brain" (6).

In order to function properly and perform these vital functions, the proper embryonic development of ENS is necessary. During its development, the precursor cells of the ENS, which have different origin, are mobilized and they migrate along the precisely established pathways to the target place where they will differentiate into neurons or glial cells (2). All these processes during the embryonic development are strictly controlled by numerous signaling pathways, which are still being actively researched, mostly in order to improve diagnostics (7) and possible modifications of their activities in different developmental disorders of the ENS, such as Hirschsprung disease (8).

The aim of this research was to present the comprehensive review of current knowledge about the proper embryonic development of ENS and potentially critical points for the development of some pathological conditions. Several recent review articles have dealt with this subject topic in a comprehensive and detailed way. (9-13).

Methods

In this review article, in order to provide a comprehensive and precise review of the embryonic development of ENS, we used literature from the MEDLINE database and the search was performed using the PubMed service. The literature in the English language that has been published in the last 10 years was obtained by searching the following key words: enteric nervous system, embryonic development, cell migration, Hirschsprung disease.

The origin of ENS cells

Traditionally, animal models are used to study the embryonic development of ENS, particularly mice, chicken embryo, zebrafish etc. The human material is being researched more and more, and different *in silico* models of ENS are being developed.

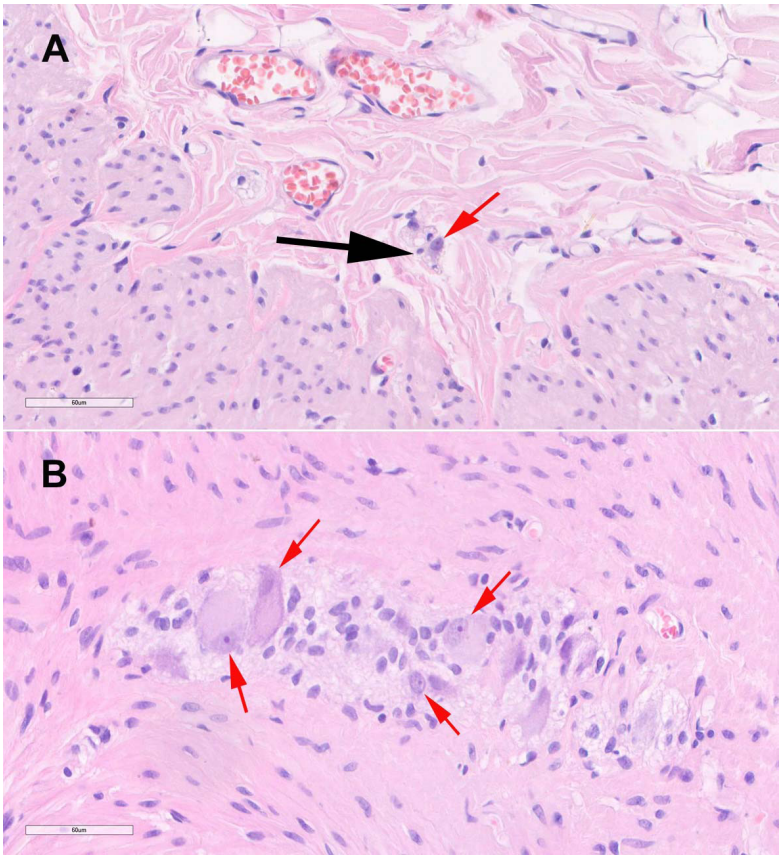
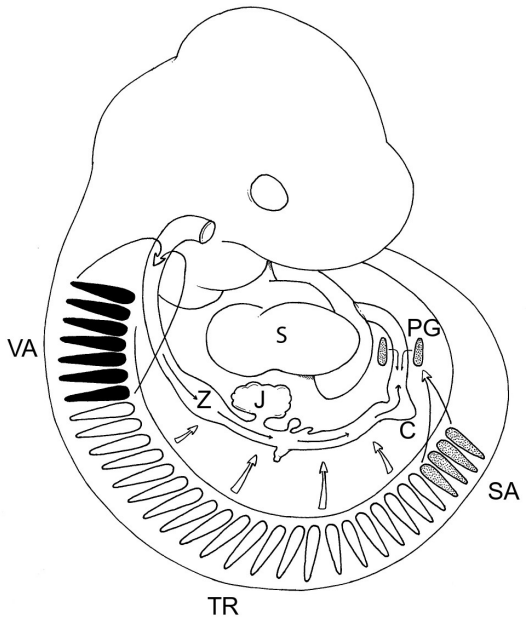


Figure 1. The microscopic view of ganglia of enteric nerve plexuses (submucosal and myenteric) (H&E, x1000).

Submucosal ganglia are small (black arrow), and contain a small number of ganglion cells (red arrow) (A), while myenteric ganglia are larger and contain larger number of larger ganglion cells with vesicular nuclei and prominent nucleoli (red arrows) (B).

na nju kada je u pitanju pomenuta hipoteza (14). Savremene studije, u kojima je vršeno praćenje ćelija poreklom nervnog grebena duž njihove migratorne putanje (*lineage tracing*), potvrdile su da ove ćelije učestvuju u izgradnji ENS (15). Nervni greben je privremena embrionalna struktura, koja se odvaja od nervne ploče prilikom zatvaranja u nervnu cev i pozicionira dorzalno u odnosu na nju. Pored perifernog nervnog sistema, tokom razvoja, ona daje i druge važne strukture kao što su vezivna tkiva glave i vrata, melanociti, srž nadbubrežnih žlezda, itd (16). U pomenutim istraživanjima (14,15) pokazano je, takođe, da najveći broj ćelija ENS vodi poreklo od vagalnog dela nervnog grebena, u nivou od 1-7. somita. Međutim, ne doprinose sve ćelije vagalnog dela nervnog grebena podjednako formiranju ENS. Smatra se da ćelije u nivou trećeg i četvrtog somita daju najveći broj neurona (17). Ove ćelije ulaze u primitivno crevo u regionu budućeg jednjaka i započinju svoju migraciju kaudalno ka budućem analnom otvoru. Ona se odvija uporedo sa rastom creva, tako da migratorna putanja postaje sve duža tokom same migracije, pa se smatra da ove ćelije prelaze najveću putanju od svih embrionalnih ćelija našeg organizma (17,18). Druga važna populacija prekursorskih ćelija ENS jesu ćelije sakralnog dela nervnog grebena, kaudalno od 28. somita kod pilećeg embriona. Ove ćelije migriraju ventromedijalno, daju prekursore budućih ganglija pelvičnog pleksusa, a zatim ulaze u region zadnjeg creva prateći ekstrinzička nervna vlakna i nastavljaju svoju migraciju rostralno, ka ćelijama poreklom od vagalnog dela nervnog grebena (slika 2) (19). Ovakva tradicionalna slika porekla ENS danas se dopunjuje novim saznanjima koja ukazuju da jedan deo ćelija ENS vodi poreklo od prekursorskih Švanovih ćelija, koje migriraju u zid creva duž nervosa vagusa (region jednjaka i želuca), odnosno drugih ekstrinzičkih nervnih vlakana (ostatak digestivnog trakta) (20), kao i da prekursorske ćelije u regionu pankreasa (endodermalnog porekla) malim delom doprinose raznovrsnosti porekla ENS (21).



Slika 2. Poreklo prekursorskih ćelija enteričkog nervnog sistema i njihovi putevi migracije tokom embrionalnog razvoja.

Najveći broj ćelija enteričkog nervnog sistema potiče od ćelija vagalnog dela nervnog grebena (VA). Preostale ćelije potiču iz sakralnog dela nervnog grebena (SA), dok mali broj potiče od ćelija nervnog grebena regiona trupa (TR), kao i prekursorskih ćelija regiona pankreasa. Ćelije vagalnog dela nervnog grebena nakon ulaska u region prednjeg creva započinju migraciju u kaudalnom smeru. Suprotno od njih, ćelije sakralnog dela nervnog grebena prvenstveno učestvuju u izgradnji prekursorapelvičnih ganglija (PG), a zatim ulaze u region zadnjeg creva i započinju migraciju u rostralnom smeru. Prekursorske ćelije regiona trupa, kao prekursoriŠvanovih ćelija, migriraju u zid creva duž ekstrinzičkih nervnih vlakana. Smerovi migracije prikazani su strelicama. S – srce, Z – želudac, J – jetra, C – cekum.

Migracija, proliferacija, diferencijacija

Da bi se pravilno formirao ENS, ćelije prekursori prolaze kroz važne procese kao što su migracija, proliferacija, neuro-glijalna diferencijacija, stvaranje ganglija i aksonskih puteva, kao i sinaptogeneza (11). Ovi procesi se odigravaju u različitom periodu embrionalnog razvoja kod različitih vrsta, međutim, ono što je zajedničko, jeste da se ove faze međusobno u velikoj meri preklapaju. Na primer, kada prekursorske ćelije poreklom od nervnog grebena uđu u region prednjeg creva i započnu kaudalnu migraciju, jedan deo ćelija će se zaustaviti ranije i započeti diferencijaciju, dok će ostale ćelije, koje neprestano proliferišu, nastaviti migraciju kaudalno kako bi naselile čitavo crevo (13).

Ćelije vagalnog dela nervnog grebena u nivou prvog i drugog somita započinju svoj drugi talas migracije ka mezenhimu ventralnog dela somita (sklerotom). Prethodno se u toku prvog talasa migracije odvajaju ćelije koje naseljavaju škržne lukove i region izlaznog trakta srca (17). Migracija ka sklerotomu somita je delom posledica odbojnog dejstva semaforina-3F koji eksprimiraju ćelije zad-

As all other parts of the peripheral nervous system, ENS cells are derived from precursor cells of the neural crest (NCCs – neural crest cells). This was confirmed by the study of Yntema and Hammond back in 1954 and all future studies relied on it in regard to the above-mentioned hypothesis (14). Contemporary studies, in which NCCs have been observed along their migratory pathway (*lineage tracing*), have confirmed that these cells take part in the formation of ENS (15). The neural crest is a temporary embryonic structure, which arises from the neural plate when the neural tube closes and it is positioned along the dorsal side of the neural tube. Besides the peripheral nervous system, during its development, it gives rise to other important structures such as connective tissues of head and neck, melanocytes, and the marrow of adrenal glands, etc (16). In the mentioned research (14,15), it has been shown that the majority of ENS cells originate from the vagal part of the neural crest, region adjacent to somite 1-7. However, not all vagal NCCs contribute equally to the formation of ENS. It is deemed that cells at the level of somites three and four give the largest number of neurons (17). These cells enter the primitive gut tube in the region of future esophagus and begin their migration caudally towards the future anal region. This occurs along with the intestine's growth, and therefore the migratory path becomes longer during migration. Therefore, it is thought that these cells pass the longest path of all embryonic cells of our organism (17,18). Another important population of ENS precursor cells are sacral NCCs, caudal from the 28th somite in the chicken embryo. These cells migrate ventromedially, give precursors of future ganglia of the pelvic plexus, and then enter the hindgut region following the extrinsic nerve fibers and continue their migration rostrally towards vagal NCCs (Figure 2) (19). This traditional picture of the origin of ENS is complemented by new knowledge that indicates that one part of the ENS cells originate from Schwann cells precursors (SCP), which migrate into the intestinal wall along vagus nerve (the region of esophagus and stomach), and other extrinsic nerve fibers (the rest of the digestive tract) (20), as well as that precursor cells in the pancreatic region (endodermal origin) contribute, to a small extent, to the heterogeneity of ENS origin (21).

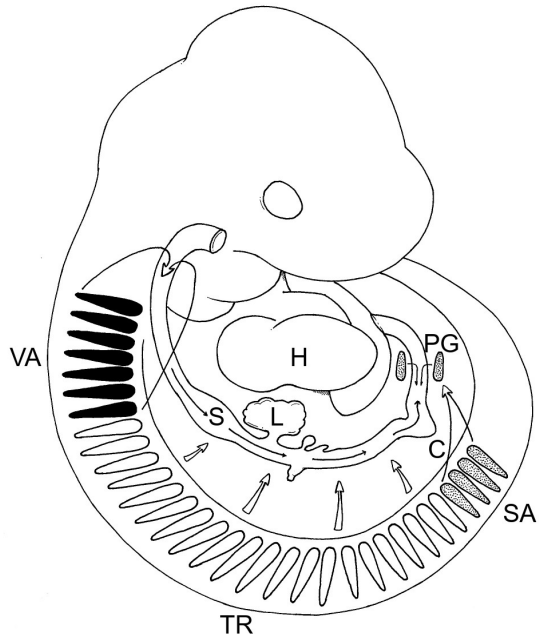


Figure 2. The origin of precursor cells of the enteric nervous system and their migration pathways during embryonic development.

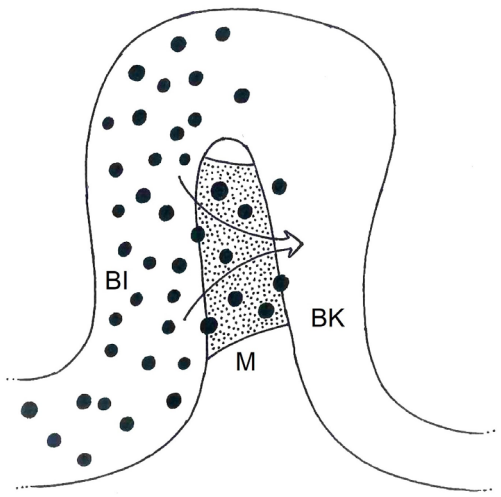
The majority of enteric nervous system precursor cells are derived from the vagal neural crest (VA). Remaining cells originate from the sacral neural crest (SA), the trunk neural crest (TR) and the pancreatic precursor cells. Vagal neural crest cells enter the foregut region and continue their migration caudally. In contrast, sacral neural crest cells primarily form the precursors of the pelvic ganglia (PG), then enter the hindgut region and begin to migrate in the rostral direction. Trunk neural crest cells, as Schwann cell precursors, migrate into the gut wall along extrinsic nerve fibers. The migration pathways are shown by arrows. H – heart, S – stomach, L – liver, C- cecum.

Migration, proliferation and differentiation

Precursor cells pass through important processes such as migration, proliferation, neuro-glial differentiation, creation of ganglia and axonal pathways, as well as synaptogenesis so that the ENS would develop properly (11). These processes unfold in different periods of embryonic development in different species, however, what they have in common is the fact that these stages are mutually interconnected. For example, when NCCs enter the foregut region and begin caudal migration, one part of these cells will be stopped earlier and start differentiation, while other cells, which proliferate all the time, will continue migration caudally in order to colonize the whole gut (13).

njeg dela somita (dermatomiotom), a koji se vezuje za neuropilin-2 receptor na ćelijama nervnog grebena (22). Jedna podgrupa ovih ćelija odvaja se i putuje ka budućem srcu, učestvujući dodatno u izgradnji izlaznog trakta. Smatra se da ovu grupu ćelija koja eksprimira CXCR4 (*CXC chemokine receptor 4*) privlači ligand SDF1 (*stromal cell-derived factor 1*) koji eksprimiraju embrionalne ćelije srca (23). Druga podgrupa daje prekursorske Švanove ćelije koje putuju duž vagusnih nervnih vlakana do jednjaka i želuca i učestvuju u izgradnji ENS ovih delova digestivnog trakta (20).

Ćelije vagalnog dela nervnog grebena u nivou 3-7. somita, koje će dati najveći deo ENS započinju migraciju ka proksimalnom delu prednjeg creva i ulaze u region budućeg jednjaka oko četvrte nedelje gestacije kod čoveka (24), a u mišjem modelu između devetog i desetog dana embrionalnog razvoja (25). Od ovog trenutka, smatra se da su opredeljene za izgradnju ENS pa se nazivaju enteričke ćelije poreklom od nervnog grebena (ENCC – *enteric neural crest-derived cells*) (9). Ove ćelije svoj put nastavljaju u kaudalnom smeru, ka budućem analnom otvoru. Prvenstveno migriraju duž prednjeg (od jednjaka do nishodnog dela dvanaestopalačnog creva u nivou buduće Vaterove papile) i srednjeg creva (od Vaterove papile sve do kraja ⅔ transverzalnog dela debelog creva) (10,11). Primećeno je da jedna subpopulacija migrirajućih ćelija zaobilazi slepo crevo, prelazeći direktno iz proksimalnog u distalni deo srednjeg creva i zadnje crevo preko mezenterijuma, koji prolazno povezuje ove delove creva, odnosno, dovodi ih u neposrednu blizinu. Transmezenterična migracija, kako je nazvana, doprinosi značajnom broju ćelija ENS budućeg debelog creva (slika 3) (26). Na daljem putu kroz zadnje crevo vagalne ENCC susrešće ćelije poreklom od sakralnog dela nervnog grebena, koje migriraju rostralno, i ubrzo završiti svoju longitudinalnu migraciju. U mišjem modelu, čitavo crevo kolonizovano je do 14. embrionalnog dana (25), dok kod čoveka, najverovatnije, do 7. nedelje embrionalnog razvoja (24).



Slika 3. Transmezenterična migracija prekursorskih ćelija enteričkog nervnog sistema.

Prilikom migracije, jedan deo prekursorskih ćelija enteričkog nervnog sistema, prelazi direktno iz regiona budućeg ileuma u region budućeg kolona preko mezenterijuma, zaobilazeći region slepog creva. Crne tačke označavaju prekursorske ćelije poreklom od nervnog grebena koje migriraju. Strelica pokazuje pravac i smer transmezenterične migracije.
BI – budući ileum, M – mezenterijum, BK – budući kolon.

Ćelije nervnog grebena ispod nivoa 7. somita (region trupa) ne ulaze u region prednjeg creva. Pretpostavlja se da mezenhimne ćelije prednjeg creva produkuju određene molekule, kao što je Slit1, koji, vezujući se za Robo receptor eksprimiran na NCC regiona trupa, sprečava migraciju ovih ćelija ka prednjem crevu. Robo receptor nije eksprimiran na vagalnim NCC (27). Stvarajući prekursore Švanovih ćelija, ove ćelije migriraju duž ekstrinzičkih nervnih vlakana, i u manjoj meri doprinose ukupnom broju ćelija ENS (20).

Grupa sakralnih ćelija nervnog grebena koja učestvuje u izgradnji ENS, kao što je rečeno, migrira ventralno ka distalnom delu zadnjeg creva. Nakon što izgrade mrežu ćelija od koje će se formirati pelvični pleksus, ove prekursorske ćelije ulaze u region zadnjeg creva duž ekstrinzičkih nervnih vlakana, gde će dati neurone i glija ćelije ENS ovog regiona. Njihova kratka migracija usmerena je rostralno (19). Iako daju morfološki i funkcionalno identične nervne i glijalne ćelije, vagalne i sakralne ENCC se u mnogo čemu razlikuju (28). Pored suprotnog smera migracije i različite lokalizacije primećene su još neke značajne razlike. Kada se vagalne ENCC implantiraju u sakralni region, one migriraju znatno ranije, u većem broju i proksimalnije od sakralnih ENCC. Pri tome pravac migracije je rostralni, kao i kod sakralnih ENCC. Zaključuje se da vagalne ENCC imaju znatno veći migratorni potencijal od sakralnih, kao i to da smer migracije

Vagal NCCs at the level of the first and the second somites begin their second wave of migration towards the mesenchyme of the ventral part of somites (sclerotome). Previously, during the first wave of migration, cells proliferate and colonize the gill's arches and region of the cardiac outflow tract (17). Migration towards the somite sclerotome is partly the consequence of the inhibiting effect of semaphorin-3F which is expressed in the posterior part of the somites (dermomyotome), and which is bound to the neuropilin-2 receptor on NCCs (22). One subgroup of these cells separates and travels towards the future heart, taking part in the development of outflow tract. It is deemed that this group of cells, which expresses CXCR4 (*CXC chemokine receptor 4*), attracts the ligand SDF1 (*stromal cell derived factor*) expressed in the embryonic heart cells (23). The second subgroup gives the SCPs that travel along vagal nerve fibers to the esophagus and stomach and participate in the development of ENS in these regions of digestive tract (20).

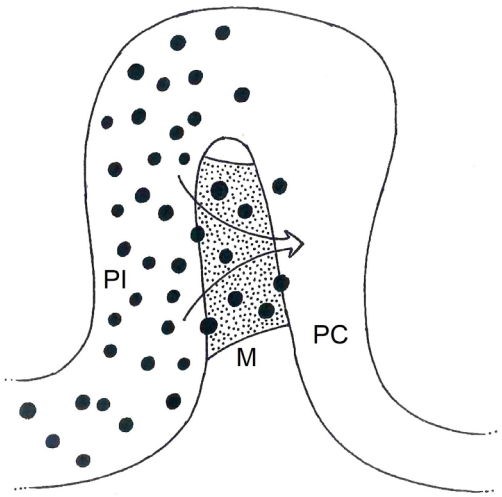


Figure 3. Transmesenteric migration of precursor cells of the enteric nervous system.

During migration, a number of precursor cells of the enteric nervous system pass directly from the region of the future ileum to the region of the future colon through the mesentery, bypassing the region of the cecum. Migrating precursor cells are shown by black dots. The arrow indicate the direction of transmesenteric migration. PI – presumptive ileum, M – mesentery, PC – presumptive colon.

Vagal NCCs at the level of somites 3-7, which would give the largest part of ENS, start their migration towards the proximal part of foregut and enter the region of future esophagus around the fourth gestational week in humans (24), and between the ninth and tenth day of embryonic development in the murine model (25). Since that moment, it is deemed that these cells are responsible for the ENS development, and therefore, they are called enteric neural crest-derived cells (ENCCs) (9). These cells continue their way caudally towards the future anal region. Primarily, they migrate along the foregut (from the esophagus to the descending part of duodenum at the level of future papilla of Vater) and the midgut (from papilla of Vater to the end of 2/3 of the transversal part of colon) (10,11). It has been noticed that one subpopulation of migratory cells avoids the appendix, passing directly from the proximal part to the distal part of midgut and to the hindgut through mesentery, which connects these parts of intestines, bringing them to the immediate vicinity. The transmesenteric migration, as it has been named, contributes to the significant number of ENS cells of the future colon (Figure 3) (26). On their way through the hindgut of the vagal ENCCs meet sacral ENCCs, which migrate rostrally, and they would soon finish their longitudinal migration. In the murine model, the whole gut is colonized until the 14th embryonic day (25), while in humans, most probably, until the 7th week of embryonic development (24).

NCCs below the level of the seventh somite (the trunk region) do not enter the foregut region. It is assumed that mesenchymal cells of the foregut produce certain molecules, such as Slit 1, which attaches to the Robo receptor, expressed on the NCCs of the trunk region, and prevents the migration of these cells towards the foregut. Robo receptor is not expressed on vagal NCCs (27). By creating the SCPs, these cells migrate along extrinsic nerve fibers, less contributing to the total number of ENS cells (20).

A group of sacral NCCs, as it has been mentioned, migrates ventrally towards a distal part of hindgut. After they build a network of cells, which the pelvic plexus will be formed from, these precursor cells enter the region of hindgut along the extrinsic nerve fibers, where they will give rise to neurons and glial

nije unapred određen već zavisi od interakcije sa okolnim ćelijama (28). Zanimljivo je da u modelu zebrica, sakralne NCC ne učestvuju u izgradnji ćelija ENS (29).

Da bi buduće ćelije ENS kolonizovale čitav digestivni trakt neophodna je kontinuirana proliferacija progenitorskih ćelija i održavanje tzv. progenitorskog pula. Pored broja inicijalnih NCC, za pravilnu migraciju i diferencijaciju od velikog značaja je gustina ENCC na tzv. migratornom frontu. Ova gustina održava se proliferacijom prekursorskih ćelija (30). Velika migratorna sposobnost ćelija migratornog fronta, koje vode migraciju u nekolonizovane delove creva, delimično je posledica međućelijske interakcije. Naime, smatra se da ćelijski kontakti posredovani kadherinima, integrinima i nekim proteinima imunoglobulinske superfamilije odbijaju ćelije jedne od drugih i primoravaju ih da pronađu put u nenaseljene delove creva (fenomen kontaktne inhibicije). Manjak ćelijske adhezije doveo bi do nemogućnosti trakcije i samog kretanja ćelija, dok bi prevelika adhezija izazvala usporenje ili prevremeni prekid migracije (31). Nesumjivo je i to da mezenhimne ćelije creva eksprimiraju molekule koji privlače ove ćelije i doprinose usmeravanju migracije. Primer ovakvog molekula je GDNF (*Glial-Derived Neurotrophic Factor*), koji se vezuje za RET (*Rearranged during Transfection*) receptor na ENCC (32).

Uporedo sa napredovanjem migracije, jedan broj ćelija lociranih iza migratornog fronta, započinje diferencijaciju u neurone ili glijalne ćelije dok druge nastavljaju ćelijski ciklus kao progenitorske ćelije, kako bi obezbedile dovoljan broj prekursorskih ćelija u rastućem crevu. Iz ovoga je jasno da je neophodna veoma precizna kontrola i balans između procesa proliferacije i diferencijacije kako bi se razvoj ENS do kraja nesmetano odigrao. Transkripcioni faktori SOX10 (*SRY-box transcription factor 10)* i PHOX2B (*Paired-like Homeobox 2B*), koji su koeksprimirani na prekursorskim ćelijama poreklom od nervnog grebena, doprinose balansu broja neurona odnosno glija ćelija. Prekursorske ćelije ENS koje su opredeljene za diferencijaciju u neurone smanjuju ekspresiju SOX10, a održavaju ekspresiju PHOX2B, dok se u ćelijama opredeljenim za glijalnu liniju dešava suprotno (9). Mutacije koje dovode do prevremene diferencijacije prekursorskih ćelija, manifestovaće se distalnom aganglionozom (Hiršprungova bolest) zbog nemogućnosti diferentovanih ćelija da značajnije migriraju. Takođe, ni kasna diferencijacija nije povoljna zbog prevelikog broja prekursorskih ćelija čija je pravilna diferencijacija potencijalno ograničena novim mikrosredinskim uslovima u razvijajućem crevu. Tokom normalnog embrionalnog razvoja, neurogeneza započinje pre gliogeneze (33). Sa eksprimiranjem neuron-specifičnih markera (pre svega specifičnih neurotransmitera), budući neuroni završavaju svoju migraciju i gube proliferativnu sposobnost (34). Specifične subpopulacije neurona koje se prve javljaju tokom razvoja su serotonergički neuroni (5-HT), holinergički neuroni (ChAT), kao i neuroni koji sadrže enkefalin. Nešto kasnije formiraju se i ostale subpopulacije neurona, kao što su neuroni pozitivni na vazoaktivni intestinalni peptid (VIP), NADPH i drugi (9).

Neophodno je da se neuroni i glija ćelije međusobno organizuju u ganglije, kako bi se pravilno formirali pleksusi ENS. Iako je pokazano postojanje više ganglijskih i neganglijskih pleksusa (2), najčešće se opisuje formiranje dva najveća i najznačajnija: mijenteričkog i submukoznog. Mijenterički pleksus razvija se pre submukoznog. Ovo navodi na zaključak da se uporedo sa longitudinalnom, odigrava i radijalna migracija prekursorskih ćelija ENS. U studiji *Memic i sar*. pokazano je postojanje velikog broja različitih signalnih molekula, odnosno transkripcionih faktora, koji, između ostalog, učestvuju i u procesu formiranja submukoznog pleksusa (35). Jedan od faktora koji doprinosi radijalnoj migraciji prekursorskih ćelija ENS ka mukozi jeste grupa molekula označenih kao netrini (*netrin-1*, *netrin-3* u mišjem modelu). Ovi molekuli ekprimirani su na epitelnim ćelijama mukoze creva, u najvećoj meri upravo u trenutku razvoja submukoznog pleksusa. Sa druge strane, prekursorske ćelije ENS eksprimiraju netrinski receptor DCC (*Detected in Colorectal Cancer*) za koji se netrini vezuju i ostvaruju svoju funkciju (36). Međutim, postavlja se pitanje šta utiče na to da se ove ćelije zaustave u submukozi gde formiraju pleksus, a ne nastave migraciju ka mukozi? Jedno od mogućih objašnjenja jeste da laminin-111 koga produkuju ćelije mukoze deluje suprotno delovanju netrina, i zaustavlja migrirajuće ćelije u blizini mukoze, odnosno u submukozi, gde će se formirati Majsnerov pleksus (37). Ovo je polje intenzivnog istraživanja i očekuje se da će u budućnosti biti otkriven veliki broj drugih molekula odnosno sig-

cells of ENS of this region. Their short migration is directed rostrally (19). Although they give morphologically and functionally identical nerve and glial cells, vagal and sacral ENCCs are different in many respects (28). In addition to the opposite direction of migration and different localization, some other significant differences have been noticed. When vagal ENCCs are implanted into the sacral region, they migrate earlier, in larger number and more proximally than sacral ENCCs. Also, the direction of migration is rostral. It is concluded that vagal ENCCs have significantly greater migratory potential than the sacral ENCCs, as well as that the direction of migration is not determined in advance, but depends on the interaction with surrounding cells (28). It is interesting that in zebrafish model, sacral NCCs do not take part in the development of ENS cells (29).

A continuous proliferation of progenitor cells and maintenance of the progenitor pool are necessary so that the future ENS cells would colonize the entire digestive tract. Besides the number of initial NCCs, the density of ENCCs on the migratory front is of great importance for the proper migration and differentiation. This density is maintained by the proliferation of precursor cells (30). A great migratory capacity of migratory front cells, which lead migration to the non-colonized parts of intestines, is partly a consequence of cell-cell interaction. Namely, it is deemed that cellular contacts, which are mediated by cadherins, integrins and some proteins of immunoglobulin superfamily, repel cells one from another and make them find their way to non-colonized parts of intestines (the phenomenon of contact inhibition). The lack of cell adhesion would lead to the impossibility of traction and movement of cells, while excessive adhesion would cause slowness or premature interruption of migration (31). Undoubtedly, mesenchymal cells in the intestines express molecules that attract these cells and determine the direction of migration. An example of such a molecule is GDNF (*Glial-Derived Neurotrophic Factor*), which is attached to RET (*Rearranged during Transfection*) receptor on ENCCs (32).

Along with the advancement of migration, a number of cells located behind the migratory front begin differentiation into neurons or glial cells, while other cells continue their cellular cycle as progenitor cells, in order to provide a sufficient number of precursor cells in the growing gut. Thus, it is clear that a precise control and balance between proliferation and differentiation processes is necessary so that the ENS development would unfold in an undisturbed way. Transcription factors SOX10 (*SRY-box transcription factor 10*) and PHOX2B (*Paired-like Homeobox 2B*), which are co-expressed on NCCs contribute to the balance of the number of neurons and glial cells. Precursor ENS cells that differentiate into neurons decrease the expression of SOX10, and maintain expression of PHOX2B, while opposite is the case in glial line precursor cells (9). Mutations that lead to the premature differentiation of precursor cells will be manifested with distal aganglionosis (Hirschsprung disease) due to the impossibility of differentiated cells to migrate more significantly. Also, late differentiation is not favorable due to the excess number of precursor cells, whose proper differentiation is potentially limited by new micro-environmental conditions in developing gut. During the normal embryonic development, neurogenesis begins before gliogenesis (33). With the expression of neuron-specific markers (primarily specific neurotransmitters), future neurons end their migration and lose their proliferative capacity (34). Specific sub-populations of neurons, which appear first during the development, are serotonergic neurons (5-HT), cholinergic neurons (ChAT), as well as neurons that contain enkephalin. Later, other subpopulations of neurons are formed, such as neurons positive to vasoactive intestinal peptide (VIP), NADPH and others (9).

It is necessary that neurons and glial cells firstly organize into ganglia, in order to form ENS plexuses properly. Although the existence of more ganglionated and non-ganglionated plexuses has been shown (2), the formation of two largest and most significant ones is described: myenteric and submucosal. The myenteric plexus develops before the submucosal. This instigates the conclusion that along with the longitudinal, radial migration of precursor ENS cells also unfolds. In the study of Memic at al. the existence of a large number of different signaling molecules and transcription factors has been shown which, among other things, take part in the process of formation of submucosal plexus (35). One of the factors, which contributes to radial migration of ENS precursor cells towards mucosa, is a group of molecules marked as netrins (*netrin-1*, *netrin-3* in the

nalnih puteva koji učestvuju ne samo u radijalnoj migraciji, već i u diferencijaciji pojedinih podtipova neurona, uspostavljanju aksonskih puteva i sinaptogenezi.

Za pravilnu funkciju ENS neophodan je pravilan embrionalni razvoj i drugih ćelija, pre svega intersticijskih (Kahalovih) ćelija i glatko-mišićnih ćelija. Kahalove ćelije su sposobne da generišu i propagiraju spore talase, zbog čega su bitne u održavanju fiziološke kontraktilnosti creva. Označene su i kao pejsmejker ćelije (8). Kahalove, kao i glatko-mišićne ćelije, nastaju od mezenhima creva, a smatra se da prekursorske ćelije ENS utiču na njihovu diferencijaciju (38,39).

Zaključak

Dve glavne populacije ćelija, poreklom od vagalnog i sakralnog dela nervnog grebena, prolazeći kroz kompleksne procese migracije, proliferacije, diferencijacije, učestvuju u izgradnji najvećeg dela ENS. Iako različite u mnogim aspektima embrionalnog razvoja, ove dve grupe ćelija daju identične tipove neurona i glijalnih ćelija koji zajednički uspostavljaju i održavaju funkciju ENS, koja je, nesumnjivo, od vitalne važnosti za naš organizam. Veliki broj različitih subtipova neurona, koji nisu organizovani u jasne formacije, kao što je to slučaj u centralnom nervnom sistemu, interakcije sa različitim ćelijama unutar i van ENS samo su neki od razloga zbog kojih su mnoge nejasnoće u strukturi i funkciji ENS još uvek prisutne. Praćenje pojedinačnih ćelija na njihovom razvojnom putu, odnosno sve bolje upoznavanje embrionalnog razvoja ENS uopšte, u velikoj meri može pomoći u razjašnjavanju ovih nedoumica.

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Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. Furness JB. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 2012; 9(5):286-294. doi: 10.1038/nrgastro.2012.32.
2. Hansen MB. The enteric nervous system I: organization and classification. Pharmacol Toxicol 2003; 92(3):105-113. doi: 10.1034/j.1600-0773.2003.t01-1-920301.x.
3. Lestarevic S, Lazic M, Jankovic R. Distribution and quantification of elements of the enteric nervous system in the distal rectum of neonates and infants: PS038. Porto Biomed J 2017; 2(5):200. doi: 10.1016/j.pbj.2017.07.059.
4. Fung C, Vanden Berghe P. Functional circuits and signal processing in the enteric nervous system. Cell Mol Life Sci2020; 77:4505-4522. doi: 10.1007/s00018-020-03543-6.
5. Furness JB, Callaghan BP, Rivera LR, Cho HJ. The enteric nervous system and gastrointestinal innervation: integrated local and central control. Adv Exp Med Biol 2014; 817: 39-71. doi: 10.1007/978-1-4939-0897-4_3.
6. Schneider S, Wright CM, Heuckeroth RO. Unexpected roles for the second brain: enteric nervous system as master regulator of bowel function. Annu Rev Physiol 2019; 81: 235-259. doi: 10.1146/annurev-physiol-021317-121515.
7. Janković R. Modern diagnostics of Hirschsprung disease and related disorders. Materia medica 2016; 32(2):1478-82.
8. Janković R. Analiza glija indeksa i Kahalovih ćelija u biopsijama debelog creva dece sa Hiršprungovom bolešću i srodnim oboljenjima [disertacija]. Beograd: Medicinski fakultet Univerziteta u Beogradu; 2016.
9. Pawolski W, Schmidt MHH. Neuron-Glia Interaction in the Developing and Adult Enteric Nervous System. Cells 2021; 10(1):47. doi: 10.3390/cells10010047.
10. Rao M, Gershon MD. Enteric nervous system development: what could possibly go wrong? Nat Rev Neurosci 2018; 19(9):552-565. doi: 10.1038/s41583-018-0041-0.
11. Nagy N, Goldstein AM. Enteric nervous system development: A crest cell's journey from neural tube to colon. Semin Cell Dev Biol 2017; 66:94-106. doi: 10.1016/j.semcdb.2017.01.006.
12. Obermayr F, Hotta R, Enomoto H, Young HM. Development and developmental disorders of the enteric nervous system. Nat Rev Gastroenterol Hepatol 2013;10(1):43-57. doi: 10.1038/nrgastro.2012.234.
13. Diposarosa R, Bustam NA, Sahiratmadja E, Susanto PS, Sribudiani Y. Literature review: enteric nervous system development, genetic and epigenetic regulation in the etiology of Hirschsprung's disease. Heliyon 2021;7(6):e07308. doi: 10.1016/j.heliyon.2021.e07308.
14. Yntema CL, Hammond WS. The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. J Comp Neurol 1954; 101(2):515-541. doi: 10.1002/cne.901010212.
15. Le Douarin NM. Cell line segregation during peripheral nervous system ontogeny. Science 1986; 231(4745):1515-1522. doi: 10.1126/science.3952494.
16. Bronner ME, Le Douarin NM. Development and evolution of the neural crest: an overview. Dev Biol 2012; 366(1):2-9. doi: 10.1016/j.ydbio.2011.12.042.
17. Kuo BR, Erickson CA. Regional differences in neural crest morphogenesis. Cell AdhMigr 2010; 4(4):567-585. doi: 10.4161/cam.4.4.12890.
18. Young HM, Bergner AJ, Simpson MJ, McKeown SJ, Hao MM, Anderson CR et al. Colonizing while migrating: how

murine model). These molecules are expressed in epithelial cells of intestinal mucosa mostly at the moment of development of submucosal plexus. On the other hand, precursor cells of ENS express netrin receptor DCC (Detected in Colorectal Cancer), which netrins bind to and perform their function (36). However, the question is what causes these cells to stop their migration in submucosa, where they form plexus? One of the possible explanations is that laminin-111 which is produced by mucosal cells acts opposite to netrin, and stops migratory in submucosa, where Meissner's plexus will be formed (37). This is the field of intense research and it is expected that a large number of other molecules or signaling pathways, which take part not only in the radial migration, but also in the differentiation of certain subtypes of neurons, establishing of axonal pathways and synaptogenesis, will be found in the future.

Proper embryonic development of other cells, like interstitial cells of Cajal and smooth muscle cells, is necessary for proper functioning of ENS. Interstitial cells of Cajal can generate and propagate slow waves, and therefore they are important for the maintenance of physiological contractility of intestines. They were marked as pacemaker cells (8). Interstitial cells of Cajal, as well as smooth muscle cells, are derived from the intestinal mesenchyme, and it is deemed that ENS precursor cells influence their differentiation (38,39).

Conclusion

Two main populations of cells, which are derived from the vagal and sacral part of the neural crest, take part in the development of the largest part of ENS. They pass through complex processes of migration, proliferation, differentiation. Although they are different in regard to many aspects of embryonic development, these two groups of cells give identical types of neurons and glial cells which establish and maintain the function of ENS, which is, undoubtedly, vital for our organism. A great number of different subtypes of neurons, not organized in clear formations, as is the case in central nervous system, interactions with different cells within and out of the ENS are just some of the reasons why many uncertainties about the structure and function of ENS are still present. Following individual cells through their development pathways, as well as better understanding of embryonic development of ENS generally, may help to elucidate some of these uncertainties.

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Competing interests

The author declares no competing interests.

Literature

1. Furness JB. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 2012; 9(5): 286-294. doi: 10.1038/nrgastro.2012.32.
2. Hansen MB. The enteric nervous system I: organization and classification. Pharmacol Toxicol 2003; 92(3): 105-113. doi: 10.1034/j.1600-0773.2003.t01-1-920301.x.
3. Lestarevic S, Lazic M, Jankovic R. Distribution and quantification of elements of the enteric nervous system in the distal rectum of neonates and infants: PS038. Porto Biomed J 2017; 2(5):200. doi: 10.1016/j.pbj.2017.07.059.
4. Fung C, Vanden Berghe P. Functional circuits and signal processing in the enteric nervous system. Cell Mol Life Sci2020; 77: 4505-4522. doi: 10.1007/s00018-020-03543-6.
5. Furness JB, Callaghan BP, Rivera LR, Cho HJ. The enteric nervous system and gastrointestinal innervation: integrated local and central control. Adv Exp Med Biol 2014; 817: 39-71. doi: 10.1007/978-1-4939-0897-4_3.
6. Schneider S, Wright CM, Heuckeroth RO. Unexpected roles for the second brain: enteric nervous system as master regulator of bowel function. Annu Rev Physiol 2019; 81: 235-259. doi: 10.1146/annurev-physiol-021317-121515.
7. Jankovic R. Modern diagnostics of Hirschsprung disease and related disorders. Materia medica 2016; 32(2):1478-82.
8. Jankovic R. Analysis of glial cell index and interstitial cells of Cajal in colorectal biopsies of children with Hirschsprung disease and related disorders [doctoral dissertation]. Belgrade: Faculty of Medicine, University of Belgrade; 2016.
9. Pawolski W, Schmidt MHH. Neuron-Glia Interaction in the Developing and Adult Enteric Nervous System. Cells 2021; 10(1):47. doi: 10.3390/cells10010047.
10. Rao M, Gershon MD. Enteric nervous system development: what could possibly go wrong? Nat Rev Neurosci 2018; 19(9):552-565. doi: 10.1038/s41583-018-0041-0.
11. Nagy N, Goldstein AM. Enteric nervous system development: A crest cell's journey from neural tube to colon. Semin Cell Dev Biol 2017; 66:94-106. doi: 10.1016/j.semcdb.2017.01.006.
12. Obermayr F, Hotta R, Enomoto H, Young HM. Development

do individual enteric neural crest cells behave? BMC Biol 2014; 12:23. doi: 10.1186/1741-7007-12-23.

19. Goldstein AM, Hofstra RM, Burns AJ. Building a brain in the gut: development of the enteric nervous system. Clin Genet 2013; 83(4):307-316. doi: 10.1111/cge.12054.
20. Espinosa-Medina I, Jevans B, Boismoreau F, Chettouh Z, Enomoto H, Müller T et al. Dual origin of enteric neurons in vagal Schwann cell precursors and the sympathetic neural crest. Proc Natl Acad Sci USA 2017;114(45):11980-11985. doi: 10.1073/pnas.1710308114.
21. Brokhman I, Xu J, Coles B, Razavi R, Engert S, Lickert H et al. Dual embryonic origin of the mammalian enteric nervous system. Dev Biol 2019; 445(2):256-270. doi: 10.1016/j.ydbio.2018.11.014.
22. Gammill LS, Gonzalez C, Gu C, Bronner-Fraser M. Guidance of trunk neural crest migration requires neuropilin2/semaphorin 3F signaling. Development 2006; 133(1):99-106. doi: 10.1242/dev.02187.
23. Escot S, Blavet C, Härtle S, Duband JL, Fournier-Thibault C. Misregulation of SDF1-CXCR4 signaling impairs early cardiac neural crest cell migration leading to conotruncal defects. Circ Res 2013; 113(5):505-516. doi: 10.1161/CIRCRESAHA.113.301333.
24. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res 2005; 319(3):367-382. doi: 10.1007/s00441-004-1023-2.
25. Anderson RB, Stewart AL, Young HM. Phenotypes of neural-crest-derived cells in vagal and sacral pathways. Cell Tissue Res 2006; 323(1):11-25. doi: 10.1007/s00441-005-0047-6.
26. Nishiyama C, Uesaka T, Manabe T, Yonekura Y, Nagasawa T, Newgreen DF et al. Trans-mesenteric neural crest cells are the principal source of the colonic enteric nervous system. Nat Neurosci 2012; 15(9):1211-1218. doi: 10.1038/nn.3184.
27. Zuhdi N, Ortega B, Giovannone D, Ra H, Reyes M, Asención V et al. Slit molecules prevent entrance of trunk neural crest cells in developing gut. Int J Dev Neurosci 2015; 41:8-16. doi: 10.1016/j.ijdevneu.2014.12.003.
28. Burns AJ, Le Douarin NM. Enteric nervous system development: analysis of the selective developmental potentialities of vagal and sacral neural crest cells using quail-chick chimeras. Anat Rec 2001; 262(1):16-28. doi: 10.1002/1097-0185(20010101)262:1<16::AID-AR1007>3.0.CO;2-O.
29. Shepherd I, Eisen J. Development of the zebrafish enteric nervous system. Methods Cell Biol 2011; 101:143-160. doi: 10.1016/B978-0-12-387036-0.00006-2.
30. Barlow AJ, Wallace AS, Thapar N, Burns AJ. Critical numbers of neural crest cells are required in the pathways from the neural tube to the foregut to ensure complete enteric nervous system formation. Development 2008; 135(9):1681-1691. doi: 10.1242/dev.017418.
31. McKeown SJ, Wallace AS, Anderson RB. Expression and function of cell adhesion molecules during neural crest migration. Dev Biol 2013; 373(2):244-257. doi: 10.1016/j.ydbio.2012.10.028.
32. Mwizerwa O, Das P, Nagy N, Akbareian SE, Mably JD, Goldstein AM. Gdnf is mitogenic, neurotrophic, and chemoattractive to enteric neural crest cells in the embryonic colon. Dev Dyn 2011; 240(6):1402-1411. doi: 10.1002/dvdy.22630.
33. Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J et al. Molecular Architecture of the Mouse Nervous System. Cell 2018; 174(4):999-1014. doi: 10.1016/j.cell.2018.06.021.
34. Lasrado R, Boesmans W, Kleinjung J, Pin C, Bell D, Bhaw L et al. Lineage-dependent spatial and functional organization of the mammalian enteric nervous system. Science 2017; 356(6339):722-726. doi: 10.1126/science.aam7511.
35. Memic F, Knoflach V, Morarach K, Sadler R, Laranjeira C, Hjerling-Leffler J et al. Transcription and Signaling Regulators in Developing Neuronal subtypes of Mouse and Human Enteric Nervous System. Gastroenterology 2018; 154(3):624-636. doi: 10.1053/j.gastro.2017.10.005.
36. Jiang Y, Liu MT, Gershon MD. Netrins and DCC in the guidance of migrating neural crest-derived cells in the developing bowel and pancreas. Dev Biol 2003; 258(2):364-384. doi: 10.1016/s0012-1606(03)00136-2.
37. Ratcliffe EM, D'Autréaux F, Gershon MD. Laminin terminates the netrin/DCC mediated attraction of vagal sensory axons. Dev Neurobiol 2008; 68(7):960-971. doi: 10.1002/cne.21027.
38. Radenkovic G, Radenkovic D, Velickov A. Development of interstitial cells of Cajal in the human digestive tract as the result of reciprocal induction of mesenchymal and neural crest cells. J Cell Mol Med 2018; 22(2): 778-785. doi: 10.1111/jcmm.13375.
39. Jankovic R, Sindjic-Antunovic S, Lukac M, Vujovic D, Jevtic J, Skender-Gazibara M. Altered Distribution of Interstitial Cells of Cajal in Normoganglionic and Transitional Zone of Hirschsprung Disease and Their Clinical Significance. Central Eur J Paed 2020; 16(1): 1-9. doi: 10.5457/p2005-114.251.

and developmental disorders of the enteric nervous system. Nat Rev Gastroenterol Hepatol 2013;10(1):43-57. doi: 10.1038/nrgastro.2012.234.

13. Diposarosa R, Bustam NA, Sahiratmadja E, Susanto PS, Sribudiani Y. Literature review: enteric nervous system development, genetic and epigenetic regulation in the etiology of Hirschsprung's disease. Heliyon 2021;7(6):e07308. doi: 10.1016/j.heliyon.2021.e07308.
14. Yntema CL, Hammond WS. The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. J Comp Neurol 1954; 101(2):515-541. doi: 10.1002/cne.901010212.
15. Le Douarin NM. Cell line segregation during peripheral nervous system ontogeny. Science 1986; 231(4745):1515-1522. doi: 10.1126/science.3952494.
16. Bronner ME, Le Douarin NM. Development and evolution of the neural crest: an overview. Dev Biol 2012; 366(1):2-9. doi: 10.1016/j.ydbio.2011.12.042.
17. Kuo BR, Erickson CA. Regional differences in neural crest morphogenesis. Cell Adh Migr 2010; 4(4):567-585. doi: 10.4161/cam.4.4.12890.
18. Young HM, Bergner AJ, Simpson MJ, McKeown SJ, Hao MM, Anderson CR et al. Colonizing while migrating: how do individual enteric neural crest cells behave? BMC Biol 2014; 12:23. doi: 10.1186/1741-7007-12-23.
19. Goldstein AM, Hofstra RM, Burns AJ. Building a brain in the gut: development of the enteric nervous system. Clin Genet 2013; 83(4):307-316. doi: 10.1111/cge.12054.
20. Espinosa-Medina I, Jevans B, Boismoreau F, Chettouh Z, Enomoto H, Müller T et al. Dual origin of enteric neurons in vagal Schwann cell precursors and the sympathetic neural crest. Proc Natl Acad Sci USA 2017;114(45):11980-11985. doi: 10.1073/pnas.1710308114.
21. Brokhman I, Xu J, Coles B, Razavi R, Engert S, Lickert H et al. Dual embryonic origin of the mammalian enteric nervous system. Dev Biol 2019; 445(2):256-270. doi: 10.1016/j.ydbio.2018.11.014.
22. Gammill LS, Gonzalez C, Gu C, Bronner-Fraser M. Guidance of trunk neural crest migration requires neuropilin2/semaphorin 3F signaling. Development 2006; 133(1):99-106. doi: 10.1242/dev.02187.
23. Escot S, Blavet C, Härtle S, Duband JL, Fournier-Thibault C. Misregulation of SDF1-CXCR4 signaling impairs early cardiac neural crest cell migration leading to conotruncal defects. Circ Res 2013; 113(5):505-516. doi: 10.1161/CIRCRESAHA.113.301333.
24. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res 2005; 319(3):367-382. doi: 10.1007/s00441-004-1023-2.
25. Anderson RB, Stewart AL, Young HM. Phenotypes of neural-crest-derived cells in vagal and sacral pathways. Cell Tissue Res 2006; 323(1):11-25. doi: 10.1007/s00441-005-0047-6.
26. Nishiyama C, Uesaka T, Manabe T, Yonekura Y, Nagasawa T, Newgreen DF et al. Trans-mesenteric neural crest cells are the principal source of the colonic enteric nervous system. Nat Neurosci 2012; 15(9):1211-1218. doi: 10.1038/nn.3184.
27. Zuhdi N, Ortega B, Giovannone D, Ra H, Reyes M, Asención V et al. Slit molecules prevent entrance of trunk neural crest cells in developing gut. Int J Dev Neurosci 2015; 41:8-16. doi: 10.1016/j.ijdevneu.2014.12.003.
28. Burns AJ, Le Douarin NM. Enteric nervous system development: analysis of the selective developmental potentialities of vagal and sacral neural crest cells using quail-chick chimeras. Anat Rec 2001; 262(1):16-28. doi: 10.1002/1097-0185(20010101)262:1<16::AID-AR1007>3.0.CO;2-O.
29. Shepherd I, Eisen J. Development of the zebrafish enteric nervous system. Methods Cell Biol 2011; 101:143-160. doi: 10.1016/B978-0-12-387036-0.00006-2.
30. Barlow AJ, Wallace AS, Thapar N, Burns AJ. Critical numbers of neural crest cells are required in the pathways from the neural tube to the foregut to ensure complete enteric nervous system formation. Development 2008; 135(9):1681-1691. doi: 10.1242/dev.017418.
31. McKeown SJ, Wallace AS, Anderson RB. Expression and function of cell adhesion molecules during neural crest migration. Dev Biol 2013; 373(2):244-257. doi: 10.1016/j.ydbio.2012.10.028.
32. Mwizerwa O, Das P, Nagy N, Akbareian SE, Mably JD, Goldstein AM. Gdnf is mitogenic, neurotrophic, and chemoattractive to enteric neural crest cells in the embryonic colon. Dev Dyn 2011; 240(6):1402-1411. doi: 10.1002/dvdy.22630.
33. Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J et al. Molecular Architecture of the Mouse Nervous System. Cell 2018; 174(4):999-1014. doi: 10.1016/j.cell.2018.06.021.
34. Lasrado R, Boesmans W, Kleinjung J, Pin C, Bell D, Bhaw L et al. Lineage-dependent spatial and functional organization of the mammalian enteric nervous system. Science 2017; 356(6339):722-726. doi: 10.1126/science.aam7511.
35. Memic F, Knoflach V, Morarach K, Sadler R, Laranjeira C, Hjerling-Leffler J et al. Transcription and Signaling Regulators in Developing Neuronal subtypes of Mouse and Human Enteric Nervous System. Gastroenterology 2018; 154(3):624-636. doi: 10.1053/j.gastro.2017.10.005.
36. Jiang Y, Liu MT, Gershon MD. Netrins and DCC in the guidance of migrating neural crest-derived cells in the developing bowel and pancreas. Dev Biol 2003; 258(2):364-384. doi: 10.1016/s0012-1606(03)00136-2.
37. Ratcliffe EM, D'Autréaux F, Gershon MD. Laminin terminates the netrin/DCC mediated attraction of vagal sensory axons. Dev Neurobiol 2008; 68(7):960-971. doi: 10.1002/cne.21027.
38. Radenkovic G, Radenkovic D, Velickov A. Development of interstitial cells of Cajal in the human digestive tract as the result of reciprocal induction of mesenchymal and neural crest cells. J Cell Mol Med 2018; 22(2): 778-785. doi: 10.1111/jcmm.13375.

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39. Jankovic R, Sindjic-Antunovic S, Lukac M, Vujovic D, Jevtic J, Skender-Gazibara M. Altered Distribution of Interstitial Cells of Cajal in Normoganglionic and Transitional Zone of Hirschsprung Disease and Their Clinical Significance. Central Eur J Paed 2020; 16(1): 1-9. doi: 10.5457/p2005-114.251.

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