PREGLEDNI RAD

UDK BROJEVI:
611.8.013
DOI: https://www.doi.org/10.5937/zdravzast51-39735

SIGNALNI PUTEVI U KONTROLI EMBRIONALNOG RAZVOJA ENTERIČKOG NERVNOG SISTEMA

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SAŽETAK

Enterički nervni sistem (ENS) obezbeđuje intrinzičku inervaciju gastrointestinalnog trakta i predstavlja najveći i najkompleksniji deo perifernog nervnog sistema. Njegove funkcije su od vitalne važnosti i podrazumevaju kontrolu motiliteta digestivnog trakta, kontrolu sekrecije, kao i razmene tečnosti i elektrolita kroz sluznicu creva. Većinu ovih funkcija ENS je sposoban da obavlja potpuno autonomno. Proučavanje najčešće kongenitalne bolesti ENS, Hiršprungove bolesti, dalo je veliki doprinos u rasvetljavanju embrionalnog razvoja ENS. Ćelije ENS potiču najvećim delom od ćelija vagalnog, a nešto manjim delom od ćelija sakralnog regiona nervnog grebena. Ove ćelije migriraju duž primitivnog creva u suprotnim smerovima, kako bi konačno kolonizovali čitavo crevo. Procesi proliferacije, migracije, neuro-glijalne diferencijacije kroz koje prolaze prekursorske ćelije ENS, regulisani su brojnim signalnim putevima. Neki od najvažnijih molekula koji učestvuju u regulaciji pravilnog razvoja ENS su GDNF (*Glial Derived Neurotrophic Fatcor*) i njegov receptor RET (*REarranged during Transfection*), endotelin 3 i njegov receptor EDNRB (*endothelin receptor type B*), transkripcioni faktori SOX10 (*SRY-box transcription factor 10*), PHOX2B (*Paired-like Homeobox 2B*), morfogeni kao što su BMP 2 i 4 (*Bone Morphogenic Proteins*) i drugi. Iako su naša saznanja o kontroli razvoja ENS poslednjih godina značajno uvećana, kompleksnost strukture i funkcije ENS ostavlja dosta prostora za dalja istraživanja. U ovom preglednom radu prikazali smo dosadašnja znanja o najvažnijim regulatornim mehanizmima i signalnim putevima koji učestvuju u razvoju ENS.

Ključne reči: enterički nervni sistem, embrionalni razvoj, signalni putevi, Hiršprungova bolest

Uvod

Najveći broj ćelija enteričkog nervnog sistema (ENS) vodi poreklo od ćelija vagalnog dela nervnog grebena, u nivou 1-7. somita (1). Prekursorske ćelije sakralnog dela nervnog grebena, kaudalno od 28. somita takođe učestvuju u izgradnji ENS distalnih delova creva (2). Ćelije vagalnog dela nervnog grebena migriraju ventromedijalno kroz mezenhim somita, ulaze u region prednjeg creva i nastavljaju migraciju kaudalno kroz prednje, srednje i zadnje crevo (3). Nasuprot tome, ćelije sakralnog dela nervnog grebena imaju suprotan smer migracije. One ulaze u zid creva u predelu zadnjeg creva i započinju migraciju rostralno. Njihova migratorna putanja je kraća, tako da već u zadnjem crevu susreću prekursorske ćelije vagalnog porekla i završavaju svoju dalju migraciju (4). Na ovim migratornim putanjama, prekursorske ćelije prolaze i kroz procese proliferacije, diferencijacije u neurone ili glijalne ćelije ENS, formiranja ganglija ENS i njihovog povezivanja u pleksuse (5,6). O važnosti ovih procesa govore brojni razvojni poremećaji sa neadekvatnim brojem (hiper/hipoganglionoze) ili potpunim odsustvom ganglija ENS (aganglionoze), kakva je Hiršprungova bolest (7-10).

REVIEW ARTICLE

UDK NUMBERS:
611.8.013
DOI: https://www.doi.org/10.5937/zdravzast51-39735

SIGNALING PATHWAYS IN THE CONTROL OF EMBRYONIC DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

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SUMMARY

The enteric nervous system (ENS) provides intrinsic innervation of the gastrointestinal tract and is the largest and most complex part of the peripheral nervous system. Its functions are vital for life and include control of motility of the digestive tract, secretion, as well as fluid and electrolyte exchange through the intestinal mucosa. ENS is capable of performing most of these functions completely autonomously. A large number of developmental and genetic studies of the most common congenital disease of the ENS, Hischsprung' s disease, has made a major contribution to the understanding of the embryonic development of the ENS. ENS cells raise from the vagal (mostly) and sacral region of the neural crest. These precursor cells migrate along the primitive gut in opposite directions, in order to colonize the entire gut. Proliferation, migration, neuro-glial differentiation, and other processes through which precursor cells of the ENS undergo, are regulated by various signaling pathways. Some of the most important molecules that participate in the regulation of the proper development of the ENS are GDNF (Glial Derived Neurotrophic Fatcor) and its receptor RET (REarranged during Transfection), endothelin 3 and its receptor EDNRB (endothelin receptor type B), transcription factors SOX10 (SRY-box transcription factor 10), PHOX2B (Paired-like Homeobox 2B), morphogens such as BMP 2 and 4 (Bone Morphogenic Proteins) and others. Although our knowledge about control of the development of the ENS has increased significantly in recent years, complexity of structure and function of the ENS requires further research. This review summarizes our current understanding of the most important regulatory mechanisms and signaling pathways involved in the development of the ENS.

Keywords: enteric nervous system, embryonic development, signaling pathways, Hirschsprung disease

Introduction

The majority of the enteric nervous system (ENS) is derived from vagal neural crest cells adjacent to somites 1-7 (1). Precursor cells of the sacral part of the neural crest, caudally from the somite 28 also take part in the development of ENS of distal parts of the gut (2). Vagal neural crest cells migrate ventromedially through the somite mesenchyme, enter the region of foregut and continue their migration caudally through the foregut, midgut and hindgut (3). Contrary to this, sacral neural crest cells migrate in opposite direction. They enter the gut wall in the region of hindgut and start their migration rostrally. Their migratory way is shorter, and therefore, they meet the vagal precursor cells in the hindgut and terminate their further migration (4). On these migratory pathways, precursor cells undergo the processes of proliferation, neuro-glial differentiation, formation of ganglia of the ENS and their interconnecting into plexuses (5, 6). Numerous developmental disorders with the inadequate number (hyper/hypoganglionosis) or complete absence of ENS ganglia (aganglionosis), such as Hirschsprung disease speak about the importance of these processes (7-10).

Dok je pravilan embrionalni razvoj zasigurno uslov za normalno funkcionisanje ENS, regulatorni mehanizmi koji učestvuju u razvoju ENS još uvek su u velikoj meri nepoznati. Ipak, ulaganjem velike istraživačke energije poslednjih godina, uloga nekih signalnih puteva u kontroli razvoja ENS je umnogome razjašnjena (11-13).

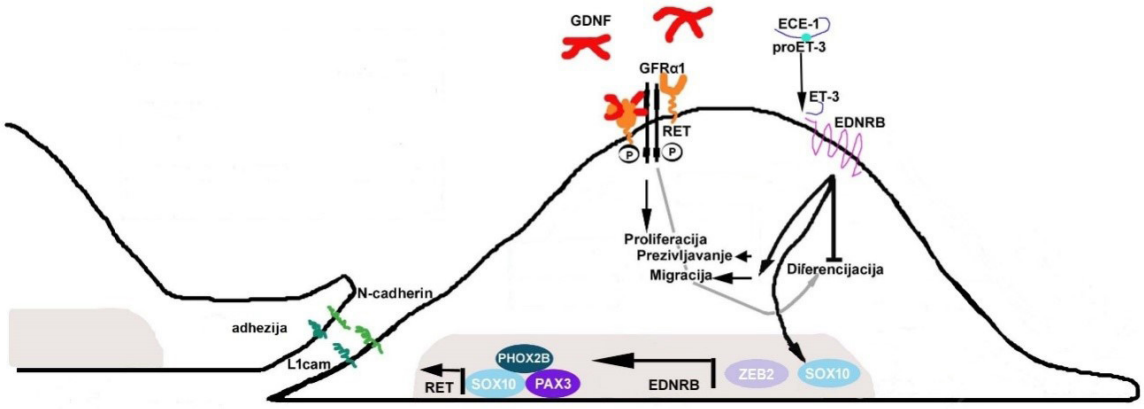
Cilj ovog rada jeste prikaz dosadašnjeg znanja o najvažnijim regulatornim mehanizmima i signalnim putevima koji učestvuju u kontroli embrionalnog razvoja ENS.

Metode

U ovom preglednom radu, korišćena je literatura dobijena pretraživanjem baze podataka MEDLINE. Literatura objavljena na engleskom jeziku, u poslednjih 10 godina, dobijena je pretraživanjem ključnih reči: enterički nervni sistem, embrionalni razvoj, signalni putevi, Hiršprungova bolest, RET, GDNF.

RET/GDNF

Najznačajniji i najbolje proučen signalni put koji učestvuje u kontroli embrionalnog razvoja ENS je RET (*REarranged during Transfection*)/ GDNF (*Glial Derived Neurotrophic Fatcor*) signalni put. Još pri prolasku prekursorskih ćelija poreklom od nervnog grebena kroz mezenhim somita, dešava se ushodna regulacija RET receptora posredovana retinoičnom kiselinom (14). Naime, mezenhimalne ćelije somita sekretuju povećanu količinu retinoične kiseline, koja aktivacijom svojih nuklearnih receptora, pre svega RARα (*Retinoic Acid Receptor alpha*), podstiče ekspresiju RET receptora na površini ćelija poreklom od nervnog grebena. Ovo će opredeliti prekursorske ćelije da migriraju ka budućem gastrointestinalnom traktu i daju ćelije ENS (15). Za ekrpesiju RET receptora na prekursorskim ćelijama ENS neophodna je aktivnost transkripcionih faktora SOX10 (*SRY-box transcription factor 10*), PHOX2B (*Paired-like Homeobox 2B*) i PAX3 (*Paired Box3*) (Slika 1) (13). RET receptor je transmembranski receptor sa tirozin kinaznom aktivnošću. Glavni ligand koji se vezuje za RET receptor je GDNF. GDNF se prethodno vezuje za koreceptor GFRα (*GDNF Family Receptor alpha*), lociran unutar lipidnih raftova ćelijske membrane, što indukuje privlačenje RET receptora, kao i formiranje i aktivaciju GDNF-GFRα-RET receptorskog kompleksa. Aktivacija RET receptora vodi autofosforilaciji intracelularnih domena i aktivaciji nishodnih signalnih puteva (16). Kao krajnji rezultat javlja se stimulacija preživljavanja, proliferacije i migracije prekursorskih ćelija ENS (17). Pored toga, dokazano je stimulativno dejstvo RET na diferencijaciju neurona *in vitro*, dok je uticaj RET na *in vivo* diferencijaciju još uvek predmet intenzivnog proučavanja (18). GDNF je eksprimiran u rastućem gradijentu



RET/GDNF signalni put stimuliše preživljavanje, proliferaciju, migraciju prekursorskih ćelija i diferencijaciju neurona ENS (*in vitro*), dok EDNRB signalni put ima iste efekte, izuzev na neuronalnu diferencijaciju, gde deluje inhibitorno. Na shemi su prikazani i adhezivni molekuli N-cadherin i L1CAM koji doprinose održavanju adekvatnog smera i brzine migracije prekursorskih ćelija tokom embrionalnog razvoja ENS. GDNF – *glial derived neurotrophic factor*; GFRα – *GDNF family receptor alpha*; RET – *rearranged during transfection receptor*; ECE-1 – *endothelin-converting enzyme*; ET-3 – endothelin 3; EDNRB – *endothelin receptor type B*; L1cam – *L1 cell adhesion molecule*; SOX10 – *SRY box transcription factor 10*; PHOX2B – *paired-like homeobox 2B*; PAX3 – *paired box 3*; ZEB2 – *zinc finger E-Box-binding homeobox 2*.

Slika 1. Uloga RET/GDNF i EDNRB signalnih puteva u kontroli razvoja enteričkog nervnog sistema (ENS).

While the normal embryonic development is certainly a condition necessary for normal functioning of ENS, regulatory mechanisms that take part in the ENS development are, to a great extent, unknown. However, a lot of energy has been devoted to research this topic in recent years, and therefore, the role of some signaling pathways in the control of ENS development has been explained (11-13).

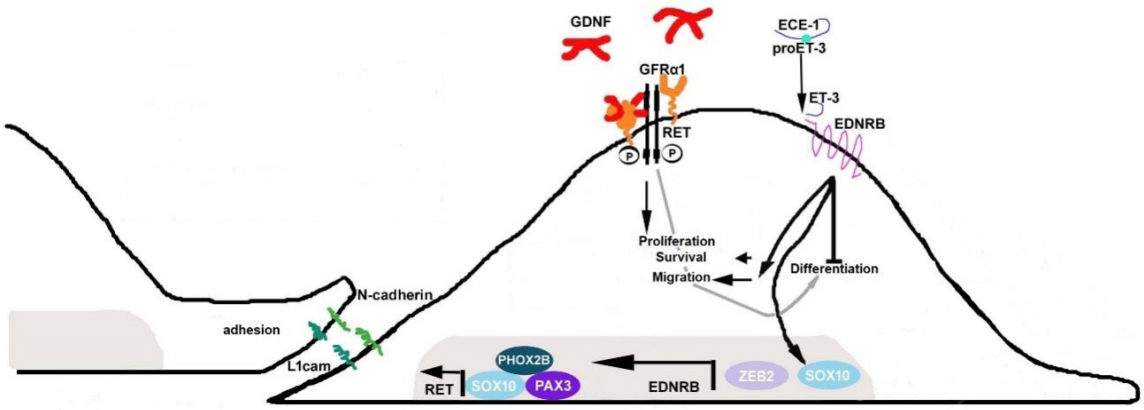
The aim of this review is to present the current knowledge about the regulatory mechanisms and signaling pathways that take part in the control of ENS development.

Materials and methods

In this review article, we used literature that was obtained through a search of MEDLINE database. 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, signaling pathways, Hirschsprung disease, RET, GDNF.

RET/GDNF

RET (Rearranged during Transfection)/GDNF (Glial Derived Neurotrophic Factor) signaling pathway is regarded as the most important and most studied signaling pathway that participates in the control of the embryonic development of ENS. When precursor cells that are derived from the neural crest pass through the mesenchyme of somites, an upward regulation of RET receptors happens and it is mediated by retinoic acid (14). Namely, mesenchymal somite cells produce higher levels of retinoic acid, which by activating its nuclear receptors, first of all RARα (Retinoic Acid Receptor α), enhances the expression of RET receptor on the surface of cells derived from the neural crest. This will make precursor cells migrate towards the future gastrointestinal tract and give ENS cells (15). The activity of transcription factors SOX10 (SRY-box transcription factor 10), PHOX2B (Paired-like Homeobox 2B) and PAX3 (Paired box 3) is necessary for the expression of RET receptors on ENS precursor cells (Figure 1) (13). RET receptor is a transmembrane receptor with tyrosine kinase activity. The main ligand that is bound to RET receptor is GDNF. GDNF is previously bound to co-receptor GFRα (GDNF Family Receptor alpha) that is located within lipid rafts of the cell membrane, which induces the activation of RET receptor, as well as the formation and activation of GDNF-GFRα-RET receptor complex. The activation of RET receptor leads to the autophosphorylation of intracellular domains and activation of downward signaling pathways (16). The stimulation of survival, proliferation and migration of precursor ENS cells



The RET/GDNF signaling pathway stimulates survival, proliferation, migration of ENS precursor cells and differentiation of ENS neurons (in vitro), while the EDNRB signaling pathway has the same effects, except for neuronal differentiation, where it has inhibitory effects.Adhesive molecules, N-cadherin and L1CAM, which contribute to maintaining the adequate direction and speed of migration of precursor cells during the embryonic development of the ENS, are also shown.GDNF – glial derived neurotrophic factor; GFRα – GDNF family receptor alpha; RET – rearranged during transfection receptor; ECE-1 – endothelin-converting enzyme; ET-3 – endothelin 3; EDNRB – endothelin receptor type B; L1cam – L1 cell adhesion molecule; SOX10 – SRY box transcription factor 10; PHOX2B – paired-like homeobox 2B; PAX3 – paired box 3; ZEB2 – zinc finger E-Box-binding homeobox 2.

Figure 1. Role of RET/GDNF and EDNRB signaling pathways in the control of enteric nervous system (ENS) development

od prednjeg ka zadnjem crevu, pospešujući na taj način migraciju prekursorskih ćelija ENS u istom smeru (17). Međutim, studija Andersona i sar. iz 2007. pokazala je da prekursorske ćelije vagalnog porekla migriraju istom brzinom u rostralnom smeru kada su implantirane u sakralni region, čime opovrgavaju uticaj gradijenta GDNF na migraciju (19). Pored GDNF, RET na isti način mogu aktivirati i drugi neurotrofni faktori (neurturin, artemin i persefin) (20). Mutacije u genu za RET receptor pronađene su u čak 50% porodičnih slučajeva i oko 20-30% sporadičnih slučajeva Hiršprungove bolesti (12,21). Animalni modeli pokazali su da je uticaj RET receptora na pravilan razvoj ENS dozno zavisan. Kod homozigotnih isključenja RET gena javlja se potpuna intestinalna aganglionoza, dok se kod heterozigota ENS razvija pravilno. Kada je nivo ekspresije RET receptora na oko 1/3, javlja se distalna aganglionoza ograničena na rektum i deo debelog creva (22). Mutacije drugih molekula uključene u RET signalni put, kao što su GDNF, GFRα, SOX10, PHOX2B, dovode do gotovo istih fenotipa (21). Sa druge strane, povećana ekspresija i aktivnost RET receptora često je udružena sa MEN sindromima, u kojima se mogu sresti ganglioneuromi ENS (MEN2B) (21,23). Neke studije ukazuju da mutacije RET gena mogu istovremeno biti udružene sa Hiršprungovom bolešću i MEN sindromima, što može zvučati paradoksalno na osnovu dosadašnjeg znanja i zahteva dalje istraživanje (23). U studiji Soreta i sar. pokazana je uloga GDNF u postnatalnoj neurogenezi kod mišjeg modela Hiršprungove bolesti, što sugeriše potencijalnu terapijsku primenu GDNF (24).

Ostali signalni putevi

EDNRB (endothelin receptor type B) signalni put

EDNRB je receptor povezan sa G proteinom i eksprimiran na prekursorskim ćelijama ENS. Za njegovu ekspresiju važni su transkripcioni faktori SOX10 i ZEB2 (*Zinc finger E-Box-binding homeobox 2*) (25). Ligand koji se vezuje za ovaj receptor i aktivira ga je endotelin-3 (EDN3), kojeg produkuju mezenhimalne ćelije creva tokom embrionalnog razvoja. Glavna uloga EDNRB signalnog puta jeste inhibicija neuronalne diferencijacije, odnosno održavanje progenitorskog stanja prekursorskih ćelija ENS, kako bi se obezbedio dovoljan broj prekursorskih ćelija za kolonizaciju čitavog creva (26). Pored toga, ovaj signalni put ostvaruje uloge slične RET signalnom putu, a to su stimulacija proliferacije i migracije prekursorskih ćelija (27). Iz ovoga se zaključuje da RET i EDNRB signalni putevi imaju sinergistički efekat na migraciju i proliferaciju, a antagonistički kada je u pitanju diferencijacija prekursorskih ćelija ENS (26,27). Mutacije u genima za EDNRB, EDN3 ili endotelin konvertujući enzim (ECE - *endothelin converting enzyme*) koji stvara END3 iz prekursorskog proteina kod miševa, dovode do Hiršprungove bolesti, najčešće u sklopu *Waardenburg* sindroma tip IV. Ovaj sindrom se još manifestuje pigmentovanim promenama na koži i senzoneuralnom gluvoćom (21,28). Kod pacijenata sa Hiršprungovom bolešću dokazane su mutacije EDNRB signalnog puta, i javljaju se u oko 5% svih slučajeva (20).

Hedgehog (Hh) i Notch signalni put

Ova dva signalna puta međusobno su tesno povezana i ostvaruju dve važne funkcije u embrionalnom razvoju ENS, a to su održavanje progenitorskog statusa prekursorskih ćelija i stimulacija gliogeneze. Ipak, indirektno, uključeni su u gotovo sve faze ravoja ENS (29,30). Ihh (*indian hedgehog*) i Shh (*sonic hedgehog*) su glavni molekuli koji pripadaju Hh familiji. Oni su eksprimirani od strane mezenhimalnih ćelija primitivnog creva. Receptori za koje se vezuju ovi molekulli označeni su kao Ptch (*Patch*) receptori i eksprimirani su na prekursorskim ćelijama ENS koje putuju duž primitivnog creva. Krajnji rezultat stimulacije Ptch receptora je ekspresija Dll1 (*Delta-like canonical notch ligand 1*) proteina na površini ćelije. Ovaj ligand vezuje se za Notch receptor susedne prekursorske ćelije ENS, što indirektno, inhibirajući ekspresiju ASCL1 (*Achaete SCute Homolog 1*) gena, stimuliše ekspresiju SOX10 (31). Kao što je već pomenuto, SOX10 je bitan za održavanje progenitorskog statusa prekursorskih ćelija ENS pre svega preko EDNRB, ali je njegova ekspresija važna i za diferencijaciju prekursorskih ćelija u glija ćelije. Upravo ravnoteža između aktivnosti ASCL1 gena, koji promoviše neurogenezu, i SOX10, koji promoviše gliogenezu, ključna je za održavanje dovoljnog broja prekursorskih, odnosno diferentovanih ćelija (Slika 2) (32).

Bone Morphogenic Proteins (BMPs)

BMP2 i BMP4 imaju važnu ulogu u gotovo svim fazama embrionalnog razvoja ENS. Ovi proteini, koji pripadaju TGFβ superfamiliji faktora rasta,

appears as the final result (17). In addition, the stimulating effect of RET on the differentiation of neurons *in vitro* has been proved, whereas the influence of RET on *in vivo* differentiation is still the subject of intense research (18). GDNF is a growth factor expressed in an increasing gradient from the foregut towards the hindgut, thus enhancing the migration of ENS precursor cells in the same direction (17). However, the study of Anderson et al. from 2017 showed that precursor cells that are derived from vagal part migrate at the same speed in the rostral direction when they are implanted into the sacral region, thus denying the influence of gradient GDNF on the migration (19). In addition to GDNF, RET receptor may be activated in the same way by other neurotrophic factors (neurturin, artemin, and persephin) (20). Mutations in the gene for RET receptor have been found in 50% of family cases and in about 20-30% of sporadic cases of Hirschsprung disease (12,21). Animal models have shown that the influence of RET receptor on the normal development of ENS is dose-dependent. In the homozygous exclusion of RET gene, complete intestinal aganglionosis appears, while in heterozygous, the ENS develops normally. When the level of expression of RET receptor is about 1/3, distal aganglionosis appears and it is limited to the rectum and part of the distal colon (22). Mutations of other molecules involved in the RET signaling pathway, such as GDNF, GFRα, SOX10, PHOX2B lead to almost the same phenotypes (21). On the other hand, the increased expression and activity of RET receptor is often associated with MEN syndromes, in which ganglioneuromas of ENS may be seen (MEN2B) (21,23). Some studies indicate that the mutations of RET gene may simultaneously be associated with Hirschsprung disease and MEN syndromes, which may sound as a paradoxical based on the current knowledge and therefore it demands further research (23). In the study of Soret et al., the role of GDNF in the postnatal neurogenesis in a murine model of Hirschsprung disease has been shown, which suggests a potential therapeutic application of GDNF (24).

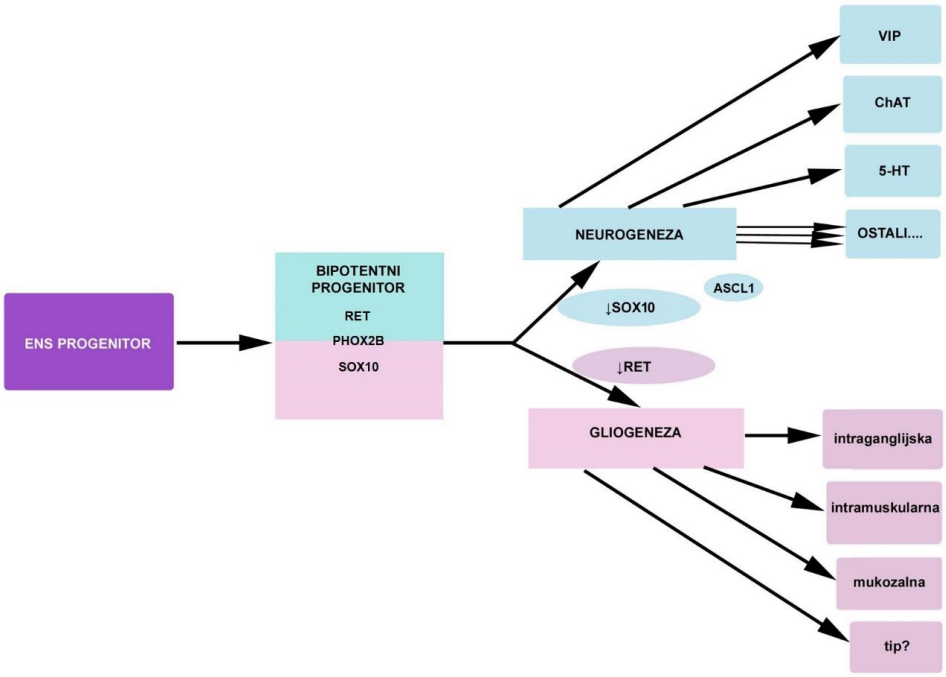
Other signaling pathways

EDNRB (endothelin receptor type B) signaling pathway

EDNRB is a G-protein-coupled receptor expressed by ENS precursor cells. Transcription factors SOX10 and ZEB2 (Zinc finger E-box-binding homeobox 2) are important for its expression (25). The ligand, which is bound to this receptor and which activates it, is endothelin-3 (EDN3) that is produced by mesenchymal intestinal cells during the embryonic development. The main role of EDNRB signaling pathway is the inhibition of neuronal differentiation, and consequently, the maintenance of progenitor state of precursor cells of the ENS, in order to secure the sufficient number of precursor cells for the colonization of the entire gut (26). In addition, this signaling pathway has roles similar to RET signaling pathway, like the stimulation of proliferation and migrations of precursor cells (27). Thus, one may conclude that RET and EDNRB signaling pathways have the synergistic effects on the migration and proliferation, while they have the antagonistic effects on the differentiation of ENS precursor cells (26,27). Mutations in genes for EDNRB, EDN3 or endothelin converting enzyme (ECE) that creates END3 from the precursor protein in mice lead to Hirschsprung disease, most frequently within Waardenburg syndrome type IV. This syndrome is also manifested by pigmented skin lesions and sensorineural hearing loss (21,28). In patients with Hirschsprung disease, mutations of EDNRB signaling pathway have been confirmed, and they appear in about 5% of cases (20).

Hedgehog (Hh) and Notch signaling pathway

These two signaling pathways are mutually interconnected and they accomplish two important functions in the embryonic development of the ENS: the maintenance of progenitor status of precursor cells and the stimulation of gliogenesis. However, they are indirectly involved in almost all stages of ENS development (29,30). *Ihh* (*Indian hedgehog*) and *Shh* (*sonic hedgehog*) are the main molecules that belong to Hh family. They are expressed by mesenchymal cells of the primitive gut. Receptors, which these molecules are marked as *Ptch* (*Patch*) receptors and they are expressed in precursor cells of the ENS that travel along the primitive gut.



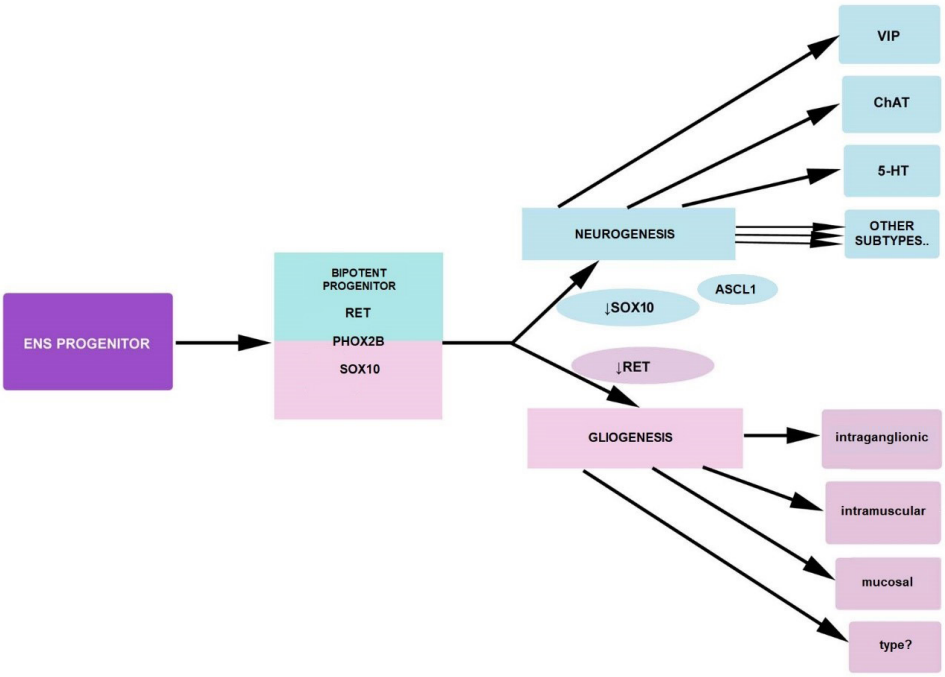
Jedan deo samoobnavljajućih progenitorskih ćelija ENS tokom razvoja daje bipotentne progenitore, koji su sposobni za dalju neuro-glijalnu diferencijaciju. U toku neurogeneze, ovi prekursori smanjuju ekspresiju SOX10 gena, dok održavaju ekspresiju RET. Suprotno se dešava tokom gliogeneze. PHOX2B ekspresija se održava u gotovo svim neuronima i nekim glija ćelijama ENS. Za sada je otkriven veliki broj različitih subtipova neurona u ENS (VIP, ChAT, 5-HT i dr.). Sa druge strane, raznolikost tipova glijalnih ćelija u ENS nije još uvek sasvim poznata, ali su glijalne ćelije morfološki sa sigurnošću prepoznate na nekoliko mesta u gastrointestinalnom traktu. VIP – vazoaktivni intestinalni peptid; ChAT – holin acetiltransferaza; 5-HT – serotonin.

Slika 2. Promena genske ekspresije u prekursorskim ćelijama enteričkog nervnog sistema (ENS) tokom neuro-glijalne diferencijacije.

vezuju se za BMP receptor. Aktivacijom receptora pokreće se nishodna kaskada i aktivacija signalnih molekula poznatih kao SMAD, koji se zatim translociraju u jedro i pokreću transkripciju brojnih gena (33). Pored pozitivne kontrole preživljavanja, migracije i proliferacije, važna je uloga, naročito BMP2, u diferencijaciji prekursorskih ćelija ENS u neurone. Kateholaminergički neuroni, zatim neuroni koji eksprimiraju nNOS i NPY diferenciraju se u prisustvu BMP2, dok uloga u diferencijaciji holinergičkih neurona i neurona koji eksprimiraju supstanciju P nije primećena. Ova diferencijacija ostvaruje se posredstvom SMAD1 (34). Dakle, BMPs zajedno sa GDNF doprinosi povećanju populacije neurona ENS. Ipak, pokazano je da BMPs nisu isključivo promoteri neuronalne, već i glijalne diferencijacije. Naime, za gliogenezu od velikog značaja su aktivacija ERBB3 receptora pomoću tzv. neuregulina, od kojih je najznačajniji GGF2 (*Glial Growth Factor 2*). Upravo pozitivnom regulacijom ovog puta, BMPs doprinose gliogenezi, odnosno uvećanju populacije glijalnih ćelija ENS (35). Inhibicijiom BMPs signalnog puta specifičnim antagonistima, kao što je nogin (*noggin*), dolazi do distalne hipoganglionoze i nepravilnog formiranja ganglija ENS. Ovim je potvrđena uloga BMPs u migraciji prekursorskih ćelija ENS, ali i gangliogenezi (36).

Semaforin 3A

Semaforin 3A i njegov receptor, neuropilin-1 (NRP1), označeni su kao negativni regulatori aksonske elongacije i sinaptogeneze (7,20). Poznato je da prekursorske ćelije ENS poreklom od sakralnog dela nervnog grebena ne ulaze u region zadnjeg creva sve dok on ne bude kolonizovan od strane prekursorskih ćelija vagalnog porekla. Pretpostavlja se da semaforin 3A doprinosi ovom odlaganju i sprečava prevremenu kolonizaciju zadnjeg creva (19). U susednim, ganglijskim delovima creva pacijenata sa Hiršprungovom bolešću pronađena je smanjena ekspresija sinapsina-1, koji je u negativnoj korelaciji sa ekspresijom semaforina 3A, što potencijalno može da bude objašnjenje postoperativnih komplikacija nakon uklanjanja aganglioznog dela creva ovih pacijenata(37). Dokazana



A number of ENS progenitor cells, during development, give rise to bipotent progenitor cells, which are capable of further neuro-glial differentiation.During neurogenesis, these precursors downregulate SOX10 gene expression, while maintaining RET expression. The opposite occurs during gliogenesis.PHOX2B expression is maintained in almost all neurons and some glial cells of the ENS.Large number of different neuron subtypes have been discovered in the ENS so far (VIP, ChAT, 5-HT, etc.). On the other hand, diversity of glial cell types in the ENS is still not completelyunderstood, but glial cells have been morphologically identified in several locations in the gastrointestinal tract. VIP – vasoactive intestinal peptide; ChAT – choline acetyltransferase; 5-HT – serotonin.

Figure 2. Gene expression alteration in precursor cells of the enteric nervous system (ENS) during neuro-glial differentiation

The final result of stimulation of Ptch receptors is the expression *Dll1* (*Delta-like canonical notch ligand 1*) protein on the cell surface. This ligand is bound to Notch receptor of the neighboring ENS precursor cell, which indirectly, stimulates the expression of SOX10 by inhibiting the expression of ASCL1 (*Achaete Scute Homolog 1*) gene. As it has already been mentioned, SOX10 is important for the maintenance of the progenitor status of precursor cells of the ENS, first of all through EDNRB, but also its expression is important for the differentiation of precursor cells into glial cells. Precisely this balance between the activity of ASCL1 gene, which promotes neurogenesis, and SOX10, which promotes gliogenesis, is of key importance for the maintenance of the sufficient number of precursor cells and differentiated cells (Figure 2) (32).

Bone Morphogenic Proteins (BMPs)

BMP2 and BMP4 have an important role in almost all phases of embryonic development of the ENS. These proteins, which belong to TGFß superfamily of growth factors, are bound to the BMP receptor. The activation of receptors instigates the downward cascade and the activation of signaling molecules known as SMAD, which are then translocated into the nucleus and trigger the transcription of numerous genes (33). In addition to the positive control of survival, migration and proliferation, especially BMP2 has an important role in the differentiation of ENS precursor cells into neurons. Catecholaminergic neurons, nNOS and NPY expressing neurons are differentiated in the presence of BMP2, while the role in the differentiation of cholinergic neurons and neurons expressing substance P has not been noticed. This differentiation is accomplished with the help of SMAD1 (34). Therefore, BPMs together with GDNF contribute to the increase in the population of neurons of the ENS. However, it has been shown that BPMs are not just promoters of neuronal, but also of glial differentiation. Namely, of great significance for gliogenesis is the activation of ERBB3 receptor with the help of the neuregulin, where GGF2 is the most important of them (Glial

je povezanost polimorfizama gena za semaforin 3A i pojave Hiršprungove bolesti (38).

Adhezioni molekuli

Tokom migracije, neophodno je da prekursorske ćelije ENS budu u kontaktu, kako bi se održali adekvatni smer i brzina migracije. Ove ćelije na svojoj površini eksprimiraju određene adhezione molekule kao što su N-kadherin, NCAM (*Neural Cell Adhesion Molecule*), kao i L1CAM (*L1 Cell Adhesion Molecule*) (Slika 1). Mutacije u genima za neki od ovih molekula mogu usporiti migraciju i potencirati nastanak distalne aganglionoze (39-41).

Zaključak

Razumevanje kontrole embrionalnog razvoja ENS značajno se poboljšalo u poslednjoj deceniji, kao posledica sve većeg interesovanja naučnika za ovaj problem i značajnim unapređenjem tehnologije u istraživanju. Otkrivanje velikog broja različitih signalnih puteva, genetskih i epigenetskih faktora uključenih u kontrolu procesa migracije, proliferacije i diferencijacije ćelija ENS doprinelo je i boljem razumevanju nekih poremećaja razvoja, pre svega Hiršprungove bolesti. Ipak, povezanost između različitih signalnih puteva i doprinos spoljašnjih faktora poremećajima razvoja samo su neki od budućih fokusa istraživanja u ovoj oblasti.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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Growth Factor 2). With positive regulation of this pathway, BMPs contribute to gliogenesis and to the increase in the population of glial cells of the ENS (35). The inhibition of BMPs signaling pathway with specific antagonists, such as noggin, leads to the distal hypoganglionosis and abnormal formation of ganglia of the ENS. Thus, the role of BMPs in the migration of precursor cells of the ENS, as well as in gangliogenesis is confirmed (36).

Semaphorin 3A

Semaphorin 3A and its receptor, neuropilin-1 (NRP-1) are marked as negative regulators of axonal elongation and synaptogenesis (7,20). It is known that precursor cells of the ENS that are derived from the sacral part of the neural crest do not enter the region of hindgut until it is not colonized by vagal precursor cells. It is assumed that semaphorin 3A contributes to this postponement and prevents the preterm colonization of hindgut (19). In neighboring, ganglionic segment of intestines of patients with Hirschsprung disease, reduced expression of sinapsin-1 was found, which is in negative correlation with the expression of semaphorin 3A which may potentially explain the postoperative complications after the removal of aganglionic portion of intestines in these patients (37). The connection between the genetic polymorphism for semaphorin 3A and the appearance of Hirschsprung disease has been proved (38).

Adhesion molecules

During migration, in order to keep the adequate direction and speed of migration, precursor cells of the ENS should necessarily be in contact. These cells on their surface express certain adhesion molecules such as N-cadherin, NCAM (*Neural Cell Adhesion Molecule*), as well as L1CAM (*L1 Cell Adhesion Molecule*) (Figure 1). Mutation in genes for some of these molecules may slow down the migration and induce the appearance of distal aganglionosis (39-41).

Conclusion

Understanding the control of the embryonic development of the ENS has significantly improved in the last decade, which is the consequence of the fact that scientists have become increasingly interested in this problem and that advances have been made regarding the technology used in research. Detecting large numbers of different signaling pathways, genetic and epigenetic factors involved in the control of migration, proliferation and differentiation processes of ENS cells has contributed to better understanding of some developmental disorders, first of all, Hirschsprung disease. However, the interconnection between different signaling pathways and the contribution of extrinsic factors to developmental disorders are only some of future focus points of research in this field.

Competing interests

Authors declare no competing interests.

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Primljen: 21.08.2022. **Revizija:** 02.09.2022. **Prihvaćen:** 08.09.2022.

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Received: 08/21/2022 **Revised:** 09/02/2022 **Accepted:** 09/08/2022