**Final Report of the NKFIH / OTKA Research Support K124740 "Extracellular matrix in enteric nervous system development and stem cell transplantation for neurointestinal diseases"**

With the help of the research grant: **K124740**, from October 2017, 20 research article (impact factor: ~ 86), 2 book chapters (in press), 2 PhD dissertations, 5 diploma theses and approx. 60 abstract (27 oral presentations, 33 posters) described the results obtained with the help of the grant. The number of publications strictly related to the research topic is 14 (impact factor: 61), but in addition to the research goals set in the application, we also achieved successful results in a number of other topics that strengthen the international collaborations of our research group.

The following specific aims were proposed: (1) Identify the extracellular matrix (ECM)-associated factors expressed by the embryonic gut and developing enteric neural crest cells. (2) Determine how ECM proteins affect the migration, proliferation, and differentiation both ex vivo and in vivo of enteric neural crest cells.

To understand how the extracellular derived factors control enteric neural crest cell (ENCC) migration, proliferation, differentiation, and survival in the developing gut, comprehensive extracellular matrix (ECM) specific immunohistochemical analysis was performed at multiple stages during gut development. We found strong expression of heparane sulphate proteoglycan proteins (HSPG) which concentrate around the developing submucosal and myenteric ganglia at embryonic day 8 (E8) in chicken embryo, and this is even more pronounced at later developmental stages. This observation led us to hypothesize a potential role of HSPGs during ENS development. Detailed immunocytochemical characterization of the developing chicken gut revealed that collagen type 18 (Col18) and agrin dynamically express during neural crest cell migration. In developing hindgut, Col18 is expressed at the migratory ENCC wavefront, while agrin expression begins later in development. Both Col18 and agrin are strongly expressed around enteric ganglia in normal gut. Results obtained from microsurgical (preganglionic chorioallantoic grafting of preganglionic guts) and genetically induced aganglionic models (Ednrb-/- mice) suggest that ENCCs are required for the periganglionic expression of Col18 and agrin; the aganglionic colon lacks both Col18 and agrin expression in the mesenchyme. Using chick-mouse intestinal chimeras and enteric neurosphere transplantations, we show that mouse and chick ENCCs secrete Col18 and agrin, and both vagal- and sacral-derived ENCCs produce these proteins. While the experiments above demonstrate that both embryonic and postnatal ENCCs express Col18 and agrin, we asked which ENS cell type produces these proteins in the postnatal intestine. Neurospheres were spontaneously generated from PLP1GFP and TauGFP/+ mouse intestine to generate ENCC cultures containing fluorescently labeled glia and neurons, respectively. After one week, neurospheres were dissociated and cultured on a fibronectin-coated surface. PLP1+ enteric glial cells co-express both Col18 and agrin. In contrast, Tau+ enteric neurons express agrin, but not Col18. Next, we have used intestinal explants, neurosphere technique, stripe-choice assay recombined with function-blocking experiments to study the role of Col18 and Agrin on ENCC migration. We found that agrin strongly inhibits ENCC migration, presumably through its interaction with its receptor, dystroglycan, which is expressed by enteric ganglia. Col18, on the other hand, is permissive to, though not essential for migration. These functional studies demonstrate that ENCCs govern their own migration by actively modifying

their microenvironment through secretion of Col18 and agrin where Col18 is permissive and agrin is strongly inhibitory to ENCC migration. Recognition of the active role of ENCCs in remodeling the intestinal ECM has important clinical and therapeutic implications. In Hirschsprung disease, for example, this implies that not only ENCCs are absent from the aganglionic segment, but that the ECM is also perturbed, as confirmed by our observation that Col18 and agrin are absent from the periganglionic areas in the distal colon of Hirschsprung model Ednrb-/- mice. This work was published by **Nagy N et al., 2018, in DEVELOPMENT**.

Confocal microscopy analysis of adult chicken and mouse myenteric ganglia stained with Col18 and agrin clearly delineates the outer border of the enteric ganglia. This strongly suggested that Col18 and agrin might have additional roles in vivo during ENS development beyond cell migration, including regulation of neuronal or glial differentiation, formation of ganglionic aggregates, and extension of neurite processes. Their persistent expression in the postnatal intestine also suggested an ongoing role for these proteins in maintenance of the mature ENS. Interestingly, the molecular structure of this barrier resembles the external glial limiting membrane of the blood-brain barrier of the central nervous system, with both possessing strong Col18 and agrin expression. In the ENC both proteins surround the ganglia and may play a role in creating a protective barrier to separate ganglia from smooth muscle or other nearby cells, to contain the cells that comprise the enteric ganglia, or to protect them from certain immune mediators. To study this hypothesis, in our recent study, we have identified and characterized a blood-myenteric barrier (BMB) consisting of ECM proteins (collagen type 18, agrin and collagen-4) and glial end-feet, that encapsulates the murine myenteric plexus. The BMB is impermeable to the passive movement of 4 kDa FITC-dextran particles. In experimentally induced (DSS treatment) intestinal inflammation, macrophage-mediated ECM degradation of the BMB disrupts its physiologic barrier function, eliminates the separation of the intra- and extra-ganglionic compartments, and allows inflammatory stimuli to access the myenteric plexus. **Dóra et al., 2021, CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY, 12: 1617-1641., Impact Factor: 9,2.**

Intestinal chimeras using green fluorescent protein (GFP)-transgenic chick embryos recombined with immunocytochemistry of the enteric ganglia of colony stimulating factor-1 receptor-(CSF1R)-GFP chicken and CX3CR1-GFP mice we have identified a novel non-neural crest-derived myeloid cell population within the Col18+/Agrin+ enteric ganglia. The novel cell type was named **intraganglionic macrophages (IGM).** These intraganglionic macrophages are highly ramified and express the hematopoietic marker CD45, major histocompatibility complex (MHC) class II antigen, and chB6, a marker specific for B cells and microglia in avians, although whether they possess a microglial function in the ENS remains to be determined. Based on embryomanipulation and histological results, we conclude that avian enteric ganglia contain, in addition to neural crest-derived neurons and glia, a third population of cells that has not been previously described. These cells are hematopoietic-derived, highly ramified, and express markers consistent with a macrophage/microglia signature: CD45+/MHCII+/CSF1R+/chB6+. This original work was published by **Dóra et al., 2018, JOURNAL OF ANATOMY**. Cover image of the October issue was selected from our paper. In addition, this article received The Journal of Anatomy Runner-Up Best Paper Prize in 2018.

Similar to the avian model system, we have also identified the CD45, CX3CR1, CD11b, Iba1, F4/80, and CSF1R expressing intraganglionic macrophages in mouse enteric ganglia, which exhibits a distinct morphology from muscularis macrophages (MMs), with extensive cytoplasmic vacuolization and mitochondrial swelling, but without signs of apoptosis in small and large intestine. IGMs can penetrate the BMB in physiological conditions and establish direct contact with neurons and glia. DSS-induced colitis leads to BMB disruption, loss of its barrier integrity, and increased numbers of IGMs in a macrophage-dependent process. Our data revealed that macrophage-mediated degradation of the ECM surrounding the enteric ganglia disrupts its physiological barrier, eliminates the separation of the intra- and extra-ganglionic compartments, and allows inflammatory stimuli to access the myenteric plexus. This process offers a mechanism for the onset of neuroinflammation in Hirschsprung disease associated enterocolitis and other intestinal pathologies with acquired ENS dysfunction. **Dóra et al., 2021, CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY, 12: 1617-1641. IF:9,2.**

The complex interaction between multiple types of intestinal macrophages, including the newly identified IGMs, and the role of ECM-ENS environment in gut neuroimmunology have been summarized, with special focus to the possible clinical implications by **Dóra et al, 2020 (ORVOSI HETILAP) and Dóra et al 2021 (invited review, APPLIED SCIENCES, under minor revision) .**

The Talpid$^2$ and Talpid$^3$ mutants are long-utilized, naturally occurring avian mutants that are characterized by limb, gastrointestinal and craniofacial phenotypes. The talpid3 gene, initially identified as the uncharacterized gene KIAA0586, was subsequently found to encode a centrosomal protein essential for primary cilia formation, the site where Sonic hedgehog (Shh) signal transduction occurs, thus strengthening the link between the phenotypic defects and hedgehog signaling. Similarly, talpid2 gene, which has a 19 bp deletion in exon 32 within C2CD3 (C2 calcium-dependent domain containing 3), has also been shown to affect ciliogenesis, hedgehog signaling, limb and craniofacial development. Previously we demonstrated that Shh patterns the ECM to control ENS development in chick embryos: Shh inhibition causes hyperganglionosis, whereas Shh overexpression causes aganglionosis owing to decreased proliferation and premature differentiation of ENCCs (Nagy et al., 2016). Embryomanipulation experiments revealed that modulating Shh activity dramatically alters the expression of ECM proteins, such as versican and collagen IX, that are known regulators of neural crest cell migration. In collaboration with **Dr. Alan Burns, UCL-Institute of Child Health (London, UK) and Dr. Samantha A. Brugmann, Divisions of Plastic Surgery and Developmental Biology (University of Cincinnati College of Medicine, USA)**, we have used the avian embryo supplemented with the analysis of the ENS of multiple transgenic mice and human foetus. In these experiments the following methodology was applied: RNAscope In Situ Hybridization, neural tube transplantation, organ cultures. ECM protein expression was characterized before and after ENS colonization of the wild type and Talpid mutants hindgut to identify ECM patterns that might suggest a role in ENS development. Gut specific ECM protein expression was also studied in human embryonic gut (normal and Joubert syndrome, bearing a homozygous null mutation in KIAA0586, the human ortholog of chicken Talpid$^3$) and Talpid$^3$ transgenic mice colon. Results showed that the GI tract of both Talpid$^2$ and Talpid$^3$ chicken embryos was reduced in length and severely malformed, with tracheoesophageal fistula or atresia and open hindgut. Gut smooth muscle was mispatterned and was not arranged in concentric layers. Likewise, ENCC

were scattered throughout the gut wall, rather than arranged in the presumptive plexuses of the ENS. Analysis of the Hedgehog signalling pathway showed altered expression of the Hedgehog receptor Patched underlying the smooth muscle defects. Analysis of the gut extracellular matrix (ECM) suggested that deficient ENCC repellent cues contributes to the ENS defects. To specifically assess the cell autonomous requirement of Talpid$^3$ during ENS development, we used intra-species neural tube grafting between Talpid$^3$ and GFP expressing chick embryos. Analysis of the chimeric embryos demonstrated that patterning of the ENS does not require Talpid$^3$, but is dependent on correct environmental cues. Interestingly, although the lack of Talpid$^3$ in vagal ENCC did not affect the gross morphology of the ENS, it affected smooth muscle and epithelial morphology in a non cell-autonomous manner. In accordance with these results, a mouse model where Talpid$^3$ was mutated conditionally in NCC also showed grossly normal ENS but smooth muscle and epithelium defects. Lastly, gut defects observed in animal models were strikingly similar to defects observed in human gut fetal tissues with Joubert syndrome, bearing a homozygous null mutation in KIAA0586, the human ortholog of chicken Talpid$^3$. Overall, our results reveal an evolutionary conserved Talpid$^3$-dependent mechanism essential for neuromuscular patterning and neuronal-ECM interactions in the developing GI tract. ***Co-first author:*** **Delalande JM, Nagy N, et al., 2021, FRONTIERS IN MOLECULAR NEUROSCIENCE.** While the Talpid$^2$ and Talpid$^3$ intestinal tracts are both significantly shorter than control embryos, the intestinal ENS phenotypes are divergent from each other as the Talpid$^2$ hindgut has an increased number of ENCCs whereas Talpid$^3$ guts have reduced numbers of ENCCs. The differences in the phenotypic presentations between Talpid$^2$ and Talpid$^3$ embryos demonstrate not only the importance of functional primary cilia for Shh signal transduction, but also the divergent effects mutations in ciliary proteins can have on Shh signaling and ECM expression during embryonic development of the ENS **(Brooks et al., 2021 JOURNAL OF DEVELOPMENTAL BIOLOGY).**

To study whether the ENCC population retains its initial ENS-forming potential with age, in collaboration with **Dr. Don Newgreen's lab (Murdoch Children's Research Institute, Royal Children's Hospital, Victoria, Australia)** we have studied the question of retention or loss of ENS-forming ability in avian model system. We have observed that ENCCs retain high ability to colonize aneural gut tissue for several days after the normal colonization phase, but this ability then rapidly declines. The age-related decline broadly matches the progressive reduction in proportion of donor ENS cells lacking neuronal and glial markers. Using embryomanipulation experiments (organ co-cultures in vitro and in chorio-allantoic membrane (CAM) grafts, and cell sorting) we have concluded that the ability of neural crest-derived cells to form ENS declines rapidly due to quantitative reduction in undifferentiated enteric neural crest like cells and also in qualitative changes in these cells. Our results was published by **Zhang D, et al 2019, DEVELOPMENTAL BIOLOGY.**

Expansion of ENCCs is an essential step to obtain adequate numbers of cells for stem cell-based therapies. We aimed to test whether non-neural crest derived stem cells and their ECM product can support the expansion of ENCCs isolated from Wnt1-Cre;R26R-tdTomato (Wnt1-tdT) reporter mice. Neurospheres generated from the dissociated smooth muscle of colon are heterogenous, containing both ENCCs and mesenchymal cells. Similar to the effect of embryonic mesenchymal cells on ENCCs, our data indicate that postnatally derived connective tissue type of stromal cells promote the proliferation of ENCCs in vitro. Common gene expression profiles of adult ENCCs and neurospheres were observed that are consistent with the characteristics of

embryonic ENCCs, including the expression of Nestin, Sox10, Sox2 and ECM compounds including Col18, Agrin and Tenascin-C, **Stavely et al. 2021, STEM CELLS.**

One of the most important mesenchymal derived signaling pathways for the regulation of ENS development is mediated by glial-derived neurotrophic factor (GDNF), which is expressed in the gut mesenchyme. GDNF binds to a receptor complex formed by Ret and the co-receptor GFRα1, expressed on the surface of migrating ENCCs. Why the distal end of the bowel is particularly at risk in Hirschsprung's disease remains unclear, and how Gdnf-Ret signaling is implicated in this distal aganglionosis is unknown. We investigated the in vivo role of GDNF on ENCC development in the colorectum using the modified catenary culture of embryonic intestines to determine the effects of GDNF on ENCC development in the distal intestine. Our results show that GDNF has pleiotropic effects during colorectal ENS development, with mitogenic, neurotrophic, and chemoattractive effects on developing ENCCs.

Using modified catenary organ culture methods we have shown that excess GDNF leads to tumors of the ENS resembling intestinal ganglioneuromas and distal intestinal aganglionosis characteristic for Hirschsprung's disease. The occurrence of these two phenotypes appeared to be paradoxical, as the former is generally believed to be because of excess RET signaling whereas the latter is felt to be caused by RET deficiency. However, our data suggest that the degree of RET stimulation is of fundamental importance to both. To study the how the treatment of GDNF leads to tumors of the ENS resembling intestinal ganglioneuromas and distal intestinal aganglionosis we introduced a novel catenary culture model of RET overactivation in both chick and mouse embryonic gut. This model recapitulates aspects of both multiple endocrine neoplasia (MEN) syndromes and Hirschsprung disease, with development of intestinal ganglioneuromas and failed migration of ENCCs to the distal hindgut. We have observed that exogenous GDNF leads to ectopic ENCC aggregates on the surface of the midgut and ceca in chick and mouse intestine. Cells in the aggregates (called ganglioneuromas) express neuronal markers Tuj1, Hu and neurofilament. In addition, embryonic guts treated with GDNF have hyperplastic enteric ganglia in both the submucosal and myenteric plexuses with significantly increased interganglionic fibers. Also, the GDNF treatment of embryonic chick gut leads to migratory arrest of ENCCs associated with accelerated neuronal differentiation. In these model systems, ganglioneuromas only arise if GDNF treatment is applied during colonization of the gut, as evidenced by the lack of tumors seen when later embryonic gut is treated. This suggests that the response of ENCCs to GDNF signaling changes after gut colonization is completed, either because of downregulation of its receptor or an intracellular milieu that responds differently to RET activation. Intriguingly, cells isolated from the ganglioneuroma-like structures stimulated by GDNF were able to reconstitute a normal-appearing ENS when transplanted into a normal developmental environment. This suggests that at least some cells contained within the intestinal ganglioneuromas retain a progenitor-like state that can be activated upon introducing them into a normal embryonic milieu. **Nagy N et al., 2020, DEVELOPMENT. https://doi.org/10.1242/dev.190900**

The multiple adaptations that have developed in rodents and avians to colonize the hindgut ENS are interesting in the context of Hirschsprung's disease, which results from incomplete colonization of the distal bowel by migrating ENCCs. Interestingly, however, the aganglionosis is limited to the colon in >90% of cases, suggesting that this distalmost end of the bowel presents

a particular challenge to ENS development. *Using avian embryomanipulation recombined with organ culture and in situ hybridization*, we have found that removal of the ceca, a paired structure present at the midgut-hindgut junction in avian intestine, leads to incomplete NCC colonization of the hindgut, suggesting that the ceca are required for ENS development in colorectum. To test this, we replaced the ceca of embryonic day 6 wild-type chicks with ceca from transgenic GFP chicks. Interestingly, the entire hindgut ENS arises from the GFP+ ceca-derived ENCC population. Ablation of the ceca buds leads to failure of NCC colonization of the hindgut. To examine molecular differences between the ceca and interceca at the critical stage as ENCCs are just about to enter this region, the ceca and intercecal region were meticulously dissected from E5 intestine to determine regional differences in transcriptional profiles by *RNA-seq.* Comparative transcriptome profiling of the cecal buds compared to the interceca region shows that the non-canonical Wnt signaling pathway is preferentially expressed within the ceca. Specifically, Wnt11 is highly expressed in the ceca, as confirmed by RNA in situ hybridization, leading us to hypothesize that cecal expression of Wnt11 is important for ENCC colonization of the hindgut. Organ cultures were prepared using E6 avian intestine, when ENCCs are migrating through the ceca, and showed that Wnt11 inhibits enteric neuronal differentiation. These results revealed an essential role for the ceca during hindgut ENS formation and highlight an important function for non-canonical Wnt signaling in regulating ENCC differentiation and thereby promoting their migration into the colon. (**Nagy N et al., 2021, November, DEVELOPMENT**).

This paper has been selected to appear as a 'Research Highlight' in the Development journal, that aim is to highlight and unpack the findings of the selected paper, and to make them accessible to all readers within the developmental biology community.

https://journals.biologists.com/dev/article/148/22/e148_e2202/273547/Seeking-out-the-role-of-caeca-in-colon

The objectives proposed in the grant application have been completed close to 90%.

Specific results achieved so far:

1.) using catenary organ culture, we demonstrated that GDNF growth factor acts as a mitogen and chemoattractant for the ENCCs. In vivo GDNF treatment induced formation of cell aggregations on the intestinal surface. These structures were named gangliospheres. Based on the location and mechanism of their formation, the gangliospheres are suitable model for modeling the development of ganglioneuromas observed in human MEN2B disease and Hirschsprung's disease.

3.) GDNF-induced cell aggregation method provides an excellent opportunity to isolate ENCCs that colonize different sections of the gut and to study its developmental potential during stem cell transplantation.

4.) We have shown that in the colorectum of Talpid2 and Talpid3 mutant chicken, Talpid 3 mouse and human embryos the abnormal ENS formation is characterized by increased chondroitin sulfate proteoglycan-type ECM production due to impaired Sonic hedgehog signaling.

5.) We demonstrated first time that ENCCs that form the ENS produce collagen type 18 and agrin-type of heparan sulfate proteoglycan molecules.

6.) A novel macrophage cell type (intraganglionic macrophage) has been discovered in mouse and chicken myenteric ganglia.

7.) The intestinal blood barrier consisting of the extracellular matrix surrounding the enteric ganglia was characterized in detail by electron microscopy and STED microscopy. We have shown that this matrix degrades upon inflammation, which is related to the activity of macrophages.

8.) In embryo manipulation experiments, in situ hybridization, RNAseq, cell and organ cultures, we demonstrated that cells derived from the coecum produce growth factors Wnt11, Wnt5a, BMP5, which determine the division and differentiation of ganglion germ cells forming the colonic nervous system.

**International collaborations supporting this project proposal** *(+recent publications, 2020-2021):*

**1.) Allan M Goldstein lab, Department of Pediatric Surgery, MHG, Harvard Medical School, Bosto.** Topic: Enteric nervous system development.

***Nagy N****, Tamas Kovacs, Rhian Stavely, Viktoria Halasy, Adam Soos, Emoke Szocs1, Ryo Hotta2, Hannah Graham2, Allan M. Goldstein. (2021). Avian ceca are required for hindgut enteric nervous system development by inhibiting neuronal differentiation via non-canonical Wnt signaling and by promoting enteric neural crest cell proliferation. DEVELOPMENT, 2021, November.*

***N. Nagy****, A. G. Richard, H. Ryo, Z. Dongcheng, F. N. Donald, H. Viktoria, K. Tamás, and M. G. Allan, "RET overactivation leads to concurrent Hirschsprung disease and intestinal ganglioneuromas," DEVELOPMENT, vol. 147, no. 21, 2020.*

*Stavely, R., Bhave, S., Ho, W., Ahmed, M., Pan, W., Rahman, A. A., Ulloa, J., Bousquet, N., Omer, M., Guyer, R.,* ***Nagy, N.,*** *Goldstein, A. M., & Hotta, R. (2021). Enteric mesenchymal cells support the growth of postnatal enteric neural stem cells. STEM CELLS, 10.1002/stem.3388.*

*Dóra Dávid ; Ferenczi Szilamér; Stavely Rhian ; Toth Viktoria E. ; Varga Zoltán V. ; Kovács Tamás ; Bodi Ildikó ; Hotta Ryo ; Kovács Krisztina ; Goldstein Allan M.,* ***Nagy Nándor****. 2021, CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY, 12: 1617-1641.*

**2.) Dr. Alan Burns, UCL-Institute of Child Health, London, UK.**

Topic: Role of Talpid3 mutation in gastrointestinal tract development

***(first authors) Jean Marie Delalande, Nandor Nagy****, Conor J. McCann, Dipa Natarajan, Julie E. Cooper[5], Gabriela Carreno[5], Alison Campbell, Nicole Lauren, Sophie Thomas[8], Caroline Alby[9], Tania Attié-Bitach, Stanislas Lyonnet, Malcolm P. Logan[1], Allan M. Goldstein, Megan G. Davey, Robert M.W. Hofstr[3], Nikhil Thapar & Alan J. Burns. Talpid[3] regulates multiple aspects of neuromuscular patterning during gastrointestinal development in animal models and human. FRONTIERS IN MOLECULAR NEUROSCIENCE.*

*https://www.frontiersin.org/articles/10.3389/fnmol.2021.757646/abstract)*

***3.)*** **Dr Sonja Hartle, Prof. Bernd Kaspers. Department of Veterinary Sciences, Faculty of Veterinary Medicine, University of Munich, Germany.**

Topic: CXCR4 signaling in hindgut.

***Nandor Nagy,*** *Florian Busaltm Viktoria Halasy, Marina Kohn, Nora Fejszak, Bernd Kaspers, Sonja Härtle (2020) In and out of the bursa - the role of CXCR4 in chicken B cell development. FRONTIERS IN IMMUNOLOGY. | doi: 10.3389/fimmu.2020.01468*

***4.)*** **Dr. Thierry Jaffredo, Université Pierre et Marie Curie, Paris, France;**

Topic: hematopoietic stem cells. ***Nagy N,*** *Dunon D, Gobel T. Jaffredo T, (2021). Development of the hematopoietic system. in: AVIAN IMMUNOLOGY. 3rd Edition, Ed. Vervelde L; Kaspers B; Schat K A.,* *https://www.elsevier.com/books/avian-immunology/kaspers/978-0-12-818708-1*

***5.)*** **Dr. Adam Balic, Prof. Lonneke Vervelde. The Roslin Institute, Edinburgh, UK**.
Topic: Transgenic chickens. ***Nagy N****, Olah I, Vervelde L. (2021). Structure of avian lymphoid system, in: AVIAN IMMUNOLOGY. 3rd Edition, Ed. Vervelde L; Kaspers B; Schat K A.*
*https://www.elsevier.com/books/avian-immunology/kaspers/978-0-12-818708-1*

**6.)** **Prof. Mike Gutnick és Nahum Shpigel, Hebrew University of Jerusalem, Israel,** Topic: Xenotransplantation of human intestine into mouse abdomen or subcutaneous tissue.

7.) **Dr. Donald Newgreen, Murdoch Children's Research Institute, Australia;** Topic: Enteric neural crest cells and intestinal stem cells.
*D. Zhang, B. N. Rollo, **N. Nagy**, L. Stamp, and D. F. Newgreen, "The enteric neural crest progressively loses capacity to form enteric nervous system," DEVELOPMENTAL BIOLOGY, vol. 446, no. 1, pp. 34–42, 2019.*

**8.)** **Dr. Samantha A. Brugmann, Divisions of Plastic Surgery and Developmental Biology, University of Cincinnati College of Medicine, USA**. Topic: Role of Talpid3 mutation in gastrointestinal tract development.
*E. C. Brooks, C. L. B. Paese, A. H. Carroll, J. N. Struve, **N. Nagy**, and S. A. Brugmann, "Mutation in the ciliary protein c2cd3 reveals organ-specific mechanisms of hedgehog signal transduction in Avian Embryos," JOURNAL OF DEVELOPMENTAL BIOLOGY, vol. 9, no. 2, 2021.*

Budapest, 5th of December, 2021.

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https://semmelweis.hu/stemcell/en/