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INCREASED GENE EXPRESSION OF SEMAPHORIN 3A IN THE ENDOTHELIN RECEPTOR-B NULL MOUSE MODEL OF HIRSCHSPRUNG'S DISEASE

Naho Fujiwara[1], Katsumi Miyahara[1], Nana Nakazawa-Tanaka[2], Chihiro Akazawa[3], Atsuyuki Yamataka[1]
[1]Department of Pediatric Surgery, Juntendo University School of Medicine, Tokyo, Japan, [2]Department of Pediatric Surgery, Juntendo Nerima Hospital, Tokyo, Japan, [3]Department of Biochemistry and Biophysics, Graduate School of Health Care Science,, Tokyo, Japan

Aims: Semaphorins have been thought to be guidance cues for developing neurons, implicated in the determination of the migratory pathway for neural crest-derived neural precursors during enteric nervous system development. Previous studies have shown that Semaphorin 3A (SEMA3A) is expressed by the hindgut and a negative regulator for migrating enteric neural crest-derived cells (ENCC). Recently, it has been reported that SEMA3A expression is upregulated in the aganglionic colon in Hirschsprung's disease (HD) patients, suggesting that increased SEMA3A expression may be a risk factor for HD. We developed a *Sox10* transgenic version of the *EDNRB* mouse to visualize ENCC with the green fluorescent protein, Venus. Thus, the aim of our study was to determine the expression of SEMA3A in the gut in this genetically modified mouse model of HD.

Methods: We harvested the mid-hindgut 2 days after birth (D2). *SOX10*-Venus+/*EDNRB*$^{-/-}$ mice were compared with *SOX10*-Venus+/*EDNRB*$^{+/+}$ mice as controls. The relative mRNA expression levels of SEMA3A were determined using real-time PCR (n=8). SEMA3A immunofluorescence was visualized using laser scanning microscopy to assess the distribution of the protein.

Results: On D2, gene expression levels of SEMA3A were significantly increased in the HD group compared to controls, throughout the whole gut (Table). Laser scanning microscopy revealed SEMA3A expression was localized within the submucosa and muscle layer of guts both in HD and controls. In HD, SEMA3A was highly expressed along the whole gut compared to controls (Figure).

Conclusion: In the present study, we demonstrated that SEMA3A expression is increased in the *EDNRB-/-* HD model on D2, suggesting that SEMA3A may interfere with ENCC migration, resulting in an absence of enteric neurons.

Table

	Ileum	Proximal colon	Distal colon
Control	0.43 ± 0.28	4.99 ± 1.31	4.42 ± 1.18
HD	0.57 ± 0.21*	9.14 ± 1.83*	9.86 ± 2.59**

*p<0.05 vs. Control **p<0.005 vs. Control

Figure