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Visualization of enteric neural crest cell migration in SOX10 transgenic mouse gut using time-lapse fluorescence imaging

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Abstract

Background: Enteric neural crest cells (ENCCs) were labeled with VENUS, an enhanced green fluoroscein protein, to record their migration in genetically engineered transgenic (SOX10-VENUS) mice.

Materials and Methods: Pregnant SOX10-VENUS mice were killed on day 12.5 of gestation. The colorectum was excised from each embryo (n = 20) and placed in tissue culture medium. Time-lapse images captured using fluorescence microscopy at 10-minute intervals for 3000 minutes were compiled into a video to display ENCC migration.

Results: At 0 minutes, VENUS$^+$ ENCC were observed to be clustered in the cecum and proximal colon (vagal ENCC), and similar cells were also seen in the rectum/sacrum (sacral ENCC). After 500 minutes, vagal VENUS$^+$ ENCC had migrated caudally from the proximal colon to the midcolon, reaching the distal colon after 800 minutes. Sacral VENUS$^+$ ENCC had migrated rostrally and transversely by 1250 minutes and had integrated with vagal ENCC by 2500 minutes.

Conclusion: We recorded the actual rostral-to-caudal migration of vagal ENCC, caudal-to-rostral migration of sacral ENCC, and their integration in the developing mouse hindgut. Such direct evidence of ENCC migration may further elucidate understanding of ENCC development, thus providing insight into the histopathology of bowel dysmotility disorders.

The enteric nervous system (ENS) is established by the migration of enteric neural crest cells (ENCCs) along the gastrointestinal tract. Normal ENS innervation requires enteric neurons and glial cells derived from the neural crest to undergo extensive migration, proliferation, differentiation, and survive. Several studies have shown there is rostral-to-caudal migration of vagal ENCC that eventually colonize the entire gut [1,2]. The presence of sacral ENCC has also been

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described [2-4], but their development and behavior have yet to be fully elucidated.

Recently, we created genetically engineered transgenic (SOX10-VENUS Tg) mice to visualize the behavior of migrating neural crest cells using time-lapse imaging of embryonic hindgut in organ culture to detect ENCC labeled with an enhanced green fluoroscein protein, VENUS [5]. This study was designed to further investigate the development of the ENS in our novel mouse model (SOX10-VENUS Tg).

1. Materials and methods

1.1. Animals

We raised a SOX10 Tg mouse line according to a protocol described by Shibata et al [5] to visualize neural crest lineage cells with VENUS (i.e., SOX10-VENUS Tg) mice. The genotype of mice and fetuses was determined by using a polymerase chain reaction protocol described elsewhere. The day a vaginal plug was detected was classified as embryonic day 0.5 (E0.5). SOX10-VENUS Tg VENUS positive ($VENUS^+$) pregnant mice were killed on E12.5 or E14.5 by cervical dislocation. All animal procedures were reviewed and approved by the Juntendo University School of Medicine Animal Care and Use Committee (Institutional Review Board No. 230033).

1.2. Time-lapse imaging

Time-lapse imaging was performed as described elsewhere [6]. Briefly, each colon/rectum from embryos (n = 20) obtained from pregnant SOX10-VENUS Tg mice was placed in DMEM/F12 tissue culture medium (Gibco, Grand Island, NY), and images were taken at 10- to 20-minute intervals. Long-term time-lapse imaging, up to 50 hours, was performed with a BZ9000 fluorescence microscope (Keyence, Osaka, Japan), and short-term time lapse imaging, up to 12 hours, was performed with an LSM 510 laser-scanning microscope (ZEISS, Jena, Germany). The images were formatted into a video to allow actual ENCC migration to be observed.

1.3. Whole-mount preparation

Whole-mount tissue was fixed with 4% paraformaldehyde for 1 hour. After washing in phosphate buffered saline (PBS), the tissue was blocked with 3% goat serum-0.1% Triton X-100 in PBS for 30 minutes, then incubated with rabbit polyclonal anti-protein gene product (PGP9.5) (1:1000; Biomol International, Plymouth Meeting, Pa) overnight at 4°C. After washing with PBS, the tissue was incubated for 30 minutes at room temperature with Alexa Fluor594-goat anti-rabbit IgG (1:300; Molecular Probes, Eugene, Ore). Immunofluorescence was detected with an LSM510 laser scanning microscope.

2. Results

2.1. General migratory behavior within the gut

We observed the migration of $VENUS^+$ ENCC in the gut from E12.5. At 0 minutes, $VENUS^+$ ENCCs were observed to be clustered in the cecum and proximal colon (vagal ENCC); similar cells were also seen in the terminal portion of the hindgut (sacral ENCC) (Fig. 1A). After 500 minutes, vagal $VENUS^+$ ENCC had migrated caudally from the proximal colon to the midcolon and had reached the distal colon after 800 minutes (Fig. 1B). Sacral $VENUS^+$ ENCC had migrated rostrally by 1250 minutes (Fig. 1C) and had integrated with vagal ENCC by 2500 minutes (Fig. 1D).

2.2. Migratory behavior of sacral ENCC

To further investigate the migratory behavior of sacral $VENUS^+$ ENCC, we observed the terminal portion of the hindgut from E14.5 using laser scanning microscopy

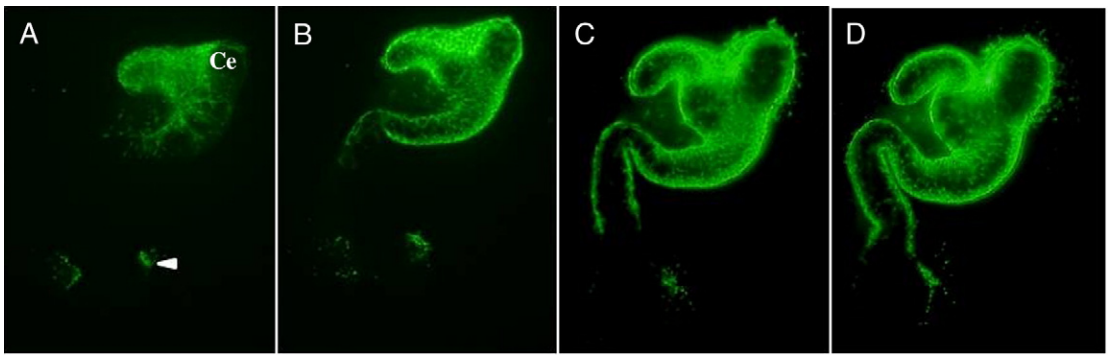

Fig. 1 Migration of $VENUS^+$ ENCC in E12.5 hindgut over time. At 1250 minutes, vagal and sacral ENCCs are still separate, but by 2500 minutes, sacral ENCC have migrated rostrally and have integrated with vagal ENCC: (A) 0 minutes, (B) 800 minutes, (C) 1250 minutes, and (D) 2500 minutes. Ce indicates cecum; arrowhead, indicates sacral ENCC (original magnification, ×5).