



c-Kit-stem cell factor signal-independent development of interstitial cells of Cajal in murine small intestine

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Received: 23 June 2019 / Accepted: 6 October 2019 / Published online: 19 November 2019
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Abstract

c-Kit receptor tyrosine kinase and its ligand stem cell factor (SCF) play critical roles in regulating the development and proliferation of various cells, including the interstitial cells of Cajal (ICC) in the gastrointestinal tract. Many subtypes of ICC are known to be lacking in c-Kit-SCF-insufficient mice, such as W/W^v and Sl/Sl^d , whereas ICC-deep muscular plexus (DMP) in small intestine are not lacking. In this study, we examine ICC-DMP development in normal and c-Kit-SCF signal-insufficient mice. In normal mice, numerous ICC-DMP labeled with c-Kit and neurokinin 1 receptor (NK1R) antibodies were observed only in the duodenum on the day of birth, in the duodenum and the jejunum on postnatal day 4 and throughout the small intestine after postnatal day 6. In W mutant mice (W/W^v , W^v/W^v , W/W), ICC-DMP investigated using c-Kit and NK1R immunoreactivities were similar to that in normal mice. c-Kit ligand SCF-deficient mice (Sl/Sl) also showed almost identical ICC-DMP development and proliferation as normal mice. These results show that the development and proliferation of ICC-DMP occur in the postnatal period independent of c-Kit-SCF signaling.

Keywords Interstitial cells of Cajal · c-Kit · Stem cell factor · Small intestine · Development

Introduction

c-Kit receptor tyrosine kinase plays critical roles in the regulation of cell development in various mammalian organs. c-Kit is activated by the binding of its ligand, stem cell factor (SCF), or c-Kit ligand (KL) that mediate the intracellular signaling cascade through the cell membrane (Lennartsson et al. 2005). In the mouse, the c-Kit gene is allelic to the white-spotting (W) locus on chromosome 5 and SCF gene is allelic to the steel (Sl) locus on chromosome 10 (Chabot et al. 1988; Geissler et al. 1988; Russell 1979). Insufficiency of c-Kit-SCF signaling during the developmental period causes defects of several types of cells, for example, the progenitors of the erythroid cells and mast cells, the stem cells of the melanocytes and the stem cells of germ cells. These results were found in mice with mutations in the W or Sl loci that cause insufficient c-Kit-SCF signaling. These mice showed anemia, insufficient

mast cells, depigmentation of the coat color and sterility. Reduction in the number of hematopoietic cells causes anemia, deficiency of melanocytes causes a white coat color and deficiency of germ cells leads to sterility (Lennartsson et al. 2005). These mice also lacked interstitial cells of Cajal (ICC) in the gastrointestinal tract (Rumessen and Vanderwinden 2003; Sanders et al. 2006).

ICC are mesenchymal cells that form a cellular network in the gastrointestinal musculature and express c-Kit specifically. The ICC contribute to the regulation of motility by generating membrane potentials (slow waves) and mediating neural signal transduction in the gastrointestinal tract (Iino and Horiguchi 2006; Rumessen and Vanderwinden 2003; Sanders et al. 2006). In order to determine the ICC functions, many researches have used small animals that are deficient in ICC and show impairment in the functions that the ICC perform. ICC depend on c-Kit-SCF signaling for their development (Maeda et al. 1992; Huizinga et al. 1995; Ward et al. 1995); therefore, ICC-deficient rodents, such as W mutant rodents, including W/W^v , W^v/W^v , W^{jic}/W^{jic} mice and Ws/Ws rats and Sl mutants, including Sl/Sl^d mouse, have been investigated (Sanders and Ward 2007).

Based on the cellular morphology and distribution patterns, ICC are classified into several subtypes, for instance, ICC-MY

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are localized in the myenteric layer, ICC-IM are intramuscular type ICC in the circular and longitudinal muscles, ICC-deep muscular plexus (DMP) are selectively localized at the small intestinal circular muscle and ICC-SM are situated at the interface between the circular muscle and submucosa in the colon (Iino and Horiguchi 2006; Rumessen and Vanderwinden 2003; Sanders et al. 2006). In the small intestine, there are two subtypes of ICC: ICC in the myenteric layer (ICC-MY) that are multipolar in shape with several processes and ICC in the deep muscular plexus layer in the circular muscle (ICC-DMP) that are bipolar in shape with long processes along the nerve bundles. Thus far, several c-Kit gene mutant animals, such as W/W^v , W^v/W^v and W^{jic}/W^{jic} mice and Ws/Ws rats that have insufficient c-Kit kinase activity are deficient in ICC-MY (Iino et al. 2007; Iino et al. 2011; Sanders and Ward 2007). Similarly, Sl/Sl^d mice that lack the SCF and normal c-Kit signaling show defects in ICC-MY (Huizinga et al. 1995; Sanders and Ward 2007; Ward et al. 1995). Therefore, the development of ICC-MY in the small intestine is strongly influenced by c-Kit-SCF signaling. In contrast, ICC-DMP seem normal in these mutant animals and are suggested to develop and differentiate without c-Kit-SCF signaling.

Current researches using GIST (gastrointestinal stromal tumor) models have revealed that the ETS family transcription factor ETV1 (ETS translocation variant 1) is highly expressed in the ICC and is required for their development (Chi et al. 2010). Mice with gain-of-function mutations in the c-Kit gene showed hyperplasia of the ICC, for example, the ICC-MY proliferate significantly in the small intestine, while ICC-DMP did not (Kwon et al. 2009). LRIG1 (leucine-rich repeats and immunoglobulin-like domains protein 1) specifically regulates the postnatal development of ICC-DMP and colonic submucosal ICC (Kondo et al. 2015). Based on these studies, ICC-DMP are a specific subset to ICC in the developmental stage and in this study, we focus on the development of ICC-DMP in normal mice and c-Kit-SCF signaling-insufficient mice.

Materials and methods

Animals

BALB/c, C57BL/6- $W^v/+$, WB- $W/+$ and WB- $Sl/+$ mice were purchased from Japan SLC (Shizuoka, Japan) and maintained in our laboratory. W/W^v mice were obtained by crossing the $W/+$ and $W^v/+$ parents. W^v/W^v , W/W and Sl/Sl mice were obtained by crossing the $W^v/+$, $W/+$ and $Sl/+$ parents, respectively (Iino et al. 2007). The use and treatment of the animals were in accordance with the guidelines for animal experiments and the regulations for Animal Research at the University of

Fukui. Every effort was made to minimize the number of animals used and their suffering.

Immunohistochemistry

For whole mount preparations, small intestines were placed in 0.01 M phosphate-buffered saline (PBS, pH 7.2) and flushed with PBS; they were then pinned to the Sylgard (Dow Corning Corporation, USA) floor of a dissecting dish and stretched before being fixed in ice-cold acetone for 15 min. The stretched tissues were washed with PBS and the mucosa was removed with sharp dissection. The musculature was removed from the Sylgard dish and washed with PBS containing 0.3% Triton X-100 (PBST) for 1 h with several changes of the solution. Non-specific antibody binding was reduced via incubation of the tissues in normal donkey serum (5% in PBST) for 1 h at room temperature. The tissues were incubated with antibodies rat anti-c-Kit (ACK2, 1:800, eBioscience, USA) and rabbit anti-neurokinin 1 receptor (NK1R, 1:2000, S8305, Sigma, USA) for 12 h at 4 °C. The tissues were then washed with PBST and incubated in secondary antibodies (Alexa Fluor-coupled donkey anti-IgG; 1:500, Molecular Probes, USA) for 1 h at room temperature. After washing with PBS, the specimens on the glass slides were mounted with PermaFluor Aqueous Mounting Medium (Thermo Fisher Scientific, CA, USA).

Fluorescent images were examined with a Leica TCS-SP2 confocal microscope using $\times 60$ oil immersion objective lens (Leica Microsystems, Germany) with excitation wavelengths of 488 nm and 543 nm. The images were collected using Leica Confocal Software (Leica Microsystems, Germany). Adobe Photoshop CS6 (Adobe Systems, CA, USA) was used to compose the final plates.

To count the ICC-DMP, confocal images of the whole mount preparations of the small intestine were collected from 5 to 10 randomly selected areas in 2 specimens from each developmental stage (0–8 days postnatal). The images were collected using $\times 40$ objective lens (image area $380 \times 380 \mu\text{m}$ square) for normal mice specimens or $\times 60$ objective lens (image area $250 \times 250 \mu\text{m}$ square) for mutant mice specimens.

Electron microscopy

For conventional electron microscopy, the tissues were flushed with PBS before being pinned to the Sylgard dissecting dish and stretched to 80% of their resting length before being fixed with the fixative containing 3% glutaraldehyde and 4% paraformaldehyde in 0.2 M phosphate buffer (PB, pH 7.4) for 2 h at room temperature. After rinsing with PB, the specimens were post-fixed with 1% OsO_4 in PB for 2 h at 4 °C. The specimens were then rinsed in distilled water, block-stained with 3% uranyl acetate solution, dehydrated in a graded series of ethyl alcohols and embedded in Epon 812 resin (Epok 812,

Oken, Japan). Ultrathin sections were cut using a Reichert ultramicrotome and double-stained with uranyl acetate and lead citrate for observation under a Hitachi H-7650 transmission electron microscope (Hitachi, Japan).

Results

Development of ICC-DMP

Using c-Kit and NK1R antibodies as specific markers for ICC-DMP (Iino et al. 2004), we were able to visualize and identify ICC-DMP by double immunolabeling in the BALB/c mouse small intestine. Although ICC-DMP have been

reported to develop mainly after birth (Torihashi et al. 1997), we observed c-Kit and NK1R immunopositive cells at embryonic day 18 (ED 18) in the proximal duodenum. Immunolabeling for both markers was still weak at this stage but was unequivocally associated with ICC-DMP. In the post-natal periods, ICC-DMP showed weak c-Kit immunoreactivity and intense NK1R immunoreactivity; therefore, we identified ICC-DMP by NK1R immunoreactivity and then confirmed ICC-DMP by c-Kit immunoreactivity. At the time of birth (P0) (Fig. 1), there were numerous c-Kit and NK1R immunopositive ICC-DMP in the duodenum (574.5 ± 16.9 cells/mm²) (Fig. 2). These ICC-DMP showed intense immunoreactivity for c-Kit and NK1R and made a cellular network as observed in the adult animals. There were a small number

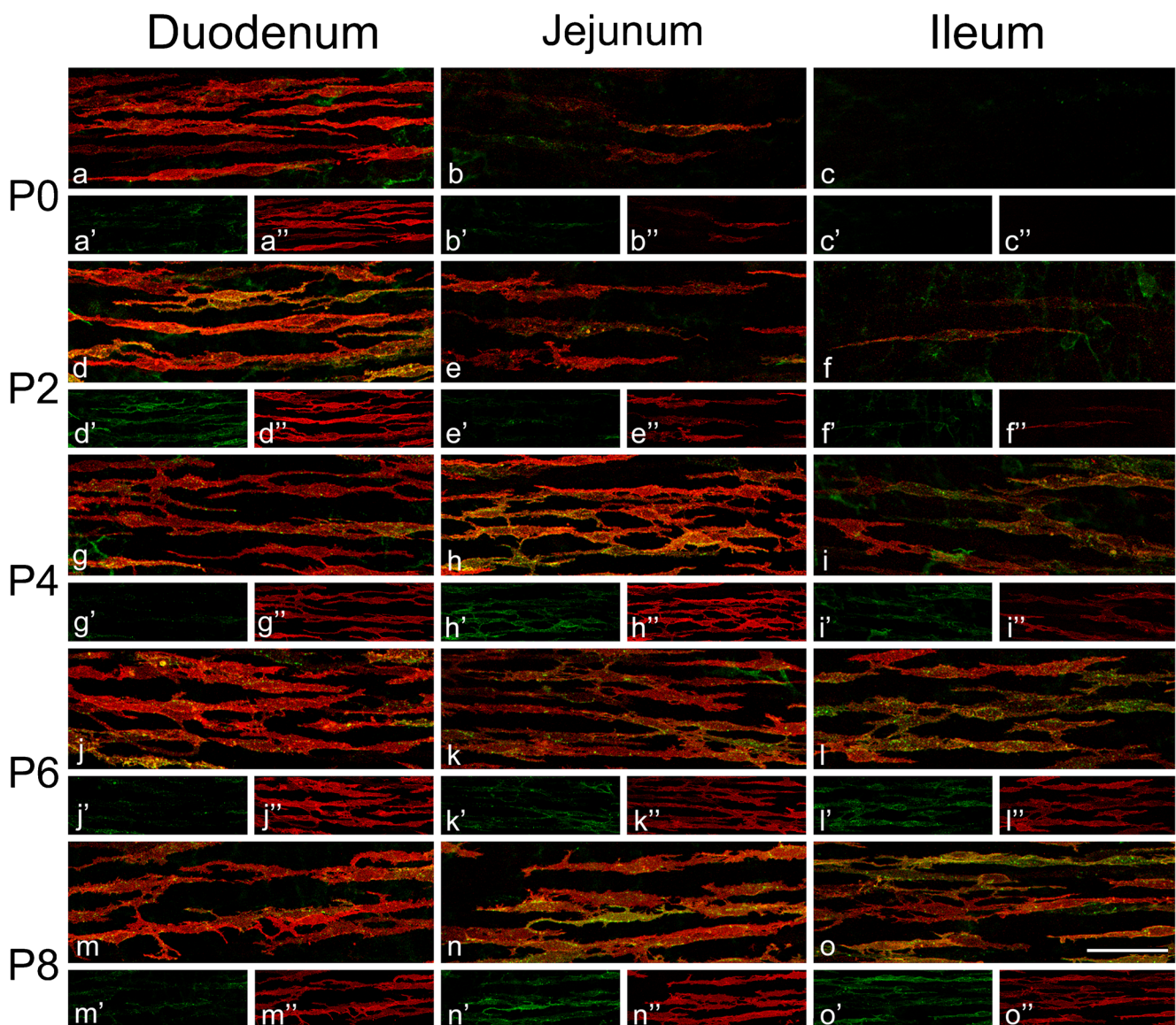
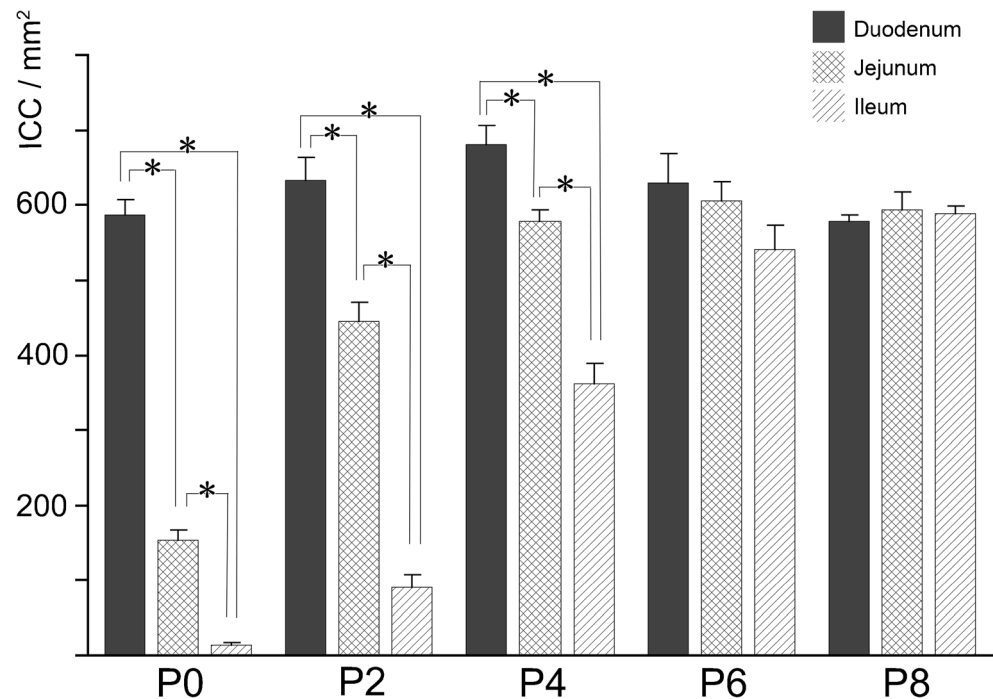


Fig. 1 Postnatal changes in the ICC-DMP in murine small intestine. The distribution of ICC in the duodenum (a, d, g, j, m), jejunum (b, e, h, k, n) and ileum (c, f, i, l, o) is shown for postnatal days 0 (P0), P2, P4, P6 and

P8. ICC-DMP were labeled with anti-c-Kit (shown in green, a'–o') and anti-NK1R antibodies (shown in red, a''–o''). NK1R immunoreactivity is apparent in ICC-DMP. Bar 50 μ m

Fig. 2 Postnatal changes in the numbers of ICC-DMP in the small intestine. The numbers of ICC-DMP labeled with anti-c-Kit and anti-NK1R antibodies on P0 to P8 were counted. On each developmental day, P0, P2 and P4, the numbers among the three regions of the small intestine (duodenum, jejunum and ileum) were significantly different (asterisks). In the ileum, there was a difference in the number of ICC-DMP on P0, P2 and P4; in the jejunum, there was a difference in the number of ICC-DMP on P2, P4 and P6



of ICC-DMP in the jejunum (154.3 ± 13.5 cells/mm²) and very few ICC-DMP in the ileum (14.3 ± 2.6 cells/mm²). The densities of ICC-DMP showed significant differences among the different sites of small intestine. ICC-DMP in the jejunum and the ileum were isolated from each other. From postnatal day 2 to postnatal day 4 (P2–P4) (Fig. 1), the number of ICC-DMP in the jejunum increased significantly (445.5 ± 25.8 cells/mm² at P2 to 578.5 ± 15.6 cells/mm² at P4). From postnatal day 4 to postnatal day 6 (P4–P6), the number of ICC-DMP in the ileum increased significantly (91.0 ± 17.2 cells/mm² at P2, 361.5 ± 27.9 cells/mm² at P4 and 540.5 ± 32.7 cells/mm² at P6) (Fig. 2). The postnatal developmental changes in the number of ICC-DMP numbers in the small intestine are shown in Fig. 2. The density of ICC-DMP in the duodenum remained unchanged from P0 to P8. In the jejunum, the density of ICC-DMP was one-third of that in the duodenum at P0 and increased significantly up to P4. In the ileum, ICC-DMP was hard to observe at P0 and increased significantly up to P6. The density of ICC-DMP in the small intestine was the same in each segment after P6.

ICC-DMP development in c-Kit mutant mice

ICC-DMP have been observed and reported in c-Kit mutant mice, such as W/W^v and W^v/W^v mice (Iino et al. 2007; Sanders and Ward 2007). Both mutant mice showed anemia with pale body color at P0 and W/W^v and W^v/W^v mutants were able to distinguish from $W/+$, $W^v/+$ and $+/+$ mice. In the mutant mice, ICC-DMP were observed in various intensities of c-Kit and NK1R immunoreactivities and immunoreactive patterns; we

carefully examined using $\times 60$ objective lens and confirmed ICC-DMP by their immunoreactivities and morphological features. At P0 (Fig. 3), both mutants had numerous c-Kit and NK1R immunopositive ICC-DMP in the duodenum (496.0 ± 91.2 cells/mm² in W/W^v and 524.8 ± 89.1 cells/mm² in W^v/W^v). In the ileum, we found it difficult to observe ICC-DMP during this period (5.3 ± 7.5 cells/mm² in W/W^v and 4.0 ± 6.9 cells/mm² in W^v/W^v). At P4 in both mutant mice (Fig. 3), we observed numerous ICC-DMP in the duodenum (588.0 ± 52.3 cells/mm² in W/W^v and 592.0 ± 68.8 cells/mm² in W^v/W^v) and the number of ICC-DMP in the ileum increased significantly as observed in normal mice (362.7 ± 39.9 cells/mm² in W/W^v and 396.0 ± 77.0 cells/mm² in W^v/W^v). At P8 in the mutant mice (Fig. 3), there were numerous ICC-DMP throughout the small intestine as observed in normal mice (656.0 ± 69.1 and 538.7 ± 45.9 cells/mm² in W/W^v duodenum and ileum, respectively and 596.0 ± 38.2 and 532.0 ± 67.3 cells/mm² in W^v/W^v duodenum and ileum, respectively). W/W^v and W^v/W^v mice had weak c-Kit kinase activity; therefore, we next observed c-Kit kinase non-active mutant W/W . W/W mutant mice showed severe anemia and high prevalence of death immediately after birth (Horiguchi et al. 2010); therefore, we carefully maintained W/W mutant mice after birth and examined them at P0, P4 and P8 (Fig. 4). At P0, there were numerous NK1R immunopositive ICC-DMP in the duodenum (597.3 ± 49.5 and 16.0 ± 13.1 cells/mm² in duodenum and ileum, respectively). The c-Kit immunoreactivity in ICC-DMP was concentrated at the perinuclear region because c-Kit molecules with W mutation could not translocate to the cell membrane. At P4 and P8, we could observe c-Kit and NK1R

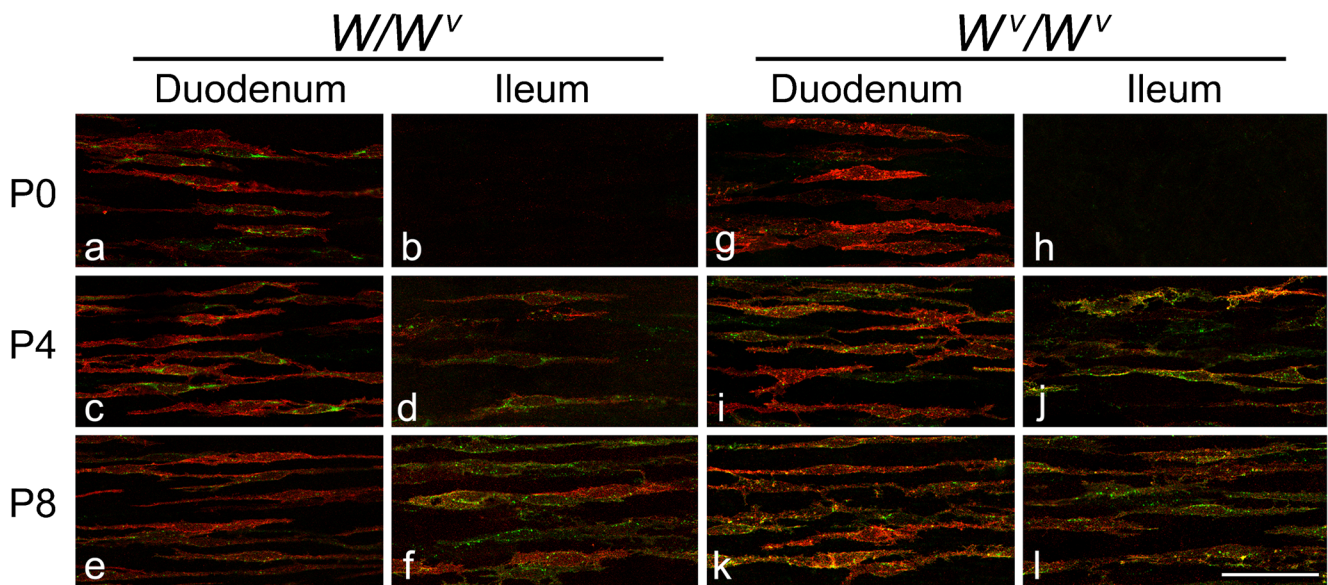


Fig. 3 Postnatal changes in the ICC-DMP in the small intestine of W/W^v and W^v/W^v mice. ICC-DMP in duodenum (a, c, e, g, i, k) and ileum (b, d, f, h, j, l) labeled with anti-c-Kit (green) and anti-NK1R (red) antibodies on

P0, P4 and P8 are shown. The intensity of c-Kit immunoreactivity (green) is weak; however, that of NK1R immunoreactivity is evident. Bar 50 μ m

immunopositive ICC-DMP not only in the duodenum (596.0 ± 52.3 and 617.6 ± 47.2 cells/ mm^2 at P4 and P8, respectively) but also in the ileum (368.0 ± 54.3 and 464.0 ± 80.3 cells/ mm^2 at P4 and P8, respectively).

In order to observe the morphological maturation of ICC-DMP without c-Kit kinase activity, we examined the ultra-structure of the ileal muscular layer of $+/+$ and W/W mice at

P5 (Fig. 5). In the $+/+$ mice, the ICC-DMP were located between the inner and outer circular muscle and were often associated with the nerve fibers. ICC-DMP showed moderate to high electron density in cytoplasm and had numerous caveolae. ICC-DMP in the W/W ileal musculature were similar to those in $+/+$. ICC-DMP had distinct caveolae and were closely associated with the nerve fibers.

Fig. 4 Postnatal changes in the ICC-DMP in the small intestine of W/W mutant mice. ICC-DMP in duodenum (a, c, e) and ileum (b, d, f) labeled with anti-c-Kit (green) and anti-NK1R (red) antibodies on P0, P4 and P8 are shown. The c-Kit immunoreactivity is observed around the perinuclear region in NK1R immunopositive cells. Bar 50 μ m

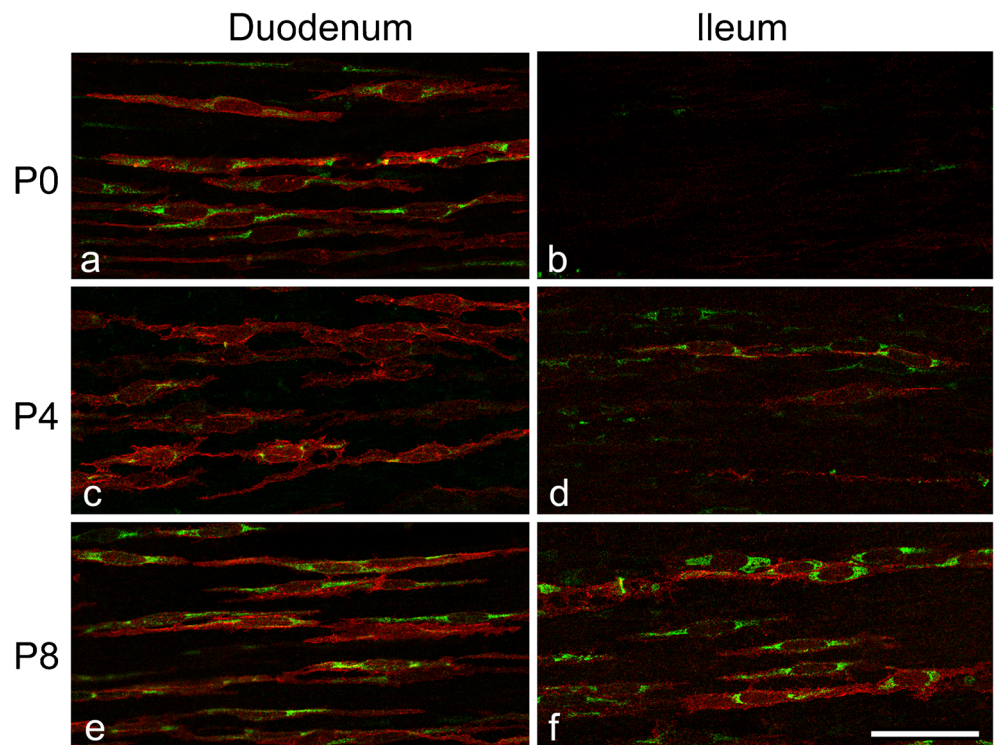
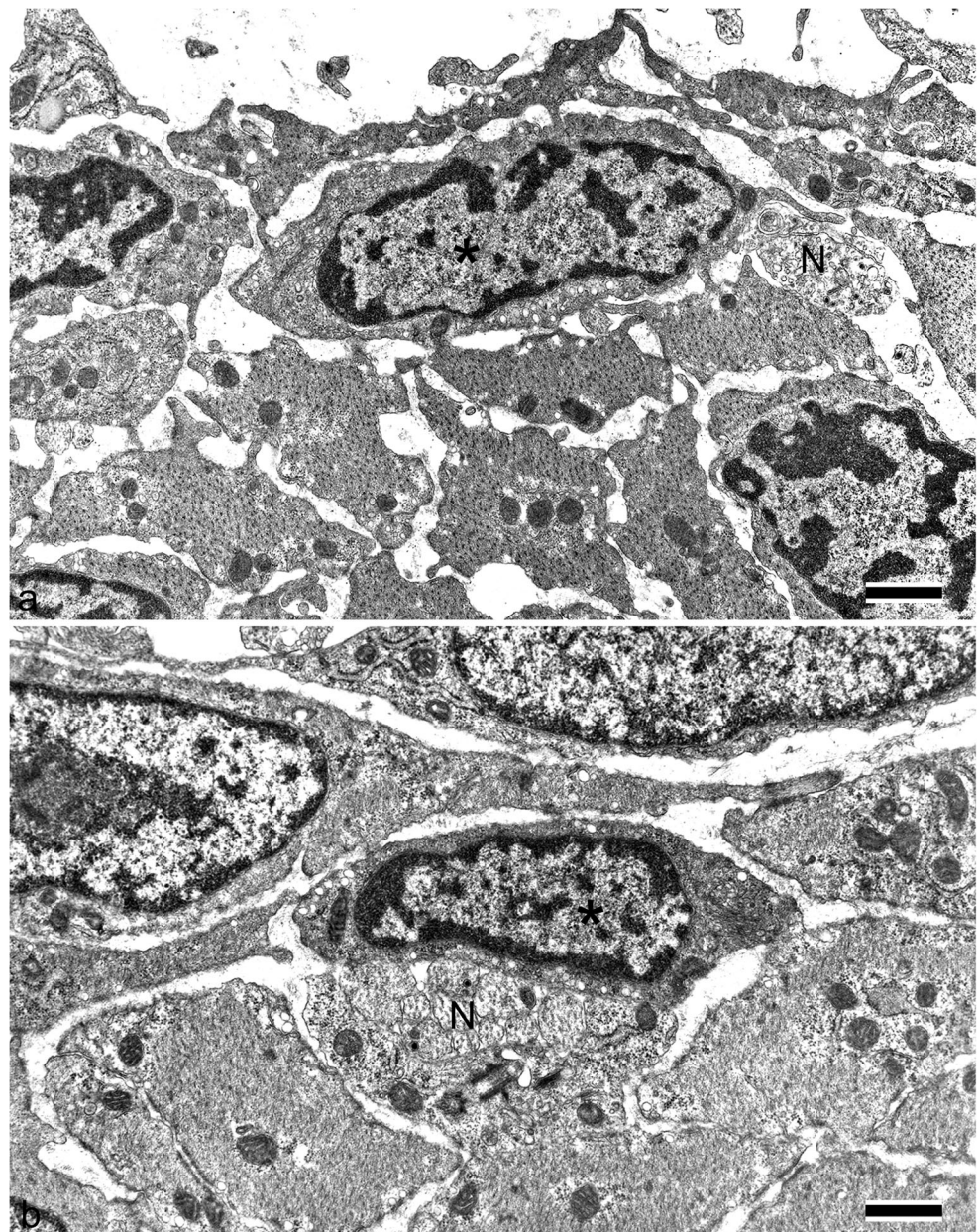


Fig. 5 Ultrastructure of the ICC-DMP in the small intestine of normal and *W/W* mutant mice. ICC-DMP in normal mouse ileum (a) and *W/W* mutant mouse ileum (b) are observed between the inner and outer sublayer of the circular muscle in postnatal day 5 (P5). ICC-DMP (asterisks) in both mice show similar morphological features and were observed in close proximity to the nerve fiber bundles (N). Bars 1.0 μ m



ICC-DMP development of SCF mutant

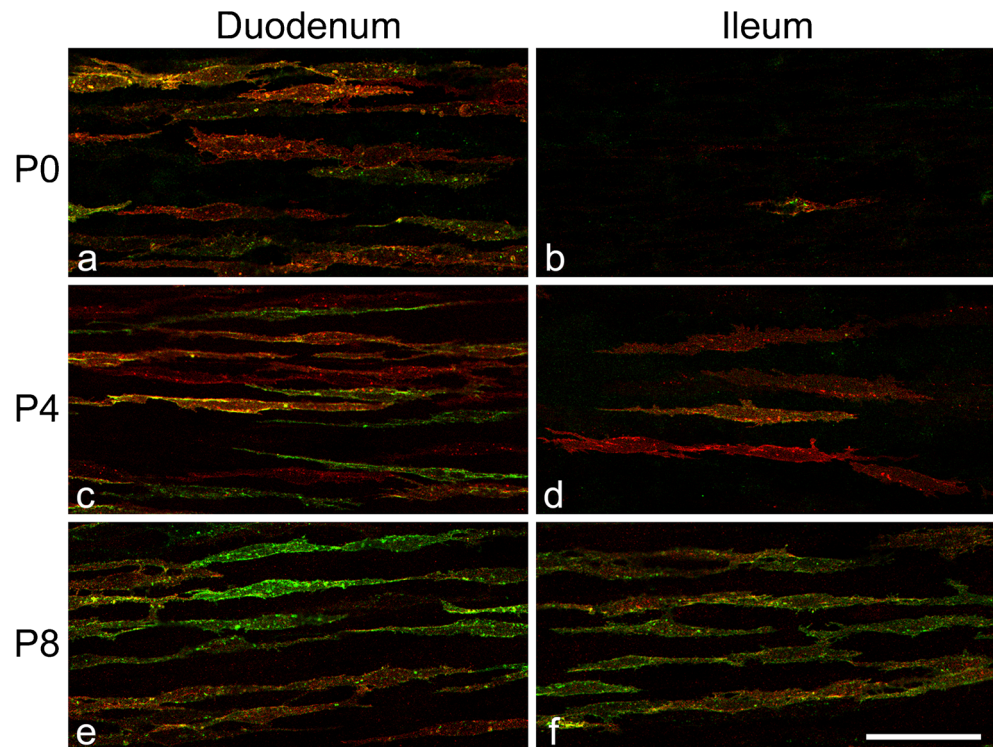
Receptor tyrosine kinase c-Kit is activated by c-Kit ligand SCF; therefore, we next examined SCF-deficient *Sl/Sl* mutant mice (Fig. 6). *Sl/Sl* mutant mice could be determined based on the pale body color with severe anemia on P0. On P0, ICC-DMP with c-Kit and NK1R immunoreactivities were observed numerous only in the duodenum (553.6 ± 66.8 and 19.2 ± 12.0 cells/ mm^2 in duodenum and ileum, respectively). On P4 and P8, the number of ICC-DMP was increased in the ileum (284.0 ± 78.7 and 596.0 ± 23.7 cells/ mm^2 at P4 and P8, respectively) and the morphological features of ICC-DMP were clearly examined with c-Kit and NK1R immunoreactivities. Especially intense c-Kit

immunoreactivity induced by upregulation of c-Kit by SCF deficiency (Yee et al. 1993) was examined.

Discussion

During the embryonic and postnatal period, the development and proliferation of the ICC are known to depend on c-Kit-SCF signaling. c-Kit is a receptor tyrosine kinase that binds to ligand SCF and transduces to downstream signaling cascades, such as phosphatidylinositol 3-kinases (PI3-kinases) signaling, Src family of tyrosine kinases (SFK) signaling, or MAP kinases signaling (Lennartsson and Ronnstrand 2012). In the murine small intestine, c-Kit proteins are first detected on

Fig. 6 Postnatal changes in the ICC-DMP in the small intestine of *Sl/Sl* mutant mice. ICC-DMP in duodenum (a, c, e) and ileum (b, d, f) labeled with anti-*c-Kit* (green) and anti-NK1R (red) antibodies on P0, P4 and P8. Bar 50 μ m



embryonic day 12.5 (E12.5) in undifferentiated cells around the intestinal mucosa (Bernex et al. 1996; Orr-Urtreger et al. 1990; Torihashi et al. 1997; Wu et al. 2000). On embryonic day 14.5 (E14.5), *c-Kit*-expressing cells around the intestinal epithelium also express smooth muscle myosin heavy chain (SMMHC) (Kluppel et al. 1998). After this period, *c-Kit*-expressing cells differentiate into *c-Kit*-positive/SMMHC-negative ICC or *c-Kit*-negative/SMMHC-positive smooth muscle cells in the intestinal musculature (Kluppel et al. 1998). Based on these findings, both ICC and smooth muscle cells differentiate from undifferentiated *c-Kit*-expressing cells and the ICC differentiate depending on *c-Kit*-SCF signaling. There are several studies regarding the insufficiency of *c-Kit*-SCF signaling that induce developmental defects in the ICC. Mutant mice with loss-of-function mutations of *c-Kit* (*W* mutants, such as *W/W^v*, *W^v/W^v*, and *W/W*) show significant defects in the ICC-MY in the small intestine and *c-Kit* ligand SCF mutations (e.g., *Sl* mutants, such as *Sl/Sl^d*) also show ICC-MY deficiency (Sanders and Ward 2007; Ward et al. 1995). These findings were confirmed in our studies (Iino et al. 2007; Iino et al. 2011), including this research. In contrast, gain of *c-Kit*-SCF signaling increases in the number of ICC in the human and murine GI tract. Gain-of-function mutations of *c-Kit* gene are crucial in the pathogenesis of GIST that show ICC hyperplasia and neoplastic transformation (Isozaki and Hirota 2006). These findings strongly indicate that ICC development and proliferation depend on *c-Kit*-SCF signaling.

In the small intestine, there are two distinct types of ICC, ICC-MY and ICC-DMP. Many researchers have observed and reported the existence of ICC-DMP in several *c-Kit* mutant mice (Horiguchi and Komuro 2000; Iino et al. 2007; Iino et al. 2011). Therefore, we considered that this type of ICC develop and differentiate independent of *c-Kit*-SCF signaling. At the beginning of this study, we immunohistochemically observed the development of ICC-DMP in the small intestine and observed the perinatal-to-postnatal development and proximal-to-distal axis development of ICC-DMP. Numerous ICC-DMP are observed in the duodenum on P0, while few are observed in the ileum. On P4, numerous ICC-DMP are observed in the jejunum and on P6, numerous ICC-DMP are observed in the ileum. Torihashi et al. (1995) reported that in the murine small intestine, ICC-DMP was weakly immunoreactive for *c-Kit* on P5 and the immunoreactivity gradually increased by P10. Vannucchi et al. (1997) reported that in the initial days after birth, extremely dispersed ICC-DMP with NK1R immunoreactivity were observed in the rat ileum and on the first 5 days of postnatal life, the ICC formed a continuous layer. Ward et al. (2006) showed that scattered distributed ICC-DMP were observed in the murine jejunum on P0 and numerous cells in the jejunum and ileum on P10. These developmental observations of ICC-DMP in the perinatal-to-postnatal period are in good agreement with the present findings. A functional study conducted by Ward et al. (2006) showed that cholinergic excitatory and nitergic inhibitory neural responses via the ICC-DMP in the jejunum on P0 were poor and, on P10, were intact; this

confirms that the distribution of ICC-DMP in the jejunum was scarce on P0 and substantial after P6. Electron microscopic observations confirm the immunohistochemical and functional studies. At birth, undeveloped ICC-DMP with caveolae and thin filaments were observed; during P5 to P10, ICC-DMP exhibited well-developed features, such as numerous caveolae and mitochondria in the electron-dense cytoplasm (Torihashii et al. 1995) as observed in this study. ICC-DMP develop from undifferentiated cells in the perinatal period and proliferate from the perinatal to postnatal period along the rostral-caudal intestinal axis.

The difference in the developmental period between ICC-DMP and ICC-MY suggests that the developmental signal on ICC-DMP is different from that on ICC-MY. Thus, c-Kit signal insufficient mice such as W/W^v and W^{jic} (Iino et al. 2011; Iino et al. 2007) have been observed to have ICC-DMP distribution in adult animals. In this study, we carefully took care of the mutant neonates and observed ICC-DMP using two markers, c-Kit and NK1R, from the day of birth (P0) to P8 in the small intestinal whole mounts. All W mutants in this study showed gradual development of ICC-DMP from that in the duodenum to that in the ileum (rostral-caudal axis of the intestine) as observed in normal mice. c-Kit ligand SCF-insufficient mutant Sl/Sl mice also showed normal development of ICC-DMP, regardless of ICC-MY deficiency (data not shown). As has been shown in adult W/W^v ICC-DMP with normal ultrastructural features (Horiguchi and Komuro 2000), there were no differences at the ultrastructural level between normal mice and W/W mice in this study. Gain-of-function mutations in the c-Kit gene in $Kit^{V558\Delta}$ mice result in ligand-independent activation of c-Kit, which increases cellular proliferation and facilitates GIST development and displayed hyperplasia of ICC-MY in the small intestine (Kwon et al. 2009). The hyperplasia of the ICC in these mice is observed to develop before birth, while ICC-DMP were not increased in number or density. The results support that ICC-DMP develop c-Kit-SCF signal independency.

The c-Kit-SCF signal independency of ICC-DMP suggests that there are specific factors for ICC-DMP development. Few reports have investigated the factors related to ICC development, except c-Kit. ETV1 is a member of the ETS family transcription factor that directly regulates gene expression and is expressed in the ICC and GIST. Chi et al. (2010) clarified that c-Kit-SCF signaling stabilizes the ETV1 cascade through the MAPK pathway and results in physiological ETV1 transcriptional output critical for ICC development. ETV1 null mice showed significant decreases in the ICC-MY and ICC-IM; ICC-DMP and ICC-SMP are preserved. Recently, Kondo et al. (2015) reported that LRIG1 regulates the postnatal development of ICC-DMP and ICC-SMP and LRIG1 null mice were defective for these ICC. LRIG1 is distributed throughout the circular muscle layer in the postnatal period and LRIG1 and SMMHC expressing

cells differentiate postnatally to ICC-DMP (LRIG1 and c-Kit positive) and smooth muscle cells (LRIG1 and SMMHC positive). Although the molecular mechanisms regarding the differentiation of these cells to ICC or smooth muscle cell are not known, ICC-DMP development does not depend on c-Kit-SCF signaling but on LRIG1 signaling in the perinatal and postnatal period in murine small intestine.

Funding information This work was supported by JSPS KAKENHI Grant Numbers JP17K10626 and JP17K09375 to K.H and S.H.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The animal experiments were approved by the Animal Research Committee, University of Fukui and in accordance with the Regulations for Animal Research at the University of Fukui.

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