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Changes in the Structure and Function of ICC Networks in ICC Hyperplasia and Gastrointestinal Stromal Tumors

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Abstract

Background & Aims—Gastrointestinal stromal tumors (GISTs) express the receptor tyrosine kinase c-kit. Approximately 90% of GISTs have gain-of-function mutations in the Kit gene, which leads to its constitutive activation and drives malignant behavior of GISTs. Interstitial cells of Cajal (ICC) express c-kit; however, it is unknown whether uncontrolled hyperplasia of ICC is responsible for GISTs. Here, we sought to determine whether gain-of-function mutations in Kit lead to hyperplasia of all classes of ICC, whether ICC hyperplasia begins before birth, and whether functional defects occur in ICC hyperplasia or the development of GISTs.

Methods—Heterozygous mutant Kit^{V558} /+ mice that develop symptoms of human familial GISTs and prematurely die from pathology of the gastrointestinal tract were utilized and compared with wild-type controls. C-kit-immunohistochemistry and intracellular electrical recording of spontaneous and nerve-evoked activity were applied to examine the density and functionality of ICC in these mutants.

Results—There was considerable hyperplasia in all classes of ICC throughout the GI tract of Kit^{V558} /+ mice, except for ICC in the deep muscular plexus of the intestine. Spontaneous electrical activity and postjunctional neural responses in hyperplastic ICC tissues appeared normal but were up-regulated in the cecum, where GISTs were commonly found.

Conclusions—Kit gain-of-function leads to hyperplasia of most classes of ICC throughout the GI tract. ICC retain normal pacemaker function and enteric neural responses well after development of hyperplasia.

Interstitial cells of Cajal (ICC) are specialized cells in the gastrointestinal (GI) tract that generate pacemaker activity and mediate enteric motor neurotransmission.^{1–3} ICC are essential for motor patterns of the GI tract. ICC express the receptor tyrosine kinase c-kit,

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Supplementary Data

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and signaling via this pathway is required for development and maintenance of ICC.^{1,2} When c-kit signaling is disrupted, ICC undergo phenotypic changes^{4,5} that result in a decreased density of these cells. Reduced numbers of ICC is a pathologic correlate often identified in GI motility disorders.^{6–8}

Approximately 94% of mesenchymal tumors are positive for c-kit receptors and are defined as gastrointestinal stromal tumors (GISTs).⁹ Thus, c-kit immunohistochemistry has become a standard for diagnosis of GISTs.^{1,10} Expression of c-kit and CD34 by GISTs suggested that these tumors are ICC neoplasms because ICC were the only cell-type expressing CD34 and c-kit.^{9,11} Expression of CD34 by ICC was challenged when it was found that CD34 positive/c-kit negative fibroblast-like cells are distinct from ICC in the human and murine GI tracts.¹² Recently, c-kit^{low}CD44⁺CD34⁺ progenitor cells have been identified that could be responsible for postnatal generation of ICC.¹³ The role of these cells in GISTs has not been evaluated. Taken together, these findings suggest that the cellular origin of GIST is ambiguous.

Human GISTs (80%–90%) have gain-of-function mutations in Kit, particularly in exon 11, encoding the juxtamembrane domain of c-kit.⁹ These mutations result in ligand-independent activation of c-kit, which increases cellular proliferation and facilitates GIST development. In kindreds with human familial GIST syndrome, a germ-line Kit gain-of-function mutation causes cells expressing c-kit to display ligand-independent activation of c-kit. Thus, ICC outside the margins of neoplastic lesions may undergo hyperplasia, as shown, for example, with the Kit^{T557A} familial mutation. ICC hyperplasia is considered a precancerous state.^{14,15} Murine models, carrying gain-of-function mutations in Kit, yield GI lesions similar to human familial GIST syndrome and provide a tool for investigating the development of GISTs and pathophysiology associated with gain-of-function mutations.^{16–19} One model, based on a mutation found in a human familial GIST, employed introduction of a Kit-activating mutation into Kit,¹⁶ Kit^{V558}. Heterozygous Kit^{V558} /+ mice developed neoplastic lesions in the cecum that were similar to human familial GIST.

Most investigators accept that GISTs develop from gain-of-function mutations in Kit, but the functional consequences of gain-of-function Kit mutations on pre-GIST ICC are unknown. We used immunohistochemistry to study the distribution of c-kit+ cells in the GI tract and characterized functions attributed to ICC (pacemaking and neural responses) in wild-type and Kit^{V558} /+ mice. We quantified hyperplasia of c-kit+ cells beyond the site of the GIST in the cecum to determine whether specific classes of ICC are more prone to hyperplasia from a Kit gain-of-function mutation. Our results provide the first comprehensive analysis of ICC structure and function in a murine GIST model with a defined gain-of-function mutation in Kit.

Materials and Methods

Animals

Mutant Kit^{V558} /+ mice were generated as previously described.¹⁶ Heterozygote males were mated with C57BL/6J females to produce Kit^{V558} /+ heterozygotes and homozygote controls. Age-matched mice on the same background as Kit^{V558} /+ mice (C57BL/6J) were

used as controls. Preterm animals were killed at embryonic day (E) 19 and adult animals between postterm day (P) 40 and P60 for morphologic and electrophysiologic studies. Animals were maintained and experiments performed in accordance with the NIH Guide for Care and Use of Laboratory Animals. All procedures were approved by the Institutional Animal Use and Care Committee at the University of Nevada.

Morphologic Analysis and Volume Rendering

Whole mounts and cryostat sections of GI tissues were processed for immunohistochemical analysis of c-kit as previously described.^{3,4} For double-labeling experiments, cryostat sections were incubated in the c-kit antibody (ACK2)^{4,5} overnight followed by phosphate-buffered saline (PBS) (0.01 mol/L; 2 hours) before incubation with a rabbit anti-smooth muscle myosin II heavy chain (1:100; Biomedical Technologies, Stoughton, MA). Sections were subsequently washed in PBS and incubated consecutively in Alexa Fluor 594-coupled goat anti-rat 594 and Alexa Fluor 488-coupled goat anti-rabbit (1:1000; for 1 hour each at room temperature).

Tissues and sections were examined with an LSM 510 Meta confocal microscope (Zeiss, Germany) using excitation wavelengths appropriate for Alexa Fluor 488 and 594. Z-series scans were collected using a $\times 63$ objective and 0.25- μm steps. The confocal aperture was set

1 Airy unit, and laser power and gain settings were kept the same between specimens. Stacks of images were attenuation corrected and deconvolved (AutoQuantX, Media Cybernetics, MD) then imported into custom in-house software (Volumetry G6b, Grant W. Herring). The depths of specific layers of ICC (eg, intramuscular ICC [ICC-IM] or myenteric plexus region ICC [ICC-MY]) were recorded. C-kit-immunofluorescent cells were thresholded (background noise inclusion, $<1\%$), and a modified marching cubes algorithm was used to calculate the subvoxel volume between adjacent optical sections throughout the stack. Both the average slice volume (expressed as percentage of the total slice volume) and the maximum slice volume (averaged over 1 μm) were calculated for each defined ICC layer. Three-dimensional surface renderings were constructed and tilted 40° in the x-axis. Double-labeled images were constructed using Zeiss LSM-5 Image Examiner software (Carl Zeiss Microimaging Inc, Thornwood, NY) and converted to .tif files for final processing in Adobe Photoshop 7.0 (Mountain View, CA) and Corel Draw 12.0 (Ontario, Canada).

Electrophysiologic Studies

Gastric fundus and antrum, jejunum, ileum, cecum, and proximal colon were removed and placed in Krebs–Ringer buffer (KRB). Strips of each organ were mounted with circular muscle upward in a recording chamber, and impalements of circular muscle cells were made with microelectrodes (80–120 M Ω). Transmembrane potentials were recorded as described previously.¹ Nifedipine (1 $\mu\text{mol/L}$) was added to reduce contractions and facilitate maintenance of impalements, except where stated. Slow waves are not affected by nifedipine, as shown previously.^{1,20} Enteric motor neurons were stimulated in muscles strips by electrical field stimulation (EFS; 0.3-ms pulse durations, 1 pulse-20 Hz, train duration of 1 second, 10–15 volts).

Solutions and Drugs

The recording chamber was perfused with oxygenated KRB (in mmol/L): NaCl 118.5; KCl 4.5; MgCl₂ 1.2; NaHCO₃ 23.8; KH₂PO₄ 1.2; dextrose 11.0; CaCl₂ 2.4. The pH of the KRB was 7.3–7.4 when bubbled with 97% O₂–3% CO₂ at 37 ± 0.5°C. Nifedipine (Sigma Chemical Co.; St Louis, MO) was dissolved in ethanol (10 mmol/L) before further dilution in KRB (1 μmol/L). Atropine, apamin, N^ω-nitro-L-arginine (L-NA) and tetrodotoxin (Sigma Chemical Co) were dissolved in deionized H₂O before dilution in KRB to desired concentrations.

Analysis of Data

Data are expressed as means ± standard errors of the mean and tested for significance with Student *t* test. *P* < .05 was taken as significant. The “n” values refer to number of animals. Several electrical parameters were analyzed: (1) resting membrane potential (RMP); (2) slow wave amplitude; (3) duration of slow wave; and (4) frequency. Responses to EFS were analyzed as excitatory junction potentials (EJPs) or inhibitory junction potentials (IJPs) or phase advancement in slow waves. Changes in slow wave duration to EFS were compared with the average of 5 spontaneous events prior to EFS.

Results

Gross Morphology

Kit^{V558} /+ mice and +/+ controls (either E19 or P40–P60) were used. Mice developed enlarged cecums and nodular masses, as previously described.¹⁶ The small intestines of these animals were grossly distended for up to 100 mm above the ileal-cecal junction. Below the cecum and above the distended region of small bowel, the remainder of the GI tract was grossly normal in appearance.

c-kit-Positive Cells in Kit^{V558} /+ Mutants vs Wild-Type Controls

c-kit-Positive cells in Kit^{V558} /+ and +/+ mice were examined using immunofluorescence techniques.

Gastric fundus—In cryostat sections and whole mount preparations of the gastric fundus, c-kit-like immunoreactive (c-kit-LI) cells were observed in the circular (CM) and longitudinal (LM) muscle layers. The cells with c-kit-LI were spindle shaped and dispersed within muscle bundles and ran orthogonal to each other in the CM and LM (Figure 1A and B), as previously described for ICC-IM.³ Density images and calculations of volume of c-kit-positive cells showed that ICC-IM increased significantly in Kit^{V558} /+ mice (Supplementary Table 1; see Supplementary material online at www.gastrojournal.org). The muscle layers were also increased in thickness in association with the expansion in c-kit-positive cells (Figure 1B).

Gastric antrum—Two populations of ICC were observed in the antrum, as previously reported.³ Spindle-shaped ICC-IM were interspersed within the CM and ran parallel to the CM fibers (Figure 1C and D). ICC-MY were present in the intermuscular plane between CM and LM (Figure 1C). c-kit-Positive cells were greatly increased in Kit^{V558} /+ mice (Figure

1D; Supplementary Table 1) to an extent that the ICC-MY developed a mat-like appearance with few intercellular spaces.

Small intestine—In samples of jejunum and ileum, c-kit-positive cells formed an anastomosing network of cells between CM and LM (ICC-MY) and within the deep muscular plexus (DMP) (ICC-DMP) near the submucosal surface of the CM (Figure 2A). ICC-DMP formed a fine network with elongated cell bodies and lateral processes that contacted adjacent ICC-DMP (Figure 2A).

Partial obstruction of the small intestine leads to hypertrophy of the bowel and loss of ICC above the obstruction.²¹ The small intestines of Kit^{V558} /+ mice were obstructed because of the masses in the cecum and greatly distended; however, c-kit-positive cells were abundant and increased in density (Supplementary Table 1). As in the stomach, ICC-MY developed a diffuse, mat-like appearance (Figure 2B). Although there was significant enhancement in ICC-MY, significant hyperplasia was not observed in ICC-DMP (Supplementary Table 1).

Cecum—c-kit-Positive cells formed networks within the CM and LM in the cecum and fine cellular processes contacted adjacent ICC-IM, forming a network. A second population of ICC generally shorter in length and possessing a dendritic morphology with numerous spiny projections was also present. ICC typically ran parallel to CM, but a few cells had no obvious orientation (Figure 3A). In many cases, cecums of Kit^{V558} /+ mice were hard and nodular, and whole mount preparations were not possible. Cryostat sections of these tissues were used to inspect cells with c-kit-LI. c-kit-Positive cells in these tissues formed a mass with no morphologic identity as ICC (Figure 3B; Supplementary Table 1).

Proximal colon—c-kit-Positive cells were located along the submucosal surface of CM (ICC-SM), within CM and LM (ICC-IM), and between CM and LM (ICC-MY; Figure 3C).^{12,22} ICC-SM were orientated parallel to the muscle fibers of CM and ICC-IM orientated parallel to muscle fibers of CM and LM and contacted adjacent ICC with thin processes. ICC-MY possessed multiple fine processes that formed an anastomosing network with adjacent ICC-MY (Figure 3C). c-kit-Positive cells were increased in density in Kit^{V558} /+ mice (Figure 3D; Supplementary Table 1). ICC-MY were increased in number to where it was hard to distinguish individual cells.

Distribution in ICC Before Birth in Kit^{V558} /+ Fetuses

c-kit-Positive cells emerge at approximately E12.5 during normal development and form a network of ICC-MY by E17.⁴ We examined E19 muscles to determine whether the hyperplasia of c-kit-positive cells develops before birth in Kit^{V558} /+ mice. As in adults, c-kit-positive cells were increased in density throughout the GI tracts of fetal tissues. Therefore, gain-of-function of c-kit in Kit^{V558} /+ mice results in hyperplasia of ICC prior to birth, and the ICC population continues to expand after birth (Figure 4).

Functional Changes in the GI Tracts of Kit^{V558} Mice vs Wild-Type Controls

Gastric fundus and antrum—RMP of fundus CM averaged -41.1 ± 0.6 mV in wild-type mice. An ongoing discharge of spontaneous transient depolarizations (unitary

potentials) was superimposed upon resting potentials, as previously described (Figure 5A; $n = 14$)^{3,23} RMPs of antral muscles averaged $+64.1 \pm 1.0$ mV, and electrical slow waves (27.4 ± 3.0 mV in amplitude, 8.9 ± 0.6 seconds) occurred at a frequency of 3.7 ± 0.3 cycles min^{-1} ($n = 14$; Figure 5B).

The electrical activity of gastric muscles of Kit^{V558} /+ mice was similar to wild-type muscles. RMPs of fundus CM averaged -41.1 ± 3.8 mV ($n = 3$), and ongoing unitary potentials were observed (Figure 5A). Antral muscles of Kit^{V558} /+ mice had RMPs of -64.2 ± 1.7 mV ($n = 7$), and slow waves (28.2 ± 4.4 mV in amplitude and 7.5 ± 0.8 seconds) occurred 5.4 ± 0.4 cycles min^{-1} (Figure 5B). There was no statistical difference in slow waves in wild-type and Kit^{V558} /+ mice.

EFS (1–20 Hz for 1 second) caused frequency-dependent IJPs in fundus muscles (Figure 5A). IJPs were reduced by L-NNA (100 $\mu\text{mol/L}$), unmasking an EJP that was inhibited by atropine (1 $\mu\text{mol/L}$). Blocking the EJP revealed a fast transient IJP that was apamin-sensitive (30 nmol/L; not shown). Postjunctional neural responses were similar in fundus muscles of Kit^{V558} /+ mice. EFS caused IJPs averaging 8.3 ± 2.3 mV in amplitude and 2.7 ± 0.2 seconds in duration. L-NNA (100 $\mu\text{mol/L}$) reduced the amplitude and duration of IJPs and unmasked an EJP that was reduced by atropine (Figure 5A).

Antral muscles responded to EFS by generating IJPs averaging 8.9 ± 1.2 mV and 1.8 ± 0.1 s (Figure 5B). The next slow wave was phase-advanced upon termination of the IJP. Kit^{V558} /+ mice also responded to EFS by generating IJPs that averaged 7.3 ± 1.4 mV in amplitude and 1.8 ± 0.2 seconds in duration. IJPs were largely blocked in antral muscles of wild-type and Kit^{V558} /+ mice by L-NNA (not shown).

Small intestine—RMPs of jejunal CM cells of wild-type muscles averaged -59.2 ± 0.6 mV, and slow waves (21.3 ± 2.6 mV in amplitude and 1.2 ± 0.1 seconds in duration) occurred at 35.4 ± 1.4 cycles min^{-1} (Figure 6A). In ileum, RMPs averaged -60.9 ± 0.3 mV, and slow waves 21.7 ± 1.3 mV in amplitude and 1.3 ± 0.1 seconds in duration occurred at 30.2 ± 0.7 cycles min^{-1} (Figure 6A). RMP of jejunal CM of Kit^{V558} /+ mice averaged -61.0 ± 0.8 mV, and slow waves (22.1 ± 2.5 mV in amplitude and 1.3 ± 0.2 seconds) occurred at 32.7 ± 2.1 cycles min^{-1} (Figure 6B). RMP of ileal CM of Kit^{V558} /+ mice averaged -60.9 ± 1.1 mV, and slow waves (29.3 ± 1.8 mV in amplitude and 1.3 ± 0.1 seconds) occurred at 29.3 ± 1.8 cycles min^{-1} (Figure 6B). The pacemaker activity of jejunums and ileums of wild-type and Kit^{V558} /+ mice was not statistically different.

EFS of small intestinal muscles caused frequency-dependent IJPs (eg, 8.6 ± 1.0 mV at 10 Hz) and attenuation of several slow waves following EFS (Figure 6C). IJPs and effects on slow waves were reduced by L-NNA (100 $\mu\text{mol/L}$), although a transient, apamin-sensitive IJP persisted after L-NNA (Figure 6C). EFS of small intestinal muscles from Kit^{V558} /+ mice also caused L-NNA-sensitive IJPs (eg, 6.8 ± 1.0 mV at 10 Hz) and attenuation of several slow waves after EFS (Figure 6D).

Cecum—CM from wild-type cecums had RMPs of -55.0 ± 0.9 mV and was electrically quiescent (Figure 7A). As described above, cecums of Kit^{V558} /+ mice varied in gross

morphology; some contained nodular masses, and others were fluid filled and distended. We were unable to make impalements in cells of tissues with nodular masses. RMP of CM in distended cecums averaged -55.9 ± 0.9 mV. In contrast to wild-type muscles, 33% of cecums of Kit^{V558} /+ mice generated spontaneous slow waves (2 muscles of 9 mice) or spike complexes (1 of 9; Figure 7B). Slow waves and spike complexes were blocked by nifedipine (1 μ mol/L; Figure 7C).

EFS of wild-type cecum muscles consisted of transient IJPs that were insensitive to L-NNA (100 μ mol/L). Neural responses of Kit^{V558} /+ cecums also consisted of L-NNA-insensitive IJPs. In contrast to wild-type muscles, rebound excitation often occurred at the break of IJPs (neural responses not shown).

Proximal colon—RMPs of CM from wild-type colonic muscles averaged -53.0 ± 1.0 mV ($n = 20$ animals). Spontaneous spike complexes (30.0 ± 3.0 mV) occurred at 2.7 ± 0.3 cycles min^{-1} , as previously reported.²² Each complex consisted of 13.8 ± 1.2 action potentials (Figure 8A). Proximal colon CM of Kit^{V558} /+ mice had RMPs of -51.0 ± 3.0 mV ($n = 10$ animals). Spontaneous activity of Kit^{V558} /+ colons was not significantly different than wild-type mice (eg, spike complexes of 35.4 ± 5.2 mV occurred at 2.3 ± 0.3 cycles min^{-1} ; Figure 8B).

EFS (single pulse, 0.3 ms) elicited biphasic IJPs (first and second components were 19.7 ± 2.3 mV and 12.0 ± 1.0 mV and 0.8 ± 0.03 and 8.1 ± 0.3 seconds, respectively; Figure 8C). Similar responses were observed in CM of Kit^{V558} /+ colons (eg, first and second components were 17.5 ± 0.1 mV and 10.7 ± 1.3 mV and 0.8 ± 0.03 and 7.6 ± 0.3 seconds, respectively; Figure 8D; $n = 10$; $P > .05$).

Discussion

We characterized and quantified the density of c-kit⁺ cells in a GIST model with many of the features of human familial GIST, including hyperplasia of ICC beyond the margins of the primary tumor.^{16,17} Extensive hyperplasia of all of the major classes of ICC was observed in the stomach and colon (ICC-IM and ICC-MY) but only ICC-MY, and not ICC-DMP, in the small intestine. Surprisingly, in spite of remarkable expansion in ICC mass, few demonstrable functional differences were observed in Kit^{V558} /+ tissues (pacemaker activity and enteric motor neuron responses). In the cecum, where primary tumors developed, abnormal spontaneous electrical rhythmicity developed in some muscles before nodular masses were found.

Enhancement in ICC was not matched by increased neural responses in Kit^{V558} /+ mice. ICC-IM are innervated by enteric motor neurons but do not require interactions with neurons for development. ICC developed normally in GDNF^{-/-} mice that lack enteric neurons distal to the proximal stomach.²⁴ Likewise, when ICC-IM are absent, varicose processes of enteric motor neurons are distributed normally within GI muscles.³ Synaptic contacts form between enteric motor neurons and ICC-IM during normal development,²⁵ and major inhibitory and excitatory neurotransmitters, nitric oxide and acetylcholine, appear to be largely confined to these junctions.^{3,26} Therefore, increasing the number of ICC-IM per se may not impact

postjunctional neural responses unless this is accompanied by parallel enhancement in nerve terminals or synaptic contacts.

It is harder to reconcile the seeming lack of effect of hyperplasia in most regions on pacemaker activity. Pacemaker activity and active propagation of slow waves are attributed to ICC-MY.^{1,2,27–29} Thus, an increase in ICC-MY as observed in the antrum, small bowel, and colon might be expected to enhance pacemaker frequency or increase the magnitude of pacemaker currents and increase slow wave amplitude. Except for the emergence of spontaneous activity in the cecum, significant effects on slow wave behavior were not observed. During hyperplasia, ICC-MY possibly undergo down-regulation of the pacemaker mechanism or electrical coupling. An evaluation of the pacemaker activity of individual ICC will be needed to determine whether down-regulation of pacemaker function is a feature of pre-GIST hyperplasia.

Resting potentials of wild-type cecum muscles showed electrical quiescence, but recordings from Kit^{V558} /+ cecums before nodular GISTs developed revealed 2 atypical patterns of electrical activity: slow wave activity, more typical of the small intestine, and spike complexes, more typical of the colon. Unlike the slow waves of small intestine, the emergent slow waves in the cecum were blocked by nifedipine. A dramatic rearrangement of ICC and development of novel properties accompanied the hyperplasia of c-kit+ cells in the cecum. Mutant mice that developed neoplasms displayed spindle-shaped c-kit+ cells that differed from ICC in cecums of animals before solid tumors developed. It is unknown whether c-kit+ cells of solid tumors retained ICC function because we were unable to impale cells in these tumors.

Kit^{V558} /+ mice develop GISTs and eventually die from GI pathology,¹⁶ possibly because of an obstruction caused by the mass that develops in the cecum. The observed distension of the small intestine is consistent with the formation of a partial obstruction. Partial obstructions of the small bowel result in distention for up to 100 mm above the obstruction,²¹ and the appearance of the bowel is grossly similar to the small bowels of Kit^{V558} /+ mice after formation of solid tumors in the cecum. In the previous study, ICC were greatly reduced above the site of obstruction.²¹ Loss of ICC was accompanied by loss of electrical slow waves and postjunctional neural responses. These findings contrast to the current study in which ICC were present (normal numbers in the DMP and expanded density in the myenteric region) and electrical slow waves and neural responses were intact in the distended small intestines of Kit^{V558} /+ mice. At present, the reasons for loss of ICC in the obstructed intestine are not fully understood, but c-kit ligand expression decreases in bowel obstruction (Hwang SJ and Ward SM, unpublished observations), and this might explain the loss of ICC in these tissues.³⁰ Reduced c-kit ligand would not be expected to affect ICC in Kit^{V558} /+ mice because Kit^{V558} is a gain-of-function in which c-kit is activated in the absence of ligand binding.¹⁶

The onset of ICC hyperplasia in Kit^{V558} /+ mice occurred prior to birth. Previous studies demonstrated that c-kit signaling prior to birth is required for establishment of the ICC phenotype³¹ and development of functional ICC.^{5,31} Others have argued that c-kit is only important for development and maintenance of the ICC phenotype after birth.^{32,33} Data

from the present study clearly show the significance of c-kit signaling for ICC during the late prenatal period because hyperplasia of ICC networks developed in Kit^{V558} /+ mice before birth.

GISTs were proposed to arise from proliferation of ICC because these were the only cells found to express c-kit and CD34 in the gut.^{11,34} This idea was later challenged because c-kit and CD34 were found to be expressed by discrete populations of cells in the human and murine GI tract,³⁵ and doubts developed about the cellular origin of GIST. Recently, a rare population of putative stem cells (c-kit^{low}CD44⁺CD34⁺) was described that may be responsible for replacement of ICC because of normal turnover and the source of cells for GISTs.¹³ c-kit^{low}CD34⁺CD44⁺ cells required stimulation by insulin-like growth factor-I for proliferation and secondary stimulation by c-kitL to develop the ICC phenotype.¹³ Constitutive activation of c-kit in Kit^{V558} /+ mice might preclude the need for c-kit-L for proliferation of ICC progenitor cells. Stimulation by insulin-like growth factor-I or other growth factors in situ might expand the progenitor cell population throughout most regions of the GI tract normally populated by ICC. It should be noted that hyperplasia of c-kit+/ICC began before birth, so abnormal expansion of this population likely results from overstimulation of early developmental pathways for ICC rather than postnatal activation of a specialized pool of adult stem cells.

We investigated previously the emergence of c-kit-positive cells and cells with the morphologic and functional phenotypes of ICC in the murine small intestine. These experiments showed that c-kit-positive cells line the exterior of the gut at E15 and develop ICC-like morphology and function by E17.³¹ We also found that early c-kit⁺ precursor cells undergo a lineage decision at approximately E15 and develop into either mature ICC or longitudinal muscle cells. We concluded that the lineage decision might depend on c-kit signaling, such that cells receiving c-kitL stimulation from opposing cells developed into mature ICC, but cells without direct contact to c-kitL developed into longitudinal muscles cells. The present study argues against this hypothesis, however, because c-kit is constitutively active in Kit^{V558} /+ and longitudinal muscle developed in these mice (although it was thin and penetrated by c-kit-positive cells in some images). These data suggest that a positive signal (besides the lack of c-kit signaling) might be necessary for early c-kit-positive precursors to develop into a smooth muscle phenotype.³⁶

In summary, Kit^{V558} /+ mice develop neoplastic lesions in the cecum that lead to obstruction, as evidenced by distension of the small intestine. Kit^{V558} /+ mice display dramatic hyperplasia of most classes of ICC, from the stomach to the colon. ICC-DMP of the small intestine, however, were not increased in numbers or density. Despite marked hyperplasia of ICC, functional characteristics are retained, and few differences were noted in the electrical behavior or responses to enteric motor neurons in Kit^{V558} /+ mice. An exception was the emergence of electrical rhythmicity in the cecum. Hyperplasia of ICC develops before birth in Kit^{V558} /+ mice, suggesting that c-kit signaling in the fetus regulates the density and normal distribution of ICC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

EFS	electrical field stimulation
EJPs	excitatory junction potentials
GIST	gastrointestinal stromal tumors
ICC	interstitial cells of Cajal
IJPs	inhibitory junction potentials
RMP	resting membrane potential

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Figure 1.

Hyperplasia of ICC in the stomachs of $\text{Kit}^{\text{V558}}/+$ mice. In each panel (A–D), cryostat cross sections (Aa–Da), whole mounts (Ab–Db), and volume-rendered micrographs (Ac–Dc; see Materials and Methods section) are shown. In each cryostat cross section (Aa–Da), c-kit-LI is labeled in *red* to distinguish intramuscular ICC (ICC-IM) and ICC of the myenteric plexus region (ICC-MY) from smooth muscle cells (SMC) that are labeled with smooth muscle myosin II heavy chain (SMMHC) in *green*. Panels Aa–c show the gastric fundus of $+/+$ mice. ICC-IM are present in both circular (ICC-IM_C; CM) and longitudinal (ICC-IM_L; LM) muscle layers. Panels Ba–c show the gastric fundus from $\text{Kit}^{\text{V558}}/+$ mice. Panels Ca–c show ICC-IM within the circular layer and ICC-MY in the gastric antrum of $+/+$ mice, and panels Da–c show ICC-IM and ICC-MY in the gastric antrum of $\text{Kit}^{\text{V558}}/+$ mice. Scale bars at bottom apply to panels above.

Figure 2. ICC hyperplasia in small intestine of Kit^{V558Δ}/+ mice. Cryostat cross sections (Aa–Ba), whole mounts (Ab–Bb), and volume-rendered micrographs (Ac–Bc) are shown. In cryostat cross sections (Aa–Ba), c-kit-LI is labeled in *red* to distinguish ICC of the deep muscular plexus (ICC-DMP) and ICC-MY from SMC labeled with SMMHC in *green*. Panel A shows images from +/+ mice, and panel B shows images from Kit^{V558Δ}/+ mice. Scale bars at bottom apply to panels above.

Figure 3.

Hyperplasia of ICC in the cecum and colon of *Kit*^{V558} /+ mice. *Panels A–D* show cryostat cross sections (*Aa–Da*), whole mounts (*Ab–Db*), and volume-rendered micrographs (*Ac–Dc*). In cryostat cross sections (*Aa–Da*), c-kit-LI is labeled in red (*ICC-IM* and *ICC-MY*) and ICC along the submucosal surface of the circular muscle (*ICC-SM*) and SMMHC is labeled in green to identify smooth muscle cells. *Panels Aa–c* show the cecum of +/+ mice. *Panels Ba–c* show images from the cecum of *Kit*^{V558} /+ mice. *Panels Ca–c* show images from the proximal colon of +/+ mice, and *Panels Da–c* show images from the proximal colon of *Kit*^{V558} /+ mice. Scale bars at bottom apply to panels above.

Figure 4. c-kit Immunohistochemistry of wholemount preparations from *Kit*^{V558} */+* and *+/+* E19 fetuses. *Panels A and B* show confocal images of ICC-IM from the fundus of *Kit*^{V558} */+* mice, and *panel C* shows ICC-IM from the fundus of an E19 *+/+* fetus. *Panels D and E* show images of ICC-IM and ICC-MY from the antrums of *Kit*^{V558} */+* E19 embryos and *panel F* from a *+/+* E19 fetus. Marked hyperplasia of ICC-IM and ICC-MY occurs prior to birth. Scale bar (100 μm) applies to all panels.

Figure 6.

Intracellular electrical recordings from small intestines of *+/+* and *Kit^{V558Δ}/+* mice. *Panels A and B* show slow waves from jejunums and ileums of *+/+* and *Kit^{V558Δ}/+* mice. *Panels C and D* show neural responses (EFS at arrowheads; 10 Hz, 0.3 ms; 1 s) from ileums of *+/+* and *Kit^{V558Δ}/+* mice. Responses consisted of a transient inhibitory junction potential (IJP) and inhibition of slow wave amplitude for several cycles following EFS. Inhibitory effects of EFS were largely blocked by L-NNA (100 μmol/L). Responses to EFS were not different in *+/+* and *Kit^{V558Δ}/+* mice.

Figure 7.

Electrical activities of cecum of +/+ and *Kit*^{V558} /+ mice. *Panel A* shows the electrical activity of the circular muscle layer of a control animal. *Panel B* shows normal and spontaneous action potentials or slow waves in the cecums of *Kit*^{V558} /+ mice in which spontaneous electrical activity consisted of small “unitary potentials.” Spontaneous action potentials or slow wave activity developed in the cecums of *Kit*^{V558} /+ mice in which solid tumors had not yet formed. Action potentials and slow wave-like depolarizations were inhibited by nifedipine (1 μmol/L; *panel C*).

Figure 8.

Electrical activity and neural responses in proximal colons of +/+ and *Kit*^{V558Δ}/+ mice. *Panels A and B* show that spontaneous electrical activity in the proximal colon was similar in +/+ and mutant animals. *Panels C and D* show neural responses in +/+ and *Kit*^{V558Δ}/+ mice. EFS (single pulse, 0.3-ms duration; *arrowheads*) caused biphasic IJPs, characterized by a rapid transient component followed by a sustained component (*dashed lines*). The sustained component was blocked by L-NNA (100 μmol/L). Atropine (1 μmol/L) in the presence of L-NNA had little effect on responses.