Plasticity of Interstitial Cells of Cajal: A Study in the Small Intestine of Adult Guinea Pigs

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ABSTRACT

Although it is well known that the reduction of interstitial cells of Cajal (ICCs) is associated with several gastrointestinal motility disorders in clinic, it is unknown whether the mature ICCs still have an active plasticity in adult mammals. This study focused on the issues of the reduction of ICCs during Imatinib administration and the recovery of ICCs following drug withdrawal in the small intestine of adult guinea pigs. ICCs were revealed by immunofluorescence on whole mount preparations with anti-Kit, α-smooth muscle actin, (α-SMA), and 5-bromo-2'-deoxyuridine (BrdU) antibodies. Moreover, the occurrence of apoptosis was also assayed. Imatinib treatment led to a gradual reduction of ICCs in number around the myenteric plexus and deep muscular plexus, which was dependent on the time but no apoptosis of ICCs was detected with the TUNEL method. During Imatinib treatment, some ICC-like cells were double labeled for Kit and α-SMA and a few ICC-like cells were only stained with α-SMA. When Imatinib was discontinued, the number of ICCs recovered to normal within 32 days. During this time, some proliferating ICCs were demonstrated by double labeling with Kit and BrdU antibodies. Our results indicated that Kit signaling was essential for the maintenance of survival and proliferation of the mature ICCs in the small intestine of adult guinea pigs. Moreover, ICCs might transdifferentiate to a type of α-SMA⁺ cells, perhaps a phenotype of smooth muscle cells, when there is a loss-of-function of Kit. Anat Rec, 292:985–993, 2009. © 2009 Wiley-Liss, Inc.

Keywords: interstitial cells of Cajal; Kit; SCF; Imatinib; α-smooth muscle actin

Interstitial cells of Cajal (ICCs) play an important role in the regulation of gastrointestinal (GI) motility (Ward and Sander, 2001). They are the pacemaker cells that generate and propagate the slow waves in the alimentary tract, and act as the mediators of inputs from the enteric nervous system to GI smooth muscles and also serve as a stretch sensor of the GI tract. Clinical studies have indicated that quite a number of GI motility disorders, such as diabetic gastroparesis (Horvath et al., 2006), slow transit constipation (Bharucha and Philips, 2001), Crohn's disease and post-operative gut dysfunctions (Porcher et al., 2002; Kiyohara et al., 2003) are...
characterized by a reduction of ICCs’ number and impairment of the cellular network. However, the underlying mechanism for ICCs loss is not yet well known. Clarification of the regulatory factors that control survival and proliferation of ICCs will promote our understanding and treatment of GI motility disorders.

ICCs express the gene product of c-kit (Ward et al., 1994; Huizinga et al., 1995), a proto-oncogene that encodes the receptor tyrosine kinase (Kit). Its ligand, stem cell factor (SCF), is produced by smooth muscle cells (SMCs) and neurons. Inactivation of kit with neutralizing antibodies (Maeda et al., 1992; Torihashi et al., 1997; Torihashi et al., 1999) or Kit blocker (Beckett et al., 2007) in fetal and neonatal animals, or nonlethal mutations of Kit (Ward et al., 1994; Huizinga et al., 1995; Nakama et al., 1998) or SCF (Ward et al., 1995) in rats and mice, leads to defects in ICCs networks and pacemaker activity of GI movement. Therefore, the Kit/SCF signal pathway is considered to be essential for the development, proliferation, differentiation, and survival of ICCs during the fetal and neonatal periods.

In vitro experiments have also shown that the cultured ICCs from the mice only before Day 6 post-partum could proliferate in response to SCF, indicating that the proliferation of ICCs is time-limited and SCF-dependent under culture conditions (Nakahara et al., 2002). However, it is unclear whether the ICCs in adult animals still possess the capacity of proliferation after the numbers of ICCs are lost. The effects of the Kit signal pathway on mature ICCs are still unknown, although ICCs keep expressing the c-kit gene and its product, Kit protein.

Imatinib is a potent inhibitor of Kit and a new clinical therapeutic agent in the treatment of gastrointestinal stromal tumors, which presumably arise from ICCs. It is noted that the use of Imatinib for the treatment of gastrointestinal stromal tumors also leads to some drastic side-effects, e.g., hematologic toxicity, hepatotoxicity, besides a side-effect that causes GI motility disorders. In vitro experiments have also shown that administration of Imatinib results in inhibition of intestinal motility in adult mice and humans (Shimojima et al., 2005; Popescu et al., 2006) and the disappearance of ICCs in organ cultures of the intestine from the fetal and neonatal murines (Beckett et al., 2007).

In view of these facts, Imatinib, as an inhibitor of Kit, may be a potentially useful tool to explore its effect on ICCs in the small intestine of adult mammals in vivo. We have investigated the effects of the Kit signal pathway on the mature ICCs in adult guinea pigs by using Imatinib. Our results indicated that ICCs still possessed plasticity in adult rodents, which involved proliferation and transdifferentiation toward a phenotype of smooth muscle cells and the Kit/SCF signal pathway was essential for the events.

MATERIALS AND METHODS

Animal Treatment

Thirty-five guinea pigs of either sex, aged 5 weeks (weight 250–300 g), were purchased from the Animal Center of the Third Military Medical University (Chongqing, China) and used in our experiments. Imatinib mesylate (Glivec®) was purchased from Novartis Pharma AG (Basel, Switzerland). Twenty-one guinea pigs were intragastrically administrated with Imatinib at a dosage of 90 mg kg⁻¹ for 0–32 days (A0d–A32d) and the animals were examined on Day 0 (A0d), A8d, A16d, and A32d, respectively. Three guinea pigs were used in each group. The Imatinib was stopped on day 32 for the other 9 guinea pigs and they were killed on Day 8, 16, or 32 after drug withdrawal (W8d, W16d, and W32d), respectively. Glucose in water was given as a control to 14 guinea pigs. All experimental animals were killed by overdose of pentobarbital and performed in accordance with our University Health Guide for the Care and Use of Laboratory Animals.

Immunofluorescence

The entire small intestine was removed and enteric contents were washed away with phosphate-buffered saline (PBS). A 10-mm piece of intestine was placed in an optimal cutting temperature (OCT) compound, and quickly frozen with liquid nitrogen. Cross sections (6–8 μm thickness) were cut with a cryostat (Leica, 1850) and fixed with 100% acetone for 15 min (4°C). To obtain whole-mount preparations, the small intestine was inflated with acetone for 30 min (4°C) and opened along the mesenteric border. Then the mucosa was removed and the longitudinal smooth muscle layer containing the ICC-MY and the circular smooth muscle layer containing the ICC-DMP were prepared with the aid of a dissection microscope respectively. The immunostaining procedures have been described previously (Komuro and Zhou, 1996). Briefly, the specimens were incubated with rat monoclonal anti-Kit antibody (ACK2, 1:100; eBioscience) or mouse monoclonal anti-α-SMA antibody (1:100; Santa Cruz), and immunoreactivity was detected by using a Cy3-conjugated secondary antibody (anti-rat IgG, 1:100; Zymed) or a FITC-conjugated secondary antibody (anti-mouse IgG, 1:100; DAKO). The control specimens were prepared in the same manner, but each primary antibody was omitted. The specimens were examined in BX51 fluorescence microscope (Olympus, Japan) or with TCS SP5 confocal laser scanning microscope (Leica, Germany) with an excitation wave-length appropriate for FITC (488 nm), or Cy3 (552 nm). The Z stacking of confocal images at 3–5-μm intervals contained all the levels of positively stained cells and processes.

Detection of ICC Apoptosis

To detect the apoptosis of ICCs, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) was done using an apoptosis detection Kit (Roche). After Kit immunostaining, the specimens were further fixed with 4% paraformaldehyde for 20 min at 25°C, washed twice with PBS for 30 min and then treated with 0.1% Triton X-100 for 20 min on ice. The sections were incubated with TUNEL reaction buffer containing 45 μL label solution and 5 μL enzyme solution at 37°C for 1 hr in a humidified atmosphere in the dark. The sections were then washed with PBS for three times (each 15 min) to remove unincorporated fluorescein-dUTP. The specimens were observed with a fluorescence microscope with an excitation wave-length in the range of 450–500 nm.

Detection of Proliferating ICCs

To identify the proliferation of ICCs, daily intraperitoneal injection of 5-bromo-2-deoxyuridine (BrdU) were
dealt out to all guinea pigs before the animals were killed for 16 days (30 mg m\(^{-2}\) day\(^{-1}\); Sigma) including each experimental animal and control animals. The whole-mount preparations were first stained for ACK2 as described earlier, and then the specimens were labeled for BrdU as follows: the specimens were further fixed in 4% paraformaldehyde in 0.1 M PB at pH 7.2 for 30 min. After rinsing with PBS, the specimens were treated with 2 N HCl for 30 min at 37°C for partial denaturation of double-stranded DNA. To reveal BrdU, the specimens were incubated with a mouse monoclonal antibody raised against BrdU (3 μg mL\(^{-1}\); DAKO) overnight at 4°C and then a FITC-conjugated secondary antibody (anti-mouse IgG, 1:100; DAKO) for 30 min (25°C).

**Measurement**

Photographs of both Kit positive cells and Kit/BrdU double labeled cells were taken in 10 random 0.2607 mm\(^2\) fields (×200 magnification) per whole-mount preparation with a digital camera (SPOT, Diagnostic Instruments) in a BX51 fluorescence microscope (Olympus, Japan). The numbers of either Kit positive cells or Kit/BrdU double labeled cells were counted with Image-Pro Plus 5.0 (Media Cybernetics). In addition, the Kit positive processes in each photograph were traced manually using the Image-Pro Plus software with hand held mouse and process length was automatically measured and recorded. All the values of process length from each sample were summed and mean process length per cell was derived by dividing the total process length by the corresponding number of Kit positive cells in the same sample. Data are expressed as means ± SEM. Five intestinal segments from each experimental animal were sampled in a systematic random manner for the same immunofluorescent staining.

**RESULTS**

Observation of the gross anatomy showed that the intestines of animals treated with Imatinib were normal in color although slightly distended and intestinal peristalsis seemed to be weaker compared with that observed in control and A0d animals.

**Alterations of ICC-MY**

The guinea pigs treated by Imatinib led to duration dependent reduction of ICC-MY in number and in length of the cytoplasmic processes. In the control and A0d groups, the ICC-MY was composed of multipolar cells with three to five primary cytoplasmic processes with secondary and tertiary branches. They formed a cellular network around the myenteric plexus in the space between the longitudinal and circular muscle layers (Fig. 1A,D). Analysis of whole mount preparations showed that the mean number of ICC-MY was 201 mm\(^{-2}\) and the mean process length was about 689 μm per cell in the A0d group. These results were similar to those of the control animals. After 8 days administration, the cell numbers decreased and the length of cytoplasmic processes also reduced. In the A16d group, ICC-MY number had decreased by about 25% and the length of the processes had shortened by nearly 40% compared to those of A0d group. In addition, their cellular networks seemed to be sparser and the cytoplasmic processes appeared to be thinner (Fig. 1B,E). By 32 days, the number of ICC-MY was reduced to about 60% of the A0d value and the cytoplasmic processes showed further shrinkage that their length was 376 μm per cell (Fig. 1C,F).

When the Imatinib treatment was discontinued, the number of ICC-MY and their cellular network were gradually restored. By W8d, the number of ICC-MY and the length of their processes seemed partially restored (Fig. 1I) and by W16d, the number of ICC-MY increased to 147 mm\(^{-2}\) and the length of cytoplasmic processes increased back to about 80% of control values and nearly formed a completed network (Fig. 1H). ICC-MY numbers showed a recovery tendency to normal values by W32d (Fig. 1G), although still less than control in density, indicating that a more than 32 day periods are required for the recovery.

**Alterations of ICC-DMP**

The ICC-DMP is associated with the deep muscular plexus located between the thin inner layer and main layer of the circular smooth muscle. ICC-DMP also showed a similar response to Imatinib as ICC-MY, but the degree of change was milder. The Kit\(^{-}\) ICC-DMP were not obviously reduced in number by A8d and A16d, respectively, until A32d of Imatinib administration at which time their numbers had fallen to about 75% of the A0d value and shrinkage of their processes was also apparent (Fig. 2A,B). After discontinuation of Imatinib, Kit positive ICC-DMP also increased in number and nearly restored to the A0d value by W32d (Fig. 2C) in a similar fashion to that observed in the ICC-MY.

**The Possible Fate of the Reduction ICCs**

No apoptosis was detected by TUNEL labeling, except several apoptotic cells were observed in the epithelium and lamina propria of the intestine. Of particular interest was the findings that a few Kit positive ICC-MY were also stained with α-SMA after administration of Imatinib for 16 days by using Kit/α-SMA double labeling, besides that a number of Kit negative/α-SMA positive ICC-like cells were also found at the level of ICC-MY (Fig. 3A–C). The α-SMA was distributed in the cytoplasm and main branches of the processes of ICC-MY (Fig. 3D–F). The ICC-DMP also showed expression of α-SMA after blockade of Kit, but this occurred later on A32d comparing to that seen in the ICC-MY. Moreover, some Kit negative/α-SMA positive ICC-like cells with short slim processes were also encountered in the same locations, which were distinct from SMCs in morphological features (Fig. 3G–I).

**The Proliferation of ICCs**

We used double immunofluorescent staining with anti-Kit/BrdU antibodies to reveal the proliferation of ICCs. Although BrdU injection was performed for an identical period of time in all experimental animals no Kit/BrdU double labeling ICCs were seen in the control or Imatinib-treated guinea pigs.
Proliferating cells were only seen in the case that Imatinib had been withdrawn for 8 or 16 days. At this time, a number of Kit/BrdU double labeling cells were seen in the ICC-MY. Double-labeled cells were often observed at low magnification (Fig. 4A–C). Among these cells, a few isolated double labeling cells with simple bipolar processes (i.e., simple stick unbranched processes) were also observed (Fig. 4D–F). Some double labeling cells were characterized by a paired distribution with closely adjacent cell bodies and relatively enriched cytoplasm and thick processes with short branches (Fig. 4G–I). Proliferating cell numbers were 8.59 ± 5.06 mm⁻² at W16d (injection of BrdU from W0d to W16d) and 16.69 ± 8.09 mm⁻² at W32d (injection of BrdU from W0d to W32d).
W16d to W32d), respectively. In the ICC-DMP, the Kit/Brdu double labeling cells were often distributed in pairs (Fig. 5A–C) and they were morphologically similar to mature cells, which were characterized by a round cell body and long slender processes (Fig. 5D–F). A few single BrdU labeling ICC-DMP were also seen with enriched cytoplasm and slim processes (Fig. 5G–I).

**DISCUSSION**

This study demonstrated that: (1) the Kit signal pathway was required for the maintenance and survival of mature ICCs in adult guinea-pigs; (2) blocking the Kit signal pathway with Imatinib caused a reduction of Kit positive ICCs in number that was not related to apoptosis but probably a trans-differentiation to a type of α-SMA positive cells, perhaps a phenotype of smooth muscle cells; (3) The proliferation of ICCs depending upon Kit/SCF signal pathway was also involved in the recovery of ICC number in the adult guinea pigs after the withdrawal beside the re-expression of Kit protein in some ICCs. Imatinib was developed as a potent inhibitor of Kit tyrosine kinase and also platelet-derived growth factor receptor kinase (PDGFR) (Manley et al., 2002) which is not reported being expressed by ICCs or smooth muscle cells, so the effects of Imatinib on the small intestine should be mainly induced by the Kit blocking action.

**The Survival of the Mature ICCs**

This study demonstrated that the effects of blocking the Kit signal pathway by Imatinib treatment were time dependent. The number of ICC-MY was clearly reduced to about two-thirds of the control value with a concomitant decrease in the length of their cytoplasmic processes. As a result, ICCs were no longer able to form a completed cellular network, a situation which, in all likelihood, would lead to a marked reduction of GI motility. A number of papers have well documented that the loss of ICCs or blockade of Kit signal would definitely lead to the motility dysfunctions. Our results suggested that the Kit/SCF signal pathway was required for the maintenance of cellular network and survival of the ICCs in adult guinea pigs. Similar results were reported previously that the signal pathway was essential for development of the ICCs during the fetal and neonatal periods (Maeda et al., 1992; Kluppel et al., 1998). In vitro study also showed that blockade of Kit or its down-stream, PI3-kinase, led to a reduction of ICCs in the bowel organ cultures from the adult mice (Ward et al., 1999). It should be noted that the ICC-DMP also showed a Kit-dependent survival response similar to that seen in the ICC-MY, but a longer period of exposure to Imatinib (>16 days) was required, indicating that the ICC-DMP was relatively less sensitive to Kit blocking when compared to the ICC-MY. Similar result was seen in the ICC-DMP of neonatal mice after injections of ACK2 (Torihashi et al., 1995) and of the mice with mutations in the Kit ligand (Ward et al., 1995) and Kit (Sanders and Ward, 2007), however, it was not clear that the different responses between the ICC-MY and ICC-DMP were due to their distinct functions or locations. A different sensitivity to blockade of the Kit/SCF signal pathway was also observed between adult and neonate or fetal animals such that, in adult animals, the ICCs' numbers were not as severely diminished as that observed in neonates after Kit antibody injection (i.e., the injection of ACK2 to neonatal mice led to 90%–100% ICCs lost) (Maeda et al., 1992; Torihashi et al., 1999). Thus, it appears that not only there are differences in sensitivity between the mature ICC-MY and ICC-DMP in the adult mammals but the mature ICCs are also not as sensitive as developing (immature) ICCs to disruption of the Kit signal pathway.

**The Fate of Reduced ICCs**

The disappearance of ICCs following Kit/SCF signal pathway inhibition raises an important question as to the
fate of ICCs during Imatinib treatment. We found no evidence of apoptosis in the ICC regions examined, indicating that cell death could not account for the diminished number of Kit positive cells. It was interesting to note that α-SMA, a structural protein of smooth muscle cells and myofibroblast, not normally found in ICCs, was observed in the remaining ICC-MY and ICC-DMP populations of the Imatinib-treated animals, indicating that the blockade of Kit signal pathway may bring ICCs to a transdifferentiation. Torihashi and colleagues (Torihashi et al., 1999) also reported similar findings in the small intestine of neonatal mice treated with a Kit-neutralizing antibody. In addition, some only α-SMA positive ICC-like cells were also found in the area of ICC-MY and ICC-DMP that were not seen in the controls or during the recovery phase. They resembled ICC-MY or ICC-DMP in morphology and distribution, suggesting that ICCs might transdifferentiate towards a phenotype of α-SMA+ cells during loss-of-function of Kit. It has been well documented that ICCs and the longitudinal smooth muscle cells originate from a same group of

Fig. 3. Confocal microphotographs showing double labeling of Kit (red) and α-SMA (green) on a whole mount preparation counterstained by DAPI (blue) in the Imatinib treated animals. Some of Kit+/α-SMA+ cells (arrowhead) and Kit-/α-SMA+ cells (arrows) were seen in the ICC-MY (A–F) and ICC-DMP (G–I) of the small intestine. Note that α-SMA distributes in the cytoplasm and main branches of (arrowheads) ICC-like cells (F, I). Scale bar: A–C = 60 μm, D–I = 20 μm.
progenitor cells that also support the possibility of transdifferentiation of ICCs (Kluppel et al., 1998). A similar result was also seen in the obstructive small intestine, which has shown a vivid plasticity of ICCs (Ekblad et al., 2001). Although current experiments could not entirely interpret the consequence of the decreasing ICCs in number when blockade of Kit signal pathway, the possibility of a transdifferentiation towards a phenotype of α-SMA⁺ smooth muscle cells may occur. Moreover, the other possibility that the drug affects the Kit transient expression and its discontinuity is followed by the reexpression of the Kit may be also involved. Further study is needed to clarify how many ICCs transdifferentiate or lost of Kit expression after Kit signal pathway blockade.

**Proliferation of the Mature ICCs**

Previous studies have demonstrated that ICCs originate from mesenchymal cells (Lecoin et al., 1996; Kluppel...
et al., 1998), and their proliferation and cytoplasmic process extension is only observed during the fetal and neonatal periods and is dependent on a functional Kit signaling in mammals (Mei et al., 2009). Our previous study provided that ICCs could restore their cellular network via regeneration of ICCs and elongation of their processes following intestinal transection and anastomosis in adult guinea pigs (Mei et al., 2006). In this study, incorporated BrdU was observed in the ICC-MY and ICC-DMP after Imatinib treatment was discontinued. The presence of adjacent labeled cell bodies with thick processes possibly represents that the cells have just finished their cellular division. The number of Kit/BrdU double labeling ICC-MY in the recovery phase accounted for about one-third to two-fifths of the increased ICCs, indicating that the ICCs in adult guinea pigs still possess the ability to divide and repopulate the network such as seen in neonatal animals (Torihashi et al., 1997; Beckett et al., 2007). A study has recently described a small group of cells which might be the ICCs progenitor in the murine stomach (Lorincz et al., 2008) and these cells probably also gave rise to the proliferation of ICCs after

Fig. 5. Confocal images showing the proliferation of ICC-DMP in adult guinea pigs after drug withdrawal. A–C: many double labeled ICC-DMP stained with Kit (red) and BrdU (green) were often distributed in pairs (arrows), and most of them were similar to mature ones (D–F, arrowheads). G–I: showing a double immunoreactive cell with an enriched cytoplasm and several slim processes (arrowhead). Scale bar A–C = 75 μm, D–I = 20 μm.
withdrawal. However, it has been reported previously that the proliferation of ICCs showed a time-limited and age-dependence and ICCs derived from Day 6 neonatal mice will not proliferate in cell culture (Nakahara et al., 2002). The difference may be related to the microenvironment, i.e., in vivo conditions supply factors for cell proliferation that are not present in the culture medium in vitro. No proliferation of ICCs were seen in the control and experimental animals during Imatinib treatment, which also suggested that ICC proliferation was very low in normal condition but this could be altered by depletion of ICCs via blockage of the Kit/SCF signal pathway. It was reported that some trophic factor could govern the proliferation of ICCs (Horvath et al., 2005). The present results suggest that the Kit/SCF signal pathway is also essential for the proliferation of ICCs in the adult animal. The increase in ICC number in these experimental animals may originate from two sources: (1) part of renewed population of ICCs might originate from the proliferation of ICCs; (2) the other possibility that a proportion of ICCs re-expressed the Kit receptor after cessation of drug treatment was also involved in the recovery phase. However, we are not able to determine which of the possibilities might be the main source of ICC repopulation at the moment.

CONCLUSION

This study indicated that ICCs in adult guinea pigs still possess potential abilities of proliferation and transdifferentiation towards a type of α-SMA+ cells. The results from our study will provide insight into understanding occurrence of GI motility disorders and will be helpful for development of anti-GI motility disorders drugs.

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LITERATURE CITED

Beckett EA, Ro S, Bayguinov Y, Sanders KM, Ward SM. 2007. Kit signaling is essential for development and maintenance of interstitial cells of Cajal and electrical rhythmicity in the embryonic gastrointestinal tract. Dev Dyn 236:60–72.

Bharucha AE, Phillips SF. 2001. Slow-transit constipation. Curr Treat Opt Gastroenterol 4:309–315.

Ekblad E, Sjuve R, Arner A, Sundler F. 1998. Enteric neuronal plasticity and a reduced number of interstitial cells of Cajal in hypertrophic rat ileum. Gut 42:836–844.

Horvath VJ, Vittal H, Ordog T. 2005. Reduced insulin and IGFI signaling, not hyperglycemia, underlies the diabetes-associated depletion of interstitial cells of Cajal in the murine stomach. Diabetes 54:1528–1533.

Horvath VJ, Vittal H, Lorincz A, Chen H, meida-Porada G, Redelman D, Ordog T. 2006. Reduced stem cell factor links smooth muscle, macrophage and loss of interstitial cells of cajal in murine diabetic gastroparesis. Gastroenterology 130:759–770.

Huizinga JD, Thuneberg L, Kluppel M, Malyss J, Mikkelsen HB, Bernstein A. 1996. W-kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 379:547–549.

Kiyohara T, Shinomura Y, Isozaki K, Nakahara M, Tsutai S, Nishiyamakura H, Miyazaki Y, Miyagawa J, Matsuzawa Y. 2003. A decreased number of c-kit-expressing cells in a patient with afferent loop syndrome. J Gastroenterol Hepatol 18:390–396.

Kruppel M, Huizinga JD, Malyss J, Bernstein A. 1998. Developmental origin and Kit-dependent development of the interstitial cells of cajal in the mammalian small intestine. Dev Dyn 211:60–71.

Komuro T, Zhou DS. 1996. Anti-c-kit protein immunoreactive cells corresponding to the interstitial cells of Cajal in the guinea-pig small intestine. J Auton Nerv Syst 61:169–174.

Lecoin L, Gabella G, Le Douarin N. 1996. Origin of the c-kit-positive interstitial cells in the avian bowel. Development 122:725–733.

Lorincz A, Redelman D, Horvath VJ, Bardsley MR, Chen H, Ordog T. 2008. Progenitors of interstitial cells of cajal in the postnatal mouse stomach. Gastroenterology 134:1083–1093.

Maeda H, Yamagata A, Nishikawa S, Yoshinaga K, Kobayashi S, Nishii K, Nishikawa S. 1992. Requirement of c-kit for development of intestinal pacemaker system. Development 116:369–375.

Manley PW, Cowan-Jacob SW, Buchdunger E, Fabbro D, Fendrich G, Furet P, Meyer T, Zimmermann J. 2002. Imatinib: a selective tyrosine kinase inhibitor. Eur J Cancer 38 (Suppl 5):S19–S27.

Mei F, Yu B, Ma H, Zhang JJ, Zhou DS. 2006. Interstitial cells of Cajal could regenerate and restore their normal distribution after disrupted intestinal transsection and anastomosis in the adult guinea pigs. Vircows Arch 449:348–357.

Mei F, Zhu J, Guo S, Zhou DS, Han J, Yu B, Li SF, Jiang ZY, Xiong CJ. 2009. An age-dependent proliferation is involved in the postnatal development of interstitial cells of Cajal in the small intestine of mice. Histochem Cell Biol 131:43–53.

Nakahara M, Isozaki K, Vanderwinden JM, Shimomura Y, Kitamura Y, Hirota S, Matsuzawa Y. 2002. Dose-dependent and time-limited proliferation of cultured murine interstitial cells of Cajal in response to stem cell factor. Life Sci 70:2367–2376.

Nakama A, Hirota S, Okazaki T, Nagano K, Kawanoto H, Horii M, Kitamura Y. 1998. Disturbed pyloric motility in Ws/Ws mutant rats due to deficiency of c-kit-expressing interstitial cells of Cajal. Pathol Int 48:843–849.

Popenoe LM, Vidulescu C, Curici A, Caravita L, Simionescu AA, Ciontea SM, Simion S. 2006. Imatinib inhibits spontaneous rhythmic contractions of human uterus and intestine. Eur J Pharmacol 546:177–181.

Porcher C, Baldo M, Henry M, Orsoni F, Jule Y, Ward SM. 2002. Deficiency of interstitial cells of Cajal in the small intestine of patients with Crohn’s disease. Am J Gastroenterol 97:118–125.

Sanders KM, Ward SM. 2007. Kit mutants and gastrointestinal physiology. J Physiol 578:33–42.

Shimojima N, Nakaki T, Morikawa Y, Hoshino K, Kitajima M. 2005. Imatinib blocks spontaneous mechanical activities in the adult mouse small intestine: possible inhibition of c-kit signaling. Pharmacology 74:96–99.

Torihashi S, Ward SM, Nishikawa S, Nishi K, Kobayashi S, Sanders KM. 1995. c-kit-dependent development of interstitial cells and electrical activity in the murine gastrointestinal tract. Cell Tissue Res 280:97–111.

Torihashi S, Ward SM, Sanders KM. 1997. Development of c-Kit-positive cells and the onset of electrical rhythmicity in murine small intestine. Gastroenterology 112:144–155.

Torihashi S, Nishi K, Tokutomi Y, Nishi T, Ward SM, Sanders KM. 1999. Blockade of kit signaling induces transdifferentiation of interstitial cells of cajal to a smooth muscle phenotype. Gastroenterology 117:140–149.

Ward SM, Burns AJ, Torihashi S, Sanders KM. 1994. Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. J Physiol 480 (Part 1):91–97.

Ward SM, Burns AJ, Torihashi S, Harney SC, Sanders KM. 1995. Impaired development of interstitial cells and intestinal electrical rhythmcity in steel mutant mice. Am J Physiol 269:C1577–C1585.

Ward SM, Brennan MF, Jackson VM, Sanders KM. 1999. Role of PI3 kinase in the development of interstitial cells and pacemaking in murine gastrointestinal smooth muscle. J Physiol 516 (Part 3):835–846.

Ward SM, Sanders KM. 2001. Physiology and pathophysiology of the interstitial cell of Cajal: from bench to bedside. I. Functional development and plasticity of interstitial cells of Cajal networks. Am J Physiol Gastrointest Liver Physiol 281:C602–C611.