Journal of Cellular and Molecular Medicine
A JOURNAL OF TRANSLATIONAL MEDICINE

J. Cell. Mol. Med. Vol 16, No 1, 2012 pp. 185-192

Two patterns of development of interstitial cells of Cajal in the human duodenum

Goran Radenkovic *

Department of Histology and Embryology, Faculty of Medicine, University of Nis, Nis, Serbia

Received: September 21, 2010; Accepted: February 15, 2011

Abstract

At the end of the embryonic period of human development, c-kit immunoreactive (c-kit IR) cells identifiable as interstitial cells of Cajal (ICC) are present in the oesophagus and stomach wall. In the small and large bowel, c-kit-IR cells appear later (in the small bowel at 9 weeks, and in the colon at 10–12 weeks), also in the MP region. The object of this study was to determine the timing of appearance and distribution of c-kit IR cells in the human embryonic and foetal duodenum. I used immunohistochemistry to examine the embryonic and foetal duodenum for cells expressing CD117 (Kit), expressed by mature ICC and ICC progenitor cells and CD34 to identify presumed ICC progenitors. Enteric plexuses were examined by way of antineuron-specific enolase and the differentiation of smooth muscle cells was studied using antidesmin antibodies. At the end of the embryonic period of development, c-kit IR cells were solely present in the proximal duodenum in the form of a wide belt of densely packed cells around the inception of the myenteric plexus (MP) ganglia. In the distal duodenum, c-kit IR cells emerged at the beginning of the foetal period in the form of thin rows of pleomorphic cells at the level of the MP. From the beginning of the fourth month, the differences in the distribution of ICC in the different portions of the duodenum were established, and this relationship was still present in later developmental stages. In fact, in the proximal duodenum, ICC of the MP (ICC-MP), ICC of the circular muscle (ICC-CM) and ICC of the septa (ICC-SEP) were present, and in the distal duodenum ICC-MP and ICC-SEP only. In conclusion, in the humans there is a difference in the timing and patterns of development of ICC in the proximal duodenum compared to the distal duodenum.

Keywords: duodenum • C-kit • immunohystochemistry • human

Introduction

Interstitial cells of Cajal (ICC) are specialized network-forming cells that play important roles in the control of digestive motility [1], including the generation of electric slow waves (pacemaker activity) that underlie the phasic contractions of muscles [2–4], nitrergic and cholinergic neurotransmission [5–7] and afferent neural signalling (stretch receptors) [8, 9]. ICC express the gene product of *c-kit*, a proto-oncogene that encodes the receptor, tyrosine kinase Kit. Labelling of Kit receptors or *c-kit* mRNA have provided efficient means of identifying ICC in a variety of preparations, including human specimens [10].

In the human intestine, the circular muscle cells are organized in lamellae, separated by connective tissue septa in continuity with both the submucous layer and the connective space between the muscle layers. The deep muscular plexus (DMP) is located in a well-defined connective tissue space between the thick outer layer and thin inner layer of circular muscle cells. ICC are classified into several subtypes based on topographic, morphologic and functional criteria, as follows: ICC of the myenteric plexus (ICC-MP); ICC of the circular muscle (ICC-CM) located within lamellae of the circular muscle layer; ICC of the septa (ICC-SEP) in the connective tissue septa which separate lamellae of the muscle; ICC of the DMP (ICC-DMP) and ICC of the longitudinal muscle (ICC-LM) [11–14]. All authors agree that ICC-IM and ICC-DMP differentiate, presumably in near-term foetuses, and their differentiation continues after birth [15–17]. Romert and Mikkelsen [18] have suggested that in the human, patterns of distribution of ICC networks are identical in the duodenum, jejunum and ileum; however, Vanderwinden and Rumessen [19] have shown that the first part of the duodenum has a distribution of ICC that departs from the rest of the intestine.

*Correspondence to: Goran RADENKOVIC,
Department of Histology and Embryology,
Faculty of Medicine, University of Nis,
Zoran Djindjic Blv 81, 18000 Nis, Serbia.
Tel.: +381-64-1523456
Fax: +381-18-238770
E-mail: radenkog@gmail.com

doi: 10.1111/j.1582-4934.2011.01287.x

At the beginning of week 4 of embryonic development, the neural crest cells enter the foregut and migrate rostrocaudally to reach the terminal hindgut by week 7, and give rise to the MP [20]. At the end of the embryonic period of human development, c-kit immunoreactive (c-kit IR) cells are present in the oesophagus and stomach wall in the form of a wide belt of cells around the inception of the MP ganglia [21, 22]. In the small and large bowel, c-kit IR cells appear later (in the small bowel at 9 weeks, and in the colon at 10–12 weeks), also in the MP region [16, 17, 23]. Whether or not ICC differentiation requires neural crest cells has not been clearly established, although some recent studies have identified ICC in the absence of neural crest cells [24, 25]. Recent studies have shown that after the emigration of neural crest cells an additional population of cells emigrates from the cranial neural tube. These cells originate in the ventral part of the hindbrain, emigrate through the site of attachment of the cranial nerves and colonize a variety of developing structures, including the gastrointestinal tract. This cell population has been named the ventrally emigrating neural tube (VENT) cells [26–29]. Prenatally, ICC develop from Kit^+ mesenchymal precursors (murine, mice) [30, 31]. ICC-IM of the foregut may also develop from VENT cells [28]. Progenitors committed to ICC have also been described during the early post-natal period [32, 33].

ICC have a central place in research examining intestinal contractions and the etiology and pathogenesis of various motility disorders [19, 34–36]. Histopathologic studies on gastrointestinal stromal tumors (GIST) showed that they were immunopositive for the c-Kit protein [37]. GIST also express CD34 [38, 39], an adhesion molecule also reported in some ICC [38], it has been proposed that a $CD34^+$ subset of ICC may give rise to GIST [40] and represent ICC progenitors. $CD34^+$ cells, mostly known as interstitial Cajal-like cells (ICLCs), are present in the submucosa of the entire human gastrointestinal tract [41]. Recently, telocytes, belonging to the group of ICLCs, were described as a distinctive type of cells [42].

The aim of this study was to investigate the timing of appearance and distribution of ICC populations in the human embryonic and foetal duodenum in parallel with differentiation of nerve structures and smooth muscle cells (SMCs).

Materials and methods

The human material was obtained after legal abortions (0.5–1 hr postmortem) and premature births because of pre-partial deaths according to the principles of the Ethical Committee of the Faculty of Medicine of the University of Nis. Both genders are represented in the sample, and no specimens had gastrointestinal disorders. Gestational ages were estimated by anatomic criteria according to the Carnegie Staging system and the crown-rump length, head circumference and foot length. Each embryo and foetal duodenum specimen was fixed in 10% neutral formalin and paraffin-embedded. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Nis.

Table 1 Antibodies

Antigen	Clone	Supplier	Dilution
C-kit	CD-117	Dako	1 : 300
CD34	QBEnd 10 N1632	Dako	Ready to use
NSE	BBS/NS VI-H14	Dako	1 : 100
Desmin	DE-R-11	Dako	1 : 100

The study material consisted of 7 human embryos and 20 human foetuses, 7–24 weeks gestational age (7 weeks, $n = 3$; 8 weeks, $n = 4$; 9 weeks, $n = 2$; 10 weeks, $n = 3$; 12 weeks, $n = 2$, 14 weeks, $n = 2$; 15 weeks, $n = 3$; 16–20 weeks, $n = 4$ and 21–24 weeks, $n = 4$). Embryos and small foetuses (9 and 10 weeks) were processed completely, sequentially sectioned at 4 μm, and stained. Immunohistochemical analysis was performed using the detection Kit-Polymer. The sections were deparaffined in xylol and a descending series of alcohol rinses ($<$1 min each), then rehydrated in distilled water. The endogenous peroxidase was blocked with 3% H_2O_2 for 10 min at room temperature. This was followed by incubation with the primary antibodies for 60 min at room temperature, rinsing in a phosphate buffered solution (0.1 M PBS, pH 7.4). The primary antibodies were dissolved in Dako antibody diluent (Cat. No. S0809; Table 1). The sections were incubated with streptavidin horseradish conjugate for 30 min at room temperature. The complex was visualised with DAKO Liquid DAB + Substrate/Chromogen System (Code No. K3468) and DAKO AEC + Substrate/Chromogen System (code no. K3469; Dako; see Figs 2H and I and 3E). Immunostaining for CD34 was then performed as previously described. All immunolabelled sections were counterstained by Mayer s hematoxylin. Immunoreactivity was absent in negative controls in which the primary antibody was omitted. Sections were examined with an Olympus BX50 microscope and photographed with an Olympus PM-C35 camera.

The primary antibodies used, and their respective dilutions, are listed in Table 1.

Results

By way of reconstruction of serial sections of each embryo and small foetus, I determined the sections of the proximal duodenal portion, immediately adjacent to the stomach, and then observed its consequential sections which extended distally.

At 7–8 weeks, c-kit IR cells were present in the wall of the proximal duodenal portion. These cells formed a wide belt of densely packed cells in the outer part of the wall (Fig. 1A–D). These cells were pleomorphic, with large oval nuclei and numerous thin processes (Fig. 2D and E). c-kit IR cells completely encircled the inception of the MP ganglia, which were c-kit negative (Fig. 1A–D). From the proximal to the distal portions, the c-kit IR cells were progressively lesser numerous (Fig. 1B and C) up to be completely absent. In this period of development, c-kit IR cells were absent in the sections of the midgut and hindgut (Fig. 1A). In the same period, CD34 IR cells were not observed

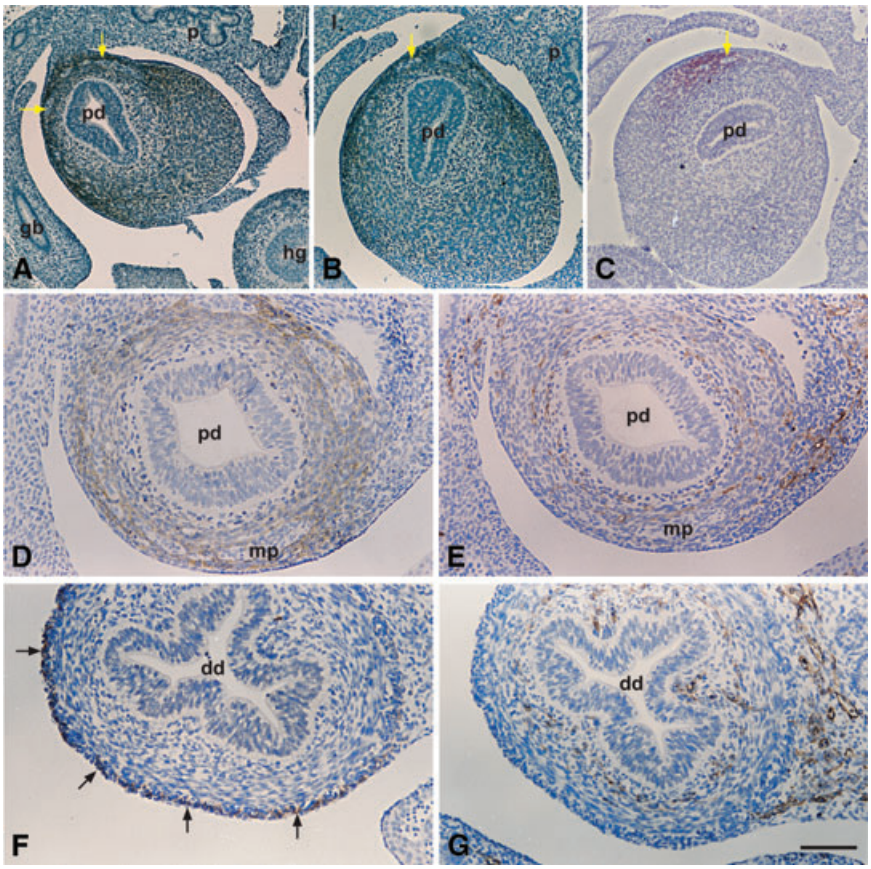

Fig. 1 c-kit (**A–D** and **F**) and CD34 (**E** and **G**) immunohistochemistry. (**A**) Seven weeks, proximal duodenum. C-kit-IR cells located in the outer layers of the developing duodenum, surrounding the presumptive myenteric ganglia (*arrows*). (**B**) Seven weeks, proximal duodenum (about 80 μm distal from section 1A). C-kit-IR cells are less numerous than in proximal sections. (**C**) Seven weeks, proximal duodenum (about 60 μm distal from sections 1B). C-kit-IR cells are present in only one portion of duodenal wall and are less numerous than in proximal sections. (**D**) Eight weeks, proximal duodenum. C-kit-IR cells are present around the myenteric ganglia, in the form of a wide belt of cells. (**E**) Eight weeks, proximal duodenum (consecutive section to 1C). CD34 IR cells located in the submucosa of the developing duodenum. (**F**) Nine weeks, distal duodenum. C-kit-IR cells (*arrows*) form thin layer of cells at the level of the MP. (**G**) Nine weeks, distal duodenum (consecutive section to 1E). CD34 IR cells are located in the submucosa. Pd: proximal duodenum; gb: gallbladder; l: liver; p: pancreas; hg: hindgut; dd: distal duodenum; mp: myenteric plexus. Bar: **A – C** = 200 μm; **D – G** = 100 μm.

at the level of the MP, but they were present in the inner part of the duodenal wall (Fig. 1E). All of the c-kit IR cells were CD34-negative (Fig. 1E).

In the foetal period, there were differences in the distribution of c-kit IR cells in particular portions of the duodenum. To more precisely illustrate the differences, I labelled the parts of the duodenum closer to the stomach (duodenum superior and proximal portion of the descendent duodenum, *i.e.* foregut derivatives) as proximal duodenum, and other parts of the duodenum as distal duodenum (midgut derivatives).

At 9–10 weeks, c-kit IR cells were detected in the proximal and distal duodenum (Figs 1F and 2A–C). In the proximal duodenum, the c-kit IR cells were not as numerous as at the end of the embryonic period. c-kit IR cells were present at the level of the MP and encircled the ganglia, but neither their bodies nor their processes were present within the ganglia (Fig. 2B). In the distal duodenum, c-kit IR cells were less numerous than in the proximal duodenum. c-kit IR cells were present in the form of a narrow band of cells, clearly located at the level of the MP (Figs 1F and 2A and C). In this period of development, CD34 IR cells were located in the submucosa and all of the c-kit IR cells were $CD34^{-}$ (Fig. 1G). At 9–10 weeks, in addition to the already described multipolar c-kit IR cells, spindle-shaped cells also appeared with two long processes originating from the opposite ends (Fig. 2F). All of these cells are designated ICC-MP.

By weeks 11–12, c-kit IR cells were present along the entire length of the duodenum (Fig. 2H and J). No differences were detected in the distribution of these cells in the proximal and distal duodenum. The c-kit IR cells were present at the MP level and enveloped the ganglia (Fig. 2I). ICC-MP were more abundant at the border of the MP with the circular muscle layer (Fig. 2H and J). In addition, I also found a large number of c-kit IR mast cells, but they were easily distinguished from the presumed ICC on the basis of their shape and granular content (Fig. 2G).

At 13–14 weeks, differences in the distribution of c-kit IR cells were observed in the different duodenal regions. In the proximal duodenum, c-kit IR cells were present within the entire circular muscle layer and at the level of the MP (Fig. 3A). In the circular layer, spindle-shaped c-kit IR cells were present, and ran parallel to the longitudinal axis of the adjacent SMCs. These cells correspond to the ICC-CM. ICC-MP are multipolar and spindle-shaped. The ICC-MP do not encircle ganglia completely and are more abundant at the MP border with the circular layer. In the distal duodenum, c-kit IR cells are present at the level of the MP only (Fig. 3B and C).

In the period from week 15–24 of development, ICC were distributed as in the previously described developmental stage. The

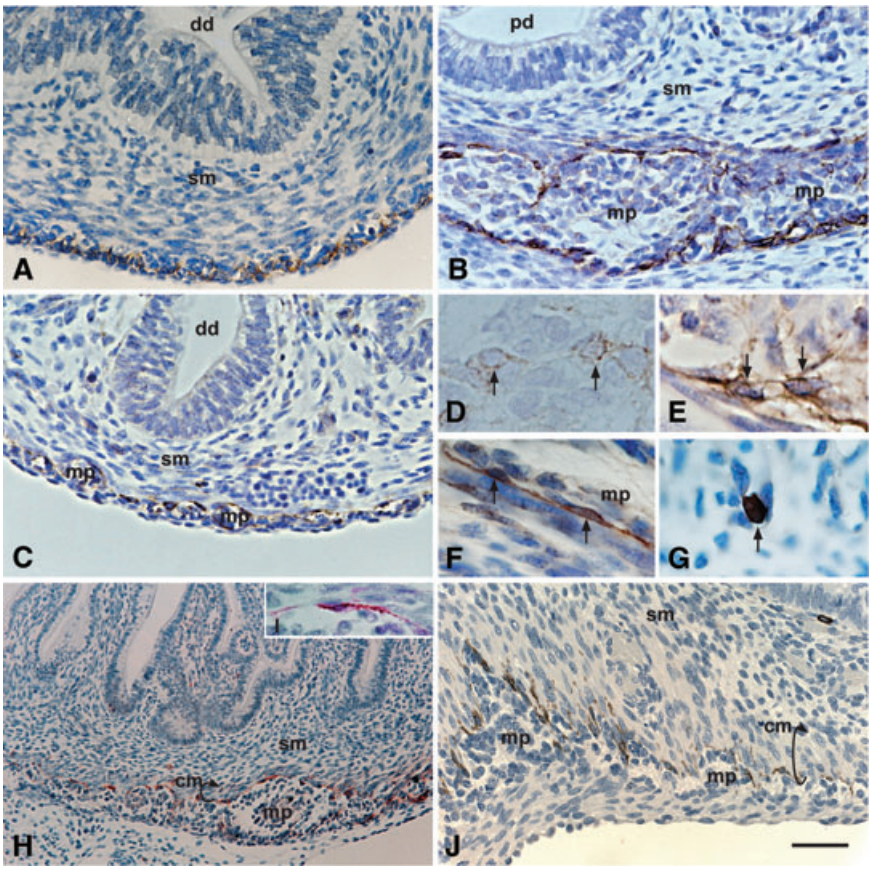

Fig. 2 c-kit immunohistochemistry. (**A**) Nine weeks, distal duodenum. C-kit IR cells are present at the level of the MP. (**B**) Ten weeks, proximal duodenum. C-kit IR cells located around the myenteric ganglia. (**C**) Ten weeks, distal duodenum. C-kit IR cells located at the myenteric plexus level. (**D**) Seven weeks, proximal duodenum. Two pleomorphic c-kit-IR cells (*arrows*). (**E**) Eight weeks, proximal duodenum. Pleomorphic c-kit-IR cells (*arrows*) with thin processes lying at the myenteric plexus level. (**F**) Ten weeks, distal duodenum. Two spindle-shaped ICC-MP appeared to be interconnected. (**G**) Ten weeks, proximal duodenum. In the submucosa, isolated oval c-kit-IR mast cell is seen. (**H**) 12 weeks, proximal duodenum. ICC-MP are located around the myenteric ganglia. (**I**) One spindle-shaped c-kit-IR cell with two processes closely apposed to a myenteric ganglion. (**J**) Twelve weeks, distal duodenum. ICC-MP are present at the level of the MP. pd: proximal duodenum; dd: distal duodenum; p: pancreas; cm: circular muscle layer; mp: myenteric plexus; sm: submucosa. Bar: **A–C, J** = 50 μm; **D–G, I** = 20 μm; **H** = 100 μm.

differences between the proximal and distal duodenum were still present (Fig. 3D and E). ICC-CM were observed only in the proximal duodenum (Fig. 3E and F). ICC were similar in shape and size to the surrounding SMCs and appeared to be interconnected in long rows, extending parallel to the major axis of SMCs (Fig. 3F). In this period, I noted c-kit IR cells inside the connective tissue septa within the circular muscle layer (Fig. 3G). The c-kit IR cells were most commonly spindle-shaped, less frequently multipolar and correspond to ICC-SEP. The c-kit IR cells appeared to be linearly interconnected within the septa and to be connected with three-dimensional networks of cells which formed ICC-MP (Fig. 3G).

At 7–8 weeks, only MP elements were present in the duodenum, and were faintly labelled. By weeks 9–10, the MP was intensely labelled, but the ganglia were more numerous and more prominent in the proximal (Fig. 4A) than the distal duodenum (Fig. 4B). Both myenteric and submucous plexuses were present in foetuses at 12–13 weeks of gestation. At 14–22 weeks, nerve structures were intensely labelled (Fig. 4C).

In the embryos aged 7–8 weeks, desmin immunoreactivity was faint in the cells which will form the circular layer. At 10–11 weeks, desmin immunoreactivity was present in the circular layer and outside the MP at the level of longitudinal muscle layer in the form of a thin row of elongated cells (Fig. 4D). In older foetuses, the muscle layers were intensely labelled and clearly identifiable (Fig. 4E).

Discussion

At the end of the embryonic period of development, in weeks 7 and 8, c-kit IR cells are present in the first portion of the small bowel corresponding to the proximal duodenum. They are distributed in an almost identical pattern as described for the human esophagus [22] and stomach [21] in the same developmental period (Fig. 5). Thus, c-kit IR cells form an uninterrupted wide belt extending throughout the esophagus, stomach (except for the fundus), to the proximal part of duodenum, that is the parts of the digestive tube originating from the foregut [21, 22]. All of the described cells are morphologically very similar. In the same period of development, c-kit IR cells are absent in other parts of the gut, emerging there in the beginning of the foetal period of development [16, 17, 23], although in a different pattern (they are less numerous, distributed in a different manner and morphologically different). At 9–10 weeks, c-kit IR cells appear in the distal

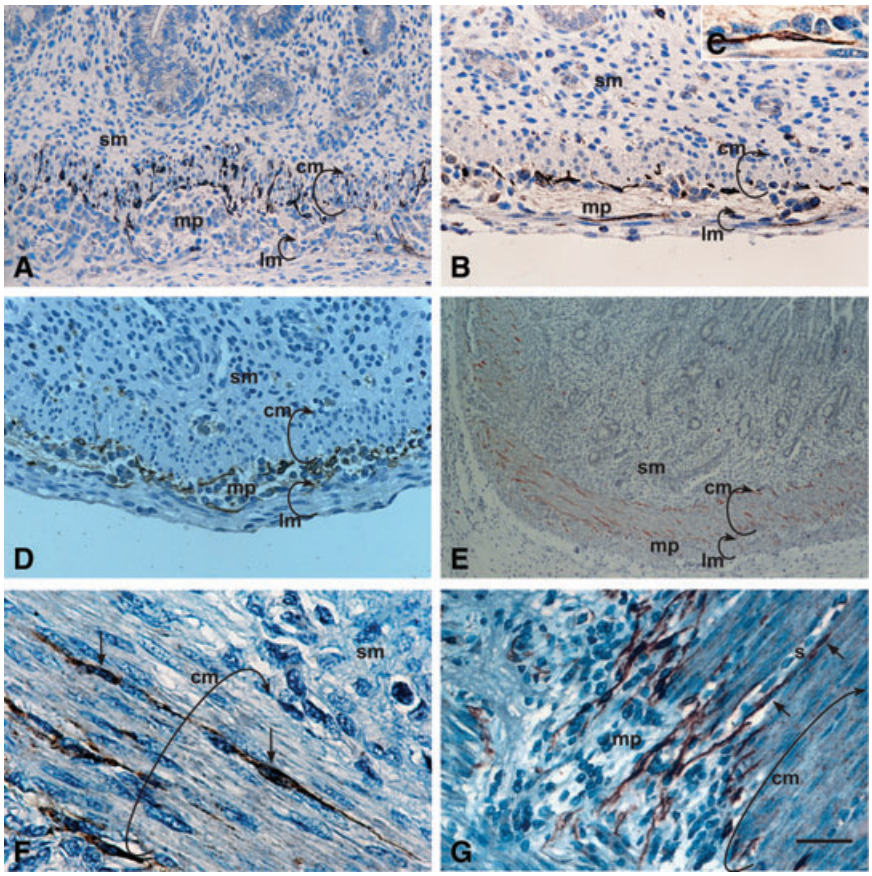

Fig. 3 c-kit immunohistochemistry. (**A**) Fourteen weeks, proximal duodenum. ICC are abundant within the circular muscle layer and around the myenteric ganglia. (**B**) Fourteen weeks, distal duodenum. ICC-MP are present at the myenteric plexus level, but there are no ICC within the circular and the longitudinal muscle layers. (**C**) Fourteen weeks, distal duodenum. One spindle-shaped c-kit-IR cell located at the MP border with the longitudinal layer. (**D**) Sixteen weeks, distal duodenum. ICC are abundant at the myenteric plexus level. (**E**) Eighteen weeks, proximal duodenum. ICC-CM and ICC-MP are numerous. (**F**) Twenty-one weeks, proximal duodenum. ICC-CM are orientated parallel to the long axis of muscle cells. (**G**) Twenty-four weeks, distal duodenum. Two elongated ICC-SEP (*arrows*) orientated parallel with long axis of septa and appeared to be interconnected. cm: circular muscle layer; lm: longitudinal muscle layer; mp: myenteric plexus; sm: submucosa; s: septa. Bar: **A, B, D, G** = 50 μm; **C, F** = 20 μm; **E** = 200 μm.

duodenum and other parts of the gut in the form of narrow linear rows of cells, situated at the level of the MP (Fig. 5). A wide belt of cells, as described earlier, is not present. At the same time, the number of c-kit IR cells in the esophagus, stomach and proximal duodenum is reduced and they are situated at the level of the MP [21, 22]. Based on these observations, the pattern of appearance of c-kit IR cells in the esophagus, stomach and proximal duodenum differs from that in the distal duodenum and other parts of the gut. The reason for these differences should be lay in the fact that the esophagus, stomach and the proximal duodenum develop from the foregut, whereas the distal duodenum and the remaining gut develop from the midgut and hindgut [43].

The results of Bockman and Sohal [26], Sohal *et al.* [28] and Dickinson *et al.* [29] show that VENT cells colonize the foregut only (chick, quail and duck), and differentiate into neurons and glial cells of the ENS, ICC and epithelium in the duodenum and stomach. They specified several possibilities why the colonization is restricted to the duodenum and stomach, and one of them was that the environment of the foregut may be ideal for the rapid differentiation of the VENT cells. It is well documented that such an arrangement exists for the neural crest cells. For example, vagal crest cells adjacent to somites one to two in chicks, and somites six to seven in mice colonize the foregut only [44–46]. The similarity with the distribution of c-kit IR cells in the human gastrointestinal tract near the end of the embryonic period of development is apparent, as well as the fact that these cells are abundant in the parts developing from the foregut. These facts suggest two possibilities. First, VENT cells are those responsible for the differences in the development of ICC in the foregut compared to other portions of the gut. Secondly, the foregut environment favours rapid proliferation of c-kit IR cells, which later, during the foetal period, migrate to other parts of the gut.

c-kit IR cells present in the wall of the proximal duodenum at the end of the embryonic period are $CD34^{-}$. According to the hypothesis by Huizinga [47], the possible pathways of ICC differentiation can be extended to the human gastrointestinal tract; my findings indicate that c-kit IR cells present in the proximal duodenum at the end of the embryonic period of development are in fact already differentiated, mature ICC [48]. In this case, the process of ICC differentiation in the proximal duodenum, as well as in the esophagus and stomach, takes place before week 7 of development. The other option is that c-kit IR cells present in the wall of the proximal duodenum at the end of the embryonic period represent the common precursors of ICC and SMCs, so that a number

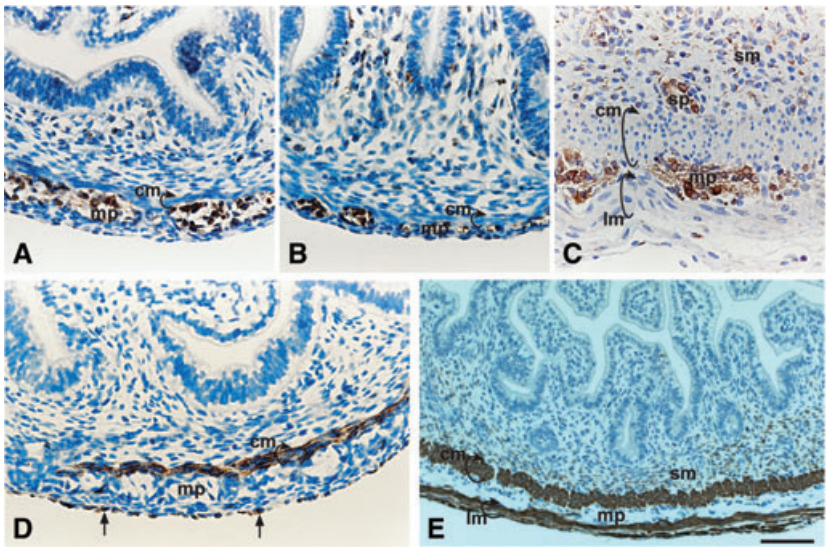

Fig. 4 neuron specific enolase (**A–C**) and desmin (**D** and **E**) immunohistochemistry. (**A**) Ten weeks, proximal duodenum. The myenteric neurons are labelled. (**B**) Ten weeks, distal duodenum. The myenteric ganglia are less prominent than in the proximal duodenum. (**C**) Sixteen weeks, proximal duodenum. Both the myenteric and submucous plexuses are present. (**D**) Ten weeks, proximal duodenum. DES-IR is present in the circular muscle layer and in the very thin longitudinal muscle layer (*arrows*). (**E**) Fourteen weeks, proximal duodenum. Both the circular and the longitudinal muscle layers are DES-IR cm, circular muscle layer; lm: longitudinal muscle layer; mp: myenteric plexus; sm: submucosa; sp: submucous plexus. Bar: **A–D** = 50 μm; **E** = 100 μm.

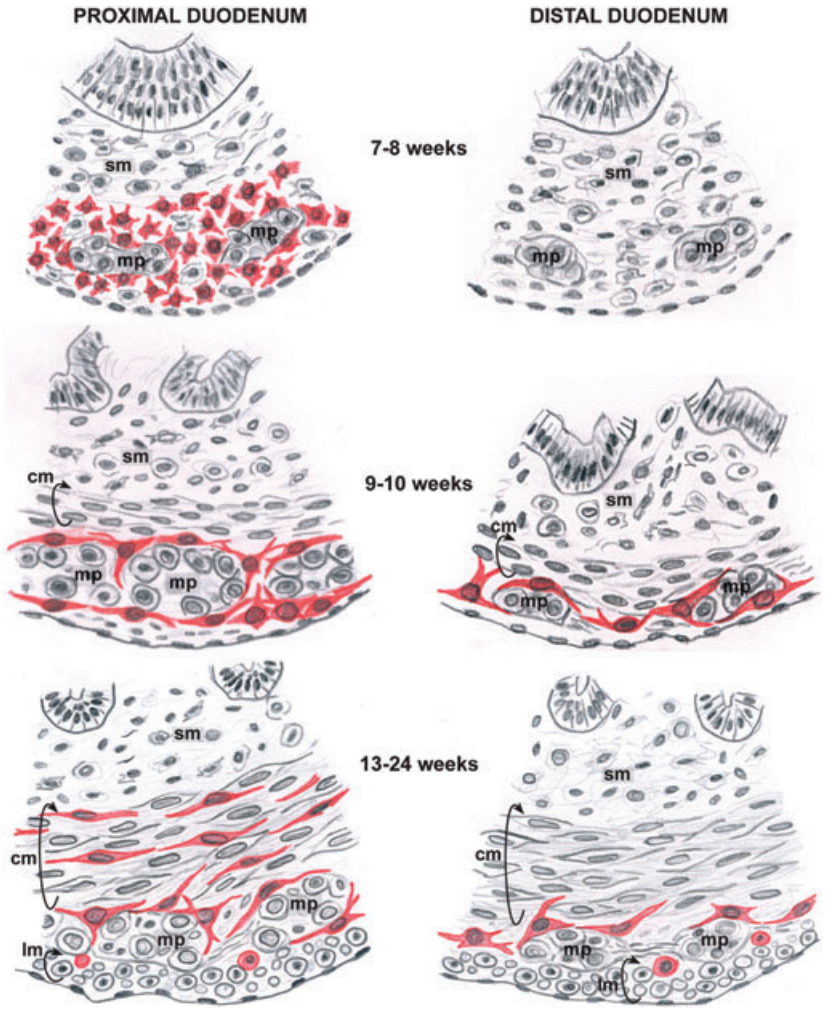

Fig. 5 Distribution of c-kit IR cells (*red colour*) in human embryonal and foetal duodenum. cm: circular muscle layer; lm: longitudinal muscle layer; mp: myenteric plexus; sm: submucosa.

of them differentiate into ICC which remain in the MP region, and the rest differentiate into SMCs. The papers of numerous authors support this hypothesis, demonstrating that ICC and SMCs share the same precursors [30, 31]. Needless to say, this is just a hypothesis requiring confirmation.

My results show that the longitudinal muscle layer appears 2–3 weeks after the circular muscle layer and that the MP develops approximately 2 weeks before the submucous plexus. This finding is consistent with earlier studies [20–23].

At the beginning of the fourth month of development, c-kit IR cells appear in the entire circular muscle layer in the proximal duodenum (Fig. 5). They correspond to ICC-CM described in the stomach and the esophagus in this period of development. This finding differs from the results obtained by Junquera *et al.* [49], who assert that ICC-CM are star-shaped (stellate). In the distal duodenum, c-kit IR cells are not present in the circular layer, which makes it similar to other parts of the small bowel [19, 50]. In particular, ICC-CM appear in the small bowel substantially later, around week 36 of development [15–17]. Lee *et al.* [51] have described ICC-MP and ICC-DMP in the rat duodenum.

The differences described could be viewed in light of the findings by Sohal *et al.* [28] regarding the role of VENT cells in ICC-CM differentiation, that is some VENT cells differentiate into ICC-CM [29]. My findings demonstrate that ICC-CM are present solely in the proximal duodenum (originating from the foregut) and that they are absent in the distal duodenum. I speculate that a limited colonization of the gastrointestinal tract with VENT cells is responsible for the differences in ICC distribution between the proximal and distal duodenum.

The differences in ICC distribution are maintained postnatally when their distribution in the proximal duodenum is very similar to gastric distribution, whereas the distribution in the distal duodenum is almost identical to the jejunal and ileal distribution [19, 36, 52, 53].

In conclusion, there is a difference in the time and pattern of development of ICC in the proximal duodenum compared to the distal duodenum, and in the fourth month of development the differences in distribution of ICC are established and maintained during later developmental period.

Acknowledgements

The author thank Mile Randjelovic for his technical assistance. The author also thank Milkica Tosic Djordjevic for her precious help in immunohistochemical procedures.

Conflict of interest

The author confirm that there are no conflicts of interest.

References

1. **Sanders KM.** A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology*. 1996; 111: 492–515.
2. **Ward SM, Burns AJ, Torihashi T, *et al*.** Mutation of the proto-onkogene c-kit blocks development of interstitial cells and electrical rhytmicity in murine intestine. *J Physiol*. 1994; 480: 91–7.
3. **Torihashi S, Ward SM, Nishikawa SI, *et al*.** C-kit-dependent development of interstitial cells and electrical activity in the murine gastrointestinal tract. *Cell Tiss Res*. 1995; 280: 97–111.
4. **Huizinga JD, Thuneberg L, Kluppel M, *et al*.** W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature*. 1995; 373: 347–9.
5. **Burns AJ, Lomax AEJ, Torihashi S, *et al*.** Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. *Proc Nat Acad Sci USA*. 1996; 93: 12008–13.
6. **Ward SM, Morris G, Reese L, *et al*.** Interstitial cells of Cajal mediate enteric inhibitory neurotransmission in the lower esophageal and pyloric sphincters. *Gastroenterology*. 1998; 115: 314–29.
7. **Ward SM.** Interstitial cells of Cajal in enteric neurotransmission. *Gut*. 2000; 47: 40–3.
8. **Thuneberg L, Peters S.** Toward a concept of stretch-coupling in smooth muscle. I. Anatomy of intestinal segmentation and sleeve contraction. *Anat Rec*. 2001; 262: 110–24.
9. **Suzuki H, Ward SM, Bayguinov YR, *et al*.** Involvement of intramuscular interstitial cells in nitrergic inhibition in the mouse gastric antrum. *J Physiol*. 2003; 546: 751–63.
10. **Maeda H, Yamagata A, Nishikawa S.** Requirement of c-kit for development of intestinal pacemaker system. *Development*. 1992; 116: 369–75.
11. **Thuneberg L.** Interstitial cells of Cajal: intestinal pacemaker cells? *Adv Anat Embryol Cell Biol*. 1982; 71: 1–130.
12. **Hanani M, Farrugia G, Komuro T.** Intercellular coupling of interstitial cells of Cajal in the digestive tract. *Int Rev Cytol*. 2005; 242: 249–82.
13. **Komuro T.** Structure and organization of interstitial cells of Cajal in the gastrointestinal tract. *J Physiol*. 2006; 576: 653–8.
14. **Iino S, Horiguchi K.** Interstitial cells of Cajal are involved in neurotransmission in the gastrointesinal tract. *Acta Histochem Cytochem*. 2006; 39: 145–53.
15. **Faussone-Pellegrini MS, Vannucchi MG, Alaggio R, *et al*.** Morphology of the interstitial cells of Cajal of the human ileum from foetal to neonatal life. *J Cell Mol Med*. 2007; 11: 482–94.
16. **Wester T, Eriksson L, Olsson Y, Olsen L.** Interstitial cells of Cajal in the human fetal small bowel as shown by c-kit immunohistochemistry. *Gut*. 1999; 44: 65–71.
17. **Kenny SE, Connell G, Woodward MN, *et al*.** Ontogeny of interstitial cells of Cajal in the human intestine. *J Pediatr Surg*. 1999; 34: 1241–7.
18. **Romert P, Mikkelsen HB.** C-kit immunoreactive cells of Cajal in the human small and

large intestine. *Histochem Cell Biol*. 1998; 109: 195–202.
19. **Vanderwinden JM, Rumessen JJ.** Interstitial cells of Cajal in human gut and gastrointestinal disease. *Microsc Res Tech*. 1999; 47: 344–60.
20. **Fu M, Tam PK, Sham MH, Lui VCH.** Embryonic development of the ganglion plexuses and the concentric layer structure of human gut: a topographical study. *Anat Embryol*. 2004; 208: 33–41.
21. **Radenkovic G, Savic V, Mitic D, *et al*.** Development of c-kit immunopositive interstitial cells of Cajal in the human stomach. *J Cell Mol Med*. 2010; 14: 1125–34.
22. **Radenkovic G, Ilic I, Zivanovic D, *et al*.** C-kit-immunopositive interstitial cells of Cajal in human embryonal and fetal oesophagus. *Cell Tiss Res*. 2010; 340: 427–36.
23. **Wallace AS, Burns AJ.** Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. *Cell Tiss Res*. 2005; 319: 367–82.
24. **Huizinga JD, Berezin I, Sircar K, *et al*.** Development of interstitial cells of Cajal in a full-term infant without an enteric nervous system. *Gastroenterology*. 2001; 120: 561–7.
25. **Wu JJ, Rothman TP, Gershon MD.** Development of the Interstitial Cell of Cajal: origin, kit dependence and neuronal and nonneuronal sources of kit ligand. *J Neurosci Res*. 2000; 59: 384–401.
26. **Bockman DE, Sohal GS.** A new source of cells contributing to the developing gastrointestinal tract demonstrated in chick embryos. *Gastroenterology*. 1998; 114: 878–82.
27. **Sohal GS, Ali MM, Galileo DS, Ali AA.** Emigration of neuroepithelial cells from the hindbrain neural tube in the chick embryo. *Int J Dev Neurosci*. 1998; 16: 477–81.
28. **Sohal GS, Ali MM, Farooqui FA.** A second source of precursor cells for the developing enteric nervous system and interstitial cells of Cajal. *Int J Dev Neurosci*. 2002; 20: 619–26.
29. **Dickinson DP, Machnicki M, Ali MM, *et al*.** Ventrally emigrating neural tube (VENT) cells: a second neural tube-derived cell population. *J Anat*. 2004; 205: 79–98.
30. **Torihashi S, Ward SM, Sanders KM.** Development of c-Kit-positive cells and the onset of electrical rhythmicity in murine small intestine. *Gastroenterology*. 1997; 112: 144–55.
31. **Kluppel M, Huizinga JD, Malysz J, Bernstein A.** Developmental origin and Kit-dependent development of the interstitial cells of Cajal in the mammalian small intestine. *Dev Dyn*. 1998; 211: 60–71.
32. **Faussone-Pellegrini MS.** Cytodifferentiation of the interstitial cells of Cajal related to the myenteric plexus of mouse intestinal muscle coat. An E.M. study from foetal to adult life. *Anat Embryol (Berl)*. 1985; 171: 163–9.
33. **Liu LW, Thuneberg L, Huizinga JD.** Development of pacemaker activity and interstitial cells of Cajal in the neonatal mouse small intestine. *Dev Dyn*. 1998; 213: 271–82.
34. **Sanders KM, Ordog T, Ward SM.** Physiology and pathophysiology of the interstitial cells of Cajal: from bench to bedside. IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal. *Am J Physiol Gastrointest Liver Physiol*. 2002; 282: G747–56.
35. **Vanderwinden JM, Liu H, De Laet MH, *et al*.** Study of the interstitial cells of Cajal in infantile hypertrophic pyloric stenosis. *Gastroenterology*. 1996; 111: 279–88.
36. **Streutker CJ, Huizinga JD, Driman DK, *et al*.** Interstitial cells of Cajal in health and disease. Part I: Normal ICC structure and function with associated motility disorders. *Histopathology*. 2007; 50: 176–89.
37. **Min KW, Leabu M.** Interstitial cells of Cajal (ICC) and gastrointestinal stromal tumor (GIST): facts, speculations, and myths. *J Cell Mol Med*. 2006; 10: 995–1013.
38. **Hirota S, Isozaki K, Moriyama Y.** Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*. 1998; 278: 577–80.
39. **Streutker CJ, Huizinga JD, Driman DK, *et al*.** Interstitial cells of Cajal in health and disease. Part II: ICC and gastrointestinal stromal tumors. *Histopathology*. 2007; 50: 190–202.
40. **Robinson TL, Sircar K, Hewlett BR, *et al*.** Gastrointestinal stromal tumors may originate from a subset of CD34-positive interstitial cells of Cajal. *Am J Pathol*. 2000; 156: 1157–63.
41. **Pieri L, Vannucchi MG, Faussone-Pellegrini MS.** Histochemical and ultrastructural characteristics of an interstitial cell type different from ICC and resident in the muscle coat of human gut. *J Cell Mol Med*. 2008; 12: 1944–50.
42. **Popescu LM, Faussone-Pellegrini MS.** Telocytes – a case of serendipity: the winding way from interstitial cells of Cajal, *via* interstital Cajal-like cells to telocytes. *J Cell Mol Med*. 2010; 14: 729–40.
43. **Grand RJ, Watkins JB, Torti FM.** Development of the human gastrointestinal tract. A review. *Gastroenterology*. 1976; 70: 790–810.
44. **Epstein ML, Mikawa T, Brown AM, *et al*.** Mapping the origin of the avian enteric nervous system with a retroviral marker. *Dev Dyn*. 1994; 201: 236–44.
45. **Durbec PL, Larsson-Blomberg LB, Schuchardt A, *et al*.** Common origin and developmental dependence on c-ret of subsets of enteric and sympathetic neuroblasts. *Development*. 1996; 122: 349–58.
46. **Burns AJ, LeDouarin NM.** Enteric nervous system development: analysis of the selective developmental potentialities of vagal and sacral neural crest cells using quail-chick chimeras. *Anat Rec*. 2001; 262: 16–28.
47. **Huizinga JD, White EJ.** Progenitor cells of interstitial cells of Cajal: on the road to tissue repair. *Gastroenterology*. 2008; 134: 1252–4.
48. **Lorincz A, Redelman D, Horvath VJ, *et al*.** Progenitors of interstitial cells of Cajal in the postnatal murine stomach. *Gastroenterology*. 2008; 134: 1083–93.
49. **Junquera C, Martìnez-Ciriano C, Castiella T, *et al*.** Immunohistochemical and ultrastructural characteristics of interstitial cells of Cajal in the rabbit duodenum. Presence of a single cilium. *J Cell Mol Med*. 2007; 11: 776–87.
50. **Vanderwinden JM, Rumessen JJ, Laet MHD, *et al*.** CD34 immunoreactivity and interstitial cells of Cajal in the human and mouse gastrointestinal tract. *Cell Tissue Res*. 2000; 302: 145–53.
51. **Lee SE, Wi JS, Min Yi, *et al*.** Distribution and three-dimensional appearance of the interstitial cells of Cajal in the rat stomach and duodenum. *Microsc Res Tech*. 2009; 72: 951–6.
52. **Lee HT, Hennig GW, Fleming NW, *et al*.** Septal interstitial cells of Cajal conduct pacemaker activity to excite muscle bundles in human jejunum. *Gastroenterology*. 2007; 133: 907–17.
53. **Belzer V, Nissan A, Freundi HR, *et al*.** Coupling among interstitial cells of Cajal in the human ileum. *Neurogastroenterol Motil*. 2004; 16: 75–80.