Smooth muscles and stem cells of embryonic guts express KIT, PDGFRRA, CD34 and many other stem cell antigens: suggestion that GIST arise from smooth muscles and gut stem cells

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Abstract: Gastrointestinal stromal tumor (GIST) is believed to original from interstitial cells of (ICC) present in Auerbach’s nerve plexus. GIST frequently shows gain-of-function mutations of KIT and PDGFRA. In practical pathology, GIST is diagnosed by positive immunostaining or KIT and/or CD34. The author herein demonstrates that human embryonic gastrointestinal tract smooth muscles (HEGITSM) and human embryonic stem gastrointestinal cells (HEGISSC) consistently express KIT, CD34, NCAM, PDGFRA and other stem cell (SC) antigens NSE, synaptophysin, chromogranin, bcl-2, ErbB, and MET throughout the embryonic development of 7-40 gestational week (GW). CK14 was negative. The author examines 42 cases (7-40 GW) of embryonic GI tract (EGI). The HEGISM, HEGIST, and gall bladder smooth muscles (SM) were consistently positive for KIT, CD34, NCAM, PDGFRA, synaptophysin, chromogranin, NSE, bcl-2, ErbB2, and MET in foregut, stomach, GB, midgut, and hindgut throughout the fetal life (7-40 GW). The stem cells (SC) were seen to create the SM, nerves, ICC, and other all structures of GI tract. In adult gastrointestinal walls (n=30), KIT, CD34, PDGFRA, and S100 proteins were expressed in Auerbach’s nerve plexus and ICC. The bronchial and vascular SM of embryos did not express these molecules. In GIST, frequent expressions of KIT (100%, 30/30), CD34 (90%, 27/30), and PDGFRA (83%, 25/30) were seen. In general, characteristics of tumors recapitulate their embryonic life. Therefore, it is strongly suggested that GIST may be originated from GI SM and/or GI SC in addition to ICC.

Keywords: GI tract, GIST, smooth muscles, stem cells, stem cell antigens

Introduction

Gastrointestinal stromal tumor (GIST) was once classified as neurogenic or myogenic tumors. However, discovery of KIT, a receptor of stem cell factor (SCF), elucidate the origin of GIST. Now, it is widely accepted that GIST originates from interstitial cells of Cajal (ICC). GIST preferentially expresses KIT and PDGFRA, and it frequently shows gain-of-function mutations of the KIT and PDGFRA genes.

To elucidate the status of smooth muscles (SM) and gastrointestinal (GI) stem cells (SC), the author investigated the expression of many molecules associated with SC antigens.

Materials and methods

The author collected 42 human embryonic GI (HEGI). They were abortions (spontaneous and artificial), intrauterine fetal death, and autopsies. The gestational ages (weeks) of the 42 HEGI were as follows: 7, 8 (n=2), 9 (n=3), 10 (n=4), 11 (n=4), 12 (n=3), 13 (n=2), 14 (n=2), 15 (n=2), 16 (n=2), 17 (n=2), 18 (n=2), 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 36, 38, and 40 gestational week (GW). Informed consent was obtained from each mother. The author obtained two cases of normal adult stomach and gut in the surgically resected specimens. The HEGI and adult GI specimens thus obtained were immediately fixed in formalin and embedded in paraffin. Many 3 μm thin sections were cut, and they were subjected to HE stain and IHC.

An IHC study was performed with the use of Dako Envision method (Dako Corp, Glostrup, Denmark). The antibodies used were as follows: NCAM(clone MOC-1, Dako Corp, Dilution=1:150),
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and KIT (CD 117) (polyclonal, Dako Corp, dilution=1:100), MET (clone SC-10, Santa Cruz Lab, Santa Cruz, CA, USA, dilution=1:100), PDGFRA (polyclonal, Santa Cruz Lab, dilution=1:100), chromogranin (clone DAK-A3, Dako Corp, dilution=1:100), CD34 (clone NU-4A1, Nichirei, Tokyo, Japan; dilution=1:200), synaptophysin (polyclonal, Dako Corp, dilution=1:150), neuron-specific enolase (NSE) (polyclonal, Dako Corp, dilution=1:100), ErbB2 (polyclonal, Dako Corp; dilution=1:100), and bcl-2 (clone 3.1, Novocastra Laboratories, NewCastle Upon Tyne, UK; dilution=1:40). Cytokeratin (CK) 14 (clone LL002, Novocastra; dilution=1:150), and vimentin (clone V9, Dako Corp; dilution=1:180). Microwave pretreatment was performed by each immunohistochemical run. All of these are highly related to HSC, STC and all SC including embryonic SC and among the antigens or markers of HSC, STC and SC of almost all types [1-17].

The author collected 30 archival specimens of GI GIST of adults. Immunostaining for KIT, PDGFRA, CD34, S100 proteins and other antigens were performed with the use of the above mentioned methods. This enables to know the expression of these molecules in non-tumorous normal GI.

Results

The embryonic gastrointestinal (GI) including foregut, midgut, hindgut, stomach, and gall bladder (GB) had well developing SM layer (Figure 1A-F) and numerous embryonic stem cells (ESC) (Figure 1A-F), the latter being par-
particularly prominent in and outside the SM. It was seen that the ESC creating the SM of the GI. Numerous huge-clusters of ESC (HC-ESC) were also seen in the submucosa and subserosa (Figure 1G and 1H). It was seen that the GI SC were derived from HC-ESC. The nerve plexuses of Meissner and Auerbach are seen to be created by ESC. ICC was seen to be created by ESC.

Figure 2. Immunohistochemical features of smooth muscles in embryos. The smooth muscles of GI were consistently positive for KIT (A), PDGFRA (B), NCAM (C), NSE (D), CD34 (E), synaptophysin (F), chromogranin, bcl-2, ErbB, and MET. CK14 was negative.

The SM of GI were positive for KIT (Figure 2A), PDGFRA (Figure 2B), NCAM (Figure 2C), NSE (Figure 2D), CD34 (Figure 2E), synaptophysin (Figure 2F), chromogranin, bcl-2, ErbB, and MET. CK14 was negative.

The ESC and HC-ESC of GI were positive for KIT (Figure 3A), PDGFRA (Figure 3B), NCAM (Figure 3C), CD34 (Figure 3D), NSE, synaptophysin
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p53, p63 and other antigens. Meissner’s nerve plexus were negative for these SC antigens. These were noises in immunostaining of KIT (mast cells), S100 (Langerhans cells and nerve fibers), CD34 (endothelial cells) and NCAM (nerve fibers).

Discussion

The author herein reported the very important findings that embryonic SM of GI were positive for KIT, PDGFRA, NCAM, NSE, synaptophysin, chromogranin, bcl-2, ErbB, and MET. CK14 was negative. The bronchial and vascular SM of embryos did not express these molecules.

In GIST, frequent expressions of KIT (100%, 30/30) (Figure 4A), CD34 (90%, 27/30) (Figure 4B), and PDGFRA (83%, 25/30) (Figure 4C) were seen. In general, characteristics of tumors recapitulate their embryonic life. Therefore, it is strongly suggested that GIST may be originated from GI SM and/or GI SC in addition to ICC.

In normal gut walls of the adults (n=30), the only positive tissue was Auerbach’s nerve plexus (intramuscular nerve plexus) including ICC (Figure 5A), which were positive for KIT (Figure 5B), CD34 (Figure 5C), PADFRA (Figure 5D), S100 proteins (Figure 5E), NCAM (Figure 5F), NSE, synaptophysin, and chromogranin, vimentin, and other SC antigens. The nerve plexuses were negatives for smooth muscle actin, CK, p53, p63 and other antigens. Meissner’s nerve plexus were negative for these SC antigens. These were noises in immunostaining of KIT (mast cells), S100 (Langerhans cells and nerve fibers), CD34 (endothelial cells) and NCAM (nerve fibers).

Figure 3. Immunohistochemical features of stem cells in human embryos. The stem cells of GI were positive for KIT (A), PDGFRA (B), NCAM (C), CD34 (D), NSE, synaptophysin (F), chromogranin, bcl-2, ErbB, and MET. CK14 was negative. The bronchial and vascular SM of embryos did not express these molecules.
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It was found that the embryonic SM are derived from ESC. This is quite a novel finding. Thus, the SC natures are present in the GI SM in embryos. Thus, it seems that the SM, neuron and ICC are closely related in embryos and probably in adults. These findings also suggest that adult GIST can arise from SM in addition to from ICC.

The close relationship between SM and SC is a very important finding. Numerous ESC and HC-ESC were seen in the GI throughout the embryonic life. They appear to create epithelium, nerves, vasculatures, submucosal tissue, subserosal tissue, ICC, Meissner's nerve plexus and Auerbach's nerve plexus. Therefore, the gut SC was the source of all gut elements.

Present study suggests strongly that GIST can arise from SM of GI. The present study showed that other elements of GI or other organs are created by embryonic SC. Thus, theoretically, GIST can arise in any areas concerned with embryonic SC. Therefore, e-GIST, which arise outside the gut, can be explained by this fact.

The development and differentiation of nerve plexus of ICC, Meissner and Auerbach in humans have been unknown. The present study revealed the ICC and both plexuses, which play a central role in gut movement, are derived from embryonic SC. That is, the SM, ICC and GI nerve plexuses have the same origin in embryonic life. These findings suggest that it is not surprisingly that GIST can arise from any site of GI and extra-GI (e-GIST). The author examined many works of e-GIST. Please refer to the author's publications.

The development of GIST is associated with mutations of KIT and PDGFRA, which are mutually exclusive. The gain of function mutations of KIT or PDGFRA are associated the development of GIST. However, mutations-negative GIST is present. Much more works of GIST genetics are mandatory. The author contributed to this.

Figure 4. Adult GIST. In GIST, frequent expressions of KIT (100%, 30/30) (A), CD34 (90%, 27/30) (B), and PDGFRA (83%, 25/30) (C) were seen. In general, characteristics of tumors recapitulate their embryonic life. Therefore, it is strongly suggested that GIST may be originated from GI SM and/or GI SC in addition to ICC.
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Figure 5. Adult normal guts. In normal gut walls of the adults, the only positive tissue was Auerbach’s nerve plexus (intramural nerve plexus) including ICC (A), which were positive for KIT (B), CD34 (C), PAFRA (D), S100 proteins (E), NCAM (F), NSE, synaptophysin, and chromogranin, vimentin, and other SC antigens. The nerve plexuses were negative for smooth muscle actin, CK, p53, p63 and other antigens. Meissner’s nerve plexus were negative for these SC antigens. These were noises in immunostaining of KIT (mast cells), S100 (Langerhan’s cells and nerve fibers), CD34 (endothelial cells) and NCAM (nerve fibers).

Please refer to the author’s publications. Also refer to the excellent works of Hirota et al.

In pathology practice, the diagnosis is GIST is very easy. However, it should be kept in mind that epithelioid GIST can mimic undifferentiated carcinoma. In contrast, the diagnosis of spindle cell GIST was very easy. In making the diagnosis, KIT positivity of GI tumor was easily diagnosed as GIST. If KIT is negative, CD34 positivity was easily diagnosed as GIST. In cases negative for both KIT and CD34, other
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neurogenic markers such as S100 and NCAM and myogenic markers such as smooth muscle actin, caldesmon, and desmin will be examined, or mutational analysis of KIT and PDGFRA are necessary. In extra-GI tumors also, e-GIST should be always kept in mind. Please refer to the author’s previous works.

In the present series, GIST, frequent expressed KIT (100%, 30/30), CD34 (90%, 27/30), and PDGFRA (83%, 25/30) were seen. In general, characteristics of tumors recapitulate their embryonic life. Therefore, it is strongly suggested that GIST may be originated from GI SM and/or GI SC in addition to ICC. The author believes that adult stem cells may give rise to GIST. This is strengthened by the present study. Please refer to the author’s previous works.

GIST is a potential malignant tumor. The malignant potentials were evaluated by tumor size, tumor necrosis, mitotic activity, Ki-67 labeling and so on. Many articles of this issue are now available. GIST occurs most often in the stomach, followed by colon. GIST of esophagus and colorectum are rare. GIST develops outside the GI (e-GIST). Clinically, imatinib methylate, a molecular targeting drug, may be effective in addition to surgical resection. The author ends this because these issues are out of focus of the present study.

The present study clearly demonstrated the clear morphology of Auerbach’s nerve plexus in adult guts. Auerbach’s nerve plexus were positive for KIT, CD34, PADFRA, S100 proteins, NCAM, NSE, synaptophysin, and chromogranin, vimentin, and other SC antigens. The nerve plexuses were negative for smooth muscle actin, CK, p53, p63 and other antigens. Thus, The histology and immunophenotype are very similar to nerve plexus and SM in embryonic like. This also suggests the close associations among GIST, SM, nerve plexus, and stem cells.

In conclusion, the author demonstrated that almost all elements of GI are produced by stem cells plentiful present in GI throughout the fetal life. Characteristically, the smooth muscles of guts are positive for KIT, CD34, NCAM, NSE and other stem cell antigens. These findings suggest that the smooth muscles in adult may give rise to GIST and e-GIST because most of tumors recapitulate their embryonic structures. The present study also suggests that some GIST are derived from stem cells. Thus, GIST may be stem cell malignancy. In developmental biology, the present study showed close associates among smooth muscles, neurons, vasculatures, and epithelial structures.

Declaration

The author has no conflict of interest.

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