

# Platelet-derived growth factor receptor $\alpha$ -positive cells in the *tunica muscularis* of human colon

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## Abstract

An obstacle to understanding motor pathologies of the gastrointestinal (GI) tract is that the physiology of some of the cellular components of the gut wall is not understood. Morphologists identified fibroblast-like cells in the *tunica muscularis* many years ago, but little is known about these interstitial cells because of inadequate techniques to identify these cells. Recent findings have shown that fibroblast-like cells express platelet-derived growth factor receptor  $\alpha$  (PDGFR $\alpha$ ) in mice and that antibodies for these receptors can be used to label the cells. We used immunohistochemical techniques to study the phenotype and intercellular relationships of fibroblast-like cells in the human colon. Fibroblast-like cells are labelled specifically with antibodies to PDGFR $\alpha$  and widely distributed through the *tunica muscularis* of human colon. These cells form discrete networks in the region of the myenteric plexus and within the circular and longitudinal muscle layers. Platelet-derived growth factor receptor  $\alpha^+$  cells are distinct from c-Kit $^+$  interstitial cells of Cajal and closely associated with varicose processes of neurons expressing substance P (excitatory motor neurons) or neuronal nitric oxide synthase (nNOS) (inhibitory motor neurons). Platelet-derived growth factor receptor  $\alpha^+$  cells express small conductance Ca $^{2+}$ -activated K $^+$  channels (SK3), which are likely to mediate purinergic neural regulation of colonic muscles. Our data suggest that PDGFR $\alpha^+$  cells may have an important role in transducing inputs from enteric motor neurons. This study identifies reagents and techniques that will allow investigation of this class of interstitial cells and help develop an understanding of the role of PDGFR $\alpha^+$  cells in the human GI tract in health and disease.

**Keywords:** interstitial cells of Cajal • fibroblast-like cell • enteric inhibitory neurotransmission • GI motility • purinergic neurotransmission

## Introduction

There are at least two types of interstitial cells, interstitial cells of Cajal (ICC) and cells referred to as 'fibroblast-like' or 'interstitial cells of Cajal-like (ICLC)', in the *tunica muscularis* of the GI tract with anatomical localizations suggestive of important physiological functions [1, 2]. Interstitial cells of Cajal express c-Kit [3], and exploiting this feature made it possible to isolate and study the function and pathophysiology of this population of interstitial cells [4–6]. Now it is recognized that ICC are pacemaker cells, provide propagation pathways for slow waves responsible for timing phasic contractions, and constitute an interface between terminals of enteric motor neurons and smooth muscle cells to mediate a portion of the motor input from the enteric nervous system [5, 7]. Several motility disorders have been

linked to loss or defects in ICC, and it is likely that gastrointestinal stromal tumours (GIST) originate from transformed ICC [8]. In contrast, investigations of 'fibroblast-like' cells or 'ICLC' have been impeded by the inability to identify these cells in living tissues or amongst the mixed cell populations resulting after enzymatic dispersion. The terms 'fibroblast-like' and 'ICLC' are vague and imply that little is known about the function of these cells and why they are located in specific anatomical niches within the *tunica muscularis*. Iino *et al.* [9] provided a critical tool for investigating these cells by showing that PDGFR $\alpha$  is a specific immunomarker for the 'fibroblast-like' cells in the murine GI tract. With this knowledge, it was possible to utilize transgenic mice with a histone-eGFP fusion protein driven by the endogenous promoter for *Pdgfra* to isolate and study the function of these cells [10]. We have suggested that naming these cells on the basis of chemical coding or molecular phenotype (*e.g.* PDGFR $\alpha^+$  cells) is a more precise method to refer to this class of interstitial cells [10].

Immunohistochemical studies had shown that PDGFR $\alpha^+$  cells in GI muscles express SK3 channels, a possible mediator of enteric inhibitory responses [11–13]. This observation, coupled with findings

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that PDGFR $\alpha$ <sup>+</sup> cells are very closely associated with enteric motor neurons and electrically coupled to smooth muscle cells [14], suggested that they, like ICC, may mediate responses to enteric inhibitory neurotransmitters. We isolated PDGFR $\alpha$ <sup>+</sup> cells from mouse colon and showed that they express receptors and ion channels required to mediate purinergic inhibitory responses. We also found that single PDGFR $\alpha$ <sup>+</sup> cells generated large outward currents *via* Ca<sup>2+</sup>-activated, apamin-sensitive K<sup>+</sup> channels in response to purinergic neurotransmitters. In comparison, smooth muscle cells were far less responsive to purines and appear to be incapable of generating purinergic inhibitory responses in intact muscles. These results demonstrated that PDGFR $\alpha$ <sup>+</sup> cells are an important component of the post-junctional receptive field for enteric motor neurotransmitters.

'Fibroblast-like cells' have also been identified in the human GI tract as shown by immunological and ultrastructural studies [12, 15], but little is known about the role of these cells in normal GI motility or pathophysiology. Some GISTs are positive for PDGFR $\alpha$  and manifest gain-of-function mutations in *Pdgfra* [16–18], so it is possible that these cells, like ICC, can be transformed. We investigated whether fibroblast-like cells in the human colon are immunoreactive for PDGFR $\alpha$ <sup>+</sup> antibodies, form a population of cells distinct from ICC, display close contacts with enteric motor neurons, and express ion channels that would allow them to participate in enteric inhibitory neurotransmission. Our data show that PDGFR $\alpha$ <sup>+</sup> cells are expressed widely in the *tunica muscularis* of the human colon, and the techniques we describe will facilitate future clinical evaluations of the status of PDGFR $\alpha$ <sup>+</sup> cells in health and disease.

## Materials and methods

### Preparation of human colonic muscles

Segments of human colon tissues were obtained as a surgical waste from three patients (ages 62–68) who underwent colon resections for neoplasms at Renown Regional Medical Center (Reno, NV, USA). Tissues from outside the disease-affected area were placed in oxygenated Krebs solution (10°C) containing (mmol/l): 118.5 NaCl; 4.2 KCl; 1.2 MgCl<sub>2</sub>; 23.8 NaHCO<sub>3</sub>; 1.2 KH<sub>2</sub>PO<sub>4</sub>; 11.0 dextrose; 1.8 CaCl<sub>2</sub> (pH 7.4). The Human Subjects Research Committees at Renown Regional Medical Center, and the Biomedical Institutional Review Board at University of Nevada, Reno approved the use of human tissues.

### Immunohistochemistry

Colon tissues were pinned at the base of the dish coated with Sylgard elastomere (Dow Corning Inc., Midland, MI, USA), and were stretched to 150% of the original length and width. The mucosa was removed by sharp dissecting the submucosal layer. The remaining tunica muscularis was fixed in Periodate-Lysine-Paraformaldehyde fixative overnight at 4°C. After fixation, tissues were washed every 30 min. for 7 hr, then washed through a sucrose gradient (5%, 10% and 15% in PBS) for 15 min. each, and were placed in 20% sucrose in PBS overnight at 4°C. Tissues were then embedded in half-and-half mixture of OCT

compound (Sakura Finetek Co., Tokyo, Japan) and 20% sucrose in PBS, frozen in liquid nitrogen, and stored at –80°C.

Frozen tissues were cut (100  $\mu$ m) using a cryostat (Carl Zeiss, Thornwood, NY, USA) and were placed in PBS (15 min.  $\times$  3 washes) to remove OCT. To block non-specific antibody binding, sections were incubated in 5% donkey serum in 0.5% Triton X-100 in PBS for 1 hr at room temperature. Following blocking, sections were incubated in primary antibodies diluted with half-and-half mixture of 0.5% Triton X-100 and 1% bovine serum albumin in PBS (48 hr at 4°C). For double-labelling, sections were washed in PBS (30 min.  $\times$  4 washes) and subsequently incubated in the second primary antibody (48 hr at 4°C). After primary antibodies, sections were incubated in appropriate secondary antibodies (1:500 in PBS, Alexa; Invitrogen, Carlsbad, CA, USA) for 1 hr at room temperature in dark. Information on primary antibody sources and concentrations is shown in Table 1. Following incubation of antibodies, sections were washed in PBS (30 min.  $\times$  8 washes) and mounted on glass slides using Thermo Aqua-Mount (Fisher Scientific, Waltham, MA, USA). Sections were examined with a confocal microscope (Zeiss LSM510 Meta; Carl Zeiss). Micrographs were constructed using Zeiss LSM 5 Image Examiner software, and are composites of Z-series scans (0.2–0.5  $\mu$ m optical sections) through a depth of 4–40  $\mu$ m.

## Results

### Distribution of PDGFR $\alpha$ <sup>+</sup> cells

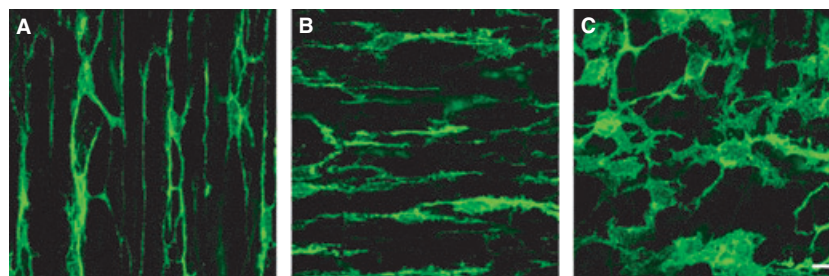
The distribution of PDGFR $\alpha$ <sup>+</sup> cells was studied by immunolabelling using an anti-human PDGFR $\alpha$  antibody on muscles of ascending colon (A colon), transverse colon (T colon) and sigmoidal colon (S colon) (Figs 1 and S1). PDGFR $\alpha$ <sup>+</sup> cells had multi-polar shapes and were interconnected to form a discrete network. In circular and longitudinal muscle layers (Figs 1A and B, S1A, B, D, E, G and H), cell bodies and major processes of PDGFR $\alpha$ <sup>+</sup> cells ran parallel to smooth muscle cells. PDGFR $\alpha$ <sup>+</sup> cells were more stellate in shape in the myenteric region (Figs 1C, S1C, F and I), and they formed a network of interconnecting cells, similar to that of ICC. There were no gross differences in the morphology of these cells or in the multi-cellular networks they formed in A, T and S colons (Fig. S1). Localization of PDGFR $\alpha$ <sup>+</sup> cells within the muscle layers and within the myenteric region recapitulates the distribution of these cells in mice, and suggests that PDGFR $\alpha$ <sup>+</sup> cells may have similar a functional role in regulation of GI motility.

### Relationship between PDGFR $\alpha$ <sup>+</sup> cells and ICC

Some of the networks of PDGFR $\alpha$ <sup>+</sup> cells were similar in general morphology to the networks formed by ICC. Therefore, we investigated whether ICC and PDGFR $\alpha$ <sup>+</sup> cells in human colon muscles are distinct populations of cells and whether they are closely associated with each other (*i.e.* occupy the same anatomical niches). Double immunolabelling for PDGFR $\alpha$  and c-Kit was performed on human A, T and S colon (Figs 2, S2, S3 and S4). The network pattern of PDGFR $\alpha$ <sup>+</sup> cells was similar to that of ICC, but the cellular distribution of the cell-specific biomarkers was distinct. No obvious differences were noted in the

**Table 1** Primary antibodies

Primary antibodies	Species	Catalogue no.	Dilution	Source
PDGFR $\alpha$	Goat	AF-307-NA	1:100	R & D systems, MN, USA
c-Kit	Rabbit	A4502	1:100	Dako, CA, USA
SK 3	Rabbit	APC-025	1:100	Alomone Labs Ltd., Israel
PGP 9.5	Rabbit	RA-95101	1:3000	UltraClone Ltd., UK
nNOS	Rabbit	SC-648	1:250	Santa Cruz Biotechnology, Inc., CA, USA
Substance P	Rat	MAB356	1:500	Millipore, MA, USA



**Fig. 1** Immunolabelling of PDGFR $\alpha$  (green) in the muscle layer of human colon. **(A)** Circular muscle layer of T colon. **(B)** Longitudinal muscle layer of A colon. **(C)** Myenteric plexus region of S colon. PDGFR $\alpha$ <sup>+</sup> cells were multi-polar cells and interconnected. In circular and longitudinal muscle layer, cell bodies of PDGFR $\alpha$ <sup>+</sup> cells ran parallel to smooth muscle cells. In myenteric region, PDGFR $\alpha$ <sup>+</sup> cells formed an extensive fine network. Scale bar is 10  $\mu$ m in all panels.

distributions of ICC and PDGFR $\alpha$ <sup>+</sup> cells or in their close proximities in A, T or S colon (Figs S2–S4). In each region, PDGFR $\alpha$ <sup>+</sup> cells and ICC were heavily intertwined, forming cable-like structures within the circular and longitudinal muscle layers (Movie S1) and a complex mesh-like structure within the myenteric region between the muscle layers (Movie S2). As PDGFR $\alpha$ <sup>+</sup> cells and ICC are distributed within the same anatomical regions, we suggest adopting the terminology typically used for ICC to distinguish different populations of these cells: *i. e.* cells running in muscle bundles as intermuscular PDGFR $\alpha$ <sup>+</sup> cells (PDGFR $\alpha$ <sup>+</sup>-IM) and cells within the region of the myenteric plexus as PDGFR $\alpha$ <sup>+</sup>-MY.

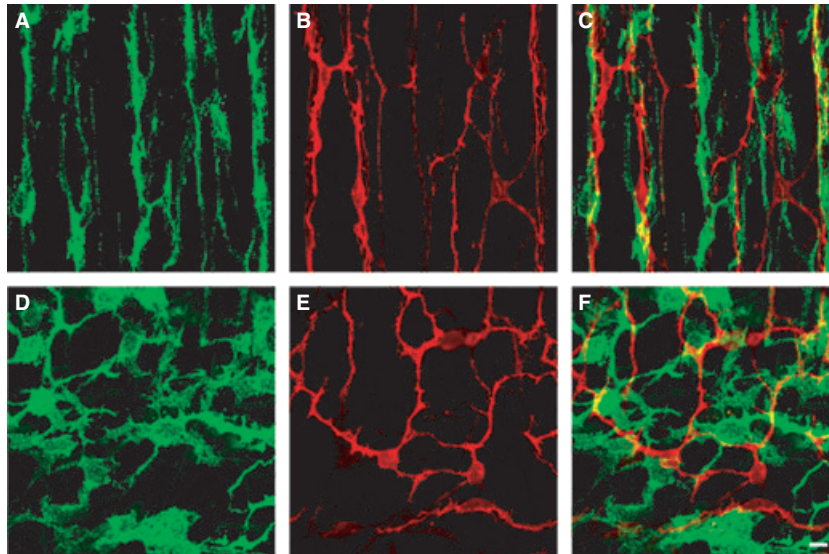
### Proximity of PDGFR $\alpha$ <sup>+</sup> cells to motor nerves

The close relationship of PDGFR $\alpha$ <sup>+</sup>-IM with ICC-IM suggests that this class of PDGFR $\alpha$ <sup>+</sup> cells may be closely associated with terminals of enteric neurons. We studied the relationship between PDGFR $\alpha$ <sup>+</sup> cells and enteric neurons, using double immunolabelling with antibodies for PDGFR $\alpha$  and protein gene product 9.5 (PGP9.5) (Figs 3, S5), nNOS (Figs 4, S6) and Substance P (Figs 5, S7). PGP9.5 labels all enteric neurons, and nNOS and substance P are markers for enteric inhibitory and excitatory motor neurons that innervate GI muscles [19]. PDGFR $\alpha$ <sup>+</sup> cells ran parallel to and were closely associated with both excitatory and inhibitory enteric neu-

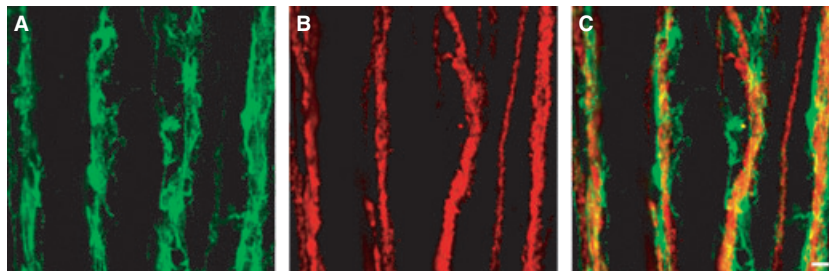
rons, and we could find no obvious differences in this pattern in A, T and S colon (Figs S5–S7). Processes of PDGFR $\alpha$ <sup>+</sup>-IM form a basket around neural processes (Fig. 3C), and when fine varicose processes of inhibitory or excitatory fibres were observed, a PDGFR $\alpha$ <sup>+</sup>-IM process was usually in close proximity [Figs 4C (arrow) and 5C (arrows)]. These results suggest that neurotransmitters released from motor neurons will be highly concentrated near PDGFR $\alpha$ <sup>+</sup> cells, and these cells may play a functional role in regulation of colonic motility.

### Expression of SK3 in PDGFR $\alpha$ <sup>+</sup> cells

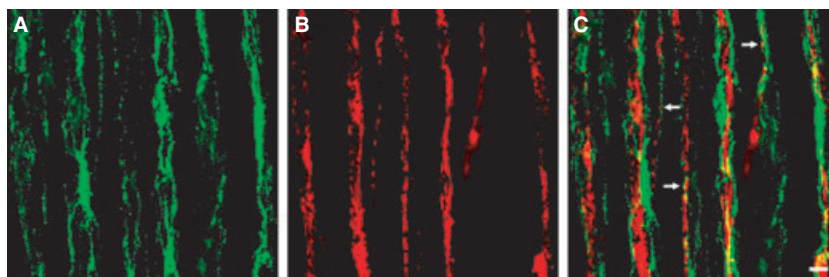
It is well known that the purinergic component of enteric inhibitory regulation of GI motility is mediated *via* apamin-sensitive, SK3 channels. Opening of these channels results in K<sup>+</sup> efflux and causes hyperpolarization of muscles and relaxation. The robust expression of SK3 channels in fibroblast-like cells has suggested that these cells could mediate purinergic inputs within the gut [10–13, 20]. We investigated SK3 expression in PDGFR $\alpha$ <sup>+</sup> cells of human colon, using double immunolabelling. PDGFR $\alpha$ <sup>+</sup> cells in human A, T and S colon muscle layer expressed SK3 (Figs 6, S8–S10), and labelling of other cells was not resolved. Expression of SK3 in PDGFR $\alpha$ <sup>+</sup> cells confirms that these cells are the fibroblast-like cells described in previous studies. These data are con-



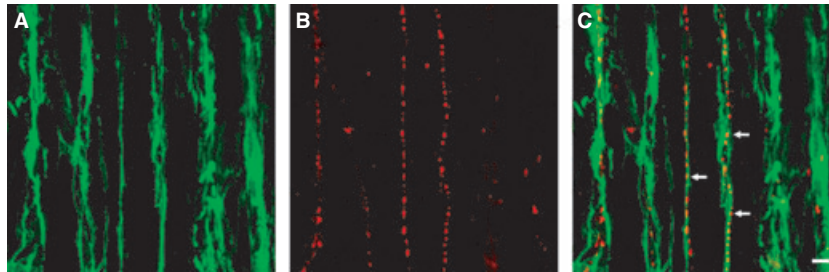
**Fig. 2** Double immunolabelling of PDGFR $\alpha$  (green) and c-Kit (red). (A–C) Circular muscle layer of A colon. (D–F) Myenteric plexus region of S colon. PDGFR $\alpha$ <sup>+</sup> cells and c-Kit<sup>+</sup> cells (ICC) formed distinct networks in the same anatomical regions and close relationships with each other. Scale bar is 10  $\mu$ m in all panels.



**Fig. 3** Double immunolabelling of PDGFR $\alpha$  (green) (A) and PGP9.5 (red) (B) in the circular muscle layer of T colon. PDGFR $\alpha$ <sup>+</sup> cells were closely associated with processes of enteric neurons, which express PGP9.5-like immunoreactivity (C). Scale bar is 10  $\mu$ m in all panels.



**Fig. 4** Double immunolabelling of PDGFR $\alpha$  (green) (A) and nNOS (red) (B) in the circular muscle of S colon. PDGFR $\alpha$ <sup>+</sup> cells were closely associated with inhibitory enteric neurons, denoted by the expression of nNOS-like immunoreactivity (C). Fine varicose processes of inhibitory enteric neurons were in close proximity to PDGFR $\alpha$ <sup>+</sup> cell processes (arrows in C), suggesting that PDGFR $\alpha$ <sup>+</sup> cells may have synapse-like communication with these neurons. Scale bar is 10  $\mu$ m in all panels.



**Fig. 5** Double immunolabelling of PDGFR $\alpha$  (green) (A) and substance P (red) (B) in the circular muscle layer of A colon. PDGFR $\alpha$ <sup>+</sup> cells were closely associated with excitatory enteric neurons, denoted by expression of Substance P-like immunoreactivity (C). Fine varicose processes of excitatory enteric neurons were in close proximity to PDGFR $\alpha$ <sup>+</sup> cell processes (arrows in C), suggesting that PDGFR $\alpha$ <sup>+</sup> cells may have synapse-like communication with these neurons. Scale bar is 10  $\mu$ m in all panels.

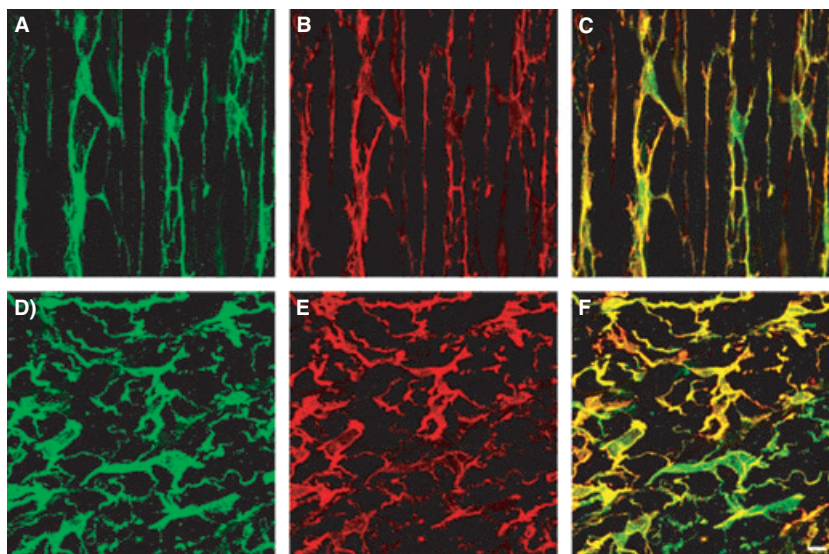
sistent with a role of PDGFR $\alpha$ <sup>+</sup> cells in enteric inhibitory hyperpolarization responses in human colon.

## Discussion

Discovering that PDGFR $\alpha$  antibodies can label cells previously referred to as ‘fibroblast-like’ interstitial cells in GI muscles has opened the door to investigations of these cells [9, 10]. Here, we verified that this approach is also useful for identification of this class of interstitial cells in the human GI tract. We showed that these cells, referred to here as PDGFR $\alpha$ <sup>+</sup> cells because of their chemical coding, are widely distributed within the myenteric region and in circular and longitudinal muscle layers throughout the colon. PDGFR $\alpha$ <sup>+</sup> cells are closely associated with, but distinct from ICC. We suggest referring to the morphologically distinct populations of cells as PDGFR $\alpha$ <sup>+</sup>-MY and PDGFR $\alpha$ <sup>+</sup>-IM, respectively, because they occupy the same ana-

tomical niches as ICC-MY and ICC-IM. PDGFR $\alpha$ <sup>+</sup>-IM networks form baskets around the processes of excitatory and inhibitory motor neurons, and ultrastructural studies have shown that this type of cell forms gap junctions with smooth muscle cells [14]. We also showed that PDGFR $\alpha$ <sup>+</sup> cells express SK3, a potential mediator for purinergic regulation of colonic motility. Establishing a biomarker for this class of cells makes it possible for pathologists and neurogastroenterologists to characterize their status in pathophysiological conditions and in animal models. Just as occurred with ICC after development of c-Kit labelling, this is a critical step in more fully understanding the role of these unique cells in health and disease.

The question of whether ICC and the class of interstitial cells referred to by others as ‘fibroblast-like’ cells or ‘ICLC’ are discrete classes of cells has been debated previously. In the seminal article showing that GIST are c-Kit<sup>+</sup> cells, it was also noted that GIST cells express CD34, a sialylated transmembrane glycoprotein [21]. Others agreed that CD34 and c-Kit labelled the same population of cells. For



**Fig. 6** Double immunolabelling of PDGFR $\alpha$  (green) and SK3 (red). (A–C) Circular muscle layer of T colon. (D–F) Myenteric plexus region of T colon. SK3-like immunoreactivity was expressed in all PDGFR $\alpha$ <sup>+</sup> cells and was not resolved in other cell types. Scale bar is 10  $\mu$ m in all panels.



example, sequential immunohistochemical labelling of 3  $\mu\text{m}$  sections of human small intestine with antibodies for c-Kit and CD34 suggested that ICC near the myenteric plexus and within the circular muscle layer of human small intestine were positive for both antigens [22]. Subsequent studies disputed these findings, however, and showed that c-Kit and CD34 labelled distinct cells in close proximity to one another [2, 23]. Pieri et al. [2] chose to refer to these cells as ICLC. It seems likely that some of the cells labelled by CD34 are PDGFR $\alpha^+$  cells, but additional cell types, including endothelial cells, are CD34 $^+$  [2], making this antigen a less specific target for cellular identification. In the present study, we showed that PDGFR $\alpha^+$  cells are distinct from c-Kit $^+$  ICC in the human colon.

The current study showed that the organization and anatomy of PDGFR $\alpha^+$  cells in human colon are similar to that in mice [9, 10]. These data suggest that mice might be good models for investigating the role of PDGFR $\alpha^+$  cells. In recent studies, we utilized animals with expression of an eGFP-histone fusion protein driven by endogenous promoters for *Pdgfra*. We showed that cellular expression of the fusion protein in the gut co-localized with PDGFR $\alpha$  by immunohistochemistry, and that PDGFR $\alpha^+$  cells could be found in enzymatic dispersions of colonic muscles. These cells expressed P2ry1 and Kcnn3 (P2Y1 receptor and SK3 channel transcripts), and, when studied with patch clamp techniques, displayed spontaneous outward currents and large outward current responses to P2Y1 receptor agonists. It should be noted that responses of smooth muscle cells to purine agonists were minor or unresolvable in comparison with responses of PDGFR $\alpha^+$  cells. ATP and  $\beta$ -NAD, a new candidate for the purinergic enteric inhibitory neurotransmitter, elicited inward currents in human and non-human primate colonic smooth muscle cells [24]. Thus, it is unlikely that smooth muscle cells can mediate post-junctional responses to purinergic neurotransmitters in the human colon. Taken together, the anatomical features of PDGFR $\alpha^+$  cells (*i.e.* proximity to varicosities of enteric motor neurons; gap junction with smooth muscle cells), molecular apparatus (*i.e.* receptors and effector channels) and outward current responses to purines (as opposed to minimal or even contradictory responses of smooth muscle cells) suggest that PDGFR $\alpha^+$  cells are the primary post-junctional mediators of the purinergic component of enteric inhibitory neurotransmission. Currently, we are unable to identify human PDGFR $\alpha^+$  cells in the heterogenous mixture of cells resulting from enzymatic dispersion of tissues. We are now looking for an effective technique to make physiological studies of these cells possible.

The anatomy, distribution and spatial relationships with other cells suggest that PDGFR $\alpha^+$  cells have a role in enteric inhibitory responses in the human colon similar to that in mouse. Proximity of these cells to neural processes with nNOS-like immunoreactivity suggests that neurotransmitters released from inhibitory neurons might activate post-junctional responses in PDGFR $\alpha^+$  cells. Purinergic responses in the human colon are characterized by rapid hyperpolarization transients (fast inhibitory junction potentials) that are inhibited by P2Y1 receptor antagonists and apamin [25–28]. We have not identified an antibody suitable for P2Y1 receptor labelling in human colon, but expression of SK3, an apamin-sensitive K $^+$  channel, PDGFR $\alpha^+$  cells would enable these cells to generate outward currents in

response to purines. Spontaneous activation of SK3 channels, as observed in mouse PDGFR $\alpha^+$  cells is also suggestive of a contribution of these cells to resting membrane potentials in colonic muscles. Thus, these cells could generally participate in the regulation of excitability responses of colonic muscles to other stimuli. It should also be noted that excitatory neurons, labelled by immunoreactivity to substance P, are also closely associated with PDGFR $\alpha^+$  cells. Little is known about a potential role for these cells in excitatory neurotransmission.

How activation of ion channels in PDGFR $\alpha^+$  cells can influence the behaviour of colonic muscles should also be discussed. Cells previously identified as ‘fibroblast-like’ cells in GI muscles form gap junctions with surrounding smooth muscle cells as shown by ultrastructural studies [14, 29]. Gap junctions provide electrical coupling between cells, such that induction of a K $^+$  current (*i.e.* outward current) in PDGFR $\alpha^+$  cells results in hyperpolarization that is conducted to coupled cells. Thus, hyperpolarization of PDGFR $\alpha^+$  cells results in net hyperpolarization of the syncytium of cells formed by smooth muscle, PDGFR $\alpha^+$  cells, and ICC. Colonic contractions are initiated by entry of Ca $^{2+}$  through voltage-dependent Ca $^{2+}$  channels in smooth muscle cells. Thus, hyperpolarization of smooth muscle cells will result in lowered open probability of Ca $^{2+}$  channels and reduce contraction.

At present, little is known about the involvement, fate or loss-of-function of PDGFR $\alpha^+$  cells in GI motor dysfunction. Certainly, changes in purinergic neural inputs could have profound effects on colonic motility. Mice with enhanced activation of PDGFR $\alpha$  develop GI fibrosis and sarcoma [30], and PDGFR $\alpha$  plays a role in the development of fibrotic diseases in several organs [31]. Gain-of-function mutations in *Pdgfra* are present in about 5% of c-Kit-negative and PDGFR $\alpha$ -positive GISTs [17, 18].

In summary, we demonstrated that the ‘fibroblast-like’ cells in the *tunica muscularis* of human GI tract can be specifically labelled with antibodies raised against PDGFR $\alpha$ , and these cells are widely distributed in the colon and distinct from ICC. PDGFR $\alpha^+$  cells are closely associated with enteric motor neurons and express important cellular effectors that mediate responses to enteric inhibitory neural regulation of the gut. This study identifies reagents and techniques that provide new opportunities for understanding the role of fibroblast-like cells in the human GI tract.

## Acknowledgements

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## Conflicts of interest

MK, YN, GWH, SMW and KMS have no commercial investments or conflicts to disclose.

## Contributions of authors

MK and YN acquired and analysed data; GWH analysed and interpreted data and developed three-dimensional movies from confocal slices; KMS, MK and SMW planned study, analysed data and wrote and critically revised manuscript.

## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1** Immunolabelling of PDGFR $\alpha$  (green) in the muscle layer of human A colon (A–C), T colon (D–F) and S colon (G–I). (A, D, G) Circular muscle layer. (B, E, H) Longitudinal muscle layer. (C, F, I) Myenteric plexus region. Scale bar is 10  $\mu$ m in all panels.

**Fig. S2** Double immunolabelling of PDGFR $\alpha$  (green) and c-Kit (red) in the muscle layer of human A colon. (A–C) Circular muscle layer. (D–F) Longitudinal muscle layer. (G–I) Myenteric plexus region. Scale bar is 10  $\mu$ m in all panels.

**Fig. S3** Double immunolabelling of PDGFR $\alpha$  (green) and c-Kit (red) in the muscle layer of human T colon. (A–C) Circular muscle layer. (D–F) Longitudinal muscle layer. (G–I) Myenteric plexus region. Scale bar is 10  $\mu$ m in all panels.

**Fig. S4** Double immunolabelling of PDGFR $\alpha$  (green) and c-Kit (red) in the muscle layer of human S colon. (A–C) Circular muscle layer. (D–F) Longitudinal muscle layer. (G–I) Myenteric plexus region. Scale bar is 10  $\mu$ m in all panels.

**Fig. S5** Double immunolabelling of PDGFR $\alpha$  (green) and PGP9.5 (red) in the circular muscle layer of human A colon (A–C), T colon (D–F) and S colon (G–I). Scale bar is 10  $\mu$ m in all panels.

**Fig. S6** Double immunolabelling of PDGFR $\alpha$  (green) and nNOS (red) in the circular muscle layer of human A colon (A–C), T colon (D–F) and S colon (G–I). Scale bar is 10  $\mu$ m in all panels.

**Fig. S7** Double immunolabelling of PDGFR $\alpha$  (green) and substance P (red) in the circular muscle layer of human A colon (A–C), T colon (D–F) and S colon (G–I). Scale bar is 10  $\mu$ m in all panels.

**Fig. S8** Double immunolabelling of PDGFR $\alpha$  (green) and SK3 (red) in the muscle layer of human A colon. (A–C) Circular muscle layer. (D–F) Longitudinal muscle layer. (G–I) Myenteric plexus region. Scale bar is 10  $\mu$ m in all panels.

**Fig. S9** Double immunolabelling of PDGFR $\alpha$  (green) and SK3 (red) in the muscle layer of human T colon. (A–C) Circular muscle layer. (D–F) Longitudinal muscle layer. (G–I) Myenteric plexus region. Scale bar is 10  $\mu$ m in all panels.

**Fig. S10** Double immunolabelling of PDGFR $\alpha$  (green) and SK3 (red) in the muscle layer of human S colon. (A–C) Circular muscle layer. (D–F) Longitudinal muscle layer. (G–I) Myenteric plexus region. Scale bar is 10  $\mu$ m in all panels.

**Movie S1** Three-dimensional image of PDGFR $\alpha$ <sup>+</sup>-IM (green) and c-Kit<sup>+</sup> cells (red) in circular muscle layer of human T colon.

**Movie S2** Three-dimensional picture of PDGFR $\alpha$ <sup>+</sup>-MY (green) and c-Kit<sup>+</sup> cells (red) in myenteric plexus region of human S colon.

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