A Functional Role for the 'Fibroblast-Like Cells' in Gastrointestinal Smooth Muscles

(J Physiol 2011;589[Pt 3]:697-710)

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Summary

The physiologic role of fibroblast-like cells (FLCs) has not been well established. Recently Kurahashi et al¹ suggested the involvement of FLCs in neurotransmission by Ca^{2+} -activated K^{+} (SK3) channels. They demonstrated that a network of more extensively branching cells with platelet-derived growth factor receptor alpha (PDGFR α) existed between the circular and longitudinal muscles in the plane of the myenteric plexus. FLCs (PDGFR α^{+}) were located in the same anatomical spaces and distributed in similar density as interstitial cells of Cajal (ICC), but PDGFR α^{+} cells were distinct from c-Kit $^{+}$ ICC, as shown by double labeling. Other double labeling experiments with anti-SK3 antibody revealed that PDGFR α^{+} cells in both muscle layers and the region of the myenteric plexus expressed SK3. Some other experiments showed that PDGFR α^{+} cells labeled with enhanced green fluorescent protein (eGFP) produced robust expression of *P2ry1* (P2Y1 receptors) and *Kcnn3* (SK3 channels).

Whole-cell patch clamp results suggested that PDGFR α^{+} cells express a high density of apamin-sensitive Ca^{2+} -activated K^{+} channels, which is consistent with the expression of SK3

channels: small amplitude and time-dependent outward currents without voltage-dependent inward currents in step depolarization, and significant reduction of this currents by apamin 300 nM and charybdotoxin (block both BK and IK conductance Ca^{2+} -activated K^{+} channels) caused a small but significant reduction in outward currents. Single channel patch recording (excised inside-out patch) to change Ca^{2+} at the intracellular surface produced the results consistent with previous reported data of Ca^{2+} -activated K^{+} channels. Pharmacological data using purines (ATP, β -NAD and ADP) evoked significant outward currents in PDGFR α^{+} cells. Apamin and MRS2500 (P2Y1 antagonist) attenuated current amplitudes.

The authors concluded that PDGFR α^{+} cells are a novel class of excitable cells with large current densities attributable to SK channels that are the molecular and ionic apparatus to mediate enteric inhibitory responses to purines in gastrointestinal (GI) muscles.

Comment

FLCs are one kind of interstitial cells in smooth muscles. The most famous interstitial cell is ICC, which serves as a pacemaker that creates the slow waves leading to peristaltic con-

Received: August 4, 2011 Revised: August 11, 2011 Accepted: August 13, 2011

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Financial support: None.

Conflicts of interest: None.

traction of the smooth muscles in the GI tract.¹ FLCs belong to the same kinase group as c-Kit receptor tyrosine kinase that is specifically expressed in the ICC of GI tract.²

PDGFR is a type III receptor tyrosine kinase structurally similar to c-Kit and is expressed in many organs as well as in the GI tract.² Thus FLCs can actually be labelled robustly with antibody of PDGFR α ,³ as ICC labelled with antibody of c-Kit. Double labelling with PDGFR α + and c-Kit antibodies shows that FLCs and ICC are distinct populations and the distribution of PDGFR α + cells is unaffected in *W* mutants (rodents to lack a specific type of ICC).²

Anatomically, FLCs were observed in the musculature in all parts of the GI tract.^{1,2} These cells were closely associated with intramuscular ICC and enteric nerve fibers. In immunohistochemical studies, FLCs are communicated with neuron and smooth muscle cells by gap junction (< 20 nm length).⁴ In the myenteric layer, FLCs formed a cellular network with their ramified processes and encompassed myenteric ganglia. Numerous PDGFR α + cells observed in the subserosal plane showed a multipolar shape.⁴ The fact that PDGFR α + cells share similar anatomical distributions with ICC suggests that the nomenclature similar to that used for different classes of ICC would be useful in order to distinguish PDGFR α + cells (eg, PDGFR α + -IM [intramuscular] and PDGFR α + -MY [myenteric]).²

Ultrastructurally, FLCs have cytoplasm of moderate to high electron density, well-developed rough endoplasmic reticulum, and nuclei with thick peripheral accumulation of heterochromatin. There were no basal lamina and caveolae along the cell membrane. It was also observed that single fibroblast-like cells formed probable small gap junctions with muscle cells.⁴

PDGFR α + -MY expresses small-conductance Ca^{2+} -activated K^{+} channels, a potential mediator of purinergic enteric inhibition.⁴ And it is closely related with inhibitory (neural nitric

oxide synthase +) and excitatory (vesicular acetylcholine transporter [vAChT] +) enteric motor neurons.¹

The development of eGFP cells enabled some studies of specific cells (ie, immunoreactivity study, patch clamp etc). Real-time PCR for relative expression of *Kit*, *P2ry1* and *Kcnn3* resulted in robust expression of *P2ry1* and *Kcnn2* and very low expression of *Kit*, which meant that FLCs were a different kind of cells from ICC.

The results of patch clamp technique were as follows; (1) dialysis of cell with Ca^{2+} -activated enormously small conductance K^{+} currents which gene blocked by apamin; (2) application of ATP, ADP or β -NAD activated large amplitude; and (3) apamin-sensitive K^{+} currents which was blocked by the P2Y1 antagonist (MRS2500). These results indicate that the whole responses are in such a close association with SK channels.

FLCs (PDGFR α +) have networks between enteric neurons and smooth muscle cells, and express many Ca^{2+} -activated K^{+} channels in the muscle layers in the GI tract. And FLCs transduce the enteric inhibitory neural input from nerve terminals to smooth muscles as a primary mediator.

References

1. Kurahashi M, Zheng H, Dwyer L, Ward SM, Don Koh S, Sanders KM. A functional role for the 'fibroblast-like cells' in gastrointestinal smooth muscles. *J Physiol* 2011;589(Pt 3):697-710.
2. Iino S, Horiguchi K, Horiguchi S, Nojyo Y. c-Kit-negative fibroblast-like cells express platelet-derived growth factor receptor alpha in the murine gastrointestinal musculature. *Histochem Cell Biol* 2009;131:691-702.
3. Sanders KM, Hwang SJ, Ward SM. Neuroeffector apparatus in gastrointestinal smooth muscle organs. *J Physiol* 2010;588(Pt 23):4621-4639.
4. Horiguchi K, Komuro T. Ultrastructural observations of fibroblast-like cells forming gap junctions in the W/W(nu) mouse small intestine. *J Auton Nerv Syst* 2000;80:142-147.