

## **DCAMKL-1 expression identifies tuft cells rather than stem cells in the adult mouse intestinal epithelium**

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Dear Sir,

In an editorial of the last issue of *Gastroenterology*, Montgomery and Shivdasani comment on the known markers of mammalian intestinal epithelial stem cells <sup>1</sup>. We wish to caution that staining for doublecortin and calcium/calmodulin-dependent protein kinase-like-1 (DCAMKL-1), one of the putative stem cell markers mentioned in this editorial, is a highly specific and robust marker of postmitotic, differentiated, tuft cells, a minority cell lineage of the intestinal epithelium, rather than a marker for intestinal epithelial stem cells. This is important since candidate markers of intestinal stem cell are scarce and DCAMKL-1 might be especially attractive to researchers because of the availability of good antibodies, which is not the case for other, functionally validated, markers, such as Lgr5 <sup>2</sup>.

DCAMKL-1 is a microtubule-associated kinase, originally identified in the developing nervous system <sup>3</sup>. It was first proposed as a gastric and small intestinal stem cell marker by Giannakis et al. after sequencing of cDNA libraries generated from laser capture microdissected adult gastric and small intestinal epithelial progenitors <sup>4</sup>. May et al. confirmed DCAMKL-1 as a putative stem cell marker, although they reported its expression in villus cells <sup>5</sup>. In both studies, it was noticed that DCAMKL-1-expressing cells do not express proliferation markers <sup>4</sup>, and do not incorporate BrdU in pulse-chase experiments <sup>4,5</sup>.

Using a commercially available antibody directed against the C-terminus of DCAMKL-1, we found scarce DCAMKL-1 expressing cells scattered throughout the adult mouse small intestinal (Figure 1A) and colon (not shown) epithelium. Cells expressing DCAMKL-1 were predominantly located on villi (50%) and at the crypt-villus junction (29%), and only 21% were found in the whole crypt region (n=353). These cells were never proliferating, irrespective of their position along the crypt-villus axis (Figure 1B). Neither the dispersion of DCAMKL-1 cells along the crypt-villus axis, nor the fact that they are never found in a proliferative state <sup>4,5</sup>, are expected features of stem cells. So, what is the identity of DCAMKL-1-expressing cells?

We could not detect co-staining of DCAMKL-1 with any of the known markers of enterocytes, goblet, enteroendocrine and Paneth cells, the main lineages constituting the intestinal

epithelium (not shown). In contrast, DCAMKL-1-expressing cells also expressed typical tuft cell differentiation markers. Tuft cells, also known as brush, caveolated, multivesicular or fibrillovesicular cells, are found in the hollow organs of the GI tract and in respiratory organs<sup>6</sup>. They are reliably distinguished from other epithelial cells by their apical tuft of stiff microvilli that protrudes in the gut lumen (hence the name). They also express molecular markers such as the cyclooxygenase (Cox) enzymes 1 and 2<sup>7</sup>, and higher levels of alpha-tubulin and villin<sup>6</sup>. DCAMKL-1-expressing cells indeed displayed Cox-1 expression and elevated expression of villin and alpha-tubulin (Figure 1C-E), as well as Cox-2 expression (not shown). Co-expression of DCAMKL-1 and Cox-1, was found in 100% (n>100) and >99% (n>100) of DCAMKL-1-positive cells located in villi and crypts, respectively. Thus, co-expression was found in all but one cells, likely because of unfavorable sectioning plane, and this cell was located in the upper crypt region, apart from the stem cell zone. In line with this, DCAMKL-1 has recently emerged from a gene signature of the mouse intestinal epithelial tuft cell, together with the Trpm5 gene encoding a cation channel involved in taste signal transduction<sup>7</sup>. Together, these results indicate that intestinal DCAMKL-1-expressing cells are *bona fide* tuft cells rather than quiescent intestinal epithelial stem cells. Therefore, we suggest not to use DCAMKL-1 as a stem cell marker in the intestinal epithelium, and to consider it with high caution as a marker of stem cells in other organs, especially those in which the presence of tuft cells has been documented, such as the digestive and respiratory organs.

### Legend to figure 1

Immunofluorescence lineage analysis of DCAMKL-1-expressing cells in the adult mouse small intestinal epithelium. Such cells (indicated by asterisks) are found along the entire crypt-villus axis of the epithelium (A) and are never in a proliferative state (B). DCAMKL-1-expressing cells co-express markers of differentiated tuft cells such as a strong apical villin immunoreactivity (C, the arrow indicates the typical villin expression associated with the apical tuft of microvilli), expression of cyclooxygenase-1 (D, D') and strong alpha-tubulin

expression (E, E'). D, E: expression in the villus compartment. D', E': expression in the crypt compartment. Each of the B, C, D, D', E and E' panels contains three parts: the merged image on the left, with nuclei stained with Hoechst, and upper right and lower right panels showing, single channel, grey scale, images. Bar: 10µm. DCAMKL-1 antibody: Abgent #AP7219b.

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