Authors' Reply

**CD117/c-kit positive
interstitial (Cajal-like) cells in
human pancreas**

Abstract

We provide evidence that interstitial Cajal-like cells, previously described in human pancreas - pICC (*J Cell Mol Med*, 9: 169, 2005), are positive for c-kit irrespective of immunohistochemical procedures used. Various sample types (fresh cryosections or formalin-fixed, paraffin-embedded specimens), various slide pretreatments (with or without heat-induced epitope retrieval) or different antibodies used (Dako polyclonal or Santa Cruz monoclonal), all showed CD117-positive pICC.

Keywords: interstitial cells of Cajal • CD117/c-kit • immunohistochemistry • microwave antigen retrieval • exocrine pancreas

We appreciate Dr. Min's letter [1], published in this issue and elicited by our previous paper [2], for bringing up the interesting issue of c-kit detection by immunohistochemistry (IHC). c-kit is currently the sole practical marker for ICC [3].

Before getting to the point of staining specificity, we would like to stress that we did not "identify pICC only by immunostains". We used IHC as an additional tool to confirm that pICC identified and characterized by other methods (mainly by transmission electron microscopy and non-conventional light microscopy) are positive for CD117/c-kit.

Regarding c-kit immunostaining with Dako polyclonal antibody A4502 (DakoCytomation, Glostrup, Denmark), our choice of antibody dilution and slide pretreatment proved satisfactory in terms of sensitivity and specificity for GIST diagnosis. It is worth mentioning that conditions we used were more stringent than those illustrated by Dr. Min (primary antibody dilution of 1:400 vs. 1:100, respectively). Moreover, after microwave antigen retrieval, c-kit immunostaining well delineated ICC and mast cells, and all criteria of enteric hypoganglionosis were easily evaluated using the above described IHC procedure [4].

However, in order to answer Dr. Min's concluding remark ("we wonder if pICC demonstrated by Popescu *et al.*, exhibited positive staining for CD117 at the more stringent conditions"), we tested again formalin-fixed, paraffin-embedded normal human pancreata using the conditions listed as optimal in a paper by Lucas DR *et al.* [5]. Thus, we skipped any antigen retrieval step and used a working antibody dilution of 1:250. Figures 1 and 2 show the results obtained for positive control (normal human large intestine) and test slides, respec-

tively. We also examined cryosections of fresh pancreas samples using a different, monoclonal antibody (clone Ab 81, code sc-13508, Santa Cruz Biotechnology, Santa Cruz, CA, USA), at a final dilution of 1:50 (Fig. 3). In both situations, pICC are revealed, showing their characteristic morphology and CD117 expression. In a rough quantitative estimation, we counted an average of about 1 pICC for 5 acini (100x, oil immersion, n = 20 microscopic fields).

We totally agree that "taking seriously" only one technique is misleading, especially when dealing with complex issues, such as origin of a lesion or characterization of a (novel) cell type. That is why we based our approach (in the paper in question [2], but also in two other studies [6, 7]), on an array of complementary methods.

Fig. 1 Positive control in human colon. Immunohistochemical staining of a paraffin-embedded tissue section for CD117. A typical CD117/c-kit-positive interstitial cell of Cajal, from the circular muscle layer, is shown (100x, oil immersion).

1. Min KW. Letter to the Editor. *J Cell Mol Med*. 2005; 9: 737.
2. Popescu LM, Hinescu ME, Ionescu N, Ciontea SM, Cretoiu D, Ardeleanu C. Interstitial cells of Cajal in pancreas. *J Cell Mol Med*. 2005; 9: 169-90.
3. Wouters M, Smans K, Vanderwinden JM. WZsGreen/+: a new green fluorescent protein knock-in mouse model for the study of KIT expressing cells in gut and cerebellum. *Physiol Genomics*. 2005; 11: 412-421.
4. Park SH, Min H, Chi JG, Park KW, Yang HR, Seo JK. Immunohistochemical studies of pediatric intestinal pseudo-obstruction: Bcl2, a valuable biomarker to detect immature enteric ganglion cells. *Am J Surg Pathol*. 2005; 29: 1017-1024.
5. Lucas DR, al-Abbadi M, Tabaczka P, Hamre MR, Weaver DW, Mott MJ. c-Kit expression in desmoid fibromatosis. Comparative immunohistochemical evaluation of two commercial antibodies. *Am J Clin Pathol*. 2003; 119: 339-45.
6. Ciontea SM, Radu E, Regalia T, Ceafalan L, Cretoiu D, Gherghiceanu M, Braga RI, Malincenco M, Zagrean L, Hinescu ME, Popescu LM. C-kit immunopositive interstitial cells (Cajal-type) in human myometrium. *J Cell Mol Med*. 2005; 9: 407-20.
7. Popescu LM, Ciontea SM, Cretoiu D, Hinescu ME, Radu E, Ionescu N, Ceausu M, Gherghiceanu M, Braga RI, Vasilescu F, Zagrean L, Ardeleanu C. Novel type of interstitial cell (Cajal-like) in human fallopian tube. *J Cell Mol Med*. 2005; 9: 479-523.

Fig. 2 A-F IHC staining of interstitial Cajal-like cells in human pancreas (pICC). Paraffin-embedded pancreas sections were incubated with polyclonal antibodies against CD 117. The bound primary antibodies were detected by a peroxidase-conjugated biotin-streptavidin system and visualized by diaminobenzidine (DAB). Nuclei were counterstained with Mayer hematoxylin. **A.** Observe the distribution of c-kit positive interstitial cells between pancreatic acini (original magnification 10x). **B-F.** Interstitial Cajal-like cells with different cell body shapes can be seen: pyriiform (**B**), triangular (**C**), fusiform (**D-F**). Note the typical long, moniliform cytoplasmic processes that 'touch' the acini (**F**). Original magnification: 100x, oil immersion.

Fig. 3 Human pancreas, unfixed specimen, cryosections. CD117/c-kit monoclonal antibodies (Santa Cruz) reveal interstitial (Cajal-like) cells among pancreatic acini. Images similar to Fig. 2. Counterstaining with methylene blue (original magnification: 40x).

**L.M. Popescu^{a,b,*}, M.E. Hinescu^{a,b},
E. Radu^{a,b}, Sanda M. Ciontea^a,
D. Cretoiu^{a,b}, M. Leabu^{a,b},
Carmen Ardeleanu^b**

^a Department of Cellular and Molecular Medicine,
"Carol Davila" University of Medicine and Pharmacy,
Bucharest, Romania

^b "Victor Babeș" National Institute of Pathology,
Bucharest, Romania

July 26, 2005

* Correspondence to: L.M. POPESCU, M.D., Ph.D.
Department of Cellular and Molecular Medicine,
"Carol Davila" University of Medicine and Pharmacy,
P.O. Box 35-29, Bucharest 35, Romania.
Tel: +40(0)744 535 298; Fax: +40(0)21-312 4885
E-mail: LMP@univermed-cdgm.ro