J Mol Hist (2007) 38:275–284
DOI 10.1007/s10735-007-9099-0

ORIGINAL PAPER

Interstitial Cajal-like cells in human gallbladder

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Received: 22 April 2007 / Accepted: 9 May 2007 / Published online: 31 May 2007
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Abstract We describe here an interstitial Cajal-like cell type (ICLC) in human gallbladder, resembling the archetypal enteric interstitial cells of Cajal. Gallbladder ICLC were demonstrated in *fresh preparations* (tissue *cryosections*) using methylene-blue, and *fixed specimens* in Epon semi-thin sections stained with toluidine blue or *transmission electron microscopy* (TEM). The positive diagnosis of gallbladder ICLC was further verified by *immunohistochemistry*: CD117/c-kit, CD34, and another 16 antigens: vimentin, desmin, nestin, α-smooth muscle actin, NK-1, S-100, PGP-9.5, tau protein, chromogranin A, NSE, GFAP, CD1a, CD62-P, CD68, estrogen and progesterone receptors. Double immunostaining was performed for CD117, CD34 and CD117 and nestin, respectively. In fresh specimens, the spatial density of gallbladder ICLC was 100–110 cells/mm^2. ICLC mainly appeared beneath the epithelium and in muscularis (about 7%, and ~5%, respectively). In toto, ICLC represent in gallbladder ~5.5% of subepithelial cells. TEM showed that diagnostic criteria were fulfilled by ICLC. Moreover, TEM indicated that the main ultrastructural distinctive feature for ICLC, the cell processes, develop into the characteristic shape at a relatively early stage of development. It remains to be established if, in humans, ICLC are involved in gallbladder (dis)functions (e.g. pace-making, secretion (auto-, juxta- and/or paracrine), intercellular signaling, or stone formation).

Keywords Interstitial cells of Cajal · CD117/c-kit · CD34 · Vimentin · Human gallbladder · Immunohistochemistry

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Introduction

It has been repeatedly shown on previous occasions that pleiomorphic and/or spindle shaped cells, immunoreactive for CD117/c-kit, were present in some malignant human gallbladder tumors (Ortiz-Hidalgo et al. 2000; Mendoza-Marin et al. 2002; Park et al. 2004; Langner et al. 2004; Furihata et al. 2005; for review see Thomas 2007). However, despite the fact that (in two case reports) it was speculated that the origin of such tumors might be Cajal-like cells (Ortiz-Hidalgo et al. 2000; Furihata et al. 2005), the presence of interstitial Cajal-like cell type (ICLC) in a *human* non-tumoral gallbladder has not yet been thoroughly documented. It appears obvious why for ethical reasons, literary accounts of human material have only been from patients with chronic cholecystitis (Hopwood and Ross 1997). Another line of evidence has been provided by a recent report about the presence of cells similar to interstitial cells of Cajal in CD1 mice (Sun et al. 2006) or guinea pig (Lavoie et al. 2007) gallbladders, respectively. The purpose of this study was to show, by means of a set of complementary techniques, the 'expected' presence, the distribution and ultrastructural characteristics, at high transmission electron microscopy (TEM) resolutions, of ICLC in human adult non-tumoral gallbladder. We applied a methodological algorithm previously used for

identification and characterization of ICLC in some other non-enteric human and/or animal regions: pancreas (Popescu et al. 2005d), myometrium (Ciontea et al. 2005; Popescu et al. 2007), Fallopian tube (Popescu et al. 2005b), mammary gland (Popescu et al. 2005a; Radu et al. 2005), human atrial (Hinescu and Popescu 2005; Hinescu et al. 2006) and ventricular myocardium (Popescu et al. 2006a).

Materials and methods

Human tissue specimens

For immunohistochemistry, histological normal areas of gallbladder, remote of primary disease, were obtained from archived paraffin-embedded tissue of adult patients that had undergone surgical procedures for non-neoplastic disease. For a few cases, fresh gallbladder was used for vital staining. Archived paraffin and Epon-embedded specimens from a 17-week-old human fetus were processed to assess by immunohistochemistry and electron microscopy, respectively, the presence of gallbladder ICLC at an early stage of development.

Ethics

The study was conducted in accordance with the moral, ethical, regulatory and scientific principles governing clinical research as set out in the Declaration of Helsinki (1989) and Good Clinical Practice guidelines (GCP). Internationally recognized regulatory and ethical standards regarding ownership of tissue samples used for research were followed for archived tissue specimens (Hakimian and Korn 2004). This study was approved by the Bioethics Committees of the ''Victor Babes'' Institute of Pathology, Bucharest and ''Carol Davila'' University of Medicine.

Toluidine blue staining

Control semi-thin sections (less than 1 μm) were stained with 0.25% toluidine blue and examined by light microscopy (Nikon Eclipse E600). Representative photomicrographs were taken using Nikon Plan 40× and Nikon Plan Fluor 100×/1.30 oil.

Electron microscopy

A classical protocol was used to obtain Epon-embedded specimens. After the gallbladder was excised it was immersed 90 min, at 4°C, in a freshly prepared solution of 2.5% glutaraldehyde in 15 mM cacodylate buffer, pH 7.2. Tissue samples (fragments of about 1 mm^3) were then fixed with 1% osmium tetroxide in 15 mM cacodylate buffer, pH 7.4, for 90 min at 4°C, and dehydrated in a series of graded ethanols. After immersion in propylene oxide (three times for 10 min each), the samples were immersed in a mixture (1:1) of propylene oxide and Epon 812 resin, overnight, and embedded in Epon 812. The regions studied were sectioned using a MT-7000 ultramicrotome (from Research Manufacturing Company Inc., Tucson, Arizona, USA) into ultrathin sections (50 nm). The sections were mounted on Formvar coated copper slot grids, stained with uranyl acetate and lead citrate, and then observed in a CM 12 Philips electron microscope at an acceleration voltage of 60 kV.

Morphometric analysis

The absolute and relative number of cells, as well as the number and length of processes emerging from the cell body were evaluated based on a combination of measurements of semithin and ultrathin sections obtained from 27 human gallbladders (from which 17 examined by TEM and 27 by light microscopy). Tissue specimens were from both male (n = 11) and female patients (n = 16), of 29 to 66-years-old. Two histopathologists (unaware of experimental design) counted the number of stained cells (excluding vascular structures) within photographs (n = 92) randomly taken on semi-thin sections. Similarly, 94 randomly taken TEM images were examined according to the ultrastructural diagnostic criteria for ICLC. Software for statistical analysis determined average values for different cellular types. Results were reported as mean plus or minus standard error.

Immunohistochemistry

The human gallbladder selected specimens were tested by immunocytochemistry for ICLC, by means of the following antibodies: *CD117*, polyclonal, 1:100 (DAKO, Glostrup, Denmark), CD34, 1:100, clone QBEND10 (Biogenex, San Ramon, CA, USA), *chromogranin A*, monoclonal, 1:50, clone LK2H10, (Novocastra, Newcastle upon Tyne, UK), *smooth muscle α-actin* (SMA), monoclonal, 1:1500, clone 1A4 (Sigma Chemical, St. Louis, MO), *S-100*, polyclonal, 1:500 (DAKO, Glostrup, Denmark), *CD68*, monoclonal, 1:50, clone PG-M1 (DAKO, Glostrup, Denmark), *NK1*, monoclonal, 1:50, clone NK1, (DAKO, Glostrup, Denmark), *vimentin*, monoclonal, 1:100, clone V9 (DAKO, Glostrup, Denmark), *desmin*, monoclonal, 1:50, clone D33 (DAKO, Glostrup, Denmark), *tau protein*, polyclonal, 1:100, (NeoMarker, Fremont, CA, USA), *nestin*, monoclonal, 1:100, clone 5326 (Santa Cruz, CA, USA), *PGP9.5*, monoclonal 1:40, clone 10A1 (Novocastra, Newcastle upon Tyne, UK), *GFAP*, monoclonal, 1:50, clone 6F2, (DAKO, Glostrup, Denmark), *CD62P*

(selectin), monoclonal, 1:25, clone 1E3 (DAKO, Glostrup, Denmark) *estrogen receptor*, monoclonal, 1:25, clone 6F11, (Novocastra, Newcastle upon Tyne, UK), and *progesteron receptor* monoclonal, 1:50, clone 16 (Novocastra, Newcastle upon Tyne, UK). All specimens were counterstained with Mayer's hematoxylin, examined and photographed on a Nikon Eclipse 600 microscope. Immunocytochemistry was performed on 3 μm-thick sections from 10% formalin fixed paraffin-embedded specimens, according to the Avidin-Biotin-Complex (ABC) method of Hsu (Hsu et al. 1981), modified (Bussolati and Gugliotta 1983) (for detailed preparative techniques see Miller 2002). Briefly, the procedure was: deparaffinization in xylene and alcohol series, rehydration, washing in phosphate saline buffer (PBS), incubation with normal serum, for 20 min, incubation with primary antibody overnight, standard labeled streptavidin–antibody biotin (LSAB) kit (DAKO), washing in carbonate buffer and development in 3-3′-DAB hydrochloride/H_2O_2. Microwave antigen retrieval (in M-citrate buffer, pH 6.0) was performed for CD117/c-kit, chromogranin A, S-100 and CD68. For CD34 and CD117 double immunostaining of the human gallbladder, an ABC indirect tristadial method was used: DAB (diaminobenzidine) chromogenic substrate developing for CD34 monoclonal antibody, and fast red alpha naphtol in Tris buffer for CD117 polyclonal antibody. Negative controls were maintained using an irrelevant primary antibody or replacing the secondary antibody with phosphate buffered saline. A positive control, a tissue known to express the marker in question, was used for every specific immunocytochemical stain. To ensure the reliability of this experimental study, internal quality control of immunocytochemical techniques was performed as a part of a certified quality assurance system.

Results

Methylene blue staining

By tradition, methylene blue staining is the first step in identifying cell profiles in a stroma (Cajal 1911). Figure 1A shows selectively-stained cells in fresh tissue samples in the human gallbladder. The characteristic features of ICLC (positioning in interstitium, networklike appearance, and thin, characteristic, very long, and moniliform cell prolongations) can be foreseen. Using the methylene blue vital staining followed by cryosectioning, we approximated a value of 100–110 cells/mm^2. This value is in the same order of magnitude as previously reported for methylene blue staining in the fallopian tube (Popescu et al. 2005b). We estimated the spatial density of ICLC in adult human gallbladder and observed a dual

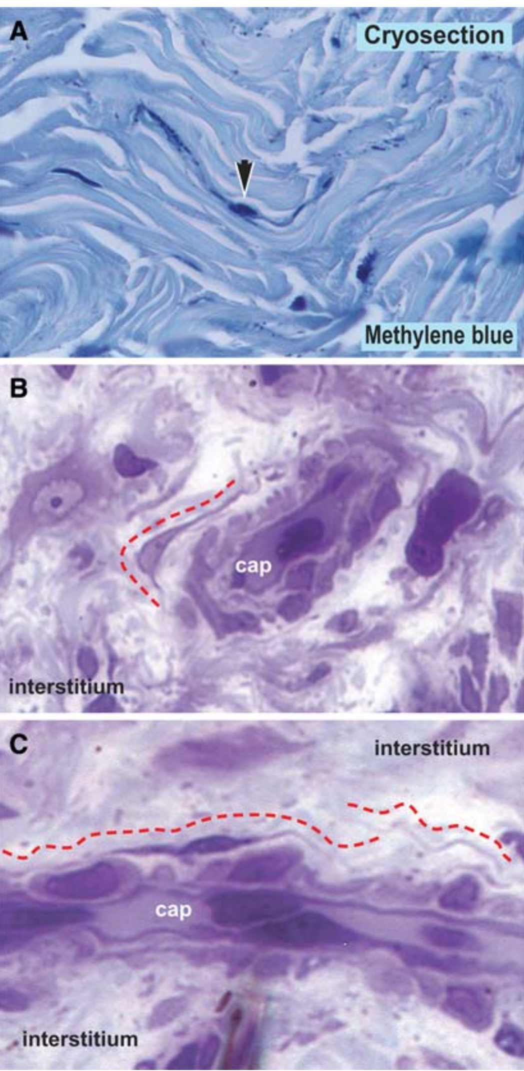

Fig. 1 Adult human gallbladder stained with methylene blue (**A**). Human gallbladder semithin sections stained with toluidine blue (non-conventional light microscopy). Interstitial cells having ICLC morphology, with thin cellular processes are illustrated. Cellular processes embrace crosssectioned (**B**) or longitudinally sectioned small diameter vessels (**C**). Long cellular processes running between smooth muscle bundles are suggestive for ICLC. Original magnification 40×. Red dot lines follow the ICLC profile in order to make it more evident

distribution: in the close vicinity of the epithelium and in the interstitial spaces between bundles of smooth muscle fibers. Sometimes 'beads' of the moniliform cytoplasmic processes were observed.

Toluidine blue staining

This approach (consisting of tissue processing as for TEM, followed by sectioning around 1 μm and examination under a light microscope) allows a rough estimation of ICLC localization, quantity, or morphological details

(e.g. cytoplasmic processes). In the human adult gallbladder, as shown in Fig. 1, ICLC are mainly placed near small vessels (Fig. 1B, C), in the subepithelial region of lamina propria and between smooth muscle bundles in muscularis. A combined analysis on semithin and ultrathin section enabled us to determine the relative proportion of ICLC in the sub-epithelial and muscularis interstitium (about 7%, and ~5%, respectively). *In toto*, we estimated that gallbladder ICLC represent ~5.5% of sub epithelial cells. Like in other organs (McCloskey et al. 2002; Bobryshev 2005; Ciontea et al. 2005; Lang and Klemm 2005; Popescu et al. 2005d; Radu et al. 2005; Harhun et al. 2005; Sergeant et al. 2006), ICLC cell shape is characteristic, with a long, thin process (several tens of μm).

Immunohistochemistry

In order further characterize ICLC, immunohistochemistry was used to correlate morphology with the cell phenotype. To this aim, 16 types of antibodies were assessed by single- or double-labeling IHC technique on formalin-fixed, paraffin-embedded sections, or cryosections from human adult lamina propria and muscularis of the gallbladder (Table 1). IHC using antibodies against CD117/c-kit revealed a high density of ICLC, mainly in the human adult gallbladder lamina propria (Fig. 2A) and mast cells. The positive control for CD117 immunoreactivity was performed on human myenteric plexus in the colon. CD117/c-kit expression was detected in cells with characteristic morphology susceptible to classification as ICLC (one or more very long, thin processes, sometimes with a beads-on-string appearance, on spindle-shaped cell bodies). In the adult human gallbladder lamina propria, ICLC constantly expressed CD34 at high levels (Fig. 2B). CD117/CD34 double immunohistochemical reaction was positive (Fig. 2C). CD117 mainly stains the cell body, while CD34 preferentially stains the cell processes. Almost a majority of ICLC, from lamina propria and muscularis, were non-reactive for α-SMA (Fig. 2D). In contrast, constant positive immunostaining was documented for vimentin (Fig. 2E), and nestin (Fig. 2G).

Scattered cells were found positive for desmin (Fig. 2F). IHC with antibodies against some additional antigens showed inconstant or weak immunostaining for S-100 (Fig. 2H), and NSE (Fig. 3A), clear negative immunostaining for CD68 (Fig. 3B), GFAP (Fig. 3C), chromogranin A (Fig. 3D), PGP9.5 (Fig. 3E) and CD62 (Fig. 3G). In contrast, *tau*-protein was constantly positive (Fig. 3F). These data support the distinction between ICLC and other cells present in interstitium or adjacent layers (fibroblasts, pericytes, macrophages, mast cells, smooth muscle cells, neuroendocrine cells, neural cells). No significant differ-

Table 1 Summary of immunohistochemical results for human gallbladder ICLC

Antibody	Source	Clone	Dilution	IHC positivity
CD117/c-kit	DAKO	Polyclonal	1:100	+ + + +
CD34	Biogenex	QBEND10	1:100	+ + +
Vimentin	DAKO	V9	1:100	+ +
Nestin	Santa Cruz	5326	1:100	+ +
Tau protein	NeoMarker	Polyclonal	1:100	+
α-SMA	Sigma	1A4	1:1500	+/–
Desmin	DAKO	D33	1:50	+/–
S-100	DAKO	Polyclonal	1:500	+/–
NSE	DAKO	BBS/NC/VI-H14	1:50	+/–
CD68	DAKO	PG-M1	1:50	–
GFAP	DAKO	6F2	1:50	–
Chromogranin A	Novocastra	LK2H10	1:50	–
PGP9.5	Novocastra	10A1	1:40	–
CD62P (selectin)	DAKO	1E3	1:25	–
CD1a	Novocastra	CD1a-235	1:30	–
NK1	DAKO	NK1	1:50	–
Estrogen receptor	Novocastra	Clone 6F11	1:25	–
Progesteron receptor	Novocastra	Clone 16	1:50	–

An adaptation of Quick score method (Lee et al. 2002) was used to semiquantitatively assess the intensity of the immunoreactivity

Intensity: Negative (no staining of any cellular part at high magnification): –; Occasionally weak positive: +/–; Low (only visible at high magnification): +; Medium (readily visible at low magnification): + +; High (strikingly positive at high magnification): + + +; Strong (strikingly positive even at low magnification): + + + +

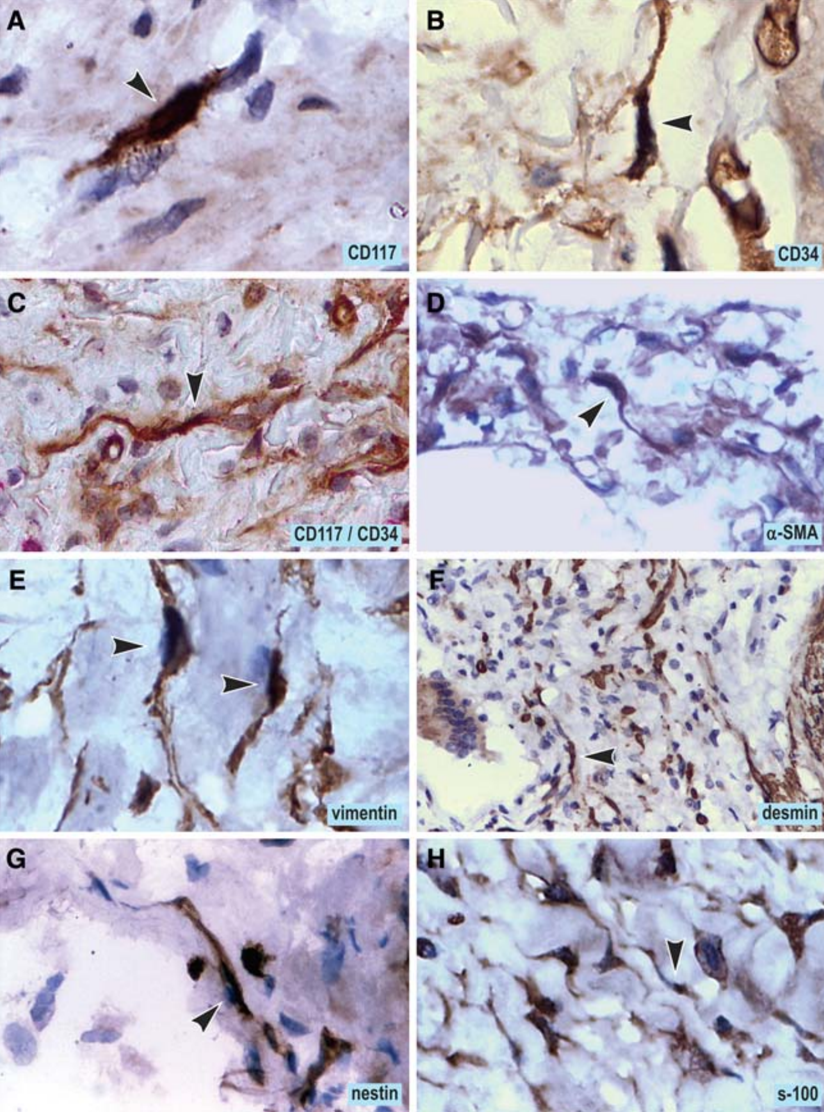

Fig. 2 Immunocytochemical reactions for CD117 (**A**), CD 34 (**B**), double staining for CD117/CD34 (**C**), smooth muscle actin (**D**), vimentin (**E**), desmin (**F**), nestin (**G**), S-100 (**H**). Mayer's hematoxilin counterstaining; original magnification A–E, G, H 40×; F 20×. Arrows indicate cells with a profile suggestive for ICLC

ences were observed when immunohistochemistry in adult human gallbladder tissue specimens was compared to data on fetal human gallbladder.

Electron microscopy

TEM images revealed the presence of two subpopulations of gallbladder ICLC (located in the sub-epithelial region and between muscular bundles) with similar ultrastructural features: (1) Location in the non-epithelial space; (2) Close contacts with targets: nerve bundles, and/or epithelia, and/or smooth muscle cells, and/or capillaries; (3) Characteristic cytoplasmic processes: i. Number (1–3, frequently: 2–3); ii. Length (tens up to hundreds of μm); iii. Thickness (uneven caliber, <0.5 μm); iv. Aspect: moniliform, usually with mitochondria in dilations; v. Presence of 'Ca^{2+} release units'; vi. Branching: dichotomous pattern; vii. Organization in network—labyrinthic system: overlapping cytoplasmic processes; (4) Gap junctions: with smooth muscle cells or with each other; (5) Basal lamina: occasionally present; (6) Caveolae: 2–3% of cytoplasmic volume; ~0.5 caveolae/μm of cell membrane length; (7) Mitochondria: 5–10% of cytoplasmic volume; (8) Endoplasmic reticulum: about 1–2%, either smooth or rough; (9) Cytoskeleton: intermediate and thin filaments, as well as microtubules, present; (10) Myosin thick filaments: undetectable. Most of these specific features of ICLC are illustrated in Fig. 4. The inset shows the characteristic labyrinth of cell prolongations. Sometimes ultrastructural complex associations of caveolae, endoplasmic reticulum and/or mitochondria (putative as Ca^{2+} release units) were observed. Ultrastructure of human gallbladder interstitial cells in early stages of development (17 weeks) is presented in Fig. 5A, B. One may observe that early in development, a twisted system of cell prolongations is already formed by ICLC. Connections between ICLC and other connective cells have been previously reported in other

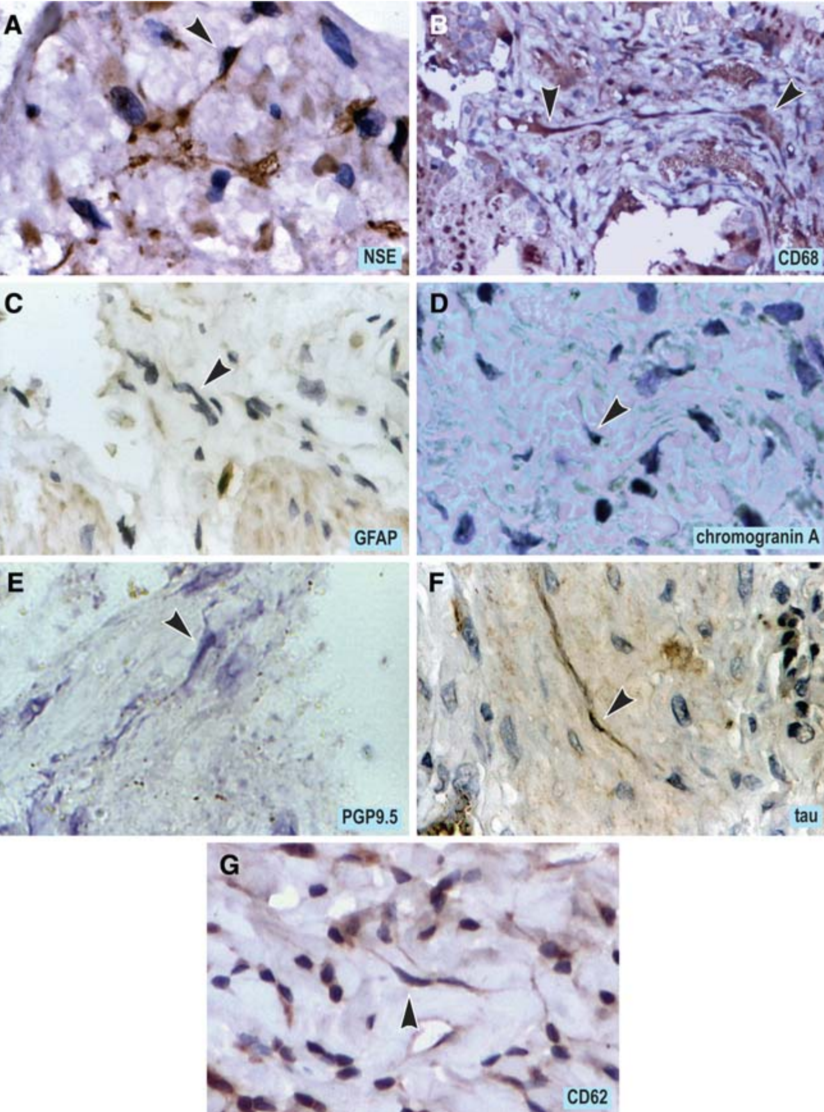

Fig. 3 Adult human gallbladder, immunostaining for: NSE (**A**), CD 68 (**B**), GFAP (**C**), chromogranin (**D**), PGP9.5 (**E**), tau protein (**F**), CD62P (**G**). Mayer's hematoxilin counterstaining; Original magnification 40×

tissues as stromal synapses (Popescu et al 2005c, 2006a). Figure 6A and B present a stromal synapse with a cleft width of 15.7 ± 2 nm, and wider inter-membrane distances (35.5–40.8 nm) observed in human adult gallbladder interstitium. At least in a few zones, it seems that the two membranes-contracting synapse are very closed together. Preliminary estimations revealed that there is an ultrastructural resemblance of gallbladder ICLC with both, interstitial cell of Cajal described in the musculature of gastrointestinal tract, and ICLC recently described in some other extra digestive organs. The only specific aspect of gallbladder ICLC seems to be the heightened twisting profile of cell processes.

Discussion

One can scrutinize the medical significance of the presence of ICLC in human gallbladder from different points of view: embryological, physiological and/or pathological.

From an *embryological point of view*, indirect evidence supports the idea that ICLC simultaneously develop in the intestine and bilio-pancreatic region. For the archetypal ICC, a kit receptor seems to be involved in the development and maintenance of the differentiated state of ICC after birth (Vannucchi 1999; Sanders and Ward 2006). As observed a long time ago, physiological disorders in the alimentary tract and unregulated secretion of bile are characteristic phenotypes of W mutant mice (Maeda et al. 1992; Isozaki et al. 1995). More recently, the fact that the biliary system, liver and pancreas might be considered as a unique organ was suggested (Frossard 2005). Molecular mechanisms which determine the organ identity in this complex area are not yet completely known (Sumazaki et al. 2004; Shen et al. 2002). The very close developmental relationships in this anatomical region might explain the transdifferentiation/plasticity of pancreas, biliary system and liver (Lardon et al. 2004) and provides a

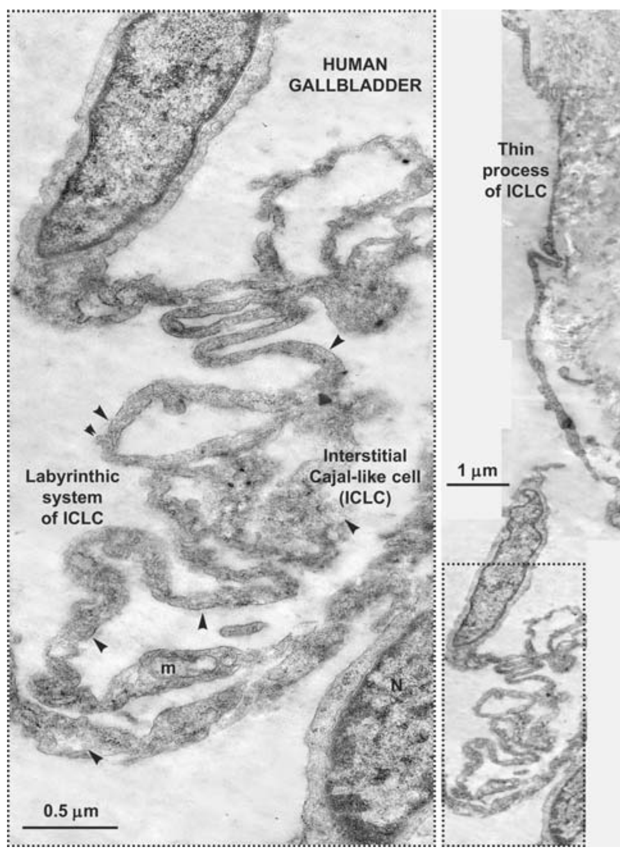

Fig. 4 Adult human gallbladder: TEM; original magnification: 40,000×. Photographic reconstruction illustrating the cell shape and a typical ICLC with long, thin, elaborate branching system of cell processes. Left image presents at a higher magnification area delimited by the thin border in the photographic reconstruction. N = nucleus; m = mitochondria; arrowheads = caveolae

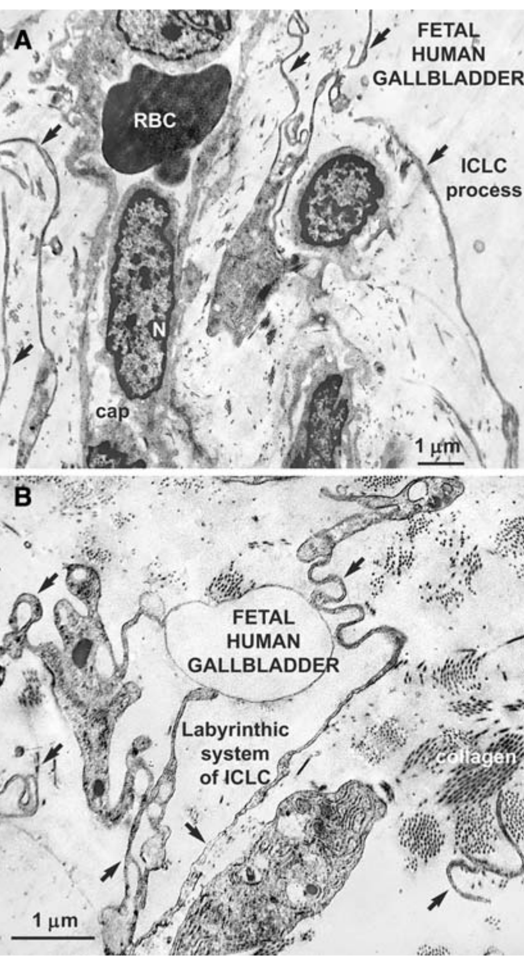

Fig. 5 Fetal (17 weeks) human gallbladder: TEM; original magnification: 3,400× (**A**); 5,700× (**B**). (**A**) illustrates the location of gallbladder ICLC in the close vicinity of a vessel, and (**B**) illustrates the very early development of the long, labirinth-forming cell prolongations. N = nucleus; cap = capillary; arrows = ICLC cell prolongations; RBC = red blood cell

rationale to hypothesize that, if present in the pancreas (Popescu et al. 2005d), ICLC might be present in the gallbladder too.

From a *histological point of view*, data presented here represent a first methodical attempt to examine if, in non-tumoral *human* gallbladder interstitium cells corresponding to the morphological and immunocytochemical criteria for ICLC can be identified. For the first time a(n) (almost) complete immunohistochemical profile for both fetal and adult human gallbladder for ICLC is provided here. Several reports indicate that many gastrointestinal stromal tumours (GISTs) are positive for c-kit and CD34 and have other features similar to those of ICC (Streutker et al. 2007). In ICLC from the human gallbladder, we documented the concomitant presence of these two antigens. Recent studies on Cajal-like cells in CD1 mice gallbladder (Sun et al. 2006) and guinea pigs (Lavoie et al. 2007) assessed the exclusive presence of CD117, from an immunocytochemical perspective. Furthermore, one group initially reported the lack of c-kit positive cell in guinea pig gallbladder (Parr et al. 2003). However, it is worthwhile to mention that human and murine ICLC were similarly distributed, mainly in the sub epithelial and muscular layers. An overview of literature shows that, when attempts were made to culture *in vitro* gallbladder tissues, little attention was paid to interstitial cells (for details see Nakanuma et al. 1997).

From a *physiological point of view*, Cajal-type of interstitial cells might represent, through analogy to the gastrointestinal tract an essential player in the physiology of a digestive cavitary organ such as gallbladder, imposing the rhythm of bile release (pace-maker cells). It is noteworthy to observe that, when a comparison of phenotypic characteristics of c-kit mutant C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ and WBB6F1-*Kit*$^{W/W\text{-}v}$ mice was made, among other modifications, a lack of (digestive) ICC was concomitant with the exhibit of bile reflux, in both strains (Grimbaldeston et al. 2005). However, whether the physiology of ICC is species-dependent or whether ICC expresses similar mechanisms and functions in animals and humans remains to be

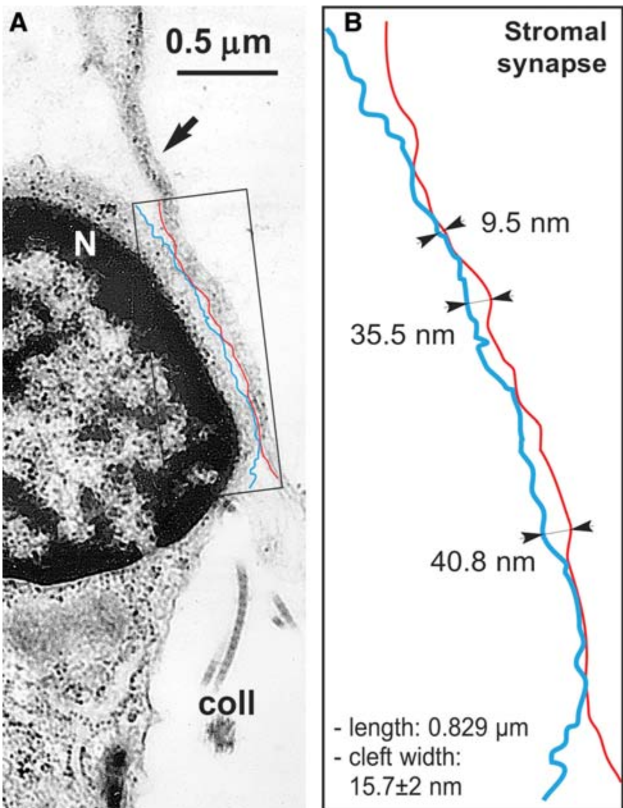

Fig. 6 Stromal synapse between an ICLC prolongation and another interstitial gallbladder cell (**A**). TEM, original magnification 10,000×. Contours of the two cell membranes in contact appear traced in (**B**). coll = collagen fibres

established (Oldham-Ott and Gilloteaux 1997). Whether recent data obtained in CD1 mice gallbladder (Sun et al. 2006) or in guinea pig gallbladder (Lavoie et al. 2007) could be easily extrapolated for humans needs to be verified. On one hand, in some species, eating only periodically, as well as in humans, bile received from the liver becomes more concentrated, and is stored for longer time periods than the bile in vertebrates that eat frequently, such as guinea pigs (Oldham-Ott and Gilloteaux 1997). On the other hand, some other species, such as pigeons, rats, and deer, which eat almost continuously, have no gallbladder. Instead, in rat, for example, a bile ductular plexus seems to have storage and bile modification functions (Oldham-Ott and Gilloteaux 1997). The effects of imatinib on generation and propagation of action potentials, reported in guinea pig gallbladder preparations (Lavoie et al. 2007) also have to be verified in the human gallbladder and compared with results in other tissues (Popescu et al. 2006b). Further functional studies are required to confirm involvement of ICLC in pace-making and/or some other (paracrine, juxtacrine, autocrine) functions cannot be ruled out *a priori* (Rumessen et al. 2001; Cretoiu et al. 2006; Faussone-Pellegrini 2006; Faussone-Pellegrini and Vannucchi 2006; Faussone-Pellegrini et al. 2006; Ye et al. 2006). Starting from an immunostaining pattern similar to that of an intestitial cell of Cajal, a human gallbladder GIST-resembling tumor was considered as potentially deriving from mesenchymal stem cells. It implies that, in this case, putative ICLC (virtually regarded as stem cells) were considered as the starting point. The speculation went further, stating that stem cells residing at different sites could display dissimilar characteristics and stem cells in the gallbladder might possess the ability to differentiate more than in a classical GIST, even in a rhabdomyomatous direction (Furihata et al 2005) (previously not described for GIST; for review see (Bussolati 2005; Heinrich and Corless 2005; Robinson et al. 2000; Sanders et al. 2002).

From a *pathological point of view*, if one accepts a pacemaking role, identification of Cajal-type cells in human gallbladder might enlarge the discussion about the role of this cell type in not only stromal tumor development (Ortiz-Hidalgo et al. 2000; Mendoza-Marin et al. 2002; Park et al. 2004; Langner et al. 2004, Furihata et al. 2005) but in human motor pathologies too. Depletion of ICC populations has been reported in tissues of patients with a variety of gastrointestinal motor disorders, including both congenital and acquired diseases (Sanders et al. 2002; Porcher et al. 2002; Lyford et al. 2002; Kubota et al. 2005; Taguchi et al. 2005, Tong et al. 2004). One might speculate that the same mechanism could be valid for gallbladder pathology. For instance, the reported depletion of ICC in patients with Crohn's disease and the increased risk of pigment gallstone formation have apparently not been correlated. Similarly, it could be of interest to assess the gallbladder ICLC population in patients with home parenteral nutrition showing a high rate of cholelithiasis (Dray et al. 2007). In this context, as pure speculation, a (more) generalized decrease in the global ICC pool might be hypothesized as a new mechanism of disease, too. Recently, ICC depletion as a potential mechanism of disease has also been reported outside the digestive tract (Solari et al. 2003). By analogy with multiple endocrine neoplasia, one can imagine a 'multiple-organ ICC/ICLC depletion'. This analogy overpasses the limits of simple assumption since, for example Hirschprung's disease (an example involving ICC depletion) has in common with multiple endocrine neoplasia mutation(s) in RET tyrosine-kinase (for review see Takahashi 2001). As mentioned earlier, other indirect evidence of the presence of Cajal-type cells in the gallbladder was repeatedly provided in the last few years, when gallbladder tumors with a phenotype typical for interstitial cells of Cajal were reported (Ortiz-Hidalgo et al. 2000; Mendoza-Marin et al. 2002; Park et al. 2004; Furihata et al. 2005). An elegant study examined whether a microarray technique could be an appropriate tool to identify

such tumors (and stated a negative conclusion) (Langner et al. 2004).

Conclusions

In this paper, we further substantiate the idea that the distribution of Cajal-type cells in humans might be on a larger scale than was originally thought (Huizinga and Faussone-Pellegrini 2005), documenting the presence of ICLC in fetal and adult non-tumoral human gallbladder interstitium. It was previously observed that heterogeneity of ICC, as well as organ or species specificity of corresponding types of ICC, have to be interpreted in a complex context, taking into account: origin (multipotency of a family of mesenchymal cells), microenvironment, mechanical force, nerve supply, relationships with muscle cells, or eating habits of animals, and movement patterns of the organ proper (Komuro 1999). Accumulating evidence suggests that this is the case for all types of ICLC as well. Cytological features exposed by ICLC depend upon the specific requirements of different tissues or organs. The gallbladder is not devoid of such peculiar requirements. Nevertheless, there is still a possibility of misdiagnosing ICC/ICLC (Streutker et al 2007). Extensive use of the available guides (Faussone-Pellegrini and Thuneberg 1999; Huizinga et al. 1997) and standards for identification for ICLC (Popescu et al. 2005b) should be encouraged in the future.

Acknowledgements Supported by CEEX 111/2006, CEEX 112/2006 and 'Program Nucleu' 06.26.01.02./2006, MCT Grants to 'Victor Babes' Institute of Pathology and ''Carol Davila'' University of Medicine and Pharmacy, Bucharest, Romania. We gratefully acknowlegde Mrs. Christine Lewis Radu for critical reading the manuscript and Dr. Dragos Cretoiu for experimental help and advice.

References

Bobryshev YV (2005) Subset of cells immunopositive for neurokinin-1 receptor identified as arterial interstitial cells of Cajal in human large arteries. Cell Tissue Res 321:45–55
Bussolati G (2005) Of GISTs and EGISTs, ICCs and Ics. Virchows Arch 447:907–908
Bussolati G, Gugliotta P (1983) Nonspecific staining of mast cells by avidin – biotin-peroxidase complexes (ABC). J Histochem Cytochem 31:1419–1421
Cajal RS (1911) Histologie du système nerveux de l'Homme et de Vertébrés. Grand sympathique, Paris, Maloine
Ciontea SM, Radu E, Regalia T, Ceafalan L, Cretoiu D, Gherghiceanu M, Braga RI, Malincenco M, Zagrean L, Hinescu ME Popescu LM (2005) C-kit immunopositive interstitial cells (Cajal-type) in human myometrium. J Cell Mol Med 9:407–420
Cretoiu D, Ciontea SM, Popescu LM, Ceafalan L, Ardeleanu C (2006) Interstitial Cajal-like cells (ICLC) as steroid hormone sensors in human myometrium: immunocytochemical approach. J Cell Mol Med 10:789–795
Dray X, Joly F, Reijasse D, Attar A, Alves A, Panis Y, Valleur P, Messing B (2007) Incidence, risk factors, and complications of cholelithiasis in patients with home parenteral nutrition. J Am Coll Surg 204:13–21
Faussone-Pellegrini MS (2006) Relationships between neurokinin receptor-expressing interstitial cells of Cajal and tachykininergic nerves in the gut. J Cell Mol Med 10:20–32
Faussone Pellegrini MS, Thuneberg L (1999) Guide to the identification of interstitial cells of Cajal. Microsc Res Tech 47:248–266
Faussone-Pellegrini MS, Vannucchi MG (2006) Substance P and neurokinin 1 receptor – expression is affected in the ileum of mice with mutation in the W locus. J Cell Mol Med 10:511–518
Faussone-Pellegrini MS, Vannucchi MG, Ledder O, Huang TY, Hanani M (2006) Plasticity of interstitial cells of Cajal: a study of mouse colon. Cell Tissue Res 325:211–217
Frossard JL (2005) Conversion of biliary system to pancreatic tissue: complete plasticity or same organ? J Hepatol 42:782–785
Furihata M, Fujimori T, Imura J, Ono Y, Furihata T, Shimoda M, Kato M, Kita J, Ohkura Y, Kubota K (2005) Malignant stromal tumor, so called ''gastrointestinal stromal tumor'', with rhabdomyomatous differentiation occurring in the gallbladder. Pathol Res Pract 201:609–613
Grimbaldeston MA, Chen CC, Piliponsky AM, Tsai M, See-Ying Tam S-Y, Galli SJ (2005) Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. Am J Pathol 167:835–848
Hakimian R, Korn D (2004) Ownership and use of tissue specimens for research. JAMA 292:2500–2505
Harhun MI, Pucovsky V, Povstyan OV, Gordienko DV, Bolton TB (2005) Interstitial cells in the vasculature. J Cell Mol Med 9:232–243
Heinrich MC, Corless CL (2005) Gastric GI stromal tumors (GISTs): the role of surgery in the era of targeted therapy. J Surg Oncol 90:195–207
Hinescu ME, Popescu LM (2005) Interstitial Cajal-like cells (ICLC) in human atrial myocardium. J Cell Mol Med 9:972–975
Hinescu ME, Gherghiceanu M, Mandache E, Ciontea SM, Popescu LM (2006) Interstitial Cajal-like cells (ICLC) in atrial myocardium: ultrastructural and immunohistochemical characterization. J Cell Mol Med 10:243–257
Hopwood D, Ross PE (1997) Biochemical and morphological correlations in human gallbladder with reference to membrane permeability. Microsc Res Tech 38:631–642
Hsu SM, Raniel L, Fanger H (1981) Use of ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580
Huizinga JD, Faussone-Pellegrini MS (2005) About the presence of interstitial cells of Cajal outside the musculature of the gastrointestinal tract. J Cell Mol Med 9:468–473
Huizinga JD, Thuneberg L, Vanderwinden JM, Rumessen JJ (1997) Interstitial cells of Cajal as targets for pharmacological intervention in gastrointestinal motor disorders. Trends Pharmacol Sci 18:393–403
Isozaki K, Hirota S, Nakama A, Miyagawa J, Shinomura Y, Xu Z, Nomura S, Kitamura Y (1995) Disturbed intestinal movement, bile reflux to the stomach, and deficiency of c-kit expressing cells in Ws/Ws mutant rats. Gastroenterology 109:456–464
Komuro T (1999) Comparative morphology of interstitial cells of Cajal: ultrastructural characterization. Microsc Res Tech 47:267–285
Kubota M, Kanda E, Ida K, Sakakihara Y, Hayashi M (2005) Severe gastrointestinal dysmotility in a patient with congenital myopathy: causal relationship to decrease of interstitial cells of Cajal. Brain Dev 27:447–450
Lang RJ, Klemm MF (2005) Interstitial cell of Cajal-like cells in the upper urinary tract. J Cell Mol Med 9:543–556
Langner C, Lemmerer M, Kornprat P (2004) Analysis of KIT (CD117) expression in gallbladder carcinomas by tissue microarray. Eur J Surg Oncol 30:847–850

Lardon J, De Breuck S, Rooman I, Van Lommel L, Kruhoffer M, Orntoft T, Schuit FF, Bouwens L (2004) Plasticity in the adult rat pancreas: transdifferentiation of exocrine to hepatocyte-like cells in primary culture. Hepatology 39:1499–1507

Lavoie BB, Onesmo B, Balemba OB, Mark T, Nelson MT, Sean M, Ward SM, Mawe GM (2007) Morphological and physiological evidence for interstitial cell of Cajal (ICC)-like cells in the guinea pig gallbladder. J Physiol 597:487–501

Lee H, Douglas-Jones AG, Morgan JM, Jasani B (2002) The effect of fixation and processing on the sensitivity of oestrogen receptor assay by immunohistochemistry in breast carcinoma. J Clin Pathol 55:236–238

Lyford GL, He CL, Soffer E, Hull TL, Strong SA, Senagore AJ, Burgart LJ, Young-Fadok T, Szurszewski JH, Farrugia G (2002) Pan-colonic decrease in interstitial cells of Cajal in patients with slow transit constipation. Gut 51:496–501

Maeda H, Yamagata A, Nishikawa S, Yoshinaga K, Kobayashi S, Nishi K, Nishikawa S-I (1992) Requirement of c-kit for development of intestinal pacemaker system. Development 116:369–375

McCloskey KD, Hollywood MA, Thornbury KD, Ward SM, McHale NG (2002) Kit-like immunopositive cells in sheep mesenteric lymphatic vessels. Cell Tissue Res 310:77–84

Mendoza-Marin M, Hoang MP, Albores-Saavedra J (2002) Malignant stromal tumor of the gallbladder with interstitial cells of Cajal phenotype. Arch Pathol Lab Med 126:481–483

Miller K (2002) Immunocytochemical techniques. In: Bancroft JD, Gamble M (eds) Theory and practice of histological techniques, 5th edn. Churchill Livingstone, London, pp 421–464

Nakanuma Y, Katayanagi K, Kawamura Y, Yoshida K (1997) Monolayer and three dimensional cell culture and living tissue culture of gallbladder epithelium. Microsc Res Tech 39:71–84

Oldham-Ott CK, Gilloteaux J (1997) Comparative morphology of the gallbladder and biliary tract in vertebrates: variation in structure, homology in function and gallstones. Microsc Res Tech 38:571–597

Ortiz-Hidalgo C, de Leon Bojorge B, Albores-Saavedra J (2000) Stromal tumor of the gallbladder with phenotype of interstitial cells of Cajal. A previously unrecognized neoplasm. Am J Surg Pathol 24:1420–1423

Park JK, Choi SH, Lee S, Min KO, Yun SS, Jeon HM (2004) Malignant gastrointestinal stromal tumor of the gallbladder. J Korean Med Sci 19:763–767

Parr EJ, Kennedy AL, Mawe GM (2003) Lack of evidence for the existence of interstitial cells of Cajal in the gallbladder. Gastroenterology 124(Suppl 1):A347

Popescu LM, Andrei F, Hinescu ME (2005a) Snapshots of mammary gland interstitial cells: methylene-blue vital staining and c-kit immunopositivity. J Cell Mol Med 9:476–477

Popescu LM, Ciontea SM, Cretoiu D, Hinescu ME, Radu E, Ionescu N, Ceausu M, Gherghiceanu M, Braga RI, Vasilescu F, Zagrean L, Ardeleanu C (2005b) Novel type of interstitial cell (Cajal-like) in human fallopian tube. J Cell Mol Med 9:479–523

Popescu LM, Gherghiceanu M, Cretoiu D, Radu E (2005c) The connective connection: interstitial cells of Cajal (ICC) and ICC-like cells establish synapses with immunoreactive cells. Electron microscope study in situ. J Cell Mol Med 9:714–730

Popescu LM, Hinescu ME, Ionescu N, Ciontea SM, Cretoiu D, Ardeleanu C (2005d) Interstitial cells of Cajal in pancreas. J Cell Mol Med 9:169–190

Popescu LM, Gherghiceanu M, Hinescu ME, Cretoiu D, Ceafalan L, Regalia T, Popescu AC, Ardeleanu C, Mandache E (2006a) Insights into the interstium of ventricular myocardium: interstitial Cajal-like cells (ICLC). J Cell Mol Med 10:429–458

Popescu LM, Vidulescu C, Curici A, Caravia L, Simionescu AA, Ciontea SM, Simion S (2006b) Imatinib inhibits spontaneous rhythmic contractions of human uterus and intestine. Eur J Pharmacol 546:177–181

Popescu LM, Ciontea SM, Cretoiu D (2007) Interstitial Cajal-like cells in human uterus and fallopian tube. Ann NY Acad Sci 1101:139–165

Porcher C, Baldo M, Henry M, Orsoni P, Jule Y, Ward SM (2002) Deficiency of interstitial cells of Cajal in the small intestine of patients with Crohn's disease. Am J Gastroenterol 97:118–125

Radu E, Regalia T, Ceafalan L, Andrei F, Cretoiu D, Popescu LM (2005) Cajal-type cells from human mammary gland stroma: phenotype characteristics in cell culture. J Cell Mol Med 9: 748–752

Robinson TL, Sircar K, Hewlett BR, Chorneyko K, Riddell RH, Huizinga JD (2000) Gastrointestinal stromal tumors may originate from a subset of CD34-positive interstitial cells of Cajal. Am J Pathol 56:1157–1163

Rumessen JJ, de Kerchove d'Exaerde A, Mignon S, Bernex F, Timmermans JP, Schiffmann SN, Panthier JJ, Vanderwinden JM (2001) Interstitial cells of Cajal in the striated musculature of the mouse esophagus. Cell Tissue Res 306:1–14

Sanders KM, Ordog T, Ward SM (2002) Physiology and pathophysiology of the interstitial cells of Cajal: from bench to bedside IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal. Am J Physiol Gastrointest Liver Physiol 282:G747–G756

Sanders KM, Ward SM (2006) Interstitial cells of Cajal: a new perspective on smooth muscle function. J Physiol 576:721–726

Sergeant GP, Thornbury KD, McHale NG, Hollywood MA (2006) Interstitial cells of Cajal in the urethra. J Cell Mol Med 10:280–291

Shen CN, Slack JMV, Tosh D (2002) Molecular basis of transdifferentiation of pancreas to liver. Nat Cell Biol 2:879–887

Solari V, Piotrowska AP, Puri P (2003) Altered expression of interstitial cells of Cajal in congenital ureteropelvic junction obstruction. J Urol 170:2420–2422

Streutker CJ, Huizinga JD, Driman DK, Riddell RH (2007) Interstitial cells of Cajal in health and disease. Part II: ICC and gastrointestinal tumors. Histopathology 50:190–202

Sumazaki R, Shiojiri N, Isoyama S, Masu M, Keino-Masu K, Osawa M, Nakauchi H, Kageyama R, Matsui A (2004) Conversion of biliary system to pancreatic tissue in Hes1-deficient mice. Nat Genet 36:83–87

Sun X, Yu B, Xu L, Dong W, Luo H (2006) Interstitial cells of Cajal in the murine gallbladder. Scand J Gastroenterol 41:1218–1226

Taguchi T, Suita S, Masumoto K, Nagasaki A (2005) An abnormal distribution of c-kit positive cells in the normoganglionic segment can predict a poor clinical outcome in patients with Hirschsprung's disease. Eur J Pediatr Surg 15:153–158

Takahashi M (2001) GDNF/RET signaling pathway and human diseases. Cytokine Growth Factor Rev 12:361–373

Thomas MB (2007) Biological characteristics of cancers in the gallbladder and biliary tract and targeted therapy. Crit Rev Oncol Hematol 61:44–51

Tong WD, Liu BH, Zhang LY, Zhang SB, Lei Y (2004) Decreased interstitial cells of Cajal in the sigmoid colon of patients with slow transit constipation. Int J Colorectal Dis 19:467–473

Vannucchi MG (1999) Receptors in interstitial cells of Cajal: identification and possible physiological roles. Microsc Res Tech 47:325–335

Ye J, Zhu Y, Khan WI, Van Snick J, Huizinga JD (2006) IL-9 enhances growth of ICC, maintains network structure and strengthens rhythmicity of contraction in culture. J Cell Mol Med 10:687–694