Identity and Roles of Interstitial Cells in the Upper Urinary Tract (UUT)

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ABSTRACT

For a long time, it was known that in the upper urinary tract (UUT), the peristaltic contractions that propel urine to the bladder are spontaneous and myogenic, supposedly generated by pacemaker atypical smooth muscle cells (ASMCs) located in the initial part of the tract and 'driven' to the contracting typical smooth muscle cells (TSMCs). The discovery in the UUT of an interstitial cell (IC) type that had a spontaneous electrical activity and morphological similarities to the interstitial cells of Cajal (ICC) that generate intrinsic electrical rhythmicity in the digestive tract smooth muscles has triggered intense and enthusiastic research, especially after the intensive use of stem cell growth factor receptor c-Kit, which was initially considered specific. This cell was named ICC-like cell (ICC-LC) with a presumed latent pacemaker activity, an integrative and modulatory role for coordinated peristalsis in the renal pelvis and ureter.

Alteration in the number of ICC-LCs and other intrinsic and extrinsic causes has been investigated in neonatal hydronephrosis due to congenital ureteropelvic junction obstruction (UPJO) and other congenital abnormalities of the kidney and urinary tract (CAKUT). Most C-Kit negative ICs were later identified, and different names and markers were proposed. After 25 years of research, the elusive nature, functions, differences, and names of the ICs in the UUT across species are still under a slower pace of investigation.

Keywords: Interstitial Cells of Cajal-like cells (ICC-LCs), telocytes (TCs), interstitial cells (ICs), atypical smooth muscle cells (ASMCs), c-Kit, PDGFRα, Upper Urinary Tract (UUT)

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1. Identity still debated: Different names for similar cells?

In histology, the interstitium corresponds to the fluid-filled spaces supported by connective tissue composed of fibers, the extracellular matrix (ECM), and different cells. The connective tissue of the kidney parenchyma, called interstitial tissue, surrounds the nephrons, ducts, blood, and lymphatic vessels.

The stroma of different organs is made of loose but also dense irregular connective tissue. It contains blood and lymphatic vessels, nerves, ducts, and smooth muscle fibers with a connective role to the parenchyma's cells that perform the tissue or organ function. Stromal and interstitial are the same but sometimes different.

The connective tissue cells can be permanent (resident) cells and wandering (transient) cells originating from blood vessels [1]. The resident cell population is represented by the fibroblasts (activated cells) or inactive (usually called fibrocytes, but this name is also used for cells derived from the bone marrow and identified as a circulating mesenchymal cell precursor [2], myofibroblasts, macrophages, mast cells, adipocytes, and adult stem cells. Lymphocytes, plasma cells, neutrophils, eosinophils, basophils, and monocytes represent the transient cell population. "Interstitial cells" is not usually used to designate these connective tissue cells.

Papers about the urinary tract still use inconsistent terminology regarding the cells located in the interstitial tissue, which may play a unique role in the contraction of urinary organs: Interstitial Cells (IC), Interstitial Cells of Cajal (ICCs), Interstitial Cells of Cajal-like cells (ICC-LCs), Cajal cells, c-Kit positive cells [3], Renal Interstitial Cells (RICs) [4], Fibroblast-like Cells FiCs [5], Sub-urothelial ICs (SICs) later renamed Basal Epithelial Cells BECs [6], Telocytes TCs [7], stromal cells, c-Kit(+) Interstitial Cells (KICs) and PDGFRα(+) Interstitial Cells (PIC1&PIC2) [8].

The name Interstitial Cells Cajal-like cells (ICC-like cells) was first used in 1998-99 [4], then in the upper urinary tract. Renal Interstitial Cells (RICs) [5] and Fibroblast-like Cells [6] were also introduced as specific cells of the renal interstitium.

Interstitial cells of Cajal (ICCs) were first indicated as intestinal pacemaker cells. Later, c-Kit(+) ICCs generating spontaneous pacemaker activity were found in the digestive tract. Structural and electrophysiologically similar cells were found in the upper urinary tract (UUT) and were named ICC-like cells [9]. Later, the term "Telocytes" was proposed to define the ICC-LCs in different organs outside the digestive tract (7).

In the urinary tract, the peristaltic contractions are spontaneous and myogenic as they continue in vivo after denervation. In uni-calyceal kidneys, the spontaneous electrical impulse originates in the most proximal regions of the pelvic-papilla border [10] and the mid and distal RP [11]. In multi-calyceal kidneys, the spontaneous electrical impulse originates in one minor calyx simultaneously, spontaneously moving between calyces [12]. The pacemaker cells fire 'pacemaker' action potentials with a higher frequency than the 'driven' action potentials of the smooth muscle cells, and 'intermediate' action potentials are fired by ICC-LCs.

The contracting 'Typical' smooth muscle cells (TSMCs) establish close appositions with 'atypical' SMCs (ASMCs) and ICC-LCs. ASMCs fire spontaneous high-frequency membrane potential oscillations, while ICC-LCs fire spontaneous low-frequency action potentials that propagate distally [5]. UUT peristalsis appears to be only myogenic in origin, with the ASMCs being the primary pacemaker cells and ICC-LCs regulating motility as secondary pacemaker cells. The initial trigger of the pacemaker signal involves the spontaneous release of Ca2+ transients from internal cell storage that can be visualized and measured using green calcium fluorescent indicators [7] or genetically encoded Ca2+ sensors [13].

During the last decade, alteration in the number or activity of the ICC-LCs, along with other intrinsic and extrinsic causes (urinary tract development, muscular and nerve defects, fibrosis, inflammation), has been investigated as one of the leading causes of congenital abnormalities of the kidney and urinary tract (CAKUT). The most studied cause is neonatal hydronephrosis due to congenital ureteropelvic junction obstruction (UPJO). Some authors have recently classified ASMCs as Ics. [13].

2. Structural identity:
2.1 Methods of cell investigation in UUT (short inventory)

Various morphological, electrophysiological, and molecular approaches are used to establish identity in the interstitium, using samples from humans and wild-type or even transgenic animals from different species.
Immunohistochemistry and immunofluorescence techniques with single or multiple labeling identify ICs in normal and pathological samples.
C-Kit (CD117) was initially considered the ICC-LCs-specific marker and was intensely used, but finally, most ICs were found to be c-Kit negative [13]. The lack of IHC or IF positivity reported in different studies was related to species, antibodies used, sensitivity to fixation methods like fresh frozen versus formalin-fixed paraffin-embedded tissue samples [14], or just non-specific reactivity. The quest for selective markers for different IC populations is necessary to highlight their identity and distribution correctly.

Transmission electron microscopy (TEM) was used to identify the ICC-LCs in the UUT [4,9] and their relation to other cells in normal or pathological samples.
A 2-photon laser scanning electron microscope (SEM) and image analysis were used to prove that in a rat's normal ureter, the predominant orientation of SMCs and parallel ICs is longitudinal [5].
Images captured by SEM of the mouse renal sample surface trimmed with a focused ion beam (FIB-SEM) were used with imaging software to reconstruct the 3D shape of ICs and visualize the spatial relationships between different cells [15].
A single-cell cell-picking microdissection was automatically performed using UVa laser-assisted cell-picking microdissection software, a microscope with a computer-controlled stage, and a UV-cut software.
Segments of UUT can be studied in organ baths using video microscopy for live cell imaging of contraction waves and electrical activity with voltage-sensitive fluorescent dyes or by measuring changes of intracellular Ca 2+ transients using fluorescence imaging with Ca2+ fluorophores (Cal-520).

Electrophysiological studies of enzymatically dispersed SMCs and ICs using intracellular microelectrodes and glass microelectrodes recording the electrical activity of spontaneous contractions combined with electron and confocal microscopy proved the existence of three different spontaneously discharging cells involved in the peristalsis of UUT: ASMCs, ICC-LCs, and TSMCs [16].
Various transgenic animal models try to mimic pathological conditions to allow further investigation of the mechanisms and potential new therapeutic approaches. For UPJO, different studies used spontaneous hydronephrosis congenital models or surgically induced complete or incomplete, permanent or reversible, unilateral or bilateral ureteral obstruction of fetal, neonate, or adult animals [10] and knockout and transgenic rodents.
Transgenic reporter strains of mice with GFP (green fluorescent protein) with fluorescent nuclei limited only to cells presenting targeted protein (PDGFRα-, ANO1-, smMHC-eGFP) were also used [13]. Other markers can be added to these cells for multiple labeling. Different populations of enzymatic dispersed cells with conjugated antibodies can be sorted and measured using FACS (fluorescence-activated cell sorting) [13].
eGFPmice expressing GECIs (genetically-encoded Ca2+ indicators) were used to measure the variation of intracellular Ca2+ levels in GFP PDGFRα-, c-Kit-, SMC-GCaMP mice. In-situ imaging of Ca2+ variations allowed the measurement of frequency, duration, and spatial spread in ICs and muscle cells in the renal pelvis of wild-type and transgenic mice [13]. The authors identify, classify, and propose to rename three types of ICs in the RP of the mouse: 2 PDGFRα(+)ICs (ASMCs as PIC1 and c-Kit(-) ICs as PIC2) and a c-Kit(+)ICs as KICs. However, the anatomical differences among species should also be considered.

2.2 Comparative anatomy of UUT (human versus laboratory animals)

There are some differences between humans and other mammals not only in size [kidney length average in humans 10-13 cm (38,39), pig 11-12 cm (40), mouse 1.3 cm (41), rat 1,5 cm, guinea pig 11-12 cm, position and shape but also in the structure and

physiology of the organs. The ureters are more similar in structure in different species. The main difference is in the kidneys' structure and the first part of the UUT -Pyelo kidney junction (PKJ) or pyelo calyx junction, calyces, and renal pelvis with UPJ. The cells generating and conducting the peristaltic waves are located in these regions.

Unilobar, uni-papillary, and uni-calyceal kidneys (a single papilla extending deep into the renal pelvis) are characteristic of small rodents: mice, rats, and guinea pigs. The porcine kidney is more similar to the human kidney than primates. The primate's kidney is different from that of a human's because it has one pyramid with variations in the shape of the papilla. For example, the macaque kidney is uni papillate, with a relatively underdeveloped papilla. The structure is similar across all the nonhuman primates except the genus Ateles (spyder monkey), which, like humans, has multi-pyramidal, multi-papillate kidneys.

The pig may be the best animal model with the closest similarity to the human urinary system regarding the structure, dimensions, and vascularization. Still, the uni papillary kidney of small laboratory animals can represent the basic unit of more complex kidneys.

It is essential to know the similarities and differences in the anatomy, structure and ultrastructure, physiology, and location of pacemakers and ICs in various species, genetically modified animals, or isolated organs to choose the best animal model for a specific human pathology.

2.3 Microscopic Comparative Anatomy of the UUT

The arrangement and distribution of tissues in the UUT of the pig and human are similar [17]. A variable number of minor calyces (5-20) are applied to each one, sometimes two or three papillae of the renal pyramids, and the urine generated by each kidney is collected. The narrow part of the minor calyces (infundibulum) opens in 2-3 significant calyces, which converge into the flattened funnel-shaped renal pelvis, or some minor calyces may open directly into the pelvis. The calyces and the first part of the pelvis are located inside the renal sinus. The central part of the renal pelvis is extrarenal and continues at the UPJ with the ureter. Sometimes, an intrinsic or extrinsic factor causes the ureteropelvic junction obstruction (UPJO), which slows or stops the flow of urine, causing hydronephrosis and destruction of the kidney. Most often, UPJO is congenital. The peristaltic contractions of the muscular coat originating from the pelvicalyceal region constantly propel boluses of urine through the UUT segments to the bladder, where urine is stored and intermittently eliminated through the urethra.

In uni-calyceal species like the rat, a long renal papilla penetrates deeply into the renal pelvis, corresponding to one minor calyx of multi-papillary kidneys. The renal pelvis (RP) attaches to the parenchyma of the kidney via some “finger-like” spokes with concave edges in between, forming some secondary pouches. The RP's most proximal region, the PKJ spoke projections, contains thin muscle strips forming more distally a continuous muscle band surrounding the inner papilla [4].

The rat renal papilla is not lined with urothelium but by a single layer of regularly shaped cuboidal cells [4], while the transitional epithelium lines the human renal papilla.

The RP of the uni-calyceal species has a thin mucosa (urothelium and lamina propria), a muscular formed by a continuous inner dense layer of circumferentially arranged bundles of TSMC, originating from the kidney parenchyma at the base of the papilla, continued by the ureter's muscular and an outer thinner layer of loosely arranged ASMCs that are considered to be the pace-maker cells that initiate peristalsis in UUT.

TSMCs are spindle-shaped and extremely elongated, mainly circumferentially oriented, densely packed, and increasing in number with distance from the papilla.

ASMCs are irregularly shaped with long, thin extensions. They are predominantly longitudinally or in a basket weave arrangement, loosely packed, close to other ASMCs, TSMCs, or ICs, and decrease in number away from the papilla.

In multi-calyceal kidneys, the structure of minor and significant calyces is the same: a tunica mucosa (thin urothelium and lamina propria), a muscular, and an adventitia with two layers of thin bundles of SMCs, but with the inner ASMCs muscle layer and the outer TSMCs layer.

The difference between the minor and major calyces is the ring arrangement of SMCs forming a sphincter around the minor calyces. ASMCs alone develop the muscle of a minor calyx and extend between the minor calyces as a loosely thin sheet of cells. Collagen fibers and axons are found between ASMCs. A thickening layer of TSMCs appears and extends to the major calyces and renal pelvis.

RP has a greater diameter and a thicker wall consisting of a tunica mucosa (urothelium and a thin loose lamina propria), a tunica muscularis (an inner longitudinal and an outer circular layer of SMCs and, TSMC are increasingly more tightly packed into closely associated bundles) and the adventitia (same as in major and minor calyces). In humans, the musculature is made of SMC bundles with circular, longitudinal, and spiral orientation both in normal and UPJO but thicker in the last one, with increased interfascicular and intrafascicular collagen that may disperse the smooth muscle. All tunics continue with those of the ureter at the UPJ.

The lamina propria (350-700 µm thick in humans and 20 µm in rat ureter) consists of loose connective tissue, ICs with processes, and the majority of the collagen fibers lying in the long axis of the ureter, with unmyelinated nerve fibers and longitudinal small blood vessels many close to and located into grooves of urothelium basal membrane. Usually, two layers of smooth muscle are described under the lamina propria: an inner longitudinal and an outer circular layer; in the distal ureter, a third outer longitudinal layer is added. However, in the human upper ureter, the various bundles of SMCs separated by loose connective tissue tend to have a random and varying spiral, predominantly longitudinal orientation, and 2-3 layers (not in young children) in the distal ureter. In comparison, in rats, the two layers are distinct. The muscle layer of the human ureter is 750 µm thick, while in rats, it is 25 µm thick.

The nerves form two continuous networks communicating with each other. The first is located within the lamina propria, and the second is within the muscular layer, from the renal pelvis to the ureter. The innervation of the renal pelvis muscle is more significant than the ureter's. The lower ureter receives denser innervation than the upper ureter [5].

Large nerves are located in adventitia with branches near arterioles. Autonomic ganglion cells or neuromuscular synapses are not identified in any layers [13]. In rats, the arterioles end before the outer muscle layer. In other lab animals (mice, guinea pigs), some arterioles penetrate the muscle coat and terminate in mucosa, associated with small nerves. In the pig, the nerves are located mainly between the muscle bundles, and just a few are intrafascicular.

2.4 Ultrastructural features of UUT's ICs

"Icc-like cell" was first described in the lamina propria of the renal pelvis and pelvicalyceal junction but not in the ureter of the guinea pig [4]. These cells were similar to the Interstitial Cells of Cajal (ICC) described in the mammalian intestine. In electron micrographs, ICC-LC was oval (3-5 µm wide) [9] or with a stellate cell body, with many narrow (0.1-0.5µm), long (more than 30 nm) processes [18], forming close appositions (20 nm) and adherent junctions [9], intermediate and gap-like junctions with same cells [4,5,9], SMCs, capillaries and hemidesmosomes with connective tissue.

The nucleus is oval, thin, long, or irregularly shaped, with clusters of peripheral heterochromatin. A light cytoplasm with few organelles surrounds the nucleus. More specifically, there are numerous caveolae and a discontinuous basal lamina or generally absent basal lamina. Numerous [9] or moderate [19] mitochondria, Golgi apparatus, and rER. Some intermediate filaments, microtubules, and dense core vesicles but no contractile filaments.

After 2010, when it was proposed to rename the ICC-LCs telocytes, many authors started using this name for ICs in different organs and tissues, including in UUT [3, 4, 7].

Without specific immune markers, the 'gold standard' method to identify the telocytes in different organs remained the TEM [1]. TCs are elongated cells with small variable-shaped cell bodies (9-15 µm) presenting a variable number (1-5) of long dichotomously branched hundreds of µm cytoplasmic projections named 'telopodes'. The telopodes have a 'beads on string' appearance,

presenting numerous thin segments - 'podomers' (75-80 nm) in alternance with dilated 'podoms' (250-300 nm) and communicate via gap junctions with the same cells and establishing close contacts with different types of cells. The elongated heterochromatic nucleus is surrounded by a small amount of cytoplasm with scarce organelles, a plasma membrane with few caveolae, numerous coated vesicles, and a discontinuous or, more often, absent basal lamina. 'Podoms' present caveolae, small mitochondria, and some rER.

Some authors have named the ICs fibroblast-like cells [6]. However, fibroblasts have a larger oval euchromatic nucleus, shorter cytoplasmic projections, and lack basal lamina, caveolae, and intermediate filaments.

The ICs present a thinner nucleus and longer light cytoplasmic projections. They have no plasma membrane thickenings, fibro nexuses (an archetypal structural marker of the myofibroblast), or contractile myofilaments, as myofibroblasts do [8].

Similar to the ICs, the ASMCs may appear on TEM images as spindle or stellate, very irregular, presenting many radiating long and narrow but also short and narrow cytoplasmic processes. They have a continuous or discontinuous basal lamina but with a rounder, larger euchromatic nucleus and bundles of myofilaments [9].

In the rat renal pelvis, few ICC-LCs were identified in the lamina propria. However, in the ureter, TCs are found in the lamina propria and between smooth muscle bundles.

In humans, the ICC-LCs were observed in the lamina propria and muscular layer of the ureteropelvic junction. However, UPJO samples contained fewer ICC-LCs than controls.

In the rodent RP, a loosely packed single layer of ICs adjacent to urothelium were named basal epithelial cells BECs (previously termed suburothelial ICs). BECs are irregular, with many short protrusions, a large round nucleus, numerous dense bodies, mitochondria, and free ribosomes, closely associated with each other, with urothelial cells and TSMCs. Collagen fibers, small blood vessels, and nerves were found between BECs [7]. Recently, BECs were described as part of the urothelium and have close appositions with ICs.

The ASMCs are spindle-shaped and irregular, presenting many long and narrow cytoplasmic processes forming a network with the same cells and some short cytoplasmic processes in close contact with adjacent TSMCs. Compared to the larger and darker TSMCs, which have more than 60% of sectional areas containing packed myofilaments, the paler ASMCs have less than 40% of widely spread myofilaments separated by areas containing small mitochondria, rER, and the Golgi apparatus [4,5].

FIB-SEM tomography followed by 3D volume rendering of cells from mouse RP revealed that the suburothelial ICs are flat, ribbon-shaped; the TSMCs are long, spindle cigar-like; the adventitial-located ASMCs are not spindle or stellate, and the thin, long, irregular projections are continuous. FIB-SEM, followed by 3D reconstruction, also revealed the ribbon-like aspect of telopodes from skin TCs, different from the 'moniliform' TEM aspect.

Immunoelectron labeling would help segregate cells with apparently the same electron micrograph features.

3. Phenotype:

Finding a reliable monoclonal or polyclonal antibody method to identify a specific cell type in tissue samples of humans and other species is essential in laboratory research and diagnosis.

3.1. c-Kit (Cd117)

More than two decades ago, Klemm M.F. and Lang R.J. identified and named for the first time the Interstitial Cells of Cajal-like Cells (ICC-LCs) in the renal pelvis of guinea-pig by using EM and electrophysiology methods [4].

C-Kit type III tyrosine kinase receptor (CD117) is considered the specific marker for ICCs in the gut and a reliable marker for identifying ICC-LCs. C-Kit-expressing cells were first identified in the ureters of mouse embryos, but in Klemm's experiments, the ICC-LCs from the renal pelvis of guinea pigs failed to express the marker.

In retrospective studies on patients with congenital UPJO and ureter obstruction experiments, increased activity and the number of ICC-LCs were expected to be found as a compensatory mechanism reflected by increased c-Kit expression compared to controls. If

the compensatory mechanisms are ineffective, decreased activity and the number of ICC-LCs are reflected in decreased c-Kit expression.

Different authors and experiments on various species gave controversial findings: ICC-LCs were either c-Kit positive or c-Kit negative. The lack of c-kit-positive ICs in other studies was interpreted to be related to the species, the c-kit antibody used, and the fixation methods.

The search for an immunohistochemical marker or set of markers to precisely identify the ICs in the urinary tract began with Klemm's article and is continuing today. No definitive answer has yet been given about the c-Kit expression in humans and other species.

Anti-c-Kit and anti-mast cell tryptase or chymase co-labeling of mast cells differentiate them from ICC-LCs, which are only c-Kit positive. Still, a few investigators used double labeling to determine c-Kit-positive ICC-LCs from mast cells.

C-Kit positive ICC-LCs forming a network in mice ureters were negative for mast cell FITC-avidin immunofluorescence

c-Kit+ spindle-shaped ICC-LCs were different from c-Kit+ rounded mast cells also positive to AA1, CC1, toluidine blue, alcian blue, CD34. ICC-LCs were negative to CD34, neuron specific S-100, enolase BBS/NC/VI-H14, and αSMA. ICC-LCs were found ubiquitously distributed in the human ureter in the lamina propria and among the inner and outer smooth muscle layers. A third type of c-Kit + cells was identified as vertical interepithelial cells between the basal cells of the urothelium. However, the majority of c-Kit+ cells were mast cells. In rodents, ICC-LCs were found more in adventitia and less in muscle and lamina propria. In all those species, the c-kit receptor was detected in spindle-shaped cells (ICC-LCs) and round cells (mast cells) found in all layers of the ureteral wall but not in the urothelium. A higher number of ICC-LCs was present in humans and pigs and the lowest in rats and mice. An extensive set of 10 monoclonal and eight polyclonal antibodies against c-Kit receptors were used. The same markers made it possible to differentiate mast cells, nerves, and muscle cells from ICC-LCs. All used anti-c-kit antibodies showed a uniform reaction in humans, but only the polyclonal antibodies reacted with the other species in various ways. There are cytological differences between the typical stag-horn appearance of the gut ICCs and the spindle-shaped with thin dendritic processes of ureteral ICC-LCs. The morphological data indicate that the pig is favorable for functional studies.

On the whole porcine urinary tract, three cell types c-Kit positive were found based on morphology and phenotype:

a. The majority were identified as mast cells with a wide range of polymorphisms found in varying amounts in all segments and layers of the urinary tract.

b. Interstitial spindle cells presenting an oval nucleus and bi-tripolar morphology with thin, branching, often long wavy processes (~100µm). Not forming networks, frequently found between smooth muscle bundles but also in the lamina propria and adventitia.

c. Basal and vertically oriented cells within the urothelium present a large oval nucleus, thin cytoplasm, and processes. In humans, lower ureter c-Kit-positive ICCs are distinguished from c-Kit-positive mast cells by their aspect and reactivity to mast cell tryptase. ICC-LCs located between the smooth muscle bundles and fewer in the lamina propria presented an oval nucleus, little perinuclear cytoplasm, and one or several processes.

Balikci et al. [20] reported a reduced number of c-Kit+ ICC-LCs in the lamina propria and muscularis of the renal pelvis and proximal ureter in humans with hydroureteronephrosis compared to the control group. Most c-Kit+ cells in the renal pelvis were mast cells. Rounded mast cells (CD117+/CD45+) are more abundant in the proximal renal pelvis and PKJ regions. In contrast, elongated c-Kit+ ICs (CD117+/CD45-/ PDGFRα-) are found predominantly in the distal renal pelvis, ureteropelvic junction, and proximal ureter of WT and transgenic eGFP+ mice [21].

From 2003 to 2021, only in 8 articles the investigators identified the c-Kit positive ICs located in the UUT using double labeling with specific markers to differentiate them from the c-Kit positive mast cells. Studies were performed on mice and human ureters or the entire pyeloureteral complex (UUT) of various species - humans, rats, mice, dogs, cats, rabbit, wild boar, swine and cow, Vietnamese pig ureters, ureteropelvic junctions after distal ureteral ligature obstruction on rats, human normal

and obstructed ureteropelvic junctions [22] and human renal pelvis and ureters with and without hydronephrosis [20], mice renal pelvis [21].

In the same period, the other 28 articles identified the c-Kit-positive ICs and differentiated them from mast cells only by their morphological aspect observed by light or fluorescent microscope without double labeling. Of those articles, the large majority (21) were studies on human renal pelvis, ureter, UPJ, and ureteropelvic junction obstruction (UPJO) made mainly by urologists, and 6 were studies on mice or rats.

In other studies, the only c-Kit-positive cells were the mast cells, the ICs being c-Kit negative in guinea pig renal pelvis [9,12]. Still, positive in mouse and rat renal pelvis [23], other ICs were negative to c-Kit but positive to other markers like PDGFRα in the human UPJ [24] or mice [21]. Other studies reported no c-Kit positive ICs in mouse and rat renal pelvis - human renal pelvis – 6, 11, 13, 21]. C-pit positivity is restricted only to mast cells and nerve bundles in rodent renal pelvis [6], and c-kit positivity is located primarily in nerve bundles. If some images of c-Kit positive elongated structures might be ambiguous, the spindle-shaped or stellate-shaped ICs in other micrographs do not look like Schwann cells or nerve fibers. Average human adult Schwann cells do not express any detectable expression of c-Kit. In all three layers of the ureter, the axons can be identified in bundles invaginated into Schwann cell's cytoplasm. In the superficial layers of the lamina propria, many bundles of unmyelinated nerve fibers are found, but never nearer than 100μm to the urothelium.

In the peripheral nerves, the telocytes (TCs) were detected in the endoneurium and have been named either endoneurial stromal cells, endoneurial fibroblasts, endoneurial fibroblast-like cells, capsular fibroblasts, CD34+ endoneurial cells, dendritic endoneurial cells, endoneurial mesenchymal cells, nerve mesenchymal precursor-like cells. In the epi-perineurium, TCs are arranged on both sides of the layers formed by the perineurial cells and can occasionally be intermixed with these cells. In nerves, in addition to CD34, TCs express PDGFRa and PDGFRb. Schwann cells express S100, nerve fibers express neurofilaments. Only mast cells express c-kit and are frequently associated with TCs.

In the human paraffin-embedded normal upper ureter, three different types of c-kit negative ICs with TCs morphology were identified by Dobra et al. according to CD105 expression: a. suburothelial spindle-shaped with long telopodes CD34(-); b.in deep lamina propria and within muscle interstitia CD34(+), smMHC(+) myoid dichotomously branched TCs and c.perivascular CD34(+) TCs. Endothelial cells were CD34(+) and CD105(+) [19].

The lack of a c-Kit positive reaction can be species-dependent and involve various antibodies, fixation (use of paraformaldehyde-fixed tissue instead of fresh frozen tissue), and other materials and methods procedures (like the antigen retrieval phase).

No c-Kit positive reaction of ICs in the absence of positive controls of ICCs in the gut and double labeling for differentiating them from mast cells or nerves does not exclude the possibility of their existence.

ASMCs, TSMCs, and ICs were examined by IHC, focused ion beam scanning electron microscopy (FIB-SEM) tomography, and Ca2+ imaging [15], also in mouse proximal renal pelvis's pacemaker region. BECs are described as part of the urothelium and FICs (previously considered ICC-LCs), are located in the sub-urothelial space and adventitia, increase in number distantly from the papilla and make close appositions with similar cells and neighboring ASMCs and TSMCs.

Volume rendering in serial sections reveals the shape of cells: FICs are long, flat, and 'ribbon-shaped' while the mostly longitudinaly and loosely arranged ASMCs have continuous projections like an irregular leaf or rim of a saucer; TSMCs are extremely long, compact, and circularly set.

ICs are a mixed population of αSMA(-) cells located sub-urothelial and in the adventitia of the mouse RP:

1. ANO1(+) and mildly Kv7.5(+) cells;
2. intensely PDGFRα(+) ICs in B6.129S4-Pdgfrα tm11(EGFP)sor/J mouse are a small proportion of αSMA(-) ICs;
3. HCN3(+), Cav3.1(+) cells [12].

3.2. PDGFRα

The platelet-derived growth factor receptor (PDGFR) is a type III tyrosine kinase receptor closely related to the c-Kit receptor. Some authors differently name

these cells: PDGFRα-positive cells, PDGFRα-positive ICs, fibroblast-like cells, and telocytes.
In the gastrointestinal tract and urinary mucosa of the human bladder, the mast cells were c-Kit(+) and PDGFRα(+) [1], but no other specific marker was used for their identification. The TCs in the bladder were PDGFRα(+), CD34(-) and some were αSMA(+), others αSMA(-).
In the normal human upper ureter samples, CD34(+) SMM(+) myoid TCs were found in the deep lamina propria together with CD34(+) SMM(-) dichotomously branching TCs that were also found within the muscle interstitium; other CD34(+) SMM(-) were perivascular TCs. The lamina propria's superficial layer, the spindle-shaped concentrical layers with long telopodes, were CD34(-) SMM(-) TCs. All those TCs were also endoglin (CD105)+ [19]. Endoglin is a type-III accessory receptor for the Transforming Growth Factor (TGF) regulating endothelial cell proliferation and migration.
PDGFRα(+) fibroblast-like ICs identified in the renal pelvis of the mutant mice strain B6.129S4-Pdgfrα tm11(EGFP)sor/J mouse (expressing the H2B-eGFP fusion gene from the endogenous Pdgfrα locus) were located sub-urothelial, in the intermuscular space and the adventitia (Hashitani et al., 2017), being a small proportion of the αSMA(-) ICs, different from the intensely ANO1(+) and mildly Kv7.5(+) cells sharing the exact location and separate from the HCN3(+), Cav3.1(+) cells located only in sub-urothelium of the proximal renal pelvis [10].
PDGFRα(+) cells are not a homocellular population because the ASMCs externally located to TSMCs (are also lightly PDGFRα(+). They were identified as a new class of spindle-shaped "fibroblast-like" ICs in the human UUT. PDGFRα(+) ICs, with a subset of PDGFRα(+), SK3(+) ICs form a discrete network in the lamina propria, between and surrounded by SMCs bundles in the human UPJ; PDGFRα(+) ICs are distinct but associated to c-Kit(+) ICC-LCs, Phalloidin(+), SK2(+) SMCs and PGP9.5(+) nerve fibers.
PDGFRα(+) cells are the significant population of ICs. In the bladder, mast cells were identified as the only c-Kit(+) cells, but as opposed to all other UUT levels, they also expressed PDGFRα [1].
Studies of ultrastructure, gene expression, and marker analysis reveal that PDGFRα(+)ICs located in the mouse bladder are fibroblasts with multiple, regionally distinct (suburothelial, outer LP, intermuscular, and serosal) populations [25].

4. Electrophysiological properties as an instrument for identification

The stretch-activated mechanically gated non-selective cationic channels permeable to Ca2+ PIEZO1+ are co-expressed in PDGFRα+, αSMA+ very thin, spindle-shaped ICs often arranged in wavy parallel bundles in the upper lamina propria. PIEZO1+ is also expressed in the smooth muscle cells and urothelium from the RP and ureter of transgenic mice that express PIEZO1 fused with a red fluorescent reporter Tomato - PIEZO1 tdT mice. These cells act with afferent nerves as mechano-sensors to urine flow and wall tension in the urinary tract [26].
APC: The mechanosensitive, Ca2+ permeable transient receptor potential vanilloid TRPV4 channels are expressed in short spindle-shaped ASMCs and TSMCs in the muscle layer of pelvicalyceal junction (PCJ) of mouse RP, ASMCs coexpressing PDGFRα+ [8].

The asynchronous spontaneous Ca2+ transients visualized using a Ca2+ fluorescent indicator (Cal-590) of the short spindle-shaped, parallel arranged lightly GFP fluorescent PDGFRα+ cells from the muscular layer of the renal calyx (proximal pelvis) indicate that these cells are ASMCs while the cells with rounded intensely green fluorescence nuclei, exhibiting slow, prolonged spontaneous Ca2+ transients located in lamina propria and adventitia are ICs [12]

ICC-CL c-kit+ and HCN3+ channels are located in distinct cell populations and are required for coordinated peristalsis in the pyelocaliceal junction and ureter.
Different ion channels have also been reported to be expressed by ICs (Hyperpolarisation-activated, cyclic nucleotide-gated cation channels (HCN), voltage-gated calcium-activated chloride channels (ANO1/TMEM16A), Ca2+ activated K+ (SK3) channels, voltage-gated potassium channels (Kv7.5 and Kv4.3), transient receptor potential ion channel

ankyrin 1 (TRPA1), stretch-activated mechanically gated channel PIEZO1.
Hyperpolarisation-activated, cyclic nucleotide-gated (HCN) are membrane cation channels that underlie pacemaker currents in excitable spontaneously active cells like cardiac cells and neurons and work as pacemaker channels in mouse colonic interstitial cells of Cajal. Studies showed that HCN channels are sufficient to transform quiescent cell types into pacemakers [14]
Hurtado and colleagues investigated the HCN isoform 3(HCN3) channel [14] in mice's PKJ and the proximal renal pelvis. Real-time video microscopy showed that the proximal renal pelvis containing PKJ initiated and coordinated peristalsis. RT-PCR showed abundant levels of HCN3 in the proximal urinary tract. IHC of paraffin sections revealed highly expressed HCN3 in PKJ and renal papilla.

Videomicroscopy and optical mapping using voltage-sensitive dye demonstrate that spontaneous electrical and contractile activity is initiated at the PKJ and propagates down the renal pelvis and ureter in a coordinated, wave-like manner that is lost after the HCN channel inhibitor ZD7288 administration. For the first time, an analogous ion channel expression profile between UUT and heart pacemakers was shown [14].

Lang et al. also investigated the presence of HCN3 marker in the mouse proximal renal pelvis and found that a subset of ICs ('Fibroblast-like cells' previously named ICC-LCs) present only in the sub-urothelial space are HCN3+, Cav3.1+, SMA - cells. Cav3.1+, SMA - ICs were present in the whole renal pelvis sub-urothelial space but never in the adventitia and are different from the PDGFRα+ ICs from the sub-urothelial, intermuscular, adventitial space as well as from the lightly SMA(+) ASMCs and intensely SMA(+) TSMCs [15].

Western blot analysis of isolated proteins from human ureters (control and ureteral tuberculosis) showed that all 4 HCN channels, mainly HCN4 and HCN1, are expressed in the human ureter. Double immunofluorescence reveals the co-localization of HCN1, HCN4, and c-Kit in urinary tract ICC-LCs suburothelial and between the SMC bundles [27].

Voltage-gated calcium-activated anion channels, known as Anoctamin 1(ANO1) or Transmembrane member 16A (TMEM16A), act as chloride channels. They are highly expressed in ICCs in the gastrointestinal tract and other cells, such as smooth muscle cells.

Immunophenotypic studies used monoclonal and polyclonal antibodies against ANO1 and Voltage-gated potassium channels (Kv7.5 and Kv4.3) on coronal sections of mouse and rat renal pelvis. c-Kit(-) ICs were ANO1(+), Kv7.5(+), Kv4.3(+), αSMA(-) while SMCs were ANO1(+), Kv4.3(+), αSMA(+) (more intense in the distal RP). The ANO1(+) αSMA(-) ICs were found in suburothelium and adventitia. Kv7.5(+) cells were evident in suburothelium but sparsely in the adventitia, forming close associations with similar cells, being elongated, single-layered in the proximal and middle RP, and clearly stellate and densely distributed in the distal RP. C-Kit (+) was most likely expressed in PGP9.5(+) nerve bundles and not in ICs; no mast cells were identified [32-35]. Not clear if all ANO1(+) ICs are also Kv7.5(+) [40,41].
The BECs and Kv7.5+ SICs are said to contribute to the transitional epithelium, but it is not clear if they are located beneath or above the epithelium's basement membrane.
There is doubt about c-Kit(+) as a specific marker for ICC-LCs, and because the ICs can be divided into several c-Kit(-) subpopulations, the molecular signature of these different cells needed to be defined more clearly [36, 42-46]. The researchers used various markers and multiple labeling, but no other specific marker has been identified yet [21, 37].

5. Instead of conclusions (Types of IC, locations, roles, clinical relevance)

Determining the phenotype of ICs in the urinary tract is not easy, as reflected by their heterogeneous nomenclature and the necessity to urgently ascertain whether those different names correspond to one or various types of cells.

After years of intense and enthusiastic research, the elusive nature, functions, differences, and names of the ICs in the UUT across species are still being investigated at a slower pace in search of a vital breakthrough finding.

The nature of primary pacemaker cells in uni and multicalyceal species is still unclear: are they ASMCs or a type of ICs? Are some ICs initiating (latent pacemaker cells) and modulating UUT peristaltic activity or are just fibroblast-like cells? What would be a more appropriate name to use for these cells? How many different types of interstitial cells are in the urinary tract, what are their roles, and how can we differentiate them? Should we name some telocytes according to their appearance, use Cajal like cells as known by urologists or find new names? [38, 39].

Because specific markers for the different types of ICs in the UUT of humans and other species have not been found yet, the investigation of IC's role in various pathologies is slowly advancing.

The most frequently used markers for ICs in UUT have been the stem cell growth factor receptor c-Kit, which was initially considered specific. However, further studies proved that it is not expressed in most ICs in humans and other species of UUT.

Investigation of UUT segments in humans and other species with c-kit, mast cells, and nerve-specific markers [48], combined with different techniques, must clarify whether the c-kit is a reliable marker since urologists are still using it to determine Cajal Cell density in the UUT and not PDGFRα (28-31, 47]).

Although SEM's resolution and magnification power are not as good as TEM's, 3D-ultrastructural analysis of human and other species' UUT segments will help identify distinct subtypes of ICs, their shapes, orientation, spatial relationships, interactions, and variations between species and layers of different segments of the ordinary and pathological UUT. Immuno-electron microscopy can also help segregate cells with apparently the same ultrastructural features.

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