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PRIMER

Emerging diverse roles of telocytes

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ABSTRACT

Since the first description of 'interstitial cells of Cajal' in the mammalian gut in 1911, scientists have found structurally similar cells, now termed telocytes, in numerous tissues throughout the body. These cells have recently sparked renewed interest, facilitated through the development of a molecular handle to genetically manipulate their function in tissue homeostasis and disease. In this Primer, we discuss the discovery of telocytes, their physical properties, distribution and function, focusing on recent developments in the functional analysis of Foxl1-positive telocytes in the intestinal stem cell niche, and, finally, the current challenges of studying telocytes as a distinct cell type.

KEY WORDS: Stem cell niche, Stroma, Telocyte

Introduction

The study of telocytes began over a century ago with the discovery of a unique population of cells by the pioneering Spanish neuroscientist and pathologist Santiago Ramón y Cajal. In his original report, Cajal identified cells with long, 'neuron-like' cytoplasmic projections in the muscle layer of the human gut. He named these cells 'interstitial neurons' because of their projections, and because these cells were found between nerve endings and smooth muscle cells (Cajal, 1911) (see Box 1 for timeline of telocyte terminology). It was not until several decades later that scientists returned to the study of these 'interstitial neurons', now using electron microscopy to investigate tissue architecture at the ultrastructural level. Faussone-Pellegrini and Thuneberg independently determined that 'interstitial neurons' were not actually neurons, and consequently re-named them 'interstitial cells of Cajal' (ICCs) (Faussone-Pellegrini et al., 1977; Thuneberg, 1982). ICCs were defined ultrastructurally by electron microscopy as cells with spindle-shaped bodies and long cytoplasmic processes, which extend from the cell body and interact with each other or other cells (Gabella, 1992; Torihashi et al., 1994). In these early stages of characterization, ICCs in the intestine were shown to act as pacemaker cells regulating gastrointestinal movement (Thuneberg, 1982), and suspected to have roles in neurotransmission (Sanders et al., 2006) and stretch sensing (Forrest et al., 2008).

It was not until 1996 that Lecoin and colleagues showed that, in chick embryos, the ICCs were of mesenchymal origin, and marked by expression of the gene encoding the cytokine receptor tyrosine kinase Kit (Lecoin et al., 1996). Over time, cells that resembled ICCs were discovered in the connective tissue of multiple organs of the vertebrate body. Like the ICCs, these cells possessed very long processes, or telopodes, which extended outwards to interact with multiple cells in the surrounding tissue. These cells were given the name 'interstitial Cajal-like cells' (ICLCs) and in 2010 they were re-named 'telocytes' (Popescu and Faussone-Pellegrini, 2010). For the remainder of the article, these cells will be referred to as telocytes for simplicity.

Telocytes have been proposed to play a role in structural support and mechanical sensing, cell-to-cell signaling by interacting with many other cell types, and regulation of immune response, although much of this remains to be proven experimentally. More recently, telocytes have been shown to function as crucial components of the stem cell niche (Cretiou et al., 2012a,b; Aoki et al., 2016; Shoshkes-Carmel et al., 2018), as discussed in detail below.

To date, telocytes have been identified in many vertebrates, including humans, mice, rats, guinea pigs and chickens, and in several organs such as the pancreas (Fig. 1A,B) (Popescu et al., 2005; Nicolescu and Popescu, 2012), and the gastrointestinal tract, including the esophagus (Chen et al., 2013), small intestine and colon (Cretoiu et al., 2012a,b). Telocytes are also present in the epicardium (Fig. 1C) (Popescu et al., 2010), endocardium (Gherghiceanu et al., 2010) and myocardium (Kostin, 2010) of the cardiac system. The reproductive system features telocytes in the prostate (Corradi et al., 2013), uterus (Duquette et al., 2005), myometrium (Cretoiu et al., 2012a,b) and placenta (Suciu et al., 2007). Telocytes in the respiratory system are largely found in the lungs (Popescu et al., 2011a,b). In addition, telocytes have also been found in the interstitium of the mammary gland (Gherghiceanu and Popescu, 2005), skeletal muscle (Popescu et al., 2011a,b), bone marrow (Li et al., 2014) and mesentery (Hinescu et al., 2008) (see Table 1 for a complete list of tissues). As more studies shed light on telocytes, we may discover new ways of understanding the complexities of tissue homeostasis and pathogenesis of human diseases. This Primer summarizes the physical properties of telocytes, their distribution across organ systems, key expression markers that define telocytes, and their suggested roles in tissue homeostasis and structure.

Physical properties

Telocytes have distinct physical features that distinguish them from other stromal cells. Such physical features include the presence of telopodes, and nuclei that are oval in shape and surrounded by minimal cytoplasm. Although the number of telopodes extending from the cell body varies across different tissues, there are typically two to three cytoplasmic processes visible in a two-dimensional tissue section (Popescu et al., 2007). These telopodes can be in contact with multiple surrounding cell types, including immune cells, muscle fibers, blood vessels and epithelial cells (Fig. 1).

Because of this very limited set of unique features, telocytes are difficult to distinguish from fibroblasts and neuronal cells, demonstrated by the fact that their discoverer thought them to be neuronal in origin. To identify telocytes accurately, Popescu and colleagues have developed a so-called 'platinum standard' set of criteria for telocytes (Popescu et al., 2007) (Table 2). To summarize these criteria: telocytes do not penetrate the epithelia, are in close contact with many target cells, have long cytoplasmic processes, and have an organelle composition distinct from that of other cells (Popescu et al., 2007). These criteria, which are all based on ultrastructural features observed by transmission electron

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Box 1. From interstitial cells of Cajal to the discovery of telocytes – timeline of terminology

1911 Neuron-like cells found in the muscle layer of the gut, named 'interstitial neurons' (Cajal et al., 1911)

1977 Neuron-like cells found to not be neurons, named 'interstitial cells of Cajal (ICCs)' (Faussone-Pellegrini et al., 1977)

2005 Cells that resemble ICCs are found in the exocrine pancreas, named 'interstitial Cajal-like cells' (ICLCs) (Popescu et al., 2005)

2010 ICLCs collectively re-named 'telocytes' (Popescu et al., 2010)

microscopy (TEM), have since been utilized to discover telocytes in various tissue types, and have helped scientists to characterize their distribution within tissues.

Despite the establishment of the platinum standard for identifying telocytes, fully understanding the complete physical structure requires certain precautions. By electron microscopy, ultrastructural features can only be characterized in 2D sections, and may not necessarily represent the true hallmarks of these cells. For instance, in 2015 Popescu's group performed 3D imaging of telocytes using focused ion beam scanning electron microscopy (FIB-SEM) tomography (Cretoiu et al., 2015) (Fig. 2). In this study, they found that telocytes in the human papillary dermis have 'ribbon-like' telopodes, drastically different from 'moniliform' (i.e. resembling a string of beads) telopodes (observed previously), which raises questions regarding the criteria for 'dichotomous' (bipartite) telopode branching (Popescu et al., 2007). Therefore, a much deeper assessment of telocytes is required to fully understand the true physical properties of telocytes, and whether these features are consistent across animal models and tissues. These problems may be circumvented in the future with a more universal use of FIB-SEM tomography and other 3D imaging techniques.

Tissue distribution

As mentioned above, telocytes are found in many tissues and are most commonly located in interstitial layers of organs. For example, in the heart, telocytes have been documented in interstitial regions of the epicardium, myocardium and endocardium (Gherghiceanu et al., 2010; Kostin, 2010; Popescu et al., 2010). Here, the telocytes' long telopodes interact with surrounding blood vessels (Fig. 1C) (Hinescu and Popescu, 2005). The mammalian gut is made up of layers, including the muscularis (muscle layer), submucosa (connective tissue layer), and mucosa (which includes epithelium and lamina propria, a layer of connective tissue that lies beneath the epithelium and contains small blood vessels and immune cells), and telocytes are found within all of these layers (Cretoiu et al., 2012a,b; Vannucchi et al., 2013; Shoshkes-Carmel et al., 2018). In the muscularis, telocytes occupy the interstitial space and interact with nerve fibers, smooth muscle cells, blood vessels and epithelial cells (Vannucchi et al., 2013).

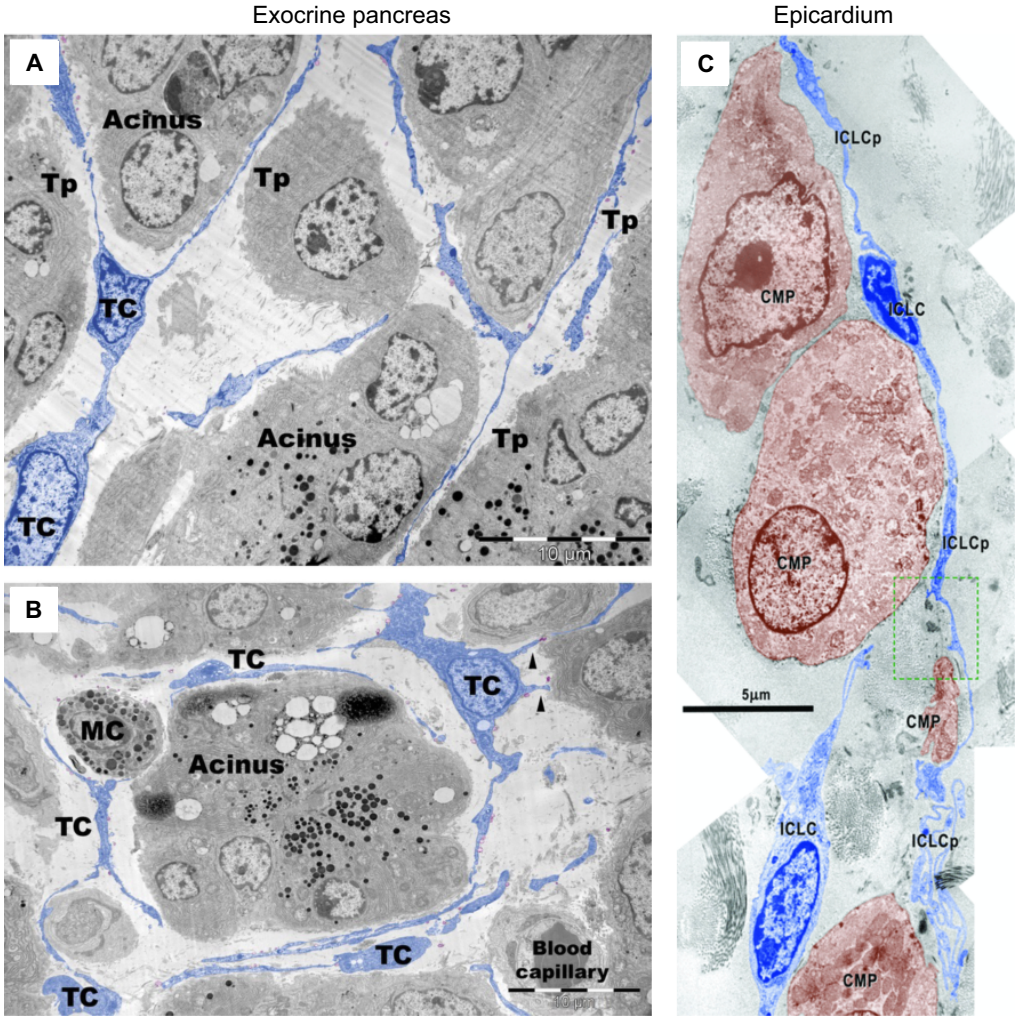

Fig. 1. TEM images of telocytes *in vivo*. (A-C) Telocytes can be seen with small cell bodies and long cytoplasmic processes that interact with each other and other surrounding cells in the exocrine pancreas (A,B) or epicardium (C). In the human exocrine pancreas, telocytes are highlighted in blue and form networks around acini, mast cells and blood capillaries. CMP, cardiomycyte progenitors; TC, telocyte; Tp, telopode; MC, mast cell; ICLC(p), Interstitial Cajal-like cell (processes). Reprinted with permission from Nicolescu and Popescu, 2012 and Popescu et al., 2009.

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Table 1. Summary of telocytes found in the body and their cell and tissue interactions

Organ	Localization	Associated cells	Reference(s)
Heart	Epicardium, endocardium, myocardium	Plasma cells, lymphocytes, capillaries, nerve fibers, satellite cells	Gherghiceanu et al., 2010; Kostin, 2010; Popescu et al., 2010
Heart valves	Apex and base of heart valves: mitral valve, tricuspid valve, aortic valve	Stem cells	Yang et al., 2014
Pulmonary veins	Myocardial sleeves of pulmonary veins	Capillaries, nerve endings	Gherghiceanu et al., 2008
Aorta, blood vessels	Muscle layer surrounding blood cells	Arterioles, venules, capillaries	Zhang et al., 2015
Trachea and bronchi	Trachea and lung stroma, between smooth muscle fibers	Smooth muscle cells, subepithelium	Rusu et al., 2012
Lungs	Bronchoalveolar junctions, in subepithelial stroma	Epithelium, stem cells	Popescu et al., 2011a,b
Meninges and choroid plexus	Choroid plexus interstitium	Putative stem cells, blood vessels, ependymal cells	Popescu et al., 2012
Striated muscle	Skeletal muscle interstitium	Nerve endings, capillaries, satellite cells, myocytes	Popescu et al., 2011a,b
Fascia lata	Among collagen fibers	Collagen fibers	Dawidowicz et al., 2015
Papillary and reticular dermis	In reticular dermis, perifollicular sheath, around sebaceous glands, eccrine sweat glands	Blood vessels, glands, arrector pili muscles, stem cells, immune cells	Ceafalan et al., 2012
Lumbus and uvea	Lamina propria of conjuctiva, limbal area, sclera, pars palana of ciliary body, iris stroma, beneath corneal epithelium	Epithelium, stromal stem cells, nerve endings, melanocytes, macrophages	Luesma et al., 2013
Esophagus	Lamina propria of esophageal mucosa, muscular layer	Lymphocytes, nerves, capillaries	Chen et al., 2013
Duodenum	Lamina propria, below mucosal crypts	Immune cells, blood vessels, nerve endings	Cretoiu et al., 2012a,b
Jejunum	Subepithelium, between smooth muscle cells of muscularis mucosae	Immune cells, epithelium, smooth muscle cells, nerve bundles	Cretoiu et al., 2012a,b
Colon	Mucosa, submucosa, muscularis, subepithelium	Muscle bundles, nerve structures, blood vessels, gastric glands, epithelium, epithelial stem cells	Cretoiu et al., 2012a,b
Salivary glands	Parotid stroma, interacinar stroma, subductal stroma	Ducts, blood vessels, parotid gland acini	Nicolescu et al., 2012
Gall bladder	Near epithelium, interstitial spaces between smooth muscle fibers	Smooth muscle cells, capillaries	Hinescu et al., 2007
Pancreas	Pancreatic exocrine	Blood vessels, nerves, acinar cells and ducts	Popescu et al., 2005
Bone marrow	Bone marrow	Arteriola, capillaries	Li et al., 2014
Mammary gland	Mammary gland stroma	Nerve fibers, capillaries, immune cells, fibroblasts	Gherghiceanu and Popescu, 2005
Fallopian tube	Fallopian tube ampular region, lamina propria, between smooth muscle fibers	Epithelium, capillaries	Popescu et al., 2007
Myometrium	Among myometrial fibers	Myocytes, nerve fibers, capillaries	Ciontea et al., 2005
Placenta	Mesenchymal tissue of villi	Blood vessels, collagen fibers, vascular smooth muscle cells	Suciu et al., 2007
Kidney	Sub-capsular space	Macrophages	Zheng et al., 2012
Ureter and urinary bladder	Between smooth muscle bundles in lamina propria	Smooth muscle cells, nerve endings, capillaries	Zheng et al., 2012
Prostate	Fibromuscular stroma	Blood vessels, nerves, immune cells	Corradi et al., 2013

Telocytes of other organs have similar patterns of networking and intercellular interactions (Table 1). Telocytes are present in the human skin surrounding the dermal connective tissue, around hair follicles, blood vessels, and the secretory and excretory parts of sweat glands (Ceafalan et al., 2012). Skin telocytes establish interlocking networks, which appear to form an incomplete sheath wrapped around the skin appendages such as hairs and sweat glands. The interacting telopodes are connected by gap junctions, and wrap around vascular smooth muscle cells, blood vessels, the epidermis and immune cells (Ceafalan et al., 2012). Lastly, in the exocrine pancreas, telocytes surround capillaries, stellate cells (myofibroblast cells), nerve fibers, ductal cells (which make up the pancreatic duct to transport enzymes), and acini (the functional units of the exocrine pancreas) (Fig. 1A) (Popescu et al., 2005). Similar to in the skin, telopodes in the pancreas appear to intertwine with each other, forming a telocyte ‘plexus’ (Nicolescu and Popescu, 2012).

Telocytes have also been observed within stem cell niches. In the same study discussed above, regarding the presence of telocytes in human skin, it was shown that telocytes form sheaths around the stem cells of hair follicles. In addition, telocytes have been observed to make direct contact with dermal stem cells (Ceafalan et al., 2012). Lastly, in the lung there are cells with telocyte morphology that contact type 2 epithelial cells, which are known to function as stem cells (Sirianni et al., 2003; Nabhan et al., 2018; Chung et al., 2018). Currently, the roles of telocytes within stem cell niches are best described for intestinal telocytes.

In *An Atlas of Ultrastructure*, published in 1963, Rhodin used electron microscopy to make observations in the lamina propria, and identified cells that featured flattened nuclei and sparse cytoplasmic organelles (Rhodin, 1963). These cells were located just below the basement membrane of the epithelium and their presence was soon confirmed by Deane in 1964, when she described

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Table 2. Ultrastructural criteria for identifying telocytes in tissues

Physical features	Description
Location	Non-epithelial space
Cellular contacts	Nerve bundles, epithelia, smooth muscle cells, capillaries
Cytoplasmic processes:	
Number	1-5; frequently 2-3 in 2D space
Length	Tens to hundreds of µm
Thickness	Uneven thickness, <0.5 µm
Aspect	Moniliform ('beads on a string'), presence of mitochondria in dilated regions
Ca^{2+} release units	Present
Branching	Dichotomous pattern
Organization in network	Labyrinthic system of overlapping cytoplasmic processes
Gap junctions	With smooth muscle cells or with each other
Basal lamina	Occasionally present
Caveolae	2-4% of cytoplasmic volume; ~0.5 caveolae/µm of membrane in length
Mitochondria	5-10% of cytoplasmic volume
Endoplasmic reticulum	1-2% of cytoplasmic volume, either smooth or rough
Cytoskeleton	Intermediate and thin filaments, and microtubules
Myosin thick filaments	Undetectable

cells with extremely attenuated processes positioned at the basement membrane of the epithelium. The cells often interlocked and formed a thin sheath layer surrounding the epithelial basal membrane (Deane, 1964). Today, we know these cells to be the subepithelial telocytes involved in supporting the stem cell niche of the intestinal crypts (Shoshkes-Carmel et al., 2018). Other studies support these findings by identification of telocytes in subepithelial regions of other tissues. In the prostate, telocytes are found in the subepithelial space forming multiple intertwining contacts through their telopodes (Corradi et al., 2013), although their specific role in epithelial stem cell regulation has not yet been established. Telocytes also form internetworking sheaths below the epithelium in the bovine uterine tube (Abd-Elhafeez and Soliman, 2017).

The discovery and identification of telocytes in numerous tissues may indicate heterogeneous roles for these cells. For example, telocytes found in the interstitial regions of organs may be important for maintaining structural integrity of the whole organ. The networks of telopodes surrounding structures such as hair follicles (Ceafalan et al., 2012), intestinal crypts (Cretoiu et al., 2012a,b) and acini (Popescu et al., 2005) may be important for structural integrity of these individual functional units. Below, we discuss in detail the many functions of telocytes that have been proposed, although not necessarily proven.

Molecular markers

Telocytes have been labeled with various immunohistological markers in different organs and tissues in the mammalian body; at present, there is no singular way of distinguishing telocytes as a cell type by expression of a single protein. However, the most commonly used markers are Kit, CD34, vimentin, platelet-derived growth factor receptor α (PDGFRA) and α-smooth muscle actin (α-SMA) (summarized in Table 3). Kit is known to be expressed in hematopoietic stem cells and multipotent progenitor cells, as well as common myeloid progenitors. In addition, CD34 is a cell surface marker suggested to play a role in early hematopoiesis; thus telocytes expressing Kit and/or CD34 may imply a hematopoietic lineage. Vimentin is a type of intermediate filament and *PDGFRA* encodes a receptor tyrosine kinase, both of which are broadly expressed in mesenchymal cells and therefore these two genes are often colocalized. α-SMA is, as the name suggests, strongly expressed in smooth muscle cells.

Currently, the methods used for identifying protein markers on telocytes are standard immunohistochemical (IHC) staining methods and immunolabeling with gold for electron microscopy. However, using standard IHC techniques limits how confidently the cells of interest are correctly identified, because IHC methods cannot visualize ultrastructural properties of a cell. On the other hand, immunolabeling for certain protein markers for electron microscopy allows not only for detection of the protein of interest, but for the assessment of the ultrastructural features, which ensures the cell satisfies the set of telocyte criteria.

PDGFRα is co-expressed with CD34 in telocytes of the esophagus, stomach, and the small and large intestine, which indicates that these cells constitute a population of stromal mesenchymal cells (Vannucchi et al., 2013). In PDGFRα-positive telocytes, in addition to immunohistochemical staining ultrastructural characterization is necessary because PDGFRα is broadly expressed in the lamina propria of the gut, but only a select few of these PDGFRα-positive cells are telocytes (Greicius et al., 2018). In such cases, it would be beneficial to have a better gene expression profile of telocytes to distinguish them from other stromal cells. Telocytes in the intestinal subepithelium have been found to express not only PDGFRα (Vannucchi et al., 2013; Greicius et al., 2018), but also CD34 (Stzepourginski et al., 2017), FOXL1 (Shoshkes-Carmel et al., 2018), GLI1 (Degirmenci et al., 2018), SOX6 (Kinchen et al., 2018) and CD90 (Karpus et al., 2019).

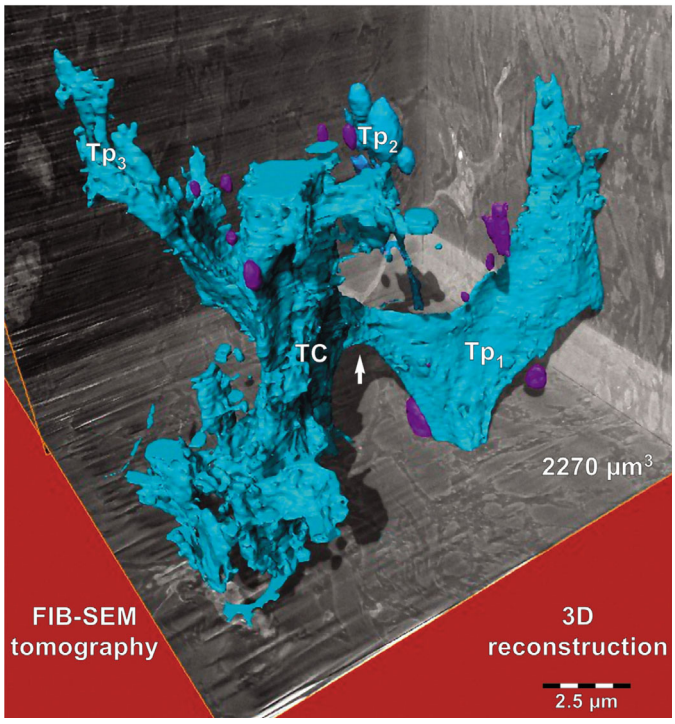

Fig. 2. Three-dimensional reconstruction of a human skin telocyte. Focused ion beam scanning electron microscopy (FIB-SEM) tomography of a human skin telocyte (blue) shows ribbon-like telopodes, different from the 'moniliform' structure used to describe physical features of telocytes. In purple are extracellular vesicles, possibly budded from the telocyte and proposed to play a role in cell-cell signaling with neighboring cells. TC, telocyte; Tp, telopode. Reprinted with permission from Cretoiu et al., 2015.

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Table 3. Molecular markers of telocytes

Organ	Markers	Reference
Heart	Kit; CD34; S100	Gherghiceanu et al., 2010
Heart valves	Kit; CD34; vimentin; PDGFRβ	Yang et al., 2014
Pulmonary veins	Kit	Gherghiceanu et al., 2008
Trachea and bronchi	Kit	Rusu et al., 2012
Lungs	Kit; CD34	Popescu et al., 2011a,b
Meninges and choroid plexus	Kit	Popescu et al., 2012
Striated muscle	Kit; caveolin 1	Popescu et al., 2011a,b
Skin	Kit or CD34; vimentin	Ceafalan et al., 2012
Gastrointestinal tract	CD34; vimentin; PDGFRα	Vannucchi et al., 2013
Gastrointestinal tract (lamina propria)	CD34; vimentin; PDGFRα; FOXL1; GLI1; SOX6; CD90	Vannucchi et al., 2013; Greicius et al., 2018; Shoshkes-Carmel et al., 2018; Degirmenci et al., 2018; Kinchen et al., 2018; Karpus et al., 2019
Salivary glands	Kit; vimentin; α-SMA	Nicolescu et al., 2012
Gall bladder	Kit; CD34; vimentin	Hinescu et al., 2007
Pancreas	Kit; CD34; vimentin; some α-SMA; some S100 positive	Popescu et al., 2005
Mammary gland	CD34; vimentin; CD10	Petre et al. 2016
Fallopian tube	Kit; CD34; S100; some vimentin	Popescu et al., 2007
Myometrium	Kit; CD34	Cretoiu et al., 2013
Placenta	Varying expression of Kit; CD34; vimentin	Suciu et al., 2007
Kidney	Kit; CD34; vimentin	Qi et al., 2012
Ureter and urinary bladder	CD34/calreticulin double positive; PDGFRα/ calreticulin double positive	Vannucchi et al., 2014

The gene markers expressed in telocytes not only vary from tissue to tissue, but also sometimes vary between different telocytes within the same tissue. For example, in the myocardium, Kit is seen in some, but not all, telocytes and CD34 is co-expressed with Kit in some telocytes. These cells are also strongly positive for vimentin expression, and some are also positive for α-SMA (Hinescu et al., 2006). All pancreatic telocytes are positive for Kit and CD34, and 40-50% of the telocytes are also positive for α-SMA or S100 (Popescu et al., 2005), a calcium-binding protein. In the urinary bladder, telocytes have been classified into three subtypes based on differential expression of PDGFRα, calreticulin (an endoplasmic reticulum-associated protein that acts as a calcium binding/storage protein), α-SMA, CD34, and Kit, which might indicate that the telocytes have region-specific roles (Vannucchi et al., 2014).

It will be important in the future to identify a marker or marker set that is unique to telocytes regardless of tissue type, to better define telocytes as a distinct cell type. Single cell RNA-seq or sequential RNA-FISH (RNA fluorescent *in situ* hybridization) (Shah et al., 2017), technologies will likely be helpful in this regard. In fact, a recent study by Simmons and colleagues identified a 'stromal 2' population by RNA-seq of human gut mesenchyme that express many of the markers identified by Shoeshkes-Carmel and colleagues in mouse telocytes, including BMP5, BMP2 and WNT5A (Kinchen et al., 2018). Therefore, data sets like these could be mined for better protein markers.

Functions

Despite the large number of publications on telocytes, their function is understudied. Many of the identifications of telocytes are documented by Popescu and his colleagues, and thus the unspecialized knowledge in the field where telocytes have been identified has resulted in disputable implications about telocyte function. For example, whereas Popescu's group describe telocytes to be involved in supporting mammalian heart stem cells (Popescu et al., 2009), the concept of stem cells in the heart is widely disputed in the field. Thus, it is important to take into consideration that much of the functions 'assigned' to telocytes are not currently based on functional evidence, and instead are mostly based on the cell types that are physically near telocytes. Of the many implications in tissue homeostasis and disease, we have highlighted below some of the proposed functions of telocytes.

Contributions to organ structure and mechanical sensing

The distinct structural features of telocytes suggest unique functional roles for these cells in their resident tissues. First, the telocytes' ability to make contact with numerous cells in their vicinity could facilitate structural support and tissue organization during development and homeostasis. In the heart, telocytes are believed to help establish the complex three-dimensional structure of the organ and guide tissue organization during morphogenesis, based on their localization and cell interactions during heart development (Bani et al., 2010). Telocytes may also be contributing to structural support at a more cellular level; the skin is made up of small components such as hair follicles and sweat glands, and telocytes have been found to form mesh-like networks surrounding these structures (Ceafalan et al., 2012). Similar patterns of telocytes are found in the exocrine pancreas (Popescu et al., 2005), and intestinal crypts where intestinal stem cells are located (Cretoiu et al., 2012a,b).

In organs that undergo constant physical stress, telocytes may play an essential role in modulating mechanical sensing. In the intestinal muscularis, for example, intercellular networks established by telocytes create a rigid structure that is resistant to deformation and thus supportive of the peristaltic movements in the gastrointestinal tract (Pieri et al., 2008). Similarly, telocytes' network within the interstitial space of the urinary bladder may provide mechanical support during stretching of the urinary bladder (Vannucchi et al., 2014). Lastly, skeletal muscle, which undergoes contraction and relaxation, may benefit from mechanical support of telocytes during motor activity (Díaz-Flores et al., 2013).

Cell-to-cell communication and signaling

Telocytes have long been postulated to be important for cell-to-cell signaling with their surrounding cells in their resident tissues. Telocytes make many direct contacts with neighboring cells by gap junctions (Gherghiceanu and Popescu, 2012), which allow molecules and ions to pass through between two cells. Furthermore, telocytes have been proposed to communicate with neighboring cells through short-range signal release. Electron microscopy images in the heart show that these cells release three types of extracellular vesicles: exosomes (i.e. extracellular vesicles produced in the endosomal compartment), ectosomes (vesicles that bud out from the cell's plasma membrane), and multi-vesicular cargos, which contain multiple endomembrane-bound vesicles (Fertig et al., 2014). The exact functions of release of these vesicles are yet to be determined, but Popescu's group suggested that they might regulate the differentiation program of cardiomyocytes in the heart during development, although this remains to be shown experimentally.

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Regulation of immune responses

Telocytes have also been proposed to be involved with regulation of immune responses in their resident tissues. Expression of cytokines in telocytes has been reported in three different systems: heart, skin and intestine. Popescu's group performed a protein secretory profile analysis of mouse cardiac telocytes and determined that these cells express interleukin 6 (IL-6), which is a pro-inflammatory cytokine activated upon infection to activate an inflammatory response (Albulescu et al., 2015). Similarly, cytokine profiling analysis in human skin telocytes have shown that, compared with fibroblasts, these cells highly express not only IL-6, but also the pro-inflammatory cytokine IL-10 (Kang et al., 2015). Finally, transcriptomic analyses of the telocytes of the lamina propria of the gut show differential upregulation of *IL-6* and *IL-10* compared with other stromal cells (Shoshkes-Carmel et al., 2018). These factors are involved in many inflammatory diseases and autoimmune diseases, therefore a functional study that deletes these genes in telocytes might shed new light on the role of telocytes in pathogenesis of immune-related diseases.

Telocytes constitute the intestinal stem cell niche

The mammalian intestine is made up of several layers including the muscularis, submucosa and mucosa. The mucosal layer consists of the lamina propria and a single layer of epithelial cells facing the lumen, and it is the epithelium that is essential for digestion and nutrient uptake, water absorption, and defense against luminal pathogens. Within the epithelium, the active intestinal stem cells (ISCs) at the base of the crypts divide and give rise to transit amplifying cells (Barker et al., 2007). The ISCs highly express leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), a receptor for R-spondin molecules. Transit amplifying cells are progenitor cells that produce mature absorptive enterocytes, which make up the vast majority of the intestinal epithelial cells and secretory cells, such as goblet, Paneth and enteroendocrine cells.

ISCs require constitutive Wnt/β-catenin signaling to maintain their undifferentiated and proliferative state (Wielenga et al., 1999). However, the source of the relevant Wnt signals remained controversial until recently. In the small intestine, Paneth cells, which produce WNT3, have been put forward as the ISC niche (Sato et al., 2011). In the small intestine, Paneth cells are descendants of LGR5-positive stem cells residing at the base of the crypt, as they are responsible for producing anti-microbial agents. However, two independent studies found no changes in ISC proliferation or maintenance upon ablation of Paneth cells or disruption of their function (Durand et al., 2012; Kim et al., 2012), challenging the model of the Paneth cell as the source of Wnt signals. Furthermore, when Wnt signaling is eliminated in the epithelium, including Paneth cells, through the genetic deletion of the obligatory Wnt-processing enzyme Porcupine (Porcn), the function of the intestinal stem cell compartment is not impaired (San Roman et al., 2014).

The first indication that telocytes constitute the intestinal stem cell niche came from mice. Telocytes, marked by the expression of the winged helix transcription factor FOXL1, were conditionally ablated using diphtheria toxin after expression of the diphtheria toxin receptor in these cells (Aoki et al., 2016). As a result, the proliferation of gut epithelial stem and progenitor cells ceased within 3 days, although the nature of the mitogenic pathway was not formally proven. In 2018, several independent studies provided evidence that the crucial Wnt signals required for stem cell maintenance and transit amplifying cell proliferation derive from a mesenchymal population. Shoshkes-Carmel and colleagues showed that the rare FOXL1-positive population of subepithelial telocytes form a continuous plexus just underneath the epithelium and express multiple Wnt signaling molecules, including WNT2B and WNT5A and the co-activator R-spondin3 (RSPO3) in a location-specific manner (Fig. 3) (Shoshkes-Carmel et al., 2018). RSPO3 is an important protein for Wnt signaling in the intestine as it can bind to LGR4-6 receptors on the cell surface, thereby blocking the Wnt signaling antagonists RNF43 and ZNRF3. Thus, increased expression of RSPO3 acts to potentiate Wnt signaling in the intestine. Crucially, Shoshkes-Carmel and colleagues showed that the population of Foxl1-positive cells is absolutely required for stem cell maintenance through conditional ablation of *Porcn*. This elimination of active Wnt signaling reduced epithelial proliferation within 24 h, and epithelial proliferation was completely eliminated within 72 h (Shoshkes-Carmel et al., 2018). Because of the loss of this epithelial renewal, the mutant mice died 3-4 days after gene ablation.

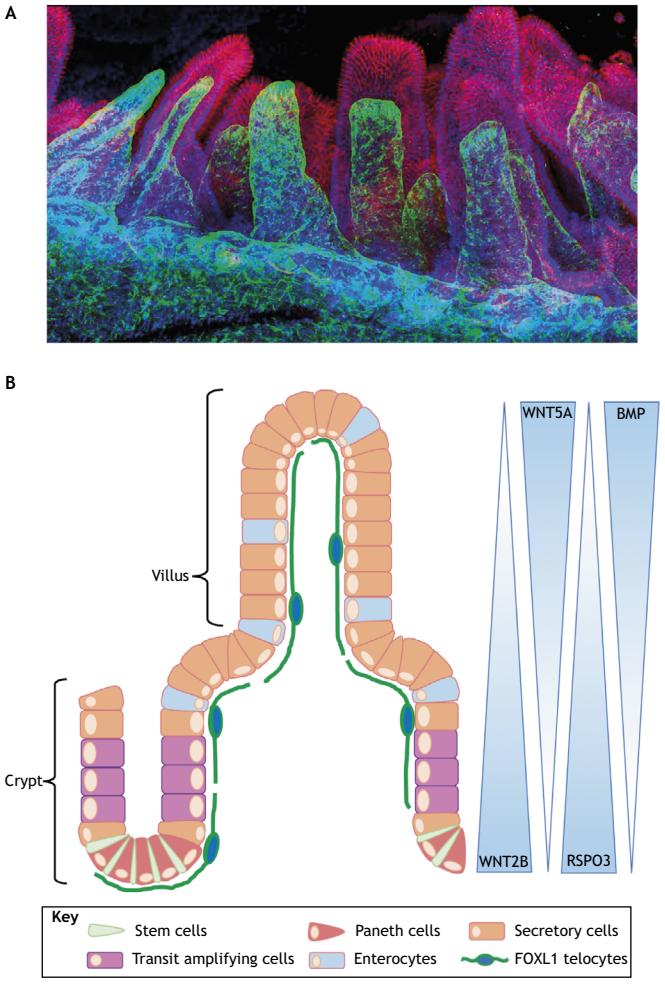

Fig. 3. Intestinal subepithelial telocytes provide Wnt signaling to the epithelium. (A) Lightsheet imaging of cleared mouse whole small intestine using X-CLARITY. Immunofluorescent staining for PDGFRα (green) and EpCAM (red) showing the subepithelial network of FOXL1-/PDGFRα-positive telocytes. Reprinted with permission from Shoshkes-Carmel et al., 2018. (B) The small intestinal epithelium consists of the crypt, which houses intestinal stem cells and transit amplifying cells, and the villus, which consists of the mature enterocytes and secretory cells. FOXL1-expressing telocytes lie just beneath the epithelium, forming a continuous plexus. Telocytes beneath the small intestinal epithelium secrete Wnt, BMP and R-spondin proteins at specific positions along the crypt-villus axis to limit Wnt activity to LGR5-expressing stem cells and transit amplifying cells. Blue wedges represent expression gradients.

Around the same time, other groups showed that a population of stromal cells expressing CD34 and PDGFRα provide Wnt ligands and Rspo3 for the ISCs (Stzepourginski et al., 2017; Greicius et al., 2018). This is in concordance with Shoshkes-Carmel's work, because FOXL1-expressing telocytes are the subepithelial subset of PDGFRα-positive gastrointestinal stromal cells. Finally, further studies have shown that that the crucial source of Wnt ligands are cells that express the Hedgehog pathway transcription factor GLI1 (Degirmenci et al., 2018). These GLI1-expressing cells include Foxl1-positive telocytes, because epithelial-derived Hedgehog signaling activates GLI1 in intestinal mesenchymal cells, which in turn binds to an evolutionarily conserved enhancer to drive *Foxl1* gene expression (Madison et al., 2009).

Conclusions and future perspectives

Although the presence of telocytes has been known for many decades, albeit by other names, they have only recently gained prominence through the discovery of their presence in numerous tissues, and for their role in structural support and cell-to-cell communication. However, there are still key unanswered questions about telocytes.

It is clear to date that telocytes are present throughout the body, but expression of molecular markers varies significantly across different tissues. Although immunohistochemical staining has shown that telocytes are a heterogeneous population expressing different combinations of markers (Popescu et al., 2005; Hinescu et al., 2006; Vannucchi et al., 2014), there are still ambiguities regarding which marker, or combinations of markers, can be employed for definitive identification of telocytes. Based on how little we know about telocytes and their function, this seems to indicate that telocytes across different tissues share minimal features besides their ultrastructural characteristics. To better define telocytes as a distinct cell type, we need a deeper examination of their gene expression profiles, functions and cell lineage. Expression of KIT and CD34 in telocytes suggests that they are of hematopoietic lineage, but do KIT/CD34 double-negative cells with telocyte morphology share the same common precursor? If not, are they the same cell type as the KIT/CD34-positive cells? In other words, do telocytes as we know them today represent a single, distinct population of cells?

Understanding telocytes as a cell type also requires more robust transcriptional profiling of these cells. Current technologies in transcriptomic analysis are limited for studying cells with delicate cell structures, such as telocytes, because they tend to disintegrate during tissue dissociation or cell sorting. However, this challenge may be circumvented using single nuclear RNA-seq technologies in which nuclei are isolated from tissue for sequencing instead of whole cells. This is used commonly by neuroscientists performing transcriptomic sequencing on neurons, because their axon projections make it difficult to isolate whole cells. To this end, scientists have expressed sortable reporters on nuclei using the nuclear envelope protein Sun1 transgenic mouse model (Mo et al., 2015). A documented gene marker for telocytes in a certain tissue may be isolated by expressing GFP in the nuclei of telocytes, so that downstream analyses, such as RNA-sequencing, can be performed. Another potential method is sequential barcoding FISH (seqFISH), which can detect over 100 genes on a 2D section (Shah et al., 2017).

Furthermore, with so much molecular heterogeneity within telocytes, it is essential to understand their function(s), and how telocytes with specific markers might differ in their roles. When considering telocyte subcategorization, it may be worth considering what types of cells the telocytes are contacting in a particular tissue. For example, based on functional telocyte studies during the past 3 years, it has been established that subepithelial telocytes are crucial for stem cell function in the intestine, and that they express CD34, PDGFRα, FOXL1 and GLI1 (Aoki et al., 2016; Shoshkes-Carmel et al., 2018; Greicius et al., 2018; Degirmenci et al., 2018). As FOXL1 and GLI1 are both expressed downstream of Hedgehog activation coming from the intestine (Madison et al., 2009), it is logical that these telocytes express FOXL1 and GLI1. It will be interesting to investigate whether other telocytes near epithelial cells, such as the skin, express similar markers and thus may be categorized as the same cell type. Similarly, telocytes that surround non-epithelial cells (nerve fibers, muscle cells, etc.) might have distinguishable transcriptomic profiles as a result of cross-talk with neighboring cells.

Ultimately, the best methods for characterizing telocyte function will be by loss-of-function studies, whether it be by ablating telocytes *in vivo* or using gene ablation models for proteins of interest. Overcoming the technical challenges will be difficult but are crucial for an improved definition of telocytes as a distinct cell type. Thus, telocyte research is an exciting field that is only beginning to change the way we study development and tissue homeostasis.

Competing interests

The authors declare no competing or financial interests.

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