Original Article

Morphological Evidence of Telocytes in Mice Aorta

Hong-Qi Zhang[1,2], Shan-Shan Lu[1], Ting Xu[1], Yan-Ling Feng[3], Hua Li[4,5], Jun-Bo Ge[5,6]

[1]Department of Anatomy, Histology and Embryology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[2]Key Laboratory of Medical Imaging Computing and Computer Assisted Intervention of Shanghai, Shanghai 200032, China
[3]Department of Pathology, Shanghai Public Health Clinical Center, Shanghai 201508, China
[4]Institute of Biochemistry and Cell Biology, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
[5]Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[6]Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China

Abstract

Background: Telocytes (TCs) are a novel type of interstitial cells, which have been recently described in a large variety of cavitary and noncavitary organs. TCs have small cell bodies, and remarkably thin, long, and moniliform prolongations called telopodes (Tps). Until now, TCs have been found in various loose connective tissues surrounding the arterioles, venules, and capillaries, but as a histological cellular component, whether TCs exist in large arteries remains unexplored.
Methods: TCs were identified by transmission electron microscope in the aortic arch of male C57BL/6 mice.
Results: TCs in aortic arch had small cell bodies (length: 6.06–13.02 μm; width: 1.05–4.25 μm) with characteristics of specific long (7.74–39.05 μm), thin, and moniliform Tps; TCs distributed in the whole connective tissue layer of tunica adventitia: TCs in the innermost layer of tunica adventitia, located at the juncture between media and adventitia, with their long axes oriented parallel to the outer elastic membrane; and TCs in outer layers of tunica adventitia, were embedded among transverse and longitudinal oriented collagen fibers, forming a highly complex three-dimensional meshwork. Moreover, desmosomes were observed, serving as pathways connecting neighboring Tps. In addition, vesicles shed from the surface of TCs into the extracellular matrix, participating in some biological processes.
Conclusions: TCs in aorta arch are a newly recognized complement distinct from other interstitial cells in large arteries, such as fibroblasts. And further biologically functional correlations need to be elucidated.

Key words: Aorta; Telocyte; Telopode

Introduction

Conventionally, cells in the large arteries, whose wall are composed of three layers including tunica intima, tunica media and tunica adventitia, are mainly comprised of endothelial cells, smooth muscle cells (SMCs), fibroblasts (FBs), and macrophages. The tunica adventitia is a thin layer of loose connective tissue containing principally collagen fibers (CFs) arranged in circular bundles and a few elastic fibers as well as FBs and macrophages.[1] Besides, the presence of other interstitial cells in large arteries was also reported, such as interstitial cells of Cajal (ICC).[2]

Telocytes (TCs) are a distinct cell type of interstitial cells which have been recently described in stroma of various tissues and organs, such as placenta,[3] endocardium,[4] lung,[5] parotid gland,[6] skin,[7] eye,[8] myometrium,[9] esophagus,[10] liver,[11] heart valves,[12] vasculature,[13] bone marrow[14], *et al*. TCs were primarily regarded as interstitial Cajal-like cells (ICLCs), whereas subsequently verified to be different from ICLCs and ICC in both ultrastructure and immunophenotype.[15] The most distinctive ultrastructural feature of TCs is the presence of special long, thin, and moniliform prolongations called telopodes (Tps), which comprise thin segments (podomers) in alternation with dilated segments (podoms), accommodating mitochondria, rough endoplasmic reticulum (rER), smooth endoplasmic reticulum, and caveolae. Their immunophenotypes mainly focus on CD34, CD117, and vimentin.[13,16,17]

Although TCs were identified in the connective tissue that surrounded rat duodenal arterioles, venules, and capillaries,[18] TCs exist in large arteries is still unknown. Here, the existence of TCs with typically structural features in mice aorta was convinced by transmission electron microscope (TEM). Furthermore, TEM indicated the ubieties of TCs in tunica adventitia, intracellular communication between Tps, and vesicles shedding from TCs into the adjacent extracellular matrix.

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Address for correspondence: Prof. Jun-Bo Ge, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai 200032, China
E-Mail: ge.junbo@zs-hospital.sh.cn

Methods

Animals

Six male C57BL/6 mice, 8 weeks old, with the weight between 15 g and 20 g (Laboratory Animal Center, Shanghai Medical College, Fudan University), were used in accordance with the local ethical guidelines. These mice were housed at 22°C under a 12 hours light/12 hours dark cycle, with free access to standard laboratory chow and tap water. Approval for this study was granted by the Institutional Ethics Board of Fudan University, according to the generally accepted international standards.

Transmission electron microscopy

The mice were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg) (Sigma, USA). The animals were fixed in the supine position with their necks extended. The thoracic cavity was cut open, and the beating heart was exposed. Then, through a puncture in the left ventricle, 15 ml of physiological saline (containing heparin sodium 240 IU/20 ml, Wanbang Biochemical Pharmaceutical Company) was perfused, followed by perfusion of 30 ml 4% paraformaldehyde (Sigma, USA) (pH 7.2) in phosphate-buffered saline at physiological pressure to fix the blood vessels *in situ*. The right auricle was cut open, and the perfusion solution was permitted to flow out from the opened incision to maintain a smooth perfusion. After the perfusion, the aortic arch samples were removed and cut into small pieces about 1 mm^3, which were washed in phosphate buffer and fixed with 4% glutaraldehyde (Sigma, USA) overnight at 4°C. After washed in 0.1 mol/L phosphate buffer for 5 minutes, the samples were postfixed with 1% OsO_4, rinsed, dehydrated in a graded series of ethanols, and then embedded in Epon 812. Ultrathin sections were made by a MT-7000 ultramicrotome (Research Manufacturing Company Inc., Tucson, AZ, USA), collected on 50-mesh grids, counterstained with 1% uranyl acetate and lead citrate for 10 min, observed and photographed under a FEI TECAI SPIRIT TEM (The Republic of Czech).

Results

Under TEM, a novel type of interstitial cell with distinctive ultrastructural features defined as TC was observed in loose connective tissue of tunica adventitia of mice aortic arch. TCs in tunica adventitia were morphologically consistent with those previously reported in other tissues and organs.

Distribution of telocytes in aortic arch

Telocytes were generally distributed in the whole connective tissue layer of tunica adventitia of the aortic arch. TCs in the innermost layer of tunica adventitia, located at the juncture between tunica media and tunica adventitia, with their long axes oriented parallel to the outer elastic membrane [Figure 1]. And no direct contacts between TCs and elastic membrane and no intercellular junctions between TCs and vascular SMCs (VSMCs) were observed [Figure 1]. TCs in outer layer of tunica adventitia were intertwined with surrounding stromal CFs, which was organized into a highly complex three-dimensional meshwork among transverse oriented fiber bundle, and longitudinal array-oriented fiber bundle [Figures 2 and 3].

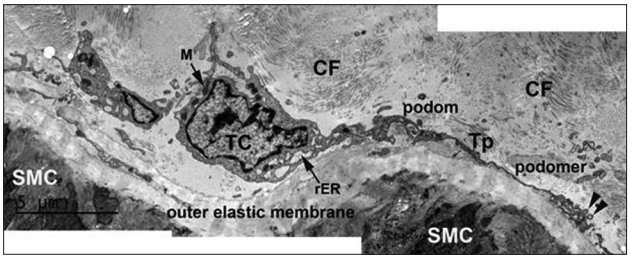

Figure 1: Transmission electron microscope image of mouse aortic arch (merged image). Telocyte (TC) with a characteristic telopode (Tp) borders the outer elastic membrane of the aortic arch. The body of TC is 6.06 μm in length and 4.25 μm in width. The cytoplasm is scare and contains a moderate amount of mitochondria (M), and rough endoplasmic reticulum (rER). The visible Tp is 14.98 μm in length, presenting moniliform respect due to the alternation of podoms and podomers (average caliber 0.09 μm). Note the shed vesicles (arrowheads) in close proximity to the distal part of Tp. CF: Collagen fibers; SMC: Smooth muscle cell; Bar = 5 μm.

Distinctive features of telocytes in tunica adventitia

The characteristics of TCs in tunica adventitia demonstrated that the cell bodies were relatively small (range from 6.06 μm to 13.02 μm in length, from 1.05 μm to 4.25 μm in width), with a high nuclear/cytoplasmic ratio [Figures 1-5]; the perinuclear cytoplasm contained some rER and mitochondria [Figure 1]; the thin and long (range from 7.74 μm to 39.05 μm) Tps were projecting from the cell body [Figures 1-5], whose number per TC was variable, with 1–3 visible Tps in a single section, generally [Figures 1-5]; and the typical morphological features of convoluted and moniliform Tps [Figure 4] occurred due to the alternation of podomers and podoms. The podomer was the thin segment whose caliber was about 0.09 μm [Figure 1], the podom was the dilated segment, which accommodated abundant organelles: rEr, Golgi apparatus, lysosomes and caveolae [Figure 5]. In addition, dichotomous branch emerged at various segment of Tps of TCs [Figures 2 and 5] and vesicles shedding from TCs were present in the adjacent extracellular matrix [Figures 1 and 4].

Cell communication between telocytes and other cells in tunica adventitia

Homocellular junctions between TCs themselves were observed under TEM. The desmosomes [Figure 6] were visible between Tps of different TCs, forming an intricate three-dimensional network in tunica adventitia. Moreover, macrophages and FB coexisted with TCs in the same region of loose connective tissue of tunica adventitia [Figure 7], where a large amount of CFs survived, whereas, no direct connections appeared among them.

Discussion

The present study indicated that TCs in tunica adventitia of mice aorta displayed the representative morphological properties defined by Popescu, and could form a

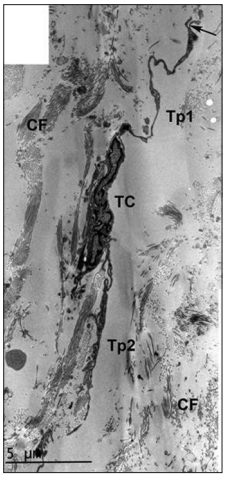

Figure 2: Telocyte (TC) in adventitia of mouse aortic arch (merged image). TC with two telopodes (Tps) exists in tunica adventitia, embedded among collagen fibers (CF). The body of TC takes on the shape of irregular thin and long ellipse with 8.15 μm in length and the average width being 1.05 μm. The length of circuitous Tp1 and Tp2 are 12.90 μm and 13.07 μm, respectively. Note a dichotomous branch (black arrow) emerging from the end of Tp1. Bar = 5 μm.

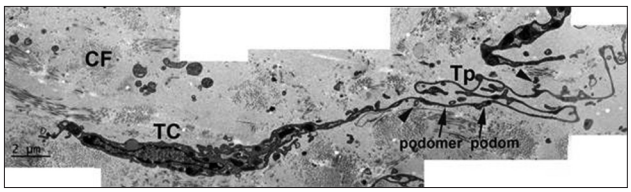

Figure 4: Distinctive feature of telopode (Tp) of telocyte (TC) in adventitia of mouse aortic arch (merged image). The Tp of a TC displays very thin, special long and convoluted aspect. The length of Tp is up to 39.05μm, more than 3 times of the macroaxis length (13.01 μm in length) of the body of TC. The moniliform aspect of Tp consists of an alternation of podomers and podoms (black arrows). Plenteous shed vesicles (arrowheads) can be seen in the neighborhood of various regions of the Tp. CF: Collagen fiber; Bar = 2 μm.

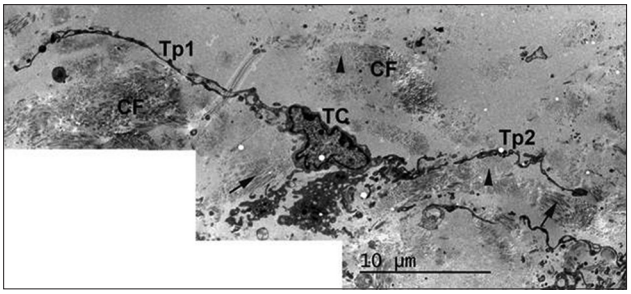

Figure 3: Telocytes (TCs) and collagen fibers (CFs) in adventitia of mouse aortic arch (merged image). A TC (length: 7.17 μm, the average width: 2.18 μm) with two characteristic telopodes (Tps) (Tp1 and Tp2 are 22.31 μm and 17.37 μm in length, respectively) is intertwined with surrounding collagen fibers, which is organized into a highly complex three-dimensional meshwork among transverse oriented fiber bundle (arrowheads), and longitudinal array-oriented fiber bundle (black arrows). Bar = 10 μm.

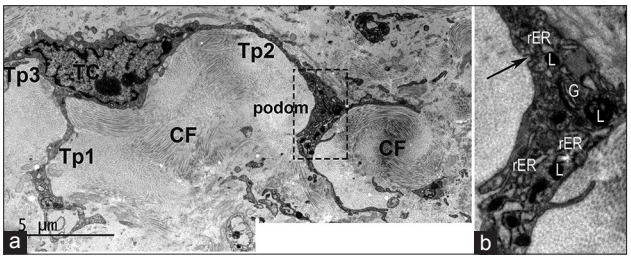

Figure 5: Ultrastructure of podom of telopode (Tp) in adventitia of mouse aortic arch (merged image). (a) A telocyte (TC) (length: 9.31 μm, the average width: 2.79 μm) with three Tps coexists with collagen fibers in adventitia of the aortic arch. Tp2 is the longest one of three, reaching 16.43 μm, presenting the typical morphological feature of alternating podomer and podom and forming bifurcation. The length of Tp1 is 7.74 μm, and Tp3 is only partially shown. (b) The higher magnification of dotted line rectangle area of A indicates that the dilated segment of Tp-podom has an irregular shape, and accommodates rough endoplasmic reticulum (rER), Golgi apparatus (G), lysosomes (L) and caveolae (black arrow). Bar = 5 μm.

three-dimensional meshwork through different Tps establishing direct contacts. The finding was also in accord with previous research that TCs located on the connective tissue of rat duodenal blood vessels including arterioles, venules, and capillaries.[18]

The tunica adventitia of the large artery is relatively thin connective tissue layer containing mainly CFs and a few elastic fibers. The principal cells are FBs and macrophages.[1] Although TEM alone allowed identification of TCs, caution should be taken to differentiate TCs from other interstitial cells in tunica adventitia. Macrophages should not give rise to diagnostic problems due to their appearance of a large Golgi apparatus, abundant lysosomes, and irregular cytoplasmic projections.[1] But attentions should be taken when in the face of FBs and TCs, which could be confused by their similar structural features. According to the results of this study, TCs were different from other interstitial cells, including FBs, by presence of Tps, which are extremely long, thin, and moniliform prolongations. The main function of FB is to synthesize CFs, elastic fibers, and some extracellular constituents. The FB body is large and pleomorphic; the Golgi complex is prominent; the rER is well developed, the cell processes are few, short, and of large caliber, thus being easily appreciable under a light microscope.[19,20] Based on these, these cells are markedly different from TCs, which present irregular cell body, large nucleus, and scarce cytoplasm, with a small quantity of Golgi apparatus, some mitochondria, few endoplasmic reticulum; and the most striking feature of special long, thin Tps.[16,19]

Numerous studies have documented the possibility of present of ICC-like cells in the blood vessels. The studies by Pucovský *et al*.[21] and Harhun *et al*.[22] described a novel cell type, which had similar morphological features of interstitial ICC in mesenteric arteries of guinea pig and rabbit portal vein, respectively. It seemed that there were some differences in both morphology and physiological roles of ICCs of rabbit portal veins and mesenteric arteries of guinea pig. Harhun *et al*.[23] utilized ICs (interstitial cells) for all subtypes of interstitial cells found in blood vessels. A study performed by Bobryshev[2] firstly showed the presence of arterial cells

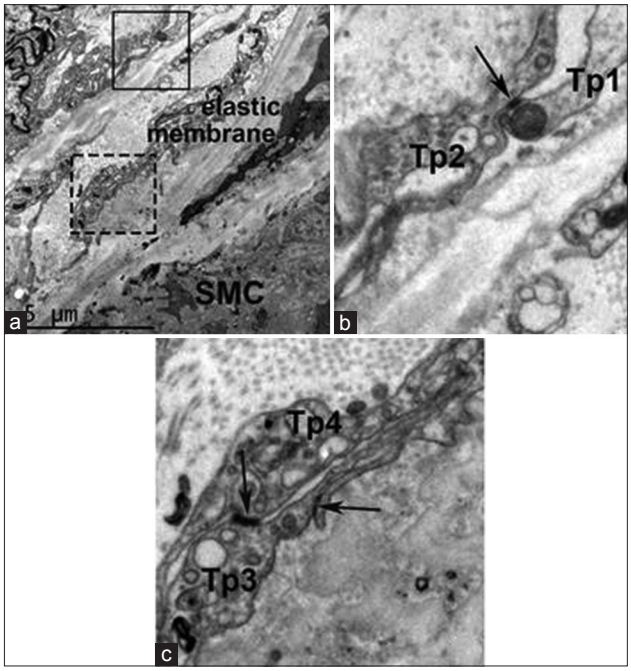

Figure 6: Cell communication between different telopodes (Tps). (a) Direct contacts come into existence between Tps of different telocytes (TCs) in adventitia of the aortic arch. (b) Local higher magnification of the line rectangular area of A shows that the end of Tp1 forms desmosome (black arrow) with Tp2 of another TC. (c) Local higher magnification of dotted line rectangular area of A demonstrates that Tp3 inserts into a cupped space formed by Tp4 of another TC, and desmosomes (black arrows) connecting Tp3 and Tp4 are visible. SMC: Smooth muscle cell. Bar = 5 μm.

with typical structural characteristics of ICC *in situ*, which was known as arterial ICC, and perhaps represent a distinct subtype within the ICC family; These cells were in direct contact with both SMCs and nerve endings at the juncture of media and adventitia of human large arteries.

According to the review of interstitial cells of blood vessels,[24] a new cell type termed interstitial cell was indicated in the tunica media of both veins and arteries. These cells possessed the characteristic of irregularly shaped, thin processes and noncontractile, which were totally different from VSMCs. The main role of portal vein ICs may tend to be a pacemaker in the wall of blood vessels; while, the physiological role of interstitial cells in arteries is still unclear, and according to their phenotypes, the arterial interstitial cells may belong to the SMC lineage.

From the research so far, ICCs or ICs of blood vessels are more likely a subset of SMC subpopulation, which share most of the features with the ICCs of the gastrointestinal tract.[25] In present study, a subset of interstitial cells with ultrastructure characteristics enabling these cells to be regarded as TCs (see in detail: http://www.telocytes.com/), was usually located in tunica adventitia of aorta, not in tunica media, and no direct intercellular junctions were found between them and SMCs. It seems to be clear that, there are plenty of difference between ICCs and TCs in blood vessels, such as ultrastructure and function.

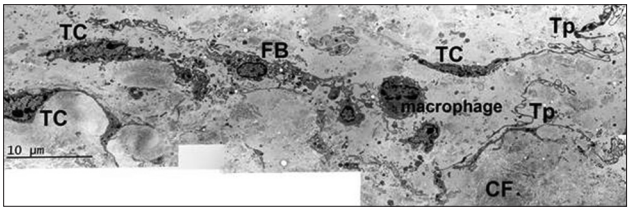

Figure 7: Relationship between telocytes (TCs) and other cells (merged image). In adventitia of mouse aortic arch, three TCs are observed to be present with fibroblast and macrophage in the same region of extracellular matrix of adventitia where a large amount of collagen fibers (CF) exist, but no direct contact among them appears. Bar = 10 μm.

Previous study by Corselli *et al*.[26] demonstrated that cells expressed mesenchymal stem cells markers resided in the outmost layer of blood vessels. Besides pericytes, which encircle capillaries and microvessels, the tunica adventitia might be another source of mesenchymal stem cells. The new finding in our current study showed the presence of TCs in the tunica adventitia of mice aorta. Since TCs had been reported in close relationship with several stem cells, such as cardiac progenitors,[27] subepithelial lung stem cells[5], skeletal muscle stem cells,[28] skin stem cell clusters.[7] There are grounds for believing that TCs play an important role in increasing the efficiency and efficacy of resident local stem cells in the process of repair/regeneration through cell-to-cell communication or shed vesicles.

In conclusion, our study provided TEM evidence for the presence of TCs with representative features in tunica adventitia of mice aortic arch. And their biologically functional significance in vasculature needed to be further explored.

Acknowledgments

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