Chapter 17
Roles of Telocytes in the Development of Angiogenesis

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17.1 Introduction

Telocytes were identified to be located on the extracellular matrix of blood vessels [1], e.g. arterioles, venues and capillaries in many organs and tissues, especially in the heart and lung. For example, the number of telocytes significantly increased around the neo-capillaries in the heart of mice with acute myocardial infarction, indicating that telocytes may contribute to the angiogenesis of capillaries [3, 5]. Telocytes have close relation with capillaries in the interstitial of lung tissues, indicating that telocytes might participate in the structure of air-blood barrier [6]. In this chapter, we try to elaborate the biological function of telocytes on the angiogenesis through studies from lung.

17.2 The Discovery of Lung Telocytes

Telocytes were firstly found, confirmed and isolated from mouse trachea and lung tissues through the methods of transmission/scanning electron microscope, immunohistochemistry and primary cell culture by Dr. Zheng et al. in 2011 [10]. Telocytes were found to be allocated in the interstitial space, between smooth muscle fibers, and/or between the cricoid cartilage and smooth muscles within the trachea (Fig. 17.1). Telocytes were observed in the interstitial space of terminal bronchioles,

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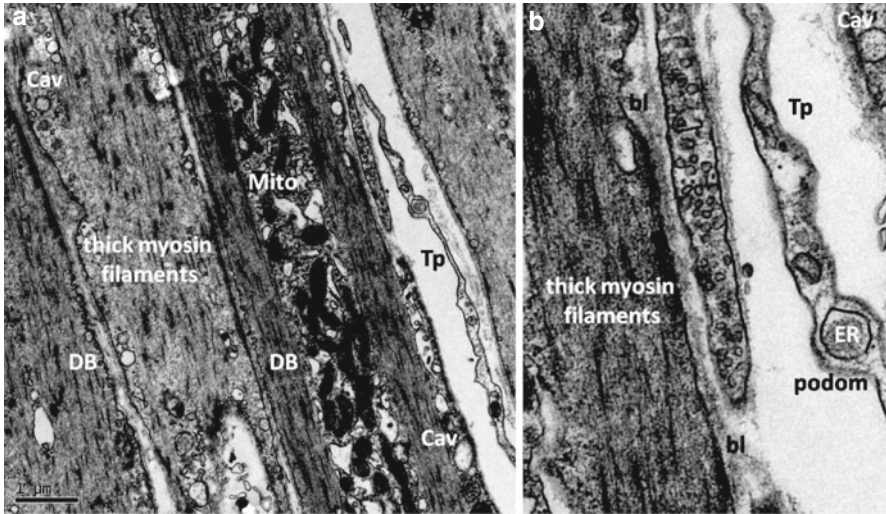e-mail: Xiangdong.wang@clintransmed.org

Fig. 17.1 (**a**) Mouse trachea; transmission electron microscopy of smooth muscle layer. Note, four muscle cells longitudinally cut and in between two fragments (one long, one short) of a Tp. It is to be mentioned the presence of thick myosin filaments in smooth muscle cells. *DB* dense bodies, *mito* mitochondria. (**b**) Higher magnification of a fragment from (**a**). Note, a smooth muscle cell with thick myosin filaments and basal lamina (*bl*); a fragment of a telopode, a podom containing element of ER. *cav* caveolae, *bl* basal lamina

of which Tps were connected with alveolar epithelial cells and other cells in the lung tissues, Telocytes were identified in the lung and were found to be related with stem-cell niches (Fig. 17.2), with positive staining of vimentin, c-kit and CD34 (Fig. 17.3).

17.3   Experience of Lung Telocyte Isolation

In order to study the biological function of telocytes, cell culture is the first step. Although several reports about the culture method have been published, there is still no detailed introduction. In this section, we try to elaborate the detailed primary culture method for telocytes according to published papers and our own study. Here we chose lung tissues of BABL/c mice as an example. After BABL/c mice were killed with an overdose of anesthetic, lung fragments were isolated under sterile conditions and collected into sterile tubes containing DMEM (Gibco, NY, USA), supplemented with 100 UI/ml penicillin, 0.1 mg/ml streptomycin (Sigma-Aldrich Shanghai Trading Co Ltd., Shanghai, China), placed on ice and transported to the cell culture laboratory. Samples were processed within 30 min. from surgery, and they were rinsed with sterile DMEM. The lung tissues were minced into fragments

Fig. 17.2 Telocytes (*TCs*) in mice lung. About four TCs with telopodes (Tps) are visible in the interstitial of lung. A putative stem cell (SC, *yellow dotted circle*) is in close contacts with telocytes, establishing heterocellular junctions, visible under electronic microscope only. The tandem TCs-SC forms, presumably, a TC-SC niche. *EC* epithelial cells, *SMC* smooth muscle cells. Magnification is 2000×, scale bar is 5 μm

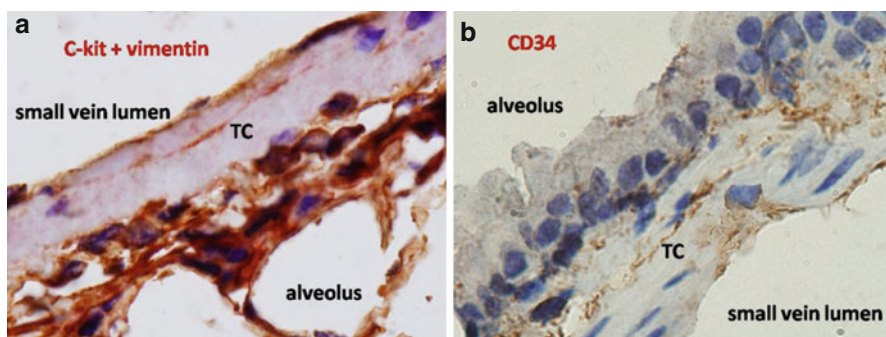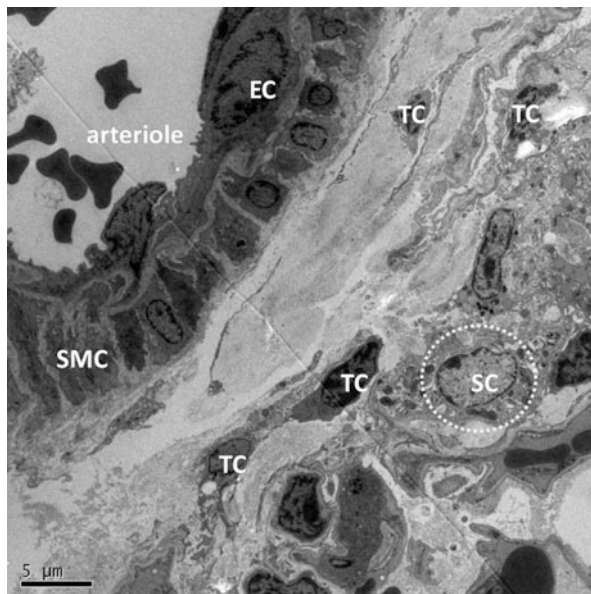**Fig. 17.3** (**a**) Immunohistochemical reaction for c-kit and vimentin in lung tissue. Long TC silhouette is seen positive near small vein lumen. (**b**) Positive reaction for CD34 of TC between airway epithelium and blood vessel endothelium. Magnification 1000×

of about 1 mm^3 and incubated on an orbital shaker for 4 h, at 37 °C, with 1 mg/ml collagenase type II (Gibco, NY, USA) in PBS (without Ca^{2+} and Mg^{2+}). Dispersed cells were separated from nondigested tissue by filtration through a 40 μm diameter cell strainer (BD Falcon, NJ, USA), collected by centrifugation at 2000 r.p.m., 5 min., re-suspended and cultured in a 75 cm^2 plastic culture flask containing DMEM, supplemented with 10 % foetal calf serum, 100 UI/ml penicillin and 0.1 mg/ml streptomycin (Sigma-Aldrich) for 2 h, and then the supernatant containing slowly adhering cells was collected and re-plated into a new 25 cm^2 plastic culture flask, at a density of 1×10^5 cells/cm^2, and maintained at 37 °C, in a

humidified atmosphere (5 % CO_2 in air) until becoming semi-confluent (usually 4 days after plating), and the adherent cells were telocytes. Culture medium was changed every 48 h, and the mixed suspension cells were deleted through the changing of the medium. Cell viability was assessed by using the Trypan blue dye exclusion test and cells were examined by phase contrast microscope [2].

17.4   Morphology of Cultured Lung Telocytes

Primarily cultured telocytes could be identified clearly from 3rd to 4th days. Telocytes appear typical Tps with long and uneven caliber, podoms and/or podomers (Fig. 17.4a). Telocytes and Tps with very long and/or uneven caliber, or dilated portions resembling "beads on a string" were observed under the staining with methylene blue (Fig. 17.4b) and giemsa (Fig. 17.4c). Janus green B is a classic vital staining with high affinity for mitochondria and used to assess viability and localize mitochondria. Figure 17.4d illustrates the TC body and Tp with
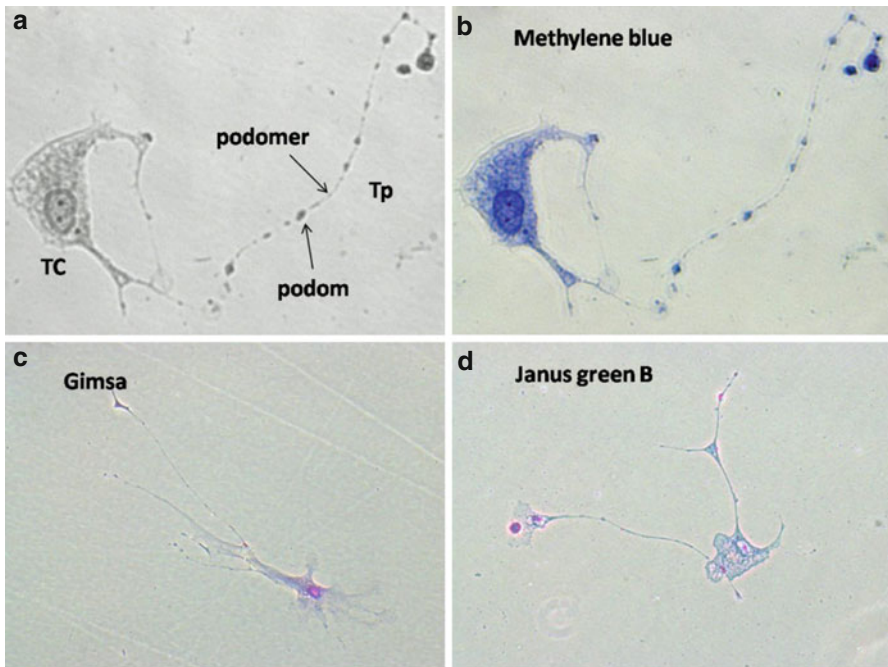

Fig. 17.4 Mouse lung telocytes (*TC*) in primary culture observed under phase-contrast microscopy. (**a**) TC is depicted with long and slender telopodes (*Tps*), which present the alternation of thick segments – podoms (*black arrows*) and thin segments – podomers (*black arrows*). Dichotomic branching of Tps is obvious. Magnification is 400×. (**b**) Methylene blue staining. Magnification is 400×. (**c**) Giemsa staining. Magnification is 100×. (**d**) Janus Green B staining Along Tp of another TC over-passed the cell body of the other Telocytes. Magnification is 100×
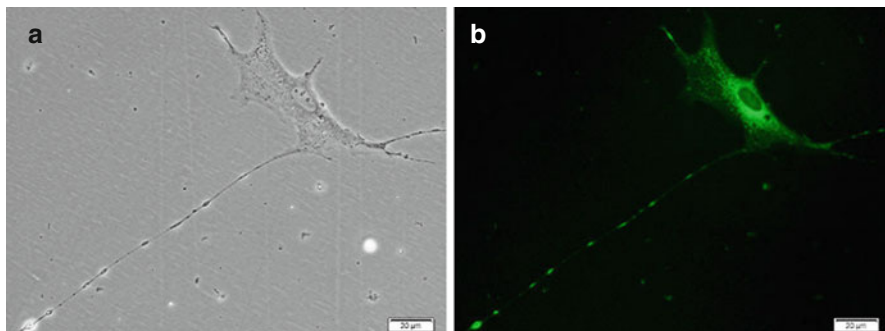
Fig. 17.5 Primary culture of human lung telocytes. (**a**) Telocytes are observed under phase-contrast microscopy. (**b**) Telocytes are observed under fluorescence microscopy. Telocytes were stained with Mito Tracker Green. The mitochondria are allocated mainly within Telocytes body and its podoms. The magnification was 400 ×, and the scale bar is 20 μm

podoms highly stained with Janus green B, indicating the rich presence of mitochondria in cell body and particularly in podoms. Mito Tracker Green FM is a molecular probe with high affinity for mitochondrial membranes and used to identify mitochondria in living cells. The strong staining of mitochondria in most Telocytes and Tps with Mito Tracker Green FM was observed (Fig. 17.5), demonstrating the accumulation of mitochondria in Telocytes and podoms. Those findings imply that the maintenance of Telocytes biological functions requires the large amount of energies, while Tps and podoms provide the energies for the extensions.

17.5   Proteomics Between Telocytes and Endothelial Cells

According to the study from Zheng et al, the proteomic analysis between telocytes and fibroblasts was studied by the method of iTRAQ and the function of protein expression profiles were analyzed with the aid of PANTHER Classification System.

By comparison between telocytes and microvascular endothelial cells, there are 38 proteins up-regulated in telocytes, especially Myosin-14, superoxide dismutase (SOD2), acid ceramidase (AC), envoplakin and epiplakin. Among these proteins, 18 of them are responsible for metabolic processes and 15 proteins in cellular processes, such as cell communication (4 proteins), cytokinesis (2 proteins), cellular component movement (2 proteins), and cell cycle (2 proteins). There are 60 proteins down-regulated in telocytes, especially cell surface glycoprotein MUC18, Ras-interacting protein 1, BTB/POZ domain-containing protein, peptidyl prolyl cis/trans isomerase and nestin and von Willebrand factor. The highly expressed proteins in telocytes are involved in important molecular functions such as: catalytic activity (17 proteins), structural molecule activity (13 proteins) compared to

microvascular endothelial cells where significantly more proteins are involved in catalytic activity (30 proteins) and 29 proteins have molecular binding function. Ten up-regulated proteins in telocytes are involved in developmental processes: anatomical structure morphogenesis (10 proteins), mesoderm development (3 proteins), system development (2 proteins) and ectoderm development (1 protein). Moreover, 10 proteins in telocytes are related to localization processes such as vesicle mediated transport (4 proteins), protein transport (4 proteins) and ion transport (3 proteins). While in microvascular endothelial cells, two up regulated proteins are involved in nucleic acid-binding transcription and one has anti-oxidant activity.

The up-regulated telocytes proteins belong to the following pathways: nicotinic acetylcholine receptor (2 proteins), inflammation mediated by chemokines (2 proteins), de novo purine biosynthesis (2 proteins), cytoskeletal regulation by Rho GTPase (2 proteins), TCA cycle (1 protein), Parkinson disease (1 protein), integrin signaling (1 protein) and blood coagulation (1 protein) . In telocytes, the up-regulated proteins are related to the following cellular components: cell part (13 proteins), organelle (12 proteins), membrane (2 proteins), cell junction (2 proteins), extracellular region (1 protein) and extracellular matrix (1 protein).

The up-regulated proteins in microvascular endothelial cells are attributed to the following protein classes: enzyme modulator (11 proteins), cytoskeletal proteins (10 proteins), oxidoreductase (7 proteins), nucleic acid binding (6 proteins), transferase, isomerase and chaperone (5 proteins each), etc. The pathways map depicted the microvascular endothelial cells proteins are related to: integrin signalling pathway (3 proteins), Huntington disease (3 proteins), cytoskeletal regulation by Rho GTPase (3 proteins), pentose phosphate pathway (2 proteins), Parkinson disease (2 proteins), inflammation mediated by chemokines (2 proteins), glycolysis (2 proteins), etc. The cellular component of microvascular endothelial cells proteome demonstrated proteins related to: cell part (12 proteins), organelle (10 proteins), extracellular region (1 protein) and extracellular matrix (1 protein) [9]. Telocytes are completely different from microvascular endothelial cells. Protein expression profile showed that telocytes play specific roles in intercellular communication and intercellular signaling. Moreover, they might inhibit the oxidative stress and cellular ageing and may have pro-proliferative effects through the inhibition of apoptosis.

17.6   Telocytes and Angiogenesis

The angiogenesis is a common phenomenon both in physiological and pathological conditions, including proliferation of vascular endothelial cells, enzymatic degradation of basement membrane and interstitial matrices by endothelial cells,

migration of vascular endothelial cells, or eventually formation of a blood vessel tube from sprouting vascular endothelial cells. Angiogenesis could be induced by activation of VEGF and EGF receptors by the binding of VEGF and EGF to induce the tube formation in the vascular endothelial cells, promote endothelial cell proliferation and vascular permeability, and maintain newly-formed blood vessels [4].

In the study of lung telocytes, Zheng et al. confirmed that lung telocytes were mainly distributed in the alveolar interstitial connected tightly with alveolar epithelia cells and participated in the structure of air-blood barrier, in the small vein and bronchioles and in the interstitial space of smooth muscle participated in the frame structure of the blood and bronchioles [7]. Telocytes are positive to CD34 and C-kit which expressed on the surface of hemopoietic stem cells, and are proposed to participate in the angiogenesis.

Distribution and structure characteristics of telocytes must fit to its biological functions. Zheng et al. also confirmed that production of VEGF and EGF from human lung telocytes increased significantly. Cultured medium of telocytes could promote the proliferation of HPMECs, and partially recover the ability of tube formation of HPMECs injured by LPS. Indicating that telocytes may participate in the angiogenesis of lung tissues, in both normal and pathological conditions and play roles as progenitor cells and nutrient cells during the regeneration and reparation of the injured tissues. Telocytes can be a new therapeutic target in lung diseases.

17.7   Perspectives

The angiogenesis is a common phenomenon both in physiological and pathological conditions, including proliferation of vascular endothelial cells, enzymatic degradation of basement membrane and interstitial matrices by endothelial cells, migration of vascular endothelial cells, or eventually formation of a blood vessel tube from sprouting vascular endothelial cells. Angiogenesis could be induced by activation of VEGF and EGF receptors by the binding of VEGF and EGF to induce the tube formation in the vascular endothelial cells, promote endothelial cell proliferation and vascular permeability, and maintain newly-formed blood vessels. Telocytes are a new type of interstitial cell with special biological functions, for the close relation with stem cells, they are also named as "stem cell helper cells". Telocyes play roles as progenitor cells and nutrient cells during the regeneration and reparation of the injured tissues, including the angiogenesis in both normal and pathological conditions through the secretion of VEGF and EGF [8]. Further studies are necessary for the application of cellular therapy with telocytes in clinic, such as lung injury, myocardial infarction, or tissue engineering.

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