

Telocytes in rat lungs: an essential pool of cells or not?

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Abstract: **Background:** The histology of the lung includes a variety of cell types. Fibrosis is a universal process, occurring in the skin, intestine, heart, muscles, kidney, blood vessels, liver, and also the lungs. Telocytes are a type of cells with a wide range of properties, which were previously described in healthy and disease-affected organs of human and animal organisms.

Aim: This study aimed to identify telocytes in the lungs of rats and discuss their possible role in the development of pulmonary fibrosis.

Methods: Tissue samples were taken from a group of ten male Wistar rats. Further histological and immunohistochemical analysis was performed. Double immunolabeling for c-kit, vimentin, CD34, and PDGFR α has revealed telocytes in the lungs.

Results: In all tissue samples, telocytes have been identified (in the area of interalveolar septa, close to blood vessels, and between the airway epithelium).

Conclusion: Telocytes might be directly and indirectly (through contact with stem cells, secretomes, and reduction in number) involved in the development of pulmonary fibrosis. The heterogeneity of the telocyte population in different pathologies and their subtypes, as well as their tendency to be common stress their important role in pathological physiology.

Key words: lungs, telocytes, fibrosis, rat, CD34.

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Introduction

The respiratory system is always requires detailed monitoring of all its parameters, not only in a pandemic. The importance of breathing for life is clear. The heterogeneity of cellular populations in the respiratory system makes possible the maintenance of local homeostasis. The histological architecture of the lung has been precisely described by Schraufnagel in 1990 [1]. The pool of cells in our respiratory tract includes basal cells, club cells (Clara cells), goblet cells, NE cells, ciliated cells, ionocytes, tuft (brush) cells, myoepithelial cells, acinar cells (mucous and serous), alveolar epithelial cells type 1 and type 2, stem cells, smooth muscle cells, myofibroblasts, peribronchial fibroblasts, pericytes, lipofibroblasts, and immune cells (dendritic cells, T- and B-lymphocytes, alveolar residence macrophages, interstitial macrophages, basophils and eosinophils) [2]. Furthermore, some of the aforementioned cells have their own subtypes and functions which are important for lung plasticity and health. Evren *et al.* demonstrated the heterogeneity of murine lung macrophages last year [3].

One more population of cells – telocytes (TCs) – seems to be involved in lung histogenesis. They have been detected in the primitive embryonic lung by day 18 of gestation in rabbits [4]. In 2011, Popescu *et al.* observed tissue specimens from human and murine lungs with a further selection of five different locations of telocytes [5] and described telocyte prevalence in the interstitial space of an intralobular bronchiole, in the terminal and respiratory bronchioles, and in alveolar ducts [6, 7]. The morphology of TCs has been described in many articles with respect to their identification and functions [8–11]. The detailed observation of its function and contacts makes sense in the context of pulmonary fibrosis development, immunosurveillance, or angiogenesis. It also gives rise to the presumption that lung telocytes may play an essential role in normal and pathological physiology as “connecting cells” [12]. Due to homo- and heterocellular contacts, TCs have been discussed by researchers as a mediator of intracellular communication and regulation [7]. The current study aimed to observe telocytes in rat lungs, as well as describe their location and contacts, with a possible explanation of their role. The study is a pilot study that will lead to further research of lung fibrosis development.

Material and Methods

Animals

Ten male Wistar rats weighing 325 ± 12 g (Jagiellonian University Medical College Animal Laboratory, Kraków, Poland) were included in the experiment. Upon arrival, the animals were housed for a week under controlled conditions — 12 h light/12 h dark cycle and temperature of $22 \pm 2^\circ\text{C}$. Transparent cages were placed adjacent to each

other to provide sight, acoustic, and odor contact. All cages contained suitable bedding materials and environmental enrichment. Animals were fed with standard dry chow: protein 25%, fat 8%, carbohydrates 67%, metabolizable energy 2.86 kcal/g (Labofeed B, Kcynia, Poland). Tap water was always available *ad libitum*.

Ethical approval

The study was conducted in accordance with the moral, ethical, regulatory, and scientific principles governing clinical research. All samples were retrieved with the approval of the Jagiellonian University Bioethical Committee using procedures that conformed to the guidelines of the Declaration of Helsinki (protocol number — 367/2020).

Morphological analysis of the respiratory system

Tissue processing

Immediately after euthanasia, tissue fragments from rat lungs were collected. Fresh tissue specimens were rinsed thoroughly with PBS (phosphate-buffered saline, 0.01 M, pH = 7.4), fixed with 9% phosphate-buffered paraformaldehyde, routinely processed and embedded in paraffin (FFPE). Solidified paraffin blocks were cut into 3–5 micrometers thick sections and placed on slides with increased adhesion (Super Frost Plus).

Routine histology

The sections were deparaffinized, rehydrated, and stained with either hematoxylin–eosin (H&E) to evaluate the gross tissue organization or Masson's trichrome staining to detect collagen deposits.

Immunofluorescence

After deparaffinization and rehydration, the slides were incubated for 30 min in PBS with appropriate normal serum at room temperature, followed by overnight incubation at 4°C in a solution of PBS with appropriate normal serum containing a mixture of primary antibodies. After 5 washes (10 min each) in PBS, the specimens were then incubated for 1 h at room temperature with a mixture of secondary antibodies diluted in PBS. Indirect double immunofluorescence for identification of telocytes in sections were performed with polyclonal rabbit anti-c-kit (1:100; code: A4502, Dako); monoclonal mouse anti-CD34 (1:100; code: M7165, Dako); polyclonal goat anti-PDGFR alpha (1;100; code: AF-307-NA, R&D Systems); monoclonal mouse anti-tryptase

(1:100; code: M7052, Dako); monoclonal mouse vimentin (1:50; code: Clone V9, Dako), monoclonal rabbit anti-CD34 (1:200; code: ab81289, Abcam). Secondary antibodies included goat anti-mouse Alexa 488-conjugated antibody (1:400; code: 115-545-146, Jackson ImmunoResearch), goat anti-rabbit Alexa 594-conjugated antibody (1:400; code: 111-585-144, Jackson ImmunoResearch), and donkey anti-goat Alexa 594-conjugated antibody (1:400; code: 705-585-003, Jackson ImmunoResearch). Negative controls were performed with omission of the primary antibodies. Finally, the slides were washed in two changes (10 min each) of PBS and cover-slipped with fluorescence mounting medium (Dako, Denmark) and covered glasses Menzel-Gläser. DAPI was performed for nuclei staining. Labeled specimens were analyzed immediately.

Microscopic examination of telocytes

Slides were examined using an MN800FL epifluorescence microscope (OptaTech, Warsaw, Poland) equipped with an Olympus DP74 digital camera. Digital images were collected at either 200× or 400× magnification. The qualitative analysis of cells was performed in 10 consecutive high-power fields (400×) using the computer-based image analysis system software Multiscan 18.03 (CSS, Warsaw, Poland). All samples were assessed by two independent specialists (each blinded to the other) without any knowledge of the clinical parameters or other prognostic factors to avoid bias. The use of mast cell tryptase staining enabled c-kit-positive mast cells to be distinguished from c-kit-positive TCs. TCs were considered cells that were c-kit-positive and tryptase-negative concurrently with characteristic morphology in tissue samples. Additionally, cells that were double positive for CD34 and PDGFR α with characteristic morphology and localization were also recognized as TCs. Double immunopositive cells for c-kit and vimentin as well as for CD34 and vimentin with typical morphology are also recognized as TCs. In all sections, the immunoreactive cells were evaluated with respect to the relative frequency (arbitrarily graded as very few = (+), few = +, moderate density = ++, high density = +++).

Results

Morphological analysis of tissue samples

The cross-sections of rat lungs were grossly characterized by the prevalence of type I and type II pneumocytes as well as macrophages. We have noted neither signs of inflammatory reaction nor the presence of pathological conditions (Fig. 1).

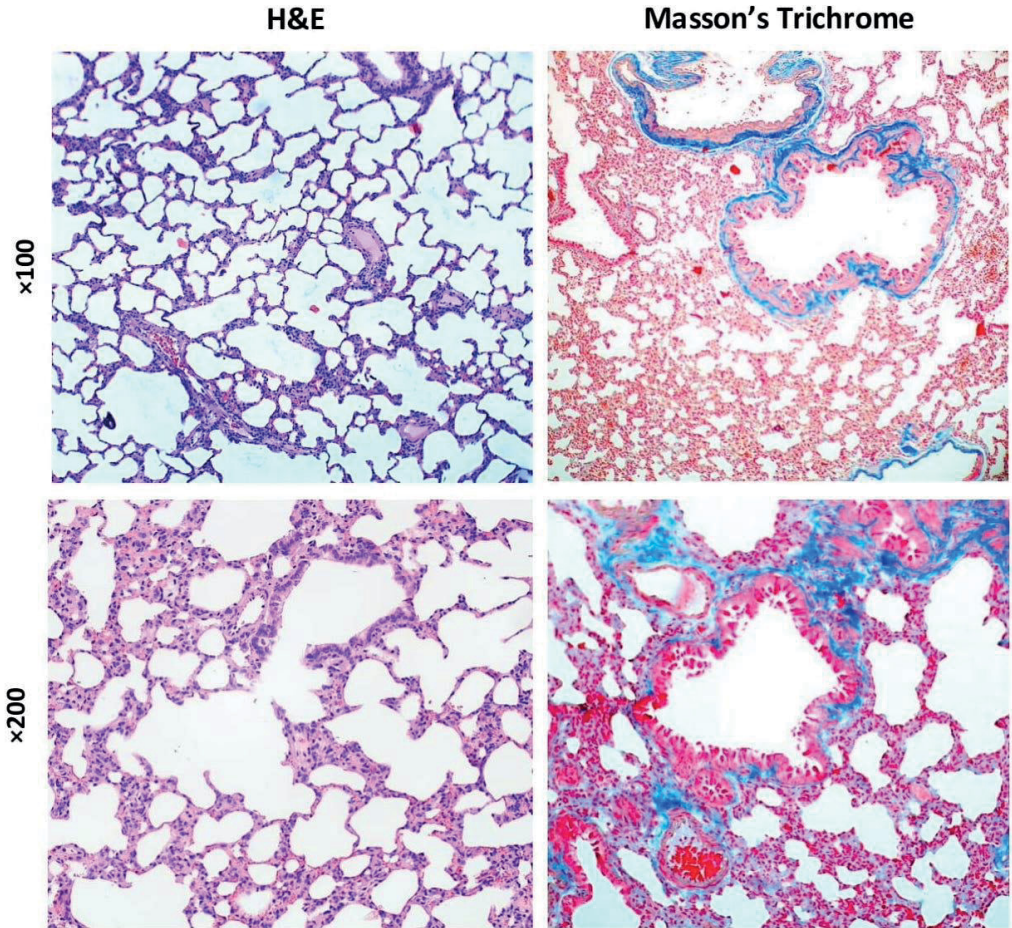


Fig. 1. Hematoxylin–eosin- and Masson’s trichrome-stained cross-sections of rat lungs. After Masson’s trichrome staining, collagen deposits were blue in color whereas muscle fibers were red in color. Total magnification: 100× and 200×.

Immunohistochemistry

In our study, lung telocytes were observed mainly in the area of the interalveolar septa, in the interstitial space between the airway epithelium, and close to blood vessels of medium and large diameter. Their prolongations (telopodes) make lines across the epithelium, sometimes make a bundle, or cross under different angles. Telocyte were present in all rat lungs samples. We used three combinations of markers for the identification of cells (vimentin/*c-kit*; CD34/PDGFR α and vimentin/CD34) (Fig. 2–6). Also, depending on the area, telocytes in the lungs have low to moderate densities (from one to two pluses).

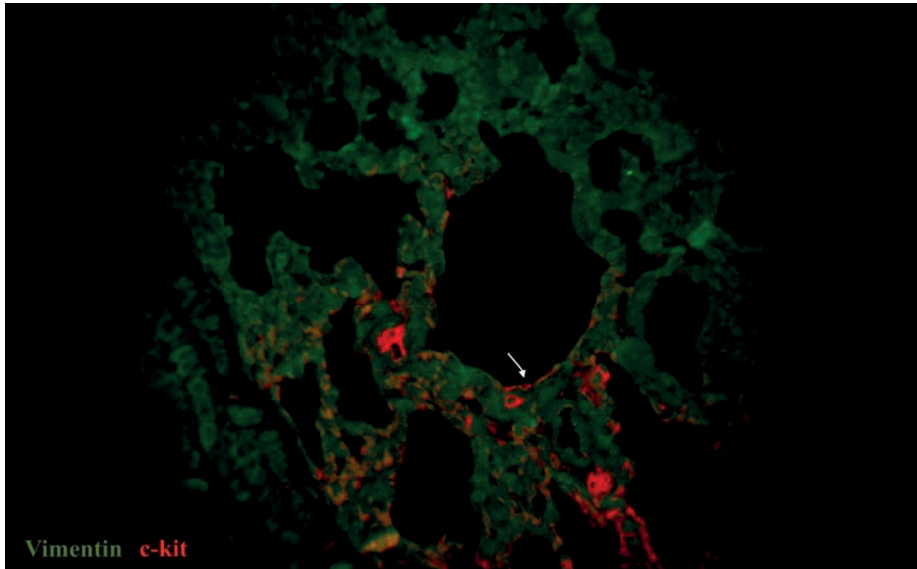


Fig. 2. Lung tissue sample stained for vimentin (green, Alexa Fluor 488) and c-kit (red, Alexa Fluor 594). Lung telocytes, which were double-positive for both markers, had oval-shaped bodies and long cellular lengths and were located close to blood vessels (indicated by arrow). Total magnification: 400 \times .

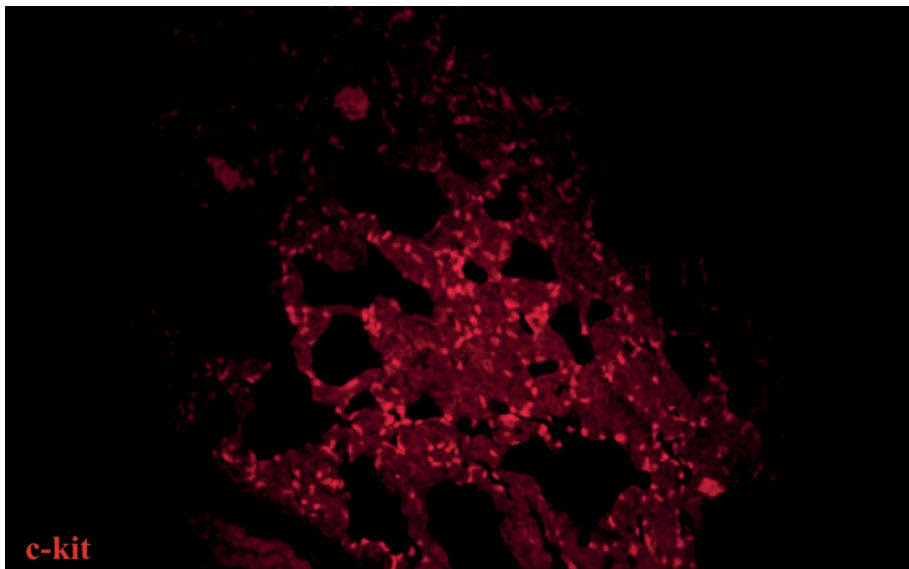


Fig. 3. Lung tissue sample stained for c-kit (red, Alexa Fluor 594). Lung telocytes, which were immunopositive for this markers, were located close to blood vessels and within interstitium. Total magnification: 400 \times .

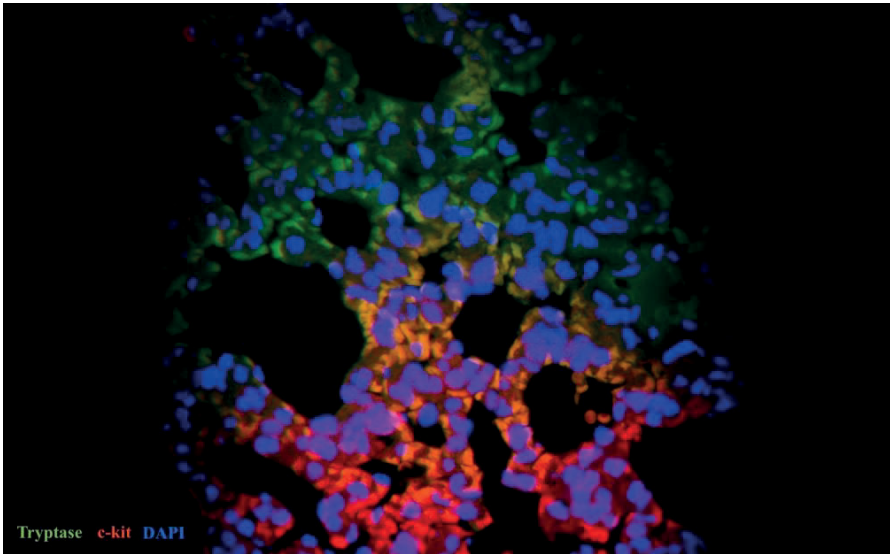


Fig. 4. Lung tissue sample stained for tryptase (green, Alexa Fluor 488) and c-kit (red, Alexa Fluor 594), DAPI nuclear stain. Total magnification: 600 \times .

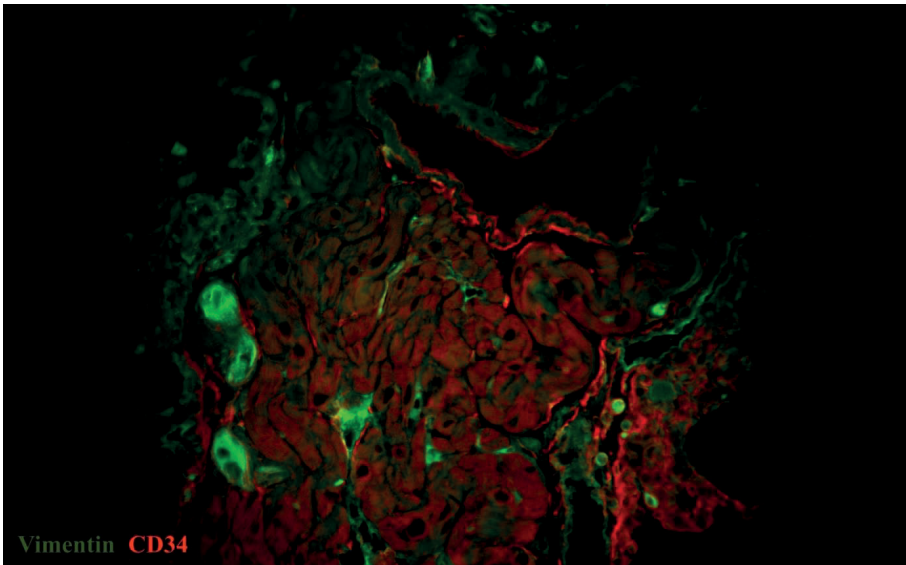


Fig. 5. Tissue sample from rat lung stained for vimentin (green, Alexa Fluor 488) and CD34 (red, Alexa Fluor 594). Telocytes, which were double-positive for both markers, had oval-shaped bodies and long cellular lengths and were located close to blood vessels. Total magnification: 400 \times .

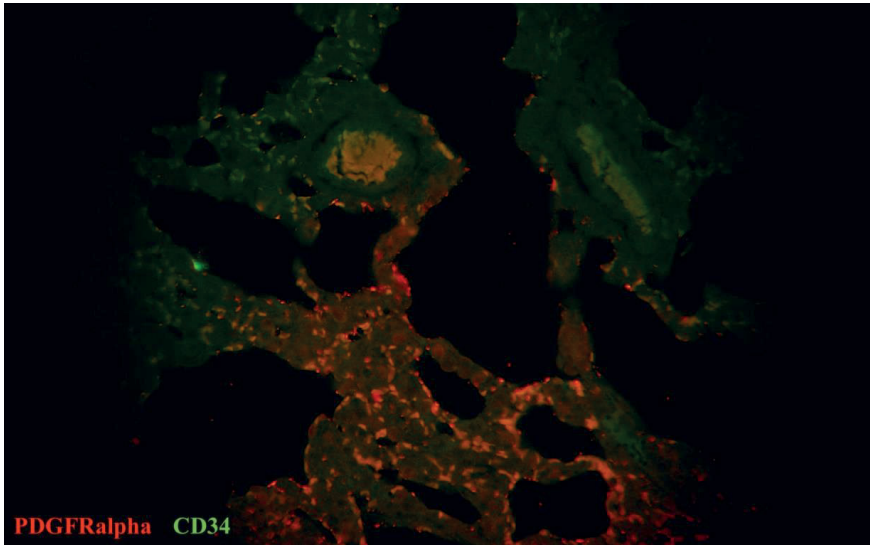


Fig. 6. Tissue sample from rat lung stained for CD34 (green, Alexa Fluor 488) and PDGFR α (red, Alexa Fluor 594). Telocytes were double-positive for both markers. Total magnification: 400 \times .

Discussion

Breathing is a vital function in all organisms using lungs for gaseous exchange. The development of pulmonary fibrosis is a major clinical problem, not only during a pandemic. Generally, fibrosis as a process is a universal mechanism of tissue remodeling occurring in all organ systems, and is always characterized by the production of extracellular matrix (ECM). This is a key factor influencing the development of fibrosis. Telocytes have been discussed in some diseases characterized by fibrotic changes. In myocardial infarction, they inhibit microvascular endothelial cell apoptosis [13] and also have been involved in the regeneration of damaged myocardium [14]. In Crohn's disease, TCs disappeared in tissue samples affected by pathological processes, while having been preserved in the control group [15]. Authors suggested that the loss of these cells correlates with disease and leads to intestinal dysmotility [16]. However, the importance of a decrease in the number of telocytes is still a matter of debate. On one hand, they are characterized by high sensitivity to hypoxia and probably the development of local inflammation leads to telocyte damage through angiogenesis and imbalance in pro- and anti-angiogenetic factors. On the another hand, homo- and heterocellular contacts of telocytes, their secretory function, immunopositivity for growth factors receptors as well as their role in the three-dimensional network of tissue organization allow us to predict that a decline in and disappearance of telocytes reflects disturbances in intracellular signaling and leads to the develop-

ment of fibrosis as a result. For example, we have the same tendency in the development of hydronephrosis [17] and uterine fibroid formation [18, 19]. We underline that due to multiple contacts with smooth muscle cells and nerves, as well as their electrophysiological activity and secretion, TCs could be a part of the motility regulation system in the ureters, the intestine, the heart, myocardium, etc. Its damage or decrease in number activates tissue repairing mechanisms. In tissue samples from patients with psoriatic lesions, TCs have degenerative features (apoptosis, membrane disintegration, cytoplasm fragmentation, and nuclear extrusion) [20].

We also know that lungs have non-respiratory functions: they can convert an inactive chemical precursor into active forms (for instance, angiotensin); have been involved in inactivating vasoactive chemical mediators (bradykinin, serotonin, and norepinephrine); neuroendocrine cells in the mucosa produce catecholamine and polypeptide hormones (calcitonin, serotonin, gastrin-releasing factor — bombesin); and the lung epithelium acts as the first line of defense for inspired air [21–23]. Telocytes are also involved in the secretion of bioactive substances and have a positive effect on growth factor receptors. In addition, they often were located close to blood vessels and nerves, which allows us to predict their role in neuroendocrinology and local homeostasis [24–26]. Stem cells in the lungs are clinically relevant in the context of aging, regeneration, or protection from carcinogenesis [27]. Telocytes make contacts with stem cells in different organs [6, 8]. The common tendency in stem cells and telocytes was the reduction in the number of both types of cells in pathological states. We also cannot omit the importance of TCs in the pathogenesis of diseases, and not only in the lungs. Their multifunctional properties participate extensively in local tissue homeostasis. Damage of such links and interactions leads to inflammation and the development of fibrosis [28, 29].

In conclusion, we want to stress that lung telocytes can be considered an essential population of cells, required for the normal physiology of the respiratory system. They have been identified in rat lungs and can be used as an “instrument” for evaluation in further experiments on pulmonary pathology in animal models. We hypothesize that development of fibrosis might be the most important implication of telocyte deficiency in the lungs.

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Conflict of interest

None declared.

Author contribution

A.G.: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content and final approval of the manuscript. V.A.: study supervision, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content and final approval of the manuscript.

References

1. *Schraufnagel D.E.*: Electron microscopy of the lung. New York: Marcel Dekker, CRC Press; 1990.
2. *Hogan B., Tata P.R.*: Cellular organization and biology of the respiratory system. *Nat Cell Biol.* 2019; <https://doi.org/10.1038/s41556-019-0357-7>.
3. *Evren E., Ringqvist E., Willinger T.*: Origin and ontogeny of lung macrophages: from mice to humans. *Immunology.* 2020; 160 (2): 126–138.
4. *Awad M., Gaber W., Ibrahim D.*: Onset of Appearance and Potential Significance of Telocytes in the Developing Fetal Lung. *Microsc Microanal.* 2019; 25 (5): 1246–1256.
5. *Popescu L.M., Gherghiceanu M., Suciuc L.C., Manole C.G., Hinescu M.E.*: Telocytes and putative stem cells in the lungs: electron microscopy, electron tomography and laser scanning microscopy. *Cell Tissue Res.* 2011; 345 (3): 391–403.
6. *Aleksandrovych V., Pasternak A., Basta P., Sajewicz M., Walocha J.A., Gil K.*: Telocytes: facts, speculations and myths (Review article). *Folia Med Cracov.* 2017; 57 (1): 5–22.
7. *Zheng Y., Li H., Manole C.G., Sun A., Ge J., Wang X.*: Telocytes in trachea and lungs. *J Cell Mol Med.* 2011; 15: 2262–2268.
8. *Aleksandrovych V., Walocha J.A., Gil K.*: Telocytes in female reproductive system (human and animal). *J Cell Mol Med.* 2016; 20 (6): 994–1000.
9. *Díaz-Flores L., Gutiérrez R., Díaz-Flores L.J.R., Gómez M.G., Sáez F.J., Madrid J.F.*: Behaviour of telocytes during physiopathological activation. *Semin Cell Dev Biol.* 2016; 55: 50–61.
10. *Hussein M.M., Mokhtar D.M.*: The roles of telocytes in lung development and angiogenesis: An immunohistochemical, ultrastructural, scanning electron microscopy and morphometrical study. *Dev Biol.* 2018; 443 (2): 137–152.
11. *Popescu L.M., Fausone-Pellegrini M.S.*: TELOCYTES — a case of serendipity: the winding way from interstitial cells of Cajal (ICC), via interstitial Cajal-like cells (ICLC) to TELOCYTES. *J Cell Mol Med.* 2010; 14: 729–740.
12. *Ibba-Manneschi L., Rosa I., Manetti M.*: Telocyte implications in human pathology: An overview. *Semin Cell Dev Biol.* 2016; 55: 62–69.

13. Liao Z., Chen Y., Duan C., Zhu K., Huang R., Zhao H., et al.: Cardiac telocytes inhibit cardiac microvascular endothelial cell apoptosis through exosomal miRNA-21-5p-targeted cdip1 silencing to improve angiogenesis following myocardial infarction. *Theranostics*. 2021; 11 (1): 268–291.
14. Zhaofu L., Dongqing C.: Cardiac Telocytes in Regeneration of Myocardium After Myocardial Infarction. *Adv Exp Med Biol*. 2016; 913: 229–239.
15. Milia A.F., Ruffo M., Manetti M., Rosa I., Conte D., Fazi M., et al.: Telocytes in Crohn's disease. *Cell Mol Med*. 2013; 17 (12): 1525–1536.
16. Ibba-Manneschi L., Rosa I., Manetti M.: Telocytes in Chronic Inflammatory and Fibrotic Diseases. *Adv Exp Med Biol*. 2016; 913: 51–76.
17. Wolnicki M., Aleksandrovych V., Gil A., Pasternak A., Gil K.: Relation between ureteral telocytes and the hydronephrosis development in children. *Folia Med Cracov*. 2019; 59 (3): 31–44.
18. Aleksandrovych V., Pasternak A., Gil K.: Telocytes in the architecture of uterine fibroids. *Folia Med Cracov*. 2019; 59 (4): 33–44.
19. Aleksandrovych V., Białas M., Pasternak A., Bereza T., Sajewicz M., Walocha J., et al.: Identification of uterine telocytes and their architecture in leiomyoma. *Folia Med Cracov*. 2018; 58 (3): 89–102.
20. Manole C.G., Gherghiceanu M., Simionescu O.J.: Telocyte dynamics in psoriasis. *Cell Mol Med*. 2015; 19 (7): 1504–1519.
21. Jin L., Wang Z., Qi X.: Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia: Case series and a review of the literature. *Medicine (Baltimore)*. 2018; 97 (52): e13806.
22. Leuenberger P.: [Clinical importance of non-respiratory functions of the lung]. *Schweiz Med Wochenschr*. 1983; 113 (29): 1006–1010.
23. Wittmann J.: [Respiratory and non-respiratory functions of the lung]. *Tierarztl Prax Suppl*. 1987; 2: 33–36.
24. Aleksandrovych V., Kurnik-Lucka M., Bereza T., Białas M., Pasternak A., Cretoiu D., Walocha J.A., Gil K.: The Autonomic Innervation and Uterine Telocyte Interplay in Leiomyoma Formation. *Cell Transplant*. 2019; 28: 619–629.
25. Song D., Cretoiu D., Cretoiu S.M., Wang X.: Telocytes and lung disease. *Histol Histopathol*. 2016; 31 (12): 1303–1314.
26. Shi L., Dong N., Chen C., Wang X.: Potential roles of telocytes in lung diseases. *Semin Cell Dev Biol*. 2016; 55: 31–39.
27. Ciechanowicz A.: Stem Cells in Lungs. *Adv Exp Med Biol*. 2019; 1201: 261–274.
28. Rokicki W., Rokicki M., Wojtacha J., Dźeljić A.: The role and importance of club cells (Clara cells) in the pathogenesis of some respiratory diseases. *Kardiochir Torakochirurgia Pol*. 2016; 13 (1): 26–30.
29. Ibba-Manneschi L., Rosa I., Manetti M.: Telocytes in Chronic Inflammatory and Fibrotic Diseases. *Adv Exp Med Biol*. 2016; 913: 51–76.