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DIABETES MELLITUS AND PULMONARY COMPLICATIONS: UNRAVELING THE INFLUENCE ON TYPE II ALVEOLOCYTES

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Introduction. *Diabetes mellitus (DM), characterized by hyperglycemia, has been extensively studied for its systemic effects. Recent attention has focused on its impact on the respiratory system, specifically on type II alveolocytes (A-II) in the alveoli. Diabetes-induced hyperglycemia has been linked to altered surfactant composition, oxidative stress, inflammation, and impaired tissue repair in A-II, potentially compromising pulmonary homeostasis.*

Aim. *To deepen our understanding of diabetes mellitus effects on the respiratory system by investigating pathological changes in A-II in experimental diabetes mellitus.*

Methods. *We performed an experimental study, involving 88 male Wistar rats distributed across intact (Group 1), control (Group 2), and experimental (Group 3) categories. Experimental diabetes was induced by administering streptozotocin (Sigma, USA) at 60 mg/kg of body weight, diluted in 0.1 M citrate buffer with a pH of 4.5. Tissue samples were gathered at 14, 28, 42, and 70-day intervals. Lung tissue fragments underwent scrutiny through electron microscopy analysis.*

Results. *Initially (14 days), electron microscopy showed baseline features in A-II cores with well-defined mitochondria. By day 28, changes indicated the onset of structural modifications: increased nuclear volume, lightened matrices, and expanded perinuclear spaces. Day 42 revealed pronounced hyperhydration in A-II cores, reflecting responses to prolonged hyperglycemia, including deformed Golgi apparatus and endoplasmic reticulum. The later stage (70 days) showed diverse cellular responses: enlarged nuclei, significant mitochondrial changes, distorted Golgi apparatus, and ongoing cellular stress.*

Conclusion. *Induction of diabetes mellitus with streptozotocin leads to pronounced alterations in A-II ultrastructure, highlighting disruptions in the pulmonary surfactant system and emphasizing the systemic impact of diabetes on lung function. Understanding these changes provides insights into the progression of respiratory complications in diabetes and suggests potential therapeutic targets for mitigating associated pulmonary issues. Further exploration of molecular mechanisms underlying these structural changes is warranted for the development of targeted strategies.*

Key words: *diabetes mellitus, lungs, experiment, type II alveolocytes, pathophysiology.*

Юлія Федорченко, Назар Саган, Ольга Антимис. Цукровий діабет і легеневі ускладнення: вивчення впливу на альвеолоцити II типу

Вступ. *Цукровий діабет (ЦД), що характеризується гіперглікемією, був детально вивчений у рамках його системних ефектів на організм. Проте останнім часом усе більше уваги було зосереджено на його впливі на дихальну систему, зокрема на альвеолоцити II типу (А-II) в альвеолах. Індукована ЦД гіперглікемія пов'язана зі зміненим складом поверхнево-активної речовини, окислювальним стресом, запаленням і порушенням відновлення тканин у А-II, що потенційно порушує легеневий гомеостаз.*

Мета. *Поглибити розуміння впливу цукрового діабету на дихальну систему шляхом дослідження патологічних змін у А-II при експериментальному цукровому діабеті.*

Методи. *Ми провели експериментальне дослідження за участю 88 самців щурів Wistar, розподілених за такими категоріями: інтактна (група 1), контрольна (група 2) та експериментальна (група 3). Експериментальний діабет індукували введенням стрептозотоцину (Sigma, США) у дозі 60 мг/кг маси тіла, розведеного в 0,1 М цитратному буфері з pH 4,5. Зразки тканин збирали через 14-, 28-, 42- і 70-денні інтервали. Фрагменти легеневої тканини досліджували за допомогою електронної мікроскопії.*

Результати. *Спочатку (14 днів) електронна мікроскопія показала звичні ознаки в ядрах А-ІІ та чітко виражені мітохондрії. На 28-й день зміни вказували на початок структурних змін: збільшення ядерного об'єму, освітлення матриксу та розширення перинуклеарних просторів. На 42-й день виявлено виражену гіпергідратацію в ядрах А-ІІ, що відображає реакцію на тривалу гіперглікемію, включаючи деформований апарат Гольджі та ендоплазматичний ретикулум. Пізніша стадія (70 днів) продемонструвала різноманітні клітинні реакції: збільшені ядра, значні мітохондріальні зміни, спотворений апарат Гольджі та постійний клітинний стрес.*

Висновок. *Індукція стрептозотоцинового цукрового діабету призводить до виражених змін ультраструктури А-ІІ, підкреслюючи порушення у системі легеневого сурфактанту та системний вплив діабету на функцію легень. Розуміння цих змін дає змогу аналізувати прогресування респіраторних ускладнень при діабеті та пропонує потенційні терапевтичні цілі для пом'якшення пов'язаних із ними легеневих проблем. Подальше дослідження молекулярних механізмів, що лежать в основі цих структурних змін, є виправданим для розроблення цільових стратегій.*

Ключові слова: *цукровий діабет, легені, експеримент, альвеолоцити ІІ типу, патофізіологія.*

Introduction

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, has been extensively studied for its systemic effects on various organ systems [1]. In recent years, attention has turned towards its impact on the respiratory system, particularly the alveoli, where type II alveolocytes (A-II) play a crucial role in maintaining pulmonary homeostasis [2].

Diabetes-induced hyperglycemia has been associated with alterations in surfactant composition and production by A-II. Changes in surfactant properties may compromise the mechanical stability of the alveoli, leading to increased susceptibility to atelectasis and impaired gas exchange [3].

Chronic hyperglycemia in diabetes contributes to oxidative stress and inflammation. A-II are not immune to these effects, as elevated levels of reactive oxygen species (ROS) may disrupt cellular function and induce inflammatory responses. This oxidative stress may compromise the structural integrity of A-II, impairing their ability to produce surfactant and maintain alveolar homeostasis[4].

Diabetes has been linked to impaired tissue repair and regeneration. A-II play a crucial role in the repair of damaged alveoli [5]. The compromised regenerative capacity of these cells in individuals with diabetes may contribute to prolonged lung injury and impaired recovery from respiratory insults [6].

Thus, we can conclude that DM exerts multifaceted effects on A-II, potentially compromising their essential functions in maintaining pulmonary homeostasis. The altered surfactant production, oxidative stress, impaired repair mechanisms, and increased susceptibility to infections collectively contribute to respiratory complications observed in individuals with diabetes.

Hence, our **objective** was to investigate the pathological changes in A-II in experimental diabetes mellitus.

Methods

In this experimental study, 88 male Wistar rats (weighing 170-210 g) were utilized. The rats were categorized into three groups: Group 1 (n=10) comprised intact rats; Group 2 (n=40) served as the control group, and Group 3 (n=38) constituted the experimental group. The experimental diabetes was induced via intraperitoneal administration of streptozotocin (Sigma, USA) diluted in 0.1 M citrate buffer with a pH of 4.5 at a dosage of 60 mg/kg of body weight. The control group was administered an equal volume of 0.1 M citrate buffer solution, maintaining a pH of 4.5, through intraperitoneal injection. All procedures were conducted under sodium thiopental anesthesia at a dose of 60 mg/kg of body weight. Tissue samples were gathered at time intervals of 14, 28, 42, and 70 days following the injection of streptozotocin.

For electron microscopy analysis, lung tissue fragments were fixed in a 2.5% glutaraldehyde solution, followed by immersion in a 1% osmium tetroxide solution for fixation. Following dehydration, the specimens were embedded in Epon Araldite. Sections, obtained using a "Tesla VS-490" ultramicrotome, were examined using a "PEM-125K" electron microscope.

All procedures and the termination of animals were conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Additionally, this study received approval from the Bioethics Commission of Ivano-Frankivsk National Medical University, as documented in protocol No. 106/19, dated February 7, 2019.

Results and Discussion

Based on the histological analysis of lung tissue biopsies, the tissue samples from the control and intact groups of laboratory animals were found to be normal. In contrast, the experimental group exhibited structural changes in A-II.

At the 14-day mark from the initiation of the experiment, nuclei of A-II displayed a matrix characterized by moderate electron density. The nucleolema exhibited minor depressions and protrusions, while

the perinuclear space showed no significant expansion. Within the cytoplasm, mitochondria with a relatively electron-dense matrix were observable. The Golgi apparatus (GA) was represented by flattened tanks and small bubbles. The granulated endoplasmic reticulum (GER) comprised tubules with well-defined ribosomes. The lamellar bodies (LB) were evident, exhibiting various degrees of maturity, size, and shape. Notably, the basal membrane exhibited no discernible structural changes.

On the 28th day of the study, certain A-II nuclei exhibited an increase in nuclear volume accompanied by a lightened matrix and the marginal placement of chromatin granules. The nuclear envelope displayed minor depressions and protrusions, and the perinuclear space showed a slight expansion. Within the cytoplasm, individual mitochondria of varying sizes and shapes were observed, featuring a lightened matrix with single disoriented cristae (Fig. 1). Simultaneously, other mitochondria displayed a matrix of moderate electron-optical density. The Golgi apparatus (AG) manifested as moderately expanded cisterns and small bubbles. Hydroelectric tubules exhibited dilation with the fibrous content inside.

The number of ribosomes on the outer surface of membranes was slightly reduced. In the cytoplasm of separate A-II, LB were evident, characterized by the presence of uneven light spaces between bimembrane osmiophilic plates. Additionally, multivesicular bodies were noted in the cytoplasm of certain cells. On the apical surface of A-II, high mosaic microvilli were observed.

After 42 days of the experiment, alterations of A-II are characterized by pronounced phenomena of hyperhydration. The nuclei of such cells have a rounded shape. The nucleoplasm is filled with a fine-grained matrix with an accumulation of chromatin granules on the periphery. The perinuclear space is significantly expanded in some areas. Mitochondria are enlarged with shortened, disorganized cristae. The constituent components of GA and GER are expanded and deformed. Fragmentation of GER membranes is also observed. The number of ribosomes on GER membranes is reduced. As a result of edema, a violation of the ultrastructural organization of the LB is also determined. At the same time, a significant part of LB is partially filled with phospholipid material with disoriented and fragmented membranes. Sometimes in individual A-II giant LBs are determined, which fill a significant part of the cytoplasm. Along with this, homogenous lipid-like inclusions of moderate electron-optical density are found in the cytoplasm of some A-II cells, which indicates the transition of such cells to the synthesis of neutral lipids. A decrease in the number of microvilli is observed on the apical surface of A-II. The basement

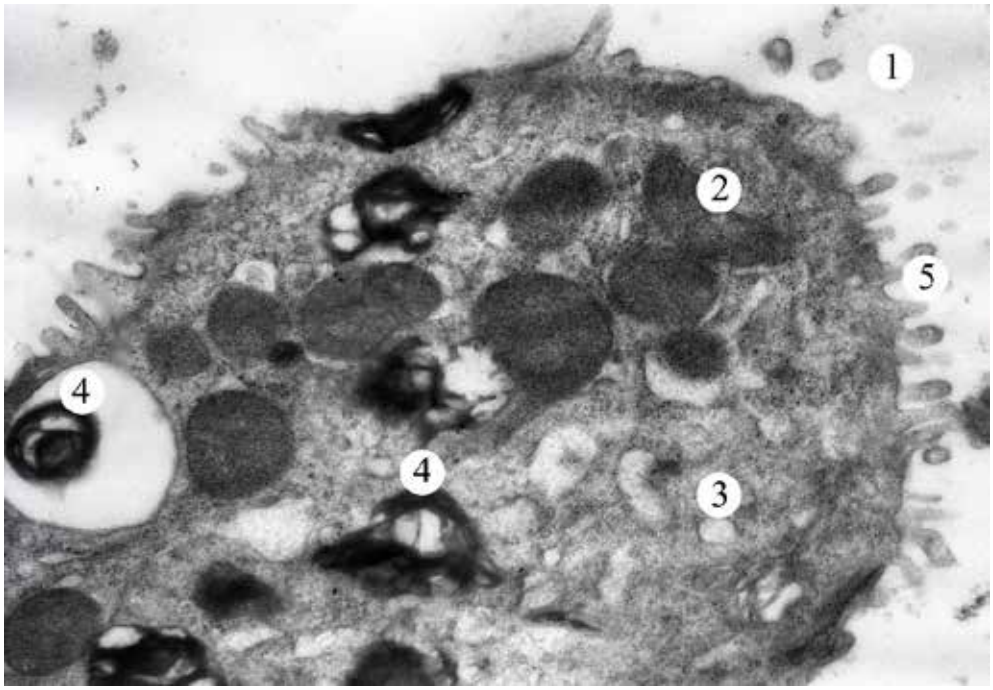

Figure 1. Submicroscopic structure of type II alveolocyte 28 days after the start of the experiment. Electron micrograph. Magnification: 12,000

Captions: 1 – alveolar lumen; 2 – mitochondria; 3 – granular endoplasmic reticulum; 4 – lamellar body; 5 – microvilli.

membrane is swollen throughout, of uneven thickness. The growing edema of A-II is accompanied by the destruction of the cell and the release of cytoplasmic structures into the lumen of the alveolus.

In the advanced stages of the experiment (at 70 days), notable changes are observed in A-II. The cell nuclei exhibit an enlargement in size with smooth contours (Fig. 2). Nucleolema displays minor depressions and protrusions, and the perinuclear space is expanded. A substantial proportion of mitochondria experiences an increase in volume, with crystals losing their parallelism and a reduced overall number. Some mitochondria exhibit complete lysis of cristae. Within the perikaryon region, the GA is visualized, represented by expanded cisterns and vacuoles of various sizes. The GER channels are, in most cases, expanded. Concurrently, there is evidence of fragmentation of GER membranes with a reduced number of ribosomes. The LBs are present but in low numbers, and they are partially filled with phospholipid material. Vacuoles with remnants of membranes are occasionally observed at the site of LB. Moreover, a decrease in the number of microvilli is noted on the plasmolemma of the apical area.

Captions: 1 – alveolar lumen; 2 – nucleus of type II alveolocyte; 3 – mitochondria; 4 – granular endoplasmic reticulum; 5 – lamellar body; 6 – microvilli.

While the lungs are not usually considered primary targets in diabetes mellitus (DM), research indicates that this condition can adversely affect respiratory functions. A study by Chance et al. highlighted a concurrent decline in lung carbon monoxide diffusion capacity and pulmonary blood flow in DM patients, evident both at rest and during near-maximal exercise [7]. This reduction might be primarily due to changes in two aspects: the efficiency of the alveolar-capillary membrane and the blood volume in pulmonary capillaries. Structural examinations have shown a thickening in the basal plate of pulmonary capillaries and the alveolar epithelium, leading to hindered gas diffusion across the alveolar-capillary membrane [8]. The thickening of the alveolar-capillary barrier and expansion of interstitial tissue results in diminished alveolar space and a constricted pulmonary capillary network. Such alterations can redistribute pulmonary blood flow, potentially causing issues with ventilation/perfusion balance and gas exchange [9].

Our study particularly examined the structural integrity of type II alveolocytes within the aero-hematic barrier. This was motivated by existing literature that identifies the secretions of alveolocytes, especially surfactant proteins, as key indicators of lung dysfunction [10, 11].

Our experimental approach provides a detailed characterization of the ultrastructural changes in A-II during experimental diabetes mellitus. The observed structural alterations underscore the intricate and dynamic responses of these cells to prolonged hyperglycemia. This study contributes to the broader understanding of diabetes-induced pulmonary complications and lays the foundation for future investigations aiming at therapeutic interven-

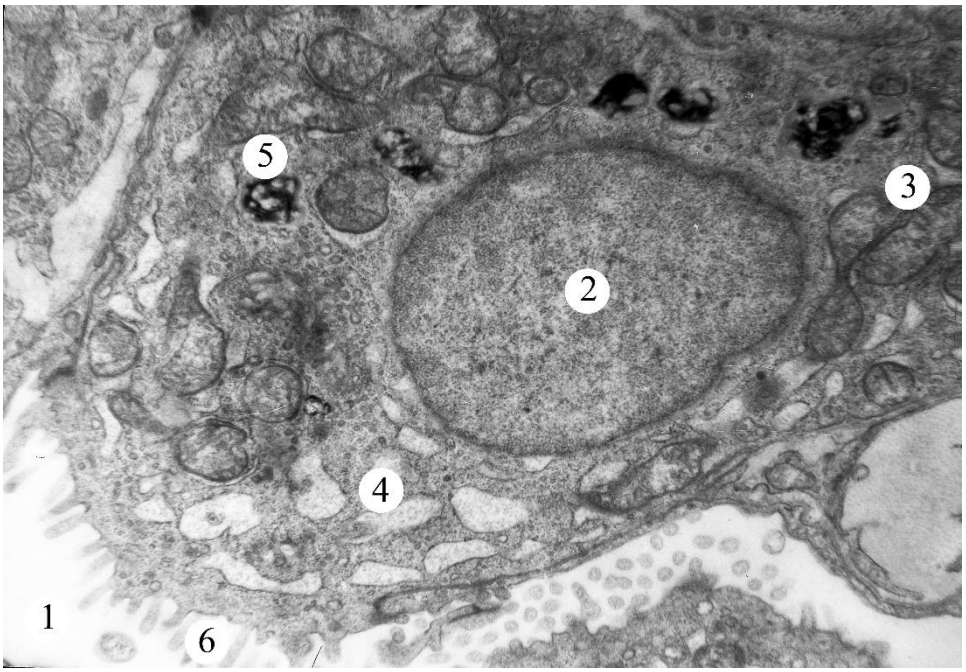

Figure 2. Ultrastructural organization of type II alveolocytes 70 days after the start of the experiment. Electron micrograph. Magnification: 8,000

tions to preserve respiratory function in individuals with diabetes.

At the early stages (14 days), electron microscopy revealed moderate electron density in A-II nuclei, indicating a baseline state. Notable features included well-defined mitochondria and a preserved basal membrane. By day 28, changes emerged, with increased nuclear volume, lightened matrices, and expanded perinuclear spaces. Mitochondria displayed diverse morphologies, and Golgi apparatus elements were observable. These early changes signify the onset of structural modifications in response to diabetes induction.

By day 42, pronounced hyperhydration phenomena were evident in A-II nuclei. Nuclear rounding, expanded perinuclear spaces, and altered mitochondrial structures highlighted cellular responses to prolonged hyperglycemia. Notably, Golgi apparatus and granulated endoplasmic reticulum components were deformed, and lipid-like inclusions suggested a shift towards neutral lipid synthesis. The deteriorating microvilli and swollen basement membrane reflected the growing cellular edema, ultimately leading to cellular destruction and cytoplasmic release into the alveolar lumen.

At the later stages (70 days), A-II nuclei exhibited substantial polymorphism, indicating diverse cellular responses to prolonged diabetes. Nuclei showed enlargement, mitochondria displayed significant volumetric changes, and the Golgi apparatus appeared distorted. Notably, the fragmented membranes of heterogeneous endoplasmic reticulum and partially filled lamellar bodies suggested ongoing cellular stress. The decreased microvilli and expanded perinuclear space indicated progressive cellular alterations.

Altered surfactant production, oxidative stress, impaired repair mechanisms, and increased susceptibility to infections collectively might contribute to the observed changes in A-II. The interplay between hyperglycemia and cellular structures, such as the GA, GER and LB of A-II, emphasizes the systemic impact of diabetes on pulmonary homeostasis.

Understanding the ultrastructural changes in A-II provides insights into the progression of respiratory complications in diabetes. The observed lipid-like inclusions and altered lamellar bodies suggest potential targets for therapeutic interventions. Further studies are warranted to explore the molecular mechanisms underlying these structural changes and to develop targeted strategies for mitigating diabetes-associated pulmonary complications.

Conclusion

The induction of diabetes mellitus using streptozotocin is associated with pronounced alterations in the ultrastructure of type II alveolocytes. These changes underscore disruptions in the pulmonary surfactant system, emphasizing the systemic impact of diabetes on lung function.

BIBLIOGRAPHY

1. Diabetes mellitus and its metabolic complications: the role of adipose tissues / Dilworth, L., Facey, A., Omoruyi, F. International journal of molecular sciences 2021. № 22(14): 7644. http://dx.doi.org/10.3390/ijms22147644

2. Potential Impact of Diabetes and Obesity on Alveolar Type 2 (AT2)-Lipofibroblast (LIF) Interactions After COVID-19 Infection / Nouri-Keshtkar, M., Taghizadeh, S., Farhadi, A., Ezaddoustdar, A., Vesali, S., Hosseini, R., Tahamtani, Y. Frontiers in Cell and Developmental Biology. 2021. № 9: 676150. http://dx.doi.org/10.3389/fcell.2021.676150

3. Alveolar tissue fiber and surfactant effects on lung mechanics – model development and validation on ARDS and IPF patients / Yuan, J., Chiofolo, C.M., Czerwin, B.J., Karamolegkos, N., Chbat, N.W. IEEE Open Journal of Engineering in Medicine and Biology. 2021. № 2. P. 44–54. http://dx.doi.org/10.1109/OJEMB.2021.3053841.

4. Oxidative stress mechanisms in the pathogenesis of environmental lung diseases / Thimmulappa, R.K., Chattopadhyay, I., Rajasekaran, S. Oxidative Stress in Lung Diseases. 2020. № 2. P. 103–137. http://dx.doi.org/10.1007/978-981-32-9366-3_5.

5. The history and mystery of alveolar epithelial type II cells: focus on their physiologic and pathologic role in lung / Ruaro, B., Salton, F., Braga, L., Wade, B., Confalonieri, P., Volpe, M.C., Confalonieri, M. International journal of molecular sciences. 2021. № 22(5): 2566. http://dx.doi.org/10.3390/ijms22052566

6. The aging lung: Physiology, disease, and immunity / Schneider, J.L., Rowe, J.H., Garcia-de-Alba, C., Kim, C.F., Sharpe, A.H., Haigis, M.C. Cell. 2021. № 184(8). P. 1990–2019. http://dx.doi.org/10.1016/j.cell.2021.03.005.

7. Diminished alveolar microvascular reserves in type 2 diabetes reflect systemic microangiopathy / Chance W.W., Rhee C., Yilmaz C., Dane D.M., Pruneda M.L., Raskin P., et al. Diabetes Care. 2008. № 31(8). P. 1596–601. http://dx.doi.org/10.2337/dc07-2323

8. State of the cellular components of the surfactant system in case of experimental diabetes mellitus / Fedorchenko Yu, Zaiats L. European Respiratory Journal. 2020. № 56:Suppl. 64. P. 2571. http://dx.doi.org/10.1183/13993003.congress-2020.2571

9. The Diabetic Lung – A New Target Organ? / Pitocco D., Fuso L., Conte E.G., Zaccardi F., Condoluci C., Scavone G., et al. The Review of Diabetic Studies. 2012. № 9. P. 23–35. http://dx.doi.org/10.1900/RDS.2012.9.23

10. Serum surfactant protein A as a surrogate biomarker of a negative heart sign among patients with interstitial lung disease / Sasaki H., Hara Y., Taguri M., Fujikura Y., Murohashi K., Yagyu H., et al. Nagoya Journal of Medical Science. 2020. № 82(3). P. 499–508. http://dx.doi.org/10.18999/nagjms.82.3.499

11. Pulmonary surfactant protein SP-B promotes exocytosis of lamellar bodies in alveolar type II cells / Martinez-Calle M., Olmeda B., Dietl P., Frick M., Perez-Gil J. The FASEB Journal. 2018. № 32(8). P. 4600–4611. http://dx.doi.org/10.1096/fj.201701462RR

REFERENCES

1. Dilworth, L., Facey, A., Omoruyi, F. (2021). Diabetes mellitus and its metabolic complications: the role of adipose tissues. International journal of molecular sciences, 22(14), 7644. http://dx.doi.org/10.3390/ijms22147644

2. Nouri-Keshtkar, M., Taghizadeh, S., Farhadi, A., Ezaddoustdar, A., Vesali, S., Hosseini, R., Tahamtani, Y. (2021). Potential Impact of Diabetes and Obesity on Alveolar Type 2 (AT2)-Lipofibroblast (LIF) Interactions After COVID-19 Infection. Frontiers in Cell and Developmental Biology, 9, 676150. http://dx.doi.org/10.3389/fcell.2021.676150

3. Yuan, J., Chiofolo, C.M., Czerwin, B.J., Karamolegkos, N., Chbat, N.W. (2021). Alveolar tissue fiber and surfactant effects on lung mechanics–model development and validation on ARDS and IPF patients. IEEE Open Journal of Engineering in Medicine and Biology, 2, 44–54. http://dx.doi.org/10.1109/OJEMB.2021.3053841.

4. Thimmulappa, R.K., Chattopadhyay, I., Rajasekaran, S. (2020). Oxidative stress mechanisms in the pathogenesis of environmental lung diseases. Oxidative Stress in Lung Diseases: Volume 2, 103–137. http://dx.doi.org/10.1007/978-981-32-9366-3_5.

5. Ruaro, B., Salton, F., Braga, L., Wade, B., Confalonieri, P., Volpe, M.C., Confalonieri, M. (2021). The history and mystery of alveolar epithelial type II cells: focus on their physiologic and pathologic role in lung. International journal of molecular sciences, 22(5), 2566. http://dx.doi.org/10.3390/ijms22052566

6. Schneider, J.L., Rowe, J.H., Garcia-de-Alba, C., Kim, C.F., Sharpe, A.H., Haigis, M.C. (2021). The aging lung: Physiology, disease, and immunity. Cell, 184(8), 1990–2019. http://dx.doi.org/10.1016/j.cell.2021.03.005.

7. Chance W.W., Rhee C., Yilmaz C., Dane D.M., Pruneda M.L., Raskin P., et al. (2008). Diminished alveolar microvascular reserves in type 2 diabetes reflect systemic microangiopathy. Diabetes Care, 31(8), 1596–601.
http://dx.doi.org/10.2337/dc07-2323

8. Fedorchenko Yu., Zaiats L. (2020). State of the cellular components of the surfactant system in case of experimental diabetes mellitus. Virtual; European Respiratory Journal, 56:Suppl.64, 2571.
http://dx.doi.org/10.1183/13993003.congress-2020.2571

9. Pitocco D., Fuso L., Conte E.G., Zaccardi F., Condoluci C., Scavone G., et al. (2012). The Diabetic Lung – A New Target Organ? Rev Diabet Stud, 9, 23–35. http://dx.doi.org/10.1900/RDS.2012.9.23

10. Sasaki H., Hara Y., Taguri M., Fujikura Y., Murohashi K., Yagyu H., et al. (2020). Serum surfactant protein A as a surrogate biomarker of a negative heart sign among patients with interstitial lung disease. Nagoya J Med Sci, 82(3), 499–508. http://dx.doi.org/10.18999/nagjms.82.3.499

11. Martinez-Calle M., Olmeda B., Dietl P., Frick M., Perez-Gil J. (2018). Pulmonary surfactant protein SP-B promotes exocytosis of lamellar bodies in alveolar type II cells. FASEB J, 32(8), 4600–4611. http://dx.doi.org/10.1096/fj.201701462RR