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UDC: 616.61-006.31:591.471.11:616-092

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THE VALUE OF PRIMARY CILIA IN THE PATHOGENESIS OF POLYCYSTIC KIDNEY DISEASE

Summary. *Polycystic kidney disease is the most common genetic disease. There are two main forms of this pathology: autosomal dominant and autosomal recessive. Polycystic kidney disease leads to the progress of terminal renal failure in 10-14% of nephrology patients.*

Key words: *polycystic kidney disease, primary cilia, cystogenesis, polycystins.*

For the first time, the structure and functions of cilia began to be studied at the end of the 17th century. In 1675, Anthony van Leeuwenhoek described mobile cilia that was structurally and functionally considered to be similar to the flagella of eukaryotic cells. In 1876 and 1898 (Langerhans, 1876; Zimmerman, 1898), another class of cilia was described. There were nonmotile (monocilia) cilia, which in 1968 (Sorokin, 1968) were renamed into primary cilia. Primary cilia have been studied for many years, however, despite the anatomical presence of them in eukaryotic cells, until recently, little was known about their specific function. During the last decade, particular attention has been paid to the study of their structure and functions, especially after linking the emergence of various forms of polycystic kidney disease (PKD) and the mutation of proteins that are part of primary cilia [2].

PKD is a group of genetic disorders characterized by the emergence and increase in the number of cysts in the kidneys. The most common type of PKD is the Autosomal Dominant Polycystic Kidney Disease (ADPKD), affecting about 12 million people worldwide. Mutations in PKD1 and PKD2 genes, that encode the polycystin-1 and polycystin-2, respectively, were identified as the cause of ADPKD. Autosomal-Recessive Polycystic Kidney Disease (ARPKD) is a severe form of PKD, typical for childhood, due to the mutation of the PKHD-1, which encodes the fibrocystin protein [5]. Almost all forms of PKD2 are connected with a disruption of the structure and functions of the primary kidney cilia, however, despite numerous studies, it is not yet fully understood how abnormal ciliogenesis contributes to the development of this disease [3].

The *purpose* of the study is to summarize the latest achievements in the study of the structure and functions of primary cilia and to investigate the interaction between the occurrence of PKD and the mutation of primary cilia proteins.

More and more works were published in recent decades, where it was noted, that the violation of the ciliary structure can be the cause of the development of many diseases, in which the emphasis is placed on the state of primary cilia. They are very common in the human body: in olfactory cells, rods and cones, cells of the renal tubular epithelium, mesenchymal cells, neurons. The process of formation of cilia (ciliogenesis) is controlled by the genes, therefore, mutations in them lead to structural anomalies of cilia and,

as a consequence, to the development of ciliopathies. Ciliopathy is a group of diseases characterized by a violation of the normal work of cilia on the surface of a number of cells, which provides reception of signals from the extracellular environment [1]. The most studied ciliopathy genes are the genes that control the process of intraflagellary transport and the genes encoding the functional proteins of primary cilia. For a better understanding of the peculiarities of the pathogenesis of PKD, it should be noted that in addition to the components of the cytoskeleton, the primary cilia include polycystins, fibrocystin, somatostatin receptors, serotonin, angiotensin, platelet factor- α , vanilloid-4 [7].

Mutations of genes attach importance to cystic kidney transformation. ADPKD is due to mutations in the genes PKD1 (chromosome 16p13.3) and PKD2 (chromosome 4q21). These genes encode PC-1 and PC-2 proteins. PKD1 mutations are found in 85-90% of ADPKD cases, and PKD2 mutations are found in 10-15% of cases. ARPKD is due to mutation of the gene of PKHD1 (chromosome 6p21) and occurs in 25% of newborns [14].

PC-1 and PC-2 are integral membrane proteins of primary cilia that play an important role in intercellular and cellular membrane interactions. PC-1 acts as a mechanoreceptor and is involved with intercellular contacts. PC-2 is a non-selective cationic channel for the transportation of Ca^{2+} ions [2]. Both proteins form a functional complex that regulates cell proliferation, adhesion, morphogenesis, and transepithelial fluid secretion [11, 12].

In the kidneys, the primary cilia return to the lumen of the tubules and are present in most cells of the nephron (on each cell on a single line, with the exception of intermediate cells of the tubes). They perform the functions of mechanoreceptors and react to the flow of fluid [13, 14]. Polycystins as part of the primary kidney cilia provide four physiological membrane effects: activation of polycystin-1 polycystin-2 and release of Ca^{2+} from the endoplasmic reticulum into the cytoplasm; receipt of Ca^{2+} ions inside the cell; the effect on the G-protein with the activation of adenylate cyclase, MAP kinase, which affects on the fluid secretion, cell proliferation and differentiation, as well as inhibition of the cell cycle by activating JAK-STAT [6,7]. The mutation of the PKD genes leads to the disturbance of the mechanosensory function of the cilia. This in turn leads to a decrease in the intracellular level of Ca^{2+} , the activation of

adenylate cyclase, and an increase in the level of cAMP. The last activates proliferative processes of the epithelium of the renal tubules, which causes cystogenesis [4, 5].

Another possibility of the influence of primary cilia and polycystins on the formation of cysts is the ability of the PC-1 to regulate the activity of mTOR (target of rapamycin), a protein involved in translational, cellular growth and proliferation. Violation of the structure of cilia or PC-1 causes a defect of the complex PC-1/mTOR, increasing the proliferation of the epithelium and promoting the formation of cysts [17].

The tail domain of PC-1 also reacts with tuberine, a product of the TSC2 gene, which mutation leads to the development of tuberous sclerosis, which is accompanied by the formation of cysts in the kidneys. In physiological conditions, tuberine inactivates Ser/Thr mTOR kinase, that depends on the rate of cell growth, apoptosis [14, 15]. In patients with ADPKD in cysts, that lick the cyst, mTOR activity is significantly increased and may become part of the PC-1 / tuberine complex. Thus, PC-1 normally suppresses mTOR activity via tuberine, and mutation of PC-1, eliminating this suppression, leads to increased growth, proliferation and differentiation of tubular epithelium cells, contributing to cystogenesis [8, 9].

Cysts with ADPKD are usually formed from the main cells of the collecting tubules and are primarily related to the maternal cell, but later this connection breaks out and the increase in cysts in capacity occurs by proliferation of cells that lining the cyst and secretion of fluid in it [1, 2]. Normally, the reabsorption process Na^+ and Cl^- occurs in the main cells of the collecting tubules. This process is provided through the activity of NaK-ATPase, which pumps Na^+ out of the cell, forms a gradient between the extra- and intracellular Na^+ concentration [3, 4]. NaK-ATPase is a heterodimer and consists of $\alpha 1$ - and $\beta 1$ -subunits. At ADPKD, there is persistent expression of fetal proteins, and if no transcription of the fetal subunit $\beta 2$ occurs, the NaK-ATPase consists of $\beta 2$ - and $\alpha 1$ -subunits. The C-terminal fragment of PC-1, changes its transport characteristics, interacting with such a structure of NaK-ATPase [12, 13]. It stops pumping Na^+ out of the cell, but begins to secrete Na^+ and water accordingly, which leads to the formation of cysts. The epithelium of cysts releases more ATP than the normal cells in the culture fluid, and the C-terminal fragment of PC-1 provides the ATP-dependent flux of Cl^- into the cyst. Liquid supplying in the cyst also provide aquaporines expressed on the epithelium of the cysts [18, 19].

Along with the secretion of electrolytes and fluid into the cyst cavity, another prerequisite for their increase is the proliferation of the cells that covers them. Normally, the proliferation of the tubular epithelium ceases after birth, but the epithelium of the proximal tubules retains the ability to recover in the case of damage [2, 3, 7]. Growth factor (EGF), which is produced in the thick ascending loop of the Henle loop, stimulates the proliferation. Since EGF receptors are located on the basolateral membrane of cells, they remain inaccessible for the EGF and in normal conditions, the cells

do not reproduce [2]. During ADPKD, EGF are localized on the apical membrane, which induces proliferation of the epithelium. Stimulation of proliferation is carried out by the interaction of EGF with receptors tyrosine kinase, mitogen activating kinase and protein kinase. The consequence of such interaction is the increasing of cell division and the transformation of the main cells of the collecting tubules from non-proliferating and capable of reabsorption in the proliferating secretory [8, 9, 10].

The main role in increasing of cyst size, have apoptosis, changes in the polarity of cystic cells, and intercellular interactions. Apoptosis with ADPKD is observed in unchanged kidney tissue and is believed to be responsible for reducing the number of active nephrons [6, 7]. The inducer of apoptosis is tumor necrosis factor α . The polarity improvement of the tubular epithelium in ADPKD patients relates not only to the above-mentioned major ion transporters, but also to other molecules. Thus, together with NaK-ATPase on the apical membrane, expression of calpastine, ankyrin, fodrin, laminin, gelatinase A, cathepsin B, FAK (focal adhesive kinase), which are normally presented in the basolateral membrane, are expressed [10, 11]. Part of the membrane proteins is placed in the cytoplasm. Such a violation of polarity is inherent in fetal tubular epithelium and indicates a violation of maturing processes. Normally, the polarity of the cell is determined by PC-1 and PC-2.

Defects of intercellular interactions during ADPKD are presented by the replacement of E-cadherin with fetal N-cadherin, which makes the ability of the reaction of B-catenin with other binding proteins of actin worse [12, 13]. All of the above mentioned changes in the renal tubules are accompanied by an increase in extracellular matrix and the development of interstitial fibrosis, which in its turn leads to the emergence and progression of chronic kidney deficiency [20].

During the ARPKD the protein fibrocystin mutates. It is known that fibrocystine is connected with the N-terminus of PC2 and is believed to be involved in the basic functions of the PC1/PC2 complex located in primary cilia [15, 16]. Obviously, fibrocystin is largely involved in the key development, differentiation, regulation of cell proliferation in the kidney tubules and bile ducts of the liver, so the manifestation of the disease affects both organs. In the formation and growth of cysts, the violation of planar polarity of cells plays a major role, unlike with ADPKD [1, 2]. At ARPKD, cysts in the kidneys are not separated from the lumen of the collecting tubes because of the fact, that the epithelial proliferation is the main factor in the growth of cysts. Cystic degeneration takes place in the cortical and medular layers of the kidney [13, 14].

Considering the main pathogenetic mechanisms of the occurrence and progress of this pathology, it becomes obvious that PKD is a serious and dangerous disease for human life, therefore it is very important to detect it early with subsequent treatment [5, 6].

Diagnostics of PKD should include the collection of

data on the presence of cystic changes in the kidneys of blood relatives and chronic renal failure of unknown etiology. The presence of family sickness cases is the basis for sonographic research performing [10, 11].

U.S. Ravine's criteria have been developed for the examination of patients with PCD, which include: ≥ 2 cysts in both kidneys at the age of less than 30 years; ≥ 2 cysts in each kidney at the age of 30-59 years; ≥ 4 cysts in each kidney over the age of 60 years. The presence of these sonographic criteria with a positive family anamnesis indicates the presence of PKD. US-parameters of cysts in PKD have characteristic features: round or oval form, smooth and thin walls, lack of calcifications and thickening, amplification of acoustic density is proportional to the size of cysts [15, 16].

Other methods of visualization as a MRI, CT are used when the date of sonographic examination is doubtful. The MRI criterion for PKDs includes: the presence of 5 or more cysts in both kidneys less than 30 years of age; 6 or more cysts in both kidneys at the age of 30-44 years; more than 6 cysts in both kidneys at the age of 45-59 for women and more than 9 cysts in both kidneys at the age of 45-59 for men [18, 19].

For prenatal screening, ARPKD does researches of the kidney fetus. A genetic study should be considered appropriate in the case of negative or questionable data of the imaging research methods. It should be assigned to all members of the family, which allows to identify preclinical cases. Conducting of population genetic screening is not feasible. The essence of the genetic researching is to identify markers (nucleotide sequences) on the 16th chromosome [1, 5, 15].

Also, patients with PKD should conduct a screening study

for the diagnosis of major extragranular manifestations of the disease, including cysts in other organs and vascular aneurysms.

Conclusions and perspectives of further development

1. The main reason for the development of PKD is the mutation of genes encoding the integral proteins of primary cilia. Changing the structure of cilia leads to loss of their functional capabilities and, as a consequence, to the formation and development of cysts.

2. In the case of ADPKD, the integral proteins of primary cilia PC-1 and PC-2 are mutating. The main pathogenetic mechanisms of the formation and development of cysts with ADPKD include: activation of proliferative processes of the tubular epithelium, changes in the structure and functional capabilities of NaK-ATPase of the renal tubules, apoptosis activation, change of the planar polarity of cells, defects in intercellular interactions, and exasperation of aquaporins, calpastine, ankyrin, laminar expression.

3. Fibrocystin protein also mutates during the ARPKD. The main role in the formation and development of cysts is the violation of planar polarity of cells, in contrast to ADPKD. This type of PKD affects not only the kidneys, but also the liver.

In recent years, more and more attention is paid to the in-depth study of the main mechanisms of development of PKD in our countries and abroad. The latest genetic research methods that will enable this pathology to be detected in the early stages, in order to prevent the development of irreversible changes in the kidneys are at the development stages.

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ЗНАЧЕНИЕ ПЕРВИЧНЫХ РЕСНИЧЕК В ПАТОГЕНЕЗЕ ПОЛИКИСТОЗНОЙ БОЛЕЗНИ ПОЧЕК

Резюме. Поликистозное заболевание почек является наиболее распространённым генетическим заболеванием. Существуют две основные формы этой патологии: аутосомно-доминантная и аутосомно-рецессивная. Поликистозное заболевание почек приводит к развитию терминальной почечной недостаточности у 10-14% нефрологических пациентов.

Ключевые слова: поликистозная болезнь почек, первичные реснички, цистогенез, полицистины.

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ЗНАЧЕННЯ ПЕРВИННИХ ВІЙОК У ПАТОГЕНЕЗІ ПОЛІКІСТОЗНОЇ ХВОРОБИ НИРОК

Резюме. Полікістозна хвороба нирок є найбільш поширеним генетичним захворюванням. Виділяють дві основні форми цієї патології: аутосомно-домінантну та аутосомно-рецесивну. Полікістоз нирок призводить до розвитку термінальної ниркової недостатності у 10-14% нефрологічних хворих.

Ключові слова: Полікістозна хвороба нирок, первинні війки, цистогенез, поліцистини.

Рецензент - д.мед.н., проф. Маєвський О.Є.

Стаття надійшла до редакції 7.06.2017

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УДК: 615.1:611.018.2:617.753.2

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ТРАНСФОРМУЮЧИЙ РОСТОВИЙ ФАКТОР β ЯК ФАКТОР РОЗВИТКУ МІОПІЇ

Резюме. Трансформуючий ростовий фактор $\beta 1$ (ТРФ- $\beta 1$) - це профібротичний цитокін, що відноситься до сімейства ТРФ- β (ТРФ- $\beta 1$, ТРФ- $\beta 2$, ТРФ- $\beta 3$), контролює синтез та склад зовнішньоклітинного матриксу (ЗКМ), пригнічує деградацію колагену, є регулятором росту склеральних хондроцитів та склеральних фібробластів. ТРФ- β приймає участь у змінах обміну ЗКМ, які виникають при міопії та відіграє значну роль у процесах ремоделювання склери, що суттєво впливає на її біомеханіку. Як наслідок, збільшується передньо-задній розмір очного яблука, що характерно для прогресуючої, так званої "злаякісної" міопії, яка проявляється у ранньому віці та часто пов'язана з важкими ускладненнями, такими як деструкція скловидного тіла, дистрофія та відшарування сітківки, субретинальні крововиливи, ускладнена катаракта.

Ключові слова: ТРФ- β , міопія, сполучна тканина, склера, зовнішньоклітинний матрикс.

Мета роботи: провести ретроспективний аналіз наукової літератури по вивченню ролі ТРФ- β у формуванні сполучної тканини, механізмів синтезу та реалізації ефекту, його ролі в розвитку міопії.

Як відомо, міопія є важливою медичною та соціальною проблемою сучасного світу, оскільки кожна третя людина на Землі страждає саме на цю патологію. Важко перебільшити значення якісного зору, який є невід'ємним фактором формування особистості, впливає на стиль життя, вибір професії, та інші важливі процеси адаптації людини в соціумі. За даними центру медичної статистики МОЗ України, у 2016 році первинна захворюваність на хвороби ока та його додаткового апарату склала 3227 на 100 тисяч населення України, що в абсолютній кількості становило 1371345 звернень, під час яких було встановлено діагноз захворювання ока та додаткового апарату. Слід відмітити, що за останні роки збільшилася кількість порушень зору внаслідок розвитку короткозорості. Так серед дитячого населення поширеність короткозорості складає 30 % зі всіх захворювань очей, серед дорос-

лого - 12 %. Проаналізувавши існуючу сучасну наукову інформацію щодо механізмів виникнення та реалізації міопії, найменш вивченою ланкою є розробка та впровадження генетичного методу ранньої діагностики та його корекції.

Важко переоцінити у цих процесах роль генетичних факторів, особливо це стосується прогресуючої міопії, яка проявляється у ранньому віці та часто пов'язана з важкими ускладненнями, такими як деструкція скловидного тіла, дистрофія та відшарування сітківки, субретинальні крововиливи, ускладнена катаракта. Ці та інші патологічні зміни, що виникають при міопії високого ступеня, є результатом механічних тисків, пов'язаних зі збільшенням очного яблука. За даними дослідників, при моделюванні міопії високого ступеня на тваринах, спостерігалось збільшення розмірів ока за рахунок сполучнотканинних змін у фіброзній оболонці очного яблука. Також було доведено і те, що активне ремоделювання склери відіграє значну роль у збільшенні очного яблука [10]. Як відомо, склера представлена волокнистою сполучною