Оригінальні дослідження

DOI: 10.31793/1680-1466.2019.24-1.5

Expression of protein kinase p70S6K in leukocytes of patients with cancer and diabetes mellitus

T.S. Vatseba[1], L.K. Sokolova[2], V.V. Pushkarev[2], O.I. Kovzun[2], V.M. Pushkarev[2], M.D. Tronko[2]

[1] SHEI «Ivano-Frankivsk National Medical University», Ivano-Frankivsk
[2] SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of NAMS of Ukraine», Kyiv

Abstract. Aim — to study the expression of p70S6K in leukocytes of patients with cancer and diabetes. **Material and methods.** P70S6K expression was determined using immune-enzyme analysis. **Results.** It has been shown that in the leukocytes of patients with cancer and type 2 diabetes, the amount of protein kinase increases, indicating an increase in the expression of p70S6K, which plays an important role in the formation of insulin resistance and tumor progression. However, in the leukocytes of patients with both cancer and diabetes, the amount of p70S6K is significantly reduced compared with the leukocytes of patients with cancer or diabetes. **Conclusion.** Possible mechanisms and the significance of p70S6K expression in leukocytes are discussed.

Keywords: type 2 diabetes, cancer, leukocytes, p70S6K, mTORC1.

The evolutionary conserved kinases of ribosomal S6 protein belong to the family of AGC kinases (PKA, PKG and PKC) and play an important role in regulation of cell growth and the metabolism [1]. S6K kinases are mTOR pathway effectors, and accumulated evidence suggests that activation of the mTOR/S6K axis stimulates protein synthesis and cell growth [2]. The mTORC1 (mammalian target of rapamycin complex 1) protein kinase controls cell growth and homeostasis, including protein synthesis, lipogenesis, glucose metabolism, autophagy, biogenesis of lysosomes, proliferation and survival, in response to environmental signals such as levels of amino acids, glucose, energy, oxygen and growth factors [3]. The main substrate of mTORC1 is protein kinase p70S6K, which controls protein synthesis and ribosomal biogenesis. The deregulation of the PI3K/Akt/mTOR/p70S6K cascade leads to serious diseases such as cancer, obesity and type 2 diabetes (T2D) [2].

The leukocytes include several types of cells that play a significant role in the development

* Адреса для листування (Correspondence): ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», вул. Вишгородська, 69, м. Київ, 04114, Україна. E-mail: pushkarev.vm@gmail.com

VERTE

of pathological conditions such as diabetes and cancer [4-6]. The pathway PI3K/Akt is involved in the activation of macrophages and lymphocytes, secretion of cytokines, initiation of inflammatory processes and immune surveillance failure [7].

The aim of the work was to determine the expression of the endpoint of the PI3K/Akt/mTORC1/p70S6K cascade in leukocytes of patients with type 2 diabetes and cancer.

Material and methods

The study was conducted in the diabetology department of the Institute. All patients signed informed consent to conduct further diagnostic and research study. Immediately after collection, the blood was layered on histopaque 1077 (Sigma, USA), centrifuged at 500 g (RT) for 15 min in the 15 ml conical Falcon™ tubes, the leukocytes collected were washed in PBS and frozen at –80 °C until use. For determination of p70S6K1 amounts ELISA kits 85-86053 (Invitrogen, USA) were used. The studies were carried out in triplets. The cells were lysed in the extraction buffer with inhibitors of proteases and phosphatases from the kit. The protein concentration in the lysate was determined using BCA protein assay kit (Novagen, USA). The measurements were carried out on a microplate reader (Bio-tek Instruments, USA) at a wavelength of 450 nm.

The calibration curve **(fig. 1)** shows satisfactory agreement of the experimental curve with the theoretical and insignificant scatter of data.

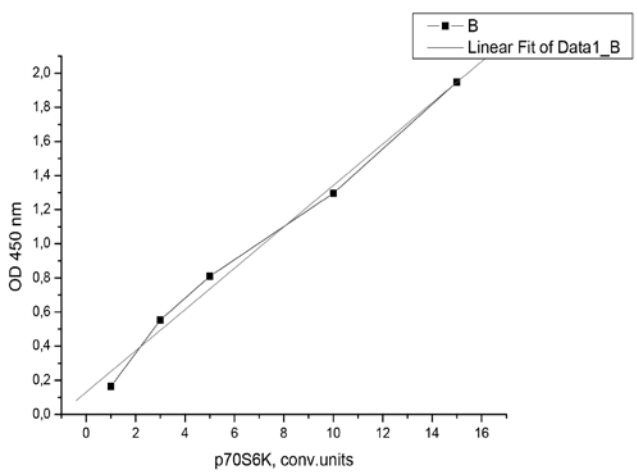

Fig. 1. Calibration curve for determination of p70S6K1 amount.

The results of the study are presented as M±SD, n=6-15. To compare the data groups, Student's *t*-test was used. Values of p≤0.05 were considered as significant.

Results and discussion

The following groups were investigated: 1 — control group (n=6) — healthy people, representative by age; 2 — patients with T2D (n=12); 3 — cancer patients (n=15); 4 — patients with both cancer and T2D (n=7). Patients with T2D used combined treatment with insulin and metformin. Patients with diabetes (groups 2 and 4) have HbA1c level — 7.4-9.2%. Most of the patients of groups 3 and 4 have uterine, breast and bowel cancers.

The amount of p70S6K1 increases in leukocytes of patients with T2D **(fig. 2)** almost one and a half times, indicating an increase in the expression of kinase or its stability. Increasing of the p70S6K level in patients with T2D, apart from changes due to the disease, is probably determined by the ratio of metformin and insulin effects that were taken by patients. The final result of the interaction of these drugs and the signaling mechanisms induced by them, obviously, is the enhancement of the expression of p70S6K.

It is known that in tissues of patients with T2D the activity of mTORC1 and its substrate —

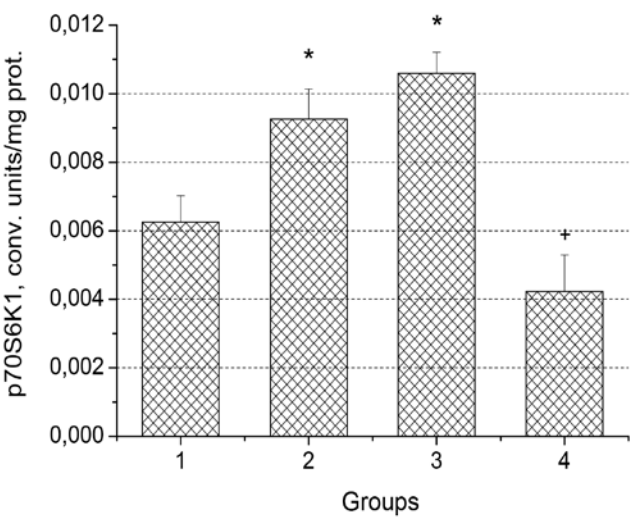

Fig. 2. Amount of p70S6K1 in leukocytes of patients with cancer and diabetes: 1 — control (n=6); 2 — patients with type 2 diabetes (n=12); 3 — cancer patients (n=15); 4 — patients with both diabetes and cancer (n=7); M±SD; * — the difference from the control group is significant, p<0.05; + — the difference from the groups 2 and 3 is significant, p<0.05.

protein kinases p70S6K is elevated, resulting in phosphorylation of IRS-1 (on S307 and other amino acid residues), insufficiency of the insulin signaling pathway, and, as a consequence, insulin resistance [8]. Obviously due to the disease there are more profound changes in the cells — at the level of transcription, as evidenced by an increase in not only activity but also the amount of p70S6K.

An even greater gain (169.5%) of the p70S6K expression was observed in leukocytes of cancer patients (fig. 2.3). Hyperactivation mTORC1 and p70S6K are often observed in sporadic cancers. The acceleration of the translation caused by aberrant activation of these kinases leads to an increase in the cells size and proliferation — two common signs of cancer, and the search for inhibitors of mTORC1 is considered a promising direction for the treatment of cancer [9]. From this point of view, an increase in the amount of p70S6K in leukocytes is of considerable interest, since it can serve as additional diagnostic marker of the disease.

It is well known that there is a relationship between diabetes (especially T2D) and cancer. Hyperinsulinemia enhances the expression of insulin and IGF receptors that causes cumulative mitogenic effect. Hyperglycemia gives the cancerous cells an excess of glucose [10]. Thus, it was expected that in the leukocytes from group of patients with both cancer and diabetes one would observe an additive effect on the expression of p70S6K. Therefore, somewhat unexpected was the decrease of p70S6K amount to 67.6% of the control level (fig. 2.4). Consequently, in patients of the latter group, the expression of p70S6K in leukocytes may be depressed, as compared to the groups of patients with diabetes (fig. 2.2) and cancer (fig. 2.3). Probable explanation for such suppression may be competition for common signaling mechanisms. Antagonistic interaction between the two main cascades controlling proliferative processes — PI3K/Akt and MAPK is also not excluded. It was shown that the MAPK/ERK signaling pathway also affects mTORC1/p70S6K [9] and an excess of insulin with T2D stimulates proliferative processes and malignant transformation through the cascade Ras/MAPK/ERK1/2 [10].

Another mechanism that can regulate the amount of p70S6K is its proteolytic cleavage by caspase-3 at Asp393 [11], and changes in protein kinase content may be due to altered stability of the protein.

The leukocytes include monocytes/macrophages (up to 11% of the total amount of leukocytes) and lymphocytes (up to 40%) involved in the processes of cellular and humoral immunity. PI3K/Akt/mTOR/p70S6K is a signaling cascade that largely determines the functioning of these blood cells in diabetes and malignant neoplasm [4-7].

p70S6K promotes several broad cellular processes: protein synthesis, cell growth/size, cell survival, cell cycle and cell motility gene transcription, adipocyte differentiation, synaptic plasticity and regulates DNA damage response. Also p70S6K regulates two transcription factors CREMt and ERα [11].

mTOR/p70S6K signaling is involved in the pathogenesis of type 2 diabetes. Obese rats have high levels of mTOR and p70S6K and reduced IRS1 expression, which are reverted by rapamycin treatment [11].

Deregulated signaling via p70S6K has been linked to various types of cancer. Many of the molecules signaling upstream of p70S6K have been shown to be either mutated or overexpressed in tumors, leading to p70S6K activation [11]. For example, p70S6K1 is significantly upregulated in cervical and gastric cancers. Furthermore, p70S6K plays an important role in metastasis formation by regulating cyclin D1, PDCD4, FAK, E-cadherin, β-catenin and tissue transglutaminase 2, which are essential for cell attachment, survival, invasion and metastasis in cancer. Also it contributes to tumor growth and angiogenesis through hypoxia-inducible factor 1α and vascular endothelial growth factor expression [12].

Thus, changes in the amount of p70S6K in leukocytes may indicate a systemic and profound effect of pathological processes on the level of control of transcription in blood cells, which may be important for the evaluation of disease progression and the effectiveness of the drugs.

References

1. Hong S, Zhao B, Lombard DB, Fingar DC, Inoki K. Cross-talk between sirtuin and mammalian target of rapamycin complex 1 (mTORC1) signaling in the regulation of S6 kinase 1 (S6K1) phosphorylation. J Biol Chem. 2014;289(19):13132-41.

VERTE

2. Tavares MR, Pavan IC, Amaral CL, Meneguello L, Luchessi AD, Simabuco FM. The S6K protein family in health and disease. Life Sci. 2015;131:1-10.
3. Yang J, Nishihara R, Zhang X, Ogino S, Qian ZR. Energy sensing pathways: Bridging type 2 diabetes and colorectal cancer? J Diabetes Complications. 2017;31(7):1228-36.
4. de Oliveira CE, Oda JM, Losi Guembarovski R, de Oliveira KB, Ariza CB, Neto JS. CC chemokine receptor 5: the interface of host immunity and cancer. Dis Markers. 2014;2014:126954.
5. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, et al. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. Oncoimmunology. 2012;1(8):1323-43.
6. Tronko ND, Pushkarev VM, Sokolova LK, Pushkarev VV, Kovzun OI. Molecular mechanisms of pathogenesis of diabetes and its complications. K.: Publishing house Medkniga, 2018. 264 p. (In Russian).
7. Dituri F, Mazzocca A, Giannelli G, Antonaci S. PI3K functions in cancer progression, anticancer immunity and immune evasion by tumors. Clin Dev Immunol. 2011;2011:947858.
8. Pushkarev VM, Sokolova LK, Pushkarev VV, Tronko MD. The role of AMPK and mTOR in the development of insulin resistance and type 2 diabetes. The mechanism of metformin action (literature review). Probl Endocrin Pathol. 2016;3:77-90. (In Russian).
9. Kim LC, Cook RS, Chen J. mTORC1 and mTORC2 in cancer and the tumor microenvironment. Oncogene. 2017;36(16):2191-201.
10. Pushkarev VM, Sokolova LK, Pushkarev VV, Tronko MD. Biochemical mechanisms connecting diabetes and cancer. Effects of methormine. Endokrynologia. 2018;23(2):167-79. (In Russian).
11. Bahrami-B F, Ataie-Kachoie P, Pourgholami MH, Morris DL. p70 Ribosomal protein S6 kinase (Rps6kb1): an update. J Clin Pathol. 2014;67(12):1019-25.
12. Akar U, Ozpolat B, Mehta K, Lopez-Berestein G, Zhang D, Ueno NT, et al. Targeting p70S6K prevented lung metastasis in a breast cancer xenograft model. Mol Cancer Ther. 2010;9:1180-7.

(Надійшла до редакції 06.02.2019 р.)

Експресія протеїнкінази p70S6K у лейкоцитах хворих на рак і цукровий діабет

Т.С. Вацеба, Л.К. Соколова, В.В. Пушкарьов, О.І. Ковзун, В.М. Пушкарьов, М.Д. Тронько

[1]ДВНЗ «Івано-Франківський національний медичний університет», м. Івано-Франківськ

[2]ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», м. Київ

Резюме. Мета — вивчити експресію p70S6K у лейкоцитах хворих на рак і цукровий діабет. **Матеріал і методи.** Експресію p70S6K визначали методом імуноферментного аналізу. **Результати.** Показано, що в лейкоцитах хворих на рак і цукровий діабет 2-го типу кількість протеїнкінази зростає, що свідчить про посилення експресії p70S6K, яка відіграє важливу роль у формуванні інсулінорезистентності та прогресії пухлин. Проте в лейкоцитах хворих і на рак, і на цукровий діабет кількість p70S6K є істотно нижчою порівняно з такою в лейкоцитах хворих на рак або цукровий діабет. **Висновки.** Обговорюються можливі механізми та значення зміни експресії p70S6K у лейкоцитах.

Ключові слова: цукровий діабет 2-го типу, рак, лейкоцити, p70S6K, mTORC1.

Экспрессия протеинкиназы p70S6K в лейкоцитах больных раком и сахарным диабетом

Т.С. Вацеба[1], Л.К. Соколова[2], В.В. Пушкарев[2], Е.И. Ковзун[2], В.М. Пушкарев[2], Н.Д. Тронько[2]

[1] ВГНУ «Ивано-Франковский национальный медицинский университет», г. Ивано-Франковск

[2] ГУ «Институт эндокринологии и обмена веществ им. В.П. Комиссаренко НАМН Украины», г. Киев

Резюме. Цель — изучение экспрессии p70S6K в лейкоцитах больных раком и сахарным диабетом. **Материал и методы.** Экспрессию p70S6K определяли методом иммуноферментного анализа. **Результаты.** Показано, что в лейкоцитах больных раком и сахарным диабетом 2-го типа количество протеинкиназы возрастает, что свидетельствует об усилении экспрессии p70S6K, которая играет важную роль в формировании инсулинорезистентности и прогрессии опухолей. Однако в лейкоцитах больных и раком, и сахарным диабетом количество p70S6K существенно снижено по сравнению с таковым в лейкоцитах больных раком или диабетом. **Выводы.** Обсуждаются возможные механизмы и значение изменения экспрессии p70S6K в лейкоцитах.

Ключевые слова: сахарный диабет 2-го типа, рак, лейкоциты, p70S6K, mTORC1.