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Activation of the protein kinase Akt in peripheral blood mononuclear cells. Association with insulin and insulin-like growth factor levels in the blood of patients with cancer and diabetes

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Abstract. Signal cascade PI3K/Akt/mTOR/p70S6K plays an important role in the pathogenesis of cancer and diabetes. Macrophages and lymphocytes are involved in the development of diabetes, diabetic atherosclerosis, the formation of tissue resistance to insulin, as well as in the immune response to cancer and tumor support. **The aim** of the study was to determine the activation of Akt by mTORC2 kinase in peripheral blood mononuclear cells (PBMC) of patients with type 2 diabetes and cancer. **Material and methods.** The following groups were studied: 1 — the control group, 2 — patients with breast cancer, 3 — patients with endometrial cancer, 4 — patients with bowel cancer, 5 — patients with pancreatic cancer. The amount of phospho-Akt (p-S473), insulin and insulin-like growth factor-1 (IGF-1) was determined using enzyme immunoassay. **Results.** Insulin and IGF-1 levels are higher in the blood of patients with breast and endometrial cancer compared with control, as well as bowel and pancreatic cancer. The change in the content of activated Akt in PMBC generally corresponds to the concentration of insulin and IGF-1 in the blood. The differences between breast/endometrial cancers and pancreatic/bowel cancers with IGF-1/insulin content in the blood and Akt activation in PMBC can be explained by the presence of hormonal background (estrogens) specific for the first two types of cancer. **Conclusion.** Chronic diseases such as type 2 diabetes and cancer can affect the signaling mechanisms in blood cells. The state of Akt phosphorylation in PMBC may indicate the activity of mTORC1 and its substrates, which may be important for the assessment of the pathological process and the effectiveness of treatment.

Keywords: Akt, insulin, insulin-like growth factor 1, peripheral blood mononuclear cells, cancer, diabetes.

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Protein kinase Akt (v-act murine thymoma viral oncogene homolog) plays a key role in the regulation of cell growth, homeostasis, survival, proliferation and metabolism [1]. Akt is activated by PDK1 via T308 phosphorylation in the T-loop of the catalytic domain and by rapamycin-insensitive mTORC2 through S473 phosphorylation in the hydrophobic region on the C-tail. Akt enhances insulin-dependent translocation of GLUT-4 and glucose transport, and activates downstream protein kinases mTORC1 and p70S6K that control protein synthesis and biogenesis of ribosomes. Dysregulation of the PI3K/Akt/mTOR/p70S6K signaling leads to severe diseases such as cancer, obesity and type 2 diabetes (T2D).

It was shown that the insulin/IGF system is often dysregulated in cancer, contributing to cancer progression, metastases, and resistance to cancer therapies [2].

The peripheral blood mononuclear cell (PBMC) include several types of cells that play a significant role in the development of pathological conditions such as diabetes and cancer [3-5]. The pathway PI3K/Akt is involved in the activation of macrophages and lymphocytes, secretion of cytokines, initiation of inflammatory processes and immune surveillance failure [6].

The aim of the work was to determine the activation of Akt, the main effector kinase of PI3K/Akt/mTORC/p70S6K cascade, in PBMC of patients with T2D and cancer.

Materials 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 (bucket-rotor of Hermle Z-300 micro-centrifuge) at 400 g (RT) for 30 min in the 15 ml conical Falcon™ tubes. The PBMC collected were washed in PBS by centrifugation at 200 g to remove platelets and frozen at –80 °C prior to use and frozen at –80 °C until use. For determination of phospho-Akt1/2/3 (p-S473) amounts ELISA kit 85-86046 (Invitrogen, USA) was 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 levels of insulin and IGF-1 were determined using the automatic analyzer Stat fax 303+ (USA) with the diagnostic kits Insulin ELISA (EIA-2935) and IGF-1600 ELISA (EIA-4140) from DRG (Germany). HbA1c was determined by ion-exchange chromatography, using the Bio-Rad D-10 analyzer and the Bio-Rad (USA) reagents.

The OD values of samples obtained are located on the calibration curve satisfactorily coinciding with a theoretical lines that indicates no scattering of the data.

The results of the study are presented as M±SD, n=6-30. To compare the data groups, Student's *t*-test was used. Values of $p \leq 0.05$ were considered as significant.

Results and discussion

The following groups were investigated: 1 — control group (n=10) — healthy people, representative by age; 2 — patients with endometrial cancer (n=8); 3 — patients with breast cancer (n=7); 4 — patients with bowel cancer (n=4); 5 — patients with pancreatic cancer (n=5). Therapy of patients included various combinations of glucose-lowering drugs and insulin. The average level of HbA1c in patients was 8.07±0.99% corresponded to the decompensation of diabetes.

The PBMC include monocytes/macrophages and lymphocytes (T cells, B cells and NK) involved in the processes of cellular and humoral immunity. PI3K/Akt/mTOR is a signaling cascade that

Table. IGF/insulin concentrations in the blood and Akt activation in the PMBC of oncological patients with type 2 diabetes

	p-Akt1/2/3, conv. units	p/t-Akt, conv. units	Insulin mkIU/ml	IGF-1 ng/ml
1. Control	0.0091± 0.0011	0.0602± 0.0066	7.56±0.81	141.57± 10.12
2. Breast cancer	0.0069± 0.0007*	0.0748± 0.0047*	15.27± 0.54*	191.17± 12.71*
3. Endometrial cancer	0.0094± 0.0017	0.0731± 0.0136	17.59± 2.76*	189.96± 15.55*
4. Bowel cancer	0.0062± 0.0007*+	0.0427± 0.0096*+	7.35±0.17+	172.75± 27.80
5. Pancreatic cancer	0.0030± 0.0018*+	0.0291± 0.0234*+	8.60±1.64+	158.16± 10.84+

*Note: * — differences from control are significant, p<0.05; + — differences from groups 2 and 3 are significant, p<0.05.*

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largely determines the functioning of these blood cells in diabetes and malignant neoplasm [3-5, 7].

As shown on the Table, the level of insulin in the blood of patients with breast and endometrial cancer is higher compared to control, as well as bowel and pancreatic cancer. Almost the same pattern we observed concerning IGF-1 concentration. Akt1/2/3 phosphorylation was lower than control in all types of cancer beside endometrial. More reliable indicator of kinase activation is normalization with respect to its total amount in the cells, that is the ratio of phospho-Akt to total. As can be seen on the Table the p/t-Akt content changes in PMBC in a whole coincide with insulin and IGF-1 concentrations in the blood.

It should be noted that Akt is phosphorylated by the mTORC2 on Ser473 in a partially IRS-independent manner, which indicates a weak connection of such phosphorylation with IRS/PI3K signaling [8, 9]. It is known that mTORC1 is regulated by the availability of nutrients, energy and growth factor signals, while mTORC2 is activated by growth factor signals [10, 11]. Thus, studying Akt phosphorylated by the mTORC2 on Ser473 we can partially separate the effect of nutrients from the effects of growth factors, and metabolic effects from mitogenic.

High levels of IGF-1 and insulin in the microenvironment provide a likely mechanism for carcinogenesis and early tumor growth through antiapoptotic signaling and metabolic reprogramming mediated by PI3K/Akt/mTOR. It is known that patients with obesity are characterized by higher levels of IGF-1 in the blood, compared to people with normal BMI [12]. This is consistent with the conclusion that diabetes and obesity increase the risk of developing types of cancer with the Warburg phenotype [13, 14].

The differences between breast and endometrial cancer on the one hand, and pancreatic and bowel cancer on the other hand, are apparently explained by the specific hormonal background that accompanies the first two types of cancer.

Early studies have shown that IGFs and estrogens are strong mitogens for breast cancer cells and that high circulating IGF-1 and estrogens are risk factors of breast cancer. Further experiments indicated that these hormones act synergistically on the pathogenesis of breast cancer. Estrogens increase the effect of IGF-1 on breast cancer cells by stimulating the expression of IGF-1 and IGF-1 receptor [15].

The insulin/IGF system and estrogens act synergistically as potent mitogens in normal breast as well as in breast tumor cells. At first it was thought that these agents act through separate pathways, but evidence was obtained that the insulin/IGF and estrogen-mediated signaling pathways are closely connected [16]. Estradiol upregulates the expression of several IGF family members, including IGF-1, IGF-2, IGF-BP2, IGF-1R, and IRS-1 [2]. It was shown that insulin receptor substrate 1 (IRS-1) has been known to be an associated factor with breast cancer progression [17].

As mentioned it is established that the insulin/IGF system is frequently impaired in cancer [2, 18]. Common alterations include overexpression of IR and IGF-1R by the malignant cells, increased IR/IGF-1R hybrid formation, deregulated autocrine secretion of IGFs, and increased IGFs secretion by the tumor stroma. IGFBPs production in the tumor microenvironment may also be dysregulated [18]. Moreover, epidemiological studies have shown that elevated IGF-1 plasma concentrations are associated with a higher risk of developing various malignancies [2].

In estrogen-induced endometrial carcinogenesis, IGF-1 plays an important role. Estrogens increase the expression of IGF-1 in tissues, and IGF-1 is required to mediate their mitogenic effects on the endometrium. In addition, estrogens modulate IGF-1 signaling by regulating the expression of other members of the IGF family, including the IRS-1 and IGF binding proteins [19].

Estrogen and insulin play a synergistic role in type 1 endometrial carcinogenesis and progression. Epidemiologic studies have found that estrogens, insulin, and IGFs are higher in patients with type 1 endometrial cancer than in healthy individuals. Steroid hormones, such as estrogen, and growth factors, such as IGF/insulin, can be major drivers of this type of cancer. Besides, insulin also promotes the development of type 1 endometrial cancer in other ways. It was shown that insulin resistance and compensatory hyperinsulinemia provoke androgen synthesis [20]. Increased free androgens supply more substrate for peripheral estrogen conversion. Also, insulin has been reported to inhibit the synthesis of sex hormone binding globulin, which tightly binds and regulates the activity of sex hormones. Thus, when insulin levels increase due to insulin resistance, this inhibition results in an increase

in free sex hormone levels (of both estrogens and androgens) and further stimulates type 1 endometrial tumorigenesis [21].

It is also important that tissue macrophage infiltration correlated positively with endometrial cancer development [22].

Conclusion

Thus, chronic diseases such as type 2 diabetes and cancer may have a systemic effect on signaling mechanisms in different tissues of the body, including blood cells.

There were the differences in patients with both cancer and diabetes between breast/endometrial cancers, and pancreatic/bowel cancers considering IGF/insulin content in the blood and Akt activation in the PMBC, that could be explained by the hormonal background of the first types of cancer.

The state of Akt phosphorylation in PBMC can indicate the activity of mTORC1 and its substrates, which may be important for the evaluation of the pathological process and the efficacy of the drugs. It also can be considered as an additional diagnostic feature for the first two types of cancer.

References

1. Manning BD, Toker A. AKT/PKB Signaling: navigating the network. Cell. 2017 Apr;169:381-405.
2. De Marco P, Cirillo F, Vivacqua A, Malaguarnera R, Belfiore A, Maggiolini M. Novel aspects concerning the functional cross-talk between the insulin/IGF-I system and estrogen signaling in cancer cells. Front Endocrinol (Lausanne). 2015 Mar;6:30.
3. Senovillan L, Vacchellin 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 Nov;1(8):1323-43.
4. de Oliveira CE, Oda JM, Losi Guembarovski R, de Oliveira KB, Ariza CB, Neto JS, et al. CC chemokine receptor 5: the interface of host immunity and cancer. Dis Markers. 2014;2014:126954.
5. Тронько НД, Пушкарев ВМ, Соколова ЛК, Пушкарев ВВ, Ковзун Е.И. Молекулярные механизмы патогенеза сахарного диабета и его осложнений. Киев: Медкн., 2018. 261 с. (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).
6. 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.
7. Kim LC, Cook RS, Chen J. mTORC1 and mTORC2 in cancer and the tumor microenvironment. Oncogene. 2017 Apr;36(16): 2191-201.
8. Copps KD, HançerNJ, Qiu W, White MF. Serine 302 phosphorylation of mouse insulin receptor substrate 1 (IRS1) is dispensable for normal insulin signaling and feedback regulation by hepatic S6 kinase. J Biol Chem. 2016 Apr;291(16):8602-17.
9. Copps KD, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1and IRS2. Diabetologia. 2012 Oct;55(10):2565-2582.
10. Rad E, Murray JT, Tee AR. Oncogenic signalling through mechanistictarget of rapamycin(mTOR): a driver of metabolic transformation and cancer progression. Cancers (Basel). 2018 Jan;10(1): E5.
11. Jhanwar-Uniyal M, Amin AG, Cooper JB, Das K, Schmidt MH, Murali R. Discrete signaling mechanisms of mTORC1 and mTORC2: Connected yet apart in cellular and molecular aspects. Adv Biol Regul. 2017 May;64:39-48.
12. Brick DJ, Gerweck AV, Meenaghan E, Lawson EA, Misra M, Fazeli P, et al. Determinants of IGF1 and GH across the weight spectrum: from anorexia nervosa to obesity. Eur J Endocrinol. 2010 Aug;163:185-91.
13. Klement RJ, Fink MK. Dietary and pharmacological modification of theinsulin/IGF-1system: exploiting the full repertoire against cancer. Oncogenesis. 2016 Feb;5: e193.
14. Fine EJ, Feinman RD. Insulin, carbohydrate restriction, metabolic syndromeand cancer. Exp Rev Endocrin Metab. 2015 Jan;10(1): 15-24.
15. Yu H, Shu XO, Li BD, Dai Q, Gao YT, Jin F, et al. Joint effect of insulin-like growth factors and sex steroids on breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2003 Oct;12(10):1067-73.
16. Bradley LM, Gierthy JF, Pentecost BT. Role of insulin-like growth factor system on an estrogen-dependent cancer phenotype in the MCF-7 human breast cancer cell line. J Steroid Biochem Mol Biol. 2008 Mar;109:185-96.
17. Kim HG, Woo SU, Kim HY, Son GS, Lee JB, Bae JW, et al. The expression of insulin receptor substrate 1 and estrogen receptor as prognostic factor on breast cancer patient. J Cancer Res Ther. 2018 Jun;14(Suppl): S494-8.
18. Samani AA, Yakar S, Le Roith D, Brodt P. The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev. 2007 Feb;28:20-47.
19. Kwasniewski W, Gozdzicka-Jozefiak A, Wolun-Cholewa M, Polak G, Sierocinska-Sawa J, Kwasniewska A, et al. Microsatellite polymorphism in the P1 promoter region of the IGF-1 gene is associated with endometrial cancer. Mol Med Rep. 2016 Jun;13(6):4950-8.
20. Suba Z. Interplay between insulin resistance and Estrogen deficiency as co-activators in carcinogenesis. Pathol Oncol Res. 2012 Apr;18:123-33.
21. Tian W, Teng F, Zhao J, Gao J, Gao C, Sun D, et al. Estrogen and insulin synergistically promote type 1 endometrial cancer progression. Cancer Biol Ther. 2017 Dec;18(12):1000-10.
22. Ning C, Xie B, Zhang L, Li C, Shan W, Yang B, et al. Infiltrating macrophages induce ERα expression through an IL17A-mediated epigenetic mechanism to sensitize endometrial cancer cells to estrogen. Cancer Res. 2016 Mar;76(6):1354-66.

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Активація протеїнкінази Akt у мононуклеарах периферичної крові. Зв'язок із рівнями інсуліну та інсуліноподібного чинника росту в крові хворих на рак і діабет

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Резюме

Сигнальний каскад PI3K/Akt/mTOR/p70S6K відіграє важливу роль у патогенезі раку та діабету. Макрофаги та лімфоцити беруть участь у розвитку діабету, діабетичного атеросклерозу, формуванні резистентності тканин організму до інсуліну, а також в імунній відповіді на рак і підтримці пухлини. **Метою** до-

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слідження було визначення активації Akt кіназою mTORC2 у мононуклеарах периферичної крові (PBMC) пацієнтів із цукровим діабетом 2-го типу та раком. **Матеріал і методи.** Вивчали такі групи: 1 — контрольна, 2 — хворі на рак молочної залози, 3 — хворі на рак матки, 4 — хворі на рак кишечника, 5 — хворі на рак підшлункової залози. Кількість фосфо-Akt (p-S473), інсуліну й інсуліноподібного чинника росту 1 (IGF-1) визначали з використанням імуноферментних наборів. **Результати.** Рівні інсуліну й IGF-1 у крові хворих на рак молочної залози й ендометрія були вищими за контрольний і в пацієнтів із раком кишечника та підшлункової залози. Зміна вмісту активованої Akt у PMBC у цілому відповідала концентрації інсуліну та IGF-1 у крові. Відмінності між пухлинами молочної залози й ендометрія та пухлинами підшлункової залози і шлунка за вмістом IGF-1/інсуліну в крові й активацією Akt у PMBC можна пояснити наявністю гормонального тла (естрогени), характерного для перших двох типів раку. **Висновки.** Хронічні захворювання, такі як діабет 2-го типу та рак, можуть впливати на сигнальні механізми в клітинах крові. Стан фосфорилювання Akt у PMBC може вказувати на активність mTORC1 і його субстратів, що є важливим для оцінки патологічного процесу й ефективності лікування.

Ключові слова: Akt, інсулін, інсуліноподібний чинник росту 1, мононуклеари периферичної крові, рак, діабет.

Активация протеинкиназы Akt в мононуклеарах периферической крови. Связь с уровнями инсулина и инсулиноподобных факторов роста в крови больных раком и диабетом

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Резюме

Сигнальный каскад PI3K/Akt/mTOR/p70S6K играет важную роль в патогенезе рака и диабета. Макрофаги и лимфоциты участвуют в развитии диабета, диабетического атеросклероза, формировании резистентности тканей организма к инсулину, а также в иммунном ответе на рак и поддержке опухоли. **Целью** исследования было определение активации Akt киназой mTORC2 в мононуклеарах периферической крови (PBMC) пациентов с диабетом 2-го типа и раком. **Материал и методы.** Изучали следующие группы: 1 — контрольная, 2 — больные раком молочной железы, 3 — больные раком матки, 4 — больные раком желудка, 5 — больные раком поджелудочной железы. Количество фосфо-Akt (p-S473), инсулина и инсулиноподобного фактора роста 1 (IGF-1) определяли с использованием иммуноферментных наборов. **Результаты.** Уровни инсулина и IGF-1 в крови больных раком молочной железы и эндометрия были выше по сравнению с такими в контроле и у больных раком кишечника и поджелудочной железы. Изменение содержания активированной Akt в PMBC в целом соответствовало концентрации инсулина и IGF-1 в крови. Различия между опухолями молочной железы и эндометрия и опухолями поджелудочной железы и желудка по содержанию IGF-1/инсулина в крови и активации Akt в PMBC можно объяснить наличием гормонального фона (эстрогены), характерного для первых двух типов рака.

Выводы. Хронические заболевания, такие как диабет 2-го типа и рак, могут влиять на сигнальные механизмы в клетках крови. Состояние фосфорилирования Akt в PMBC может указывать на активность mTORC1 и его субстратов, что важно для оценки патологического процесса и эффективности лечения.

Ключевые слова: Akt, инсулин, инсулиноподобный фактор роста 1, мононуклеары периферической крови, рак, диабет.