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Activation of extracellular signal-regulated kinase-1/2 in blood mononuclear cells of patients with diabetes and autoimmune thyroiditis

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Резюме. The peripheral blood mononuclear cells (PBMC) mainly includes monocytes and lymphocytes involved in the development of diabetes mellitus and other autoimmune diseases. Ret/Ras/Raf/MEK/ERK is a signaling cascade that controls cellular processes such as proliferation, survival, angiogenesis, cell growth and motility. **The aim** of the work was to study the activation of the main effector protein kinase of this cascade — ERK1/2 in PBMC of patients with diabetes and autoimmune (Hashimoto's) thyroiditis. **Material and methods.** The material of this work was the blood of healthy subjects, patients with type 2 diabetes, with type 2 diabetes and AIT, with type 2 diabetes and cancer (papillary thyroid carcinoma), with type 1 diabetes, with type 1 diabetes and AIT. Enzyme-linked immunosorbent assay kit 85-86012 («Invitrogen», USA) were used to determine the amount of phospho-ERK1/2 (p-Thr202/Thr204, Thr185/Tyr187, respectively). **Results.** Patients were divided into 6 groups: 1 — control — healthy subjects, representative for age and BMI, 2 — patients with type 2 diabetes, 3 — patients with type 2 diabetes and AIT, 4 — patients with type 2 diabetes and thyroid cancer, 5 — patients with type 1 diabetes, 6 — patients with type 1 diabetes and AIT. It was shown that activation of ERK1/2 in PBMC of patients with type 2 diabetes and cancer was not observed, while in patients with type 1 diabetes or autoimmune thyroiditis it increased significantly. However, in patients with type 1 diabetes with autoimmune thyroiditis, the activation of ERK1/2 in PBMC decreased to a control level, which can be explained by the competition between the two autoimmune processes for common signaling pathways. **Conclusion.** In PBMC of patients with autoimmune diseases (type 1 diabetes or AIT), MAPK/ERK cascade is activated.

Keywords: peripheral blood mononuclear cells, extracellular signal-regulated kinase-1/2, type 1 and type 2 diabetes, cancer, autoimmune thyroiditis.

Peripheral blood mononuclear cells (PBMC) mainly include monocytes/macrophages and lymphocytes — extremely flexible cells that are involved in cellular and humoral immunity. In particular, lymphocytes and macrophages are involved in pathogenesis of diabetes mellitus and other autoimmune diseases [1, 2].

Type 1 diabetes (T1D) is an autoimmune disease, in which pancreatic β-cells are destroyed by autoreactive T cells and inflammatory processes.

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In type 2 diabetes (T2D) macrophages, T-cells, B-cells, NK and other immune cell subtypes infiltrate the metabolic tissues, initiating a low-level inflammatory process [2].

Autoimmune (Hashimoto's) thyroiditis (AIT) is associated with the formation of auto-antibodies, which appear mostly in the presence of lymphocytes in the thyroid. Lymphocytes produce antibodies to thyroid peroxidase, thyroglobulin, and thyroid-stimulating hormone receptor and other proteins. AIT is characterized by wide invasion of the thyroid with lymphocytes and macrophages, which generates autoreactivity associated with T- and B-cells [3-5].

Ret/Ras/Raf/MEK/ERK is a signaling cascade which connects growth factor signals at cell membrane receptors with transcription factors, which regulate the expression of genes that control proliferation, survival, angiogenesis, cell growth and motility [6]. This signaling pathway is considered to be the principal in control of cell division [7]. It largely determines the functioning of blood cells in various diseases, including diabetes and its complications. Therefore, it was important to study the activity in the PBMC of the main effector protein kinase of this cascade — ERK1/2.

Material and methods

The study was conducted at the Department of Diabetology of the Institute. All patients signed informed consent for the use of biomaterials in further diagnostic and scientific studies. Immediately after sampling, the blood was diluted in 2-fold with PBS (pH 7.4), and PBMC were isolated as described previously [8]. The collected PBMC were washed with PBS by centrifugation at 200 g to remove platelets and frozen at –80 °C until use. To determine the amount of phospho-ERK1/2 (p-Thr202/Thr204, Thr185/Tyr187, respectively) kits for enzyme immunoassay 85-86012 («Invitrogen», USA) were used. Cells were lysed in extraction buffer containing protease and phosphatase inhibitors. The studies were performed in triplets. The protein concentration in the lysate was determined using Novagen (USA) kits (BCA protein assay kit). Measurements were made on a microplate reader from Bio-tek Instruments (USA) at a wavelength of 450 nm. The calibration curve **(Fig. 1)** indicates a satisfactory agreement of the experimental curve with the theoretical one and a slight data scatter.

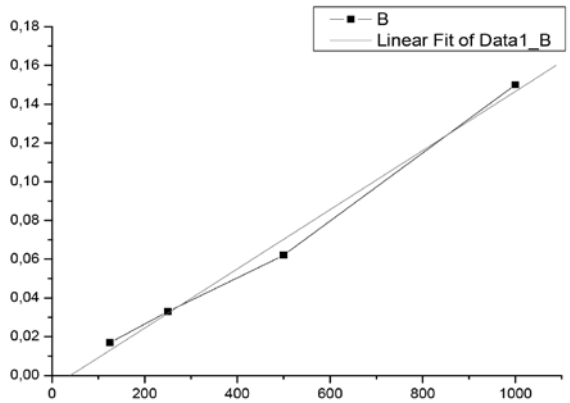

Fig. 1. Calibration curve for the determination of phospho-ERK1/2 by enzyme-linked immunosorbent assay: B — experimental line.

The results of the experiments were presented as M±m, n=3-13. Student's *t*-test and one-way ANOVA were used to compare the data groups. P values ≤0.05 were considered as significant.

Results and discussion

All patients were divided into 6 groups: 1 — control (n=3) — healthy subjects, representative for age and BMI, 2 — patients with type 2 diabetes (n=13), 3 — patients with type 2 diabetes and AIT (n=3), 4 — patients with type 2 diabetes and cancer (papillary thyroid carcinoma) (n=4), 5 — patients with type 1 diabetes (n=7), 6 — patients with type 1 diabetes and AIT (n=3).

It can be seen from the **table** that PBMC mainly consists of monocytes/macrophages and lymphocytes (T-cells, B-cells and NK) [9], which play a key role in the pathogenesis of T1D and T2D, diabetic complications and AIT [2, 4].

Phosphorylation of ERK1 (p44) on the residues Thr202/Thr204 and ERK2 (p42) on Thr185/Tyr187, means their activation. We determined the total phosphorylation of both protein kinases in the blood cells of the above groups.

Table. Cells that form the basis of human PBMC

Human PBMC composition	Percentage of total
Monocytes	10-30%
Lymphocytes	70-90%
All T cells (CD3+)	45-70%
Helper CD4+ T cells	25-60% of all T cells
Cytotoxic CD8+ T cells	5-30% of all T cells
B-cells	5-15%
NK cells	5-10%
Dendritic cells	1-2%
Stem cells (CD34+)	0,1-0,2%

Fig. 2-2 shows that there is no activation of ERK1/2 in PBMC of patients with T2D compared with control, whereas in PBMC of patients with T1D it increased more than 1.5 times **(Fig. 2-5)** that may be associated with the intensity of autoimmune processes in type 1 diabetes, in which PBMC are involved. In patients with T2D and AIT, activation of ERK1/2 in PBMC increased almost 2-times compared with control and more than 2 times compared with group 2 **(Fig. 2-2 and 2-3)**. There were no changes in amount of phosphorylated ERK1/2 in PBMC of patients with type 2 diabetes and cancer **(Fig. 2-4)**. The most interesting was the group of patients with T1D and AIT **(Fig. 2-6)**, where the decrease in protein kinase activation compared with T1D group (Fig. 2-5) down to the control level was observed.

Thus, in PBMC of patients with T2D, and with T2D + cancer (Fig. 2-2 and 2-4), which are characterized by intense infiltration of macrophages and lymphocytes into metabolic and cancer tissues, we did not observe activation of proliferative processes. It is possible that division of these cells may occur after infiltration. Autoimmune processes characteristic of patients with T1D or AIT (Fig. 2-3 and 2-5) cause enhanced ERK1/2 activation, which is probably related to increased lymphocytes/macrophages proliferation and cytokine secretion [10]. The decrease of ERK1/2 activation in PBMC of T1D and AIT patients to the control level may be associated with the imposition of two unidirectional processes on ERK1/2 activation. Activation of ERK1/2 is known to be a prerequisite for cell proliferation,

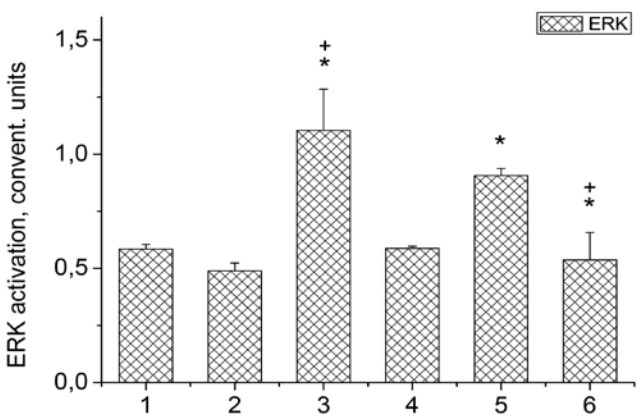

Fig. 2. Activation of ERK1/2 in PBMC of patients with diabetes mellitus and autoimmune thyroiditis: 1 — control (n=3), 2 — type 2 diabetes (n=13), 3 — type 2 diabetes and AIT (n=3), 4 — type 2 diabetes and cancer (n=4), 5 — type 1 diabetes (n=7), 6 — type 1 diabetes and AIT (n=3); M±m;
* — difference from control value is significant, p≤0.05;
+ — differences between groups 2 and 3, and 5 and 6 are significant, p≤0.05.

however, its hyperactivation sometimes leads to inhibition of the protein kinase through special cell-initiated mechanisms [11-13]. Because both AIT and T1D are associated with lymphocyte activation, proliferation, infiltration, and antibody formation, it is possible that the common signaling mechanisms initiated by pathogenic processes in these diseases compete with each other regarding ERK1/2 activation.

PBMC are critical components of the immune system because they form a response to foreign biomaterials entering the human body and to the existing cells transformed into a tumor type. PBMC are an extremely sensitive system that responds to stresses, diseases and other numerous pathological changes in homeostasis of the body, and the MAPK/ERK signaling cascade plays an important role in these reactions [14-18]. Researchers and clinicians use PBMC in areas relating to immunology, infectious disease, hematological malignancies, transplant therapy, personalized medicine, and toxicology.

Conclusion

Therefore, studying key signaling pathways in blood mononuclear cells may be important for disease diagnosis, prognosis, and evaluation of treatment efficacy.

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Активація кінази, що регулюється позаклітинними сигналами-1/2 (ERK1/2), у мононуклеарах крові людини за діабету та автоімунного тиреоїдиту

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Резюме. До складу мононуклеарних клітин периферичної крові (PBMC) в основному входять моноцити і лімфоцити, які беруть участь у розвитку цукрового діабету (ЦД), та інших автоімунних захворювань. Ret/Ras/Raf/MEK/ERK є сигнальним каскадом, який контролює такі клітинні процеси, як проліферація, виживання, ангіогенез, ріст і рухливість клітин. **Метою** роботи було дослідження активації в PBMC головної ефекторної протеїнкінази цього каскаду — ERK1/2. **Матеріал і методи**. Матеріалом роботи була кров здорових осіб, пацієнтів із діабетом 2-го типу, з діабетом 2-го типу й автоімунним тиреоїдитом (АІТ), із діабетом 2-го типу та раком щитоподібної залози, із діабетом 1-го типу, із діабетом 1-го типу й АІТ. Для визначення кількості фосфо-ERK1/2 (ф-Thr202/Thr204 і Thr185/Tyr187 відповідно) використовували набори для імуноферментного аналізу 85-86012 («Invitrogen», США). **Результати**. Пацієнтів розподілили на 6 груп: 1 — контроль — здорові особи, репрезентативні за віком та ІМТ, 2 — пацієнти з діабетом 2-го типу, 3 — пацієнти з діабетом 2-го типу й АІТ, 4 — пацієнти з діабетом 2-го типу та раком щитоподібної залози, 5 — пацієнти з діабетом 1-го типу, 6 — пацієнти з діабетом 1-го типу і АІТ. Показано, що активації ERK1/2 у PBMC хворих на діабет 2-го типу та рак не спостерігалося, тоді як у хворих на діабет 1-го типу або з АІТ вона суттєво зростала. Натомість у хворих на діабет 1-го типу з АІТ активація ERK1/2 у PBMC знижувалася до контрольного рівня, що можна пояснити конкуренцією між двома автоімунними процесами за спільні сигнальні шляхи. **Висновок**. У пацієнтів з автоімунними захворюваннями (діабет 1-го типу або АІТ) у PBMC відбувається активація MAPK/ERK-каскаду.

Ключові слова: мононуклеарні клітини периферичної крові, кіназа, що регулюється позаклітинними сигналами-1/2, діабет 1-го та 2-го типів, рак, автоімунний тиреоїдит.

Активация регулируемой внеклеточными сигналами киназы-1/2 (ERK1/2) в мононуклеарах крови человека при диабете и аутоиммунном тиреоидите

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Резюме. В состав мононуклеарных клеток периферической крови (PBMC) в основном входят моноциты и лимфоциты, участвующие в развитии сахарного диабета и других аутоиммунных заболеваний. Ret/Ras/Raf/MEK/ERK является сигнальным каскадом, который контролирует такие клеточные процессы, как пролиферация, выживание, ангиогенез, рост и подвижность клеток. **Целью** работы было исследование активации в PBMC главной эффекторной протеинкиназы этого каскада — ERK1/2. **Материал и методы.** Материалом работы была кровь здоровых лиц, пациентов с диабетом 2-го типа, диабетом 2-го типа и аутоимунным тиреоидитом (АИТ), с диабетом 2-го типа и раком щитовидной железы, с диабетом 1-го типа, с диабетом 1-го типа и АИТ. Для определения количества фосфо-ERK1/2 (ф-Thr202/Thr204 и Thr185/Tyr187 соответственно) использовали наборы для иммуноферментного анализа 85-86012 («Invitrogen», США). **Результаты.** Пациентов распределили на 6 групп: 1 — контроль — здоровые лица, репрезентативные по возрасту и ИМТ, 2 — пациенты с диабетом 2-го типа, 3 — пациенты с диабетом 2-го типа и АИТ, 4 — пациенты с диабетом 2-го типа и раком щитовидной железы, 5 — пациенты с диабетом 1-го типа, 6 — пациенты с диабетом 1-го типа и АИТ. Показано, что активации ERK1/2 в PBMC больных диабетом 2-го типа и раком не наблюдалось, тогда как у больных диабетом 1-го типа или АИТ она существенно возрастала. Однако у больных диабетом 1-го типа с АИТ активация ERK1/2 в PBMC снижалась до контрольного уровня, что можно объяснить конкуренцией между двумя аутоиммунными процессами за общие сигнальные пути. **Вывод.** У пациентов с аутоиммунными заболеваниями (диабетом 1-го типа или АИТ) в PBMC происходит активация MAPK/ERK-каскада.

Ключевые слова: мононуклеарные клетки периферической крови, регулируемая внеклеточными сигналами киназа-1/2, диабет 1-го и 2-го типов, рак, аутоиммунный тиреоидит.