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DNA COPY NUMBER VARIATIONS (14 CANCER-ASSOCIATED GENES) IN NON-SMALL CELL LUNG CANCER

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Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers, 15-30% of which is squamous cell carcinoma, 54% is adenocarcinoma. The copy number variations (CNVs) as one of the factors affecting gene transcription activity is necessary to assess the role of genetic variation in pathological processes. The purpose of our study was to research the relative number of copies of 14 onco-associated genes: *APC, AURCA, CCND1, GKN1, PIK3CA, NKX2-1, ERBB2, SOX2, EGFR1, BRCA1, BRCA2, TP63, CDKN2A, MDM2,* in lung tissue samples as tumor markers of lung cancer. The study included 72 patients with Slavic and Crimean Tatar (Crimean) population, aged 46-78 (median 64) years with a diagnosis of lung cancer T1-1aN0-2M0-1 (stage I-IV). The relative copy number variation of genetic loci was assessed by the RT-qPCR method. In our study, statistically significant CNV change events ($p<0.05$) were recorded for the *CCND1*, *GKN1*, *PIK3CA*, *EGFR1*, *SOX2*, *BRCA2*, *TP63*, *MDM2* genes in squamous cell carcinoma samples and NKX2-1 in lung adenocarcinoma samples. Thus, these genes can be used as differentiating and diagnostic biomarkers in NSCLC.

Keywords: lung cancer, copy number variations, squamous cell lung cancer, lung adenocarcinoma, biomarkers.

Introduction. Lung cancer is associated with poor prognosis and is the leading cause of cancer death [4]. Non-small cell lung cancer (NSCLC) accounts for 85% of all types of lung cancer, 15-30% of which is squamous cell lung cancer, 54% is lung adenocarcinoma [16]. Despite studies of various treatment options, patients diagnosed with NSCLC (all stages) have a mortality rate of over 50% at 1 year and an overall 5-year survival rate of less than 18% [20].

The molecular basis of lung cancer is the gradual accumulation of genetic and epigenetic changes in the cell nucleus. These changes lead to a weakening of the DNA structure and its greater susceptibility to subsequent mutations. Due to the tumor process in the cells, the mechanisms that control their division and location are violated. This is caused by disturbances in the regulation of the cell cycle (mutations of proto-oncogenes and suppressor genes) and disturbances in the processes of repair of damaged DNA. Further changes, such as increased expression of growth factors, sustained angiogenesis, avoidance of apoptosis (mutations of anti-apoptotic and pro-apoptotic genes), limitless replicative potential and tissue invasion and metastasis, affect tumor progression [14].

Among other changes, lung cancer is characterized by genomic instability leading to a high frequency of somatic mutations and extensive genomic changes in individual genomes [9]. Change in the number of copies (English copy number variations, CNV) means a change in genomic DNA, characterized by a change in the DNA sequence numbers in the normal (diploid) genome. These DNA changes can affect individual genes, chromosomal regions, or entire chromosomes. CNVs have been shown to be associated with lung cancer as well as a number of other malignancies [7]. Generally, in cancer, a decrease or increase in DNA copy number can affect tumor suppressor genes and oncogenes, respectively. CNVs play an important role in the etiology of the disease. Understanding the association of CNV with diseases will help in the early detection and prognosis of the outcome of these diseases, and will also determine the most effective treatment strategies for patients.

Purpose of the study. To study the copy number of 14 cancer-associated genes APC, *AURCA*, *CCND1*, *GKN1*, *PIK3CA*, *NKX2-1*, *ERBB2*, *SOX2*, *EGFR1*, *BRCA1*, *BRCA2*, *TP63*, *CDKN2A*, *MDM2* in lung tumor tissue relative to conditionally healthy tissue as potential lung cancer tumor markers.

Materials and methods. The study included 72 Caucasoid patients living in the Republic of Crimea, aged 46-78 years (median 64) diagnosed with lung cancer T1-1aN0-2M0-1 (stage I-IV), who underwent planned treatment at the Medical Academy named after S.I. . Georgievsky, Federal State Autonomous Educational Institution of Higher Education "KFU named after W.I. Vernadsky" in 2015-2020 (Table 1). All patients voluntarily signed an informed consent to the processing of personal data and the transfer of information constituting a medical secret, as well as to the transfer of biological material. The study was carried out in accordance with the ethical principles of biomedical research, reflect-

S.I. Georgievsky Medical Academy FSAI HE V.I. Vernadsky Crimea Federal Uniersity: **ALNSOUR Rashed D.** – postgraduate student of the Department of Oncology, xitrilis@mail.ru, **OLEKSEENKO Victor V.** – MD, Associate Professor, Head of the Departmen, FSBI NMRC of oncology, Ministry of Health of the Russian Federation, Rostov-on-Don: **PETRUSENKO Natalya Alexandrovna** – junior researcher; **MAKSIMOV Alexey Yurievich** – MD, Prof. Deputy Gen. Director, **STATESHNY Oleg Nikolayevich** – oncologist.

ed in the Declaration of Helsinki of the World Medical Association.

Extraction of total DNA from paraffin blocks of tumor and apparently healthy tissue (tissue samples fixed in 10% buffered formalin) was performed using the DNA-sorb-B kit (AmpliSens) [1]. The relative copy number variation of 14 genetic loci: *APC, AURCA, CCND1, GKN1, PIK-3CA, NKX2-1, ERBB2, SOX2, EGFR1, BRCA1, BRCA2, TP63, CDKN2A,* and *MDM2* was assessed by RT-qPCR. Each 25 µl PCR mixture contained 10 ng of genomic DNA, 0.2 mM dNTP's, 600 nM forward and reverse primers, 2.5 mM MgCl2, 1x PCR buffer, 0.05 u/µl Thermus aquaticus DNA polymerase (Synthol ", Russia). EvaGreen (Biotium, USA) was used as a dye. Amplification of each of the samples was carried out in triplicate using a CFX96 thermal cycler (Bio-Rad, USA) according to the following program: 95°C for 3 min, and 40 cycles at 95°C for 10 s, 60°C for 30 s (reading the FAM optical signal for EvaGreen) and 72°C for 15 s. Analysis of primary RT-qPCR data was performed using Bio-Rad CFX Manager (ver. 2.1) software [2]. GAPDH and B2M genetic loci were used as reference ones. Primer sequences for all genetic loci were designed using the NCBI GenBank database in the Primer-BLAST program (Table 2).

The relative copy number variation of the genetic locus (RCQ) was calculated using the formula 2-ΔCt. The dose of the studied locus was considered equal to the diploid set (2n) if the tumor/normal RCQ ratio was ~1. If the RCQ tumor/normal ratio was >1.5 or <0.5, the locus dose was considered increased (>3n) or decreased (<1n), respectively. Statistical analysis and assessment of the significance of differences were performed using the Mann-Whitney test, chi-square test, OR with an indication of the confidence interval (95% CI) and the upper and lower limits, using the Statistica v.10 program. The results of the analysis were considered statistically significant for the Mann-Whitney test and chi-square at $p<0.05$.

Results and discussion. The results obtained in this study are illustrated in Table 3 and Figure 1.

In our study, both an increase in the relative copy number and a loss for genes in lung tumor tissues relative to conditionally healthy ones were noted. Thus, for lung adenocarcinoma, a statistically significant increase in the dose of genes was observed: *NKX2-1* (p=0.049), *SOX2* (p=0.041), *BRCA1* (p=0.032), and losses for the *TP63* gene (p=0.043). In squamous cell lung cancer, amplification of genetic loci was noted: *CCND1* (p=0.007), *PIK3CA* (p=0.005), *NKX2-1* (p=0.006), *ERBB2* (p=0.045), *SOX2* (p=0.039), *TP63* (p= 0.005) (table 3, figure 1).

According to the obtained data on the relative copy number variation of genes, squamous cell carcinoma is characterized by a significant increase in the relative copy number for the *CCND1, GKN1, PIK3CA, EGFR1, SOX2, BRCA2, TP63, MDM2* genes, occurring respectively in 5.2; 5; 7.2; 3.4; 13.1; 4.8; 4.2; 4.8 times more often than with adenocarcinoma. And for adenocarcinoma of the lung, a characteristic increase in the relative copy number of the *NKX2-1* gene is 3.3 times more frequent than in squamous cell carcinoma (Table 4).

The *PIK3CA, SOX2,* and *TP63* genes located in the chromosomal region 3q were amplified more frequently in squamous cell carcinoma than in lung adenocarcinoma in this study. It is known that the most significant events in the course of carcinogenesis of squamous cell carcinoma include amplification of 3q and losses in the 3p and 9p chromosomal regions. 3q amplification is also associated with tumor progression [15].

The frequency of *SOX2* amplification in our study was 59.6% of cases of squamous cell carcinoma, 10% of cases of adenocarcinomas. In their work, Erdem et al also showed that *SOX2* amplification was predominantly observed in squamous cell carcinoma with mutations in the *TP53* gene, in addition, there was a correlation between *SOX2* and T53 mRNA levels in different samples [5]. SOX2, as a transcription factor, mainly manifests its oncogenic activity by changing gene expression. In this context, the study by Fukazawa et al. showed that SOX2 suppresses the expression of CDKN1A (a cell cycle inhibitor) and, through this mechanism, supports the growth of squamous lung tumor cells [6]. There is also evidence that high levels of SOX2 amplification are associated with a better prognosis in squamous cell lung cancer [12].

In the present study, amplification of the *PIK3CA* gene was more often observed in squamous cell lung cancer (64.3%). *PIK3CA* encodes the catalytic subunit p110 α of phosphatidylinositol 3'-kinase (PI3K). PI3K is a protein kinase that phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to form phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 is the second messenger that activates protein kinase B (Akt), which is a serine/threonine-specific protein kinase. Akt inhibits apoptosis and promotes cell proliferation. The PIK3CA gene is oncogenic and its aberrant changes have

Table 1

Clinical characteristics of groups of patients with lung cancer

Characteristic	Variables	Lung adenocarcinoma (n=30), абс.(%)	Squamous cell lung cancer (n=42), абс.(%)
Age of patients (years)	<50	3 (10)	3 (7.1)
	51-60	4 (13.3)	12 (28.6)
	61-70	16 (53.3)	23 (54.8)
	>70	7 (23.3)	4 (9.5)
Sex	men	19 (63.3)	33 (78.6)
	women	11 (36.7)	9 (21.4)
TNM	T1-1aN0-2M0	4 (13.3)	3 (7.1)
	T2-2bN0-2M1	18 (63.3)	34 (81)
	T3N0-2M0	5 (16.7)	4 (9.5)
	T4N0-2M0	2 (6.7)	1 (2.4)
The degree of tumor differentiation	G1	2 (6.7)	2 (4.8)
	G2	18 (60)	26 (61.9)
	G3	10 (33.3)	14 (33.3)

Table 2

A panel of primers for identification the relative copy number of genes

№	Gene	Chromosomal location	F (forward)	R (reverse)
1	*APC*	5q22	ATTCCCGGGGCAGTAAAGAG	TGCCTCTCTTGTCATCAGGC
2	*AURCA*	20q13.2	TGAAATTGGTCGCCCTCTGG	CTGAGCTGATGCTCCACTCC
3	*CCND1*	11q13.3	GGTGAACAAGCTCAAGTGGAAC	CCGGCCAGGGTCACCTAA
4	*GKN1*	2p13.3	CAACAATGCTGGAAGTGGGC	CAGGAGTCCCATCCGTTGTT
5	*PIK3CA*	3q26.3	GCTTGGGAGGATGCCCAAT	GCTGTGGAAATGCGTCTGGA
6	*NKX2-1*	14q13.3	ACCAAGCGCATCCAATCTCA	CCCTAGCGTGGAAAACCCAT
7	*ERBB2*	7p11.2	CAAGGACCACTCTTCTGCGT	CTTGAATGGCAACGCTCCTC
8	*SOX2*	17q12	TTTGTCGGAGACGGAGAAGC	CCGGGCAGCGTGTACTTAT
9	*EGFR1*	3q26.3-q27	GCCAAGTAAGGGCGTGTCT	GGCCGAAGAACGAAACGTC
10	*BRCA1*	17q21.31	GTAGCCCCTTGGTTTCCGTG	CCCTTTCCCGGGACTCTACT
11	*BRCA2*	13q13.1	TGCATCCCTGTGTAAGTGCAT	ACGTACTGGGTTTTTAGCAAGC
12	*TP63*	3q28	GCACAAGGTTGATGTAAAGTGGC	GGGATGCCTTTTGTAGCTCTTG
13	*CDKN2A*	9p21.3	GCCACATTCGCTAAGTGCTC	CAAATCCTCTGGAGGGACCG
14	*MDM2*	12q15	TCTTTGGGACCCATCTACCCT	AGAATGCTTTAGTCCACCTAACCTT
15	*GAPDH*		GCTGAACGGGAAGCTCACT	GCAGGTTTTTCTAGACGGCAG
16	*B2M*		TGAGTGCTGTCTCCATGTTTGA	ATTCTCTGCTCCCCACCTCT

been noted in many types of cancer. For example, taking into account the mutational status of PIK3CA with the CNV status is important in predicting the outcome in patients with cervical cancer [13].

In our study, the TP63 gene was amplified in 64.3% of squamous cell carcinoma tumors, the RCQ level (RCQ=1.96, p=0.005) was 3.6 times higher than in adenocarcinoma. Thus, losses in the TP63 gene were observed in lung adenocarcinoma (RCQ=0.54, p=0.043). The TP63 gene may be a differentiating marker for squamous cell lung cancer and lung adenocarcinoma. It has been noted that the TP63 gene is often amplified or overexpressed in primary squamous cell carcinomas of the head and neck [11].

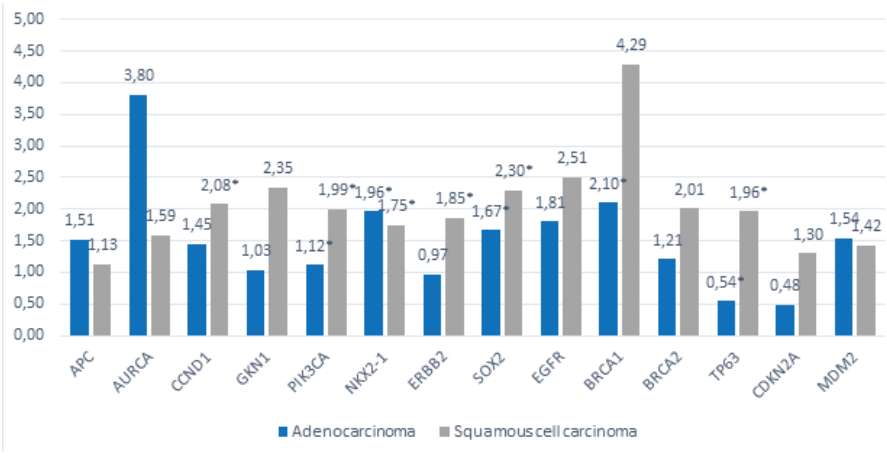
We observed a statistically significant increase in the dose of the BRCA1 gene (60%) in lung adenocarcinoma. The identification of inherited mutations in BRCA1 and BRCA2 has led to the successful use of PARP inhibitory therapy in breast and ovarian cancer. Detection of germline mutations in lung cancer may also be useful, similar to the benefit obtained from screening for pathogenic mutations in *BRCA1* and *BRCA2* [17].

In the present study, *CCND1* gene was amplified in 69% of squamous cell tumors and its RCQ level was 1.4 times higher than in lung adenocarcinoma (p=0.007). CCND1 regulates over 35 different transcription factors. The oncogenic role of CCND1 has been demonstrated in various studies, with overexpression of *CCND1* noted in numerous human cancers, including thyroid cancer, adenocarcinoma of the lung, liver, colon, and prostate [19]. CCND1 activates the MAPK/PI3K-AKT signaling pathway, and overexpression of the corresponding gene neutralizes the FGFR1 effect on MAPK/PI3K-AKT signaling, suggesting that FGFR1 partially inhibits the MAPK/PI3K-AKT signaling pathway by down-

Table 3

Frequency of amplifications and gene losses in a group of patients with non-small cell lung cancer

Gene	Adenocarcinoma (n=30)		Squamous cell carcinoma (n=42)	
	RCQ>1,5 абс. (%)	RCQ<0,5 абс. (%)	RCQ>1,5 абс. (%)	RCQ<0,5 абс. (%)
APC	12 (40)	3 (10)	10 (23.8)	2 (4.8)
AURCA	15 (50)	6 (20)	13 (31)	4 (9.5)
CCND1	9 (30)	3 (10)	29 (69)	0
GKN1	3 (10)	6 (20)	15 (35.7)	4 (9.5)
PIK3CA	6 (20)	3 (10)	27 (64.3)	4 (9.5)
NKX2-1	18 (60)	3 (10)	13 (31)	4 (9.5)
EGFR1	9 (30)	0	25 (59.6)	4 (9.5)
ERBB2	6 (20)	3 (10)	15 (35.7)	4 (9.5)
SOX2	3 (10)	3 (10)	25 (59.6)	0
BRCA1	18 (60)	3 (10)	15 (35.7)	8 (19.)
BRCA2	6 (20)	9 (30)	23 (54.8)	6 (14.3)
TP63	9 (30)	3 (10)	27 (64.3)	4 (9.5)
CDKN2A	15 (50)	3 (10)	23 (54.8)	4 (9.5)
MDM2	6 (20)	3 (10)	23 (54.8)	2 (4.8)



Показатели относительной копийности генов при немелкоклеточном раке легкого. * Статистически значимые различия от условно нормальной ткани с использованием критерия Манна-Уитни (p<0,05).

regulating CCND1. Yang et al showed that expression of the nuclear proteins CCND1 and FGFR1 is correlated and unregulated in squamous cell lung cancer. Also, CCND1 was not associated with overall survival in lung adenocarcinoma, but was associated with poor prognosis in squamous cell lung cancer [19].

In our study, the RCQ level of the *ERBB2* gene was statistically significantly higher by 1.9 times in squamous cell lung cancer. *ERBB2* has been extensively studied in breast cancer. Its amplification or overexpression of the encoded protein has become a biomarker for anti-ERBB2 targeted therapy in breast cancer. However, mutations in this gene are also common in lung cancer. ERBB2 consists of an extracellular domain that contains two -L receptor domains and a furin-like cysteine-rich domain, a trans-

Table 4

Results of the statistical analysis of the search for associations between the relative copy number variation of genes in adenocarcinoma and squamous cell lung cancer

	RCQ>1,5			RCQ<0,5		
	OR	CI 95% (lower - upper limit)	χ 2, p	OR	CI 95% (lower - upper limit)	χ 2 , p
APC	2.133	0.770-5.909	2.162. p=0.142	2.222	0.348- 14.199	0.743. p=0.389
AURCA	2.231	0.846-5.882	2.672. p= 0.103	2.375	0.607- 9.295	1.606. p= 0.206
CCND1	**0.192**	0.069-0.532	**10.706.** p= 0.002	н/р	н/р	4.383. p= 0.037
GKN1	**0.200**	0.052- 0.771	**6.171.** p= 0.013	2.478	0.632- 9.724	1.606. p=0.206
PIK3CA	**0.139**	0.046- 0.415	**13.825.** p<0.001	1.056	0.218- 5.105	0.005. p= 0.947
NKX2-1	**3.346**	1.255- 8.921	**6.023.** p= 0.015	1.056	0.218- 5.105	0.005. p= 0.947
EGFR1	**0.291**	0.108- 0.788	**6.120.** p= 0.014	н/р	н/р	3.025. p= 0.082
ERBB2	0.450	0.151- 1.345	2.818. p= 0.094	1.056	0.218- 5.105	0.005. p=0.947
SOX2	**0.076**	0.020- 0.289	**18.060.** p<0.001	н/р	н/р	4.383. p=0.037
BRCA1	1.700	0.621-4.657	**4.157.** P=0.042	0.472	0.114-1.953	1.107. p=0.293
BRCA2	**0.207**	0.070-0.609	**8.243.** p=0.005	2.571	0.802-8.242	2.620. p=0.106
TP63	**0.238**	0.087-0.650	**8.229.** P=0.005	1.056	0.218-5.105	0.005. p=0.947
CDKN2A	0.826	0.323-2.112	0.159. P=0.690	1.056	0.218-5.105	0.005. p=0.947
MDM2	**0.207**	0.070-0.609	**8.791.** p=0.004	2.222	0.348-14.199	0.743. p=0.389

Note: statistically significant differences are highlighted in bold; n/r - not calculated, because there was no group with the studied trait for a certain gene in the sample.

membrane domain (TMD) and an intracellular structure that contains a tyrosine kinase domain (TKD) and a carboxy-terminal tail. Wei et al showed that a non-TKD mutation accounted for more than half of the *ERBB2* mutations, a significant proportion of which were oncogenic. A mutation in the *ERBB2* gene was a poor prognostic factor in non-small cell lung cancer. A mutation in the *ERBB2* gene that is not associated with TKD can also be used as a therapeutic target in ERBB2-targeted therapy [18].

A statistically significant increase in the relative copy number variation of the *MDM2* gene occurred 4.8 times more often in squamous cell lung cancer than in adenocarcinoma. The *MDM2* gene is an oncogene that promotes cell growth, survival, invasion, and therapeutic resistance. MDM2 is a protein with multiple functions, of which the most widely studied function is E3 ubiquitin ligase. The main function of MDM2 is the regulation of p53. Clinical studies have shown that *MDM2* gene amplification occurs in several tumor types and tends to correlate with the presence of wild-type p53 [10].

The *NKX2-1* gene in our study was amplified 3.3 times more often (60%) in lung adenocarcinoma. The *NKX2-1* (*TTF-1*) gene encodes thyroid transcription factor 1 (TTF-1), a homeodomain-containing transcription factor for lung morphogenesis and lung epitheliocyte differentiation. Hokari S. et al. in their study also showed that *TTF-1* is expressed in 75-80% of cases of lung adenocarcinoma [8]. Thus, the NKX2-1 gene may be a marker that differentiates lung adenocarcinoma from squamous cell lung cancer.

Conclusions. The treatment of NSCLC has evolved over the past 10 years as a result of a better understanding of the heterogeneity of lung cancer and the molecular abnormalities that underlie this heterogeneity, with the consequent development of targeted therapies and immunotherapies that ushered in the era of personalized medicine [3]. Our study demonstrated statistically significant CNV (RCQ) change events for nine of the 14 studied genes that differentiated squamous cell carcinoma (*CCND1, GKN1, PIK3CA, EGFR1, SOX2, BRCA2, TP63, MDM2*) and lung adenocarcinoma (*NKX2-1*). Accordingly, these genes can be used as NSCLC biomarkers and promising targets for targeted therapy.

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THE EFFECT OF OBESITY ON SEXUAL DIMORPHISM OF IRISIN LEVELS

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The aim of this study is to conduct a comparative analysis of average irisin levels between female and male (with normal weight and obese) to assess sexual dimorphism. Circulating levels of irisin in the blood of 279 Yakuts (185 female, 94 male, average age 19.8 ± 2.03 years) were determined. A comparative analysis of irisin levels between male and female in three BMI groups (underweight, normal weight, overweight/obesity) was carried out. The average level of irisin in the blood plasma in female was 8.33 ± 2.74 mcg/mL, and in male 7.76 ± 1.86 mcg/mL. Sexual dimorphism (p = 0.02) was detected in Yakuts with normal weight, where the level of irisin was higher in women (8.42 = 2.92 mcg/mL) compared to men (7.51 = 1.61 mcg/mL). Conducted a comparative analysis of irisin levels between male and female based on global data, were including this analysis are 2132 people. The age of the participants ranged from 18 to 61 years old. The meta-analysis was carried out for two different BMI groups: the first group included people with normal weight (18.5-24.9 kg/m^2), the second group included people with varying degrees of obesity (>30 kg/m^2). Comparative analysis of irisin levels in a large sample revealed statistically significant sexual dimorphism, where irisin levels were also higher in female compared to male, only in a sample of obese people (p = 0.02), in a sample of people with normal weight, no sexual differences were found (p = 0.09). Thus, the influence of obesity on sexual dimorphism was revealed.

Keywords: irisin, obesity, adipose tissue, Yakut population.

Introduction. During the previous decade, adipose tissue and skeletal muscles were recognized as endocrine organs secreting hormones adipokines and myokines, respectively. It is believed that there is a certain relationship between muscles and adipose tissue [24], which may be crucial for the regulation of body weight and metabolism, but specific metabolic pathways and mediators remain unclear [12]. Irisin is a short-lived myokine and is produced by proteolytic cleavage of fibronectin type III domain-containing protein (FNDC5) in response to the activation of gamma coactivator 1 of the alpha receptor activated by the proliferator peroxisome (PGC-1 α) [1]. Although irisin is primarily known as a myokine, it can also be an adipomyokine, since it is produced in adipocytes [1]. There are several factors known to affect the levels of irisin circulating in the blood, such as physical activity and diseases such as obesity and type 2 diabetes mellitus (DM2).

Irisin is mainly produced by skeletal muscles during aerobic exercises (running, swimming and treadmill workouts) [19-23, 33]. With intense physical exertion, an increase in the concentration of irisin occurs after 30-60 minutes [23], but after 90 minutes of training, irisin levels no longer increase [2]. In addition, prolonged training leads to a general decrease in the level of circulating irisin, so athletes have rather low levels compared to people who lead a sedentary lifestyle [5].

On the other hand, irisin can be produced by adipocytes, and numerous studies show that the levels of irisin circulating in the blood are significantly higher in obesity and in a prediabetic state [13, 15, 16, 18, 25, 31]. It is believed that in obesity and in a prediabetic state, irisin may be involved in general cycles of compensatory mechanisms, in which the recorded increased amount of irisin is explained by its increased secretion in an attempt to increase energy consumption due to browning of white adipose tissue or other, as yet unidentified effects in skeletal muscles [4, 23, 28]. Sesti et al., [27] suggested that in obesity, an increased level of irisin is an adaptive mechanism for preserving beta cells from overload, and can compensate for impaired insulin sensitivity. However, in T2D, irisin levels begin to decrease over time and become significantly lower in the decompensation stage than in people without diabetes [4, 14, 34]. This pattern is probably related to the dysfunction of β-cells, due to the depletion of their adaptive abilities to insulin resistance [3, 9].

Sexual dimorphism of irisin levels remains an open question, as there are many contradictory results. Some studies do not find differences in irisin levels between male and female [4, 5, 10-13,