ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2018. Vol. 34. N 6. P 445–460
doi: http://dx.doi.org/10.7124/bc.000990

UDC 577.218+616.65

Expresion patterns of genes, regulating lipid metabolism in prostate tumors

G. V. Gerashchenko[1], O. A. Kononenko[2], Yu. M. Bondarenko[3], E. O. Stakhovsky[2], V. I. Kashuba[1,4]

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03680

[2] National Cancer Institute
33/43, Lomonosova Str., Kyiv, Ukraine, 03022

[3] State Institution «Institute of Urology of NAMS of Ukraine»
9-a, Yu. Kotsubyns'koho Str., Kyiv, Ukraine, 04053

[4] Karolinska Institutet
Stockholm SE-171 77, Sweden
g.v.gerashchenko@imbg.org.ua

Aim. To assess relative expression (RE) levels of genes involved in lipid metabolism in prostate tumors. To define clinically significant specific alterations on the basis of the expression pattern. **Methods.** RE levels were analyzed in 37 samples of prostate cancer tissues by quantitative RT-PCR. The tumors were of a different Gleason score (GS) and various stages; the paired conventionally normal prostate tissue (CNT) samples and 20 samples of prostate adenomas were also analyzed. **Results.** Increased RE levels of *FASN* and *COX2* were found in an adenocarcinoma group and in adenocarcinomas with GS=7 compared to the adenoma group. Four genes, namely *FASN*, *LDLR*, *HMGCR* and *COX2*, demonstrated significant RE alterations in the adenocarcinoma groups at different stages compared to the adenoma and CNT groups. Expression of three genes (*LDLR*, *HMGCR*, *COX2*) showed a negative correlation with stage and GS in the adenocarcinoma group. For *FASN*, *LDLR*, *HMGCR*, several positive correlations of RE with levels of the epithelial cell markers were found. *CPT1C* and *COX2* demonstrated positive correlations of RE with expression of mesenchymal, fibroblast and inflammation markers in the adenocarcinoma group. **Conclusions.** The studied genes controlling lipid metabolism showed differential RE in prostate cancer samples. RE levels of *FASN*, *HMGCR* and *COX2* might be used as markers of sensitivity and efficacy of inhibitory drugs. Further studies are needed to confirm these data in a larger patient cohort.

Keywords: prostate tumors, relative gene expression, lipid metabolism, pharmacological markers.

© 2018 G. V. Gerashchenko *et al.*; Published by the Institute of Molecular Biology and Genetics, NAS of Ukraine on behalf of Biopolymers and Cell. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited

Introduction

Alterations in a lipid metabolism pathway serve to metabolic reprogramming of cancer cells [1, 2]. Lipid metabolism in cancer cells includes the next major steps: import of lipids (Low density lipoprotein (LDL) and high density lipoprotein (HDLR) are involved in this step); catabolism (fatty lipid oxidation (FAO) and tricarboxylic acid cycle (TCA cycle)); lipogenesis; cholesterol synthesis; export of lipoproteins and cholesterol and lipid storage. All these processes are usually altered upon tumor development and depend on signals of tumor microenvironment [3, 4].

The uptake of exogenous cholesterol and LDL functions via a low density lipoprotein receptor (LDLR) [2]. Actually, this major pathway mediates the tumor growth. Low density lipoprotein (LDL) is normally bound to the cell membrane. This protein is internalized, ending up in lysosomes where LDL is degraded; then, cholesterol can repress a microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGCR*). HMGCR is the rate-limiting enzyme in the cholesterol synthesis. Cholesterol plays important role in steroid synthesis, in regulation of several cell functions and pathways, including regulation of the PI3K/Akt pathway [5].

Numerous genes, involved in the cholesterol-related pathways and FAO, are crucial in tumor growth [6]. There is a FAO-limiting enzyme, the carnitine palmitoyltransferase 1 (*CPT1*). *CPT1* has three isoforms, that are expressed in different tissues and have own fermentative kinetics [7]. Usually, *CPT1A* is expressed in liver and *CPT1C* — in brain [8]. It is known that both isoforms, A and C (*CPT1A* and *C*) are overexpressed in many tumor types [7]. The *CPT1C* upregulation, induced by AMPK and p53, can protect cancer cells from the death under the deprived glucose and oxygen conditions [9]. This makes cancer cells resistant to glucose- and oxygen-deprivation [7]. Moreover, despite *CPT1C* is quite seldom expressed in metastases of epithelial tumors, it might be involved in the regulation of metastasizing [10]. Knocking down of *CPT1* sensitizes cancer cells to radiotherapy and to drugs, inducing apoptosis [11].

One of the key enzymes in the lipids synthesis is Fatty acid synthase (*FASN*) [1]. Its overexpression predicts a poor survival of cancer patients [2]. *FASN* is detected in the precancerous lesions and it persists in metastatic breast and prostate tumors [12]. *FASN* might function as an oncogene [13].

A special place in the development of epithelial cancers belongs to the metabolism of prostaglandins, which are lipid messengers with many functions, for example, regulation of a cancer-stroma interaction [14]. Prostaglandins are derivatives of arachidonic acid, which are synthesized by prostaglandin-endoperoxide synthase (*PTGS*), also known as cyclooxygenase. It is a key enzyme in the prostaglandin biosynthesis. There are two isozymes of PTGS with different regulation of expression and tissue distribution: constitutively expressing *PTGS1* (*COX1*) and inducible *PTGS2* (*COX2*) [15]. COX2 produces prostaglandins through the stimulation signals [16]. It is known for hundreds of years, that COX2 inhibitors evoke anti-inflammatory effects [15], and only 15 years ago the anti-proliferative activity of COX2 inhibitors was demonstrated, also in prostate

carcinogenesis [17, 18]. Now many COX2 inhibitors are proposed for the treatment of several diseases, usually targeting inflammation and pain [15].

Many proteins, involving in lipid metabolism are the putative targets for anti-cancer therapy. Some drugs with anti-inflammatory and immunomodulation effects, approved by the FDA for non-cancerous diseases, demonstrate an anti-cancer effect [11, 13, 15]. For example, Cerulenin, Orlistat, C75, Triclosan and EGCG, that were developed as FASN inhibitors, are now in pre-clinical trials for solid tumors [1, 19]. Moreover, Orlistat has already demonstrated efficacy in the treatment of prostate cancer [20, 21].

Another group of equally well-known drugs, namely Statins, is now tested in the anti-cancer treatment. These compounds are inhibitors of a 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) [22], and are now in clinical trials. The statin designated as Simvastatin [23], is tested now at a preclinical stage for the treatment of prostate cancer [1]. Noteworthy, *LDLR* could be a target for the anticancer drug delivery [24]. CPT1 inhibitors Etomoxir, Ranolazine and ST1326 are in clinical trials to treat prostate cancer and leukemia, respectively [25, 26, 27]. Celecoxib, the COX2 inhibitor, known as the drug, targeting lipid mediators of tumor-stroma interaction is used now in clinical trial for breast cancer [28].

Summaring, it is important to assess RE of the above mentioned genes and to understand if the described drugs would be effective for treatment of prostate cancer. We think, that the RE levels of these genes could serve as ”pharmacological markers”, describing the probable drug sensitivity and treatment efficiency. These data would also help to choose the treatment method.

Materials and Methods

Prostate tissue collection. Tissues of prostate cancer (T) and the paired conventionally normal prostate tissues (CNT, or N from a side, opposite to cancer) were frozen in liquid nitrogen directly after surgery. All samples were collected at National Cancer Institute (Kyiv, Ukraine). Benign prostate tumors (prostate adenoma samples) were collected with the same procedure at the Institute of Urology (Kyiv, Ukraine) after radical prostatectomy. The samples were collected in accordance with the Declaration of Helsinki and the guidelines, issued by the Ethic Committee of the Institute of Urology and the National Cancer Institute of a National Academy of Medical Sciences of Ukraine and an Ethic Committee of the Institute of Molecular Biology and Genetics of NAS of Ukraine. 37 prostate adenocarcinomas of different GS and stages, 37 paired CNT and 20 samples of benign prostate tumors (A, adenomas) were studied. The Tumors were characterized, according to the International System of Classification of Tumors based on the tumor-node-metastasis (TNM) and the World Health Organization (WHO) criteria classification. The clinical and pathological characteristics (CPC) of the prostate cancer samples were described earlier [29].

Total RNA isolation and cDNA synthesis. 50–70 mg of frozen prostate tissues were mashed to powder in liquid nitrogen. Total RNA was extracted by TRI-reagent (SIGMA), according to the manufacturer’s protocol. The total RNA concentration was analyzed by a spectrophotometer (NanoDrop Technologies

Inc. USA). The quality of the total RNA was determined in a 1 % agarose gel by band intensity of 28S and 18S rRNA (28S/18S ratio). cDNA was synthesized from 1 µg of the total RNA, treated with RNase free DNase I (Thermo Fisher Scientific, USA), using RevertAid H Minus M-MuLV Reverse Transcriptase (Thermo Fisher Scientific, USA), according to the manufacturer's protocol.

Quantitative PCR (qPCR). The levels of a relative gene expression (RE) of 5 genes were assessed by qPCR, using a Maxima SYBR Green Master mix (Thermo Fisher Scientific, USA) on a Bio-Rad CFX96 Real-Time PCR Detection System (USA) under the following conditions: 95°C — 10 min, following 40 cycles of 95°C — 15 s, 60°C — 30 s, elongation 72°C — 30 s. Primers for all genes were selected from the qPrimerDepot (https://primerdepot.nci.nih.gov/) database and confirmed, using the https://www.ncbi.nlm.nih.gov/tools/primer-blast/ algorithm.

Four reference genes (*TBP*, *HPRT*, *ALAS1* and *TUBA1B*) were used for [the] gene expression normalization [30]. Two main models ($2^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$ methods), described earlier [29, 31] were used for the calculation and analysis of RE levels.

Statistical analysis. The Kolmogorov-Smirnov test was applied to assess the normality of distribution. The Wilcoxon Matched Pairs test was performed for the comparison of RE levels in prostate adenocarcinomas and paired normal tissues samples [29]. The Benjamini-Hochberg procedure with false discovery rate (FDR) 0.10-0.25 was used under multiple comparisons detection [32]. The Kruskal-Wallis test was used to determine differences between experimental groups. The Dunn-Bonferroni post hoc test for multiple comparisons was performed to determine RE differences between pairs of prostate samples. The Spearman's rank correlation test was used to find possible correlations between the gene RE and CPC of prostate tumors [31].

Results

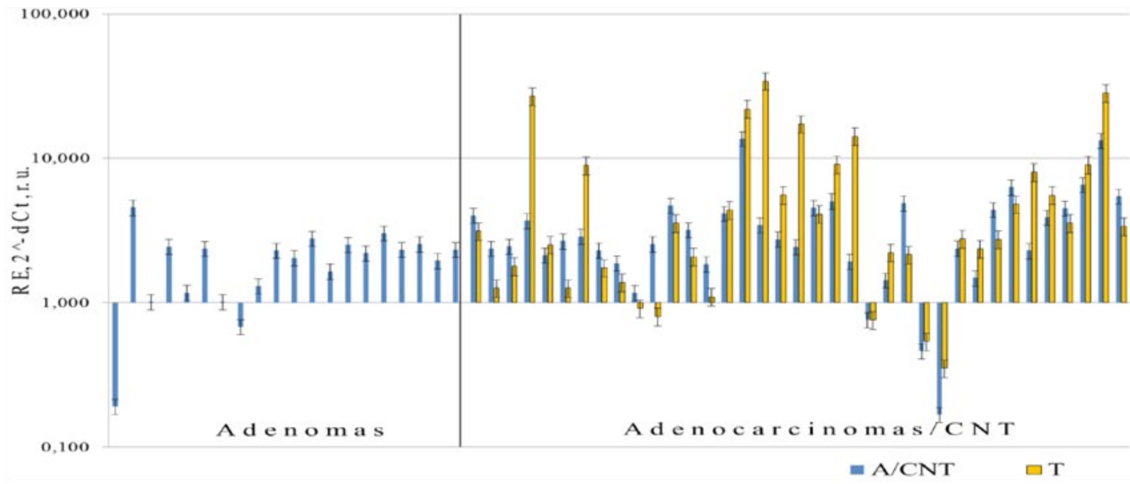
We have analyzed RE levels of 5 genes in prostate T, CNT and A (Figure 1A-E). T samples are arranged, according to an increase of tumor stage and GS. Two of the studied genes, namely *FASN* and *COX2* were expressed at the high levels, whereas other three, namely *LDLR, HMGCR, CPT1C* showed moderate and low RE. The lowest RE level was detected for the *CPT1C* gene.

Comparison of the RE levels of these five genes in 37 paired samples of T/CNT did not show any significant difference. Importantly, comparison between A, T and CNT groups identified some significant changes in the RE levels of genes, involved in control of lipid metabolism (Table 1 A, B). The Kruskal-Waillis test has shown a significant difference in RE for two genes: *FASN* ($p = 0.0253$) and *COX2* ($p = 0.0079$).

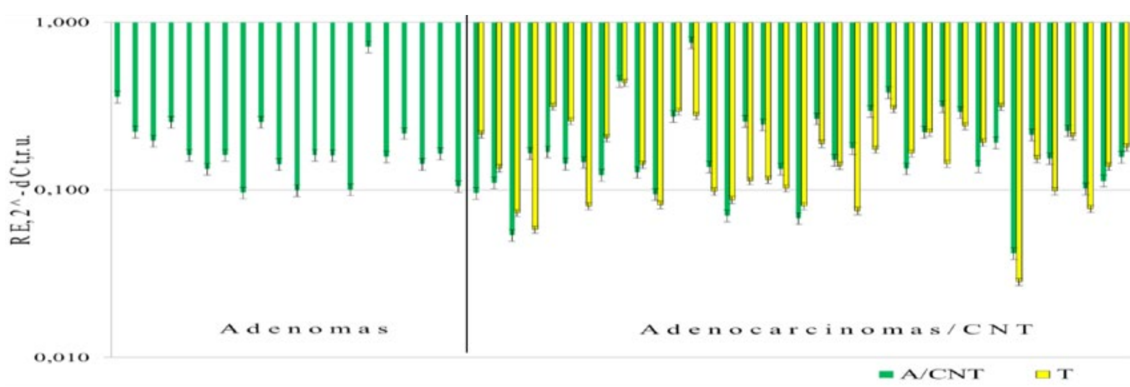
The following Dunn-Bonferroni post hoc test has clarified the sample groups with RE differences (Table 1 B). RE of *FASN* increased significantly in the T group, compared with the A group ($p = 0.0311$). The *COX2* expression was higher in both, the A ($p = 0.0191$) and CNT ($p = 0.0109$) groups. Other genes were equally expressed in the described groups.

All T samples were divided into two groups, taking into consideration the stages of the disease. The first group is T at the stages 1–2 (28 samples) and in the second group the sam-

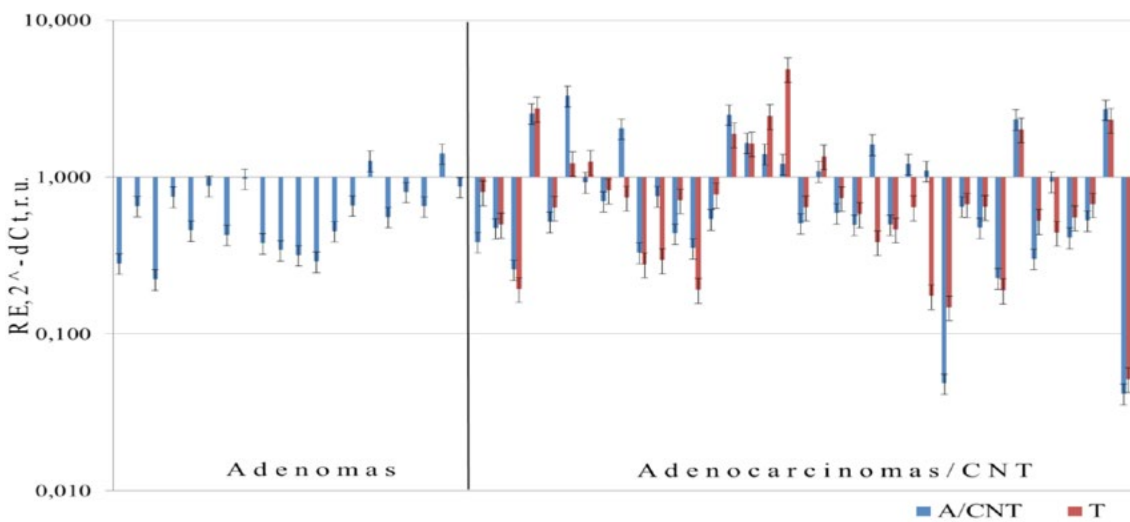
A.
FASN



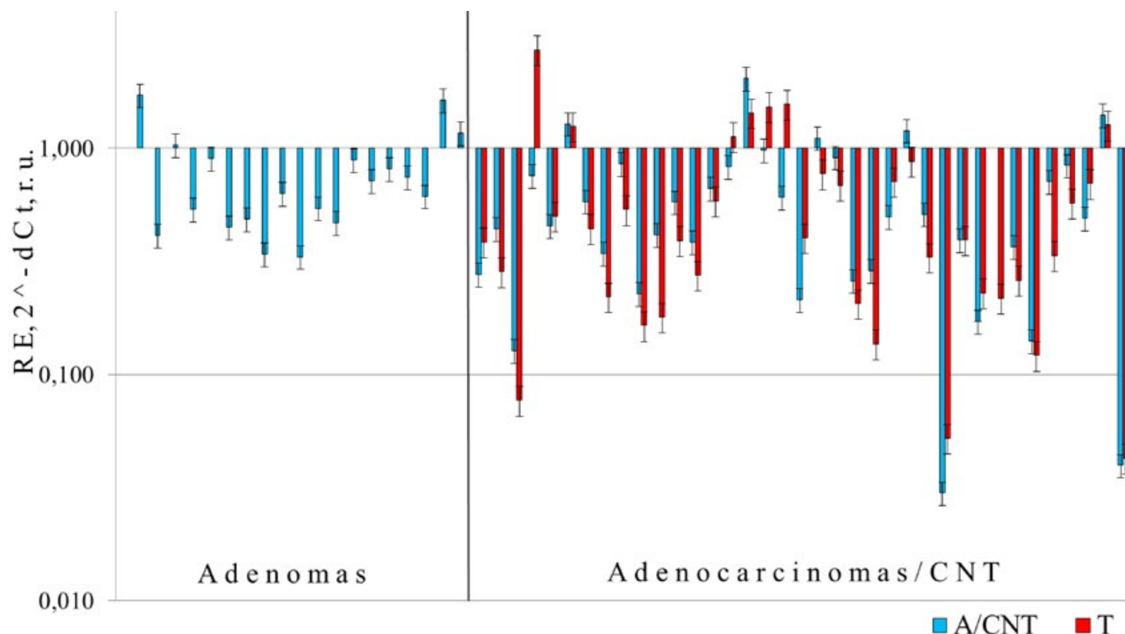
B.
CPT1C



C.
LDLR



D.
HMGCR



E.
COX2

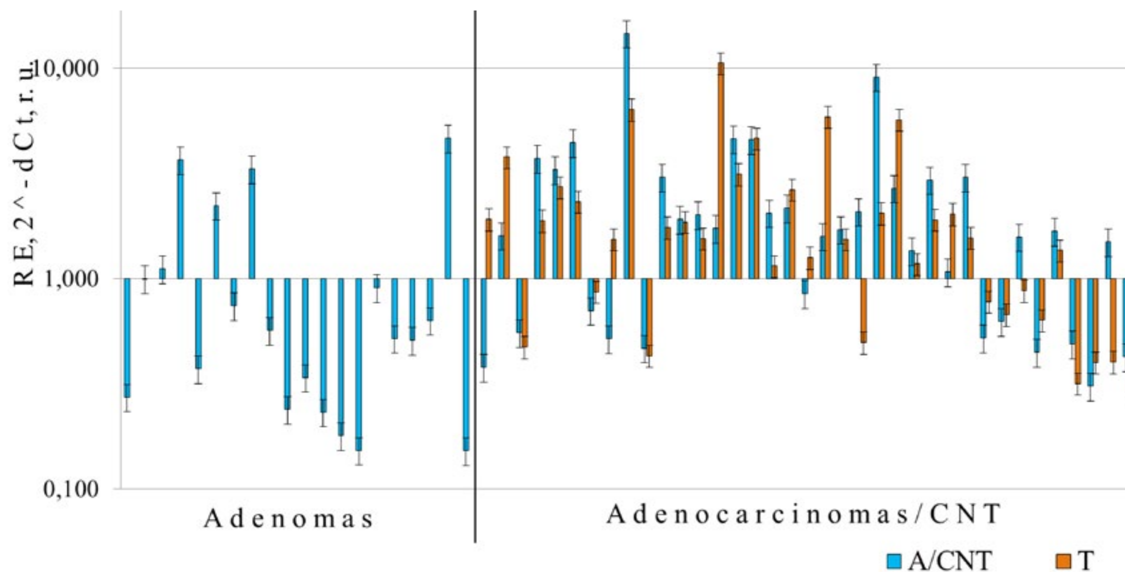


Fig. 1. RE profiles of the genes, involved in the control on lipid metabolism in prostate adenoma (A), adenocarcinoma (T) and the paired CNT prostate tissue samples: *A* — *FASN*; *B* — *CPT1C*; *C* — *LDLR*; *D* — *HMGCR*; *E* — *COX2*.

ples of T at the stages 3–4 were included (9 samples). The paired CNT was divided into two groups as well (N stages 1–2 and N stages 3–4). Descriptive statistics and p-values are shown in Table 2 A, B. The Kruskal-Waillis test revealed the significant differences in the

Table 1. **Descriptive statistics of groups of T, CNT and A, calculated by the Kruskal-Waillis test with FDR=0.2 (A); the Dunn-Bonferroni post hoc method for multiple comparisons (B)**

A

Genes	Group	Median	Minimum	Maximum	25-th percentile	75-th percentile	p-value*
FASN	T	3.132	0.351	34.230	1.744	8.048	0.0253
	N	2.750	0.068	13.687	2.132	4.511	
	A	2.252	0.191	4.567	1.241	2.485	
CPT1C	T	0.143	0.028	0.435	0.098	0.215	
	N	0.154	0.042	0.754	0.122	0.245	
	A	0.161	0.096	0.714	0.133	0.217	
LDLR	T	0.647	0.052	4.890	0.443	1.227	
	N	0.589	0.042	3.300	0.437	1.217	
	A	0.605	0.223	1.415	0.360	0.835	
HMGCR	T	0.393	0.043	9.724	0.221	0.712	
	N	0.489	0.000	2.037	0.278	0.831	
	A	0.631	0.331	1.717	0.469	0.901	
COX2	T	1.550	0.291	10.554	0.777	2.319	0.0079
	N	1.672	0.308	14.623	0.623	2.945	
	A	0.542	0.152	4.647	0.256	1.053	

B

Genes	Group pairs with differences	p-value**
FASN	T/A	0.0311
COX2	T/A	0.0191
	N/A	0.0109

Note: * — Kruskal-Waillis test with FDR=0.2;
** — Dunn-Bonferroni post-hoc test for multiple comparisons.

RE levels for 4–5 genes in such groups. Only *CPT1C* was expressed at the similar levels in all studied groups.

Three out of four genes showed difference in the RE levels in T or CNT, compared with A, according to the Dun-Bonferroni post hoc test. The RE levels of *FASN* and *COX2* were increased in T, stage 1–2, compared with the A group ($p = 0.0372$, $p = 0.0385$, respectively). Besides, *COX2* was up-regulated in N, stage 1–2, compared with the A group ($p = 0.0245$). *HMGCR*, in contrast, was down-regulated significantly in T, stage 3–4, compared with the A group ($p = 0.0211$). Noteworthy, the highest expression of *HMGCR* was observed in the A group. *LDLR* increased dramatically in T, stages 1–2 and CNT 1–2 stages, compared with T, stages 3–4 ($p = 0.0156$, $p = 0.0194$, respectively). Noteworthy, however, that it was a high level of heterogeneity of RE in tumor samples for the majority of the studied genes. The same phenomenon we observed earlier, for other genes, though [29, 31].

Next parameter used for the group of patients was GS. Among 37 prostate T, there

Table 2. **Descriptive statistics of the groups of the paired T and CNT of different tumor stage and A, calculated by the Kruskal-Waillis test with FDR=0.2 (A) and the Dunn-Bonferroni post hoc method for multiple comparisons (B)**

A

Genes	Group	Median	Minimum	Maximum	25-th percentile	75-th percentile	p-value*
FASN	T 1-2 st	3.345	0.801	34.230	1.913	8.967	0.0161
	T 3-4 st	2.228	0.351	9.091	0.761	3.560	
	N 1-2 st	3.040	1.169	13.687	2.340	4.476	
	N 3-4 st	2.293	0.068	5.461	0.761	4.511	
	A	2.252	0.191	4.567	1.241	2.485	
CPT1C	T 1-2 st	0.136	0.028	0.435	0.084	0.227	
	T 3-4 st	0.174	0.073	0.303	0.143	0.208	
	N 1-2 st	0.140	0.042	0.754	0.112	0.218	
	N 3-4 st	0.221	0.054	0.381	0.157	0.295	
	A	0.161	0.096	0.714	0.133	0.217	
LDLR	T 1-2 st	0.726	0.190	4.890	0.610	1.501	0.0027
	T 3-4 st	0.386	0.052	0.732	0.174	0.526	
	N 1-2 st	0.729	0.227	3.300	0.486	1.533	
	N 3-4 st	0.412	0.042	1.616	0.258	0.589	
	A	0.605	0.223	1.415	0.360	0.835	
HMGCR	T 1-2 st	0.422	0.165	9.724	0.268	1.002	0.0141
	T 3-4 st	0.137	0.043	0.712	0.077	0.573	
	N 1-2 st	0.533	0.000	2.037	0.355	0.844	
	N 3-4 st	0.288	0.030	0.911	0.128	0.511	
	A	0.631	0.331	1.717	0.469	0.901	
COX2	T 1-2 st	1.554	0.399	10.554	0.869	2.679	0.0232
	T 3-4 st	1.533	0.291	5.667	0.472	2.023	
	N 1-2 st	1.704	0.308	14.623	0.774	3.028	
	N 3-4 st	1.077	0.425	9.087	0.488	2.688	
	A	0.542	0.152	4.647	0.256	1.053	

B

Genes	Group paires with differences	p-value**
FASN	T 1-2 st/A	0.0372
LDLR	T 1-2 st/T3-4 st	0.0156
	T 3-4 st/N 1-2 st	0.0194

Genes	Group paires with differences	p-value**
HMGCR	T 3-4 st/A	0.0211
COX2	T 1-2 st/A	0.0385
	N 1-2 st/A	0.0245

Note: * — Kruskal-Waillis test with FDR=0.2;
** — Dunn-Bonferroni post-hoc test for multiple comparisons.

Table 3. **Descriptive statistics of prostate T, CNT with different GS and A groups by the Kruskal-Waillis test with FDR = 0.2 (A) and the Dunn-Bonferroni post hoc method for multiple comparisons (B)**
A

Genes	Group	Median	Minimum	Maximum	25-th percentile	75-th percentile	p-value*
FASN	T<7	1.744	0.801	26.882	1.261	3.132	0.0163
	T=7	4.395	1.101	34.230	3.559	17.312	
	T>7	3.390	0.351	28.300	2.228	8.048	
	N<7	2.455	1.169	4.015	2.132	2.882	
	N=7	3.452	1.845	13.687	2.750	4.546	
	N>7	3.905	0.068	13.264	1.481	5.039	
	A	2.252	0.191	4.567	1.241	2.485	
CPT1C	T<7	0.141	0.058	0.435	0.080	0.257	
	T=7	0.113	0.080	0.293	0.098	0.188	
	T>7	0.165	0.028	0.313	0.138	0.208	
	N<7	0.127	0.054	0.444	0.096	0.165	
	N=7	0.245	0.068	0.754	0.133	0.266	
	N>7	0.177	0.042	0.381	0.138	0.225	
	A	0.161	0.096	0.714	0.133	0.217	
LDLR	T<7	0.741	0.193	2.735	0.295	1.227	
	T=7	1.359	0.191	4.890	0.711	1.879	
	T>7	0.554	0.052	2.316	0.386	0.668	
	N<7	0.702	0.258	3.300	0.386	2.045	
	N=7	1.094	0.353	2.512	0.509	1.407	
	N>7	0.530	0.042	2.693	0.412	1.097	
	A	0.605	0.223	1.415	0.360	0.835	
HMGCR	T<7	0.385	0.077	9.724	0.179	0.536	0.0148
	T=7	0.773	0.275	1.570	0.403	1.434	
	T>7	0.330	0.043	1.268	0.206	0.688	
	N<7	0.441	0.128	1.283	0.278	0.755	
	N=7	0.665	0.214	2.037	0.578	0.982	
	N>7	0.393	0.000	1.400	0.172	0.711	
	A	0.631	0.331	1.717	0.469	0.901	
COX2	T<7	1.880	0.429	6.348	0.864	2.717	0.0035
	T=7	2.640	1.144	10.554	1.550	4.638	
	T>7	0.874	0.291	5.667	0.497	1.558	
	N<7	1.601	0.377	14.623	0.517	3.713	
	N=7	2.005	0.845	4.610	1.736	2.166	
	N>7	1.497	0.308	9.087	0.520	2.085	
	A	0.542	0.152	4.647	0.256	1.053	

Continued Table 3

B

Genes	Group paires with differences	p-value **
FASN	T=7/A	0.0491
COX2	T=7/A	0.0124
	N=7/A	0.0462

Note: * — Kruskal-Waillis test with FDR=0.2;
** — Dunn-Bonferroni post-hoc test for multiple comparisions.

were 11 samples of tumors with GS < 7 (T < 7), 9 samples — with GS = 7 (T = 7) and 17 samples — with GS > 7 (T > 7). The paired CNT were grouped accordingly. The descriptive statistics and p-values of statistical tests for samples with different GS are shown in Table 3A, B. According to the Kruskal-Waillis test, 3 genes showed significant differences in the RE levels: *FASN* (p = 0.0163), *HMGCR* (p = 0.0148) and *COX2* (p = 0.0035). However, the Dunn-Bonferroni post hoc method confirmed the differences in RE only for two genes (Table 3 B). *FASN* was upregulated in T with GS = 7 (T = 7), compared with the A group (p = 0.0491).

COX2 showed a 3–4 fold increase in the RE levels in T and CNT with GS = 7, compared to the A group (p = 0.0124, p = 0.0462, respectively). Also, the *HMGCR* RE levels showed a trend to decrease in the T groups with GS < 7 and GS > 7, compared with the T group with GS = 7. This tendency is also observed for CNT groups.

The Spearman rank correlation analysis of adenocarcinoma samples, comparing RE and CPC, revealed three unexpected negative correlations (Table 4A).

The RE levels of *COX2* showed negative correlation with the GS (r^s = –0.3401, p < 0.05). The RE levels of *LDLR* and *HMGCR* correlated negatively with the stage (r^s = –5411, p < 0.01 and r^s = –0.4204, p < 0.05, respectively).

Table 4. **The Spearman rank correlation analysis of T group, comparing RE of genes, regulating lipid metabolism genes and CPC (A); correlation between RE levels of all studied genes (B)**

A.

CPC/genes	*FASN*	*CPT1C*	*LDLR*	*HMGCR*	*COX2*
GS	0.1704	0.0166	–0.2368	–0.1106	–0.3401
Stage	–0.2948	0.1528	***–0.5411***	–0.4204	–0.1880
PSA ng/ml	0.0699	–0.1667	–0.2553	–0.2435	–0.2963
Age	–0.0013	0.1009	0.0837	0.1507	0.1778

B.

Genes	*FASN*	*CPT1C*	*LDLR*	*HMGCR*
CPT1C	–0.4248			
LDLR	***0.5747***	–0.2800		
HMGCR	0.4881	–0.0624	**0.7558**	
COX2	–0.3177	0.2527	0.2449	0.3566

Note: p < 0.05 – red; ***p < 0.01*** – red bold italic; **p < 0.001** – red bold

Table 5. **The Spearman rank correlations analysis of possible interaction of genes, involved in regulation of lipid metabolism and genes, controlling EMT, and extracellular matrix, associated with prostate cancer**

№	Genes	*FASN*	*CPT1C*	*LDLR*	*HMGCR*	*COX2*
1	*CDH1*	**0.682**	-0.174	**0.643**	**0.637**	0.073
2	*CDH2*	-0.215	***0.578***	-0.078	0.268	0.254
3	*AR 1 isof*	0.324	0.329	0.306	0.521	0.217
4	*AR 2 isof*	0.308	0.269	0.369	0.469	0.077
5	*PRLR*	0.257	0.111	0.412	**0.618**	0.050
6	*PRL*	-0.394	0.199	-0.295	-0.284	-0.010
7	*FN1*	-0.333	0.388	-0.107	0.074	0.450
8	*VIM*	-0.346	0.166	-0.435	***-0.529***	0.117
9	*OCLN*	**0.645**	-0.082	0.434	0.486	-0.013
10	*MMP2*	***-0.546***	***0.586***	***-0.562***	***-0.526***	0.037
11	*MMP9*	-0.254	0.058	-0.372	-0.338	0.005
12	*VDR*	0.124	0.306	0.226	0.318	0.239
13	*NKX3-1*	**0.719**	0.008	**0.717**	**0.815**	0.070
14	*PCA3*	**0.668**	***-0.595***	***0.500***	0.370	-0.160
15	*PSA*	**0.748**	-0.472	0.431	0.238	-0.446
16	*HOTAIR*	-0.374	0.430	-0.104	0.175	0.250
17	*SCHLAP1*	0.438	-0.359	-0.100	-0.243	**-0.746**
18	*KRT18*	**0.694**	***-0.511***	0.212	-0.008	***-0.566***
19	*MKI67*	0.344	0.101	0.370	0.304	-0.043
20	*CASP3*	0.136	0.304	0.353	***0.551***	0.336
21	*XIAP*	0.323	0.237	0.460	0.706	0.346
22	*TMPRSS2/ ERG*	0.416	-0.173	0.215	0.071	-0.317
23	*PTEN*	-0.147	***0.575***	-0.215	-0.094	0.214
24	*GCR AG*	-0.344	0.497	-0.173	0.085	0.366
25	*GCRinsAG*	-0.381	***0.577***	-0.155	0.005	0.423
26	*GCRinsB*	0.096	0.255	0.376	0.496	0.477
27	*ESR1*	-0.081	0.318	-0.017	0.207	0.438
28	*ESR2*	-0.368	0.363	-0.022	-0.019	0.429
29	*INSRA*	0.343	0.205	0.494	***0.511***	0.298
30	*INSRB*	-0.174	0.361	0.249	0.376	**0.687**
31	*IGF1R*	0.436	0.018	***0.567***	***0.532***	0.251
32	*SRD5A1*	0.234	-0.068	0.099	-0.172	-0.090
33	*SRD5A2*	-0.056	0.253	0.215	0.248	0.360
34	*THY1*	-0.252	0.431	-0.107	0.042	0.127
35	*ACTA2*	***-0.590***	***0.503***	-0.212	-0.078	0.470
36	*CXCL12*	***-0.516***	***0.583***	-0.390	-0.142	0.330
37	*CXCL14*	0.129	-0.087	-0.084	-0.062	-0.013
38	*CTGF*	-0.237	0.074	0.103	0.086	***0.531***
39	*HIF1A*	0.276	-0.046	***0.579***	***0.511***	0.371
40	*S100A4*	***-0.506***	0.467	***-0.548***	-0.460	-0.027
41	*FAP*	-0.118	0.473	-0.207	0.040	0.194
42	*CD68*	0.099	0.275	0.171	0.256	0.288
43	*CD163*	-0.407	0.277	**-0.621**	***-0.534***	-0.076
44	*CCR4*	-0.007	0.176	0.144	0.216	0.316
45	*CCL17*	-0.068	0.073	-0.409	-0.367	-0.282
46	*CCL22*	0.042	0.231	0.092	0.141	0.112
47	*NOS2A*	0.145	0.224	0.307	0.167	0.329
48	*CIAS1*	-0.018	0.281	0.283	0.360	***0.633***
49	*CTLA4*	-0.008	-0.064	0.068	0.142	0.278
50	*KLRK*	-0.121	0.145	-0.053	0.226	0.187
51	*IRF1*	0.157	0.129	0.455	0.446	0.433
52	*IL1RL1*	-0.208	0.302	0.252	0.238	0.364
53	*IL1R1*	0.243	0.330	0.441	***0.589***	0.380
54	*IL2RA*	-0.079	-0.052	-0.087	-0.093	-0.034
55	*MSMB*	0.080	-0.069	0.147	0.085	-0.006
56	*HLA-G*	-0.202	0.062	**-0.603**	***-0.562***	-0.245

Note: $p < 0.05$ – red; ***p < 0.01*** – red bold italic; **p < 0.001** – red bold; 1-33 – EMT and prostate cancer associated genes, 34–41 — CAF-associated genes; 42–47 — TAM-associated genes; 48–56 — immune-associated genes.

When the Spearman rank correlations analysis was applied to all the studied genes, we found few genes with [the] coordinated RE levels (Table 4 B). The most significant correlation was found between the *HMGCR* and *LDLR* genes ($r^s = 0.7558$, $p < 0.001$). Notably, the largest number of correlations (three correlations) were found for *FASN* and *HMGCR*.

The *FASN* expression correlated with *LDLR* ($r^s = 0.5747$, $p < 0.01$), *HMGCR* ($r^s = 0.4881$, $p < 0.05$) and *CPTC1* ($r^s = –0.4248$, $p < 0.05$) levels. The *HMGCR* levels correlated only with *FASN*. *COX2* has shown the strongest correlation with *LDLR* ($r^s = 0.3566$, $p < 0.05$).

Recently, we have studied the RE levels of several genes, controlling EMT and extracellular matrix formation associated with prostate cancer [29, 31, 33]. Now we have calculated any putative correlations between genes, involved in [the] regulation of metabolism and 56 previously studied genes/transcripts, using the Spearman rank correlations analysis. These results are presented in Table 5.

We have found 113 significant correlations. There are 15 correlations with the highest indexes ($r^s > 0.600$, $p > 0.001$). The *NKX3-1* and *CDH1* genes, investigated earlier show the strongest positive correlations ($r^s > 0.637$, $p > 0.001$) with the *FASN, LDLR* and *HMGCR* genes, involved in lipid metabolism. These genes (*NKX3-1* and *CDH1*) are considered as markers of prostate epithelial cells, hence, we might speculate that the expression of *FASN, LDLR* and *HMGCR* is associated with this cell type. This is further confirmed by negative correlations of the expression of *FASN, LDLR, HMGCR* with the levels of the *ACTA2* and *CXCL12*, expressed in cancer-associated fibroblast (CAF), and also with the expression of *CD163* and *CCL17,* markers of tumor-associated macrophages (TAM). *CPT1C* and *COX2* showed an inverse correlation dependence with the described *CAF* and *TAM* genes.

Discussion

As mentioned above, the] alterations in lipid metabolism are one of the hallmarks of carcinogenesis [2]. Many efforts have been made in recent years to understand the molecular mechanisms, underlying the influence of lipid metabolism on the cancer treatment. In the case of prostate tumors, the FDA has improved only five new drugs over the past 8 years of cancer treatment [34]. From the other hand, there is another approach, so called drugs repositioning, when the already known drugs are used for clinical studies. Statins (HMGCR inhibitors) [22, 23] and COX-2 inhibitors are the examples of such drugs [28].

In the present work, high levels of heterogeneity in RE of all studied genes were observed, especially in tumor samples. So, it is possible that the tumors, despite being in one group, can show the different drug sensitivity, considering their stage or the GS. Hence, it is important to identify a set of genes and an RE threshold, when cancers will respond to the drug treatment most efficiently [25, 28].

Unexpectedly, only *FASN* and *COX2* were upregulated in the common T group, in comparison with adenomas. Noteworthy, the RE levels of *COX2* were elevated in the CNT group as well, which could be an indicator of inflammation in prostate [13, 14]. Our previous studies have demonstrated a high dispersion of investigated genes RE for example EMT and prostate cancer-associated genes [29, 31, 33] in adenocarcinoma groups like, the RE of lipid metabolism genes, i.e. *FASN* and *COX2*. It means that in the same group we have samples with both high and low gene RE. Statistical analysis of adenocarcinoma groups with different stages and GS has shown an increased RE only in T at stage 1–2 and with GS = 7. These groups have the highest number of samples with an increased expression. Probably, T at stage

1–2 and with GS = 7 could respond to chemotherapy in this case. However, even in these groups there are samples with a decreased RE which is lower, than the median of the A group. Here a question arises, whether such samples can be less sensitive to the specific drugs for these genes? Therefore, we can say that the sensitivity to drugs is higher in these groups, although it is preferable to determine the individual RE values, which corresponds to the approaches of personalized medicine.

LDLR was significantly down-regulated in more advanced tumors (stage 3–4) and with the higher GS, compared with T, stage 1–2. *LDLR* showed no differences in RE, compared with [the] adenoma group. Our data are supported by other works [35]. We expect [a] decrease in the drug sensitivity in advanced prostate cancer (stage 3–4).

HMGCR is down-regulated in T, stage 3–4, compared with the A group. On the one hand, this fact may be an obstacle to the statins use [22], on the other, it may be a good reason for treatment of patients with enzalutamide: it was reported that resistance to enzalutamide usually developed, when *HMGCR* is expressed at high levels [36]. Importantly, enzalutamide is recently approved for the management of castration-resistant prostate cancer [37]. Therefore, we think that *HMGCR* is a significant pharmacological marker. Moreover, its levels should be assessed prior to the decision about the treatment of prostate cancer patients.

In contrast to the data reported by the authors who demonstrated high expressed RE of *CPT1C* in aggressive prostate tumors [7, 10], we have shown low *CPT1C* RE in all investigated tumor groups. We have not detected any differences in the levels of *CPT1C* RE, and it was expressed at low levels in T, CNT and A. This should be investigated further, i.e. the special isoform *CPT1A* should be compared with the *CPT1C* levels.

Importantly, we found that expression of the *FASN, LDLR* and *HMGCR* genes follows the expression pattern of markers of epithelial cells and prostate cancer-associated genes, namely *CDH1, AR 1isof, KRT18, OCLN, NKX3-1 etc.* whereas At the same time, the RE levels of *COX2* and *CPT1C* show the same trend in expression, as CAF markers (*ACTA2, CXCL12, CTGF*), mesenchymal cell markers (*CDH2, FN1*) and inflammation-associated genes (*CIAS, IRF1 et al.*). Negative RE correlations of *COX2* with epithelial cell markers (*PSA, KRT18*) have been shown.

It may reflect differences in expression of these genes in various types of cells, and also suggest that the expression of these genes is controlled by different mechanisms, as was shown earlier [1, 14].

Conclusions

The genes, controlling lipid metabolism show a differential expression in the prostate cancer samples. *FASN* and *COX2* are upregulated in the adenocarcinoma groups, including the stage and the GS compared with the adenoma group. *LDLR* and *HMGCR* have demonstrated significant changes in RE only in tumor samples of the different stage. The *LDLR, HMGCR* and *COX2* levels decreased upon tumor progression, and this should be considered in the treatment of patients with advanced stage of disease. The RE levels of *FASN, HMGCR* and *COX2* could be considered as pharmacological markers of sensitivity and efficiency of the inhibitory drugs. Further experiments are need-

ed to confirm the described results in a larger patient cohort.

REFERENCES

1. *Beloribi-Djefaflia S, Vasseur S, Guillaumond F.* Lipid metabolic reprogramming in cancer cells. *Oncogenesis.* 2016;**5**:e189.
2. *Cheng C, Geng F, Cheng X, Guo D.* Lipid metabolism reprogramming and its potential targets in cancer. *Cancer Commun (Lond).* 2018;**38**(1):27.
3. *Santi A, Caselli A, Ranaldi F, Paoli P, Mugnaioni C, Michelucci E, Cirri P.* Cancer associated fibroblasts transfer lipids and proteins to cancer cells through cargo vesicles supporting tumor growth. *Biochim Biophys Acta.* 2015;**1853**(12):3211–23.
4. *Ashida S, Kawada C, Inoue K.* Stromal regulation of prostate cancer cell growth by mevalonate pathway enzymes HMGCS1 and HMGCR. *Oncol Lett.* 2017;**14**(6):6533–6542.
5. *Roy M, Kung HJ, Ghosh PM.* Statins and prostate cancer: role of cholesterol inhibition vs. prevention of small GTP-binding proteins. *Am J Cancer Res*. 2011;**1**(4):542–61.
6. *Cruz PM, Mo H, McConathy WJ, Sabnis N, Lacko AG.* The role of cholesterol metabolism and cholesterol transport in carcinogenesis: a review of scientific findings, relevant to future cancer therapeutics. *Front Pharmacol.* 2013;**4**:119.
7. *Casals N, Zammit V, Herrero L, Fadó R, Rodríguez-Rodríguez R, Serra D.* Carnitine palmitoyltransferase 1C: From cognition to cancer. *Prog Lipid Res.* 2016;**61**:134–48.
8. *Roomets E, Kivelä T, Tyni T.* Carnitine palmitoyltransferase I and Acyl-CoA dehydrogenase 9 in retina: insights of retinopathy in mitochondrial trifunctional protein defects. *Invest Ophthalmol Vis Sci.* 2008;**49**(4):1660–4.
9. *Sanchez-Macedo N, Feng J, Faubert B, Chang N, Elia A, Rushing EJ, Tsuchihara K, Bungard D, Berger SL, Jones RG, Mak TW, Zaugg K.* Depletion of the novel p53-target gene carnitine palmitoyltransferase 1C delays tumor growth in the neurofibromatosis type I tumor model. *Cell Death Differ.* 2013;**20**(4):659–68.
10. *Nath A, Chan C.* Genetic alterations in fatty acid transport and metabolism genes are associated with metastatic progression and poor prognosis of human cancers. *Sci Rep.* 2016;**6**:18669.
11. *Schlaepfer IR, Rider L, Rodrigues LU, Gijón MA, Pac CT, Romero L, Cimic A, Sirintrapun SJ, Glodé LM, Eckel RH, Cramer SD.* Lipid catabolism via CPT1 as a therapeutic target for prostate cancer. *Mol Cancer Ther.* 2014;**13**(10):2361–71.
12. *Kuhajda FP.* Fatty acid synthase and cancer: new application of an old pathway. *Cancer Res.* 2006;**66**(12):5977–80.
13. *Wu X, Daniels G, Lee P, Monaco ME.* Lipid metabolism in prostate cancer. *Am J Clin Exp Urol.* 2014;**2**(2):111–20.
14. *Su CW, Zhang Y, Zhu YT.* Stromal COX-2 signaling are correlated with colorectal cancer: A review. *Crit Rev Oncol Hematol.* 2016;**107**:33–38.
15. *Brune K, Patrignani P.* New insights into the use of currently available non-steroidal anti-inflammatory drugs. *J Pain Res.* 2015;**8**:105–18.
16. *Yang P, Cartwright CA, Li J, Wen S, Prokhorova IN, Shureiqi I, Troncoso P, Navone NM, Newman RA, Kim J.* Arachidonic acid metabolism in human prostate cancer. *Int J Oncol.* 2012;**41**(4):1495–503.
17. *Hussain T, Gupta S, Mukhtar H.* Cyclooxygenase-2 and prostate carcinogenesis. *Cancer Lett.* 2003;**191**(2):125–35.
18. *Lin DW, Nelson PS.* The role of cyclooxygenase-2 inhibition for the prevention and treatment of prostate carcinoma. *Clin Prostate Cancer.* 2003;**2**(2):119–26.
19. *Flavin R, Peluso S, Nguyen PL, Loda M.* Fatty acid synthase as a potential therapeutic target in cancer. *Future Oncol.* 2010;**6**(4):551–62.
20. *Souchek JJ, Davis AL, Hill TK, Holmes MB, Qi B, Singh PK, Kridel SJ, Mohs AM.* Combination Treatment with Orlistat-Containing Nanoparticles and Taxanes Is Synergistic and Enhances Microtubule Stability in Taxane-Resistant Prostate Cancer Cells. *Mol Cancer Ther.* 2017;**16**(9):1819–1830.
21. *Wright C, Iyer AKV, Kaushik V, Azad N.* Anti-Tumorigenic Potential of a Novel Orlistat-AICAR Combination in Prostate Cancer Cells. *J Cell Biochem.* 2017;**118**(11):3834–3845.

22. *Hajar R.* Statins: past and present. *Heart Views.* 2011;**12**(3):121–7.
23. *Kang M, Lee KH, Lee HS, Jeong CW, Ku JH, Kim HH, Kwak C.* Concurrent treatment with simvastatin and NF-κB inhibitor in human castration-resistant prostate cancer cells exerts synergistic anti-cancer effects via control of the NF-κB/LIN28/let-7 miRNA signaling pathway. *PLoS One.* 2017;**12**(9):e0184644.
24. *Zhang N, Li S, Hua H, Liu D, Song L, Sun P, Huang W, Tang Y, Zhao Y.* Low density lipoprotein receptor targeted doxorubicin/DNA-Gold Nanorods as a chemo- and thermo-dual therapy for prostate cancer. *Int J Pharm.* 2016;**513**(1-2):376–386.
25. *Dheeraj A, Agarwal C, Schlaepfer IR, Raben D, Singh R, Agarwal R, Deep G.* A novel approach to target hypoxic cancer cells via combining β-oxidation inhibitor etomoxir with radiation. *Hypoxia (Auckl).* 2018;**6**:23–33.
26. *Samudio I, Harmancey R, Fiegl M, Kantarjian H, Konopleva M, Korchin B, Kaluarachchi K, Bornmann W, Duvvuri S, Taegtmeyer H, Andreeff M.* Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. *J Clin Invest.* 2010;**120**(1):142–56.
27. *Ricciardi MR, Mirabilii S, Allegretti M, Licchetta R, Calarco A, Torrisi MR, Foà R, Nicolai R, Peluso G, Tafuri A.* Targeting the leukemia cell metabolism by the CPT1a inhibition: functional preclinical effects in leukemias. *Blood.* 2015, 126(16):1925–9.
28. *Perroud HA, Alasino CM, Rico MJ, Mainetti LE, Queralt F, Pezzotto SM, Rozados VR, Scharovsky OG.* Metastatic breast cancer patients treated with low-dose metronomic chemotherapy with cyclophosphamide and celecoxib: clinical outcomes and biomarkers of response. *Cancer Chemother Pharmacol.* 2016;**77**(2):365–74.
29. *Gerashchenko GV, Mankovska OS, Dmitriev AA, Mevs LV, Rosenberg EE, Pikul MV, Marynychenko MV, Gryzodub OP, Stakhovsky EO, Kashuba VI.* Expression of epithelial-mesenchymal transition-related genes in prostate tumours. *Biopolym Cell.* 2017, 33(5):335–55.
30. *Schmidt U, Fuessel S, Koch R, Baretton GB, Lohse A, Tomasetti S, Unversucht S, Froehner M, Wirth MP, Meye A.* Quantitative multi-gene expression profiling of primary prostate cancer. *Prostate.* 2006;**66**(14):1521–34.
31. *Gerashchenko GV, Mevs LV, Chashchina LI, Pikul MV, Gryzodub OP, Stakhovsky EO, Kashuba VI.* Expression of steroid and peptide hormone receptors, metabolic enzymes and EMT-related genes in prostate tumors in relation to the presence of the TM-PRSS2/ERG fusion. *Exp Oncol.* 2018;**40**(2):101–8.
32. *Benjamini Y, Hochberg Y.* Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society.* 1995, 57(1):289–300.
33. *Gerashchenko GV, Rynditch AV, Kashuba VI.* Molecular profiling of prostate tumors. *Dopov Nac Acad Nauk Ukr.* 2018;**6**:113–9.
34. *New Drugs at FDA: CDER's New Molecular Entities and New Therapeutic Biological Products.* 2018
35. *Stopsack KH, Gerke TA, Andrén O, Andersson SO, Giovannucci EL, Mucci LA, Rider JR.* Cholesterol uptake and regulation in high-grade and lethal prostate cancers. *Carcinogenesis.* 2017;**38**(8):806–811.
36. *Kong Y, Cheng L, Mao F, Zhang Z, Zhang Y, Farah E, Bosler J, Bai Y, Ahmad N, Kuang S, Li L, Liu X.* Inhibition of cholesterol biosynthesis overcomes enzalutamide resistance in castration-resistant prostate cancer (CRPC). *J Biol Chem.* 2018;**293**(37):14328–14341.
37. *FDA approves enzalutamide for castration-resistant prostate cancer.* 2018, 07,

Патерни експресії генів, що регулюють ліпідний метаболізм у пухлинах передміхурової залози

Г. В. Геращенко, О. А. Кононенко,
Ю. М. Бондаренко, Е. О. Стаховський,
В. І. Кашуба

Мета: Встановити рівні відносної експресії (ВЕ) генів, що задіяні у ліпідному метаболізмі у пухлинах передміхурової залози. На основі цих патернів виявити клінічно-значущі специфічні порушення. **Методи:** ВЕ була встановлена у 37 зразках раку передміхурової залози (П) методом ПЛР у реальному часі. Пухлини були з різним ступенем Глісону (СГ) та різними ста-

діями. Крім того ВЕ виявлена у парних умовно-нормальних тканинах (УНТ) та аденомах (А) передміхурової залози. **Результати.** Підвищені рівні ВЕ *FASN* та *COX2* знайдено у групі аденокарцином та аденокарцином зі СГ=7 порівняно з групою аденом. Чотири гени, а саме *FASN, LDLR, HMGCR* та *COX2* продемонстрували значущі порушення ВЕ у групах аденокарцином з різними стадіями у порівнянні з групою аденом та УНТ. Три гени (*LDLR, HMGCR, COX2*) показали значущі негативні кореляції зі стадіями та СГ у групі аденокарцином. Для генів *FASN, LDLR, HMGCR* виявлена низка позитивних кореляцій ВЕ з маркерами епітеліальних клітин, тоді як для генів *CPT1C* та *COX2* знайдено ряд позитивних кореляцій ВЕ з маркерами мезенхімальних клітин, фібробластів та запалення у групі аденокарцином. **Висновки:** Досліджені гени, що залучені до контролю ліпідного метаболізму показали диференційну експресію у пухлинах передміхурової залози. Рівні ВЕ генів *FASN, HMGCR* та *COX2* можуть бути маркерами чутливості та ефективності препаратів-інгібіторів експресії генів. Для підтвердження отриманих результатів необхідні додаткові дослідження на більшій вибірці пацієнтів.

Ключові слова: рак передміхурової залози, відносна експресія генів, ліпідний метаболізм, фармакологічні маркери.

Паттерны экспрессии генов, регулирующих липидный метаболизм в опухолях простаты

А. В. Геращенко, А. А. Кононенко,
Ю. Н. Бондаренко, Э. А. Стаховский,
В. И. Кашуба

Цель: Установить уровни относительной экспрессии (ОЭ) генов, участвующих в липидном метаболизме в опухолях простаты. На основе этих паттернов выявить клинически-значимые специфические нарушения. **Методы:** ОЭ была установлена в 37 образцах рака простаты (О) методом ПЦР в реальном времени. Были использованы опухоли с разной степенью Глиссона (СГ) и различными стадиями заболевания. Кроме того ОЭ установлена в условно-нормальных тканях (УНТ) и аденомах (А) простаты. **Результаты.** Повышенные уровни ОЭ *FASN* и *COX2* выявлены в группах аденокарцином и аденокарцином с СГ=7 по сравнению с группой аденом. Четыре гена, а именно *FASN, LDLR, HMGCR* и *COX2* показали значимые изменения ОЭ в группах аденокарцином с разными стадиями по сравнению с группой аденом и УНТ. Три гена (*LDLR, HMGCR, COX2*) показали значимые негативные корреляции со стадией и СГ в группе аденокарцином. Для генов *FASN, LDLR, HMGCR* выявлен ряд позитивных корреляций ОЭ с маркерами эпителиальных клеток, тогда как для генов *CPT1C* и *COX2* найден ряд позитивных корреляций с маркерами мезенхимальных клеток, фибробластов и воспаления в группе аденокарцином. **Выводы.** Исследованные гены, участвующие в контроле липидного метаболизма, показали дифференциальную экспрессию в опухолях простаты. Уровни ОЭ генов *FASN, HMGCR* и *COX2* могут быть маркерами чувствительности и эффективности препаратов-ингибиторов экспрессии. Для подтверждения полученных результатов необходимы дополнительные исследования на большей выборке пациентов.

Ключевые слова: рак простаты, относительная экспрессия генов, липидный метаболизм, фармакологические маркеры.

Received 24.08.2018