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Programming Of Energy Metabolism In Prostate Carcinoma: In Silico Analysis

Prostat Karsinomunda Enerji Metabolizmasının Yeniden Programlanması: İn Siliko Analiz

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ABSTRACT

Prostate carcinoma is known to be a hypoxic and lipogenic solid tumor, exhibiting a remarkable oncogenic modulated metabolic programming. Increasing intake of glucose and aerobic glycolysis, called the Warburg effect, are main metabolic changes in hypoxic tumors. Protein,nucleid acid , and lipid biosynthesis are the other metabolic processes associated with cancer metabolic rewiring. In addition to “Warburg effect” in prostate carcinoma, fatty acids, glutamine, and mitochondrial oxidative phosphorylation in alternative metabolic pathways are considered main contributors to tumorigenesis. The aim of this study is to investigate reprogramming of energy metabolism in well and poorly differentiated prostate carcinomas with seminal vesical invasion. The GSE32448 gene’s microarray data were downloaded from the "Gene Expression OmniBus". Differences in gene expression levels were generated by re-analyzing the mRNA transcripts of tissues obtained from 40 patients specimens. "Biobase", "Limma" and "Geoquery" libraries were obtained with bioinformatics analysis using R program. Statistically significant differences were found in genes related to fatty acid metabolism. Increased awareness of the role of lipid metabolism in prostate cancer can lead to developing better treatment strategies against this malignancy.

Keywords: Bioinformatic, Metabolic Reprogramming, Molecular Pathology, Prostate Cancer.

ÖZ

Prostat karsinomunun, dikkate değer bir onkojenik modüle edilmiş metabolik programlama sergileyen, hipoksik ve lipojenik bir solid tümör olduğu bilinmektedir. Glukoz alımının ve Warburg etkisi olarak bilinen aerobik glikolizin artması hipoksik tümörlerin ana metabolik değişiklikleridir. Protein, nükleik asit ve lipid biyosentezi, kanserin metabolik yeniden programlanması ile ilişkili diğer metabolik süreçlerdir. Prostat karsinomunda “Warburg” etkisine ek olarak, alternatif metabolik yolaklarda yağ asitleri, glutamin ve mitokondriyal oksidatif fosforilasyon tümörün progresyonunda önemli katılımcılar olarak kabul edilir. Bu çalışmanın amacı, az diferansiye (seminal vezikül invazyonlu) ve iyi diferansiye prostat karsinomlarında enerji metabolizmasının programlanmasını araştırmaktır. GSE32448 mikrodizi verileri “Gene Expression OmniBus" tan indirildi. Gen ekspresyon seviyesindeki farklılıklar, 40 prostat kanseri örneğinden elde edilen dokuların mRNA transkriptlerinin yeniden analiz edilmesiyle üretildi. R programı kullanılarak biyoenformatik analiz ile "Biobase", "Limma" ve "Geoquery" kütüphaneleri elde edildi. Yağ asidi metabolizması ile ilgili genlerde istatistiksel olarak anlamlı farklılıklar bulunmuştur. Prostat kanserinde lipid metabolizmasının rolüne ilişkin artan farkındalık, bu maligniteye karşı daha iyi tedavi stratejilerinin geliştirilmesine yol açabilir.

Anahtar Kelimeler: Biyoinformatik, Metabolik Yeniden Programlama, Moleküler Patoloji, Prostat Kanseri.

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INTRODUCTION

Normal cells gradually develop a sequence of biological skills, known as hallmarks of cancer, during the multistep development of human tumors.[1]

Among these skills, oncometabolic rewiring that fuels tumorigenesis is defined by upregulation of glycolysis, phosphogluconate pathway, generation of new mitochondria, glutaminolysis, lipid metabolism.

Metabolic reprogramming is a fundamental requirement for tumor cells to survive in the nutrient and oxygen-depleted tumor microenvironments during carcinogenesis. [2]

Nonetheless, for accelerated growth and proliferation, cancer cell requires metabolism alterations due to a well-established biological phenomenon, tumor hypoxia which is frequently observed in solid malignant tumors, which include head and neck, cervix, liver, lung, and prostate cancer.[3]

Due to its highly heterogeneous properties, every cancer has its own metabolic features.

The efficient production of ATP in some cancer cells can be achieved by switching from mitochondrial oxidative phosphorylation to aerobic glycolysis (the "Warburg effect"). [4]

Warburg effect is favored for producing ATP while still maintaining Krebs cycle and mitochondrial oxidative phosphorylation due mainly to the rapid proliferation of cancer cells, adaptation to the hypoxic tumor microenvironment, increased invasion and metastasis of cancer cells in this acidic microenvironment, and the production of less reactive oxygen species.

Apart from the Warburg effect, oxidative phosphorylation is used as a pathway of energy production, especially in cervical and breast cancer cell lines.[5]

Furthermore, recent cancer metabolism research findings have shown that cells reprogram their mitochondria to enhance lipid synthesis for carcinogenesis and cancer cell survival. [6]

The significance of altered fatty acid metabolism in cancer, in particular, has been revived because they are essential secondary messengers and also can be used as fuels for energy production in addition to their main function as structural components of the membrane matrix.

Lipid accumulation in cytosolic lipid droplets will satisfy this strong demand for lipids in cancer cells.

Besides, lipid droplets may provide energy for cancer cells during nutrient and oxygen depletion periods.[7]

Lung cancer, triple-negative breast cancer, acute myeloid leukemia, hepatocellular carcinoma, glioma, and low-grade astrocytoma exhibit a high activity of fatty acid oxidation enzymes.[8]

Prostate cancer is the most common male cancer. One of the best predictors of devastating prostate cancer's biological behavior is Gleason score, a pathological indicator of the differentiation of tissue of the prostate tumor.[9]

Prostate cancer is considered as a lipogenic tumor. Under hypoxic conditions, prostate cancer cells were reprogrammed their lipid metabolism as a way to proliferate. In spite of that, metabolic reprogramming in prostate cancer has not been clearly elucidated yet.[10]

In this study, we aimed to explore the re-lationship between metabolic reprogramming and Gleason score in prostate cancer.

MATERIALS AND METHODS

Microarray Data of Gene Expressions

GSE32448 was generated and downloaded from the National Center for Biotechnology Information's website. GSE32448 dataset contained 40 prostate carcinoma tissues and corresponding normal tissues.

Patient Selection Criteria

From over 300 patients treated radical prostatectomy, laser-capture microdissection compatible 40 prostate cancer specimens were selected from age and race (Caucasians) matched poorly differentiated or well-differentiated patients with no family history of prostate cancer.

Differentially Expressed Genes Analysis

In this analysis, raw data files were used, including Affymetrix chip CEL files.

Differentially expressed genes (DEGs) were determined using R program with GEO query and limma packages.

The P value <0.05 and log fold change (FC)> 1.0 or log FC <− 1.0 were considered to be statistically significant.

Classical t test was used for screening DEGs.

DEGs with statistical significance were selected between the prostate carcinoma samples and corresponding normal tissues samples.

Probe sets unmatched with gene symbols were excluded, and genes which have more than one probe set were averaged.

Gene Ontology (GO) and Pathway Enrichment Analysis

DEGs were uploaded to DAVID 6.8 to identify GO categories and pathway categories.

P value of <0.05 and FDR value of <0.05 were considered statistically significant. GO analysis and KEGG pathway enrichment analysis were performed.

Gene Interaction Network Construction

DEGs list was uploaded to STRING database with interaction network chart with a combined score> 0.4.

Ethical Aspect of Research

All data was freely accessible from GEO database (www.ncbi.nlm.nih.gov/geo/), and none of the authors carried out any experiment on animals or humans.

Limitation of Research

It needs further specific gene silencing experiments to verify and confirm the metabolic reprogramming in prostate cancer.

Besides, immunohistochemical staining and Western Blotting can be performed in human prostate cancer specimens for protein expression of selected genes that differ significantly in microarray analysis.

The fact that our research population is Caucasian and the lack of racial/ethnic diversity is another limiting aspect of our study.

RESULTS AND DISCUSSION

Identification of DEGs

420 DEGs were identified by integrated bioinformatics analysis, including 41 upregulated and 379 downregulated DEGs in well-differentiated prostate cancer tissue compared with matched normal prostate glands.

221 DEGs were identified by integrated bioinformatics analysis, including 67 upregulated and 154 downregulated genes in poorly differentiated prostate cancer tissue compared with matched normal prostate glands.

Remarkable upregulated and down-regulated genes in well-differentiated and

poorly differentiated prostate cancers are shown in Table 1.

Table 1. Remarkable Metabolic Rewiring Genes in Prostate Cancer

Well-differentiated prostate cancer	
Up-regulated	ACSM1, AMACR, PLA2G7, OR51E2, OR51E1, CAMKK2
Down-Regulated	PDK4, ALAD, UCP2, PTGS2, SLC2A5, INSIG1, MSMO1, FDFT1, EBP, HMGCS1, SLC16A5, ALOX15B
Poorly differentiated prostate cancer	
Up-Regulated	APOE, HSD17B4, PLA2G7, IMPA1, TWIST1 ACSM1, AMACR, LYPLA1, FABP5, PLA2G7, CAMKK2, NEDD4L, SLC25A6, MAPK9, OR51E2, PEX10, OR51E1
Down-Regulated	PDK4, UCP2, SLC2A5, SLC16A5, INSIG1, SCD, FDFT4, ALOX15B, SEC14L2

GO and Pathway Enrichment Analysis

Gene ontology analysis was performed after candidate DEGs were identified.

The results in terms of programming of energy metabolism indicated that the downregulated DEGs in poorly differentiated prostate cancer were "Lipid modification", "Lipid oxidation" and are shown in Figure1.

The results in terms of programming of energy metabolism indicated that the upregulated DEGs in poorly differentiated prostate cancer were mainly enriched in mainly enriched in lipid biosynthetic process,

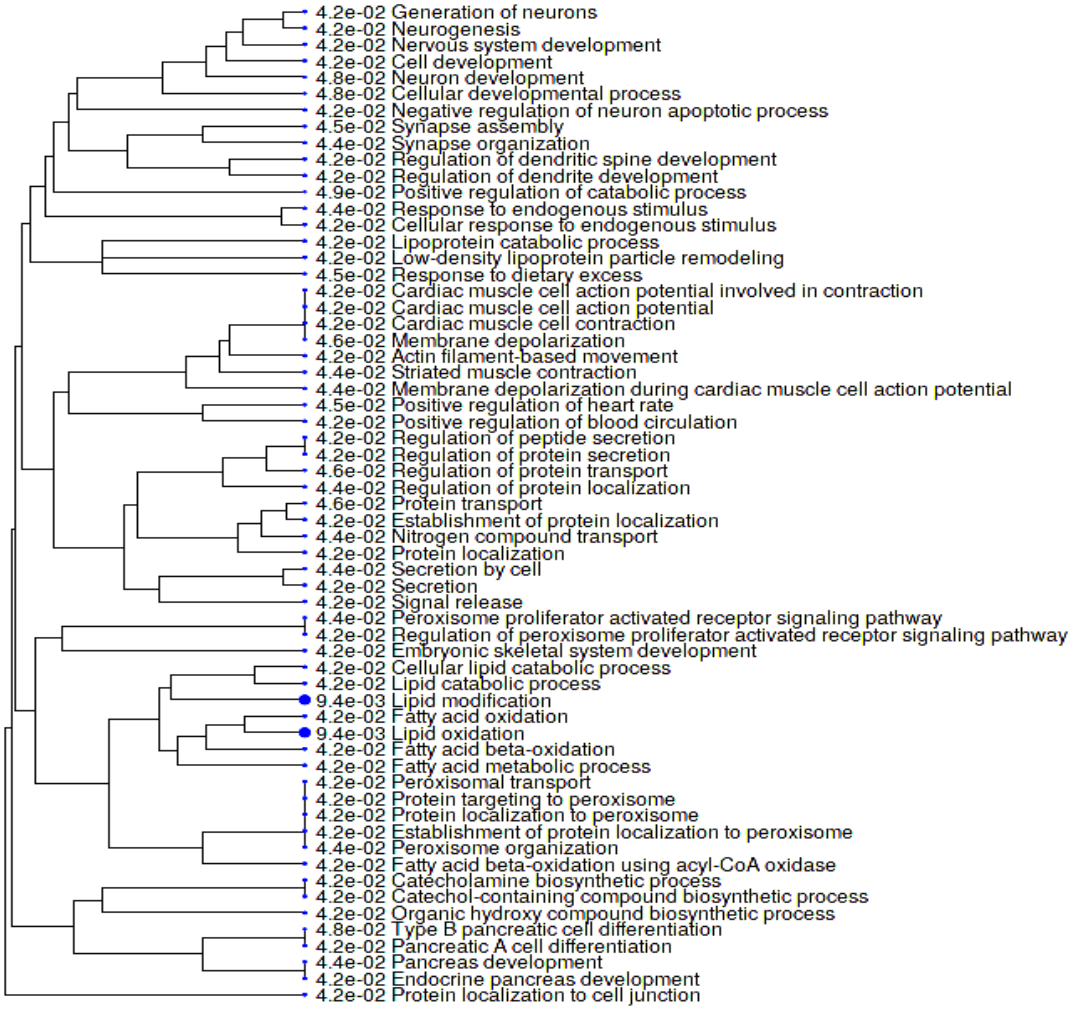

Figure 1. Upregulated Genes of Poorly Differentiated Prostate Adenocarcinoma Are Summarized By A Hierarchical Clustering Tree.

The results in terms of programming of energy metabolism indicated that the upregulated DEGs in well-differentiated prostate cancer were mainly enriched in "Lipid oxidation"," Regulation of cellular process", "Ether lipid metabolism", "Regulation of lipolysis in adipocytes".

The results in terms of programming of energy metabolism indicated that the downregulated DEGs in well-differentiated prostate cancer were mainly enriched in "Lipid synthetic process" and "Insulin receptor signaling pathway", "Cholesterol biosynthetic process".

Protein-Protein Interaction Network Analysis

The relationship between enriched pathways in upregulated genes of poorly differentiated prostate adenocarcinoma are shown in Figure2.

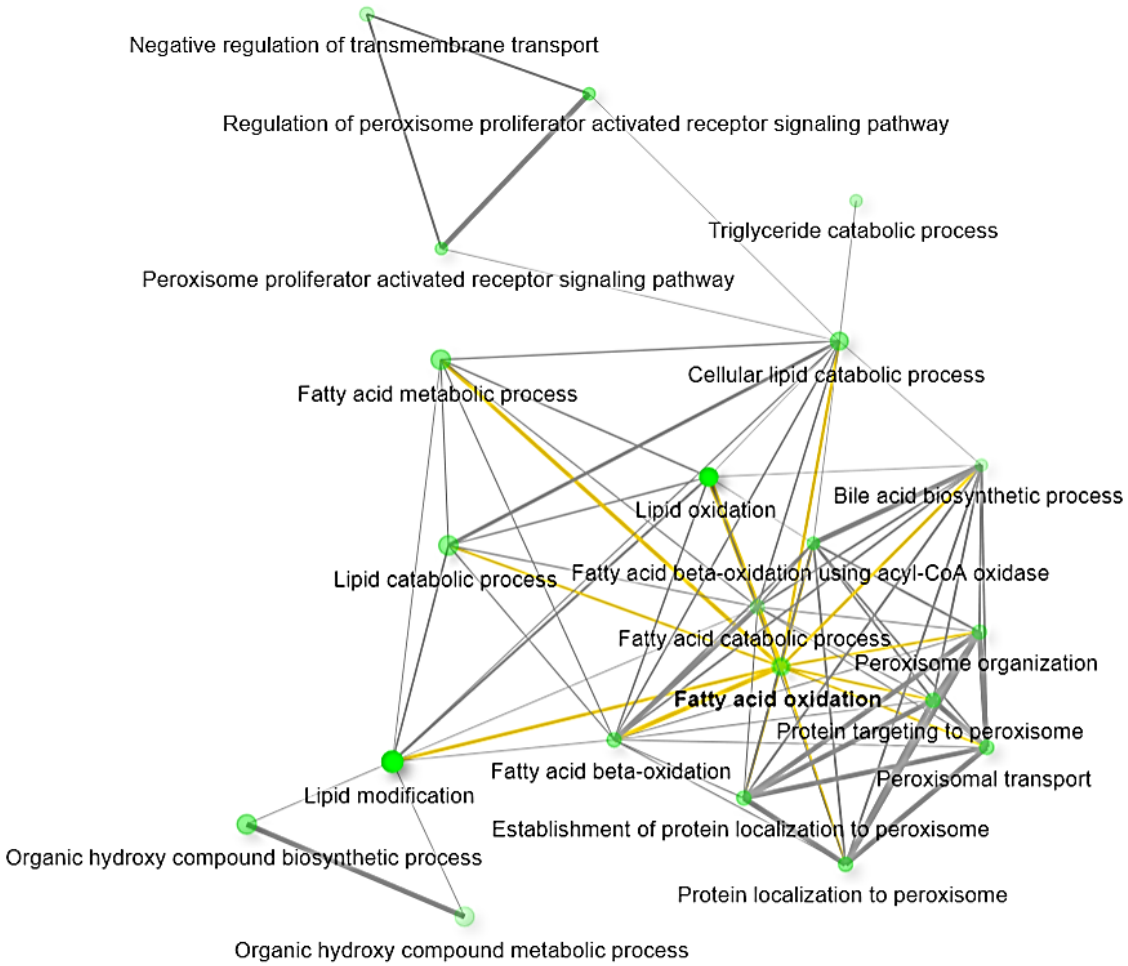

Figure 2. The Relationship Between Enriched Pathways In Upregulated Genes Of Poorly Differentiated Prostate Adenocarcinoma.

Upregulated Remarkable Metabolic Reprogramming Genes

Medium-chain acyl-CoA synthetases (ACSM) use medium-chain fatty acids as a substrate for beta-oxidation.[11] An essential cofactor for mitochondrial metabolism, ACSM1, involved in the activation of lipoic acid.[12]

Alpha-methylacyl-CoA racemase (AMACR), which converts branched-chain fatty acids for β-oxidation, has a crucial role for the oxidation of fatty acid and upregulated in prostate.[13]

Phospholipase A_2 hydrolyze phospholipids into fatty acids and is encoded by the PLA2G7 gene. It was found that the PLA2G7 gene was significantly linked to the

altered phospholipid metabolism of prostate cancer and played a key role in pathogenesis.[14]

OR51E2 is upregulated in prostate cancer. Short chain fatty acids seemingly stimulate OR51E2.[15] The modulated mitochondrial complex V activity by OR51E1 and OR51E2 increases production of ATP by the stimulation of mitochondrial AKT.[16]

By activating AMPK, CAMKK2 induces glycolysis, and altering the cell metabolism with increasing fatty acid synthesis.[17,18]

Upregulation of ApoE is previously reported in several cancers including prostate cancer.[19] APOE has been involved in lipid transfer to glioblastoma cells. APOE is upregulated in the PC-3 cell lines and the Gleason score is directly associated with its expression.[20]

HSD17B4 has a role in the peroxisomal beta-oxidation pathway for lipids.[21] Compared to benign epithelium, HSD17B4 is significantly overexpressed in prostate cancer.[22]

The fatty acid-binding proteins (FABPs) have a crucial role for uptake and transport of lipid. Energy metabolism, is controlled by FABP5 in prostate cancer cells.[23]

Upregulation of Twist1 is previously documented in breast, liver, prostate, gastric cancers.[24] In prostate cancers overexpression of Twist1 is correlated with more aggressive and metastatic disease.[25] Twist1 inhibits glycolysis but stimulates fatty acid oxidation.[26]

Downregulation of NEDD4L increases the mitochondrial oxygen consumption and decreases extracellular glutamine uptake.[27]

Adenine nucleotide translocase is encoded by SLC25A6, has a crucial role in importing ADP from the cytosol and exporting ATP produced in the mitochondrial matrix.[28]

Downregulated Remarkable Metabolic Reprogramming Genes

Downregulation of PDK4 reduces the use of glucose and increases lipid metabolism.[29] Fatty acid synthesis is increased by knockdown of PDK4.[30]

Glucose utilization and proliferation in normal cells are increased by downregulation of uncoupling protein 2 (Ucp2). On the cancer cells front, downregulation of UCP2 switches their metabolism from oxidative phosphorylation to glycolysis. UCP2 seems to be a crucial cellular metabolism regulator.[31]

CONCLUSION AND RECOMMENDATIONS

Metabolic reprogramming of prostate cancer cells is mainly characterized by decreasing glycolysis and increasing fatty acid oxidation. In addition, it has been concluded that genes associated with lipid metabolism are more common in poorly differentiated tumors than well-differentiated tumors.

Our results may strengthen our understanding of the molecular background in the metabolic rewiring of prostate cancer.

In order to promote new treatment strategies, and diagnostic tools, it is therefore important to understand metabolic reprogramming of prostate cancer.

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