OTJHS
Online Turkish Journal of Health Sciences

e-ISSN: 2459-1467

OTSBD
Online Türk Sağlık Bilimleri Dergisi

Online Turkish Journal of Health Sciences 2023;8(4):497-502 *Online Türk Sağlık Bilimleri Dergisi 2023;8(4):497-502*

Investigation of Protein Expressions of PLA2G7, UCP2 and NEDD4L Genes Associated with Fat Droplet Formation in Prostate Cancer

Prostat Kanserinde Yağ Damlacık Oluşumu ile İlişkili PLA2G7, UCP2 ve NEDD4L Genlerinin Protein Ekspresyonlarının Araştırılması

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ABSTRACT

Objective: Prostate cancer (PCa) is characterised by dysregulated lipid metabolism. PCa cells store lipids as lipid droplets and use them to facilitate proliferation and growth. In this study, we aim to investigate the expression levels of PLA2G7, UCP2 and NEDD4L proteins, which are involved in lipid droplet metabolism, in PC3 (advanced metastatic) and DU145 (intermediate metastatic) PCa cells.

Materials and Methods: DU145 and PC3 cells were cultured in a high-glucose DMEM medium containing 10% FBS, 1% penicillin-streptomycin, and 1% non-essential amino acid. The expression levels of PLA2G7, UCP2, and NEDD4L protein were assessed with ELISA assay.

Results: There was no significant difference in the protein level of PLA2G7 between the PC3 and DU145 cells ($p>0.05$), while the protein level of UCP2 increased in the PC3 cell line significantly ($p<0.05$). The protein level of NEDD4L decreased significantly in the DU145 cell line when compared to the PC3 cell line ($p<0.05$).

Conclusions: As a result of this study, the UCP2 gene might play a role in the progression of prostate cancer, and there could be a relationship between NEDD4L and cell proliferation control.

Keywords: Lipid droplet, NEDD4L, PLA2G7, Prostate cancer, UCP2

ÖZ

Amaç: Prostat kanseri (PCa) düzensiz lipid metabolizması ile karakterize edilen bir kanserdir. PCa hücrelerinin; lipitleri lipid damlacıkları şeklinde depoladığı ayrıca proliferasyon ve büyümeyi kolaylaştırmak amacıyla membran sentezi için yapıtaşı olarak kullandığı gözlemlenmiştir. Bu çalışmada amacımız PC3 ve DU145 prostat kanseri hücre hatlarında lipid damlacık metabolizmasında görev alan PLA2G7, UCP2 ve NEDD4L proteinlerinin ekspresyon seviyelerini incelemektir.

Materyal ve Metot: Prostat kanseri hücre hatları orta seviye metastatik DU145 ve ileri metastatik PC3 %10 FBS, %1 penisilin-streptomisin, %1 non-esansiyel amino-asit içeren yüksek glukoz DMEM besi yerinde çoğaltılmıştır. Daha sonra PLA2G7, UCP2 ve NEDD4L proteinlerinin ekspresyon düzeyleri ELISA testi ile incelenmiştir.

Bulgular: Bu çalışmada, PC3 ve DU145 hücrelerinde PLA2G7 protein seviyeleri açısından anlamlı bir fark olmadığı ($p>0,05$) ancak UCP2 protein seviyelerinin PC3 hücrelerinde anlamlı derecede arttığı gösterilmiştir ($p<0,05$). Bunun aksine NEDD4L protein seviyeleri DU145 hücre hattında anlamlı derecede düşmüştür ($p<0,05$).

Sonuç: Çalışmanın sonucunda, UCP2'nin prostat kanserinin ilerlemesinde rolü ve NEDD4L ile hücre proliferasyonu kontrolü ile arasında bir ilişki olabilir.

Anahtar Kelimeler: Lipid damlacığı, NEDD4L, PLA2G7, Prostat kanseri, UCP2

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Yayın Bilgisi / Article Info:
Gönderi Tarihi/ Received: 08/09/2023
Kabul Tarihi/ Accepted: 12/11/2023
Online Yayın Tarihi/ Published: 18/12/2023

Atıf / Cited: Atakol D and et al. Have Investigation of Protein Expressions of PLA2G7, UCP2 and NEDD4L Genes Associated with Fat Droplet Formation in Prostate Cancer. *Online Türk Sağlık Bilimleri Dergisi* 2023;8(4):497-502. doi: 10.26453/otjhs.1330334

INTRODUCTION

PCa is a slow-growing and hormone-dependent cancer. It uses lipid oxidation for energy.[1] Normal cells can organise intracellular lipid levels by regulating lipid uptake of synthesis and degradation. In contrast, cancer cells can get more lipids and raise lipid synthesis owing to increased energy demand. When cellular lipids are in excess amount, they can be converted to triglycerides and cholesterol esters in the ER and stored as lipid droplets (LD). Lipids stored in LD are broken down to meet the cell's energy needs in starvation.[2] LD controls both the uptake of lipids and their use in response to cellular needs.[3]

There are genes involved in the regulation of these processes. In this study, PLA2G7, UCP2 and NEDD4L genes that play a role in the regulation of these processes were examined. Phospholipase A2 Group 7 (PLA2G7), a 45 kDa monomeric protein, is also named lipoprotein-associated phospholipase A2 (LpPLA2) because two-thirds of the enzyme circulates in theplasma bound to LDLs and the remaining is associated with HDLs and other lipoproteins.[4]PLA2G7 contributes to creating the pool of free fatty acids required for LD synthesis.[5] Uncoupling Proteins (UCPs) are proteins belonging to the carrier family SLC25 (The solutioncarrier family 25) located in the mitochondrial inner membrane.[6] They dissipate oxidationenergy in the form of heat by functioning as separating oxidative phosphorylation from ATPproduction. Uncoupling Protein 2 (UCP2) is a member of UCPs and plays a role in LDformation due to its relation with fatty acid synthase (FASN). FASN is responsible for LD accumulation in the cell.[7] In addition, it is known that inhibition of UCP-2 leads to down-regulation of FASN. So, UCP-2 is thought to have essential roles in LD formation.[2]

Neural precursor cells expressed developmentally downregulated gene 4-like (NEDD4L) is an E3 ubiquitin ligase regulating ubiquitination and protein degradation.[8] Spartin protein, which is involved in the turnover of LD and disruption of the epidermal growth factor receptor, interacts with NEDD4L and organises lipid droplet metabolism.[9]

The study aims to show the relationship of these proteins with increased metastasis potential by showing the expression levels of PLA2G7, UCP2 and NEDD4L proteins, which are involved in LD metabolism in PC3 and DU145 prostate cancer cell lines.

MATERIALS AND METHODS

Ethics Committee Approval: Ethics committee approval is not required for studies to be conducted on commercially available human cadavers, cadaver parts and other biological materials. Since a commercially available cell line was used in our study, ethics committee approval was not required.

***Culture of Cells*:** The human prostate carcinoma cell line PC3 and DU145 were obtained from Muğla Sıtkı Koçman University Research Laboratories Center Coordinators andYeditepe University Department of Genetics and Bioengineering, respectively. Cells were cultured in high glucose DMEM containing 10% FBS, 1% penicillin-streptomycin and 1% non-essential amino acid (L-glutamine) and were passaged when they reached 80-90% confluency.

Cell Lysate Preparation: Firstly, the medium in the flask was removed and washed with PBS. Then, the collected trypsinised cells were centrifuged at 1000 rpm at +4 °C for 5 minutes and later on, the supernatant was removed. After that, the cells were kept at -80°C for 5 minutes andat 37°C for 5 minutes, respectively. This process was repeated 3 times. Finally, the bursted cellmixture was centrifuged at 1500 rpm for 10 min at +4 °C. The supernatant was collected in aneppendorf tube, and the ELISA protocol was applied.

Elisa Test: For ELISA experiments, the NEDD4L ELISA kit (ShanghaiCoon Koon Biotech Co., Ltd., 20522), UCP2 ELISA kit (ShanghaiCoon Koon Biotech Co., Ltd., 13901), and Lipoprotein-associated phospholipase A2 (Lp-PLA2) ELISA kits (ShanghaiCoon Koon Biotech Co., Ltd., 12314) were used.

While applying the ELISA protocol, 50µL standard solution, 10 µL of sample and 40 µL of sample diluent were added to the wells, respectively. They were incubated at 37°C for 60 minutes. After incubation, chromogen A and chromogen B solutions were added and incubatedagain at 37°C for 15 minutes. Finally, 50 µL of stop solution was added, and the measurement was made at 450 nm absorbance in the Epoch Reader Spectrophotometer device.

Statistical Analysis: GraphPad Prism 9 program was used for statistical analysis. Comparisonswere made using the unpaired t-test. The significance level was accepted as $p<0.05$.

RESULTS

ELISA tests were used to determine the level of each protein (PLA2G7, UCP2 and NEDD4L)in PC3 and DU145 prostate cancer cells. The expression level of PLA2G7 protein was found to be 2,18 ng/mL in the PC3 cell line, while it was 3,11 ng/mL in the DU145 cell line. There was no statistically significant difference in PLA2G7 level between the two cell lines ($p>0.05$)(Figure 1).

The expression level of protein UCP2 was found to be 221,3 pg/mL in the PC3 cell line, whileit was 39,3 pg/mL in the DU145 cell line. It was deter-

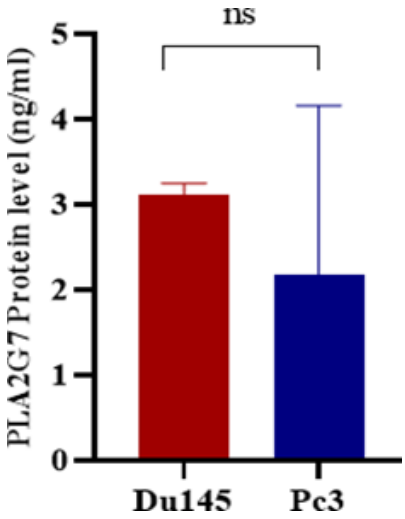

Figure 1. The expression level of PLA2G7 protein in DU145 and PC3 cells (ns:p> 0.05).

mined that the expression level of UCP2protein was higher in PC-3 cells than in DU145 cells ($p<0.05$) (Figure 2).

The expression level of NEDD4L protein was found to be 0,76 ng/mL in the PC3 cell line, whileit was 1,4 ng/mL and was found in the DU145 cell line. The expression level of NEDD4L protein was found to be higher in DU145 cells than in PC3 cells ($p<0.05$) (Figure 3).

DISCUSSION AND CONCLUSION

Cancer cells require adaptation across multiple metabolic processes to provide the energy needed for their enhanced rate of proliferation. Irregularity in lipid metabolism is one of the metabolic changes in cancer.[10] These changes are involved in several mechanisms, including LD accumulation, increased lipid uptake, de novo lipid synthesis, and regulation of lipolysis. LD is involved in the proliferation, growth and stress response of cancer cells [11]. Lipid

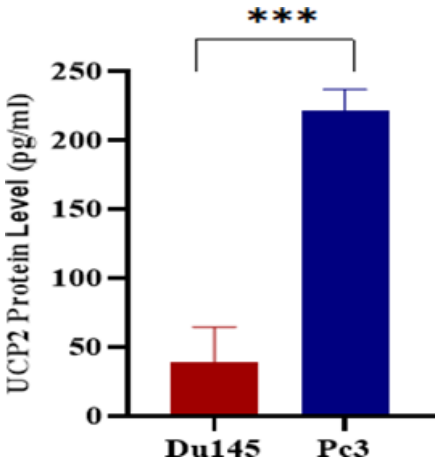

Figure 2. The expression level of UCP2 protein in DU145 and PC3 cells (***: p≤ 0.001).

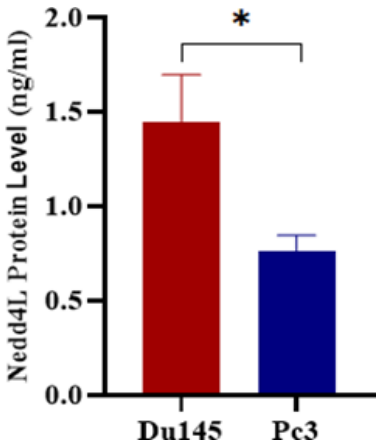

Figure 3. The expression level of NEDD4L protein in DU145 and PC3 cells (*: p≤ 0.05).

accumulation in LD is a hallmark of prostate cancer cells.[12] In this study, the expression levels of PLA2G7, UCP2 and NEDD4L proteins were investigated.

Lipoprotein-associated phospholipase A2, or platelet -activating factor (PAF), contributes to thecreation of the pool of free fatty acids required for LD synthesis.[5] Scientific research on this subject has shown that LD causes tumour development in tumours such as breast cancer, ovariancancer and multiple myeloma.[13] The expression of PLA2G7 is related to hormone receptor negativity and poor prognosis, especially in metastatic breast cancer. It has been shown to support metastasis and invasion, as well as its role in the regulation of epithelial-mesenchymaltransition (EMT) in breast cancer. Also, inhibition of PLA2G7 expression resulted inreduced vimentin expression, enhanced E-cadherin expression, and reduced cell invasion.[14] Substrate hydrolysis catalysed by PLA2G7 also generates platelet-activating factor (lysoPAF)/lyso phosphatidylcholine (lysoPC) and oxidised fatty acids. Stafforini et al. have reported that PLA2G7 has pro-inflammatory and pro-oxidative activities.[15] In addition, it contributes to the formation of the pool of free fatty acids needed for LD synthesis.[5] Vainio et al. indicated that PLA2G7 is a new biomarker in 50% of primary prostate cancers and 70% ofmetastatic prostate cancers. It has also been associated with aggressive prostate cancer.[16] Benliet al. found higher PLA2G7 activity in prostate cancer patients than in the control group.[13] Kispert reported that increased PLA2G7 expression reduces the motility of tumour cells.[17] In our study, it was found that the expression level of PLA2G7 protein was lower in the PC3 celllinet than in the DU145 cell lines, but it was not statistically significant ($p>0.05$).

UCP2 is a mitochondrial transporter that is associated with the changes in cell proliferation [18].Recent advances in cellular glucose and lipid metabolism research described UCP2 as an important regulator of cellular fuel utilisation and lipid metabolism.[7] Pecqueur et al. showed that the inactivation of UCP2 caused a decrease in mitochondrial fatty acid oxidation and an increase in glucose metabolism.[19] Esteves et al. demonstrated that targeting mitochondrial function through UCP2 could reverse reprogramming, leading to a return toward less glycolysis and higher oxidative phosphorylation capacity.[20] In the research by Burch et al., higher UCP2 expression levels were found in non-malignant RC77N/E and malignant RC77T/E cells from prostate adenocarcinoma cells. As a result of RT-PCR sequencing and Western Blotanalysis, the expression level of UCP2 protein was found to be considerably increased in malignant RC77T/E cells compared to non-malignant RC77N/E cells.[21] In our study, it was determined that the level of UCP2 in PC3 cells was higher than in DU145 cells ($p<0.05$). Increased UCP2 level suggests that it might promote tumour growth and metastasis in prostate cancer. In addition, these results show that this protein can be used as a potential new drug targetand biomarker.

NEDD4L is an E3 ubiquitin ligase that has been reported to attend cellular procedures by regulating substrate ubiquitination and protein degradation.[8] Until today, many E3 ubiquitin ligases, including NEDD4L, were identified as the regulators of lipid mechanism.[22] In the studyby Hooper et al., spartin protein, which is involved in the turnover of lipid droplets and disruption of the epidermal growth factor receptor, has been shown to interact with E3 ubiquitin ligases belonging to the NEDD4 family.[23] In addition, in the study by Alberts et al., it has beenreported that spartin activates NEDD4 family ligases for the ubiquitination and degradation oflipid droplet proteins like adipophilin.[9] Hu et al. evaluated radical prostatectomy (RP) samples from 56 patients with clinically localised PCa and benign prostatic hyperplasia (BPH) samples from 31 patients in terms of NEDD4L expression. They reported that NEDD4L is considerablydecreased in PCa in comparison with benign prostate tissue. In the same study, 86% of cancers with a Gleason score ≥ 8 had negative NEDD4L expression.[24] In our research, it was determinedthat the expression level of NEDD4L protein in DU145 cells was higher than in PC3 cells ($p<0.05$). These results show that regulation of NEDD4L expression may play a role in the development of prostate cancers.

In conclusion, it was aimed to show the expression levels of PLA2G7, UCP2 and NEDD4L proteins, which are involved in LD formation and storage in prostate cancer. It was determinedthat the expression level of PLA2G7 protein did not have a statistically significant difference. Therefore, further research is needed in the PLA2G7. It was determined that the expression level of UCP2 protein in PC3 cells was higher than in DU145 cells. As the expression level ofUCP2 protein increases in association with advanced tumour aggressiveness, examination of serum UCP2 protein can inform clinicians about disease progression. It can be thought that UCP2 protein might support the progression of the disease in prostate cancer and could also betherapeutic in targeted therapies against altered tumour metabolism. In addition, the expression level of NEDD4L protein in DU145 cells was higher than in PC3 cells. The decreased expression level of NEDD4L protein in PCa, especially in more aggressive cells, suggests a relationship between the control of cell proliferation and the NEDD4L protein. The expressionlevel of NEDD4L protein might be a prognostic marker for prostate cancer development. Detailed

investigations should be conducted to comprehend the role of NEDD4L in tumorigenesis better. A better understanding of NEDD4L's involvement in prostate cancer progression could provide more effective clinical therapy.

Ethics Committee Approval: An ethical approval for the study is not required. In this study, secondary cell culture was used.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – MEE, EŞ; Materials – DA, HY; Data Collection and/or Processing – DA, HY; Analysis and/or Interpretation – DA, ET; Writing –DA; Supervision – EŞ.

Peer-review: Externally peer-reviewed.

Other information: This study was presented as an oral presentation at the 2nd International Graduate Studies Congress (Igscong'22) (June 2022).

Financial Support: This research was supported by Ankara Yıldırım Beyazıt University Scientific Research Projects Unit with project number 2307.

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