*Research Article / Araştırma Makalesi*

# Expression Analysis in the Transcription Level of PTGS2, CALR, MAGE-A3 Genes in Non-Small Cell Lung Cancer

## Küçük Hücreli Dışı Akciğer Kanserinde PTGS2, CALR, MAGE-A3 Genlerinin Transkripsiyon Düzeyinde İfade Analizi

Büşra KÖSE [1], Süreyya BOZKURT [2]

1 Biochemistry and Molecular Biology, Institute of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
2 Department of Medical Biology, Faculty of Medicine, Istinye University, Istanbul, Turkey

Abstract

Background: In lung cancer, expression changes in genes that indirectly trigger carcinogenesis are a remarkable issue. In addition to mutations, detection of expression of genes that affect cell division, apoptosis, invasion and cell migration is important for studies to develop new inhibitor or monoclonal antibody drugs. Aim of this study was to investigate the mRNA expression levels of PTGS2, CALR and MAGE-A3 genes in the A549 cell line which is modeling lung adenocarcinoma compared to the BEAS-2B cell line which is modeling healthy bronchial epithelium.
Materials and Methods: Quantitative Real-time PCR was performed to obtain the mRNA expression level changing.
Results: In the results of the study, the mRNA level expression difference in A549 cell line compared with BEAS-2B cell line 229,13-fold increase in PTGS2 gene; 4,03-fold increase in CALR gene; 3,41-fold increase in MAGE-A3 gene were observed. These three genes are known to play roles in cancer cell division, apoptosis resistance, invasion, and angiogenesis.
Conclusions: As a result of this study, these three genes are thought to have jointly effect on the NF-κB signaling pathway. It may be beneficial to investigate on protein level for these three genes to illuminate at this point.

Key Words: Lung Cancer, PTGS2 gene, CALR gene, MAGE-A3 gene

Öz.

Amaç: Akciğer kanserinde dolaylı yoldan karsinogenezi tetikleyen genlerdeki ifade değişimleri dikkat çekici bir konudur. Mutasyonların yanı sıra hücredeki bölünme, apoptoz, invazyon ve hücre göçleri üzerinde etkili olan genlerin ifade artışının saptanması, yeni inhibitör veya monoklonal antikor ilaçları geliştirme çalışmaları için önem arz etmektedir. Bu çalışmanın amacı, akciğer adenokarsinomunu modelleyen A549 hücre dizisinde PTGS2, CALR ve MAGE-A3 genlerinin, sağlıklı bronş epitelini modelleyen BEAS-2B hücre dizisine kıyasla ilgili genlerin ifade düzeylerini araştırılmasıdır.
Materyal ve Metod: mRNA ifade seviye değişikliğini belirlemek için kantitatif Gerçek zamanlı PCR yapılmıştır.
Bulgular: Çalışmanın sonucunda, A549 hücre dizisinde mRNA ekspresyon seviye farkı, BEAS-2B hücre dizisi ile karşılaştırıldığında PTGS2 geninde 229,13 kat artış; CALR geninde 4,03 kat artış; MAGE-A3 geninde 3,41 kat artış gözlendi. Bu üç genin kanser hücresi bölünmesinde, apoptoz direncinde, invazyon ve anjiyogenezde rol oynadığı bilinmektedir.
Sonuç: Bu çalışmanın bir sonucu olarak, bu üç genin NF-κB sinyal yolu üzerinde birlikte etkisi olduğu düşünülmektedir. Bu üç genin bu noktada aydınlanması için protein seviyesinde araştırma yapmak faydalı olabilir.

Anahtar kelimeler: Akciğer kanseri, PTGS2 geni, CALR geni, MAGE-A3 geni

Sorumlu Yazar / Corresponding Author

Süreyya BOZKURT

Istinye University, Faculty of Medicine, Department of Medical Biology
Maltepe Mahallesi. Edirne Çırpıcı yolu. No:9
Zeytinburnu. İstanbul, Turkey.

Tel:05373180565
e-mail:sbozkurt@istinye.edu.tr

**Geliş tarihi / Received:** 14.02.2020

Kabul tarihi / Accepted: 15.04.2020

DOI: 10.35440/hutfd.689002

*Bu çalışma Büşra Köse'nin "Yüksek Lisans Tezinden" üretilmiştir.*

## Introduction

Lung cancer is a malignant tumor with uncontrolled cell division in lung tissue. This growth usually occurs because of mutation accumulation, changes in gene expression or troubles of post-translational modifications or abnormal protein inhibition and epigenetic factors. Lung cancer can be divided into two major types based on the morphology of cells called Small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) (1,2).

The prostaglandin endoperoxide synthase-2 (PTGS2) or cyclooxygenase-2 (COX2) gene is the key enzyme for prostaglandin biosynthesis. It converts arachidonic acid to prostaglandin-E2 (PGE2). PGE2 stimulates the activity of transcription factors in the Nuclear Factor-kappa B (NF-κB) signaling pathway that remains cellular survival. The stimulated NF-κB signaling pathway increases the transcription of the BCL2 anti-apoptotic protein, which leads to inhibition of apoptosis. Thus, abnormally increasing PTGS2 expression indirectly contributes to carcinogenesis by increasing the amount of PGE2 (3).

The calreticulin gene is localized on human chromosome 19 and codes calreticulin protein which is a chaperone protein involved in the proper folding of synthesized proteins. It is not only involved in protein processing, but also in transport. In addition, it acts as a calcium-binding agent and plays a role in maintaining intracellular calcium ion concentration (4). One of the nuclear activities of the Calreticulin (CALR) protein is that interacting with the transcription factor NF-κB signaling pathway to increase the production of pro-inflammatory cytokines. It has also been shown to induce maturation of dendritic cells. Moreover, it has been shown to support the adaptive immune response against cancer by triggering the activation of effector memory T cells and dendritic mature cell infiltration in cancer (5,6).

The MAGE-A3 gene, member 3 of the melanoma-associated antigen gene family, is localized in Xq28 and encode MAGE-A3 protein. MAGE-A3 has been identified in many tumors, including all melanomas, liver, NSCLC and hematologic cancers (NCBI, Gene ID: 4102). NSCLC has been reported to encode a protein that causes immune response in the body in testicular cancer, head and neck cancers, liver cancer, and melanomas and is associated with the NF-κB signaling pathway (7,8).

PTSG2, COX2 and MAGE-A3 genes are known to play a role in cancer cell division, apoptosis resistance, invasion, and angiogenesis. Moreover, these three genes are thought to have jointly effect on the NF-κB signaling pathway. Aim of this study was to determine and compare the expression of PTGS2, CALR, and MAGE-A3 genes at transcription levels in the A549 lung epithelial squamous cell adenocarcinoma cancer cell line and the BEAS-2B healthy bronchial epithelium cell line.

## Materials and Methods

### Cell Culture

In this study, A549 human lung epithelial squamous cell adenocarcinoma and BEAS-2B human lung bronchial epithelial cell lines were used. They were purchased from American Type Tissue Culture Collection (ATCC).

Cells were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate, and 6 mM glutamine at 37°C in a humidified incubator atmosphere containing 5% CO2. Cells were seeded in 175-cm2 flasks with ventilated cap at a density of 2 million cells per flask and grown until 85% cell confluency. After 24 hours, the cells were washed once with PBS and detached using trypsin–EDTA. Viable cells were counted by staining with trypan blue (all chemicals purchased from Sigma-Aldrich, St. Louis, MO, USA). The cells were pelleted at 300 ×g for 5 min and then stored at −80°C until further processed.

### Reverse transcription polymerase chain reaction (RT PCR) and quantitative (q)PCR

Total RNA Kit-I was purchased from Omega Bio-tek, Inc. (Norcross, GA, USA). First-strand cDNA synthesis kit and SYBR green Real-time Quantitative PCR (RT-qPCR) kit were purchased from Qiagen, Inc. (Germantown, MD, USA). Total RNA was isolated with Total RNA isolation kit, following to produce cDNA using the first-strand cDNA synthesis kit, which were then served as templates for RT-qPCR amplification with the SYBR green qPCR kit.

The cDNA synthesis was done (65 °C, 5 min; 4 °C, 5 min; 25 °C, 10 min; 50 °C, 60 min; 85 °C, 5 min) in a 30 µL reaction mixture containing 5X Reaction buffer, 1,5 M dNTP randomized primers, 50 mM RNase inhibitor, 1,25 M DTT, 1 M RTE, and 10 uL RNA samples. The qPCR reaction was done (94 °C, 4 min; 95 °C, 30 sc; 58 °C, 30 sc; 72 °C, 30 sc; 72 °C, 10 min) in 25 µl reaction mixture containing 10 µl 2X SYBR Green with reaction buffer, 1,5 µl forward and reverse primer, 4 µl cDNA template and 4 µl ddH2O. The qPCR primers are given in Table 1. Beta-actin was amplified as an internal control. Fold changes were calculated relative to BEAS-2B cells. All experimental conditions were performed in technical triplicates.

## Results

In this study, gene expression at transcription level of PTGS2, CALR, and MAGE-A3 genes in A549 cell line and BEAS-2B cell line with healthy bronchial epithelium were compared comparatively. The expression levels of PTGS2, CALR and MAGE-A3 genes were given in the A549 cell line, according to the BEAS-2B cell line, including the housekeeping gene Beta-actin in Table 2. Calculation results of Ct values and expression change rates of the studied genes can be seen in Table 2.

According to the real-time PCR analysis results, an increase in the expression level of all three genes was observed. Delta delta Ct (ΔΔCt) calculation of the results of the study showed that the expression increase rates of genes were 229,13 times in the PTGS2 gene for A549 cell line; 4,03 times in the CALR gene, and in MAGE-A3 gene, it was determined as 3.41 times. Expression fold-changes of the genes can be seen in Figure 1.

Table 1. qPCR primers

| qPCR primers | | |
|---|---|---|
| Gene Name | | Primer sequences |
| PTGS2 | Forward | ATCATTCACCAGGCAAATTGC |
| PTGS2 | Reverse | GGCTTCAGCATAAAGCGTTTG |
| CALR | Forward | AAGTTCTACGGTGACGAGGAG |
| CALR | Reverse | GTCGATGTTCTGCTCATGTTTC |
| MAGEA3 | Forward | AAGCCGGCCCAGGCTCGGT |
| MAGEA3 | Reverse | GCTGGGCAATGGAGACCCAC |

Table 2. Expression fold changes of the genes

| | A549 | BEAS-2B (control) | Delta Ct **ΔCt** (A549) | Delta Ct **ΔCt** (BEAS-2B) | Delta Delta Ct Calculation **ΔΔCt** | Expression Change 2^-**ΔΔCt** |
|---|---|---|---|---|---|---|
| | Average CT value | Average CT value | | | | |
| Beta-Actin (house-keeping gene) | 31,25 | 31,62 | | | | |
| PTSG2 | 17,93 | 26,14 | 13,32 | 5,48 | -7,84 | 229,13 |
| CALR | 14,37 | 16,75 | 16,88 | 14,87 | -2,01 | 4,03 |
| MAGE-A3 | 22,78 | 24,92 | 8,47 | 6,70 | -1,77 | 3,41 |

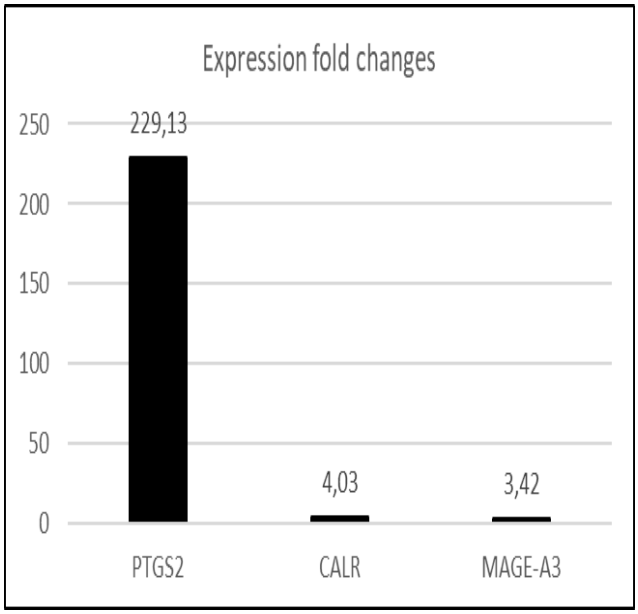


Figure 1. Expression fold changes of the genes

The analysis was carried out as a set of three replicate experiments, and the average of the three was taken for the value to be based on.

## Discussion

In this study, gene expression of PTGS2, CALR and MAGE-A3 genes in the A549 cell line modeling lung adenocarcinoma and BEAS-2B cell line with healthy bronchial epithelium were compared comparatively. In the studies in the literature, it is stated that the expression of the PTGS2 gene increases in many cancerous tissues and indirectly plays a role in angiogenesis and apoptosis resistance (9,10). In addition, increased expression in lung cancer has been associated with metastasis and invasion (11,12). In this study, expression of PTGS2 gene was determined by qPCR method in NSCLC adenocarcinoma cell line A549 and healthy lung epithelial cell line BEAS-2B. Accordingly, in accordance with the results of previous studies in the literature, 229.13 fold increase in expression of PTGS2 gene was detected in NSCLC A549 cell line compared to BEAS-2B, which is a healthy lung epithelial cell line. Similarly, in 2014, Li and his team found that PTGS2 expression was very high in the A549 cell line. In this study, butein substance obtained from Rhus verniciflua and Butea monosperma plants, whose secondary metabolites are used in traditional treatments in China, was used as PTGS2 inhibitor. Butein treatment has been shown to reduce PTGS2 mRNA and protein levels and promote cells to apoptosis (13). In our study, the other gene with high expression level in A549, which is the NSCLC adenocarcinoma cell line, is the CALR gene. Expression of the CALR gene was found to be 4.03 fold increased compared to the healthy lung cell line BEAS-2B. There are studies that have been suggested to play a role in the control of CALR protein, immune response, wound healing, cell growth and division, cell migration, cell adhesion and apoptosis. (14). In addition, there are study results showing that CALR protein plays an anticancer role in the immune response and the increase in expression in cancer cells gives the cells apoptosis resistance. A study conducted in China in 2012 suggested that CALR may be a biomarker for lung cancer as a result of the immunohistochemical experiment with CALR monoclonal antibody (15). Contrary to the conclusion found in our study; In a study published in Germany in 2009 by Bergner and his team, it was revealed that CALR expression decreased in lung cancer and hepatocellular cancer compared to healthy lung epithelial cells and Calcium homeostasis was very different between normal lung epithelial cells and cancerous lung cells (16). Another gene studied was MAGE-A3, and expression of the MAGE-A3 gene increased by 3.41 times in the A549 NSCLC cell line compared to the BEAS-2B cell line. A study similar to our results in the literature is a study published by Thongprasert et al. In Taiwan in 2016. In the study, MAGE-A3 expression was

found in 26% of 377 samples taken from patients with East and Southeast Asian NSCLC. (17). In a study by Chen and his friends in the USA in 2016, it was observed that the expression of MAGE-A3 in NSCLC was related to the prognosis (18). In another clinical study conducted in Belgium in 2013, a 27-month 13-dose MAGE-A3 immunotherapy was performed on 182 NSCLC patients. In patients with high MAGE-A3 expression, a 35% regression in cancer was detected with this method, which was applied in the process after the surgical approach, and it was suggested that this application was at the minimum toxicity level and it could be initiated in the third phase studies (19). MAGE-A3 protein is known as "lung cancer antigen". In the name of lung cancer treatment development studies, "MAGE-A3 vaccine" was applied in 2004. In the clinical trial applied in 122 NSCLC patients with high expression of MAGE-A3 in diagnosis and in the early stages, 33% successful responses were obtained (20).

In our study, in parallel with the studies in the literature, the expression of the MAGE-A3 gene increased by 3.41 times in A549 NSCLC cell line compared to BEAS-2B cell line. Our study results are in line with other study results in the literature.

PTGS2, CALR and MAGE-A3 genes have relationships with the NF-κB pathway. NF-κB is a transcription factor directly related to cytokine production and cell survival in the immune response. It plays a primary role in vital functions such as immunity, cellular proliferation, inflammation and apoptosis. Dysfunction is directly related to inflammation, autoimmune diseases and cancer. The increased expression of the MAGE-A3 gene is thought to lead to the immortality of the cell, keeping this signal path always open. High PTGS2 expression increases the activation of the NF-κB pathway by increasing the amount of PGE2. Increased activation NF-κB signal path triggers BCL2 transcription and creates apoptosis resistance in the cell (3). On the other hand, the CALR protein has been reported to initiate an anti-inflammatory and anti-cancer response that activates the immune response, and even acts as a kind of extracellular chaperone for the surrounding cancerous cells (21). As an example of its role in the anti-inflammatory response, it has been reported that it interacts with the NF-kinB signaling pathway to increase the production of pro-inflammatory cytokines. CALR also performs chaperone protein function via the NF-κB signal pathway (5)

Based on all this information, it can be speculated that increased expressions of PTGS2 and MAGE-A3 genes can play a role in the cancer process by interacting with the NF-κB signal pathway, by the proliferation of cancer cells and inhibition of apoptosis. In order to develop new treatment approaches in NSCLC, more studies are needed regarding PTGS2, CALR and MAGE-A3 genes and proteins, which are thought to induce carcinogenesis directly or indirectly.

*Ethics Committee Approval: No ethical Approval required for cell culture study*

*All authors declare that there is no conflict of interest.*

## References


1. Nasim F, Sabath BF, Eapen GA. Lung cancer. Med Clin North Am. 2019; 103(3): 463-73.
2. Mao M, Wu Z, Chen J. MicroRNA-187-5p suppresses cancer cell progression in non-small cell lung cancer (NSCLC) through down-regulation of CYP1B1. Biochem Biophys Res Commun. 2016; 478(2): 649-55.
3. Park JY, Pillinger MH, Abramson SB. Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases. Clin Immunol Immunopathol. 2006; 119(3): 229-40.
4. Gold LI, Eggleton P, Sweetwyne MT, Van Duyn, LB, Greives MR, Naylor SM, et al. Calreticulin: non-endoplasmic reticulum functions in physiology and disease. FASEB J.2010; 24(3): 665-83.
5. Bajor A, Tischer S, Figueiredo C, Wittmann M, Immenschuh S, Blasczyk R, et al., Modulatory role of calreticulin as chaperokine for dendritic cell-based immunotherapy. Clin Exp Immunol.2011;165(2): 220-34.
6. Fucikova J, Becht E, Iribarren K, Goc J, Remark R, Damotte D, et al., Calreticulin Expression in Human Non–Small Cell Lung Cancers Correlates with Increased Accumulation of Antitumor Immune Cells and Favorable Prognosis. Cancer Res. 2016;76(7):1746-56.
7. The Human Protein Atlas. MAGE family member A3. Available from https://www.proteinatlas.org/ENSG00000221867-MAGEA3/pathology.
8. Lu YC, Parker LL, Lu T, Zheng Z, Toomey MA, White D E, et al., Treatment of patients with metastatic cancer using a major histocompatibility complex class II-restricted T-cell receptor targeting the cancer germline antigen MAGE-A3. J Clin Oncol. 2017; 35(29): 3322-29.
9. Daniel TO, Liu H, Morrow JD, Crews BC, Marnett LJ. Thromboxane A2 is a mediator of cyclooxygenase-2-dependent endothelial migration and angiogenesis. Cancer Res. 1999; 59(18): 4574-77.
10. Dempke W, Rie C, Grothey A, Schmoll HJ. Cyclooxygenase-2: a novel target for cancer chemotherapy?. J Cancer Res Clin Oncol.2001; 127(7): 411-17.
11. Hida T, Yatabe Y, Achiwa H, Muramatsu H, Kozaki KI, Nakamura S, et al., Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res. 1998; 58(17): 3761-64.
12. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimäki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res. 1998; 58(22):4997-01.
13. Li Y, Ma, C, Qian M, Wen Z, Jing H, Qianet D, et al., Butein induces cell apoptosis and inhibition of cyclooxygenase-2 expression in A549 lung cancer cells. Mol Med Rep.2 014; 9(2): 763-67.
14. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al., Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013; 369(25): 2379-90.
15. Lu YC, Weng WC, Lee H. Functional roles of calreticulin in cancer biology. Biomed Res Int. 2015; 32:1-9.
16. Bergner A, Kellner J, Tufman A, Huber R. Endoplasmic reticulum Ca 2+-homeostasis is altered in small and non-small cell lung cancer cell lines. J Exp Clin Cancer Res.2009; Feb 24;28:25.
17. Thongprasert S, Yang PC, Lee JS, Soo R, Gruselle O, Myo A, et al., The prevalence of expression of MAGE-A3 and PRAME tumor antigens in East and South East Asian non-small cell lung cancer patients. Lung Cancer.2016; 101:137-44.
18. Chen X, Wang L, Liu J, Huang L, Yang, Gao Q, Malinowski W, et al., Expression and prognostic relevance of MAGE-A3 and MAGE-C2 in non-small cell lung cancer. Oncol Lett. 2017; 13(3): 1609-18.
19. Vansteenkiste J, Zielinski M, Linder A, Dahabreh J, Gonzalez E E,

et al., Adjuvant MAGE-A3 immunotherapy in resected non–small-cell lung cancer: phase II randomized study results. J Clin Oncol.2013; 31(19):2396-03.
20. Raez LE, Fein S, Podack ER. Lung cancer immunotherapy. Clin Med Res 2005; 3(4): 221-28.
21. Clarke C, Smyth MJ. Calreticulin exposure increases cancer immunogenicity. Nat Biotechnol. 2007; 25(2): 192-93.