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Evaluation of gene expression levels in the diagnosis of lung adenocarcinoma and malignant pleural mesothelioma

Akciğer adenokarsinomu ve malign plevral mezotelyoma tanısında gen ekspresyon düzeylerinin değerlendirilmesi

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ABSTRACT

Background: This study aims to evaluate gene expression levels in the diagnosis of lung adenocarcinoma and malignant pleural mesothelioma both which have a distinct treatment and prognosis.

Methods: Between January 2012 and January 2014, 12 newly diagnosed patients with a lung adenocarcinoma, 12 patients with malignant pleural mesothelioma, and eight healthy individuals as the control group were included. After treatment of the fresh samples of lung adenocarcinoma stored at -80°C for ribonucleic acid isolation, and paraffin-embedded tissues of patients with malignant pleural mesothelioma were deparaffinized, complementary deoxyribonucleic acid synthesis and expression of 84 genes associated with deoxyribonucleic acid repair were analyzed via real-time polymerase chain reaction assay. According to the expression of tumor cells, expression of each fold change was calculated.

Results: The BRCA1, BRCA2, CDK7, MLH3, MSH4, NEIL3, SMUG1, UNG, XRCC2, and XRCC4 genes showed more than five-fold higher expression in the patients with lung adenocarcinomas, compared to the control group. The patients with malignant pleural mesothelioma showed a five-fold higher expression in the APEX2, BRCA1, BRCA2, CDK7, MLH1, MLH3, MSH3, MSH4, NEIL3, PARP2, PARP3, PMS1, RAD50, RAD51, RAD51B, RAD51D, RAD52, RPA3, SMUG1, UNG, XPA, XRCC2, and XRCC4 genes, compared to the control group. Comparing malignant pleural mesothelioma with lung adenocarcinoma cases, we found that CDK7, MLH1, TREX1, PRKDC, XPA, PMS1, UNG, and RPA3 genes were overexpressed.

Conclusion: Our study results showed differences between expression profiles of deoxyribonucleic acid repair genes in lung adenocarcinoma and malignant pleural mesothelioma cells. Based on our study results, we suggest that TREX1, PRKDC, and PMS1 genes may play a key role in the differential diagnosis of these two entities.

Keywords: Adenocarcinoma, gene expression, lung, malignant, mesothelioma.

ÖZ

Amaç: Bu çalışmada tedavi ve prognozları birbirinden farklı olan akciğer adenokarsinomu ve malign plevral mezotelyomanın ayırıcı tanısında gen ekspresyon düzeylerinin değerlendirilmesi planlandı.

Çalışma planı: Ocak 2012 - Ocak 2014 tarihleri arasında akciğer adenokarsinomu ile yeni tanı konan 12 hasta, malign plevral mezotelyomalı 12 hasta ve kontrol grubu olarak sekiz sağlıklı birey çalışmaya alındı. Ribonükleik asit izolasyonu için -80°C'de saklanan taze akciğer adenokarsinom dokuları işlendikten ve malign plevral mezotelyomalı hastaların parafine gömülü dokuları deparafinize edildikten sonra, tamamlayıcı deoksiribonükleik asit sentezi ve deoksiribonükleik asit onarımı ile ilişkili 84 genin ekspresyonu gerçek zamanlı polimeraz zincir reaksiyon testi ile çalışıldı. Her kat değişiminin ekspresyonu tümör hücrelerinin ekspresyonuna göre hesaplandı.

Bulgular: BRCA1, BRCA2, CDK7, MLH3, MSH4, NEIL3, SMUG1, UNG, XRCC2 ve XRCC4 genleri, kontrol grubuna kıyasla, akciğer adenokarsinomlu hastalarda beş kattan daha fazla ekspresyon sergiledi. Kontrol grubuna kıyasla, malign plevral mezotelyomalı hastalarda APEX2, BRCA1, BRCA2, CDK7, MLH1, MLH3, MSH3, MSH4, NEIL3, PARP2, PARP3, PMS1, RAD50, RAD51, RAD51B, RAD51D, RAD52, RPA3, SMUG1, UNG, XPA, XRCC2 ve XRCC4 genlerinde beş kat daha fazla ekspresyon izlendi. Malign plevral mezotelyomalı ve akciğer adenokarsinomlu olguların karşılaştırmasında, CDK7, MLH1, TREX1, PRKDC, XPA, PMS1, UNG ve RPA3 genlerinin aşırı eksprese olduğu tespit edildi.

Sonuç: Çalışma sonuçlarımız, malign plevral mezotelyoma ve akciğer adenokarsinom hücrelerinde deoksiribonükleik asit onarım genlerinin ekspresyon profilleri arasında farklılıklar olduğunu gösterdi. Çalışma sonuçlarımıza göre, TREX1, PRKDC ve PMS1 genleri bu iki patolojinin ayırıcı tanısında önemli bir rol oynayabilir.

Anahtar sözcükler: Adenokarsinom, gen ekspresyonu, akciğer, malign, mezotelyoma.

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The prevalence of lung cancer is on the rise due to the increased smoking rates world wide.[1] Several studies on this subject have demonstrated that smoking, the main factor, genetic predisposition, occupational exposures (i.e., radiation, nickel, asbestos), and sequelae of previous pulmonary diseases increase the development risk of lung cancer.[2]

In recent years, in Turkey, it is reported that lung adenocarcinomas (LADCAs) are diagnosed more often. Malignant pleural mesothelioma (MPM) is also a common type of cancer caused by 70 to 90% asbestos exposure.[1-3] Malignant pleural effusions can be noticed at the time of diagnosis of cancer and primary tumor localization may not be found in 5 to 15% of the cases. A total of 15% of LADCAs and 90% of MPMs present with malignant pleural effusion.[4] The definite diagnostic difference of LADCAs and MPM can not be made and diagnostic aid of cytology constitutes 4 to 77%.[5] Lung cancers have different life expectancies in different subgroups, and genetic alterations also suggests that lung cancers should have different disease profiles and treatments. Therefore, it has been proposed that gene expressions ratio plays a decisive role in the diagnosis and treatment, and analysis of gene expression ratio is the most useful molecular method in the discrimination of MPM from LADCAs.[6]

It has been established that various tumor suppressor genes and oncogenes play important direct or indirect roles in cell cycle (a part of vital mechanisms) progression and regulation in lung cancers. Lung cancers share similar chromosomal changes and these chromosomal alterations have typical structures that are special to some histological types. Previous studies have shown a loss in the chromosomal arms of 1q, 3p, 8p, 9p, 13q, 17p at non-small cell lung cancer.[7-10]

Cell cycle control and deoxyribonucleic acid (DNA) repair mechanisms, important oncogenes such as RAS gene family, Myc oncogenes, growth factors and their receptors, and angiogenesis factors and telomerase activity are components of other important neoplastic processes. As a member of RAS family, KRAS conducts the signals received from receptor tyrosine kinases. Specific RAS gene mutations are seen in various cancer cells and codon 12, 13, and 61 are detected almost in all cases. These mutations cause a delay in GTP-Ras inactivation due to a significant decrease in GTPase activity. This is characterized with the excessive cellular response given to the signals coming through the receptors. Epidermal growth factor receptor (EGFR) amplifications in lung cancers are one of them.[11] The Myc gene is localized on 8q24 region and encodes a nuclear protein which is effective in cell proliferation. During re-organizations, exon 1 of Myc gene often disappears. However, this does not cause a change in Myc functions, as this exon does not play a role in synthesis of proteins. An uncontrolled cell proliferation, related to the over expression of Myc gene product, is seen after the translocation of the Myc region with one of immunoglobulin genes.[12]

Ongoing DNA micro-array and mass spectrometry technologies enables analysis of gene expressions. A relationship between gene expression profiles of lung cancers, expression patterns special to histological type, heterogeneity of LADCA, specific expressions, and clinical outcomes has been discovered.[13] The most frequently described amplification regions in lung cancers include Myc, telomerase reverse transcriptase (TERT), CCND1, and EGFR and many different amplifications, although less frequent, have been described. The 14q13.3 region is particularly described for LADCA which is associated with NKX2-1 (also known as TTF-1) and MBIP genes.[14]

Asbestos fibers are mechanically hazardous by interfering with cell cycle abnormalities of chromosomes to the mitotic process, leading to aneuploidy. Additional release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can lead to DNA damage. Consequently, it is thought to cause the expression of various transcription factors and cancerogenicity. In a few recent studies, genetic alterations caused by mutations in oncogenes and tumor suppressor genes have been described.[15-17]

A progress has been achieved in the treatment and differential diagnosis of LADCAs from MPM, and the methods which are still in use for histopathological diagnosis is not adequate. Due to have less knowledge about genetic alterations associated with MPM, different mechanisms are still under investigation. In the present study, we aimed to evaluate gene expression levels in the diagnosis of LADCA and MPM which have a different treatment and prognosis.

PATIENTS AND METHODS

Between January 2012 and January 2014, 12 newly diagnosed patients with a LADCA, 12 patients with MPM, and eight healthy individuals as the control group were included. All LADCA cases were diagnosed at Dokuz Eylül University, Department of Pulmonology and operated at Department of Thoracic Surgery. Fresh samples were obtained from these cases. The paraffin-embedded tissues of patients with MPM were obtained from Dicle University, Faculty of Medicine, in Diyarbakir province of Turkey. A written

informed consent was obtained from each participant. The study protocol was approved by the Dokuz Eylül University Faculty of Medicine, Ethics Committee (Date: 02.06.2011, No: 2011/16-18). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data including demographic, clinical, pathological characteristics and gene expressions of the cases were collected. During routine procedures, complete blood count samples of the control group were collected in ethylenediaminetetraacetic acid (EDTA) tubes and sterility was maintained. Collected bloods were diluted at a ratio of 1:1 with phosphate-buffered saline (PBS). Then, peripheral blood mononuclear cells (PBMCs) were centrifuged at 1,650 rpm for 15 min using Sigma 3-16K centrifuge (DJB Labcare Ltd, Buckinghamshire, England) with histopaque-density solution 1,077 and the cells were separatedbased on their density gradient. After the washing procedure, ribonucleic acid was isolated from these PBMCs.

Before starting mononuclear cell isolation from blood, PBS which would be utilized for the isolation was prepared. For this purpose, one box of dusted Sigma P3883 was added into 1 L of distilled water and homogeneity of the mixture was provided. In total, 4 mL histopaque (Sigma-Aldrich 1077 d: 1.077) was taken into a 15 mL tube. Collected bloods were diluted at a ratio of 1:1 with PBS at room temperature. The PBS and blood were made homogenous with the help of Pasteur pipettes. The blood diluted with PBS was slowly delivered into a histopaque containing 15 mL tube (Sigma-Aldrich 1077 d: 1.077) at 45° angle in a way that blood run down through the wall of the tube. This procedure could be completed at three times. A special care was paid to avoid formation of bubbles. Centrifugation was made at 1,600 rpm for 20 min. The goal was to separate different phases. After the centrifugation, serum was on top, as a thin layer mononuclear cells were in the middle, below them histopaque and red blood cells were at the bottom. The sterile Pasteur pipette was dipped, until the level of mononuclear cell layer and cells were collected by the help of pipette (while plunger was pushed). During the procedure, the tube was hold in tilted position and a dark colored paper was put behind the tube, and thus, the cells could be seen more easily. After the cells were collected in a 15 mL tube, approximately 5 mL of PBS was added into the tube. Centrifugation was made at 1,200 rpm for five min. A liquid which was above the cells collected by centrifugation was discarded by the Pasteur pipette. The PBS up to 8 to 9 mL was added on the cellsand centrifuged at 1,200 rpm for fivemin. This procedure was repeated three times. Therefore, PBMC isolationwas achieved. Samples were taken from tumoral sites of archival paraffin blocks of mesothelioma casesto compare them with hematoxylin-eosin stained microscope slides. Samples were taken in a 2×2×2 mm sized Eppendorf tube. Paraffin xylol was removed via passing through the descending alcohol series and were, then, kept waiting in proteinase k for one night and RNA isolation process was started next. Lung cancer sites were carefully sampled from the specimens excised from LADCA cases by a pathologist during surgeryin sterile conditions. These samples were transferred to the oncology laboratory within RPMI and transfer medium containing 1% penicillin & streptomycin. After imprinting, Giemsa staining and tumor confirmation,a 2×2×2 mm sized mechanical tissue was cut with a sterile lancet and taken in a sterile Eppendorf tube to keep at an -80°C cold freezer. It was spitted with mechanical vibration before the isolation of RNA. The rest of the procedure was performed as follows: washing with PBS, centrifugation, removal of supernatant, and RNA isolation.

Isolation and measurement of RNA

Basic principles of RNA isolation management include fragmentation of cells with lysis solution and DNA extraction using phenol. Current protocols are the modified versions of designated RNA isolation protocol designated by Chomczynski and Sacchi in 1987.[18] Cells should be isolated and examined immediately to obtain maximum efficiency from RNA isolation. Firstly, isolated cells should be flash-freeze in liquid nitrogen approximately for five min (-196°C) and, then, RNA isolation process should be started. The main goal is to enhance effectivity of RNA which would be obtained by the destruction of cell wall. After the dilation process, the isolated PBMC is counted on the TOMA cell counting slide. Accordingly, about 15 µg RNA extract was obtained out of 1×10^6 cells. The Macherey-Nagel™ kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used for RNA and ready-made buffers were provided. As for RNA quality, any kind of contamination was strictly avoided and special attention was paid to accurate pipetting and sterility.

Complementary DNAsynthesis

Complementary DNA (cDNA) synthesis is the process of DNA copying from a RNA molecule by the help of reverse transcriptase enzyme. When the DNA of targeted cells are considered, expressed or non-expressed, it includes all genes. Therefore, messenger RNA (mRNA) which is the expressed part of a cell is used. Thus, only expressed genes are present, when

Table 1. Genes with expression analysis

Base excision repair (BER): APEX1, APEX2, CCNO, LIG3, MPG, MUTYH, NEIL1, NEIL2, NEIL3, NTHL1, OGG1, PARP1, PARP2, PARP3, POLB, SMUG1, TDG, UNG, XRCC1.

Nucleotide excision repair (NER): ATXN3, BRIP1, CCNH, CDK7, DDB1, DDB2, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC8, LIG1, MMS19, PNKP, POLL, RAD23A, RAD23B, RPA1, RPA3, SLK, XAB2, XPA, XPC.

Mismatch repair (MMR): MLH1, MLH3, MSH2, MSH3, MSH4, MSH5, MSH6, PMS1, PMS2, POLD3, TREX1.

Double-strand break (DSB) repair: BRCA1, BRCA2, DMC1, FEN1, LIG4, MRE11A, PRKDC, RAD21, RAD50, RAD51, RAD51C, RAD51L1, RAD51L3, RAD52, RAD54L, XRCC2, XRCC3, XRCC4, XRCC5, XRCC6.

DNA repair related other genes: ATM, ATR, EXO1, MGMT, RAD18, RFC1, TOP3A, TOP3B, and XRCC6BP1.

Table 2. Fold change values obtained DNA repair associated 84 genes which shows LADCA and MPM expressions

Gene	LADCA/control fold change	MPM/control fold change	(LADCA/control fold change/ MPM/control fold change) *p*
APEX2	1.6763	6.1243	>0.05 / >0.05
BRCA1	9.5919	20.2646	>0.05 / 0.01558
BRCA2	4.3804	10.8169	>0.05 / 0.012071
CCNH	2.3922	4.4773	>0.05 / 0.032102
CDK7	3.909	15.4192	>0.05 / 0.019161
LIG4	2.2608	13.7822	0.044834 / >0.05
MLH1	2.5581	7.5515	>0.05 / 0.013792
MLH3	5.9579	15.4275	>0.05 / >0.05
MSH3	2.2494	10.2785	>0.05 / >0.05
MSH4	6.5356	12.0767	>0.05 / >0.05
NEIL3	14.3334	80.5092	0.015299 / >0.05
PARP1	0.9019	2.0543	>0.05 / >0.05
PARP2	4.7127	7.4604	0.043874 / 0.009579
PARP3	2.3613	8.2119	>0.05 / 0.049911
PMS1	1.8236	7.3582	>0.05 / 0.039034
RAD50	2.6223	10.2765	>0.05 / 0.03758
RAD51	2.6223	10.2765	>0.05 / >0.05
RAD51B	4.3683	16.7622	>0.05 / >0.05
RAD51D	3.2769	7.0688	>0.05 / >0.05
RAD52	2.0126	5.1099	>0.05 / >0.05
RPA3	2.8581	7.9353	>0.05 / >0.05
PRKDC	0.5309	2.9778	>0.05 / >0.05
SMUG1	6.7053	18.0914	>0.05 / >0.05
TREX1	0.5115	4.1669	>0.05 / >0.05
UNG	7.422	26.6752	0.027669 / 0.009662
XPA	2.0485	8.8342	>0.05 / >0.05
XRCC2	5.6758	17.6367	>0.05 / >0.05
XRCC4	5.9765	17.0836	>0.05 / >0.05

DNA: Deoxyribonucleic acid; LADCA: Lung adenocarcinoma; MPM: Malignant pleural mesothelioma.

cDNA is obtained from mRNA. For this purpose, RNA isolation is performed before cDNA synthesis. While mRNA is being processed, introns are expelled, and thus, exons remain behind. The RNA is used in for the synthesis of cDNA. Reverse transcriptase enzyme which is used for cDNA synthesis, anchors itself to primary poly-A tail (essential for the initiation). The presence of poly-A tail in the enzyme provides a predominance in reverse transcription phase. Then, reverse transcriptase uses mRNA as a template, while facilitates elongation by the help of its primary, and it produces a copy of a single cDNA strand. For one plate, one microgram of RNA is supposed to be used. Therefore, amount of required RNA was calculated. cDNA synthesis was implemented using conventional PCR device (ATC 401 model NY-X Technique Inc., CA, USA).

Real-time polymerase chain reaction (RT-PCR) array analysis

Real-time polymerase chain reaction is a PCR method which gives quantitative data by measuring fluorescence signals that become stronger with DNA amplifications. The kit which we used for RT-PCR serial analysis (QIAGEN GmbH, Hilden, Germany) is a human-based standard commercial kit prepared for genes encoding DNA repair enzymes. Obtained RNAs were transformed to cDNA and cDNAs were added into the PCR mixtures of DNA and contamination was avoided.

Statistical analysis

Statistical analysis was performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). The increase or decrease in expression of each condition according to gene expression was calculated by fold change. These analyses were performed on the free of charge data analysis expression page of SA Bioscience (Greenwich Biosciences Inc., Carlsbad, CA, USA). Gene expression analyses with heat maps and clustergram were supported. The t-test based p value was calculated using this site (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php) In the univariate analysis, the Fisher's exact test was used to compare the variables specified by counting and the Mann-Whitney U test was used to compare the variables specified by the measurement. The Kaplan-Meier was used for survival analysis. Two life curves were compared using the log-rank test. A *p* value of <0.05 was considered statistically significant.

RESULTS

In this study, we chose to evaluate the DNA repair gene expression levels in the diagnosis of LADCA and MPM. House-keeping genes of this study included ACTB, B2M, GADPH, and RPLP0. In the results, the BRCA1, BRCA2, CDK7, MLH3, MSH4, NEIL3, SMUG1, UNG, XRCC2, and XRCC4 genes showed more than five-fold higher expression in the patients with LADCAs, compared to the control group. The patients with MPM showed a five-fold higher expression in the APEX2, BRCA1, BRCA2, CDK7, MLH1, MLH3, MSH3, MSH4, NEIL3, PARP2, PARP3, PMS1, RAD50, RAD51, RAD51B, RAD51D, RAD52, RPA3, SMUG1, UNG, XPA, XRCC2, and XRCC4 genes, compared to the control group. Comparing MPM with LADCA cases, we found that CDK7, MLH1, TREX1, PRKDC, XPA, PMS1, UNG, and RPA3 genes were over expressed. Genes with expression analysis are presented in Table 1. Among the genes listed in Table 1, increased gene expressions in MPM and LADCA were investigated, and we observed that there were differences in expression of DNA repair genes in LADCA and MPM tumor cells in the TREX1, PRKDC, and PMS1 genes. Comparative results of LADCA and MPM cases with the control group based on gene expression analyses with RT-PCR array analysis are shown in Table 2 and Table 3. The fold changes of the genes detected in the analysis are also shown on the clustergram in figures 1 and 2, and the comparison of the fold changes of the genes that show significant difference between LADCA and MPM is shown in Figure 3 on the graph. Our study suggests that the TREX1, PRKDC, and PMS1 genes, which are the DNA repair genes, would be significant in favor of MPM in the differential diagnosis of MPM-LADCA.

DISCUSSION

The differential diagnosis between pleural mesothelioma and pleural effusions of LADCA is

Table 3. MPM to LADCA tumor cells which genes "fold change" value of statistical significance (p<0.05)

Gene	MPM/LADCA fold change	*p*
CDK7	4.39	<0.05
MLH1	5.32	<0.05
TREX1	9.29	<0.05
PRKDC	7.64	<0.05
XPA	5.54	<0.05
PMS1	5.19	<0.05
UNG	4.93	<0.05
RPA3	2.97	<0.05

MPM: Malignant pleural mesothelioma; LADCA: Lung adenocarcinoma.

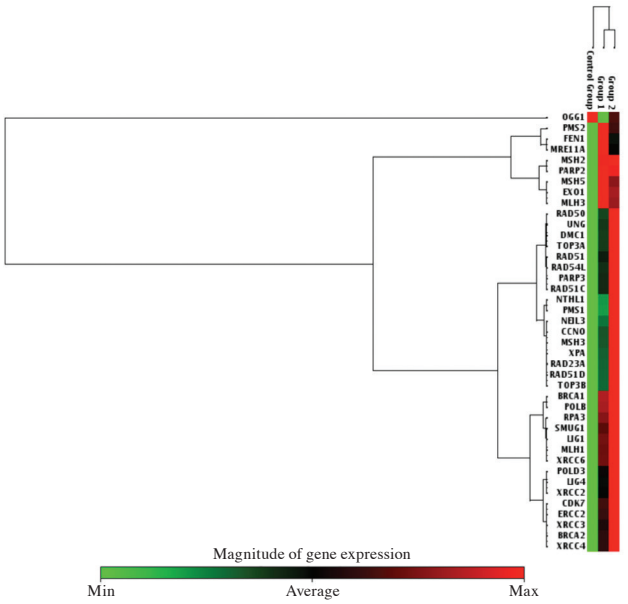

Figure 1. Clustergram of patient groups (group 1= LADCA, group 2 = MPM, control group).
MPM: Malignant pleural mesothelioma; LADCA: Lung adenocarcinoma.

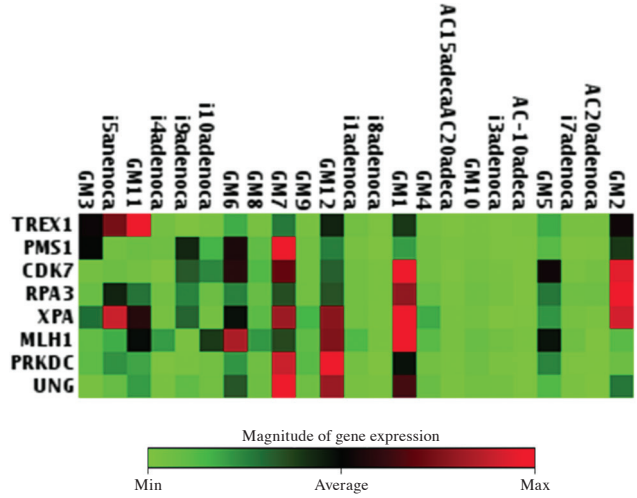

Figure 2. Clustergram of cases with significant genes.

essential. In particular, epithelial-type MPM has a differential diagnosis. Malignant pleural effusions can be detected at the time of diagnosis of cancer and approximately 15% of LADCAs and 90% of MPM are detected by pleural fluid.[4] Some tumor markers, CEA, mesothelin, calretinin, cytokeratin and TTF-1 were developed to reach a differential diagnosis.[19-23] In particular, mesothelin increase displays 48 to 84% sensitivity and 70 to 100% specificity for MPM; however, negative results do not exclude the diagnosis. [24] Other markers such as increased hyaluronic acid in mesothelial cells of MPM or CDKN2A loss have been investigated.[24,25] Immunohistochemical positivity of calretinin, cytokeratin and vimentin, negativity of CEA, and Leu-M1 and TAG 72 supports the MPM diagnosis. However, several studies demonstrate that these markers can be positive both in MPM and in LADCA. Therefore, reaching a differential diagnosis still remains difficult.[3] These diagnostic limitations directed attentions to the gene expressions.[26] Using PCR, Gordon et al.[27] discovered four different gene expressions in solid tumor cases and they suggested that the expressions had a significant role in the differential diagnosis of LADCA and MPM. Previous studies on cases with LADCA and MPM with synchronic pleural effusion showed that some gene

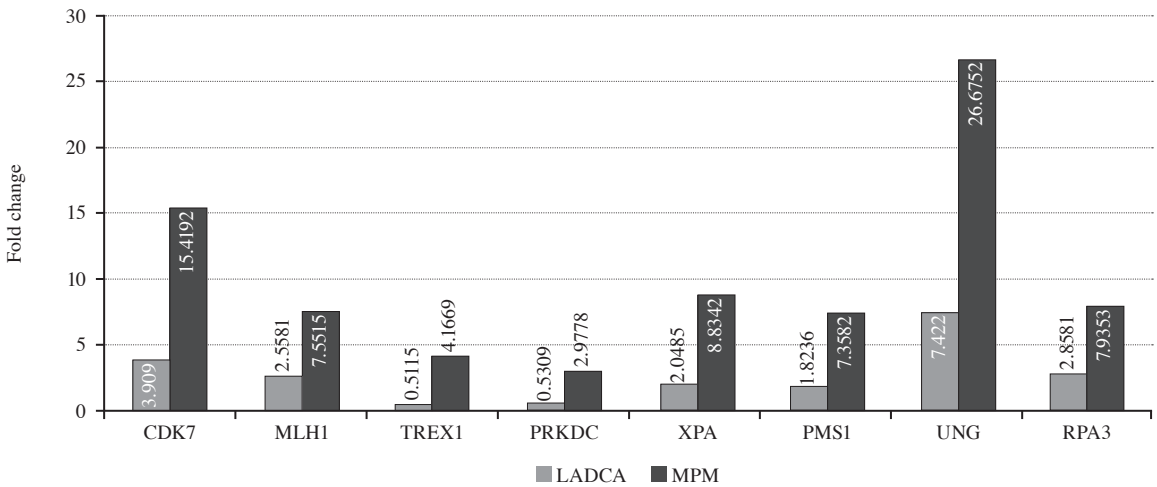

Figure 3. Fold increase of the genes that expressions were statistically significant in MPM and LADCA tumor cells.
LADCA: Lung adenocarcinoma; MPM: Malignant pleural mesothelioma.

expressions had a significant difference between these two cancer types.[28]

The diagnostic limitations of LADCA and MPM directed interest to gene expression analysis.[5,26] Gordon et al.[27] developed a predictive model for describing the overall survival times in two different groups using mRNA expression profile information from surgically collected tissue samples from MPM patients who developed a gene expression rate-based prognostic and diagnostic test for MPM. Among the two groups, the genes showing a significant correlation between the two groups were identified and evaluated from a prognostic point of view. They, then, formed a profile of four genes independent of the histological type. These samples were taken by fine needle biopsy and, then, analyzed the expression of RNA isolation by RT-PCR and the expression of six genes (CALB2, CLDN7, ANXA8, EPCAM, CD200, and NKX2-1) and calculated the expression ratios of three different gene pairs. In the gene expression analysis with pleural effusions, significant gene expression differences between LADCA and MPM were observed (GAS6, SEMA3C, KIBRA, GFPT2, S1-5, RALDH2). There were significant differences in gene expression between LADCA and MPM, and gene expression values were found to be significant in predicting treatment response ratesfor MPM.[28]

In the literature, there are few studies about MPM genetics, particularly in the sarcomatoid-type mesothelioma. 1p36, qp21.3, 3p21.3, 4q22, 6q25, 9p21.3, 13q and 22q deletions and 1q and 8q increase, and CDKN2A and CDKN2B are the most common. 9p deletions were also seen. These results are also reported to be associated with poor prognosis and recurrence of the disease.[29,30] The presence of p53 was found to be significant to show that mesothelial cells were malignant in the samples taken from the pleura.[31] In some studies, the present findings were common in lung cancers and were not specific to MPM which had no contribution to the separation of benign and malignant processes.[32,33] Although BAP-1 is the most commonly associated gene with MPM, neurofibromatosis type 2 (NF2) 22q12 deletions and TERT mutations have been also investigated.[34,35]

In recent years, it has been investigated whether gene expression assays which are thought to be significant in the diagnosis of both malignancies can be used in differential diagnosis between MPM and LADCAs. The DNA methylation 1413 autosomal CpG locus-associated 773 cancer-associated genes were screened and 60% more DNA methylation was detected in LADCAs.[36] When the methylation of 6157 CpG islet is evaluated in parallel with comparative genomic hybridization and chromatin immunoprecipitation; Kazal-Type Serine Peptidase Inhibitor Domain 1 (KAZALD1), Mitogen-Activated Protein Kinase 13 (MAPK13) and Transmembrane Protein 30B (TMEM30B) genes were found to be hypermethylated. Methylation status analysis of the promotor regions of nine candidate genes was performed; E-cadherin (71.4%) and FHIT (78%) genes were found to be very high according to ACP1A (14.3%), RASSF1A (19.5%), and DARK (20%) genes.[37]

MicroRNA (miRNA) expression can be also used to differentiate mesothelioma and LADCA, although the biological basis of this technology has not been sufficiently elucidated yet but it is thought that it can contribute to reveal the differences in pathogenesis between diseases by miRNA expression.[38,39] In a study, that a panel of miRNAs from the miR-200 gene family was generated with the quantitative RT-PCR, which was compared between MPM to LADCA by a more sensitive detection method, and it has been shown that miRNAs were all downregulated in MPM compared to LADCA.[38] The specificity of these changes was validated in 100 MPMs and 32 LADCAs. The analyses suggested that these miRNAs might be used as biomarkers. It was also reported that they were regenerators in the Wnt signaling pathway and they could play a role in tumor progression and create a choice for targeted therapies.[38]

Defects which occur during DNA repair leads to genetic instability and this is one of the most important causes of cancer. Also, in many cancer cells, increased DNA repair can be associated with developing resistance to cancer treatment. Changes in the structure of DNA leads to more significant results than such RNA and protein changes in the other components of the cell from changes. In our study we found that the TREX1, PRKDC, and PMS1 genes of DNA repair, would be significant in favor of MPM in the differential diagnosis of MPM and LADCA.

PMS1, DNA mismatch repair encodes a protein belonging to the MUTL/HEXB family. This protein is thought to play a role in DNA mismatch repair.[40] Formation of the HNPCC phenotype, also known as Lynch syndrome, and the PMS1 gene have been found among genes that are significant in whole-genomesequencing in lung cancers.[41] Due to the mutations in DNA repair genes such as PMS1

and BRCA1, BRCA2, ATM, SLX4, FANCC, FANCI, PALB2, FANCF, and XPC, it is thought that DNA damage caused by asbestos can not be repaired and the process of carcinogenesis has initiated.[42]

The PRKDC gen is known as DNA-dependent protein kinase (DNA-PK) and is localized on the long arm of chromosome 8. It is involved in the coding of the catalytic subunit of DNA-PK. The DNA functions with the Ku70/Ku80 heterodimer protein in double chain fracture repair and recombination. This protein encodes a member of the PI3/PI4-kinase family. It is currently under investigation that inhibition of the PRKDC gene may be significant in Myc-associated lung cancers.[43]

The TREX1 is localized in the short arm of chromosome 3 and encodes DNA exonuclease in the 3' >5' local direction in human cells. It is a non-processive exonuclease with error repair capability for human DNA polymerase. It is also a component of the SET complex (the endoplasmicreticulum-associated complex) and plays a role in the rapid decay of the three complex end of the DNA throughout the cell death of the granzyme A (apoptosis activity in caspase-independent cell death). Mutations in this gene have been associated with autoimmune diseases; Aicardi-Goutières syndrome overlaps with systemic lupus erythematosus (SLE), resulting in Chil blain Lupus. The TREX1 is associated with mismatch repair, also has been associated with drug resistance in pancreatic malignancies. The TREX1 gene is one of the primary exonuclease DNA repair genes and is reported to be low in lung cancer.[44]

In conclusion, deoxyribonucleic acid repair genes were selected for the differential diagnosis of lung adenocarcinoma and malignant pleural mesothelioma, as the effects of asbestos (an epidemiologic agent) on malignant pleural mesothelioma is well-known. Scientific questions such as "Which deoxyribonucleic acid repair genes do play role in a possible damage?", "Are these deoxyribonucleic acid repair genes can be used for differential diagnosis, if they are inactive in lung adenocarcinomas?" were sufficiently answered in this study and it was studied from fresh surgical material of lung adenocarcinoma and archive paraffin-embedded blocks of pleural tissue in malignant pleural mesothelioma. Gene expression increases were investigated and our results showed that TREX1, PRKDC, and PMS1 genes were most likely to increase expression in malignant pleural mesothelioma. Based on these results, we believe that it would be appropriate to investigate these genes in ribonucleic acid and protein levels in the differential cases and pleural fluids.

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