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Determination of Genetic Alteration in Pancreatic Ductal Adenocarcinoma Tissues by Analysis of Gene Expression Data

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Abstract

Objective: It is very important to determine the molecular infrastructure of pancreatic ductal adenocarcinoma, which has a very high mortality rate, limited treatment options, and does not have an option for targeted therapy, and to understand the disease by clinicians. Therefore, in this study, the gene expression dataset was used to determine the differences in transcriptome levels between tissues with pancreatic ductal adenocarcinoma and normal tissues.

Methods: In the current study, gene expression data set obtained from 10 pancreatic ductal adenocarcinoma tissues and 5 normal tissues were used. The limma package available in the R programming language was used to identify transcripts with differential expression in pancreatic ductal adenocarcinoma compared to normal tissues. The log2FC and adj-p values were used to identify genes that showed differential (up or down) regulation.

Results: According to the results of gene expression analysis, 7098 transcripts showed different regulation in pancreatic ductal adenocarcinoma tissue compared to normal tissue. With the UMAP graph, normal and pancreatic ductal adenocarcinoma tissues are distributed differently from each other, indicating that there is a difference in transcript between these two tissues.

Conclusion: As a result of the gene expression analysis performed in the study, transcripts differing between pancreatic ductal adenocarcinoma tissues and normal tissues were found. With the help of studies with these transcripts, targeted treatment strategies can be developed for the treatment of the disease, and the status of this disease, which has a very high mortality rate, can be changed.

Keywords: Pancreatic ductal adenocarcinoma, gene expression, differential expression

Pankreatik Duktal Adenokarsinom Dokularında Genetik Değişikliklerin Gen Ekspresyon Verileri Analizi ile Belirlenmesi

Özet

Amaç: Ölüm oranı çok yüksek, tedavi seçenekleri kısıtlı ve hedefe yönelik tedavi seçeneği bulunmayan pankreatik duktal adenokarsinomun moleküler altyapısının belirlenmesi ve hastalığın klinisyenler tarafından anlaşılması oldukça önemlidir. Bu nedenle, bu çalışmada, pankreatik duktal adenokarsinomlu dokular ile normal dokular arasındaki transkriptom seviyelerindeki farklılıkları belirlemek için gen ekspresyon veri seti kullanılmıştır.

Metod: Bu çalışmada, 10 pankreatik duktal adenokarsinom dokusu ve 5 normal dokudan elde edilen gen ekspresyon veri seti kullanılmıştır. R programlama dilinde bulunan limma paketi, normal dokulara kıyasla pankreatik duktal adenokarsinomda diferansiyel ekspresyona sahip transkriptleri tanımlamak için kullanılmıştır. Log2FC ve adj-p değerleri, diferansiyel (yukarı veya aşağı) düzenleme gösteren genleri tanımlamak için kullanılmıştır.

Bulgular: Gen ekspresyon analizi sonuçlarına göre, 7098 transkript pankreatik duktal adenokarsinom dokusunda normal dokuya kıyasla farklı düzenleme göstermiştir. UMAP grafiği ile normal ve pankreatik duktal adenokarsinom dokularının birbirinden farklı dağılım göstermesi, bu iki doku arasında transkript farklılığı olduğunu göstermektedir.

Sonuç: Çalışmada yapılan gen ekspresyon analizi sonucunda pankreatik duktal adenokarsinom dokuları ile normal dokular arasında farklılık gösteren transkriptler bulunmuştur. Bu transkriptler ile yapılacak çalışmalar sayesinde hastalığın tedavisi için hedefe yönelik tedavi stratejileri geliştirilebilir ve ölüm oranı çok yüksek olan bu hastalığın durumu değiştirilebilir.

Anahtar kelimeler: Pankreatik duktal adenokarsinom, gen ekspresyonu, diferansiyel ekspresyon

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INTRODUCTION

Pancreatic cancer has a 5-year overall survival (OS) rate of approximately 10%, and is the cancer with the worst prognosis among commonly known solid malignancies (1). With this survival rate, it is observed that the chance of survival of the patients is very low and the number of deaths from this disease continues to increase day by day. It has also been reported that the recent increase in pancreatic cancer overshadows breast cancer, which ranks third in cancer deaths (2). The most common type of pancreatic cancer is pancreatic ductal adenocarcinomas (PDAC) with a rate of 95%. PDACs are exocrine cell tumors and are known for their high mortality rates. Endocrine pancreatic cancers, on the other hand, are slower-growing tumors that generally have a better prognosis than exocrine pancreatic cancers (3).

There are 4 main factors underlying the high mortality rate observed in PDAC. The first has to do with the location of the pancreas. The pancreas is an organ located deep in the upper abdomen, behind the stomach, between the aorta and the upper abdominal branches. Not only does its location make tumors difficult to detect, but because the cancerous area mostly develops around these vessels, only 15%-20% of cases are suitable for surgical resection based on curative therapy (4). The second reason is that PDAC metastasizes early and exhibits aggressive biology. More than half of the patients have distant metastatic disease at the time of admission to the hospital, and most of the patients undergoing resection have metastases within 4 years following surgery. In this case, it is thought that individuals with apparently localized tumors may actually have micrometastases (1, 5, 6). The third reason is that the physiological effects of PDAC greatly weaken patients by limiting their resistance to withstand aggressive treatment. Up to 80% of patients with PDAC have cachexia exhaustion syndrome at the time of diagnosis, which is further complicated by exocrine and endocrine pancreatic dysfunction (7-9). The last major factor is that PDAC can progress very rapidly even in the treatment with the most effective systemic agents and in the application of radiotherapy, and it shows high resistance to many antineoplastic treatments with very low response rates (10). Taken together these 4 main elements, PDAC, as with other tumor types, is

suitable for multiple trials using targeted agents gave unsuccessful results (11, 12). With the development of effective targeted therapy approaches on PDAC, this tumor, which is quite aggressive, may result in fewer deaths in the future. Better elucidation of PDAC biology, molecular development, and underlying genetic basis with various studies will lead to the understanding of the disease by clinicians and may encourage the emergence of new targeted and immune-based therapies that may be available for patients with PDAC in the near future (13). Therefore, the need for genetic-based studies on PDAC is increasing day by day.

In the current study, it was aimed to determine the differences in the transcription level in patients with Pancreatic Ductal Adenocarcinoma. For this reason, open access gene expression data obtained from patients with pancreatic ductal adenocarcinoma and normal tissue were used in the study. With the data set used, the transcripts that created expression differences in pancreatic ductal adenocarcinoma tissues compared to normal tissues were determined as a result of bioinformatic analysis.

METHODS

Dataset

Within the scope of the current study, gene expression sequence analyses were performed in order to examine the factors that may be associated with pancreatic cancer at the transcriptomic level. Therefore, by determining the expression differences between 10 pancreatic ductal adenocarcinoma samples and 5 normal tissue samples, the most expressed (up-down) RNAs were determined. Affymetrix HTA2.0 Array was used to determine expression differences. Afterward, bioinformatic analyzes were performed. The data set used in the study was obtained from the National Center for Biotechnology Information (NCBI). Data were obtained from Gene Expression Omnibus (GEO) with the code "GSE132956".

Bioinformatics and gene expression analysis

Bioinformatics is the collection, storage, organization, archiving, analysis, and presentation of results based on theory and practice in a discipline such as biology, medicine, behavioral, or health sciences. Furthermore, it is focused on the research and development of computational tools and methodologies to broaden the use and processing of data obtained through studies or the application of recognized procedures. Obtained as a consequence of research or the use of well-known methodologies. Analyses in bioinformatics are performed by selecting a database and a program that allows bioinformatic analysis to be performed in accordance with the biological question, molecule, or structure to be analyzed. The data and results obtained as a result of the analyzes are brought together and the evaluations are analyzed analytically in the light of the previous information in the literatüre (14).

Changes in an organism's or cell's physiology will be accompanied by changes in the pattern of gene expression, making gene expression analysis important in many fields of biological research. The still-in-development DNA microarray method is used to study gene expression by hybridizing mRNA to a high-density array of immobilized target sequences, each corresponding to a specific gene. The effect of chemicals on gene expression, for example, can reveal functional and toxicological qualities. Expression studies on clinical samples, both healthy and sick, may lead to the identification of novel biomarkers (15).

Bioinformatics analysis phase

In this study, gene expression analyses were performed on trancriptomic data obtained from 10 pancreatic ductal adenocarcinoma samples and 5 normal tissue samples. In the investigation, the limma package, which is accessible in the R programming language and permits expression analysis, was employed (16). Limma (Linear Models for Microarray Analysis) is a library for evaluating gene expression microarray data, with a focus on the use of linear models for analyzing specified experiments and determining differential expression. The packet's functions are applicable to all gene expression technologies, such as microarrays, RNA-seq, and quantitative PCR. With the Limma package, it is also possible to provide stable results even when the number of sequences is low, thanks to Empirical Bayes methods. As a result of the bioinformatic analysis, Lof2FC was obtained, which shows the fold change of expression differences of the genes listed in order of importance. Up-regulated genes are identified using log2FC>1 while down-regulated genes are identified using log2FC<-1.

Box-plot graphs and expression density graphs were used to see the distribution of the data used in the study. In the graphs used samples with the same characteristics are shown with the same color. Uniform Manifold Approximation and Projection (UMAP) graph was preferred in order to visualize the relations of the samples in the study with each other. Finally, the volcano plot was preferred to show differentially expressed genes (up and down). The volcano graph depicts significance vs fold-change in log2 base on the y- and x-axes to quickly identify differentially expressed genes. In the graph, the red color indicates the genes that are up-regulated, the blue color the down-regulated genes, while the black color indicates the genes that do not differ.

RESULTS

Distribution graphs for 10 pancreatic ductal adenocarcinoma samples and 5 normal tissue samples used in the study are given in Figure 1 and Figure

The UMAP graph, where we can see the relationships of the samples with each other, is given in figure 3. With this graph, it is seen that

the samples with the same characteristics are clustered together. In the graph, green dots show pancreatic ductal adenocarcinoma samples, while purple dots show normal tissues.

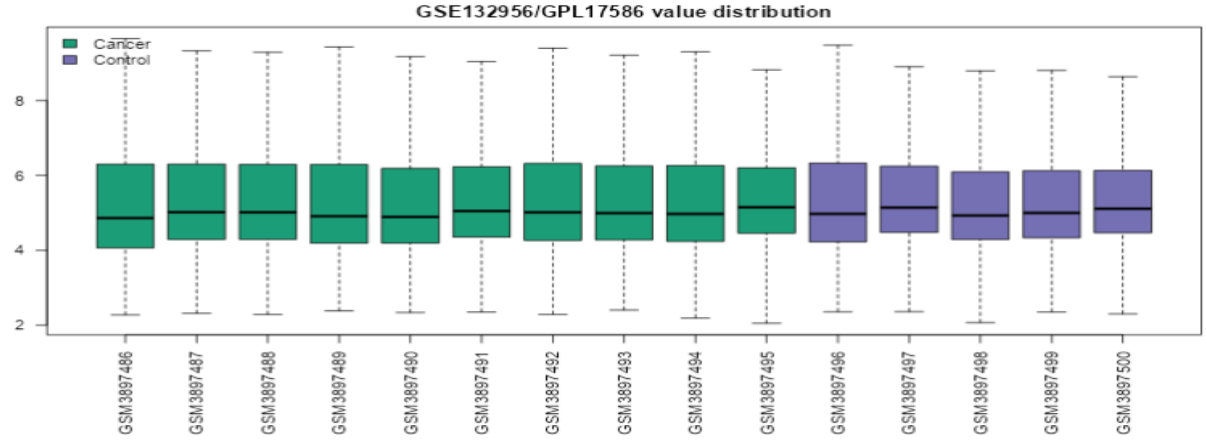

Figure 1: The distribution of the values of the selected samples.

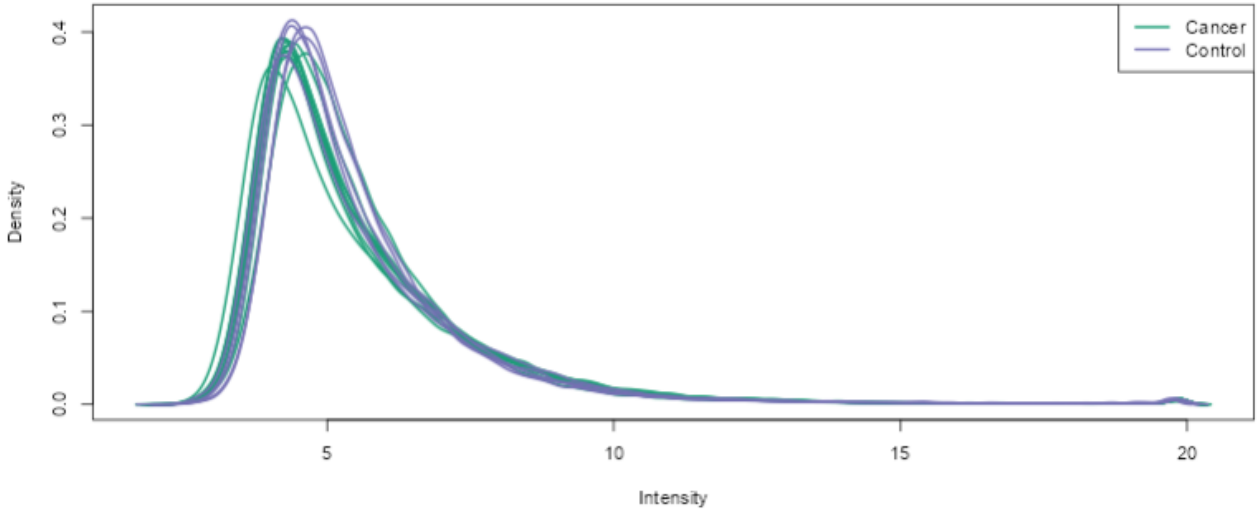

Figure 2: The expression density graph of the selected samples.

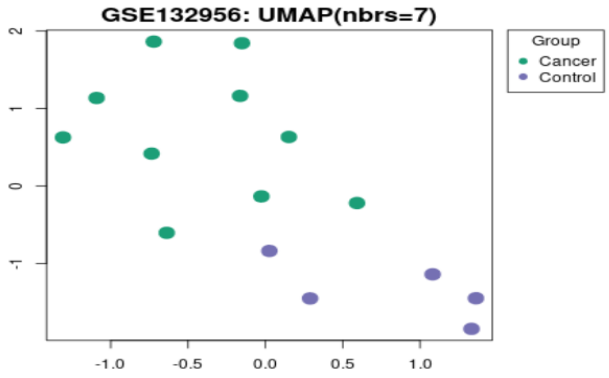

Figure 3: UMAP plot of the samples

According to gene expression analysis, 7098 expressions with statistically significant differences in gene expression levels were found between the two groups.(|log2FC| > 1.0, P 0.05). Information on the first 15 genes showing up-regulate and down-regulate in expression between the two groups are given in Table 1 and Table 2.

Table 1: Transcripts whose expression level is up-regulated in pancreatic ductal adenocarcinoma samples relative to normal tissue

ID	Adj.P Val	P Value	t	B	Log2FC
TC10001390.hg.1	0,014861	5,32E-04	4,39	-0,06672	9,124919
TC03003290.hg.1	0,002162	4,51E-06	6,97	4,476732	8,975542
TC21000141.hg.1	0,000359	5,32E-08	9,96	8,497688	8,647183
TC03003289.hg.1	0,002117	4,11E-06	7,03	4,565055	8,199274
TC05001556.hg.1	0,00234	5,23E-06	6,89	4,3384	7,586244
TC15000461.hg.1	0,00775	1,11E-04	5,19	1,436824	7,446171
TC07002463.hg.1	0,00299	1,04E-05	6,48	3,688199	7,267876
TC17001751.hg.1	0,010834	2,48E-04	4,77	0,666021	7,264719
TC12002256.hg.1	0,005437	4,77E-05	5,64	2,247014	6,828612
TC07003086.hg.1	0,006079	6,11E-05	5,5	2,010041	6,756634
TC05002808.hg.1	0,016737	7,10E-04	4,24	-0,34377	6,679631
TC10001389.hg.1	0,033297	3,20E-03	3,51	-1,78341	6,630858
TC21000779.hg.1	0,014837	5,30E-04	4,39	-0,06279	6,593831
TC12000365.hg.1	0,006259	6,69E-05	5,45	1,923044	6,218943
TC19000584.hg.1	0,010548	2,31E-04	4,81	0,736559	6,149568

Table 2: Transcripts whose expression level is down-regulated in pancreatic ductal adenocarcinoma samples relative to normal tissue

ID	Adj.P Val	P Value	t	B	Log2FC
5	0,004437	2,85E-05	-5,92	2,736069	-10,5878
TC09000767.hg.1	0,006627	7,52E-05	-5,39	1,81114	-10,0377
TC07000780.hg.1	0,002918	9,94E-06	-6,51	3,735494	-9,65693
TC07000809.hg.1	0,01186	3,01E-04	-4,67	0,48041	-9,62033
TC16000923.hg.1	0,009443	1,64E-04	-4,98	1,061781	-9,5312
TC10000844.hg.1	0,021786	1,27E-03	-3,96	-0,90177	-9,35428
TC01000277.hg.1	0,001332	1,04E-06	-7,89	5,837119	-9,32311
TC01000278.hg.1	0,001795	2,84E-06	-7,26	4,910037	-9,17262
TC01003928.hg.1	0,003291	1,43E-05	-6,3	3,393631	-8,99115
TC10000845.hg.1	0,013219	4,04E-04	-4,53	0,199158	-8,93247
TC10000846.hg.1	0,012668	3,65E-04	-4,58	0,296364	-8,85196
TC19001513.hg.1	0,004477	2,97E-05	-5,89	2,697251	-8,71258
TC12002042.hg.1	0,010591	2,34E-04	-4,8	0,721621	-8,6297
TC06003618.hg.1	0,016431	6,73E-04	-4,27	-0,29121	-8,52873
TC01000188.hg.1	0,002392	5,45E-06	-6,86	4,299571	-8,42689

Figure 4 depicts the volcano plot used to visualize the differentially expressed genes between groups.

DISCUSSION

Pancreatic cancer is a well-known fatal disease with similar mortality and morbidity. While its incidence continues to increase with each passing year, the 5-year survival rate is the lowest among all cancers (about 10%) (17). In addition, because pancreatic cancer can usually be detected at an advanced stage, treatment options are limited, and most patients experience recurrence and metastasis after curative resection. This increases the lethality of the disease. Recent advances in surgical approaches and various new chemotherapy regimens have not improved the poor prognosis of the disease in recent years (18, 19). PDAC, the most common type of pancreatic cancer, is one of the deadliest malignancies among all cancers and has a worse prognosis than other types of pancreatic cancer (20). Treatment options for this aggressive cancer type, which can be diagnosed most often

at an advanced stage, are quite limited, sometimes even surgery cannot be applied, and it is limited to systemic chemotherapy with modest clinical responses (13).

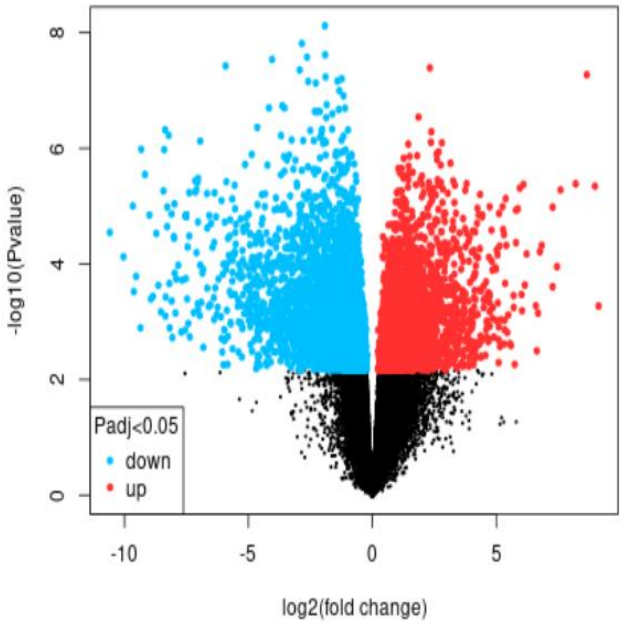

Figure 4: Volcano plot of genes with up-regulated (red dots) and down-regulated (blue dots) gene expression among the group of pancreatic ductal adenocarcinoma samples and normal tissue

For this reason, there is a great need for up-to-date studies investigating new therapeutic methods for this cancer type with a very high mortality rate. The relationship between neoplastic characteristics and individual treatment responses is highlighted by targeted therapy. It is based on genetics and biomarker expression, implying that genomic alterations, as well as their changed downstream pathways, might be relevant pharmaceutical targets or prognostic indications. Researchers can now quickly discover genetic variations between tumor cells and normal cells because of advances in genome sequencing (18).

The aim of this study is to perform bioinformatic analyzes with open access gene expression dataset to determine the expression differences between tissue samples with pancreatic ductal adenocarcinoma and normal tissue samples. Thus, with the results obtained, transcripts with different expressions in tissue with pancreatic ductal adenocarcinoma compared to normal tissues will be determined.

According to the results obtained from the bioinformatic analysis, 7098 transcripts showed different expression in pancreatic ductal adenocarcinoma tissues compared to normal tissues. Information on how much fold the most expressed (up or down) transcripts fold differ is the following.

When the p values of the transcripts are examined, it is seen that these transcripts differ in pancreatic ductal adenocarcinoma samples and normal tissue samples. These transcripts are also transcripts with log2FC values greater than 1 and less than -1, with different regulation in pancreatic ductal adenocarcinoma samples and normal tissue samples. The transcript with id TC10001390.hg.1 showed 556.40 fold up-regulation in pancreatic ductal adenocarcinoma samples compared to normal tissue samples. Likewise, the transcripts with id TC03003290.hg.1, TC21000141.hg.1, TC03003289.hg.1, TC05001556.hg.1,

TC15000461.hg.1, TC07002463.hg.1, TC17001751.hg.1, TC12002256.hg.1, TC07003086.hg.1, TC05002808.hg.1, TC10001389.hg.1, TC21000779.hg.1, TC12000365.hg.1, and TC19000584.hg.1 had up-regulated gene expression of 501.46, 398.93, 292.03, 191.34, 173.64, 154.02, 153.27, 112.98, 107.63, 101.82, 99.04, 96.33, 74.02, 70.52 fold, respectively. Moreover the transcript with id TC09002315.hg.1 showed 1530.72 fold down-regulation in pancreatic ductal adenocarcinoma samples compared to normal tissue samples. Likewise, the transcripts with id TC09000767.hg.1, TC07000780.hg.1, TC07000809.hg.1, TC16000923.hg.1, TC10000844.hg.1, TC01000277.hg.1, TC01000278.hg.1, TC01003928.hg.1, TC10000845.hg.1, TC10000846.hg.1, TC19001513.hg.1, TC12002042.hg.1, TC06003618.hg.1, and TC01000188.hg.1 had down-regulated gene expression of 1045,51, 803.41, 786.88, 739.29, 652.57, 639.14, 576.02, 508.46, 487.75, 461.44, 418.76, 393.44, 367.09, 342.50 fold, respectively.

Transcripts with this expression difference obtained show that there are genetic changes in pancreatic ductal adenocarcinoma tissues compared to normal tissues. Targeted treatment strategies can be developed and overall survival may be increased for this highly lethal type of cancer, by examining the background of these differences and addressing them with further analyses such as determining which pathway/pathway they are associated with. Or, metastases after treatment with surgery can be reduced and mortality rates can be reduced in the same way by controlling the patients.

As a result, it has been determined that the genetic structure changes in the case of PDAC with the information obtained, and it may be possible to increase the therapeutic efficacy of the disease with comprehensive and various genetic studies to be carried out considering these changes. Appropriate treatment for PDAC can be developed and the disease can be treated before it progresses with biomarkers used in the diagnostic phase of the disease. With the new oncological treatment selection developed in this way, the treatment may reach the target, and mortality rates can be reduced.

Ethics Committee Approval: Ethics committee approval is not required in this study

Peer-review: Externally peer-reviewed

Author Contributions: Concept: ZK, İPÇ, Design: ZK, İPÇ, Literature Search: ZK, İPÇ, Data Collection and Processing: ZK, İPÇ, Analysis or Interpretation: ZK, İPÇ, Written: ZK, İPÇ.

Conflict of Interest: The authors declared no conflicts of interest.

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