ORIGINAL ARTICLE

Eastern Journal of Medicine
East J Med 28(2): 334-351, 2023
DOI: 10.5505/ejm.2023.79446

Evaluation of Serum MicroRNA Levels and Mutations Using Next-Generation Sequencing of Liquid Biopsies From Metastatic Pancreatic Cancer Patients: A Turkish Pilot Study

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ABSTRACT

Pancreatic cancer is highly aggressive and at the time of diagnosis, 80% of patients are in the metastatic stage. Personalized therapy guided by genomic biomarkers for pancreatic cancer has only recently begun to be investigated.This study aimed to comprehensively identify possible activating and pathological mutations in metastatic pancreatic cancer with NGS of liquid biopsies and to investigate miRNAs and mutations as therapeutic targets as we evaluated their relationships and effect on prognoses

Seventeen patients and 20 healthy volunteers were included in the study. Blood samples were taken from the patients, cell-free DNA was isolated from the serum, and mutation profiles were analyzed by NGS. Serum miRNA levels were analyzed by isolating circulating miRNA from the same samples from all groups.

In patients with G288G synonymous mutants in the HNF1A gene compared to non-mutant patients, miR-17, miR-24, and miR-150 levels were found to be statistically significantly lower ;The miR-17 and miR-146a levels of patients with a TP53 gene Pro72Arg mutation were found to be statistically significantly higher than that of the non-mutant group Survival was lower with the c.2472 C>T mutation located in exon 18 of the PDGFRA gene; higher in patients with P72R mutations in the TP53 gene and those with miRNA 17 and 146 upregulation carrying this mutation.

This study is clinically meaningful in revealing possible pathogenic mutations detected in tumor cells circulating in metastatic pancreatic cancer, both in terms of prognostic value and whether the information can be used to target treatment, including gene therapy.

Keywords: Pancreatic cancer, MicroRNA, next-generation sequencing, liquid biopsies

Introduction

Pancreatic cancer has an extremely poor prognosis due to late diagnosis, high metastatic potential, lack of effective treatment methods, and resistance to chemotherapy. Diagnosis and treatment of pancreatic cancer are very challenging. It represents the fourth leading cause of cancer deaths in the United States, with 57,600 new cases and 47,050 deaths recorded in 2020 (1). It is an aggressive cancer, and at the time of diagnosis, 80% of patients are in the metastatic stage. The five-year survival rate for those patients is 3% (2). As a result of these characteristics, pancreatic cancer is one of the most researched cancers in both basic and clinical sciences.

As in many other cancers, genome stability is impaired in pancreatic cancer. Approximately 97% of pancreatic cancers have gene irregularities defined by point mutations, amplifications, deletions, translocations, and inversions (3). Recently, gene aberrations as biomarkers to identify therapeutic strategies have been demonstrated in various types of cancer. Detection of driver mutations and targeted drugs, especially in advanced lung cancer, have increased survival. On the other hand, personalized therapy guided by genomic biomarkers for pancreatic cancer has only recently begun to be investigated. To realize precision medicine for pancreatic cancer guided by genomic biomarkers, the profiles of somatic gene aberrations associated with this disease must be thoroughly investigated.

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Received: 30.11.2021, **Accepted:** 18.07.2022

Aberrations of the KRAS, CDKN2A, TP53, and SMAD4 genes are well-known drivers of pancreatic carcinogenesis (4). Recent genome-wide sequencing studies detected tens of genes mutated in pancreatic cancer tissues (5). However, mutation profiles could not be defined comprehensively in these studies due to the cellular scarcity of tumor tissue. Liquid biopsy methods and next-generation sequencing (NGS), which allow more comprehensive profiling, become especially important when tumor tissue cannot be obtained. The number of newly described cancer-associated genes has grown exponentially since the advent of NGS technology. The mutations discovered in liquid biopsies reflect the mutations found in tumors. Because circulating tumor cell analysis originates from the entire tumor area and/or metastasis present in the body, a formalin-fixed paraffin-embedded (FFPE) specimen can detect more genetic changes than analysis of a specific area in tumor tissue (6).

MicroRNAs (miRNAs) are small, non-protein-coding RNA molecules, approximately 19-24 nucleotides long, that regulate gene expression at the translational level (7). A single miRNA can interact with multiple target genes with oncogenic or tumor-suppressive functions and potentially regulate multiple cellular pathways (8). Various studies have demonstrated the role of miRNAs in the diagnosis, proliferation, progression, metastasis, and chemotherapy resistance of various cancers (9-12). Levels of miRNA are of increasing importance in clinical oncology as they can be both prognostic and predictive biomarkers and ultimately a therapeutic target.

This study aimed to comprehensively identify possible activating and pathological mutations in metastatic pancreatic cancer with NGS of liquid biopsies. We also aimed to investigate miRNAs and mutations as therapeutic targets as we evaluated their relationships and effect on prognoses.

Materials and Methods

Study Groups: This study was carried out with the approval of XXX University Ethics Committee (Approval no: E-60116787-020-66963, date: 23.06.2021). A total of 17 patients with de-novo metastatic pancreatic adenocarcinoma, a performance score of 3-4, and no treatment were included in the study. Patients who were operated on, not de-novo metastatic, previously treated, with brain metastases, a history of a second cancer or any known cancer in the family, and without a pathological diagnosis were excluded from the study. 20 healthy volunteers, who do not have a chronic disease, any previous pathology of the pancreas (pancreatitis, cyst, benign neoplasia, operation, etc.) and malignancy (pancreas or other) and regular drug use were recruited. Blood samples (7 ml) were taken from the patients, cell-free DNA (cfDNA) was isolated from the serum, and mutation profiles were analyzed by NGS. Serum miRNA levels were analyzed by isolating circulating miRNA from the same samples from all groups.

Next Generation Sequencing: According to the manufacturer protocol, cell-free DNA (cfDNA) was extracted from those liquid biopsies with the QIAamp DNA Mini kit (QIAGEN, Germany, Cat No./ID: 51304) and was eluted in 60 µL volume. Concentration of extracted DNA samples was measured by the Qubit dsDNA High Sensitivity (HS) assay kit (Life Technologies-Fisher Scientific, Cat No: Q32851). Testing quality and amplifiability of the extracted cfDNA samples was done by quantitative PCR (qPCR) technology. All samples with ΔCq value below 5 can be selected for further use. Following the manufacturer instructions, the libraries were prepared using accel-amplicon 56G oncology Panel v2 (Swift biosciences, US, Cat. No: AL-56248) which is a targeted next generation sequencing assay detecting of 56 genes (ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, DDR2, DNMT3A, EGFR, ERBB2, ERBB3, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MAP2K1, MET, MLH1, MPL, MSH6, NOTCH1, NPM1, NRAS, PDGRA, PIK3CA, PTEN, PTPN11, RB1, RET, STK11, SMAD4, SMARDCB1, SMO, SRC, TP53, TAC1 AND VH1). Quality of the libraries was checked out by Agilent 2100 Bioanalyzer device utilizing the DNA 1000 reagents and Chips (Agilent Technologies, Santa Clara, California, Cat. Code: 5067-1504). The expected PCR product is 186–277 bp, which indicates successful library amplification. Patients' libraries together with PhiX control library were normalized and equal volumes were pooled to form the terminal sequencing library. The AmpliSeq™ for accel-amplicon 56G oncology Panel v2 achieves detection limits of 5% variant allele frequency across 207 amplicons with> 95% of bases covered at ≥ 500 [14]. Sequencing was done using Cancer Hotspot Panel v2 Nano kit on NextSeqDx device

(llumina). Bioinformatics and data analysis start from checking each run quality through assessing the specifications based on Illumina PhiX control library which support cluster densities between 865–965 k/mm² clusters passing filter for v2 chemistry. The second item is the quality score (Q-score) which is a prediction of the probability of an error in base calling. The percentage of bases > Q30 is averaged across the entire run. The quality scores for v2 chemistry> 80% bases higher than Q30 at 2 150 bp. Image processing and fastq file generation were further analyzed by Sophia-DDM-v4 programme. Each variant is linked to numerical identifier in Catalogue of Somatic Mutations in Cancer (COSMIC) database. The likely impact of amino acid changes was determined with In Silico Predictions (Sift & PolyPhen) and Functional Analysis through Hidden Markov Models (v2.3) (FATHMM) prediction. The variants were categorized as benign or pathogenic according to ClinVar database. Mutations with low depth, which indicate ≤ 10 depths variant were filtered out.

miRNA analyses: The serum samples were treated with 1/5 QIAzole, and miRNA isolation was performed using a miRNeasy serum/plasma kit (cat: 217184 Qiagen, Hilden, Germany). After miRNA isolation was completed, cDNA was obtained from all samples using a miRNA cDNA Synthesis Kit with Poly (A) Polymerase Tailing (cat: G903 ABM, Richmond, Canada). An adenylation kit with an oligo adapter was used for miRNA cDNA translation. At the end of the reaction, spectrophotometric measurements were made and stored at -20°C. Based on the literature, 20 candidate miRNAs were identified. We obtained cDNA abm miRNA (hsa-miR-17, hsa-miR-21, hsa-miR-24, hsa-miR-34a, hsa-miR-93a, hsa-miR-93b, hsa-miR-125a, hsa-miR-145, hsa-miR-146, hsa-miR-150, hsa-miR-199b, hsa-miR-210, hsa-miR-221, hsa-miR-636, hsa-miR-936, hsa-let-7a, hsa-let-7b, hsa-let-7c, hsa-let-7d and hsa-let-7e) with the BIO-RAD CFX96 instrument using BrightGreen miRNA qPCR MasterMix (abm, Richmond, Canada). The cycle threshold (CT) was determined for each miRNA, and a control (cel-miR-39-3p). The relative abundance of each miRNA transcript was then determined using delta delta CT method. The delta delta CT was used to evaluate the relative expression levels of miRNA genes in the samples of patients and healthy controls. When we compared patient serum miRNA levels with those of healthy volunteers, a statistically significant increase was found in the expression of all miRNA ($p < 0.05$). Receiver operator characteristic (ROC) curve analysis, performed with the same two groups, found the sensitivity and specificity to be statistically significant ($p < 0.05$).

Statistical Analysis: Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 17 software (IBM Corp., Armonk, NY, USA). Descriptive data are expressed as mean and percentages. The Student's t-test for continuous variables were used. Categorical variables were compared by a chi-square test (Fisher's exact probability test). Survival curves were formed using the Kaplan-Meier compared by the log rank test. Statistical significance was defined as a P value of <0.05.

Using CT values in the patient and healthy group, the increase or decrease ratio of expression levels serum miRNA genes and the cel-miR-39-3p expression levels were calculated according to the following formula.

$2^{-\Delta\Delta CT}$ where ΔCT= CT target gene - CT reference gene

All data is evaluated parameters using the program with graphpad prism 7.0 version, unpaired t test and one-way anova test were applied and the graphs were analyzed. All mutations variant were compared serum miRNA levels in patients. The same data sets were confirmed using SPSS 17.0 program

Results

Patient Characteristics: Of the 17 cancer patients, 6 (35%) were female, and 11 (65%) were male. Their mean age was 58 (34-73). The mean age of healthy volunteers was 57 (42-66). Of the 20 volunteer patients, 8 (40%) were female, and 12 (60%) were male. All cancer patients were palliative care patients not receiving any treatment (chemotherapy, targeted therapy, immunotherapy). Their histopathological diagnosis was based on their metastatic foci (10 liver, 7 intra-abdominal lymph nodes).

Serum cfDNA Results: Hot-spot mutations of 56 genes were screened with cfDNA obtained from 7-ml serum samples. The results are summarized in table 1. All mutation profiles are available in table 2 Pathogenic mutations in the Functional Analysis through Hidden Markov Models (FATHMM) score

P6045S missense mutation in ATM gene in 1 patient, Q787Q synonymous mutation in EGFR gene in 13 patients, T785P missense mutation in 1 patient, R347H missense mutation in FBXW7

Table 1: List of Mutations Detected In 17 Metastatıc Pancreatic Cancer Patients

Gene	Variant	Consequence	Chr	Exons	dbSNP ID	HGVSc	HGVSp	No. Of patients	In silico predictions		FATHMM prediction	ClinVar	COSMIC ID
									Sift	polyphen			
ATM													
	SNV	Missense	11	12	rs2227922	c.1810C>T	p.(Pro6045Ser)	1	LP	LP	patojenik	bening	COSM22499
APC													
	SNV	synonymous	5	16	rs41115	c.4479G>A	p.(Thr1493THr)	14	V	V	neutral	vus	COSM3760869
	INDEL	frameshift	5	16	rs1114167560	c.4666dupA	p.(thr1556Asn*3)	1	P	P	n/a	Patojenik	COSM18561
CSF1R													
	SNV	3'UTR	5	22	rs3866935	c*35_*36delinTC		14	V	V	n/a	vus	n/a
EGFR													
	SNV	synonymous	7	20	rs1050171	c.2361G>A	p.(Gln787Gln)	13	V	V	pathogenic	vus	COSM1451600
	SNV	synonymous	7	15	rs1729016	c.1839C>T	p.(Ala613Ala)	1	V	v	neutral	bening	COSM5019977
	SNV	missense	7	20	n/a	c.2347A>C	p.(Thr783Pro)	1	n/a	n/a	n/a	n/a	n/a
	SNV	missense	7	20	n/a	c.2353A>C	p.(Thr785Pro)	1	p	p	pathogenic	n/a	COSM133628
	SNV	missense	7	20	n/a	c.2452T>G	p.(Cys818Gly)	1	n/a	n/a	n/a	n/a	N/A
	SNV	missense	7	20	n/a	C.2312a<c	P.(Asn771Thr)	1	n/a	n/a	n/a	n/a	n/a
	SNV	missense	7	20	n/a	c.2454T>G	p.(Cys818Trp)	1	p	p	neutral	n/a	COSM24272
ERBB2													
	INDEL	intronic	17	8	n/a	c.950_102+19dup		2	LP	LP	n/a	Vus	n/a
ERBB4													
	SNV	intronic	2	3	rs839541	c.421+58A>G		10	LP	V	neutral	bening	COSM9583227
	SNV	intronic	2	3	rs839540	c.421+95A>G		1	LP	n/a	neutral	vus	COSN28832712
FBXW7													
	SNV	missense	4	8	rs1057519	c.1040G>A	p.(arg347His)	1	P	P	Pathogenic	Patogenic	COSM22965
FGFR3													
	SNV	synonymous	4	14	rs7688609	c.1953G>A	p.(Thr651Thr)	17	p	p	Pathogenic	Bening	COSM4533173
	INDEL	intronic	4	16	n/a	c.2099_2168+16dup		4	n/a	n/a	n/a	n/a	n/a
	INDEL	3'UTR	4	18	n/a	c.2384_*53dup		1	n/a	n/a	n/a	n/a	n/a
	SNV	intronic	4	14	rs3135898	c.1959+22G>A		1	Lp	n/a	neutral	bening	COSN17152601
	SNV	synonymous	4	7	rs223909	c.882T>C	p.(Asn294Asn)	1	v	v	neutral	bening	COSM1428695

	SNV	missense	4	16	n/a	c.2155T>C	p.(Cys719Arg)	1	P	P	n/a	n/a	n/a
FLT3													
	SNV	intronic	13	11	rs2491231	c.1310-3T>C		14	n/a	n/a	neutral	n/a	COSM3999060
	SNV	intronic	13	16	rs75580865	c.2053+23A>G		2	LP	LP	neutral	bening	COSN17148966
HNF1A													
	SNV	synonymous	12	4	rs56348580	c.864G>C	p.(Gly288Gly)	7	v	v	pathogenic	bening	COSM5019699
	INDEL	frameshift	12	4	rs766191969	c.863_864insC	p.(Pro289Alafs*28)	1	n/a	n/a	n/a	n/a	COSM1476243
HRAS													
	SNV	synonymous	11	2	rs12628	c.81T>C	p.(His27His)	8	n/a	n/a	neutral	LB	COSM249860
	SNV	5'UTR	11	2	rs41294870	c-10C>T		1	n/a	n/a	not functionally	bening	COSN26961179
IDH1													
	SNV	missense	2	4	rs121913499	c.394C>T	p.Arg132Cys	1	P	P	pathogenic	pathogenic	COSM28747
	SNV	synonymous	2	4	rs11554137	c.315C>T	p.(Gly105Gly)	1	v	v	pathogenic	bening	COSM1741220
IDH2													
	SNV	synonymous	15	4	n/a	c.474A>C	p.(Pro158Pro)	2	n/a	n/a	n/a	n/a	n/a
JAK3													
	SNV	missense	19	4	rs3212723	c.394C>A	p.(Pro132Thr)	1	p	p	pathogenic	LB	COSM34216
	SNV	intronic	19	4	rs3212725	c.420+40T>C		1	n/a	n/a	n/a	n/a	n/a
	SNV	intronic	19	4	rs3212726	c.420+59A>G		1	n/a	n/a	n/a	n/a	n/a
KDR													
	SNV	3'UTR	4	30	rs4421048	c.*27T>C		16	n/a	n/a	n/a	n/a	n/a
	SNV	intronic	4	19	rs869246746	c.2615-37dupC		8	n/a	n/a	n/a	n/a	COSN17154192
	SNV	missense	4	11	rs1870377	c.1416A>T	p.(Gln472His)	9	p	p	neutral	bening	COSM149673
	SNV	missense	4	7	rs2305948	c.889G>A	p.(Val297Ile)	4	p	p	pathogenic	vus	COSM1131107
	SNV	synonymous	4	30	rs35961234	c.4008C>T		1	n/a	n/a	neutral	n/a	COSM35855
KIT													
	SNV	synonymous	4	10	rs55986963	c.1638A>G	p.(Lys546Lys)	1	v	v	pathogenic	bening	COSM21983
	SNV	synonymous	4	18	rs3733542	c.2586G>C	p.(Leu862Leu)	2	b	b	neutral	LB	COSM1325
	SNV	missense	4	10	rs3822214	c.1621G>C	p.(Met541Leu)	1	p	v	pathogenic	pathogenic	COSM28026
	SNV	synonymous	4	17	rs55789615	c.2394C>T	p.(Ile798Ile)	1	n/a	n/a	pathogenic	Bening	COSM1307
KRAS													
	SNV	missense	12	2	rs121913529	c.35G>A	p.(Gly12Asp)	2	p	p	pathogenic	pathogenic	COSM521
MAP2K1													
	SNV	synonymous	15	7	rs17586159	c.711G>A	p.(Gly237Gly)	1	v	v	neutral	bening	COSM5020742
MET													

	SNV	missense	7	14	rs56391007	c.3029C>T	p.(Thr1010Ile)	1	p	p	pathogenic	vus	COSM707
	SNV	missense	7	2	rs35775721	c.534C>T	p.(Ser178Ser)	1	n/a	n/a	n/a	bening	COSM1579024
MSH6													
	SNV	intronic	2	5	rs2020911	c.3438+14A>T		14	v	v	neutral	bening	COSN26959343
	SNV	missense	2	5	n/a	c.3329C>T	p.(Pro1110Leu)	1	p	p	n/a	n/a	n/a
NOTCH1													
	SNV	synonymous	9	26	n/a	c.4764C>T	p.(Ser1588Ser)	1	n/a	n/a	n/a	n/a	n/a
PDGFRA													
	SNV	synonymous	4	12	rs1873778	c.1701A>G	p.(Pro567Pro)	17	v	v	neutral	bening	COSM7410554
	SNV	synonymous	4	18	rs2228230	c.2472C>T	p.(Val824Val)	2	v	v	pathogenic	bening	COSM22413
PIK3CA													
	SNV	missense	3	7	rs2230461	c.1173A>G	p.(Ile391Met)	1	p	p	n/a	bening	COSM328028
PTEN													
	SNV	intronic	10	4	rs370918174	c.210-39A>G		1	v	v	neutral	bening	COSM5933
RET													
	SNV	synonymous	10	13	rs1800861	c.2307G>T	p.(Leu769Leu)	13	v	v	pathogenic	bening	COSM4418405
	SNV	synonymous	10	15	rs1800863	c.2712C>G	p.(Ser904Ser)	6	n/a	n/a	neutral	bening	COSM3751779
SMARCB1													
	SNV	intronic	22	10	rs5030613	c.1092-41G>A		4	v	v	neutral	bening	COSM1090
SMAD4													
	SNV	intronic	18	8	rs948588	c.955+58C>T		1	n/a	n/a	neutral	n/a	COSN26960043
SMO													
	SNV	synonymous	7	3	rs56334250	c.582A>G	p.(Glu194Glu)	1	v	v	neutral	bening	COSM7226231
STK11													
	SNV	intronic	19	4	rs2075606			8	v	v	neutral	bening	COSN6666958
TP53													
	SNV	missense	17	4	rs1042522	c.215C>G	p.(Pro72Arg)	14	p	v	neutral	bening	COSM250061
	SNV	synonymous	17	6	rs1800372	c.639A>G	p.(Arg213Arg)	2	n/a	n/a	neutral	bening	COSM249885
	SNV	intronic	17	2	rs1642785	c.74+38C>G		3	b	b	neutral	bening	COSN6661040
	SNV	missense	17	7	rs864622237	c.700T>A	p.(Tyr234Asn)	1	p	p	pathogenic	LP	COSM43956
	SNV	missense	17	10	rs587782529	c.1009C>T	p.(Arg337Cys)	1	p	p	pathogenic	pathogenic	COSM11071
	SNV	missense	17	1	rs1064794311	c.749C>T	p.(Pro250Leu)	1	p	p	pathogenic	Vus	COSM10771
	SNV	intronic	17	9	rs77697176	c.993+352C>T		1	n/a	n/a	n/a	n/a	n/a

gene in 1 patient, T651T synonymous mutation in FGFR3 gene in 17 patients, G288G synonymous mutation in 7 patients in HNF1A gene, IDH1 in 1 patient R132C missense mutation, G105G synonymous mutation in 1 patient, P132T missense mutation in 1 patient in JAK3 gene, V297I missense mutation in 4 patients, K546K synonymous mutation in the KIT gene, M541L missense mutation in 1 patient, I798I synonymous mutation in 1 patient, G12N missense mutation in 2 patients in KRAS gene mutation, a T1010I missense mutation in 1 patient in the MET gene, V824V synonymous mutation in 2 patients in the PDGFRA gene, L769L synonymous mutation in 13 patients in the RET gene, Y234N missense mutation in one patient in the TP53 gene, R337C missense mutation in 1 patient, and P250L missense mutation in 1 patient.

Novel Mutations Not Included In Any Database: C818G missense mutation in EGFR gene in 1 patient, N771T missense mutation in 1 patient, c.2099_2168+16dup intronic mutation in 4 patients in FGFR3 gene, c.2384_*53dup mutation in 3'UTR region in 1 patient, C719R missense mutation in 1 patient, P289Af in HNF1A gene in 1 patient *28 frameshift mutations, P158P synonymous mutation in 1 patient in the IDH2 gene, c.420+40T>G intronic mutation in one patient in the JAK3 gene, c.420+59A>G intronic mutation in the same patient, c.*27T in the 3'UTR region of the KDR gene in 16 patients >C mutation, c.2615-37dupC intronic mutation in 8 patients, a P1119L missense mutation in 1 patient in MSH6 gene, S1588S synonymous mutation in 1 patient in NOTCH1 gene, c.993+352C>T intronic mutation in 1 patient in TP53 gene. These mutations are the first encountered and unidentified in the literature.

Neutral Mutations in the FATHMM Score: T1493T synonymous mutation in the APC gene in 14 patients, A613A synonymous mutation in 1 patient in EGFR gene, C818W missense mutation in 1 patient, c.421+58A>G intronic mutation in ERBB4 gene in 10 patients, c.421+95A>G intronic mutation in 1 patient, in FGFR3 gene N294N synonymous mutation in 4 patients, c.1959+22G>A intronic mutation in 1 patient, c.1310-2T>C intronic mutation in FLT3 gene in 14 patients, c.2053+23A>G intronic mutation in 2 patients, H27H synonymous in 8 patients in HRAS gene mutation, Q472H missense mutation in 9 patients in KDR gene, T1336T synonymous mutation in 1 patient, L862L synonymous mutation in KIT gene in 2 patients, G237G synonymous mutation in MAP2K1 gene in 1 patient, c.3438+14A>T intronic mutation in MSH6 gene in 14 patients, PDGFRA P567P in 17 patients synonymous mutation, c.210-39A>G intronic mutation in 1 patient in the PTEN gene, c.1092-41G>A intronic mutation in 4 patients in the SMARCB1 gene, c.955+58C>T intronic mutation in 1 patient in the SMAD4 gene, E194E in 1 patient in the SMO gene synonymous mutation in STK11 gene in 8 patients c.465-51T>C intronic mutation, P72R missense mutation in 14 patients, R213R synonymous mutation in 2 patients, c.74+38C>G intronic mutation in 3 patients were detected in the TP53 gene.

Relationship Between Serum Mirna Results And Mutation Profile: Serum miRNA levels of the 17 patients were higher compared to that of the 20 healthy volunteers, and the difference was statistically significant ($p < 0.05$) (figure 1). The ROC curve analysis between the two groups was also statistically significant (figure 2).

In patients with G288G synonymous mutants in the HNF1A gene compared to non-mutant patients, miR-17, miR-24, and miR-150 levels were found to be statistically significantly lower (miR17 [mutant] 2.06±0.37- [non-mutant] 2.933±0.16 - p:0.037, miR-24 [mutant] 2.45±0.35- [non-mutant] 3.61±0.33-p:0.035, miR-150 [mutant] 3.51±0.36- [non-mutant] 6.63±0.78- p:0.006). The miR-17 and miR-146a levels of patients with a TP53 gene Pro72Arg mutation were found to be statistically significantly higher than that of the non-mutant group (miR-17 [mutant] 2.81±0.2- [non-mutant] 1.47±0.16- p:0.010, miR-146a [mutant] 6.09±0.57- [non-mutant] 2.7±0.75-p:0.021) (figure 3).

Relationship Between Survival And Mutations: The mean progression time of the patients was 2.1±1.09 months, and overall survival was 3.9±0.25 months. Survival in all patients with V824V mutants in the PDGFRA gene was statistically significantly lower than in those without (mutants 2±1 months, non-mutants 4.1±0.2, p: 0.002). With the intronic c.3438+14 A>T mutation in the MSH6 gene and the P72R mutation in the TP53 gene, overall survival was statistically significantly higher than non-mutants (4.2±0.19 months for MSH6 mutants, 2.3±0.6 p for non-mutants, 0.001, 4.2±0.19 in TP53 mutants, 2.3±0.6, p: 0.001 in non-TP53 mutants). The patients with high expression of serum miR-17 and miR-146a with a TP53 mutation had statistically significantly more prolonged survival than those with low miR-17 and miR-146a expression (figure 4).

Table 2: Each Identified Variant In Each Patient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
ATM						c.1810C>T p.(Pro604Ser)											
APC	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4666dupA p.(Thr1556Asnfs*3)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)		c.4479G>A p.(Thr1493=)	c.4479G>A p.(Thr1493=)	
CSF1R	c.*35_*36delinsTC		c.*35_*36delinsTC	c.*35_*36delinsTC	c.*35_*36delinsTC	c.*35_*36delinsTC	c.*35_*36delinsTC	c.*35_*36delinsTC	c.*35_*36delinsTC	c.*35_*36delinsTC		c.*35_*36delinsTC	c.*35_*36delinsTC	c.*35_*36delinsTC		c.*35_*36delinsTC	c.*35_*36delinsTC
EGFR	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=) c.1839C>T p.(Ala613=)	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)	c.2347A>C p.(Thr783Pro) c.2353A>C p.(Thr785Pro) c.2452T>G p.(Cys818Gly) c.2312A>C p.(Asn771Thr) c.2454T>G p.(Cys818Trp) c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)			c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)	c.2361G>A p.(Gln787=)
Erbb2							c.950_1021+19dup	c.950_1021+19dup									
ERBB4		c.421+58A>G c.421+95A>G	c.421+58A>G	c.421+58A>G	c.421+58A>G	c.421+58A>G	c.421+58A>G	c.421+58A>G		c.421+58A>G		c.421+58A>G			c.421+58A>G		

FBXW7		c.1040G>A p.(Arg347His)																
FGFR3	c.1953G>A p.(Thr651=)	c.1953G>A p.(Thr651=) c.882T>C p.(Asn294=)	c.1953G>A p.(Thr651=) c.2384_*53dup c.2099_2168+16dup c.1959+22G>A	c.1953G>A p.(Thr651=) c.882T>C p.(Asn294=)	c.1953G>A p.(Thr651=) c.882T>C p.(Asn294=)	c.1953G>A p.(Thr651=)	c.1953G>A p.(Thr651=)	c.1953G>A p.(Thr651=) c.2099_2168+16dup	c.1953G>A p.(Thr651=) c.2099_2168+16dup c.2155T>C p.(Cys719Arg)	c.1953G>A p.(Thr651=) c.2099_2168+16dup	c.1953G>A p.(Thr651=)	c.1953G>A p.(Asn294=) c.882T>C p.(Asn294=)	c.1953G>A p.(Thr651=)	c.1953G>A p.(Thr651=)	c.1953G>A p.(Thr651=)	c.1953G>A p.(Thr651=)	c.1953G>A p.(Thr651=)	
GNA11	c.1310-3T>C	c.1310-3T>C	c.1310-3T>C	c.2053+23A>G c.1310-3T>C	c.1310-3T>C	c.1310-3T>C	c.1310-3T>C		c.1310-3T>C	c.1310-3T>C		c.1310-3T>C		c.1310-3T>C	c.1310-3T>C	c.2053+23A>G c.1310-3T>C	c.1310-3T>C	
HNF1A			c.863_864insC p.(Pro289Alafs*28) c.864G>C p.(Gly288=)	c.864G>C p.(Gly288=)			c.864G>C p.(Gly288=)	c.864G>C p.(Gly288=)		c.864G>C p.(Gly288=)		c.864G>C p.(Gly288=)	c.864G>C p.(Gly288=)					
HRAS			c.81T>C p.(His27=)		c.81T>C p.(His27=)		c.81T>C p.(His27=)	c.81T>C p.(His27=)	c.81T>C p.(His27=)	c.81T>C p.(His27=)		c.-10C>T			c.81T>C p.(His27=)	c.81T>C p.(His27=)	c.81T>C p.(His27=)	
IDH1		c.394C>T p.Arg132Cys					c.315C>T p.(Gly105=)											
IDH2	c.474A>C p.(Pro158=)					c.474A>C p.(Pro158=)												
Jak3														c.394C>A p.(Pro132Thr) c.420+40T>C c.420+59A>G				

KDR	c.*27T>C c.1416A>T p.(Gln472His) c.2615-37dupC	c.*27T>C c.1416A>T p.Gln472His c.2615-37dupC	c.*27T>C c.1416A>T p.(Gln472His) c.2615-37dupC	c.*27T>C	c.*27T>C c.1416A>T p.(Gln472His) c.889G>A p.(Val297Ile)	c.*27T>C c.1416A>T p.(Gln472His) c.2615-37dupC	c.*27T>C c.1416A>T p.(Gln472His) c.2615-37dupC	c.*27T>C c.1416A>T p.(Gln472His) c.2615-37dupC c.4008C>T p.(Thr1336=)	c.*27T>C c.889G>A p.(Val297Ile)	c.*27T>C c.889G>A p.(Val297Ile)			c.*27T>C	c.*27T>C	c.*27T>C c.1416A>T p.(Gln472His) c.2615-37dupC c.889G>A p.(Val297Ile)	c.*27T>C	c.*27T>C c.1416A>T p.(Gln472His) c.2615-37dupC c.1638A>G p.(Lys546=) c.2586G>C p.(Leu862=) c.1621A>C p.(Met541Leu)	c.*27T>C
KIT			c.2586G>C p.(Leu862=)			c.2394C>T p.(Ile798=)												
KRAS													c.35G>A p.(Gly12Asp)				c.35G>A p.(Gly12Asp)	
MAP2K1													c.711G>A p.(Gly237=)					
MET										c.3029C>T p.(Thr1010Ile) c.534C>T p.(Ser178=)								
MSH6	c.3438+14A>T	c.3438+14A>T		c.3438+14A>T		c.3438+14A>T	c.3438+14A>T	c.3438+14A>T	c.3438+14A>T c.3329C>T p.(Pro1		c.3438+14A>T	c.3438+14A>T	c.3438+14A>T	c.3438+14A>T	c.3438+14A>T	c.3438+14A>T	c.3438+14A>T	c.3438+14A>T

									110Leu)								
NOTCH1										c.4764C>T p.(Ser1588=)							
PDGFRA	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=) c.2472C>T p.(Val824=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=) c.2472C>T p.(Val824=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)	c.1701A>G p.(Pro567=)
PIK3CA															c.1173A>G p.(Ile391Met)		
PTEN															c.210-39A>G		
RET	c.2307G>T p.(Leu769=) c.2712C>G p.(Ser904=)	c.2307G>T p.(Leu769=)	c.2307G>T p.(Leu769=) c.2712C>G p.(Ser904=)	c.2307G>T p.(Leu769=) c.2712C>G p.(Ser904=)	c.2307G>T p.(Leu769=)	c.2307G>T p.(Leu769=)	c.2307G>T p.(Leu769=) c.2712C>G p.(Ser904=)	c.2307G>T p.(Leu769=)	c.2307G>T p.(Leu769=) c.2712C>G p.(Ser904=)	c.2307G>T p.(Leu769=)			c.2307G>T p.(Leu769=) c.2712C>G p.(Ser904=)	c.2307G>T p.(Leu769=)	c.2307G>T p.(Leu769=)		c.2307G>T p.(Leu769=)
SMARCB1				c.1092-41G>A		c.1092-41G>A			c.1092-41G>A				c.1092-41G>A				
SMAD4										c.955+58C>T							
SMO								c.582A>G p.(Glu194=)									
STK	c.465-51T>C	c.465-51T>C		c.465-51T>C		c.465-51T>C		c.465-51T>C				c.465-51T>C	c.465-51T>C		c.465-51T>C		

TP53	c.215C>G p.(Pro72Arg)	c.215C>G p.(Pro72Arg) c.993+352C>T	c.749C>T p.(Pro250Leu)	c.215C>G p.(Pro72Arg)	c.215C>G p.(Pro72Arg) c.639A>G p.(Arg213=)	c.215C>G p.(Pro72Arg)	c.215C>G p.(Pro72Arg)	c.215C>G p.(Pro72Arg)	c.215C>G p.(Pro72Arg) c.74+38C>G	c.215C>G p.(Pro72Arg) c.74+38C>G	c.215C>G p.(Pro72Arg) c.1009C>T p.(Arg337Cys)	c.215C>G p.(Pro72Arg) c.74+38C>G	c.215C>G p.(Pro72Arg)	c.215C>G p.(Pro72Arg) c.700T>A p.(Tyr234Asn)	c.215C>G p.(Pro72Arg) c.639A>G p.(Arg213=)

Discussion

This study initially aimed to reveal the mutation profile of metastatic pancreatic cancer by performing mutation screening on tumor cfDNA. In addition, the relationship between gene mutations and non-coding miRNAs involved in eukaryotic gene regulation was also explored. The effects of the resulting expression changes on overall survival were investigated. This paper is the first clinical publication on this subject related to metastatic pancreatic cancer.

The application of liquid biopsy technology in solid tumors has become widespread in recent years. This capability is especially important for driver mutation screening and targeted drug therapy. In addition to driver mutations, it is necessary to distinguish between passenger mutations. It is predicted that mutations can help not only in the formulation of treatments but also explain possible metastatic mechanisms of cancer and radiological images (Kassem 2021).

Pathogenic mutations included in FATHMM scoring in the Catalogue of Somatic Mutations In Cancer (COSMIC) database and found in our study are shown in Table 1. Genes with driver mutations associated with pancreatic cancer, as reported by Korc, are the same as some genes detected in our study (13). However, these mutations need to be supported by cell culture studies to understand whether they are driver mutations or passenger mutations.

The following mutations were also found in our study ERBB4 gene, C.421+58A>G in the 3rd intron stated as neutral in the COSMIC database, c.81T>C in the HRAS gene in exon 2, and c.1416A>T in the KDR gene in exon 11, c.4479A> in the APC gene in exon 16. G, FGFR3 gene is c.882T>C in exon 7, FLT gene in c.1310-3T>C and c.2053T>C in exon 4, the KIT gene is c.2586 G>C in exon 18, RET gene is c in exon 15 .2712 C>G, SMARCB1 gene in exon 1092-41 G>A, STK 11 gene in exon 4 c.465-51T>C, in TP53 gene c.74+38 C>G in exon 2 and c in exon 6 .639 A>G. However, there is no data for these mutations in relation to pancreatic cancer in the database. It is important that we found these mutations for the first time in cfDNA associated with pancreatic cancer; however, the effect of these mutations on overall survival was not statistically significant in our study.

Without data in the COSMIC database CSF1R gene c*35_*36 in exon 22, FGFR3 gene c.2099_2168+16dup in exon 16, IDH2 gene c.474A>C in exon 4, and KDR gene c.2615 in exon 19. -37dupC mutations have been described for the first time. Since the overall survival effect of these mutations was not statistically significant in our study, we can define the effect of these mutations as neutral.

The Pro72Arg mutation in the TP53 gene is indicated as neutral in the Cosmic database. It has been reported that the P72R mutation in the TP53 gene increases apoptotic activity and that the conversion of proline to the amino acid arginine reduces the binding of TP53 protein to DNA (14). The Garziera article stated that the Pro72Arg mutation is germline heterozygous, and the second hit occurs in tumor cells based on the Life Origination Hydrate (LOH)-hypothesis. (15). In our results, the variant frequency was 95% and homozygous. Overall survival with the TP53 gene P72R mutation was statistically significantly higher than for non-mutants. Specific TP53 mutations can be used as prognostic markers in some cancers. One study in hepatocellular carcinomas showed the effect of the R249S mutation on survival comparing groups with and without TP53 mutations. The median survival time with the p53 R249S mutation was 11.30 months compared to 45.07 months for TP53 mutations (including R249S) and 60.84 months without TP53 mutations. Since the difference in survival time between the three groups was statistically significant, this specific mutation indicates a poor prognosis in hepatocellular carcinoma (16). Additional investigations can determine whether it will be a prognostic factor in more patient groups. In our study, patients with a TP53 gene Pro72Arg mutation were found to have higher serum miR-17 and miR-146a levels compared to those without this mutation. Yan et al. showed that miR-17-92 cluster expression was increased in TP53 mutant colon cancer cell lines and that these cells were more sensitive to apoptosis, including six miRNAs for the miR-17-92 cluster, including miR-17 (17). In our study, serum miR-17 expression in mutant TP53 patients was found to be statistically significantly higher. Sandhu et al. (2014) published a negative relationship between miR-146a and wild-type TP53 in their study with basal-like breast cancer cell lines (18). Li et al. reported that miR-146a expression suppressed proliferation in pancreatic cancer cell lines, while Meng et al. reported increased cell proliferation and sensitivity to gemcitabine treatment in pancreatic cancer ductal carcinoma (19,20).We found miR-146a to be upregulated in those with a Pro72Arg mutation, and the relationship between miR-17 and miR-

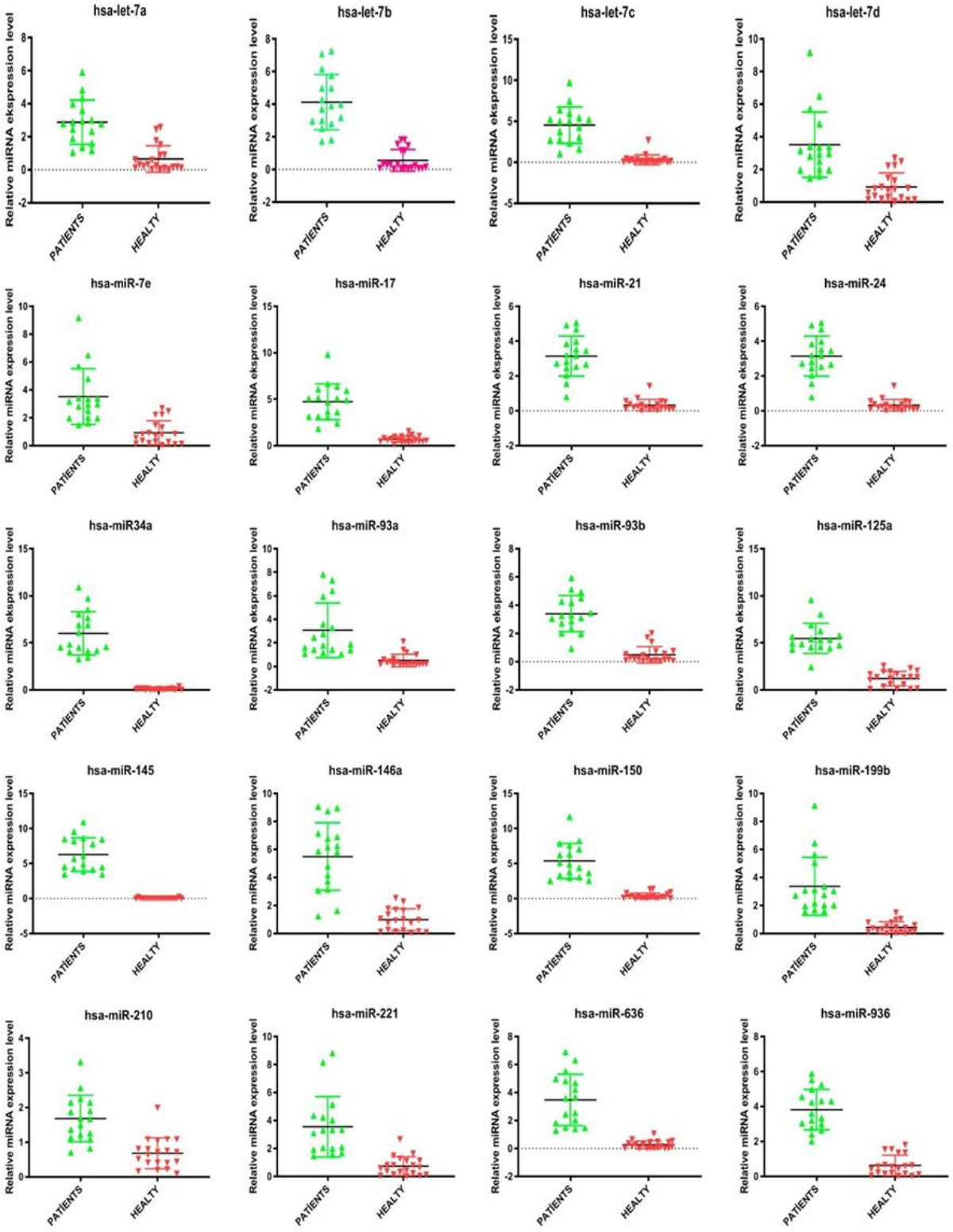

Fig. 1. Serum miRNA Levels In Healthy Volunteers and Patients

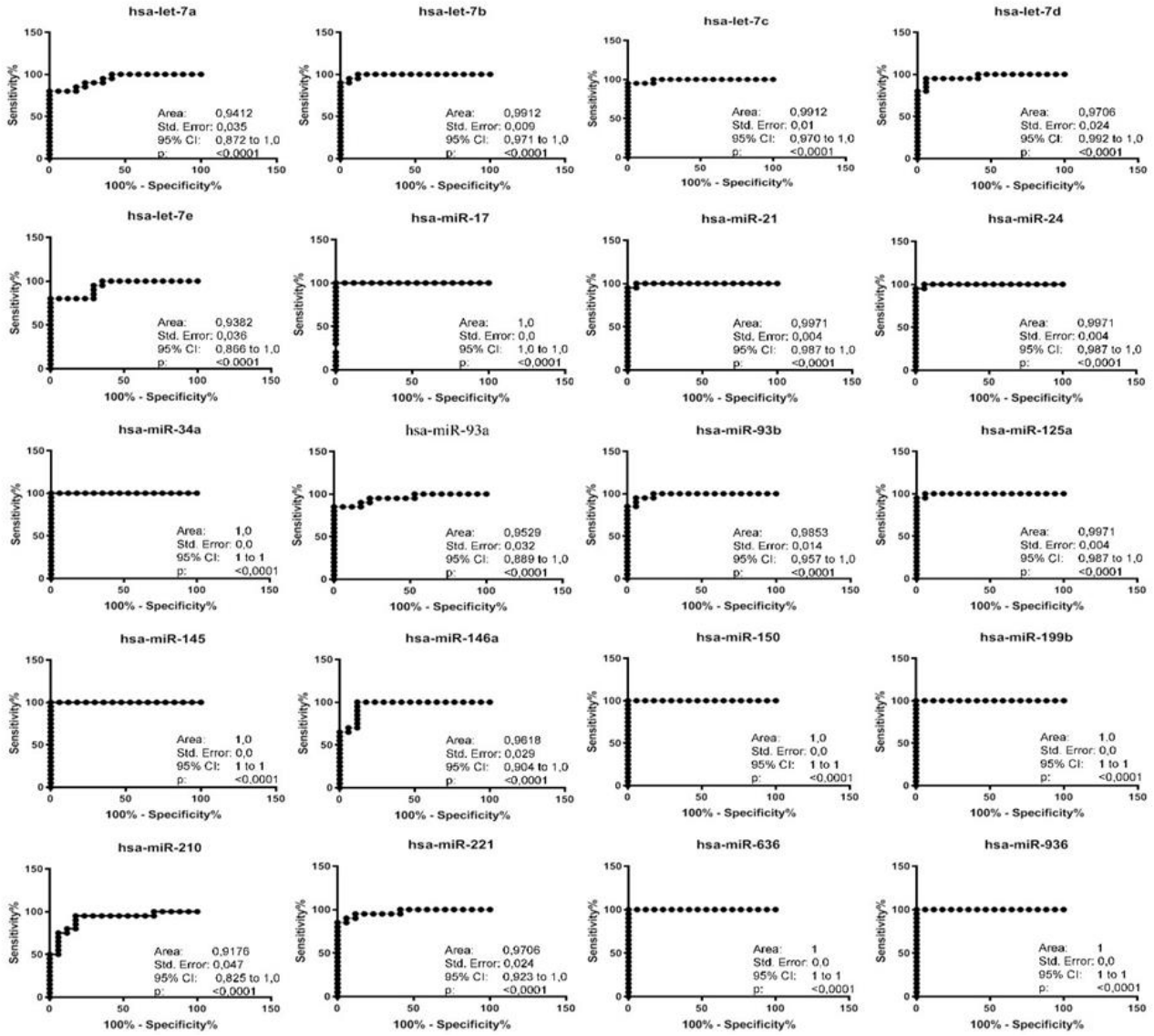

Fig. 2. The ROC Curve Analysis Between The Two Groups

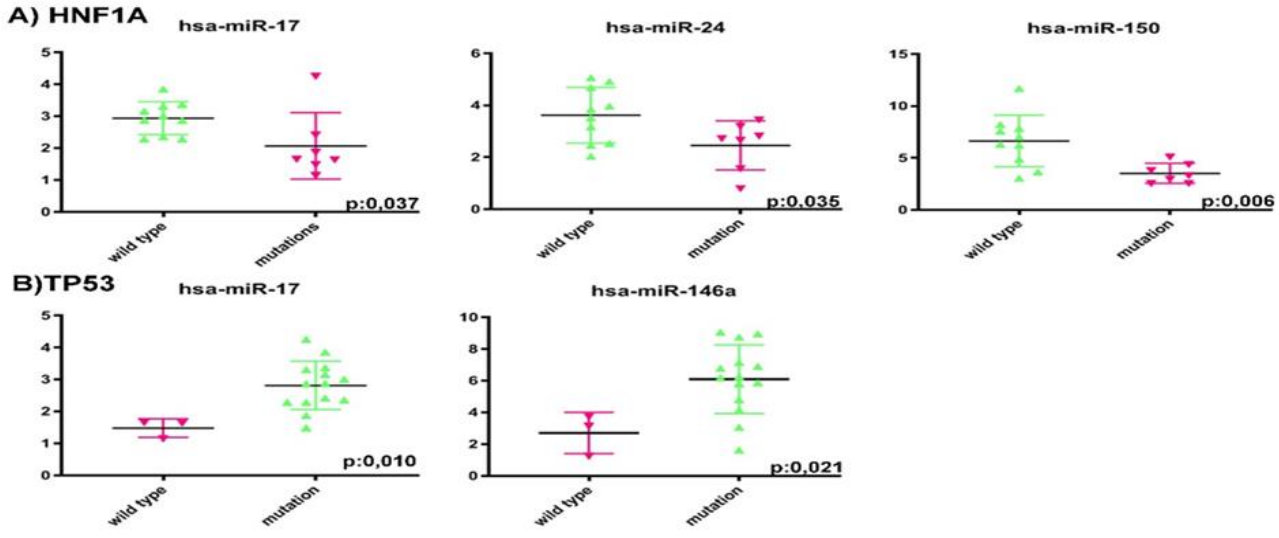

Fig. 3. Serum miRNA Levels in Patients With HNF1A and TP53 Mutations

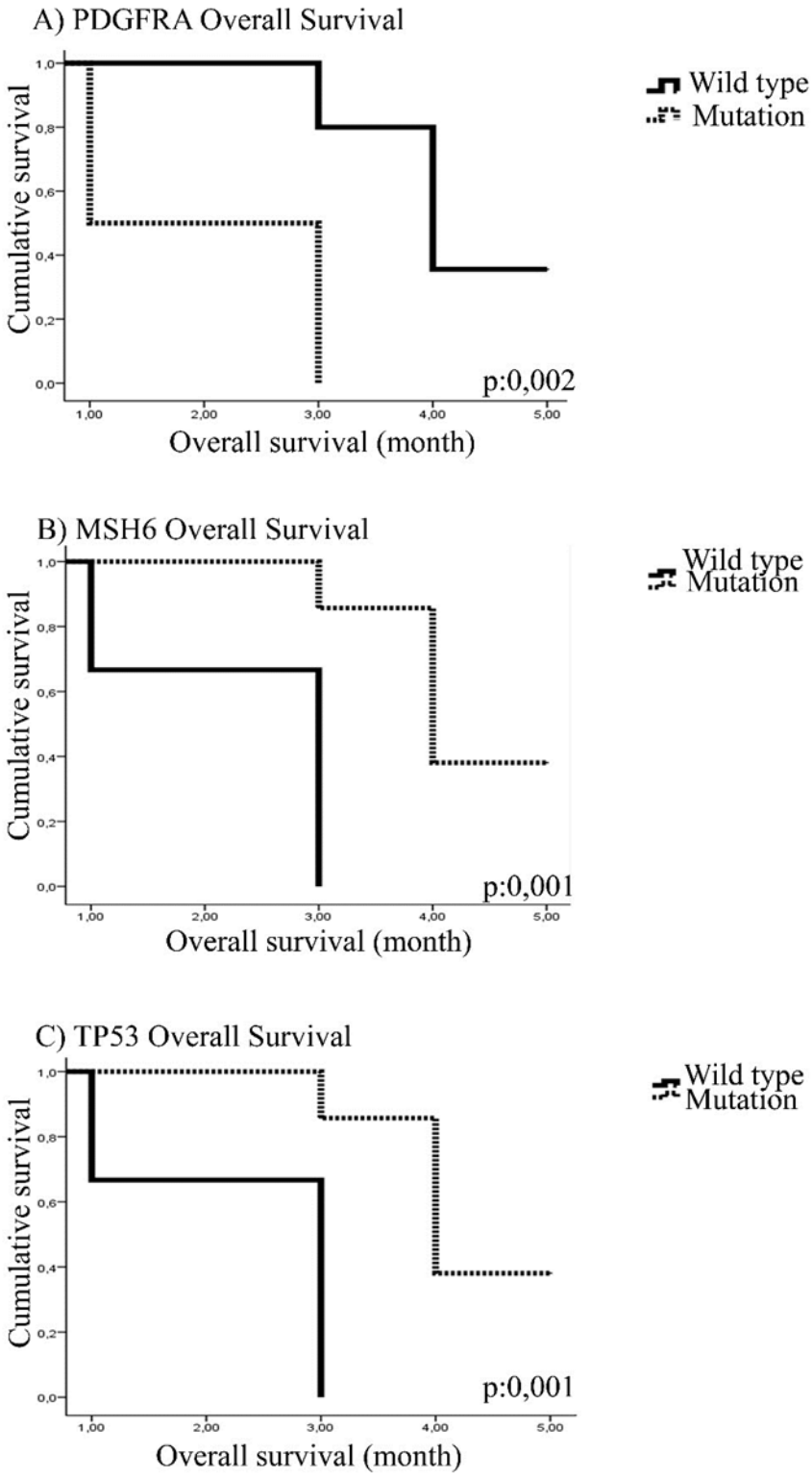

Fig. 4. Overall survival in patients with PDGFRA, MSH6 and TP53 mutations

146a upregulation and overall survival was found to be statistically significant (p: 0.001). The statistically high overall survival makes it possible to hypothesize that Pro72Arg mutation may have a protective effect and that upregulated miR-17 and miR-146 may increase susceptibility to apoptosis, causing cell death and increasing survival. The relationship between wild-type TP53 and the negative regulator miR-146a with TP53 with a P72R mutation is an issue that needs to be explained further.

The c.2472 C>T synonymous mutation in exon 18 of the PDGFRA gene is expressed as pathogenic in the COSMIC database. Organ-specific results have emerged in the literature regarding this mutation. For example, it has been associated with decreased mRNA stability and good prognosis in acral melanoma, whereas it has been associated with poor prognosis in renal cell carcinoma and no difference in colon carcinoma (21). There are no data on pancreatic cancer. Our study revealed a result of reduced overall survival, such as that for renal cell carcinoma. Statistically, significantly higher overall survival was found in patients carrying the non-mutant variant (p: 0.002). These results support the pathogenic mutation expression in the COSMIC database. The molecular mechanisms to explain this situation are not known. No statistically significant correlation was found between this mutation and serum miRNA levels.

The c.3438+14A>T mutation in the 5th intron of the MSH 6 gene is neutral in the COSMIC database, where literature on this variant in intestinal, colon, and lung tumors is limited. While the change in miRNA level as a result of this variant is not included in the literature, there is also no information with regard to in silico variant analysis. In our study, the overall survival of patients with this mutation was statistically significantly higher (p: 0.001). These results associated with this mutation of the MSH 6 gene need to be supported by in-vitro studies. No statistically significant correlation was found between this mutation and serum miRNA levels.

The small number of patients and the inability to work simultaneously in tumor tissue and cell lines are limitations of our study. However, this study is clinically meaningful in revealing possible pathogenic mutations detected in tumor cells circulating in metastatic pancreatic cancer, both in terms of prognostic value and whether the information can be used to target treatment, including gene therapy. In our study, survival was lower with the c.2472 C>T mutation located in exon 18 of the PDGFRA gene; thus, anti-PDGFRA agents may improve survival in patients with metastatic pancreatic adenocarcinoma with this mutation. Survival was higher in patients with P72R mutations in the TP53 gene and those with miRNA 17 and 146 upregulation carrying this mutation. A strategy that can increase the expression of these miRNAs in association with this mutation may also be a promising therapeutic approach for this tumor type.

Acknowledgment: There are no acknowledgments.

Funding Statement: The authors received no specific funding for this study

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Hyuna Sung, Jacques Ferlay, Rebecca L. Siegel et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-249.
2. Rebecca L. Siegel, Kimberly D. Miller, Ahmedin Jemal. Cancer Statistics, 2020. CA Cancer J Clın 2020; 70: 7–30.
3. Cicenas,J., Kvederaviciute, K., Meskinyte,I.,et al.. KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 Mutations in Pancreatic Cancer. Cancers 2017: 9(42*)*;
4. Hidalgo M, Von Hoff DD. Translational therapeutic opportunities in ductal adenocarcinoma of the pancreas. Clin Cancer Res.2012; 18: 4249–4256.
5. Christine A. Iacobuzio-Donahue, Victor E. Velculescu, Christopher L. Wolfgang and Ralph H. Hruban. Genetic basis of pancreas cancer development and progression: insights from whole-exome and whole-genome sequencing. Clin Cancer Res 2012; 18: 4257–4265.
6. Kassem,N., Kassem, H., Kassem,L. and Hassan, M. Detection of activating mutations in liquid biopsy of Egyptian breast cancer patients using targeted next-generation sequencing: a pilot study. Journal of the Egyptian National Cancer Institute 2021; 33: 10. https://doi.org/10.1186/s43046-021-00067-3
7. Hiroyuki Asama, Rei Suzuki, Takuto Hikichi, Tadayuki Takagi, Atsushi Masamune. MicroRNA let-7d targets thrombospondin-1 and inhibits the activation of human pancreatic stellate cells. Pancreatology 2019; 19: 196-203.

8. 8. Manmeet Rawat , Kavita Kadian , Yash Gupta et al. MicroRNA in Pancreatic Cancer: From Biology toTherapeutic Potential. Genes 2019; 10: 752.
9. Yu F , Yao H, Zhu P, et al. Let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell 2007; 131: 1109-1123.
10. Ohshima K, Inoue K, Fujiwara A. Let-7 microRNA family is selectively secreted into the extracellular environment via exosomes in a metastatic gastric cancer cell line. PloS One 2010; 5: 13247.
11. Esquela-Kerscher A, Trang P, Wiggins JF, et al. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. Cell Cycle 2008; 7: 759-764.
12. Boyerinas B, Park SM, Murmann AE, et al. Let-7 modulates acquired resistance of ovarian cancer to Taxanes via IMP-1-mediated stabilization of multidrug resistance 1. Int J Canc 2012; 130: 1787-1797.
13. Korc, M. Driver mutations: A roadmap for getting close and personal in pancreatic cancer. Cancer Biology & Therapy 2010; 10(6): 588-591.
14. Patrick Dumont, J. I-Ju Leu, Anthony C. Della Pietra II, Donna L. George, Maureen Murphy. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nature Genetics 2003; 33: 357-365.
15. Garziera, M., Cecchin, E., Giorda, G., Sorio, R., Scalone, S. Clonal Evolution of TP53 c.375+1G>A Mutation in Pre- and Post- Neo-Adjuvant Chemotherapy (NACT) Tumor Samples in High-Grade Serous Ovarian Cancer (HGSOC). Cells 2019; 8: 1186. doi: 10.3390/cells8101186
16. Victor D. Li, Karen H. Li , John T. Li. TP53 mutations as potential prognostic markers for specific cancers: analysis of data from The Cancer Genome Atlas and the International Agency for Research on Cancer TP53 Database. Journal of Cancer Research and Clinical Oncology 2019; 145: 625–636.
17. Yan, H., Xue,G., Mei,Q., Wang, Y., Ding, F.,et al. Repression of the *miR-17-92* cluster by p53 has an important function in hypoxia-induced apoptosis. The EMBO Journal 2009; 28: 2719–2732.
18. Sandhu, R., Rein, J., D'Arcy, M. , et al. Overexpression of miR-146a in basal-like breast cancer cells confers enhanced tumorigenic potential in association with altered p53 status. Carcinogenesis, 2014; 35(11): 2567–2575.
19. Li, Y., VandenBoom, TG., Wang, Z., et al. miR-146a Suppresses Invasion of Pancreatic Cancer Cells. Cancer Res 2010; 70(4):1486-1495.
20. Meng, Q., Liang, C., Hua1, J., et al. A miR-146a-5p/TRAF6/NF-kB p65 axis regulates pancreatic cancer chemoresistance: functional validation and clinical significance. Theranostics 2020; 10(9): 3967-3979.
21. Jemii, NB., Tounsi‐ Kettiti, H., Yaiche, H., et al. Dysregulated PDGFR alpha expression and novel somatic mutations in colorectal cancer: association to RAS wild type status and tumor size. J Transl Med 2020; 18: 440.