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Research Article

Detection of Tp73 Gene Polymorphism, Sequencing and Gene Expression in Women Suffered From Breast Cancer in Ninewa - Mosul, Iraq.

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Abstract **–**Two novel members of the P53 family were discovered in 1997 and given the names p73, and p63. p73, have numerous isoforms. Based on the resultant structure, two classifications can be created for all p73 isoforms, (TAp73), which activate transcription that exhibits tumor-suppressor activity. (DNp73) that act as dominant-negative repressors and operate as proto-oncogenes.
It was identifying new variations of the TP73 gene, which may be directly or indirectly affecting the activity of this gene, which is the cause of Among the main reasons for the development of breast tumour diseases, as it appeared in the results of sequencing tests to amplify the TP73 gene, the presence of previously unrecorded or unregistered differences in the sequences of a number of nucleotides. The results of the reaction of the Tetra ARMS PCR showed that there is a relationship between patients suffering from breast tumours and the genetic variation of the TP73 gene at the site (TP73G4C14-A4T14), as it was clear from the results of the PCR reaction that the genetic variation appeared The TP73 gene was found in three different genotypes, in different proportions. The results of the response of the Tetra ARMS PCR reaction showed, that there is a relationship between patients suffering from breast tumours and the genetic variation of the TP73 gene at the site (rs1801173), as it was clear from the results of the PCR reaction that the genetic variation of the gene TP73 in three different genotypes, in different proportions.

Keywords – TP73, Polymorphism, Gene Expression, Breast Cancer, SNP.

I. INTRODUCTION

Two novel members of P53 family were discovered in 1997 and given the names p73 [1] , and p63 [2],[3]. P53's extraordinary structural similarity to p63's and p73's led to immediate enthusiasm and rapid anticipation regarding the biological activities of these proteins. Both genes produce proteins with p53-related functions as well as completely new functions. Additionally, the p53-related functions either work synergistically with p53 or interfere with it. More than 60% of the amino acids between p63 and p73 and the DNA binding domain of p53 are conserved in both proteins, including all DNA contact and structural residues that are hotspots for p53 mutations in human malignancies. Additionally, p73 shares 29% of its DNA with the p53 transactivation domain (TA) and 38% of its DNA with the p53 tetramerization domain. The p73 and p63 genes in vertebrates are related to p53 and may have

descended from a single p63/p73 archetype [4], [5]. In addition p53 and p73, share the same DNA binding site, have similar domain structure and overlapping signaling pathways that regulate their activity [6]. Due to alternate transcriptional start sites and alternative splicing, all members of the p53 protein family, including p73, have numerous isoforms [7]. Based on the resultant structure, two classifications can be created for all p73 isoforms. Alternative transcription start sites produce long transcripts with transcriptional activator (TA) at the N-terminus, resulting in TA- isoforms (TAp73), which activate transcription [8], or shorter transcripts driven by the intragenic promoter, resulting in so-called DN- isoforms (DNp73), which lack transcriptional activator domains but retain their DNA binding domains, so their protein products primarily act as dominant-negative repressors [9],[10], But DN- isoforms can also make transcription active ([11]–[13]). The TA-domain is followed by the DNA binding domain and the tetramerization domain in the normal domain arrangement of the p53 family members [14]. To regulate mostly overlapping gene sets, all members of the p53 family can bind the same consensus DNA to create heterotetrameric complexes [15], [16]. Additionally, p73 includes the sterile alpha motif (SAM) domain, which is present in p73 but lacking in p53, and which prevents interaction with p300/CBP, hence repressing transcription [17]. The genomic organization is more complex in p73, largely the result of an alternative internal promoter generating NH2-terminally deleted dominant-negative proteins that engage in inhibitory circuits within the family. Deregulated dominant-negative p73 isoforms might play an active oncogenic role in some human cancers. The COOH-terminal extensions specific for p73 enable further unique protein-protein interactions with regulatory pathways involved in development, differentiation, proliferation, and damage response. Thus, p53 family proteins take on functions within a wide biological spectrum stretching from development (p63 and p73), DNA damage response via apoptosis and cell cycle arrest (p53, TAp63, and TAp73), chemosensitivity of tumors (p53 and TAp73), and immortalization and oncogenesis (Δ Np73) [18], so that In a number of cancers, including breast cancer, certain p73 isoforms are overexpressed and affect prognosis [19].

Both TP63 and TP73 have two promoters: P1 is located in the 5 end of the untranslated region before the non-coding exon 1, and P2 is located in the intron 3's 23 kb. The two opposing groups of proteins produced by P1 and P2 promoters are those that include the TA (TAp63 and TAp73) and those that do not (DNp63 and DNp73). Humans and mice both have DNp63 and DNp73. Additionally, additional isoforms without the transactivation are produced by alternative exon splicing of the P1 transcripts of TP63 and TP73 [5].

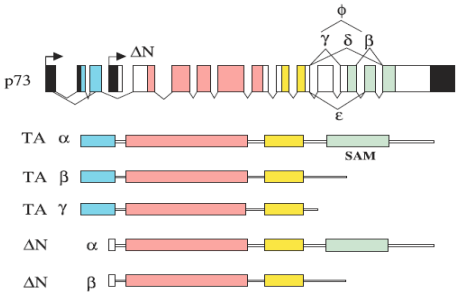

Fig 1: shows the genotype of a p73 tumor suppressor gene that contains a TA, DBD, OD, and SAM domain (Moll and Slade, 2004) [18]. (TA : Transcriptional activator, DBD : DNA binding domain, OD : oligomerization domain, SAM : sterile alpha motif)

II. Materials and Method

This study contained (80) samples taken from women, which we separated into (40) tumor tissue biopsy samples from women with breast cancer and (40) blood samples from women as

a control group in the same age group who did not have a problem with breast cancer, The blood venous was collected with an EDTA tube for DNA extraction and Genotyping test.

DNA extraction from tissue biopsy

The tissue pieces were crushed using a Ceramic pestle and by adding the MO-X solid material as a substitute for liquid nitrogen, according to the package prepared by Genaid.

DNA purity and concentration measurements

The Nanodrop instrument is used to assess the DNA extraction's purity.

Detection of TP73 Polymorphism at (rs1801173) location by Tetra ARMS PCR

The presence of the T→C mutation was detected in the site rs1801173, as 4 microliters were added, which is equivalent to (100 Nanogram) of the template DNA, and 1 microliter, which is

equivalent to (10 Picompl) from each primer that is specific to the mutation rs1801173, which was designed by the researcher and used for the first time on this type of gene as shown in Table No. (1). Which was prepared by the Korean company Macrogen to the contents of the master mix.

Table 1 : shows the design of the primer of (rs1801173) polymorphism for TP73 gene by using PCR technique.

primer	Sequence	Band size	Annealing
F-outer	CAGAGCTGCCCACCTGGCCTTC	390 bp	68
R-outer	AGTTAGCCCAGCGAAGGTGGCTGAG		
F-inner	TCCTGCAGAGCGAGCTGCCATC	240 bp	
R-inner	CTTCCCCACGCCGGCCTACA	190 bp	

After that, the reaction tubes were inserted into the thermocycler to conduct the double reaction through its special reaction program, as shown in Table No. (2).

Table 2 : Shows the Multiplication Reaction And Its Special Program

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	5 min.	1
2.	Denaturation	95	45 sec.	
3.	Annealing	68	1 min.	35
4.	Extension	72	1 min.	
5.	Final extension	72	7 min.	1
6.	Stop reaction	4	5 min	1

After that, the reaction products were transferred to the PCR using an agarose gel at a concentration of [2%].

Detection of TP73 Polymorphism at (TP73G4C14-A4T14**) location by Tetra ARMS PCR**

The presence of the GC → AT mutation at site TP73G4C14-A4T14 was detected by adding 4 microliter (100 nanogram) of template DNA and 1 microliter (10 picompl) of each primer of the mutation TP73G4C14 - which was designed by the researcher and used for the first time on this type of gene, as shown in Table No. (3) which was prepared by the Korean company Macrogen, to the contents of the master mix.

Table 3 : shows the design of the primer of (TP73G4C14-A4T14) polymorphism for TP73 gene by using PCR technique.

Primer	Sequence	Band size	Annealing
F-outer	5'-CCA CGG ATG GGT CTG ATC C-3'	430 bp	60
R-outer	5'-GGC CTC CAA GGG CAG CTT-3'		
F-inner	5'-CCT TCC TTC CTG CAG AGC G-3'	270 bp	
R-inner	5'-TTA GCC CAG CGA AGG TGG-3'	193 bp	

After that, the reaction tubes were inserted into the thermocycler to conduct the double reaction through its special reaction program, as shown in Table No. (4)

Table 4 : Shows The Multiplication Reaction And Its Special Program

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	5 min.	1
2.	Denaturation	95	45 sec.	
3.	Annealing	60	1 min.	35
4.	Extension	72	1 min.	
5.	Final extension	72	7 min.	1
6.	Stop reaction	4	5 min	1

After that, the reaction products were transferred to the PCR using an agarose gel at a concentration of [2%].

Determine the nucleotide sequence of the amplified pieces based on the DNA Sequencing technique

The sequences of the nitrogenous bases of the TP73 gene were determined for the samples TP73G4C14-A4T14 in the current study, in order to ensure the correctness of the designed primer sequence that was used in the ARMS-PCR technique, as well as to detect the presence of any additional variations or variations of the gene in the current study as shown in Table No. (5). The PCR of the aforementioned genes with all the primers of the resulting bundles, and the sequences of these genes were read based on the American company Psomagene.

The process of matching these gene sequences with the sequences of genes documented in the National Center for Biotechnology Information "NCBI" was carried out, and these results were analyzed based on the BLAST program.

Table 5 : shows the design of the TP73 primer of (TP73G4C14-A4T14) polymorphism for TP73 gene by using DNA-Sequencing technique.

TP73	Primer	Sequence
	Forward	5'-CCA CGG ATG GGT CTG ATC C-3'
	Reverse	5'-GGC CTC CAA GGG CAG CTT-3'

III. RESULTS

Detection of TP73 Polymorphism at (TP73G4C14-A4T14) location by Tetra ARMS PCR Technique.

The results of the reaction of the Tetra ARMS PCR showed, as shown in Picture No. (2) that there is a relationship between patients suffering from breast tumors and the genetic variation of the TP73

gene at the site (TP73G4C14-A4T14), as it was clear from the results of the PCR reaction that the genetic variation appeared The TP73 gene was found in three different genotypes, as shown in Table (6) in different proportions.

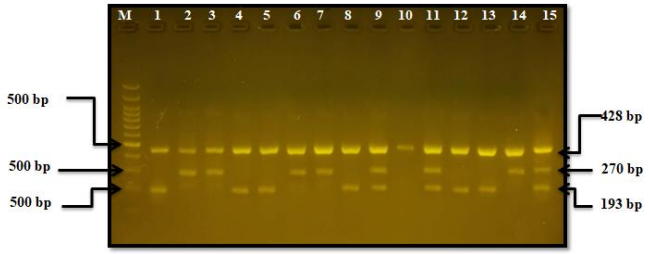

Fig 2:Shows the results of the T-ARMS-PCR reaction for the genetic variation of the TP73 gene at the site (TP73G4C14-A4T14) are shown.

From the results of the interaction in Fig 2, contained three bands according to the following:

1. The first band was 428 bp in size for the main gene segment.
2. The second band was 270 bp for the normal allele.
3. As for the third band, its size was 193 bp for the mutant allele.

Table 6 : shows the distributions of the percentage of observed alleles and the genotype of the TP73 gene at the locus (TP73G4C14-A4T14) between the "healthy" control group and breast tumor patients, noting that the GC allele is a normal allele, and the AT allele is a mutant allele.

Genotypes SNP1	Patients		Control		P Value	OR	(95%CI)
	NO.	%	NO.	%	P = 0.433	2.000	0.3402 to 11.7563
GC/ GC	30	75	32	80			
GC/AT	6	15	8	20			
AT/AT	4	10	0	0			
Alleles	NO.	%	NO.	%	P Value	OR	(95 % CI)
GC	66	82.5	72	90	P = 0.173	1.91	0.7527 to 4.8419
AT	14	17.5	8	10			

Detection of TP73 Polymorphism at (rs1801173) location by Tetra ARMS PCR

The results of the reaction of the Tetra ARMS PCR showed, as shown in Figure (3), that there is a relationship between patients suffering from breast tumors and the genetic variation of the TP73 gene at the site (rs1801173), as it was clear from the results of the PCR reaction that the genetic variation of the gene TP73 in three different genotypes, as shown in Table 7, in different proportions.

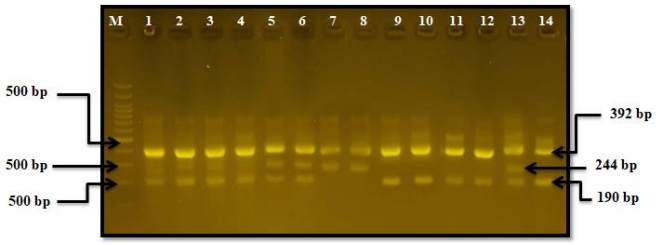

Fig 3: The results of the T-ARMS-PCR reaction for the genetic variant of the TP73 gene at the site (rs1801173) are shown.

The results of the interaction contained three packages of the two agencies:

1. The first band was 392 bp in size for the main gene segment.
2. The second band was 244 bp in size for the normal allele.
3. As for the third band, its size was 190 bp for the mutant allele.
4. Therefore, sample No. (7-8) has a normal CC genotype.

Table 7: shows the percentage distributions of observed alleles and genotypes of the TP73 gene at locus (rs1801173) between the "healthy" control group and breast tumor patients, knowing that the C allele is a normal allele, and the T allele is a mutant allele.

Genotypes SNP2	Patients		Control		P Value	OR	(95%CI)
	NO.	%	NO.	%			
CC	14	35	40	100	P = 0.001	16.7143	1.8458 to 151.3535
CT	20	50	0	0			
TT	6	15	0	0			
Alleles	NO.	%	NO.	%	P Value	OR	(95%CI)
C	48	60	80	100	P < 0.0001	12.6667	4.2144 to 38.0706
T	32	40	0	0			

Determine the nucleotide sequence of the amplified pieces, based on the DNA Sequencing technique

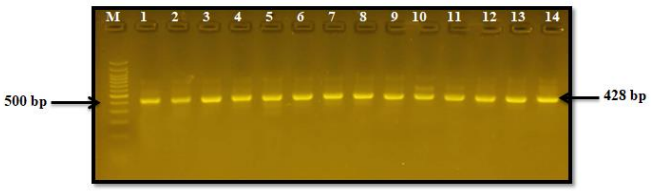

Fig 4: shows the product of the PCR reaction for the TP73 gene, with a reaction yield of 428 bp, and the size index M is 100 bp, which was prepared by Biolabs, and the separation was done with an agarose gel, which was prepared at a concentration of [2%].

The aim of conducting nucleotide sequence determination tests is to confirm conclusively that

the primers used in this study belong to the TP73 gene, in addition to identifying new variations or variations of this gene, which may be directly or indirectly affecting the activity of this gene, which is the cause of Among the main reasons for the development of breast tumor diseases, as it appeared in the results of sequencing tests to amplify the TP73 gene, the presence of previously unrecorded differences in the sequences of a number of nucleotides, and these differences or variations were explained in the following figure No. (5).

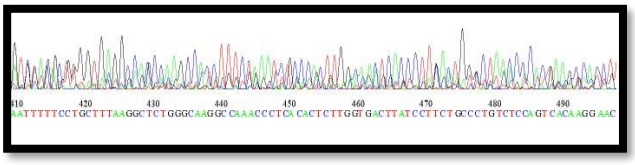

Fig 5: shows the Determination of the nucleotide sequence of TP73 gene in DNA-Sequencing.

The results of matching the nucleotide sequence of the TP73 gene to the models in the current study showed that the match rate reached 100% with the nucleotide sequences at the NCBI site, and this indicates the accuracy of the initiator design, which was used for the first time in the current study, as shown below in Figure 6.

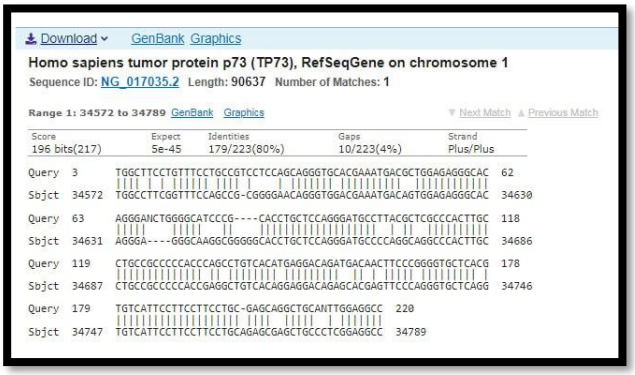

Fig 6: shows the results of matching the nucleotide sequence of the TP73 gene with the nucleotide sequences at the NCBI site.

Table 8: shows the types of variations and their locations on the exons of the protein and gene TP73 after sequencing testing and comparing them with the protein and gene sequences in the NCBI site.

Sequence ID	Nucleotide	Location	Mutation type	Identity	Gaps
(NG_017035.2)	C ⟶ T	(34576)	Transition	(80%)	(4%)
(NG_017035.2)	T ⟶ C	(34578)	Transition	(80%)	(4%)
(NG_017035.2)	G ⟶ T	(34580)	Transversion	(80%)	(4%)
(NG_017035.2)	A ⟶ T	(34587)	Transversion	(80%)	(4%)
(NG_017035.2)	-- ⟶ T	(34592)	Addition	(80%)	(4%)
(NG_017035.2)	G ⟶ C	(34594)	Transversion	(80%)	(4%)
(NG_017035.2)	G ⟶ T	(34595)	Transversion	(80%)	(4%)
(NG_017035.2)	G ⟶ C	(34596)	Transversion	(80%)	(4%)
(NG_017035.2)	G ⟶ C	(34597)	Transversion	(80%)	(4%)
(NG_017035.2)	A ⟶ G	(34599)	Transition	(80%)	(4%)

IV. DISCUSSION

From the results of the interaction in Fig 2, sample No. (2, 3, 6, 7, 14) has a normal genotype (GC/ GC). The samples (9, 11, 15) have a heterogeneous genotype (GC/AT). And the samples (1, 4, 5, 8, 12, 13) have a genotype of a AT/AT mutant, and the size index M is 100 bp in size, which was prepared by Biolabs, and the separation was done by agarose gel, which was prepared at a concentration of [2%].

From the results of the interaction in Fig 3, The samples (5, 6, 13) have a heterogeneous genotype (CT). The samples (1, 2, 3, 4, 9, 10, 11, 12, 14) have a TT mutant genotype, and the size index M is 100 bp in size, which was prepared by Biolabs, and the separation was done by agarose gel, which was prepared at a concentration of [2%].

According to what appeared in the results of the current study, Table No. (6) shows the percentage of allelic observations and the recurrence of different genotypes of the TP73 gene at the site (TP73G4C14-A4T14), as the results of breast tumor patients showed that the recurrence value of the normal and healthy genotype Wild genotype "GC / GC It was 75%, which is the highest percentage compared to what appeared in the hetero-genotype "GC/AT", which was 15%. As for "AT/AT" mutant genotype, its rate was equivalent to 10%. Compared with the results of the control group, the percentage of different genotypes was 80% for the normal genotype, 20% for the variant genotype, and 0% for the mutant genotype. In

addition from the results of the current study, Table No. (6) showed that the frequency of the mutant allele was 17.5 %, compared to 82.5 % for the natural allele, which is considered as one of the main reasons leading to the loss of the biological function of the P73 protein due to a defect in the gene expression process of the TP73 gene.

According to what appeared in the results of the current study, Table No. (7) shows the percentage of allelic observations and the recurrence of different genotypes of the TP73 gene at the site (rs1801173). The results of breast tumor patients showed that the recurrence value of the normal and healthy wild genotype "CC" was 35%. while the recurrence value of the "CT" hetero-genotype was 50%, which is the highest percentage compared to other genotypes. As for the mutant genotype "TT" the rate was equivalent to 15%. Compared with the results of the control group, the percentage of different genotypes was 100% for the normal genotype, 0% for the variant genotype, and 0% for the mutant genotype. In addition from the results of the current study, Table No. (7) also showed that the frequency of the mutant allele was 40%, compared to 60% for the natural allele, which is considered as one of the main reasons leading to the loss of the biological function of the p73 protein, due to a defect in the gene expression process of the gene.

From the results of the current study, Table No. (8) shows the different types of genetic variations or variations and their locations on the exons of the protein and gene TP73 after sequencing testing and comparing them with the protein and gene sequences on the NCBI site

When observing Table No. (8) after conducting sequencing tests and matching them to the TP73 gene in the studied models with the "healthy" control sample, and then matching them with the gene sequences on the NCBI global website, it became clear to us that there are many different genetic variants, which are divided into There are two basic types, they are either Transition Transversion, or Addition variations and their locations are depending on the type of divergent or heterogeneous bases, and these variations may lead to a decrease in the effectiveness of the TP73 gene in manufacturing the p73 tumor suppressor protein and thus increase the risk factors for the breast diseases such as breast cancer and or its tumors.

CONCLUSION

1. The studied genetic variation of the p73 gene is one of the main causes of oncology and breast cancer among the study samples.
2. Recording a new genotype for the TP73 gene and the p73 protein in the NCBI website.

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