Analysis of TP53 Gene Using Bioinformatics Tools

Tp53 Geninin Biyoinformatik Araçlarla Analizi

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Abstract

Background: The human TP53 gene, also known as p53, encodes for the tumor protein 53 (p53), regulates the cell cycle and hence functions as a tumor suppressor. This study aimed to investigate some properties of the TP53 gene and its products, such as the homologous protein sequences in different species, the common transcription factor binding sites on their promoters, their phylogenetic relationship, conserved domains, and their expression profiles by in silico biology approach.

Methods: We investigated the homology, conserved domain, promoter and expression profiles of the TP53 gene in various species using bioinformatics approaches.

Results: Our results revealed that which investigated p53 molecules among all organisms are conserved. They have three conserved domains (p53_TAD, p53 DNA_binding, and p53 tetramerization motif), some of which have full and truncated sub-domains. Human p53 proteins is similar to those of Pan troglodytes, Macaca mulatta, Macaca fascicularis and Chlorocebus aethiops. In contrast, t Monodelphis domestica protein is the most diverse of human p53. With the multiple alignment strategy, protein and domain sequences of Equus asinus, Muntiacus muntjak vaginalis and Monodelphis domestica are predicted to have a truncation. The comparative screening of the promoters demonstrated that TP53 genes do not seem to have any common conserved transcription factor binding sites.

Conclusion: This study demonstrated that, p53 molecules from various species are well conserved in the process of evolution. Comparative screening of the promoter sequences of the human p53 and its homologues found in human and NCBI database revealed that there was no any common transcription factor binding sites. Phylogenetic trees constructed using the neighbor-joining method (NJ) revealed a close evolutionary relationship of p53 in various species.

Key words: TP53, bioinformatics, comparative genomics, phylogenetics, in silico biology

Özet

Amaç: p53 olarak bilinen insan TP53 geni, hücre siklusunu düzenleyen ve tümör baskılayıcı olarak görev gören tumor protein 53'ü (p53) kodlar. Bu çalışmada, çeşitli türlerde TP53 geni ve onun ürünleri olan homolog protein dizileri, promotorlarda genel transkripsiyon faktör bağlanma alanları, filogenetik akrabalıkları, korunan alanları (p53) ve ekspresyon profilleri gibi özellikleri in silico biyoloji yaklaşımı ile araştırılması amaçlandı.

Metodlar: Biyoinformatik uygulamaları kullanarak çeşitli türlerde TP53 genlerinin homolojisini, korunmuş alanlarını, promotorlarını ve ekspresyon profillerini araştırdık.

Bulgular: Sonuçlarımız, p53 moleküllerinin araştırılan tüm organizmalar arasında korunduğunu göstermiştir. Bunların üç korunan alanı (p53_TAD, p53 DNA_binding ve p53 tetramerization motif) vardır,

bazılarının tam ve bazılarının kesikli korunan alt bölgeye sahip oldukları belirlendi. İnsan p53 proteini, Pan troglodytes, Macaca mulatta, Macaca fascicularis ve Chlorocebus aethiops'dakilerine en yakındır. Tam tersine, insan p53, Monodelphis domestica proteinine en uzaktır. Çoklu dizileme stratejisine göre, Equus asinus, Muntiacus muntjak vaginalis ve Monodelphis domestica'nın protein ve domain dizilerinin kesintili olduğu gösterilmiştir. Promotorların karşılıklı taramaları, TP53 genlerinin herhangi bir korunmuş genel transkripsiyon faktor bağlama bölgelerinin olmadığını göstermiştir.
Sonuçlar: Bu çalışma, çeşitli türler arasında p53 moleküllerinin, evrim sürecinde iyi korunduğunu göstermektedir. İnsan p53 ve NCBI databazında bulunan homologlarının promotor dizilerinin karşılaştırmalı taramaları, genel transkripsiyon faktör bağlama bölgelerinin olmadığını göstermiştir. Neighbor-joining metodu (NJ) kullanılarak filogenetik ağaçlar, çeşitli türlerde p53'ün yakın bir evrimsel akrabalığının olduğunu açığa çıkarmıştır.

Anahtar kelimeler: TP53, biyoinformatik, karşılaştırmalı genomik, filogenetik, in silico biyoloji

Introduction

The *TP53* gene is a key regulator of the cellular response to stress and plays a critical role in preventing cancer progression. The activation of p53 in response to DNA damage or cellular stress leads to cell cycle arrest, apoptosis, or senescence, depending on the cellular context (1).

The human *TP53* gene, also known as *p53,* localized on chromosome 17 (17p13.1), comprises 11 exons encoding a 393 amino-acid protein with a molecular weight of 53 kDa. p53 protein acts as a transcription factor and serves as a key regulator of the cell cycle (2).

The human p53 protein has four domains, N-terminal domain, core domain (DNA-binding domain), oligomerization domain and nuclear localization domain (1). The C-terminus of p53 tumor suppressor contains a DNA binding motif. The oncogenic activity of p53 C-terminus required both the DNA damage recognition motif and the repair enzyme-associating domain (3).

Human p53 molecule shows strong homology to several organisms, Spalax and mouse of several organisms, including those of Spalax and mouse. At the amino acid level, Spalax p53, with a 391-aa protein, an identity of 85.4% to human, and 81.9% to mouse p53 proteins is observed. Besides, in the p53 DNA-binding domain region, there is a nucleotide sequence homology of 88.1% to humans and 86.1% to mice, whereas amino acid homology is 95.8% for humans and 89.5% for mice (4).

It has been shown that the relative levels of mRNA specific for the mouse p53 cellular tumor antigen were determined in various normal adult tissues, embryos, and tumors. All tumors studied contained concentrations of *TP53* mRNA well above those present in most normal tissues. In most normal tissues, the levels of these transcripts were very low, but spleen cells contained much higher quantities of *TP*53 mRNA. Nevertheless, the spleen did not overproduce p53, owing to the exceptionally rapid turnover of the protein in this organ (5).

In this study, we aimed to analyze the *TP53* genes in different species *in silico* biology. Specifically, their p53 domains, the transcription factor binding sites on their promoters, the tissue expression profile, homology level and phylogenetic tree among mammalian TP53 genes using bioinformatics tools.

Materials and Methods

Homology search

The search for homologous protein sequence to human p53 was carried out using the BLASTp

program (6,7) at NCBI (http://www.ncbi.nlm.nih.gov) using human p53 amino acid sequence (GI: 8400737) as query against the SwissProt protein databases. Full protein and tumor protein p53 domains sequences of human and other species were downloaded and then aligned using the ClustalW (8) program at EBI (http://www.ebi.ac.uk).

Promoter Analysis

We used Genomatix software (http://www.genomatix.de) for analysis of TP53 gene promoters in various species. These nucleotide sequences were downloaded and then were aligned using the ClustalW program. Then common transcription factor binding sites were searched with the Dialign TF program in Genomatix software for all of TP53 promoters present in the database.

Evolutionary Analysis

We used amino acid sequences of p53 proteins to construct phylogenetic trees using the neighbor-joining method (NJ) with Jones-Taylor-Thomton (JTT) distances. NJ searches were conducted by using MEGA5 (9) and 500 bootstrap replicates were assessed for the reliability of internal branches; sites with gaps were ignored in this analysis.

In silico Expression Analysis

The DigiNorthern database (10) was used to analyze the expression of TP53 mRNAs based on EST data. The DigiNorthern collects all ESTs for a query gene and categorizes these ESTs based on the types of tissues and their histological status. Pairwise comparisons of relative frequencies were performed with the Fisher's exact test using SPSS 11.0 for Windows.

Results

Homology Search

BLASTp results revealed that p53 molecule is found in various species (Table 1). The homology search indicated that the p53 sequences of P. troglodytes (99), M. mulatta (95%), M. fascicularis (95%), and Chlorocebus aethiops (95%), have the highest homology to that of human. In contrast, the one with the lowest homology human p53 protein was that of Monodelphis domestica (70%) (Table 1 and Figure 1).

Table 1. BLASTp results of vertebrate p53 molecules and their homology

Species	Common name	AC Number	Length (amino acid)	% Identity with human TP53
Homo sapiens	Human	NM000546	393	100
Pan troglodytes	Chimpanzee	XP001172091	393	99
Macaca mulatta	Rhesus monkey	NP001040616	393	95
Macaca fascicularis	Crab-eating macaque	AAB91535	393	95
Chlorocebus aethiops	Cercopithecus aethiops	P13481	393	95
Tupai belongeri chinensis	Northern tree shrew	Q9TTA1	393	92
Equus asinus	Ass	Q29480	207	87
Delphinapterus leucas	beluga whale	Q8SPZ3	387	87
Marmota monax	Woodchuck	O36006	391	86
Muntiacus muntjak vaginalis	Muntiacus muntjak vaginalis	AAX12845	217	86
Oryctolagus cuniculus	Rabbit	Q95330	391	86
Spalax judaei	Spalax judaei	CAH03844	391	85
Canis familiaris	Dog	NP_001003210	381	83
Sus scrofa	Pig	NP999310	386	81
Bos taurus	Cattle	NP776626	386	81
Ovis aries	Sheep	NP001009403	382	81
Felis catus	Cat	NP001009294	386	81
Cavia porcellus	Domestic guinea pig	Q9WUR6	391	78
Mesocricetus auratus	Golden hamster	AAB41344	396	77
Mus musculus	House mouse	P02340	390	77
Mastomys natalensis	African soft-furred rat	AAB41831	378	77
Cricetelus griseus	Chinese hamster	O09185	393	76
Rattus norvegicus	Norway rat	P10361	391	76
Meriones unguiculatus	Mongolian gerbil	BAB69969	390	76
Monodelphis domestica	Gray short-tailed opossum)	CAD10682	258	70

ClustalW alignment elucidated the presence of well conserved three domains: p53_TAD (p53 transactivation motif) on the side of N-termini position, p53 DNA-binding domain on the center position, and p53_tetramer (P53 tetramerization motif) on the side of C-termini, which are well conserved especially in some localized areas (Figure 1). The first of human p53_TAD motif is fully conserved among H. sapiens, P. troglodytes, and T. b. chinensis. The second conserved domain, p53 DNA-binding domain is well conserved between H. sapiens and P. troglodytes. However, the third motif, p53_tetramer is only conserved among H. sapiens, P. troglodytes, M. mulatta, M. fascicularius, Chlorocebus aethiops, and Delphinapterus leucas (Figure 1). The p53 DNA binding domain is most probably functionally important and any mutations in them are deleterious, as implicated by their evolutionary conservation.

Figure 1. Multiple alignments of human p53 and its conserved domains, and its homologous in different species. (The p53 conseved domains are highlighted with black background. Amino acid residues are numbered on the right. The conserved amino acid residues are shown by an asterisk and amino acids with similar properties are shown by: under the alignment.)

Multiple alignment results of human p53 and its homologous revealed that this molecule is yet uncharacterized in five species, which M. natalensis (N-termini), and E. asinus, M. muntjak vaginalis, M. domestica (N-, and C- termini), and O. aries (C-termini), probably due their possible truncation (Figure 1).

Promoter Analysis

We found by the database search that the orthologous p53 gene promoters do not include any common transcription factor binding sites (TFBs) among H. sapiens, M. mulatta, R. norvegicus, C. familiaris, D. rerio in Database of Genomatix software. However, we observed that the similarity (value 1.000) and the number of identical nucleic acids (in % of short sequence segments) was 28% between the TP53 promoters of H. sapiens and M. mulatta for each pairwise alignment; however, this does not necessarily mean that the two sequences are identical.

Evolutionary Analysis

From the phylogenetic trees constructed by MEGA5 we found that p53 molecules are more closely grouped among H. sapiens, P.troglodytes, C. aethiops, M. mulatta, and M. fascicularius species (Figure 2).

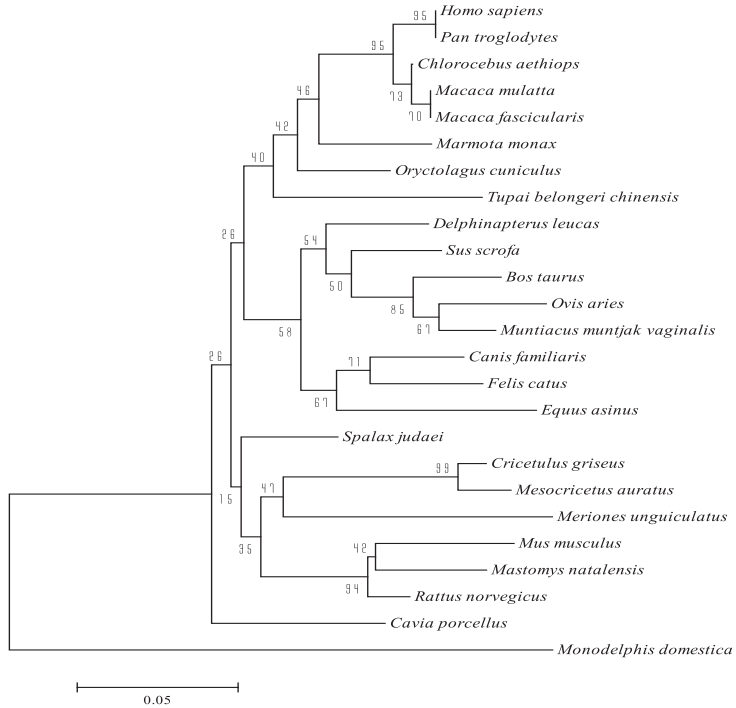

Figure 2. Phylogenetic tree of mammalian *TP53*. Phylogenetic trees were constructed by the MEGA5 program. Species names are indicated on the figure. Branch lengths indicate evolutionary relationship.

Multiple alignment results of human p53 and its homologous revealed that this molecule is yet uncharacterized in four species, which *M. natalensis*, *E. asinus*, *M. m. vaginalis*, and *M. domestica*, probably due their possible truncation. Ignoring the molecules of these four foregoing species, the p53 DNA-binding domains of the other species are very well conserved between *H. sapiens* and *P. troglodytes* through evolution (Figure 3).

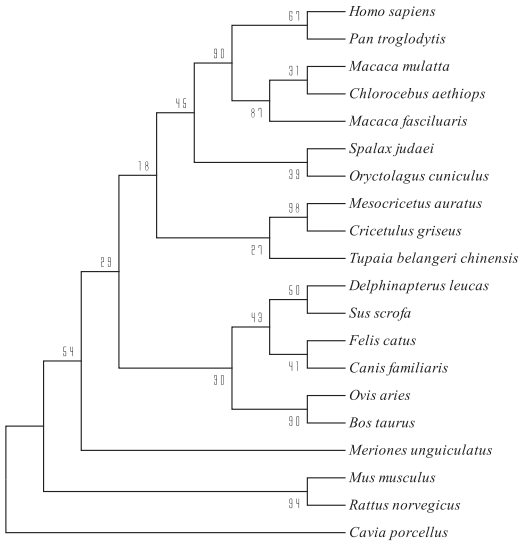

Figure 3. Phylogenetic tree of mammalian p53 DNA-binding domain. Phylogenetic trees were constructed by the MEGA5 program. Species names are indicated on the figure. Branch lengths indicate evolutionary relationship.

In Silico Expression Analysis

The distribution of p53 in the cDNA library database was analyzed using the DigiNorthern program. In columns 2 and 3 of Table 2, relative frequencies of p53 in the cDNA libraries from normal and tumor tissues respectively are shown both as absolute numbers as well as normalized values per 10^6 cDNAs (in parenthesis). Its normal as well as cancerous tissue expression profile was compared and the significance in its expression pattern was accessed with the Fisher's exact test (p value 0.05) (Table 2). The *TP53* gene seems to be expressed at a low level or not at all in some tissues while highly expressed in some others. The tissue distribution and differential expression pattern in normal and cancerous human tissues displayed somehow different frequencies. The expression of *TP53* gene in some of the normal human tissues, such as bone, cartilage, gastrointestinal tract, genitourinary, head and neck, heart, kidney, liver, lymphoreticular, muscle, nervous, ovary, pancreas, salivary gland, and spleen was not detected. In contrast, its expression seems to be lost in some of the normal and cancerous tissues such as gastrointestinal tract and synovium. Compared to the normal tissues, its expression is significantly increased in cancerous brain (0.044<p), head and neck (0.024<p), kidney (0.010<p), nervous (0.019<p), placenta (0.024<p), prostate (0.006<p), testis (0.001<p), and uterus (0.05=p) (Table 2).

Table 2. The distribution of relative frequencies of TP53 cDNA in cDNA library database.

Tissue/organ type	EST Data		p-value
	Normal	Cancer	
Adrenal cortex	0/0 (0)	0/7572 (0)	1.000
Bone	0/7929 (0)	1/45730 (22)	0.852
Brain	**6/257019 (23)**	**12/201219 (60)**	**0.044**
Cartilage	0/13369 (0)	1/39893 (25)	0.749
Cerebrum	6/103896 (58)	0/0 (0)	1.000
Cervix	1/1157 (864)	2/44671 (45)	0.074
Colon	2/28085 (71)	22/220946 (100)	0.482
Eye	1/85966 (12)	2/49827 (40)	0.305
Gastrointestinal tract	0/796 (0)	0/14690 (0)	1.000
Genitourinary	0/1687 (0)	5/39698 (126)	0.812
Germ cell	0/0 (0)	1/56605 (18)	1.000
Head and neck	**0/55508 (0)**	**9/107902 (83)**	**0.024**
Heart	0/69026 (0)	0/0 (0)	1,000
Kidney	**0/74917 (0)**	**8/96375 (83)**	**0,010**
Liver	0/73021 (0)	4/81780 (49)	0,078
Lung	3/129822 (23)	10/207630 (48)	0,198
Lymph node	4/97096 (41)	5/54341 (92)	0,187
Lymphoreticular	0/15679 (0)	4/56791 (70)	0,377
Mammary gland	16/71315 (224)	22/124006 (177)	0,289
Muscle	0/90941 (0)	1/45799 (22)	0,335
Nervous	**0/15506 (0)**	**18/63270 (284)**	**0,019**
Ovary	0/11587 (0)	7/109344 (64)	0,494
Pancreas	0/8656 (0)	1/84351 (12)	0,907
Pancreatic islet	2/95891 (21)	0/0 (0)	1,000
Placenta	**10/248276 (40)**	**6/43818 (137)**	**0,024**
Peripheral nervous system	1/30154 (33)	0/1220 (0)	0,961
Pooled tissue	6/373366 (16)	2/55060 (36)	0,275
Prostate	**2/82545 (24)**	**12/81283 (148)**	**0,006**
Salivary gland	0/414 (0)	1/20747 (48)	0,980
Skin	0/49729 (0)	5/137037 (36)	0,213
Spleen	0/20451 (0)	0/0 (0)	1,000
Stem cell	17/184378 (92)	0/0 (0)	1,000
Stomach	5/26066 (192)	29/140405 (207)	0,555
Synovium	0/278 (0)	0/1554 (0)	1,000
Testis	**2/122158 (16)**	**8/44649 (179)**	**0,001**
Thymus	1/5359 (187)	0/201 (0)	0,964
Uncharacterized tissue	8/88784 (90)	13/105216 (124)	0,316
Uterus	**0/36080 (0)**	**15/163186 (92)**	**0,050**
Vascular	2/31425 (64)	0/0 (0)	1,000
Whole body	2/73648 (27)	0/0 (0)	1,000
Total No. of ESTs Found	**97/2681980 (36)**	**226/2546816 (89)**	**0,001**

(Relative frequencies are normalized per 10^6 cDNAs; p-values are for comparison of relative frequencies of p53 in normal versus tumor tissues, using the Fisher's exact test. The data for tissues with significant or suggestive significant higher or lower frequency of p53 in the tumor and normal tissues are shown in bold.)

Discussion

Human *TP53* gene (also called p53) is located on chromosome 17p13.1 and recently identified as a tumor suppressor gene in which its mutation can lead to Li-Fraumeni syndrome-1 (LPS), characterized by autosomal dominant inheritance and early onset of tumors, multiple tumors within an individual, and multiple affected family members (11). The open reading frame of p53 is 393 amino acids long, with the central region containing the p53 DNA-binding domain. This proteolysis-resistant core is flanked by a C-terminal end mediating oligomerization, and an N-terminal end containing a strong transcription activation signal also identified a *Drosophila sp*. p53 homolog and demonstrated that it can activate transcription from a promoter containing binding sites for human p53 (12, 13). We found in this study that the human p53 protein has three important regions: p53_TAD motif on N-terminal side (at position 5-29 amino acides), core domain (p53 DNA-binding domain) on the center (at position 95-289), and the p53_tetramerization motif on the C-terminal side (at position 324-359 amino asids). Both the motifs on the N- and the C-terminal sides are not conserved among all investigated species, but p53 DNA_binding domain is well conserved between *H.sapiens* and *P.troglodytes*. This domain is important, and a proline-rich domain that mediates p53 response to DNA damage through apoptosis. It is where most of the *TP53* mutations are found on p53 DNA binding domain. Mutation in the core domain disrupts the p53 DNA-binding capability and hence causes p53 to lose its function as transcription factor (1).

In this study, the p53 molecules among human, monkey, and *Cercopithecus aethiops* are very similar (95-99% homology). Direct comparison of human and chimpanzee cancer genes indicates that they are highly conserved, showing 99.38% identities at the protein level, and 99.19% at the nucleotide level, what is similar to the average amino acid identities between both organisms (99.38%) (14). Likewise, our BLASTp results indicate that p53 is found in various species of vertebrates and these molecules have 70-99% conservation degree in the total amino acid sequences (Table 1). The human p53 molecule has highest homology to those of *P. troglodytes* (99%), *M. mulatta* (95%), and *Chlorocebus aethiops* (95%), and lowest homology to that of *Monodelphis domestica* (70%). So, these results indicate that the *TP53* gene has been evolutionary well conserved (Table 1). Additionally, we also examined the phylogenetic trees of p53 in different species using MEGA5 program. We observed that human p53 shows closest homology to those of *P. trogylodytes,* and then *M. mulatta, and M. fascicularis* (Figure 2).

The expression of *TP53* in different tissues was analyzed using the DigiNorthern program. Its expression patterns in normal and cancer tissues displayed somehow different frequencies in human. In some normal tissues, such as adrenal cortex, bone, cartilage, Gastrointestinal tract, Genitourinary, germ cell, head and neck, heart, kidney, liver, lymphoreticular, muscle, nervous,ovary, pancreas, salivary gland, skin, spleen, synovium, and uterus, and in cancerous tissues, such as adrenal cortex, cerebrum, gastrointestinal tract, heart, pancreatic islet, peripheral nervous system, spleen, stem cells, synovisium, thymus, vascular, and whole body, their expressions seem to be expressed at a very low level or not at all. In contrast, its expression is significantly increased in cancerous brain, head and neck, kidney, nervous, placenta, prostate, testis, uterus tissues (Table 2). Transcriptional regulation of *TP53* under different conditions is related to vast number of biological events in response to various cellular stresses including cell-cycle progression, carcinogenic stimulation, and so forth (15, 16). At the same time, the expression level of *TP53* varies in the context of different cell functions (17, 18). Studies have shown that the differential regulation of *TP53* in cell cycle control or cell-type specific tumorigenesis is reflected by elements of transcriptional control (16,19). Multiple sequence alignment and phylogenetic analysis of the human p53 mRNA sequence was performed which showed its relationship and pattern of variations among different organisms (20).

We suspect that *TP53* the change in its expression pattern in different tissues may be related to the role for pathogenesis of some sporadic cancers. The availability of the comprehensive data generated by high-throughput functional genomic approaches, mainly expressed

sequence tag (EST) and serial analysis of gene expression (SAGE), provides the feasibility to study gene expression through *in silico* analysis (21). In normal tissues, p53 levels are low, but p53 protein accumulates after exposure to DNA-damaging agents or during the onset of various physiological processes (22). Comparative analysis showed that, although highly tissue-specific genes tend to exhibit similar expression profiles in human and mouse, there are significant exceptions, indicating that orthologous genes, while sharing basic genomic properties, could result in distinct phenotypes (23).

Since the completion of the human genome, cataloging transcription factor binding sites (TFBSs) has been critical for understanding gene regulation. We used Dialign TF program in Genomatix software for predicting transcription factor binding sites (transcriptional elements) of all orthologous *TP53* promoters that present in the database. Dialign TF results revealed that *TP53* orthologous promoters had no common conserved transcriptional elements. Our results indicate that the binding sites of different transcription factors might have located on different parts of the promoter or promoter vicinity in various species.

Basic bioinformatics techniques are powerful tools in terms of leading to the discoveries and analysis of novel genes (24). Recently we identified and further characterized two novel genes using bioinformatics tools (25). Even though the results from bioinformatics studies are very helpful in directing and designing the experiments, they need to be supported and confirmed by further experimentation. By choosing suitable bioinformatics analysis on these data, more discoveries will be made. Bu çalışma 2007 yılında Adana'da "*III. Çukurova Kolo-Proktoloji ve Stoma-terapi sempozyumu"*nda poster bildirisi olarak sunulmuştur.

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