Research Article

Phylogenetic Analysis of Human TP73 Gene using Unweighted Pair Group Method with Arithmetic Mean and Neighbor-Joining Methods

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ABSTRACT

The p73 is an active member of p53-family (also known as a family of tumor suppressor genes), which has been identified to control a wide range of cellular process, including regulation of cell cycle, development, differentiation and apoptosis. Numerous factors such as DNA damage, viral infection and transcriptional regulators can regulate TP73 gene expression at transcriptional level leads to the alteration in various cellular process, results uncontrolled cell growth. Aberrant regulation of p73 has been linked to various pathologies, including cancer. The current work is focused to understand the distribution pattern of genetic variation in p73 gene found in different species of mammals, including Homo sapiens. This study is also aimed to construct phylogenetic tree of p73 gene among different mammal species, including human, chimpanzee, monkey rat, mouse, guinea pig, arboreal lizard, zebra finch, grey wolf, panda, horse and other cattle. Phylogenetic analysis and multiple sequence alignment of the human Tp73, transcript variant mRNA sequence through various phylogenetic trees were performed which showed its pattern of variations and relationship among different organisms especially with rat, mouse and chimpanzee. We have identified that 17 sequences of p73 gene were 100% identical with Homo sapiens chromosome 1p73 (TP73) gene while Ailuropoda melanoleuca tumor protein p73 (tp73), mRNA were the most dissimilar sequences with 86 % identity. The phylogenetic tree revealed the relationships and percent similarity of Homo sapiens TP73 gene sequence among different organisms especially with rat, mouse and chimpanzee. Our study also demonstrated that positive selection of TP73 gene during the divergence of different species during evolution.

Keywords: p73 gene; phylogenetic analysis; BLAST; MEGA7; UPGMA; Neighbor joining.

INTRODUCTION

Cancer is a multi-step cellular process in which cell loses the control of growth and differentiation. Initiation of cancer development could be regulated by a wide range of factors, including exposure of radiation and carcinogenic chemicals, living environmental condition, life style of an individual, age, gender, infectious agents and others risk factors [1]. In addition, various genetic and epigenetic factor has been identified which are actively involved in the regulation of cancer development. In majority of cancers, aberrant expression of several genes have been identified by different scientific groups. The p53-family members include p53, p63 and p73 which are actively linked to several cellular functions, such as regulation of cell cycle, development, differentiation, apoptosis and tumorigenesis [2]. The p53 mainly acts as a tumor suppressor in stress situations and p63 is vital for ectoderm development. p73 seems to take part in regulating both, the stress responses and developmental processes [1-3].

p73 transcription factor has become one of the most extensively studied proteins because of the possibility of replacing p53 with p73 in p53-defective cells due to its structural and functional homologies with p53. p73 has cellular activities similar to those of p53, including binding and transactivation of p53-responsive genes. It regulate the induction of apoptosis and cell cycle arrest, which confer the tumor-suppressive activities of p73 [2, 3]. The TP73 gene is located on chromosome 1p36, a region frequently deleted in neuroblastoma, melanoma and breast cancer[3]. The TP73 gene is rarely mutated in tumors (less than 0.5%) and in neuroblastoma the remaining P73 allele only shows a mutation frequency of 1% [4, 5]. Inactivation of TP73 due to promoter hypermethylation and subsequent loss of mRNA expression has been reported in hematological malignancies like acute lymphoblastic leukemia and Burkitt's lymphomas [6, 7]. Concerning imprinting of the remaining P73 allele, many studies have found that P73 has a balletic expression and higher p73 mRNA levels in tumor tissue compared to the surrounding normal tissue, for instance, breast, lung, prostate, ovarian, colorectal and esophageal cancers have increased p73 mRNA levels [8-10].

Although, p73 is rarely mutated, altered expression levels of p73 and its abnormal splicing variants were identified in several human cancers [11]. In majority, splicing occur at 3'

end and form proteins that have different C-terminals. The p73 genes encode numerous different isoforms protein formed by alternative splicing at C-terminal end of that protein and give rise to six different p73 terminal variants. (ά to ζ) expressed in both normal and cancer cells [12, 13].

Decreased levels of p73 were identified in pancreatic adenocarcinoma, breast cancer, thyroid cancer and osteosarcoma [14-17]. In some hepatocellular carcinoma, increased levels of p73 expression were identified and this was correlated with a lower mean survival time for the patients [18-19]. ΔNp73 isoforms are frequently upregulated in some tumors compared with their normal tissue of origin [20]. ΔNp73 isoforms express pro-oncogenic properties and act as dominant negative inhibitors over pro-apoptotic TAp73 and p53 by physically interacting with them [21]. ΔNp73 isoforms functions as oncoproteins and can contribute to the cancer progression. In several cancers, altered levels of p73 expression were identified and low or high levels of p73 can significantly alter the biological activities regulated. Stimuli, such as DNA damage, viral factors and transcriptional regulators can regulate P73 gene expression at the transcriptional level [22-24].

The current study focused to explore the distribution pattern of genetic variation in p73 gene found in different organisms, including Homo sapiens. This study also emphasized to identify the evolutionary dynamics and molecular evolution of the genes contained within the p73 network. Analytical studies were performed by generating comparative transcriptomes across various species, and by comparing pathway evolution between different species of mammals and others. The study also explores the phylogenetic analysis and comparison of p73 gene in human, chimpanzee, monkey rat, mouse, guinea pig, arboreal lizard, zebra finch, grey wolf, panda, horse and other cattle.

METHODS

Sequence retrieval

Homo sapiens tumor protein p73 (Tp73), chromosome 1 p73 (TP73) gene, complete cds sequence (AH007820.2) was retrieved from the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) databases in FASTA format.

Local sequence alignment

Basic local Alignment Search Tool (BLAST) was performed for the tumor protein p73 (Tp73), chromosome 1 p73 (TP73) gene, complete cds sequence retrieved from NCBI to identify its relatives in different organisms including human using the online NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This software takes the data in FASTA format and produces the BLAST table.

Phylogenetic analysis

Phylogenetic analysis of tumor protein p73 (Tp73), chromosome 1 p73 (TP73) gene, complete cds sequence through UPGMA and Neighbor Joining were carried out using MEGA7 software [25]. Phylogenetic tree were constructed by the software showing the ancestral relationship among the sequences. The UPGMA phylogeny tree gives different clusters showing their evolutionary relationship with each other and Neighbor Joining phylogeny tree reveals different clade showing their evolutionary distance with in the different species. The sequences which lie in the same cluster are closely related.

RESULTS

Sequence retrieval

H. sapiens tumor protein p73 (TP73), gene, complete cds was retrieved from the NCBI in FASTA format. The sequence of the gene, complete cds (AH007820.2) is as follows (Table 1):

Table 1: *Homo sapiens* chromosome 1 p73 (TP73) gene, complete cds (AH007820.2)

CAGGAGGACAGAGCACGAGTTCCCAGGGTGCTCAGGTGTCATTCCTTCCT
TCCTGCAGAGCGAGCTGCCCTCGGAGGCCGGCGTGGGGAAGATGGCCC
AGTCCACCGCCACCTCCCCTGATGGGGGCACCACGTTTGAGCACCTCTGG
AGCTCTCTGTGAGTGCGCTTGGCTGGCCAGAGCTGGGGGCCCCCCTGGG
AGGCACTCTGGGCTAGCCTCAGCCACCTTCGAAGAACAGGAGACGTAGG
CCTCCCCAGGCGTGTCAGGCATAGCTTTGAGTTATGGGCGTGCGAGGTAT
TGGGTTGACACCCAAACTTGGGACTGACGTTTCTATTTTCCTCTCCCTGCC
CCAGGGAACCAGACAGCACCTACTTCGACCTTCCCCAGTCAAGCCGGGG
GAATAATGAGGTGGTGGGCGGAACGGATTCCAGCATGGACGTCTTCCAC
CTGGAGGGCATGACTACATCTGTCATGGTGAGTGGGGGGGCTGCCCTCT
GCAAGAGGACTGGAGTGGGGACAACAAATGTGGCCTGTCCCTAGACGG
GACAGGACGACTGACTGTGGTGTGTTTCCCCCTCCCTCCTCCCCTTTCCCG
CGCCAGGCCCAGTTCAATCTGCTGAGCAGCACCATGGACCAGATGAGCA
GCCGCGCGGCCTCGGCCAGCCCCTACACCCCAGAGCACGCCGCCAGCGT
GCCCACCCACTCGCCCTACGCACAACCCAGCTCCACCTTCGACACCATGTC
GCCGGCGCCTGTCATCCCCTCCAACACCGACTACCCCGGACCCCACCACTT
TGAGGTCACTTTCCAGCAGTCCAGCACGGCCAAGTCAGCCACCTGGACG
GTGAGTTCCCCTAGTCCCTGAGGGCTGCCAGCTGCGGGCTGCGGGCTGG
AGAGGAGGTGGCTGCGTCTGGACAGGGGTGCAGTTGGGACCACTGGTC
TCACCCGCTCCCTCTCCCCCACTCCAGTACTCCCCGCTCTTGAAGAAACTCT
ACTGCCAGATCGCCAAGACATGCCCCATCCAGATCAAGGTGTCCACCCCG
CCACCCCCAGGCACTGCCATCCGGGCCATGCCTGTTTACAAGAAAGCGGA
GCACGTGACCGACGTCGTGAAACGCTGCCCCAACCACGAGCTCGGGAGG
GACTTCAACGAAGGTGAGGACCCCCAGCTCCTCTGCCCACGGTGAGCACT
TTGCCCAGCATCCCGGACAGCAGGGCTGGGCACCTCTCTGCACCTAGCAC
AAGGGGTGGGCACCTCTATGCACCTCTCTGAAGTGTCGACCCCTCCTGGC
AGGACAGTCTGCTCCAGCCAGCCACCTCATCCGCGTGGAAGGCAATAATC
TCTCGCAGTATGTGGATGACCCTGTCACCGGCAGGCAGAGCGTCGTGGT
GCCCTATGAGCCACCACAGGTAGGCCAAGAAGCCAGGCTGTGCCCAGGG
CCCTGCAGTCAGCTGTACGGGTCGGGGGAGGGGTCCCCTGAGGCAGCCC
CTGTCCCTCCTCAGTTGGCGGGGCTGCCACCTTAGTGGATTGGGGCTGCG
TGCTGATGCTAGCCCCTCTCCCTGCTCCCCCATGTGCAGGTGGGGACGGA
ATTCACCACCATCCTGTACAACTTCATGTGTAACAGCAGCTGTGTAGGGG
GCATGAACCGGCGGCCCATCCTCATCATCATCACCCTGGAGATGCGGGA
GTGAGTCCCGGGCACACGGGGTGGAGGTGGGACAGGGCTGGGCAGGC
ACGGCCGGGGGAGAAGGGGAGCTGTCATGGGAAGGTGGGCAGGTTGA
GGGTGGGCAGGGTTGAGCTCACAATTCTTGGCTGTGCCCACCCGACAGT
GGGCAGGTGCTGGGCCGCCGGTCCTTTGAGGGCCGCATCTGCGCCTGTC
CTGGCCGCGACCGAAAAGCTGATGAGGACCACTACCGGGAGCAGCAGG
CCCTGAACGAGAGCTCCGCCAAGAACGGGGCCGCCAGCAAGCGTGGTGA
GCGGCCGGCCGGGGGAACTGGACGCGTGTGGGAGGGGAAGGGGACAC
ATTGGCAGGACACCCTCTGGTCCTGCCTGCTCACCCCGCCTGCCCTGCCTG
GCCTTCCAGCCTTCAAGCAGAGCCCCCCTGCCGTCCCCGCCCTTGGTGCC
GGTGTGAAGAAGCGGCGGCATGGAGACGAGGACACGTACTACCTTCAG

GTGAGTGTGTGCTCCTACACGGCAGCCGGGAGACCTGCCTCACCTCTGTC
GTCTGCTGAGCCCAGGCTGGGCCATGGGGAGGGACTCTGGAGGCCACAC
CCCTCCCATGGGGGTCTGGGGCACGTGGGCAGAGATCTGCTCCTCTGTGC
TCAGGTGCGAGGCCGGGAGAACTTTGAGATCCTGATGAAGCTGAAAGAG
AGCCTGGAGCTGATGGAGTTGGTGCCGCAGCCACTGGTGGACTCCTATC
GGCAGCAGCAGCAGCTCCTACAGAGGCCGTGAAGTCAGCCCTAGCCCAC
CATCAGTGTGGGGAAGGAGGACATGGCTTAACCCCCCAGGAGAAGCATC
ACGGGCATGGGTGGTCGGTGGGCACGAGGCCTTGCTTCCCACCCATGCG
AGCCGTTGCTTCTGAGCAGGAGTCACCTACAGCCCCCGTCCTACGGGCCG
GTCCTCTCGCCCATGAACAAGGTGCACGGGGGCATGAACAAGCTGCCCT
CCGTCAACCAGCTGGTGGGCCAGCCTCCCCCGCACAGTTCGGCAGCTACA
CCCAACCTGGGGCCCGTGGGTGAGTCCTTGGGCAGTGCGGGCCCACGGG
CAGGGCGGGGAGGCCCACTGGGGCGCCTAGCTCAGGACACACCACCCA
GCTCTGTCTGGAGCCTGGGTGGAGGCTGCACCTGGATGCCCAGCCTGGC
TGCCCTGATGGCCCCACCTGCCTCTCACCCAGGCCCCGGGATGCTCAACA
ACCATGGCCACGCAGTGCCAGCCAACGGCGAGATGAGCAGCAGCCACAG
CGCCCAGTCCATGGTCTCGGGGTCCCACTGCACTCCGCCACCCCCCTACCA
CGCCGACCCCAGCCTCGTCAGGTGCGTGGGCTGCCGAGGGCCTGAGCAT
GTGCTGTCACCCTGTCTGTTCACCTCTGTCCTTCTGGCCATGNCAGCCAGG
CCACTCTCAGAGACGGGGGCTCGCGCAGCCCTGTGCTCGGTAGCTAATG
CTGCTTCCTTTCTCAAATTCTCTCTGCAGTTTTTTAACAGGATTGGGGTGTC
CAAACTGCATCGAGTATTTCACCTCCCAAGGGTTACAGAGCATTTACCACC
TGCAGAACCTGACCATTGAGGTAACGCCCGGGTGGACCCCGCTCTGCAG
AGGCAGTAGCTGGAGGGGCCCCTGTCCGGAGGGCAAAGAGCCTTCTCTT
CCTTGCTCTCGTGGCTGTGCTCTCCCTGCTCCACTGCCCCCTGCCCCTAAT
GCGCCGGCCTCTCGCAGGACCTGGGGGCCCTGAAGATCCCCGAGCAGTA
CCGCATGACCATCTGGCGGGGCCTGCAGGACCTGAAGCAGGGCCACGAC

TACAGCACCGCGCAGCAGCTGCTCCGCTCTAGCAACGCGGCCACCATCTC
CATCGGCGGCTCAGGGGAACTGCAGCGCCAGCGGGTCATGGAGGCCGT
GCACTTCCGCGTGCGCCACACCATCACCATCCCCAACCGCGGCGGCCCAG
GCGGCGGCCCTGACGAGTGGGCGGACTTCGGCTTCGACCTGCCCGACTG
CAAGGCCCGCAAGCAGCCCATCAAGGAGGAGTTCACGGAGGCCGAGATC
CACTGAGGGCCTCGCCTGGCTGCAGCCTGCGCCACCGCCCAGAGACCCA
AGCTGCCTCCCCTCTCCT

Local sequence alignment

The *Homo sapiens* tumor protein p73 (Tp73), transcript variant mRNA was retrieved from the NCBI (https://www.ncbi.nlm.nih.gov/nuccore) in FASTA format, with accession number AH007820.2. *H. sapiens* tumor protein p73 (Tp73), chromosome 1 p73 (TP73) gene, complete cds was studied for its similarity patterns and BLAST was therefore performed by feeding the data of sequence into the online NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). After performing BLAST, the NCBI BLAST tool produced BLAST table (list of the aligned sequence) showing the accession numbers, percent similarity, etc. (Table 2).

Table 2: BLAST table of Human p73.

S/N	AN	Organism	Desccription	Sequence Length	%Pair Wise
1	AH007820.2	*Homo sapiens*	*Homo sapiens* chromosome 1p73 (TP73) gene, complete cds	7070	100
2	NG_017035.2	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73), RefSeqGene on chromosome 1	6854	100
3	AL136528.11	*Homo sapiens*	Human DNA sequence from clone RP5-1092A11 on chromosome 1p36.2-36.33, complete sequence	6854	100
4	NM_001204190.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73), transcript variant 6, mRNA	2901	99
5	NM_001126241.2	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 3, mRNA	3181	99
6	NM_001204187.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 11, mRNA	3319	99
7	NM_001204184.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73), transcript variant 8, mRNA	3599	99
8	AB055066.1	*Homo sapiens*	*Homo sapiens* hDNp73B mRNA for deltaNp73beta, complete cds	3187	99
9	NM_001204192.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 13 , mRNA	3358	100
10	NM_001204191.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 7,mRNA	2813	100
11	NM_001126242.2	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 4, mRNA	3078	100
12	NM_001126240.2	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 2,mRNA	3358	100
13	NM_001204188.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 12, mRNA	3231	100
14	NM_001204185.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 9, mRNA	3496	100
15	NM_005427.3	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73),transcript variant 1, mRNA	3776	100
16	AK304784.1	*Homo sapiens*	*Homo sapiens* cDNA FLJ52399 complete cds, Tumor protein p73	3358	100
17	AK295669.1	*Homo sapiens*	*Homo sapiens* cDNA FLJ50534 complete cds, Tumor protein p73	1960	100
18	BC117253.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 mRNA (cDNA clone MDC:150862 IM AGE:40125804),COMPLETE CDS	3781	100
19	AY040829.	*Homo sapiens*	*Homo sapiens* DN p73 gamma (TP73) mRNA, complete cds, alternatively spliced	3084	100
20	AB055065.1	*Homo sapiens*	*Homo sapiens* hDNp73A mRNA for deltaN p73 alpha, complete cds	3363	100
21	Y11416.1	*H.sapiens*	H.sapiens mRNA for p73	3781	100
22	NM_001204189.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73), transcript variant 5,mRNA	2634	100
23	NM_001204186.1	*Homo sapiens*	*Homo sapiens* tumor protein p73 (TP73), transcript variant 10,mRNA	3052	100
24	XM_004024528.2	*Gorilla gorilla*	Gorilla gorillagorilla tumor protein p73 (TP73), mRNA	3663	99
25	AH006898.2	*Homo sapiens*	*Homo sapiens* chromosome 1p73 gene, complete cds	6626	100
26	XM_016952823.1	*Pan troglodytes*	Pan troglodytes tumor protein p73 (TP73), transcript variant X3, mRNA	3481	98
27	XM_016952816.1	*Pan troglodytes*	Pan troglodytes tumor protein p73 (TP73), transcript variant X2, mRNA	3201	98
28	XM_008970749.2	*Pan paniscus*	Pan paniscus tumor protein p73 (TP73), transcript variant X4, mRNA	2899	98
29	XM_003805381.3	*Pan paniscus*	Pan paniscus tumor protein p73 (TP73), transcript variant X2, mRNA	3063	98

30	XM_008970733.2	*Pan paniscus*	Pan paniscus tumor protein p73 (TP73), transcript variant X1, mRNA	3464	98
31	XM_016952824.1	*Pan troglodytes*	Pan troglodytes tumor protein p73 (TP73), transcript variant X4, mRNA	3378	98
32	XM_016952814.1	*Pan troglodytes*	Pan troglodytes tumor protein p73 (TP73), transcript variant X1, mRNA	3658	98
33	XM_008970761.2	*Pan paniscus*	Pan paniscus tumor protein p73 (TP73), transcript variant X6, mRNA	3637	98
34	XM_008970755.2	*Pan paniscus*	Pan paniscus tumor protein p73 (TP73), transcript variant X5, mRNA	3240	98
35	XM_008970745.1	*Pan paniscus*	Pan paniscus tumor protein p73 (TP73), transcript variant X3, mRNA	3641	98
36	AK305292.1	*Pan troglodytes*	Pan troglodytes for tumor protein p73, complete cds, clone: PtsC-58-5_H08	2808	98
37	AC196698.5	*Rhesus Macaque*	Rhesus Macaque BAC CH250-329 () complete sequence	5554	95
38	AC196671.6	*Rhesus Macaque*	Rhesus Macaque BAC CH250-243C8() complete sequence	5554	95
38	AK302118.1	*Homo sapiens*	*Homo sapiens* cDNAFLJ52358 complete cds, highly similar to Tumor proteinp73	3272	100
40	XM_015138176.1	*Macacamulatta*	Macacamulatta tumor protein p73 (TP73), Mrna	3340	95
41	XM_017882442.1	*Rhinopithecusbieti*	Rhinopithecusbieti tumor protein p73 (TP73), transcript variant X2, mRNA	2887	95
42	XM_005544972.2	*Macacafasicularis*	Macacafasicularis tumor protein p73 (TP73), mRNA	3340	95
43	XM_017956509.1	*Papio Anubis*	Papio Anubis tumor protein p73 (TP73), transcripts variant X5, mRNA	2773	94
44	XM_017882441.1	*Rhinopithecusbieti*	Rhinopithecusbieti tumor protein p73(TP73), transcript variant X1, mRNA	3305	95
45	XM_011956842.1	*Colobus angolensispall*	Colobus angolensispallatus tumor protein p73n(TP73), transcript variant X3, mRNA	2777	94
46	HQ258345.1	*Homo sapiens*	Synthetic construct *Homo sapiens* done IMAGE 100072654 tumor protein p73 (TP73) gene, encodes complete protein	3593	100
47	XM_010361799.1	*Rhinopithecus*	Rhinopithecusroxellana tumor protein p73 (TP73), transcript variant X5, mRNA	2786	94
48	XM_010361791.1	*Rhinopithecus*	Rhinopithecusroxellana tumor protein p73 (TP73), transcript variant X3, mRNA	2848	94
49	AB590585.1	*Homo sapiens*	Synthetic construct DNA, clone: pFN21AB7872, *Homo sapiens* TP73, without stop codon, in Flexi system	3591	94
50	XM_017956506.1	*Papio Anubis*	Papio Anubis tumor protein p73 (TP73), transcriptvariant X3, mRNA	2933	94
51	XM_009216652.2	*Papio Anubis*	Papio Anubis tumor protein p73 (TP73), transcriptvariant X2, mRNA	3292	94
52	XM_01795650.1	*Papio Anubis*	Papio Anubis tumor protein p73 (TP73), transcriptvariant X1, mRNA	3301	94
53	XM_011767199.1	*Macacanemestrina*	Macacanemestrina tumor protein p73 (TP73), transcriptvariant X3, mRNA	3075	94
54	XM_011767198.1	*Macacanemestrina*	Macacanemestrina tumor protein p73 (TP73), transcriptvariant X2, mRNA	3338	94
55	XM_011767197.1	*Macacanemestrina*	Macacanemestrina tumor protein p73 (TP73), transcriptvariant X1, mRNA	3067	94
56	XM_011956841.1	*Colobus angolensis*	Colobus angolensis tumor protein p73 (TP73), transcriptvariant X2, mRNA	2942	94
57	XM_011956840.1	*Colobus angolensis*	Colobus angolensis tumor protein p73 (TP73), transcriptvariant X1, mRNA	3292	94
58	XM_010361793.1	*Rhinopithecusroxellana*	Rhinopithecusroxellana tumor protein p73 (TP73), transcriptvariant X4, mRNA	2760	94
59	XM_010361785.1	*Rhinopithecusroxellana*	Rhinopithecusroxellana tumor protein p73 (TP73), transcriptvariant X2, mRNA	2952	94
60	XM_010361777.1	*Rhinopithecusroxellana*	Rhinopithecusroxellana tumor protein p73 (TP73), transcriptvariant X1, mRNA	3321	94
61	XM_012078575.1	*Cercocebusatys*	Cercocebusatys tumor protein p73 (TP73), transcriptvariant X7, mRNA	2766	94
62	XM_012078574.1	*Cercocebusatys*	Cercocebusatys tumor protein p73 (TP73), transcriptvariant X6, mRNA	3139	94
63	XM_012078572.1	*Cercocebusatys*	Cercocebusatys tumor protein p73 (TP73), transcriptvariant X5, mRNA	2926	94
64	XM_012078571.1	*Cercocebusatys*	Cercocebusatys tumor protein p73 (TP73), transcriptvariant X4, mRNA	3292	94
65	XM_012078570.1	*Cercocebusatys*	Cercocebusatys tumor protein p73 (TP73), transcriptvariant X3, mRNA	3290	94
66	XM_012078569.1	*Cercocebusatys*	Cercocebusatys tumor protein p73 (TP73), transcriptvariant X2, mRNA	3292	94
67	XM_012078568.1	*Cercocebusatys*	Cercocebusatys tumor protein p73 (TP73), transcriptvariant X1, mRNA	3292	94
68	XM_007980863.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X9, mRNA	2712	93
69	XM_007980861.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X8, mRNA	2602	93
70	XM_007980864.1	*Homo sapiens*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X10, mRNA	2872	93
71	XM_007980959.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X6, mRNA	2743	93

72	XM_007980858.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X5, mRNA	2762	93
73	XM_007980857.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X4, mRNA	3128	93
74	XM_007980856.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X3, mRNA	3126	93
75	XM_007980855.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X2, mRNA	3128	93
76	XM_007980854.1	*Chlorocebussabaeus*	Chlorocebussabaeus tumor protein p73 (TP73), transcriptvariant X1, mRNA	3136	93
77	XM_009235751.1	*Pongo abelii*	Pongo abelii tumor protein p73 (TP73) , mRNA	2775	98
78	Y11419.1	*C. aethiops*	C. aethiops mRNA for p73	3125	93
79	AK310432.1	*Homo sapiens*	*Homo sapiens* cDNA , FLJ17474	566	99
80	XM_017542690.1	*Cebuscapucinus*	Cebuscapucinus imitator tumor protein p73 (TP73), mRNA	2907	92
81	LT160000.1	*Macacafascicularis*	Macacafascicularis complete genome, chromosome chr1	4876	95
82	XM_012460991.1	*Aotusnancymaae*	Aotusnancymaae tumor protein p73 (TP73), transcriptvariant X2, mRNA	2305	95
83	XM_012460990.1	*Aotusnancymaae*	Aotusnancymaae tumor protein p73 (TP73), transcriptvariant X1, mRNA	2717	95
84	XM_012498267.1	*Nomascusleucogenys*	Nomascusleucogenys tumor protein p73 (LOC100594205),mRNA	1178	95
85	XM_012498533.1	*Nomascusleucogenys*	Nomascusleucogenys tumor protein p73 (LOC105738061),mRNA	862	95
86	AK304177.1	*Homo sapiens FLJ52392*	*Homo sapiens* cDNA FLJ52392 complete cds, highly similar to tumor protein p73	2018	99
87	XM_003793216.1	*Otolemurgarnettii*	Otolemurgarnettii tumor protein p73(TP73), transcript variant X2, mRNA	1572	90
88	XM_003793215.1	*Otolemurgarnettii*	Otolemurgarnettii tumor protein p73(TP73), transcript variant X1, mRNA	1712	90
89	XM_012660085.1	*Propithecuscoquereli*	Propithecuscoquereli tumor protein p73(TP73), mRNA	1944	90
90	XM_005339217.1	*Ictidomystridecemlineatus*	Ictidomystridecemlineatus tumor protein p73(TP73), transcript variant X2, mRNA	1265	89
91	XM_001915365.2	*Equuscaballus*	Equuscaballus tumor protein p73 (TP73), mRNA	3043	89
92	XM_003434677.1	*Canis lupus*	Canislupustumor protein p73(TP73), transcript variant 2, mRNA	1274	88
93	XM_003434676.1	*Canis lupus*	Canislupustumor protein p73(TP73), transcript variant 1, mRNA	1533	88
94	XM_001083217.2	*Macacamulatta*	Macacamulatta tumor protein p73 (TP73), mRNA	3368	88
95	XM_002196490.1	*Taeniopygiagutata*	Taeniopygiaguttata similar to transformation related protein 73, transcript variant 1 (LOC100220605), mRNA	3247	87
96	XM_003471247.3	*Caviaporcellus*	Caviaporcellus tumor protein p73 (Tp73), mRNA	4674	86
97	NM_001108696.1	*Rattusnervegicus*	Rattusnorvegicus tumor protein p73 (Tp73), mRNA	8770	86
98	Y19235.1	*Mus musculus*	Mus musculus mRNA for P73 delta-N protein (P73 gene)	3980	86
99	XM_002919424.1	*Ailuropodamelanoleuca*	Ailuropodamelanoleuca tumor protein p73 (TP73), mRNA	4245	86

It is clear from the results that Homo sapiens tumor protein p73 (TP73), Ref Seq Gene on chromosome 1 (NG_017035.2), Human DNA sequence from clone RP5-1092A11 on chromosome 1p36.2-36.33, complete sequence (AL136528.11), Homo sapiens tumor protein p73 (TP73),transcript variant 13 , mRNA (NM_001204192.1), Homo sapiens tumor protein p73 (TP73),transcript variant 7,mRNA (NM_001204191.1), Homo sapiens tumor protein p73 (TP73),transcript variant 4, mRNA (NM_001126242.2), Homo sapiens tumor protein p73 (TP73),transcript variant 2,mRNA (NM_001126240.2), Homo sapiens tumor protein p73 (TP73),transcript variant 12, mRNA (NM_001204188.1), Homo sapiens tumor protein p73 (TP73),transcript variant 9, mRNA (NM_001204185.1), Homo sapiens tumor protein p73 (TP73),transcript variant 1, mRNA (NM_005427.3), Homo sapiens cDNA FLJ52399 complete cds, highly similar to Tumor protein p73 (AK304784.1), Homo sapiens cDNA FLJ50534 complete cds, highly similar to Tumor protein p73 (AK295669.1), *Homo sapiens* tumor protein p73 mRNA (cDNA clone MDC:150862 IM AGE:40125804), COMPLETE CDS (BC117253.1), *Homo sapiens* DN p73 gamma (TP73) mRNA, complete cds, alternatively spliced (AY040829), *Homo sapiens* hDNp73A mRNA for deltaN p73 alpha, complete cds (AB055065.1), *H. sapiens* mRNA for p73 (Y11416.1), *Homo sapiens* tumor protein p73 (TP73), transcript variant 5,mRNA (NM_001204189.1), *Homo sapiens* tumor protein p73 (TP73), transcript variant 10,mRNA (NM_001204186.1), *Homo sapiens* chromosome 1p73 gene, complete cds (AH006898.2), *Homo sapiens* cDNAFLJ52358 complete cds,

highly similar to Tumor proteinp73 (AK302118.1) and Synthetic construct Homo sapiens done IMAGE 100072654 tumor protein p73 (TP73) gene, encodes complete protein (HQ258345.1) were 100% identical with H. sapiens tumor protein p73 (AH007820.2). *H. sapiens* tumor protein p73 (Tp73), transcript variant 1,2,4,5,7,9,10,11,12,13, mRNA are also 100% identical with *H. sapiens* tumor protein p73 (Tp73), gene compete cds (AH007820.2), while tumor protein p73 (TP73), gene of *Caviaporcellus* (XM_003471247.3), *Rattusnervegicus* (NM_001108696.1), *Mus musculus* (Y19235.1), *Ailuropoda melanoleuca* (XM_002919424.1) were the most dissimilar sequences with 86 % identity (Table 2).

Phylogenetic analysis

Phylogenetic analysis of TP73 gene sequence was performed using MEGA7 software, based on Tamura-Nei Algorithm [26]. UPGMA and NJ methods were adopted for the construction of phylogenetic tree.

The UPGMA rooted tree diagram of *H. sapiens* tumor protein p73 (TP73), transcript mRNA sequences showed different clusters formation. Organism that originated from same ancestors with 99%-100% pair wise identity, are placed in same clusters whereas those which are distant from each other are placed in separate clusters (Figure 1 and 2).

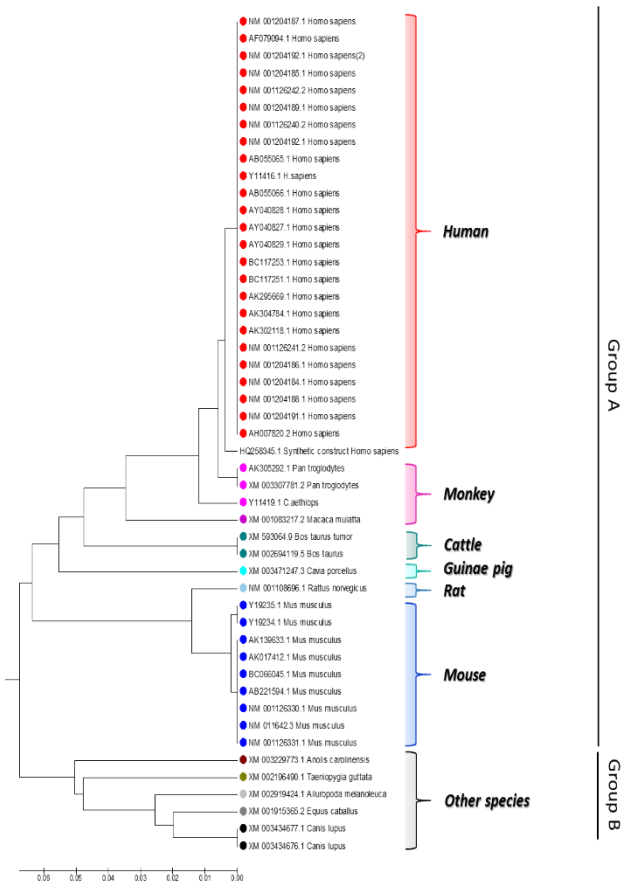

Figure 1: Phylogenetic tree to estimate evolutionary relationships of TP73 gene using UPGMA method.

The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.53459987 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 48 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 264 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Majority of human p73 sequences are lying in the same clusters. The genes lie in mainly two major groups; Group A and B. Group A includes human, monkey, cattle, guinea pig, rat and mouse. However, other species such as lizard, bird, horse, bear and wolf combined together as a Group B, which are totally distinct group compared to Group A.

Figure 2: Phylogenetic tree to understand evolutionary distance of TP73 gene Neighbor-Joining method.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.56817190 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used

to infer the phylogenetic tree. Evolutionary analyses were conducted in MEGA7.

Phylogenetic tree was also analyzed using neighbor joining (NJ) method to determine the evolutionary distance between the TP73 genes in the different species. The principle of NJ method is to find pairs of operational taxonomic units (OTUs; neighbors) that minimize the total branch length at each stage of clustering of OTUs starting with a star like tree. The branch lengths as well as the topology of a parsimonious tree can quickly be obtained by using this method [27]. We have retrieved TP73 gene of selected species human, chimpanzee, monkey rat, mouse, guinea pig, arboreal lizard, zebra finch, grey wolf, panda, horse and other cattle. NJ tree shows the more similarity between human and chimpanzee/monkey, while mouse sequence was distantly related (Figure 1 and 2). TP73 genes of human, mouse, rat and chimpanzee encoded appropriate similar protein depicted as Group A in figure 1. However, TP73 gene of group B is variable compare to Group A. Group B cluster indicates homology of TP73 gene in guinea pig, arboreal lizard, zebra finch, grey wolf, panda, horse and other cattle. During the study, our finding showed that, Group A cluster member species had more conserved TP73 gene sequence compare to other species.

DISCUSSION

Recent update in genome sequencing projects allow us to utilize the genome sequences for further studies including comprehensive analysis of the evolutionary origins, population migration and phylogenetic relationships of the specific genes. The p53 superfamily, includes p63 and p73. P73 protein is actively involved in regulating a series of pathways in a wide range of pathologies, particularly in the development of different type of cancers, including breast cancer, colorectal cancer and neuroblastoma. Recent studies suggest that p73 is an important protein which play a key role in cancer development. Current approaches are actively involved to control p73 function, and which would be helpful to develop better diagnostics and therapeutics tool via manipulating downstream regulation.

Evolutionary studies are of principal importance in the field of biological research since for a very long time as provided the basis for comparative genomics. Many biologists agreed that a phylogenetic tree of relationships should be the central underpinning of research in many areas of biology. Comparisons of human species or gene sequences in a phylogenetic context can provide the most meaningful insights into biology. This important realization is now apparent to researchers in diverse fields, including ecology, molecular biology, and physiology.

Phylogenetic reconstruction is an attempt to discern the ancestral relationship of a set of sequences. It involves the construction of a tree, where the nodes indicate separate evolutionary paths. Phylogenetic analysis is an important method and it enriches our understanding of how genes, genomes, species and molecular sequences more generally evolve. Through phylogenetic tree, we learn not only how the sequences came to be the way they are today, but also general principles that enable us to predict how they will change in the future. This is not only of fundamental importance but also extremely useful for numerous applications (Figure 1 and 2).

Purpose of the phylogenetic analysis often includes the search for evidences of directional selection in molecular evolution [28-30]. Evolution of the TP73 was studied in different organisms and adaptive changes were in the sequences, such as *Caviaporcellus* (XM_003471247.3), *Rattusnervegicus* (NM_001108696.1), *Mus musculus* (Y19235.1), *Ailuropoda melanoleuca* (XM_002919424.1) tumor protein p73 gene sequences are the 86% homology to *H. sapiens* tumor protein p73 complete cds (AH007820.2). Additionally, the phylogeny of the TP73 gene was also studied previously by different research groups and similar methodology were adopted [31-33].

CONCLUSION

The observations based on phylogenetic analysis of TP73 gene using UPGMA and NJ method revealed the relationships and percent similarity of TP73 gene within human and others species. Phylogenetic analysis and multiple sequence alignment of the *Homo sapiens* chromosome 1p73 (TP73) gene, complete cds through various phylogenetic tree were performed which showed its pattern of variations and relationship among different organisms especially with rat, mouse and chimpanzee. This study demonstrated that positive selection of TP73 gene during the divergence of different species during evolution. It is known that homoiothermy and viviparity first appeared among synapsids. These evolutionary acquirements have made necessary changes in the genetic control of ontogeny, and this, in turn, might have caused adaptive changes in the p73 gene. This current study will help in modern research strategies through the manipulation and exploitation of p73, as its pathways are promising and one can predict its extensive clinical to control the regulation of p73 in cancer development and biological use in the future for the human benefit around the world.

List of abbreviations:

TP73: Tumor Protein P73,

NJ: Neighbor-Joining,

UPGMA: Unweighted Pair Group Method with Arithmetic Mean,

BLAST: Basic Local Alignment Search Tool,

NCBI: National Center for Biotechnology Information,

mRNA: messenger ribonucleic acid,

DNA: Deoxyribonucleic acid,

CDS: Coding Sequence

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