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The interaction of p53 and MDM2 genes in cancers, *in silico* studies and phylogenetic analysis

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Abstract

The normal cell cycle process is a crucial process and is generally mediated by a number of regulatory genes. One of the most important regulators is the tumor suppressor p53, which in turn is regulated by MDM2 gene. The expression of p53 and MDM2 is found to be frequently altered in many cancers and metastasis/ relapses .This is the first report to look at the evolutionary history of these genes to decipher the role of these genes in the tumorigenesis process using *in silico* methods. We also found that they showed high degree of sequence similarity across the mammalian species, indicating that these species probably share parallel cancer causing mechanisms. Their individual unrooted phylogenetic tree formed 5 clusters each; however, p53 gene was found in a large number of species whereas MDM2 was found in smaller number of species. The role of MDM2 is therefore limited and occurs in fewer species across the mammalian species. It is evident that these molecules play an important role in the cancer process, perhaps responsible for relapses and hence need to be explored further as therapeutic targets. Such studies that are based on evidence from paleontology and genetics suggest that mechanisms of cancer are embedded deeply throughout evolution. Understanding the phylogenetic evolution of these genes could help in furthering our knowledge on the mechanisms involved in cancer.

Keywords: p53; MDM2; genes; cancer; *in silico* studies; phylogeny; cell cycle regulation.

Introduction

Neoplasia is a heterogeneous disease affecting mankind at all stages of life. To better understand the etiology of the disease, it is necessary to understand the molecular mechanisms that lead to the production of cancer cells. Abnormalities in the cell cycle process have largely been implicated in the prognosis of cancer. The cell cycle process plays a crucial role in the development of an organism as it controls cellular growth, differentiation and proliferation processes. Any change in the cell cycle would successively alter these processes and thus lead to cancer. The normal cell cycle process thus becomes a crucial process and is generally mediated by a number of regulatory genes. These genes include cyclins, cyclin dependent kinases and their inhibitors, many tumor suppressor genes, which ensure the proper progression of the cell cycle from one phase to another and also ensure the appropriate termination of the cell cycle process. In a previous study, Khan and Jamil (2008) carried out cancer metasignature gene analysis and reported that genes involved in tumorigenesis are involved in multiple pathways essential for cell survival and hence

contribute to sustained proliferation of cancer cells.

The expression of p53 tumor suppressor gene and other genes that participate in the p53 pathway are found to be frequently altered in many cancers. The p53 gene encodes a 53 kDa protein that participates in a plethora of functions. It functions as a transcription factor, regulating the expression of many genes that are essential for checkpoint response due to DNA damage and also genes that participate in apoptotic pathways (Vousden and Lane, 2007). Initial levels of p53 in cells are fairly low but gradually increase in response to genotoxic stress signals. When DNA damage has been signaled, p53 protein interrupts G1 phase of cell division and allows for repair of the damaged DNA and also activates apoptosis to remove cells that are irreparably damaged (Kastan et al., 1991). Altered expression of p53 through mutations or deletions results in change in apoptosis process and might also contribute to resistance to drug therapies (Liu et al., 2002). Though p53 alterations are infrequent during diagnosis of ALL, in 16% to 28% of children suffering from relapse in ALL point mutations in p53 have been observed (Zhou et al., 1995),

indicating a critical role of p53 in the tumorigenesis process. However, these mutations are much higher in solid tumors and other malignancies.

Since p53 has such diverse and critical functions, there exist mechanisms in the normal cells systems which regulate p53 expression so that it does not malfunction in the absence of external stress stimuli. One of the most important regulators is the MDM2 gene. The protein encoded by this gene, which is an ubiquitin ligase, binds to the N-terminal transactivation domain of the p53 molecule and thus acts as a negative regulator of p53 expression. On receiving stress stimuli, the p53-MDM2 interaction is broken allowing p53 to carry out its functional activities (Sakaguchi et al., 1998; Craig et al., 1999). MDM2 itself is as a proto-oncogenic protein wherein its overexpression leads to suppression of p53 activitv thus disrupting apoptosis. Overexpression of MDM2 and its subsequent inactivation of p53 activity has been reported in several cases of childhood ALL (Marks et al., 1997; Gustafsson et al., 2001). MDM2 also participates in cell cycle regulation, proliferation and apoptosis independent of p53. MDM2 expression levels affect these processes, and are thought to contribute to its role in tumorigenesis process (Zhang and Zhang, 2005). Overexpression of MDM2 has been reported in association with early relapse and drug resistance in pediatric leukemia (Zhou et al., 2000).

The tumorigenesis process involves unlimited cell proliferations due to deregulation of pathways involved in cellular activities. Many of these pathways are not clearly understood in normal cells, and so information about how changes in these pathways lead to malignancies is even more difficult to decipher. Recent advances in evolutionary biology has helped provide tools so that the pathways can be studied in an evolutionary context and thus provide more information regarding the effect of alterations of genes involved in these pathways and their role in tumorigenesis. Weinberg (2007) has indicated that cancer causing genes are highly conserved and those genes that are responsible for multicellularity are most often altered leading to cancer. In previous studies, Khan and Jamil (2008) have used phylogenetic approaches and functional divergence to understand evolutionary history of MTHFR gene and its SNPs, which is reported in association with many diseases including leukemia. Their studies have implicated that SNPs in MTHFR occur in sites under functional constraint and these sites might be fixed in a particular population.

In the current study, our aim was to look at the evolutionary history of p53 and MDM2 genes through the construction of phylogenetic trees, since it may further our knowledge to predict the cell cycle process in cancers as these genes are involved in the cell division process, and further it may be possible to unfold some of the mechanisms of tumorigenesis which are evolutionary.

Materials and Methods

DNA sequence data and sequence alignment The sequences for alignment were retrieved from the NCBI GenBank database (Benson et al., 2011) (available at www.ncbi.nlm.nih.gov) and saved in fasta file format for further analysis. These sequences were imported into the alignment explorer of MEGA version 4 software (Tamura et al., 2007). The ClustalW (Thompson et al., 1994) algorithm was used to perform an initial multiple sequence alignment. This alignment was again manually edited and realigned using ClustalW with default parameters for Gap Opening, Gap Extension Penalty and DNA weight matrix to obtain optimal global sequence alignment. Phylogenetic trees were then built using this multiple sequence alignment file.

Phylogenetic tree building

Phylogeny tree building was carried out using MEGA version 4.0. The Neighbour-Joining (Saitou and Nei, 1987) method, which builds trees using a distance matrix, was chosen for phylogeny reconstruction of the sequences. Kimura 2-parameter (Kimura, 1980) distance model, which assumes uniform rate of substitution among sites, was selected as the nucleotide substitution model. To further increase the reliability of the phylogenetic tree obtained, 1000 Bootstrap replications were performed.

Functional divergence

During evolution many of the residues are subjected to functional constraints. Identification of these sites is important in studies that relate to understanding evolutionary history and can be estimated through functional divergence. In the current study, we calculated functional used as input for the Diverge software. Using

this input, the software was used to build a p-

distance Neighbour-Joining tree to delineate clusters. These clusters were then used to estimate statistical parameters such as site specific profile, which is useful to predict the amino acid residues which are vital for functional divergence. Residues estimated to have a functional divergence value greater than 0.2 were highlighted in the sequence alignment.

S. No.	Organism	Common Name	Nucleotide Accession Number	Protein Accessior Number
1.	Homo sapiens	Human	NM 000546	BAC16799
2.	Cavia porcellus	Domestic Guinea Pig	NM 001172740	NP 001166211
3.	Macaca mulatta	Rhesus Monkey	NM_001047151	AAB91534
4.	Cercopithecus aethiops	African Green Monkey	X16384	CAA34420
5.	Macaca fascicularis	Crab-Eating Macaque	AF456343	AAB91535
6.	Sus scrofa	Pig	NM 214145	NP 998989
7.	Ovis aries	Sheep	NM 001009403	ACP19318
8.	Marmata monax	Woodchuck	AJ001022	CAA04478
9.	Delphinapterus leucas	Beluga Whale	AF475081	AAL83290
10.	Oryctolagus cuniculus	Rabbit	NM 001082404	NP_001075873
11.	Tupaia belangeri chinensis	Chinese Tree Shrew	AF175893	AF175893
12.	, Bos indicus	Zebu	U74486	AAB51214
13.	Felis catus	Domestic Cat	NM_001009294	BAA05653
14.	Xenopus laevis	African Clawed Frog	NM 001088098	CAA54672
15.	Rattus norvegicus	Norway Rat	NM 030989	NP 112251
16.	Danio rerio	Zebrafish	NM 131327	NP 571402
17.	Bos Taurus	Cattle	NM 174201	CAA57348
18.	Oncorhynchus mykiss	Rainbow Trout	NM 001124692	NP_001118164
19.	Oryzias latipes	Japanese Medaka	NM 001104742	AAC60146
20.	Nothobranchius kuhntae	Beira Killifish	EU391597	ACB30549
21.	Meriones unguiculatus	Mongolian Gerbil	AB033632	BAB69969
22.	Mus musculus	House Mouse	EU031806	AAA39883
23.	Canis lupus familiaris	Dog	AF060514	BAA78379
24.	Cricetulus griseus	Chinese Hamster	U50395	AAC53040
25.	Xiphophorus maculates	Southern Platyfish	AF043947	AAC31134
26.	Tetraodon miurus	Congo Puffer	AF071571	AAD34213
27.	Platichthys flesus	European Flounder	Y08919	CAA70123
28.	lctalurus punctatus	Channel Catfish	AF074967	AAC26824
29.	Barbus barbus	Barbel	AF071570	AAD34212
30.	Drosophila melanogaster	Fruit Fly	DQ191318	NP 996268
31.	Xenopus (Silurana) tropicalis	Western Clawed Frog	NM 001001903	NP 001001903
32.	Mesocricetus auratus	Golden Hamster	 U07182	
33.	Oreochromis niloticus	Nile Tilapia	GU594898	ADE21938
34.	Spalax judaei	Blind Subterranean Mole Rat	AJ783406	CAH03844
35.	Bombyx mori	Silkworm	NM_001177410	NP_001170881
36.	Entamoeba histolytica			CAD32988
37.	Mya arenaria	Softshell	AF253323	ACK28179
38.	Xiphophorus helleri	Green Swordtail	AF043946	AAC31133
39.	Coregonus lavaretus	Common Whitefish	EU978857	ACH73252
40.	Loligo forbesi	Northern European Squid	U43595	AAA98563
41.	Tigriopus japonicas	Crustaceans	GQ327969	ADG86236
42.	Homo sapiens (mutant p53)		FJ207420	ACI25593
43.	Rattus norvegicus (mutant p53)		U90328	AAB80959

Table 1: Sequence details of p53.

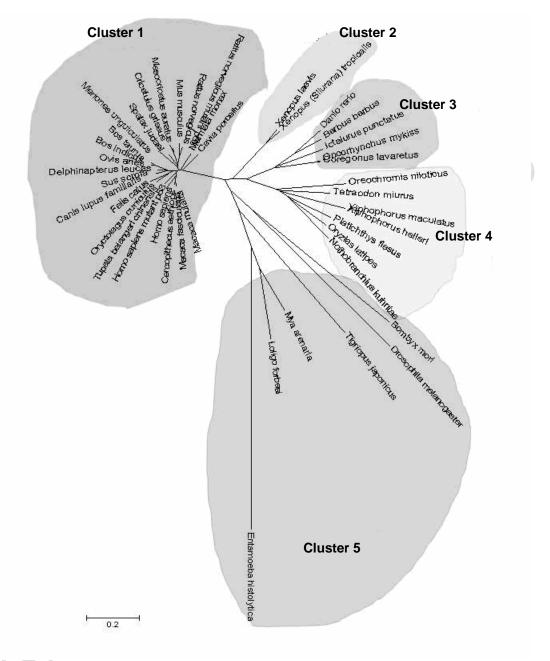


Figure 1: Phylogenetic tree of p53.

Results

We found that p53 and MDM2 showed some sequence similarity across the mammalian species, indicating that these genes probably regulate the normal cell division processes in these species as well. Details of the sequence alignment of p53 and MDM2 showing variable sites are presented in annexure-I and II.

Phylogenetic analysis of p53

A phylogenetic radiation tree constructed with p53 sequences of 41 species and a sequence

each of mutant p53 in Humans and Rats, showed five clusters – a cluster consisting of mammals, a cluster of amphibians, two clusters with different species of fish, a fifth cluster of various other organisms. Information about the gene can be accessed in GeneCards database with GCid: GC17M007565

Phylogenetic analysis of MDM2

Phylogenetic reconstruction of thirteen sequences of MDM2 from various organisms showed five clusters - Humans along with six other species formed the first cluster, Norway Rat and House Mouse form another cluster, Red Jungle Fowl forms an isolated cluster, a fourth cluster of Amphibians, another isolated cluster with Zebra fish. GeneCards database can be queried with GCid: GC12P069201 for information about the gene.

S. No.	Organism	Common Name	Nucleotide Accession Number	Protein Accession Number
1.	Canis lupus familiaris	Dog	AB031276	BAB11975
2.	Mus musculus	House Mouse	U47934	AAB09031
3.	Homo sapiens	Human	GQ848196	ACX31156
4.	Sus scrofa	Pig	EU119401	ABV09038
5.	Bos taurus	Cattle	NM_001099107	NP_001092577
6.	Equus caballus	Horse	NM_001081844	NP_001075313
7.	Felis catus	Domestic Cat	NM_001009346	NP_001009346
8.	Danio rerio	Zebra fish	AF010255	AAB64176
9.	Rattus norvegicus	Norway Rat	NM_001108099	NP_001101569
10.	Xenopus laevis	African Clawed Frog	NM_001092601	NP_001086070
11.	Gallus gallus	Red Jungle Fowl	NM_001199384	NP_001186313
12.	Xenopus (Silurana) tropicalis	Western Clawed Frog	NM_203912	NP_989243
13.	Pongo abelii	Sumatran Orangutan	NM_001131213	NP_001124685
	·			

Table 2: Sequence details of MDM2.

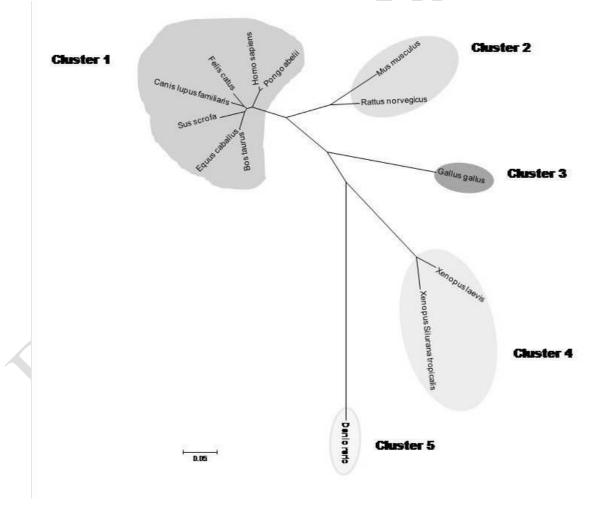


Figure 2: Phylogenetic tree of MDM2.

Functional divergence of p53 and MDM2

We used Diverge 1.04 to calculate the functional divergence of p53 and MDM2 proteins. Neighbour-Joining Tree with p-distance model was used for constructing trees to delineate clusters for both proteins. For the p53 protein, functional divergence was calculated by dividing the species into two clusters. The first cluster contains Mammalian species and the second cluster contains the rest of the organisms. The coefficient of functional divergence between cluster 1 and cluster 2 was 0.23. Further, using a posterior probability of 0.2 as a cutoff value we found 152 residues to be significantly divergent.

For the MDM2 protein also cluster 1 contained the Mammalian species and cluster 2 contained all other species. The coefficient of functional divergence between the two clusters was 0.1 and on applying a posterior probability value of 0.2, six functionally divergent residues were observed. In both the proteins, Mammalian sites were more or less similar to each other while differing from those in the second cluster.

Discussion

Neoplasia is characterized by accumulation of genetic abnormalities in the malignant cells. These changes lead to accumulation and proliferation of malignant cells and affect the patient's ability to fight infections. Though cure rates of 80% have been achieved, many patients still suffer due to therapy resistance and relapse (Pui et al., 2006; Pieters and Carroll, 2008; Bhojwani et al., 2006). p53 and MDM2 genes have been associated with treatment resistance and have been observed to have altered expression levels during relapse especially in leukemia which affect children. The p53 tumor suppression pathway is frequently deregulated in many cancers (Vogelstein et al., 2000). Since the genes in this pathway are functionally active in important cellular activities such as cell growth and proliferation, apoptosis, tumor suppression, they represent important targets to understand disease mechanisms. The key genes in this pathway, p53 and MDM2, have been observed to have a considerable effect in failure of chemotherapy in patients and hence the expression of these genes and their alterations need to be decoded. Phylogenetic studies are increasingly being used in recent years to

understand the evolution of disease genes. The information obtained from phylogenetic study can further be used to construct better models of drug-gene interactions through homology modeling and affinity modeling (Sabitha et al., 2008). Studies conducted by Khan and Jamil (2010) on E2 ubiquitin enzymes, using phylogenetic approaches and through identification of sites under functional constraints, have reported that these enzymes are in a state of active evolution and have indicated that this information could be useful in understanding their role in neoplasia. Further, Shaik et al. (2009 a, b) carried out the phylogenetic analysis of genes related to the toxicological parameters and showed how this method could be useful in determining the evolutionary history of genes or even metaanalysis of polymorphic genes could be useful in predicting solid tumors.

In our study, we have used phylogenetic reconstruction methods to look at the evolutionary history of the p53 and MDM2 genes. A further study of evolutionary history can be done by analyzing the functional diversity of a protein/gene family. In our analysis for proteins of p53 and MDM2 genes, we obtained a coefficient of functional divergence (θ_{MI}) value greater than 0 indicating type I functional divergence. This implies that these select residues/sites are subjected to different evolutionary rates. Most of the sites in these proteins have a posterior probability less than 0.5, implying that there exists functional similarity across the two clusters. This functional similarity could help in identifying the exact mechanism of change which the genes encoding these proteins undergo, resulting in their association in the tumorigenesis process. We observed that these are largely conserved in the mammalian species; the presence of these genes in various species could indicate that these genes could have similar functions or could have evolved due to natural selection process. It is also possible that the presence of these genes in other lower species could indicate that similar disease conditions might exist in those species. A study of these genes in mammalian species that are similar to humans might help elucidate how these genes acquired the function of regulating the cell division.

p53:

	1	1111111112	2222222223	333333334	444445555	6666777777	7777888888	8888999999
Homo sapiens		1234567890 EPPLSQETFS			2346890489			
Marmota_monax		EPPLSQETFS	DLWNLLPENN	VLSPVLS		PPMDDL	LLSSEDVENW	FDKGPDEA
Nomo_sapiens_mutant_p53	MEEPQSDPSV	EPPLSQETFS	DEWKLLPENN	VLSPLPS		QAMDDL	MLSPDDIEQW	FTEDPGPDEA
Rattus_norvegicus_mutant_p53		ELPLSQETFS	CLWKLLPPDD	ILPTTATGS-		PNSMED	LFLPQDVAEL	LEGPEEALQV
Cavia_porcellus Macaca fascicularis		EPPLSQETFS EPPLSQETFS	DEWKELPENN	VISDSLS		PPMDHL	MISPDDIAOM	LGENPDGD LTEDECEDEA
Macaca mulatta		EPPLSQETFS	DLWKLLPENN	VLSPLPS		QAVDDL	MLSPDDLAQW	LTEDPGPDEA
Xenopus_laevis		DPPLSQETFE	DLWSLLPDPL	OTVTCRLDN-				
Delphinapterus_leucas		EPPLSQETFS	DLWKLLPENN	LLSSELS		PAVDDL	LLSPEDVANW	LDERPD
Mus_musculus		ELPLSQETFS	GLWKLLPPED	ILPS-		PHCMDD	LLLPQDVEEF	FEGPSEALRV
Bos_taurus Sus scrofa		EPPLSQETFS	DLWKLLPENN	LLSSELS		LAAVND	LLLSP-VTNW	LDENPD
Cercopithecus_aethiops	MEEPQSDPSI	EPPLSQETFS	DLWKLLPENN	VLSPLPS		QAVDDL	MLSPDDLAQW	LTEDPGPDEA
Ovis_aries		EPPLSQETFS	DLWNLLPENN	LLSSELS		APVDDL	LPYSEDVVTW	LDECPN
Oryctolagus_cuniculus		EPPLSQETFS	DLWKLLPENN	LLTTSLN		PPVDDL	LS-AEDVANW	LNEDPEEG
Tupaia_belangeri_chinensis Bos indicus	MEEPQSDPSV	EPPLSQETFS EPPLSQETFS	DEWKLEPENN	VLSPLPS		QAMDDL	MLSPDDIEQW	FPEDPGPDEA
Felis catus		EPPLSQETFS	FLWNLLPENN	VLSSELS		SAMNEL	PLS-EDVANW	LDEAPD
Rattus_norvegicus		ELPLSQETFS	CLWKLLPPDD	ILPTTATGS-		PNSMED	LFLPQDVAEL	LEGPEEALQV
Danio_rerio		MAQNDSQEFA	ELWEKNLIIQ	PPGGGSCWDI		INDEEY	LPGSFDPNFF	ENVLEEQPQP
Oncorhynchus_mykiss		SLPLSQESFE DLPESQGSFQ	DLWKMNLNLV	AVQPPETESW		VGYDNF	MMEAPLQVEF	DPSLFEVSAT
Oryzias_latipes Nothobranchius kuhntae		RHDSFHDMWM	DLKDNVYBAL	ESPPIPVTYP		DGSDVP	DETWVDOSOM	PLLD
Meriones_unguiculatus	MEEPQSDLSI	EPPLSORTES	DLWKLLPPKN	LLSA-		LEPMED	LLLPQDVTSW	LGDADEALFV
Canis_lupus_familiaris		DPPLSQETFS	ELWNLLPENN	VLSSELC		PAVDEL	LLP-ESVVNW	LDEDSD
Cricetulus_griseus			DLWKLLPPNN	VLSTLFS-		SDSIEE	LFLSENVTGW	LEDSGGALQG
Xiphophorus_maculatus Tetraodon miurus		TLPLSQDTFH -LPLSQDTFQ	DEWENVELST	ENESLPPPEG TSTIOTANE		N	-FAMDAFDOM	NMMCNE
Platichthys flesus		ILPGSQDSFS	ELWASVOTPS	IATIAEEFDD		N	-LAWFALKON	-HLGNL
Ictalurus punctatus		VEPPDSQEFA	ELWIRNLEVR	DNSL		WGKEEE	IPDDLQEVPC	DVLLSDMLQP
Barbus_barbus		VAESOFFA	FINTENDER	OFAG-TOWEL		TN-DEY	LESSEDENTE	DNVLTFOPOP
Drosophila_melanogaster		KESTDSEDDS	TEVPIKEPIP	KTVEVSGS		EL	TTEPMAFLQG	LNSGNLMQFS
Xenopus_(Silurana)_tropicalis Mesocricetus_auratus		ELPLSQETFS	DEWELLPPL	VISTIP		SDSIFF	L FL SENVACW	LEDRGEALOG
Oreochromis_niloticus		SLPLSQESFP	DEWANVVMPT.	STICTADIN-		RP	TGSWVASLTM	ALMD
Spalax_judaei	MEEQQSDLSI	EPPLSQETFS	DLWKLLPONN	VLSTPLSP		NSMEDL	LLSPEDVANW	LDDPDEA
Bombyx_mori		VATCEDDIVN	IDLNFIPDEA	LFQGGIH		DQV	DIGVLDDIPY	IISDVS
Entamoeba_histolytica Mya arenaria		IKQVNKMSII NQPMSQETFE			ELDSDDSGQV			
Mya_alenalia Xiphophorus hellerii	MEEADI.	TLPLSODTFH	DEWNVELST	ENESLAPPEG	ETD2DD2GŐA	EKENQERTDV	SDEEMPIIGI	15555M5ED5
Coregonus_lavaretus	MADLVENV	TLPLSQDTFH SLPLSQESFE	DLWKMNLNIM	EVQPPVTEAW		VEYDNF	MMEAPLQGEF	DQSLFEVSAP
Loligo_forbesi	MSQG	TSPNSQETFN	LLWDSLEDVT	AN-EYTQIHE	GVGEHEAQEI	SAYAYGRSES	YDLLNPIINQ	IPAPMPIADT
Tigriopus_japonicus	MSHKLGVFRK	QQPKISVMVP	PKGPMVPRAT	KSEDEYLEVK		PESV	LNLSCDNSES	SSEVPKLSLK
	111111	1111111111	11111111111	11111111111	11111111111	1111111111	1111111111	1111111111
		0000111111				AAAAEEEEEE	FEFCECCCCCC	CC777777777
		6789012345		678901-345	6789012245	CTHASTAK CTHSGTAK CTHSGTAK CTHSGTAK CTHSGTAK CTHSGTAK CTHSGTAK CTHSGTAK CTHSGTAK CTHSGTAK	7 8901245 67	89 012 34567
Homo_sapiens		PPVAPAPAAP		WPLSSSVP\$Q	6789012745 KTYQGSYGFR	LGFLHSGTAK	SVTCTYPALN	KIFCQLAKTC
Marmota_monax Homo sapiens mutant p53		APKAPTPAAS PRVAPAPAAP		WPLSSEMPSQ	NTYPGVYGFR KTYQGSYGFR	CHUNCTAK	SVICIYPSIN	KLFCQLAKTC
Rattus norvegicus mutant p53		AAQEPGTEAP		WPLSSSVP\$O	KTYOSNYEFH	LGFLOSGTAK	SVMCTYISLN	KLFCOLAKTO
Cavia_porcellus		VSEAPTSAGP		WPLSS SVPSH	KPYRGSYGFE KTYHGSYGFR KTYHGSYGFR	VIITIKSGTAK	SVTCTYPGLN	KLFCQLAKTC
Macaca_fascicularis		PPMAPTPAAP		WPLSSBVPSQ	KTYHGSYGFR	LGFLHSGTAK	SVTCTYPDLN	KHECQLAKEC
Macaca_mulatta		PPMAPTPAAP		WPLSSSVPSQ	KTYHGSYCFR	LOFINSGTAK	SVTCTYPDLN	KINFCOLAKTC
Xenopus_laevis Delphinapterus leucas		YPLAADMSVL MPEPPAPAAP			DDYAGKYGLQ KTYPGSYGFH	LOPTHSGTAK	SVTCTYPELN	KLECOLAKIC
Mus musculus		AAQDPVTETP		VPLSS TVPSQ	KTYPGSYGFH KTYQCNYGFH KTYPGNYGFR KTYPGSYDFR KTYPGSYDFR KTYPGNYGFR	CHOSCIAK	SVMCTYPPLN	KLFFCLAKTO
Bos_taurus		MPEPSAPAAP		WPLSS TVPSQ	KTYPGNYGFR	LGTIQSGTAK	SVTCTYPSLN	KLFCQLAKTC
Sus_scrofa		VPAPPAATAP		WPLSSFVPSQ	KTYPGSYDFR	LGFLHSGTAK	SVTCTYPSLN SVTCTYPALN SVTCTYPDLN	KLFCQLAKTC
Cercopithecus_aethiops		PHMAPTPAAP MPEPP		WPLSS SMPSQ	KTYHGSYGFR	LOTHSCTAK	SVTCTYPDLN	KINFCQLAKTO
Ovis_aries Oryctolagus cuniculus		APEAPAPAAP					SVTCTYPSLN SVTCTYPCLN	KLECOLAKTC
Tupaia belangeri chinensis		PPVAPAPAAP		WPLSSSVPSQ	KTYQGSYGFR KTYPGNYGFR KTYPGAYGFH	LGFLHSGTAK	SVTCTYPDLN	KLFCOLAKTC
Bos_indicus		MPEPSAPAAP		WPLSS FVPSQ	KTYPGNYGFR	LGFLQSGTAK	SVTCTYPDLN SVTCTYPSLN	KLFCQLAKTC
Felis_catus		MSAVPAPAAP AAQEPGTEAP		WPLSS FMPSQ	KTYPGAYGFH KTYQGNYGFH	LGFLQSGTAK	SVTCTYPPLN SVMCTYISLN	KLFCQLAKTC
Rattus_norvegicus Danio rerio				T D D T C T L D T T	CDVDCDUCED	I DEBOCCENC.	SVTCTYPDLN	KLECOLAKTO
Oncorhynchus mykiss	EP	AP	QPSISTLDTG	SPETSTVETT	SDYPGALGFQ	RELOSSTAK	SVTCTYPDLN	KLFCQLAKTC
Oryzias_latipes	LS	GTFDDKIFDI	PIEPVPTNEV	NPPPTTMPT	TDYPGSYLLE	LRECKSGTAK	SVISIYETLN	KLYCQLAKTS
Nothobranchius_kuhntae	S	AP GTFDDKIFDI QTYNQLISEL AEG-PAPEAP MPATSAPTAP TAEDPVTETP LLSONMDFWE	PVDMPQKDCI	LPTSSTMPYT	TDHFGDYOLE	LRECKSGTTK	SVTCTYPDLN SVTCTYPDLN SVTSTYETLN SVTSTFEQLN SVTSTFEQLN SVTCTYPSLN SVTCTYPSLN SVTCTYPSLN	KLFCRLAKTT
Meriones_unguiculatus Canis lupus familiaris	CI AP	MPATSAPTAP	GPAPS	NPLSSEVPEP	KTYPGTYCFR	GRUHSGTAK	SVICTIPSEN	KLECOLAKIC
Cricetulus_griseus	VAAAAAS	TAEDPVTETP	AFVASAPATE	WPLSESVPSY	KTFQGDYGFR	GFLHSGTAK	SVTCTYPSLN	KLECCLAKTC
Xiphophorus_maculatus		LLSQNMDFWE	DFETMQETKN			DETTI PROTING	OATOT TATCHO	TITT A MERITE T
Tetraodon_miurus		STFNEALFNL			TDYPGEY6FK	LRECKSGTAK	SVTSTYEILN	KLYCQLAKTS
Platichthys_flesus	LQ	NGFDMNLFEL	PPEMVAKUSV	ED DITERVIDUT	SDYPGULUET	HEARSCEAR	SVTSTFELLK SVTCTYPDLN	K FCOLAKTS
Ictalurus_punctatus Barbus_barbus			ST	SPETASMEVA	TDYPGENGEK	LEFTQSGTAK	SVICTYSDLN	KLFCQLAKTO
Drosophila_melanogaster	QQ	SV	LREWWIGDIG	L QANL' DEKLE	NHNIGGYCHS	NVEDEPE	SVTCTYSDLN KSLWMYIPLN SVTCTYTDLN SVTCTYPSLN	KLYI RMNKAF
Xenopus_(Silurana)_tropicalis				TVTSSAVPST	EDYAGSYGLK	LEFOONGTAK	SVTCTYTDLN	KLFCQLAKTC
Mesocricetus_auratus		AAEDPVAETP		WPLSS SVPSY	KTYQGDYGFR	LGHLHSGTAK	SVTCTYPSLN	KLFCQLAKTC
Oreochromis_niloticus Spalax_judaei		PDLN-CLFEL ITGDPVTETS		NDI SERVIDEO	TDHPGEYDFK KTYQGSYGFR	GETHSGTAK	SVTCTYPPIN	KLECCLARTC
Bombyx mori				LGPREPPR	KDDPGQYTES	VEINS-KDTH	KKKFLLHKLN	RIYVNMETDE
Entamoeba_histolytica	QG	VNYIVEI	KDEKMFNEFK	RKMVKKOERE	KDDPGQYLFS EDNKDIYNII	EMINKKEIIN	KKKFLLHKLN KMIIHISSIN STTWTYDILK	KERKEYNQKE
Mya_arenaria		SPYQEMTLTS		SPIPLVPAN	TNALGUAGLE	STATSKETK	SETWTYDILK	KLYVRMATTC
Xiphophorus_hellerii Coregonus lavaretus	0	LLSQNMDFWE	-PSISTINC	SPETS TIPT	SNYAGEHEEN	LEINDSGTAK	SVTSTYVKLG	KLFCQLAKTT
Loligo_forbesi	QNNPLVNH	CPYEDMPVSS	TP-YSPHDHV	QSPOPEVPSN	IKYPGEYVFE	MSFAQSKETK	STTWTYEKLD	KLYVRMATTO
Tigriopus_japonicus		LTQTNATK		RGASVTHLAT	DIWRGGYDFD	TITNDDQSK	AKNVVYKMLN	KLEVDVNKLF
						0 0	2	

Homo_sapiens Marmota_monax Homo_sapiens_mutant_p53 Rattus_norvegicus_mutant_p5: Cavia_porcellus Macaca_fascicularis Macaca_mulatta Xenopus_laevis Delphinapterus_leucas Mus musculus Mus_musculus Bos_taurus Sus_scrofa Cercopithecus_aethiops Cercopithecus_aethiops Ovis_aries Oryctolagus_cuniculus Tupata_belangeri_chinensis Bos indicus Felīs_catus Rattus_norvegicus Danio_rerio Oncorhynchus_mykiss Oryzias_latipes Nothobranchius_kuhntae Meriones uncuiculatus Nothobranchius_kunntae Meriones_unguiculatus Canis_lupus_familiaris Cricetulus_griseus Xiphophorus_maculatus Tetraodon_miurus Platichthys_flesus Laticus_processus Platichthys_flesus Ictalurus_punctatus Barbus_barbus Drosophila_melanogaster Xencpus_(Silurana)_tropidal: Mesocricetus_auratus Oreochromis_niloticus Spalax_judaei Bombyx_mori Entamoeba_histolytica Mya_arenaria Xiphophorus_hellerii Goregonus_lavaretus Coregonus_lavaretus Loligo_forbesi Tigriopus_japonicus Homo_sapiens Marmota_monax Homo_sapiens_mutant_p53 Rattus_norvegicus_mutant_p5: Cavia_porcellus Macaca_fascicularis Macaca_mulatta Xenopus_laevis Delphinapterus_leucas Mus musculus Mus_musculus Bos_taurus Sus_scrofa Cercopithecus_aethiops Cercopithecus_aethiops Ovis_aries Oryctolagus_cuniculus Tupaia_belangeri_chinensis Bos_indicus Felis_catus Rattus_norvegicus Danio_rerio Oncorhynchus_mykiss Oryzias_latipes Nothobranchius_kuhntae Nothobranchius_kuhntae Meriones_unguiculatus Canis_lupus_familiaris Cricetulus_griseus Xiphophorus maculatus Tetraodon_miurus Platichthys_flesus Flatichthys_flesus
Ictalurus_punctatus
Barbus_barbus
Drosophila_melanogaster
Xenopus_(Silurana)_tropical:
Mesocricetus_auratus
Oreochromis_niloticus
Spalax_judaei
Bombyx_mori
Entamoeba_histolytica
Mva arenatia

Mya_arenaria Xiphophorus_hellerii Coregonus_lavaretus Loligo_forbesi Tigriopus_japonicus

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	PVQLWVES PVQLWVES	TPPPGTRVRA	MAIYKKSQHM MAIYKOSQHM	TEVNERCPEH	ERCSD	SDGIAPPOHI. SDGIAPPOHI	IRVEGNI	. RAEYLDERNT
53	PVQLWVIS	TPPPGTRVRA PPPPGTRVRA	MAIYKKSQHM	TEVMERCEN	ERCSD ERCSD	GDGLAPPOHL	IRVEGN	? YAEYIDIRQT
	PVQVWVES PVQLWVDS	TPPPGSRVRA	LAIYKKSQHM MAIYKOSQHM	TEVMERCPER	ERCSD	SDGIAPPOHL SDGIAPPOHL	IRVEGNI	
	PVQLWVES PLLVRVES	TPPPG5RVRA PPPRG5ILRA	MAIYKOSQHM FAVYKKSEHV	TEVMFRCPIH AEVMFRCPIH	EPCSD EPSVEP	SDGLAPPOHL GEDAAPPSHL	IRVEGNI MRVEGNI	QAYYMEDVNS
	PVQLWVSS	PPPPGTRVRA TPPAGSRVRA	MAIYKKSEYM MAIYKKSQHM	TEVMFRCPHH TEVMFRCPHH	ERCSDYD	SDGLAPPOHL GDGLAPPOHL	IRVEGNI IRVEGNI	S RAEYLDERNT S YPEYLEERQT
	PVQLWVDS PVQLWVSS	PPPPGTRVRA PPPPGTRVRA	MAIYKKLEHM MAIYKKSEYM	TEVMFRCPEH TEVMFRCPEH	EPSSDY	SDGLAPPOHL SDGLAPPOHL	IRVEGNI	L RAEYLDIRNI L RAEYLDIRNI
	PVQLWVES PVQLWVES	TPPPGSRVRA PPPPGTRVRA	MATYKQSQHM MATYKKLEHM	TEVMERCERE	ERCSD	SDGLAPPOHL SDGLAPPOHL	TRVEGNI	RVEYSDERNT
	PVQLWVES PVQLWVES	TPPPGERVRA APPPGERVRA	MAIYKKSQHM MAIYKQSQYV	TEVMPROPER	ERCSD	SDGLAPPOHL SDGLAPPOHL	IRVEGNI	L RAEYLDERNE
	PVQLWVDS	PPPPGTRVRA PPPPGTCVRA	MAIYKKLEHM	TEVVERCPEH	ERSSDY	SDGLAPPQHI.	IRVEGNI	RAEYLDERNT
	PVQLWVIS	TPPPGTRVRA	MAIYKKSQHM	TEVMERCPEH	ERCSD	GDGLAPPOHL	IRVEON	P YAEYIDIRQT
	PVQMVVEV PVQIVVEH	APPQG5VVRA PPPPGAVVRA	faiykksenv laiykklsdv		ERTPDG QSTSENN	-DNIAPAGHL -EGPAPRGHL	IRVEGN	2 RSEYMEIGNT
	PIEVRVSK PVEVLVSF	EPPKGAILRA EPPQNAILRA	FAVYKKTEHV FAVYKKSEHV	ADV PROPER AEAMPROPER	QNEDSV	EHRSHL DNKSHL	IRVEGS	2 LACYFELPYT 2 LACYFELPFT
	PVQLWVSS PVQLWVSS	APPPGTRVRA PPPPNTCVRA	MAIYKNSQHM MAIYKKSEFV	TEVMPROPERI TEVMPROPERI	ERCSENEASD ERCSDS	PRGRAPPOHL SDGLAPPOHL	IRVEGNI	L HAEYVDERQT RAKYLDERNT
	PVQLWVNS PIGVLVNE	TPPPGTRVRA EPPOGAVIRA	MAIYKKLQYM FAVYKKTEHV	TEVMERCPER	ERSSE QSEDLS	GDSIAPPOHL DNKSHL	IRVEGNI	- HAEYLDIKQI
	LVEVLIGK PVEVLISK	DPPMGAVLRA EPPQGAVLRA	TAIYKKTEHV TAVYKKTEHV	AEVVERCEEL ADVVERCEEL	QNEDSA QTEDTA	EHRSHI	IRMEGSF	E RAQYFEHRHT 2 RALYFEDRHT
	PVLMAVSS PVQMVVNV	SPPPGSVLRA APPQGSVIRA	TAVYKRSEHV FAIYKKSEHV	AEVAFROPER	ERSNDSS	-DGPAPPGHL -DGIAPAAHL	LRVEGNS	8 RAVYQELGNT
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lis	PLLVRVEP PVQLWVSS	PPPLGSTLRA TPPPGTRVRA	TAVYKKSEHV MAIYKKLQYM		ERSVEPE	GDDPAPPSHI. SDGLAPPOHL	MRVEGNS IRVEGNN	4 HAEYLDIKQI
	PVEVLVSK PVQLWVES	EPPKGALLRA TPPPGTRVRA	favykksenv Maiykksohm	AEAMPROPER TEVMPROPER	QNEDSV ERCSD	EHRSHL SDGLAPPQHL		L RAEYLDEKHT
		LAATOMYVRA KRNNENIIND	EVVFEDESQA LMKIRMNDMF	EKRWERCIQH SKYLETKPTS	KLCSSDKGQD CIVFKKS	RVVSENVLRS -SLYQIIPFS	SRPLGTN NKLNGYLITI	
	PVRFKTIF PIGVLVKE	QPPPGCVIRS EPPOGAVIRA	MPIFMKPEHV ESVYKKTEHV	QEAMPROPIE GEVMPROPIEI	ATSKEFN	-ENHPAPNHL DNKSHL	VRCEHK	- VSKYVELPYT
	PVQIVVEH PVRFKTAF	PPPPGAVVRA PPPSGCQIRA	LAVYKKLSDV MPIYMKPEHV	ADVVPRCPEH QEVVPRCPEH	QSTSENN	-EGFAPRGHL -EKHPAPLHI		2 RAEYMEDGNT - LAKYHEDKYS
	QIRYYIKSG-	CNLSDFOVRA	IMVYSDIASY	VEPMIRCPER	QAQESE	-ETKHKNAHV	LWADSN	- DAYYDEDITS
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lis	666677777 967941271 977427127 97815777727 97815777722 97815777722 97815777722 97815777722 97815777722 97815777722 97815777722 97815777722 97815777722 978157772222 978157772222 9781577772222 9781577772222 9781577772222 9781577772222 9781577772222 9781577772222 9781577777777222 9781577772222 9781577772222 9781577772222 9781577772222 97815777772222 97815777772222 97815777772222 97815777772222 9781577777777777777777777777777777777777	7778888888 5690 [2,459 P	888999999 780026789 7110000000 71100000000 7110000000000	0000001 DE DI 567890 SM GGMI REP SM GGMI REP	11111111112 1234557890 ILTIIFLEDS	222222223 123 D 67 500 SGN GRISP SGN GRISP	3333333 123 B 4 89 123 B 4 89 124 B 4 8	1 444444445 1 1234367890 0 DRM TERNIR 0 DRM TERNIR

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these forest series		1234667890		1234567890			234567 8901	2045678901
Homo_sapiens Marmota_monax		PGSTKRALPN	NTS-SSPORK GTS-SSPORK	К	KPLDGEYF		ARFEMFRELN	EALELKIAQA EALELKIAOA
Homo_sapiens_mutant_p53	KKGEPHHELP	PGSTKRALPN	NTS-SSPOPK	K	KPLDGEYF	TLQIFGR		EALELKDAQA
Rattus_norvegicus_mutant_p53		PGSAKRALPT			KILDGEYF		ERFEMERELN	
Cavia_porcellus Macaca_fascicularis	KKGGLCPEPT KKGEPCHOLP		STS-SSPORK NTS-SSPORK	K	KPLDAEYF		KNFEILREIN ERFEMFRELN	
Macaca_mulatta	KKGEPCHQLP	PGSTKRALPN	NTS SSPOPK	K		TLQIPGR	ERFEMERELN	EALELKDAQA
Xenopus_laevis	KKRGLKPS	GKRELAH		KRLV				DALELQESLD
Delphinapterus_leucas Mus musculus	KKEVLCPELP	TGSAKRALPT PGSAKRALPT		К	KPLDGEYF		ERFEMFRELN	
Bos_taurus	KKGQSCPEPP			K		TLQIPGF	KRYEMFRELN	DALELKDALD
Sus_scrofa Cercopithecus_aethiops	KKGQSCPEPP KKGEPCHELP			K	KPLDGEYF KPLDGEYF		ERFEMFRELN	
Ovis aries	KKGQSCPEPP		STS-SSPOOK		KPLDGEYF			EALELMDAQA
Oryctolagus_cuniculus			TTTDSSPORK			ILKINGR		EALELKDAQA
Tupaia_belangeri_chinensis Bos_indicus	KKGQSCPEPP	TGSIKRALPT PRSTKRALPT	GSS-SSPOPK NTS-SSPOPK	K	KPLDEEYF		ERFEMIREIN	DALELKDALD
Felīs_catus	KKGEPCPEPP	PGSTKRALPP	STS-STPPQK		KPLDGEYF	TLOIPGR	ERFEMFRELN	
Rattus_norvegicus Dania_raria			STS-SSPOOK KESSSATIRP				ERFEMERELN	
Danio_rerio Oncorhynchus_mykiss		TTTGTKRSLV TKPADCIKRA	MKEASLPAPQ	EGSKKAKGS- PGASKKTKSS	-PAYSDDEIY		ERYEILKKLN EKYEMLKKFN	
Oryzias_latipes		KKRK/TPNTS	SKRKKS		EEDNREVF		ERYEFLKKIN	
Nothobranchius_kuhntae Meriones_unguiculatus	KKESGSKQTQ KKQ-RCPELP		SLTTPAKKMK NTS-SSPCSK	SSSSG R	EDEDKEMI KPADGEYF		NLMKRIS KRFEVFRELN	
Canis_lupus_familiaris	KKGEPCPEPP		STS-SSPPQK	К	KPLDGEYF	TLOIPGR		
Cricetulus_griseus	KKGEPCPELP		NTS-SSPPPK		KTLDGEYF		ERFKMFQELN	
Xiphophorus_maculatus Tetraodon miurus	KSGTK KMQNDAKDAK		PDTSTAKKSK PDSTTIKKSK	SASSG TASSA	EDEDKELY		NRYLWFKSLN KRYEMLKKIN	
Platichthys_flesus	KTPNGPKQTK	KRKQAPSNSA	PHITTVMKSK	SSSSA	EEEDKEVF	TVLVKGR	ERYEIIKKIN	EAFEGAAEK-
Ictalurus_punctatus Barbus barbus	KQQEPKTSGK KDQETKTLDK		-SMEDPPSHP KDSTSSVPRP		SSDDEIY SSDEEIY		ERYEFLKKIN ERYEMLKKIN	
Drosophila melanogaster		AEEDEPSKVR			RECODSAA		DYRLAITCPN	
Xenopus_(Silurana)_tropicalis				KRLV	EEDDEETF	TLLENGR	SRYEMIKKLN	DALELQESLD
Mesocricetus_auratus Oreochromis niloticus			NTS-SSPORK SLTTPAKKMK		KTLDGEYF EPEDKEVF		ERFKMFDELN GRYEMFKKIN	
Spalax_judaci		PGSTKRALPT		K	KPLDGEYF	TLKINGR		
Bombyx_mori		RAVAPPOEDO			RDPHPAA	GGSSRSD	QHWTIALNTT	
Entamoeba_histolytica Mya arenaria		DMKLPRTLKI SQMPKFSMGT		RK			SKRLLFUSLL	
Xiphophorus_hellerii	KSGTK	QTKKRKSAPA	PDTSTAKKSK	SASSG	EDEDKEIY	TLSINGR	NRYLWFKSLN	DGLELMDKTG
Coregonus_Lavaretus Loligo forbesi			VKEASLPAPR NDITKITPKK		SPAYSDDIY IDDECF	TLOIPGK	EKYEMLKKLN ENYEILCKLR	
Tigriopus_japonicus		RKRKAPSSVG			NNKMSSSDIY		KTLNKFAEFL	
	444444444	444444455	5555555555	5555555555	5555555555		-	10
	3333333344	4444999900	0000000011	1111111122	222222233			
Homo_sapiens				2345678901 -QSTSRHKKL				
Marmota_monax				-QSTSRHKKI				
Homo_sapiens_mutant_p53				-QSTSRHKKL -QSTSRHKKP				
Rattus_norvegicus_mutant_p53 Cavia porcellus				-QSTSCHKKL				
Macaca_fascicularis	GKEPAGSBAH	SSHL	-KSKKG					
			ROMAC		MFKTEGPDSD			
Macaca_mulatta Xenopus laevis	GKEPAGSRAH	SSHL		-QSTSRHKKF	MFKTEGPDSD MFKTEGPDSD			
Xenopus_laevis Delphinapterus_leucas	GKEPAGSRAH QQKVT GKEPGESRAH	SSHL IKCR SSHL	-KCRDE -KSKKG	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD			
Xenopus_laevis Delphinapterus_leucas Mus_musculus	GKEPAGSRAH QQKVT GKEPGESRAH TEESGDSRAH	SSHL IKCR SSHL SSLQ	-KCRDE -KSKKG -PRAFQ	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL -ALIKEESPN	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD C			
Xenopus_laevis Delphinapterus_leucas Mus_musculus Bos_taurus	GKEPAGSRAH QQKVT GKEPGESRAH TEESGDSRAH	SSHL IKCR SSHL SSLQ SSHL	-KCRDE -KSKKG -PRAFQ -KSKKR	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD C MLKREGPDSD			
Xenopus_laevis Delphinapterus_leucas Mus_musculus Bos_taurus Sus_scrofa Cercopithecus_aethiops	GKEPAGSRAH QQKVT GKEPGESRAH TEESGDSRAH GREPGESRAH ARESGENRAH GKEPAGSRAH	SSHL IKCR SSHL SSLQ SSHL SSHL SSIIL	-KCRDE -KSKKG -PRAFQ -KSKKR -KSKKG -KSKKG	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL -ALIKEESPN -PSPSCHKKP -QSPSRHKKF -QSTSRHKKF	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD C MLKREGPDSD MFKREGPDSD MFKTEGPDSD			
Xenopus_laevis Delphinapterus_leucas Mus_musculus Bos_taurus Sus_scrofa Cercopithecus_aethiops Ovis_aries	GKEPAGSRAH QQKVT GKEPGESRAH TEESGDSRAH GREPGESRAH ARESGENRAH GKEPAGSRAH GREPGESRAH	SSHL IKCR SSHL SSHL SSHL SSHL SSHL	-KCRDE -KSKKG -PRAFQ -KSKKR -KSKKG -KSKKG	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL -ALIKEESPN -PSPSCHKKP -QSPSRHKKP -QSTSRNKF -PSPSCHKKP	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD MLKREGPDSD MFKREGPDSD MFKTEGPDSD MFKTEGPDSD			
Xenopus_laevis Delphinapterus_leucas Mus_musculus Bos_taurus Sus_scrofa Cercopithecus_aethiops	GKEPAGSRAH QQKVT GKEPGESRAH TEESGDSRAH GREPGESRAH ARESGENRAH GKEPAGSRAH GREPGESRAH EKEPGGSRAH	SSHL IKCR SSHL SSHL SSHL SSHL SSHL SSHL SSHL SSH	-KCRDE -KSKKG -PRAFQ -KSKKR -KSKKG -KSKKG -KAKKG	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL -ALIKEESPN -PSPSCHKKP -QSPSRHKKF -QSTSRHKKF	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD MFKREGPDSD MFKREGPDSD MFKREGPDSD MFKREGPDSD			
Xenopus_laevis Delphinapterus_leucas Mus_musculus Sus_scrofa Cercopithecus_aethiops Ovis_aries Oryctolagus_cuniculus Tupaia_bolangeri_chinensis Bos_indicus	GKEPAGSRAH QC	SSHL IKCR SSHQ SSHL SSHL SSHL SSHL SSHL SSHL SSHL	-KCRDE -KSKKG -PRAFQ -KSKKG -KSKKG -KSKKG -KSKKG -KSKKG	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL -ALIKEESPN -PSPSCHKKP -QSTSRHKKF -QSTSRHKKF -QSTSRHKKF -QSTSRHKKF -QSTSRHKKL -SSPSCHKKF	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD MFKREGPDSD MFKREGPDSD MFKREGPDSD MFKREGPDSD MFKREGPDSD MFKREGPDSD			
Xenopus_laevis Delphinapterus_leucas Mus_musculus Bos_taurus Sus_scrofa Cercopithecus_aethiops Ovis_aries Oryscolagus_cuniculus Tupaia_belangeri_chinensis Bos_indicus Felis_catus	GKEPAGSRAH QCKVT GKEPGESRAH TEESGDSRAH GREPGESRAH GKEPAGSRAII GREPGESRAH GKEPAGSRAH GREPGESRAH GKEPGGSRAH	SSHL IKCR SSHL SSHL SSHL SSHL SSYL SSHL SSHL SSHL SSHL	- KCRDE - KSKKG - FRAFQ - KSKKR - KSKKG - KSKKG - KSKKG - KSKKG - KSKKG	-QSTSRHKKF -IKPKKGKKL -QSPSRHKKL -ALIKEESPN -PSPSCHKKF -QSTSRHKKF -QSTSRHKKF -QSTSRHKKF -QSTSRHKKL -SSFSCHKKF -QSTSRHKKF	MFKTEGPDSD MFKTEGPDSD LVKDEQPDSE MFKREGPDSD MFKREGPDSD MFKTEGPDSD MFKTEGPDSD MFKREGPDSD MFKTEGPDSD MLKREGPDSD MLKREGDSD			
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				5678123456				
Canis lupus familiaris				KDTYKEVIFY				
Mus musculus	NMSVSEGAAS	TSQIPASEQT	RKPLLKKSVQ	NDTYKEIIFY	IQTREKQYSN	LDVFPSHKIY	AMYVAVSQQD	SGTSLSE
Homo_sapiens	NMSVPDGAVT	TSQIPASEQ-		TLVLFY	LQTREKQYSN	LDLFPSHKIY	TMYVVVNQQE	SSDSGTSVSE
Sus_scrofa	NMSVSDGAVS	TSQIPASEQT	RKPLLKKSVQ	KDTYKEVTFY	LQTREKQYSN	LDLFPSHKIY	TMYIVVNQQE	PSDSSTSVSE
Bos_taurus				KDTYKEVIFY				
Equus_caballus				KDTYKEVIFY				
Felis_catus				KDTYKEVIFY				
Danio_rerio				KDVEKEVMFY				
Rattus_norvegicus				TLIIFY				
Xenopus_laevis				KETEKEVIYH				
Gallus_gallus				KDTEKEVIFY				
Xenopus_Silurana_tropicalis				KDTYKEVLFY				
Pongo_abelii	NMSVEDGAVI	TOQTEASEQT	RELLERSVQ	ADI IKEV LFI	LQIREAQISM	LDLFFSHKLT	IMIAAANQQE	SSDSGISVSE
	111111111	1111111111	1111111111	11111111111	11111111111	11111111111	1111222222	22222222222
				5556666666				
				7890123456				
Canis lupus familiaris				PSTSSRRRAI				
Mus musculus				LSTSSRRRSI				
Homo sapiens				PSTSSRRRAI				
Sus scrofa				PSTSSRRRAV				
Bos taurus				PSTSSRRRAV				
Equus caballus	NRCHLEGGSN	QKDLVELQEE	KPSSSDMVSR	PSTSSRRRAV	SETENSD	ELPGERQHKS	NISEALCVIR	EICCERSSSE
Felis_catus	NRCHLEGGSD	QKDPVELQEE	KPSSSDLVSR	PSTSSRRRTI	SETEHSD	ELPGERQHKS	SISEALCVIR	EICCERSSSE
Danio rerio	SQSTF	SEPRSSEPDR	GPGDTDSDSR	SSTSQQQRRR	RRSSDPSSSA	EDESRERHKS	SFTDSWCVIG	GLHRE-RGNE
Rattus_norvegicus	SRCQPEGGSD	LKDPVASQEE	KPSSSDVVSR	PSTSSRRRAI	SETENID	ELPGERQHRA	SEGLCVLR	EICCERSSSE
Xenopus_laevis	VCCFPDKQSS	QKEKLELPDK	LIAPASDSKP	CNLSQRKSSN	ETEISSV	DHPAEQQHKS	SFTESWWVIS	GLRCD-RNSE
Gallus_gallus	AKFRLEKENV	LKESMELEEK	QTSSNATS	QPTTSRRRTH	SESENSS	DDLHSDRHKS	SITESWCVVS	GLCRDRSNSD
Xenopus_Silurana_tropicalis	VCSFPDKQKS	QKELLELPEK	VIAPAYDSKP	CNSSQRKSNN	ETVCVEISSV	DHPAEQQHKS	SITESWWVIS	GLRCD-RNSE
Pongo_abelii	NRCHLEGGRD	QKDLVELQEE	KPSSSHLVSR	PSTSSRRRAI	SETENSD	ELSGERQHKS	SISEALCVIR	EICCERSSSE
					L			
				2222222222				
				7777777778				
				1234567890				
Canis_lupus_familiaris				SEEGQELSDE				
Mus_musculus				SDEGHELSDE				
Nome_sapiens				SEEGQELSDE				
Sus_scrofa Bos taurus				SEEGQELSDE				
Equus_caballus				SEEGQELSDE				
Felis_catus				SEEGQELSDE				
Danio_rerio				NDVDSVPGEN				
Rattus_norvegicus				SDEGHELSDE				
Xenopus_laevis				SGDEHGVSEE				
Gallus gallus	STESVIPLDA	SSLSENSDWF	DHGVQIYEDH	NEEGQELTDE	DDVQLIYQDE	DDSDSNEPSL	PESEMRHHRA	LEDDEKSDKL
Xenopus_Silurana_tropicalis	STETSNPPEK	HTVDDNS	EQDDQVYDDP	SGDEHCISEE	EEVQVIYEAE	DETNADVTSE	SEEEISHHRA	LKDEESKKEL
Pongo_abelii				SEEGQELSDE				
				3333333333				
				8888899999				
1 1 1 1 1 1 1 1 1				5678901345				
Canis_lupus_familiaris				SRESCAEEID				
Mus_musculus				AKEPCAEDSE				
Homo_sapiens				SRESCVEEND				
Sus_scrofa				SRESCAEEND				
Bos_taurus				SRESCVEEND				
Equus_caballus Folic_catus								
Felis_catus Danio_rerio				SRESCAEEND SPLPETD				
Danio_rerio Rattus_norvegicus				AKESSAEDSE				
Xenopus_laevis				SQDTNVDKKE				
Gallus gallus				DKEPAVEENE				
Xenopus_Silurana_tropicalis								
Pongo abelii				SKESCVEEND				
	4444444444							
	4444555577							
	6789012906							
Canis_lupus_familiaris	FPLNAIEGKA							
Mus_musculus	FSLNAIEGKS							
Homo_sapiens	LPLNAIEGKA	FKKPQMIFP						

Canis_lupus_familiaris	FPLNAIEGRA	FKKFQMIFF
Mus musculus	FSLNAIEGKS	FKKPQMIFN
Homo sapiens	LPLNAIEGKA	FKKPQMIFP
Sus scrofa	FPLNAIEGKA	FKKPQMIFP
Bos taurus	FPLNAIEGKA	FKKPQMIFP
Equus caballus	FPLNAIEGKA	FKKPQMIFP
Felis catus	FPHNAIEGKA	FKKPQMIFP
Danio rerio	LPATCLESRA	YKNLESVMS
Rattus_norvegicus	FSLNAIEGKS	FKKPQMIFN
Xenopus laevis	LPLTSIDTRA	YKKPEMIFS
Gallus gallus	LPVSSIESKS	FRKPQMIFG
Xenopus Silurana tropicalis	LPLTSVETRA	YKKPEMIFS
Pongo_abelii	LPLNAIEGKA	FKKPQMIFP

10

Apoptosis is a key process that is initiated to remove damaged cells from the system, thus ensuring the well-being of the species. Inhibition of this pathway due to alterations of genes that activate and regulate this pathway is a major characteristic feature of many malignancies. p53 is an important gene because of its close involvement in the activation of apoptotic pathways upon detection of damage. p53 activates the expression of its key regulator, MDM2 which in turn binds to p53 and regulates activity of p53 and so these two proteins control each other. Altered expression of either of these genes can thus have an inherent effect on cell death leading to uncontrolled cell proliferation and result in nonremoval of damaged cells. The alterations of these genes also contribute to drug resistance since chemotherapeutic moieties function by activating apoptotic pathways to destroy leukemic cells.

Through phylogenetic study, we have determined that these genes share a high degree of sequence similarity across the Mammalian species and to a lesser extent with other animals, implying that they might share a common ancestor. Also, since they share sequence similarity it could be possible that the tumorigenic potential of these genes could be due to a buildup of changes during their evolution. This could entail that the mechanisms that lead to overexpression of these genes in solid tumors and leukemia could probably be deciphered by observing their genetic structure and function in the other species and by applying this information to human neoplasms. These data indicate the need to better understand not only how each of these gene are altered in disease but also how their interaction can contribute to malignancy. Interactions studies could also help determine why these genes play a more significant role in relapse than during initial disease development. This type of study could probably explore the design and development of drug moieties that could target the interaction of these two genes, instead of a single gene, and could thus prove to be an effective therapeutic strategy.

Conclusion

In conclusion, this is the first report of the phylogenetic analysis of p53 and MDM2 genes which revealed the sequence similarity shared with many species. The study of these two genes – oncogene (p53) and proto-oncogene (MDM2) - suggests that the mechanism of

neoplasia is deeply rooted throughout evolution. This evolutionary history could help determine how these genes are altered in cancers and that MDM2-p53 interaction might play a very important role in the Tumorigenesis and Leukemogenesis processes during relapse in cancer patients. Thus, these molecules are indeed important for furthering our understanding of the cancer process.

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