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Comparative Study of Cellular Tumor Antigen p53 Protein of Fishes and Analysis of its Protein Interaction Network using Computational Approach

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Abstract Progress in the field of Bioinformatics has been facilitated to understand the global network of genes and their protein products. In present study, comparative analysis of Cellular tumor antigen p53 proteins of nine fishes were carried out using Bioinformatics tools. Cellular tumor antigen p53 acts as a tumor-suppressor and having role in apoptosis, genomic stability. The results of this study indicate that, most of physico-chemical properties were almost same in Q92143 (*Xiphophorus maculates*) and O57538 (*Xiphophorus helleri*). In order to understand global network of Cellular tumor antigen p53, we have used STRING 9.1 tool and speculated that this protein interacting with several other protein but functional node -CHEK1, BCL2, MDM4 were common in *Danio rerio*, *Oryzias latipes*, *Tetraodon miurus* with high confidence score. The strong association interaction has seen between mdm2 and p53 with a good high score in *Danio rerio.* We also studied the molecular docking between Cellular tumor antigen p53 and Mdm2 of Zebrafish. Also, we have investigated conserved region present in all nine different protein sequences which specifies, that region maintained by evolution despite speciation. The present study will further support to understand the roles and associated proteins in various cellular pathways in fish. This work is also useful for the study of structural and functional analysis of p53 protein.

Keywords Cellular tumor antigen p53; Sequence analysis; Protein interaction network; Conserved region; Bioinformatics; Physico-chemical properties; Mdm2

Introduction

Although, Cellular tumor antigen p53 has been discovered about thirty years ago, but remains concern most of the consecration in the fields of cancer research (Kruse and Gu, 2009; Lu et al., 2009). The Cellular tumor antigen p53 or p53 is a protein encoded by the TP53 gene which is most important a tumor suppressor gene. This gene has been well studied in humans and mammals except in some fishes. It is also called tumor suppressor p53 or phosphor-protein p53 or antigen NY-CO-13 or p53 or Transformation-related protein 53 (TRP53) which play an important role in apoptosis i.e. programmed cell death in tumor development and genomic stability(Kruse and Gu, 2009; Storer and Zon, 2010).

The tumor suppressor p53 protein acts as a transcription factor to control expression of many genes in its interaction network, which consists of upstream regulators and downstream target genes (Fields and Jang, 1990). With its ability to respond to stress, p53 combats tumorigenesis and protects the individual at both a cellular and organismal level. P53 is a site-specific DNA-binding protein (Kern et al., 1991), that transactivates genes in its network(Fields and Jang, 1990; Lu et al., 2007). Hence, if p53 is mutated, cell growth ensues resulting in tumor formation. The activity and expression of p53 are monitored by numerous layers of regulation, mainly by ubiquitin ligases such as Mdm2 and Mdm4 at the post-translational level (Le et al., 2009). Mdm2 protein binds to p53 and inactivates it. The Mdm2 is an E3 ubiquitin ligase which up-regulated in the occurrence of active p53, where it poly-ubiquitinates tumor suppressor p53 for proteasome targeting (Oren, 1999). This is reported that mdm2 deficient zebrafish embryos show growth retardation and high levels of

apoptosis (Storer and Zon, 2010) due to off-target effects of the mdm2 morpholino (Robu et al., 2007). In case of mammals, the stability and function of p53 is regulated by a number of post-translation modifications whereas in Zebrafish, regulation at both the mRNA and protein a level in response to different types of stress has been described (Brooks and Gu, 2003; Langheinrich et al., 2002; Storer and Zon, 2010). The mutation in p53 gene will inactivate its tumor suppression mechanism and also other factors, which will lead to tumor. The single amino substitution will also affect the expression of p53 (Petitjean et al., 2007). The loss of function of p53 due to mutations has been well studied in mouse model (Leng et al., 2003; Olive et al., 2004). Thus, its function regulated by post-translational regulation along with interacting p53 binding protein such as mdm2 and E3 ubiquitin ligase of p53.

In present study, we have used bioinformatics tools for comparative analysis of reported cellular tumor antigen p53 protein sequences of nine different fish. We have explore the mechanism of tumor suppressor p53 gene and its protein sequence among fishes, because several works has been done on p53 in humans and mammals, but in case of fishes very little work has been reported. In recent era of Bioinformatics, several tools and algorithms has been developed for understanding biological molecules up to atomic level and predicting underlying mechanism. Further understanding of tumor suppressor p53 regulation in fishes using cellular mechanism along with protein modifications will facilitate to understand *in vivo* basic mechanisms that regulate the tissue specific response of p53.

1 Material and Methods

We have used different bioinformatics tools for studying the tumor suppressor p53 protein which listed along with specified purpose**.**

1.1 Collection of data

The UniProt is easily accessible database of protein sequence (http://www.uniprot.org/). We have retrieved total nine protein sequences from nine different fishes for our study; all reviewed (Table 1). We have retrieved protein sequences in a FASTA format.

Table 1 List of Cellular tumor antigen p53

S.No.	Accession no.	Name of protein	Organism
1.	P79734	Cellular tumor antigen p53	*Danio rerio*
2.	Q92143	Cellular tumor antigen p53	*Xiphophorus maculatus*
3.	O57538	Cellular tumor antigen p53	*Xiphophorus helleri*
4.	P79820	Cellular tumor antigen p53	*Oryzias latipes*
5.	O93379	Cellular tumor antigen p53	*Ictalurus punctatus*
6.	P25035	Cellular tumor antigen p53	*Oncorhynchus mykiss*
7.	O12946	Cellular tumor antigen p53	*Platichthys flesus*
8.	Q9W679	Cellular tumor antigen p53	*Tetraodon miurus*
9.	Q9W678	Cellular tumor antigen p53	*Barbus barbus*

1.2 Physico-chemical Characterization

ProtParam (http://web.expasy.org/protoparam/) is expasy tool which is useful for computation of physical and chemical parameters of given protein based on sequence. We have calculated several physico-chemical properties such as theoretical isoelectric point (pI), molecular weight, and total number of positive and negative residues, extinction coefficient, half-life, instability index, aliphatic index and grand average hydrophathy (GRAVY) of all nine retrieved protein sequences using ProtParam tool.

1.3 Alignment and Phylogenetic study

In order to study the comparison among different protein sequences, we have used global multiple sequence alignment (MSA) program for analysis of p53 protein sequences from different fishes. Now a day, multiple sequence alignment (MSA) method is widely used for assessing sequence conservation and conservation of protein domains in protein study. In this step, Clustal Omega(Sievers et al., 2011) tool was used for MSA analysis. Understanding phylogenetic relationship among different protein sequences, we

have delineated evolutionary relationship of these sequences by cladogram. The Prosite, ScanProsite (de Castro et al., 2006) tool was used to identify the no. of hit for the predicted motif (http://prosite.expasy.org/scanprosite/).

1.4 Analysis of Gene ontology and protein-protein interaction network of p53

We further studied the gene ontology of p53 for biological, molecular functions which identified using Uniprot (http://www.uniprot.org/). The STRING (Franceschini et al., 2013) (Search Tool for the Retrieval of Interacting Proteins) was used for studying the protein-protein interaction network of the p53 (http://string-db.org/).

1.5 Three dimensional structure analysis and molecular docking

The homology modeling was used to build p53 3D model based on homologous structure model. The structural templates that have highest sequence homology with our target template were identified by using PSI-BLAST (NCBI, http://blast.ncbi.nlm.nih.gov/Blast) against 3D structure available in PDB databank. The criteria used such as percent sequence identity, e-value, chain length and query coverage. The model was built by SWISS-Model using target-templates alignment. The SAVES (Structural Analysis and Verification Server) is integrated server was used for verification of models (http://nihserver.mbi.ucla.edu/SAVES/).The molecular docking was performed between p53 and Mdm2 using PatchDock followed by refine the structures using FireDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/). The Patchdock based on surface patch matching and more reliable docking tools with fast search for filtering and scoring. It uses advanced data structures and spatial search pattern. It resulted into several structures, thus further filtered through FireDock. Docking score and atomic contact energy (ACE) of the both complexes were calculated using Patch Dock.

2 Results and Discussion

For comparative analysis of the Tumor suppressor antigen p53 from different fishes, we have used computational algorithms. The p53 is well studied in mammalian system along with some model fishes. Value of most of physico-chemical properties were almost same in Q92143 (*Xiphophorus maculates*) and O57538 (*Xiphophorus helleri*) like length, theoretical pI, positive R group, negative R group and aliphatic index, etc (Table 2). The value of an instability index of all protein was above 40 which indicate that all nine proteins were unstable. The Extinction coefficients (EC) value of p53 was calculated, which help in the protein- ligand and protein-protein interaction study**.** The pI value of Q9W679 and Q9W678 were greater than 7 which indicate that both proteins are basic in nature, rest of proteins were acidic in character. All the p53 protein sequences of nine different fishes were hydrophobic in nature.

Table 2 Physico-chemical properties of protein sequences

S.No.	Accession no.	Length	Molecular weight	Theoretical pI	Total number of negatively	Total number of positive	Extinction coefficients	Instability index:	Aliphatic index	GRAVY
1.	P79734	373	41899.1	6.37	54	52	30410	60.61	63.75	-0.785
2.	Q92143	342	37957.7	6.06	48	44	27305	50.64	67.84	-0.682
3.	O57538	342	37947.7	6.06	48	44	27305	50.08	67.84	-0.679
4.	P79820	352	39752.8	6.24	51	48	29255	57.98	66.36	-0.761
5.	O93379	376	41989.2	6.48	54	52	26525	65.53	63.96	-0.809
6.	P25035	396	43966.1	6.96	51	51	26525	61.75	73.33	-0.547
7.	O12946	366	40619.0	6.73	50	49	16305	56.32	70.03	-0.607
8.	Q9W679	367	41266.6	7.60	49	50	24910	51.65	65.91	-0.734
9.	Q9W678	369	41233.5	7.04	52	52	26400	54.36	67.59	-0.711

Multiple Sequence Alignment (MSA) can give insight into sequence conservation across several species and thus allow identification of those sections of the sequence most critical to protein function (Jankun-Kelly et al., 2009). Further, performing MSA, we have seen that "MCNSSCMGGMNRR" is the conserved region (identical region) in all nine different protein sequences of p53 which indicates that this peptide sequence may have been maintained by evolution despite speciation (Figure 1). Our study of p53 phylogenetic analysis revealed that Q92143 (*Xiphophorus maculates*) O57538 (*Xiphophorus helleri*) were much closer to each other. Using Scan Prosite, we have perceived total 42 numbers of hits of "MCNSSCMGGMNRR" motif (Table 3) (Figure 2).

Table 3 No. of hits predicted by using "MCNSSCMGGMNRR"

S. No.	Name of protein	Accession no.	Species
1.	Cellular tumor antigen p53	Q9W678	*Barbus barbus* (*Barbel*) (*Cyprinus barbus*)
2.	Cellular tumor antigen p53	P67938	*Bos indicus* (*Zebu*)
3.	Cellular tumor antigen p53	P67939	*Bos taurus* (*Bovine*)
4.	Cellular tumor antigen p53	Q29537	*Canis familiaris* (*Dog*) (*Canis lupus familiaris*)
5.	Cellular tumor antigen p53	Q9WUR6	*Cavia porcellus* (*Guinea pig*)
6.	Cellular tumor antigen p53	P10360	*Gallus gallus* (*Chicken*)
7.	Cellular tumor antigen p53	P13481	*Chlorocebus aethiops* (*Green monkey*) (*Cercopithecus aethiops*)
8.	Cellular tumor antigen p53	O09185	*Cricetulus griseus* (*Chinese hamster*) (*Cricetulus barabensis griseus*)
9.	Cellular tumor antigen p53	P79734	*Danio rerio* (*Zebrafish*) (*Brachydanio rerio*)
10.	Cellular tumor antigen p53	Q8SPZ3	*Delphinapterus leucas* (*Beluga whale*)
11.	Cellular tumor antigen p53	Q29480	*Equus asinus* (*Donkey*)
12.	Cellular tumor antigen p53	P41685	*Felis catus* (*Cat*) (*Felis silvestris catus*)
13.	Cellular tumor antigen p53	P79892	*Equus caballus* (*Horse*)
14.	Cellular tumor antigen p53	P04637	*Homo sapiens* (*Human*)
15.	Isoform 2 of Cellular tumor antigen p53	P04637-2	*Homo sapiens* (*Human*)
16.	Isoform 3 of Cellular tumor antigen p53	P04637-3	*Homo sapiens* (*Human*)
17.	Isoform 4 of Cellular tumor antigen p53	P04637-4	*Homo sapiens* (*Human*)
18.	Isoform 5 of Cellular tumor antigen p53	P04637-5	*Homo sapiens* (*Human*)
19.	Isoform 6 of Cellular tumor antigen p53	P04637-6	*Homo sapiens* (*Human*)
20.	Isoform 7 of Cellular tumor antigen p53	P04637-7	*Homo sapiens* (*Human*)
21.	Isoform 8 of Cellular tumor antigen p53	P04637-8	*Homo sapiens* (*Human*)
22.	Isoform 9 of Cellular tumor antigen p53	P04637-9	*Homo sapiens* (*Human*)
23.	Cellular tumor antigen p53	O93379	*Ictalurus punctatus* (*Channel catfish*) (*Silurus punctatus*)
24.	Cellular tumor antigen p53	P56423	*Macaca fascicularis* (*Crab-eating macaque*) (*Cynomolgus monkey*)
25.	Cellular tumor antigen p53	P61260	*Macaca fuscata fuscata* (*Japanese macaque*)
26.	Cellular tumor antigen p53	P56424	*Macaca mulatta* (*Rhesus macaque*)
27.	Cellular tumor antigen p53	O36006	*Marmota monax* (*Woodchuck*)
28.	Cellular tumor antigen p53	Q00366	*Mesocricetus auratus* (*Golden hamster*)
29.	Cellular tumor antigen p53	P02340	*Mus musculus* (*Mouse*)
30.	Cellular tumor antigen p53	P25035	*Oncorhynchus mykiss* (*Rainbow trout*) (*Salmo gairdneri*)
31.	Cellular tumor antigen p53	P79820	*Oryzias latipes* (*Medaka fish*) (*Japanese ricefish*)
32.	Cellular tumor antigen p53	Q9TUB2	*Sus scrofa* (*Pig*)
33.	Cellular tumor antigen p53	O12946	*Platichthys flesus* (*European flounder*) (*Pleuronectes flesus*)
34.	Cellular tumor antigen p53	Q95330	*Oryctolagus cuniculus* (*Rabbit*)
35.	Cellular tumor antigen p53	P10361	*Rattus norvegicus* (*Rat*)
36.	Cellular tumor antigen p53	P51664	*Ovis aries* (*Sheep*)
37.	Cellular tumor antigen p53	Q64662	*Spermophilus beecheyi* (*California ground squirrel*) (*Otospermophilus beecheyi*)
38.	Cellular tumor antigen p53	Q9W679	*Tetraodon miurus* (*Congo puffer*)
39.	Cellular tumor antigen p53	Q9TTA1	*Tupaia belangeri* (*Common tree shrew*) (*Tupaia glis belangeri*)
40.	Cellular tumor antigen p53	P07193	*Xenopus laevis* (*African clawed frog*)
41.	Cellular tumor antigen p53	O57538	*Xiphophorus helleri* (*Green swordtail*)
42.	Cellular tumor antigen p53	Q92143	*Xiphophorus maculatus* (*Southern platyfish*) (*Platypoecilus maculatus*)

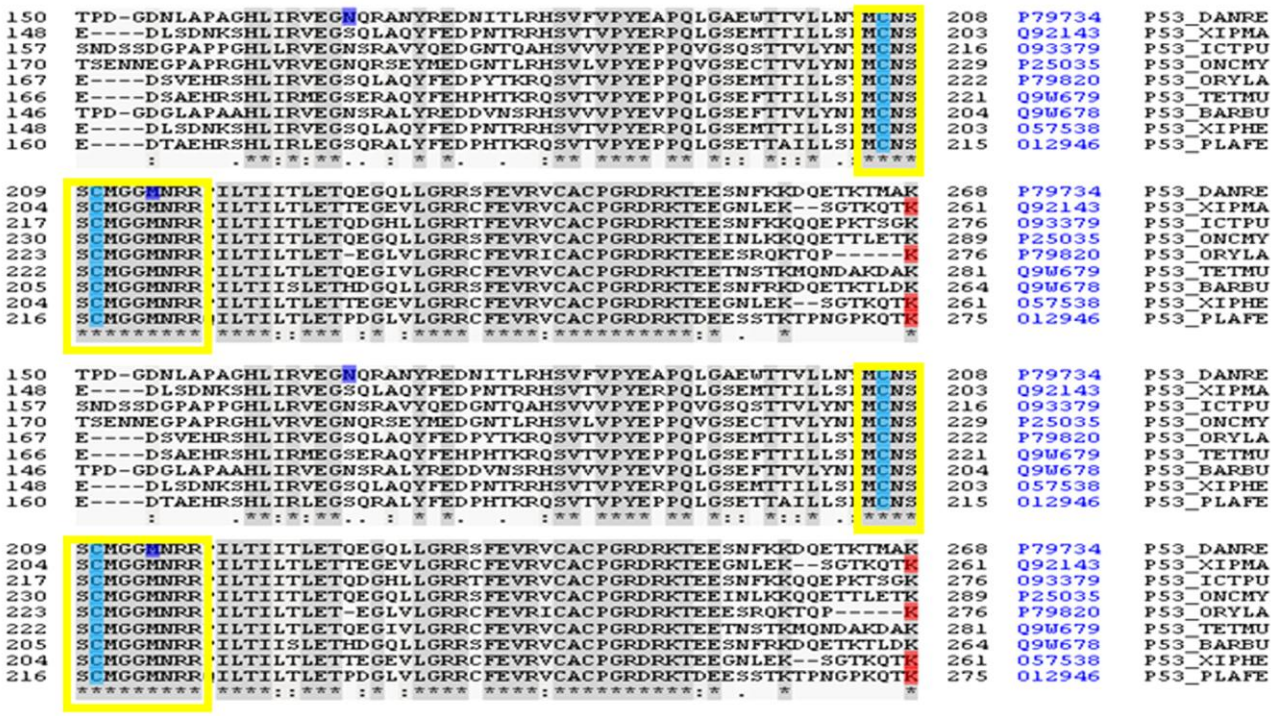

Figure 1 The snapsort of MSA result. Here, "*" indicates identical in all sequences in the alignment; ":" indicates conserved substitutions; "." indicates semi - conserved. Red color indicates the motif; dark grey color indicates the similarity; light blue color indicates metal binding; purple color indicates mutagenesis. Selected conserved region is highlighted by yellow box

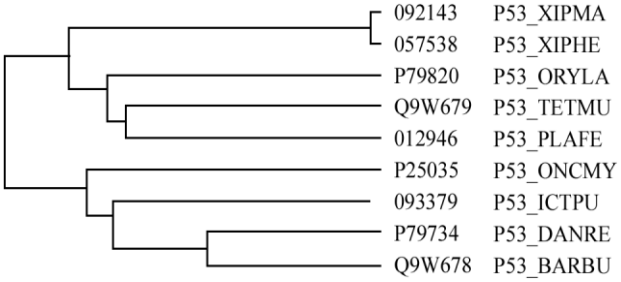

Figure 2 Phylogenetic tree show the Evolutionary relationships of retrieved protein sequences by cladogram

Protein-protein interaction investigation is a wide-ranging approach to know the organization of desire proteome. The functional network protein study will be helpful for drug discovery, to understand metabolic pathways and to predict or develop genotype-phenotype associations (Wang et al., 2009; Wang and Moult, 2001). In order to understand network of p53 protein, we performed analysis using STRING 9.1 and revealed that functional node -CHEK1, BCL2, MDM4 were common in *Danio rerio*, *Oryzias latipes*, *Tetraodon miurus*. The interaction of mdm2 and p53 indicates the good high score in *Danio rerio*. Protein-protein interaction networks are major part for the system-level understanding of cellular processes. We have studied the all nine protein sequence one by one for getting protein-protein interaction network. Here our interest to know that which functional node is common of p53 network in different fishes. We have revealed protein-protein interactions network only from three different fish of p53 i.e. *Danio rerio*, *Oryzias latipes*, *Tetraodon miurus* (Table 4, 5 and 6) and functional node -CHEK1, BCL2, MDM2 were common in p53 protein network along with high confidence score. In STRING, the functional interaction was analyzed by using confidence score. Interactions with score < 0.3 are considered as low confidence, scores ranging from 0.3 to 0.7 are classified as medium confidence and scores > 0.7 yield high confidence(Franceschini et al., 2013). In *Danio rerio*, p53 protein network showing functional association with 10 proteins and they are Cdkn1a, Mdm2, atm, Chek1, bcl2, Mdm4, Wu:fa96e12, Chek2, LOC792573, Ep300a (Figure 3).

In the interaction network, there is no black line between mdm2 & p53 which indicates that there was no co-expression. We have speculated the occurrence of result to check that all 10 proteins were conserved in *Danio rerio*, *Oryzias latipes*, *Tetraodon miurus* or not and also found, that all nodes indicated 100% sequence conservation. 4, represents the occurrence result of zebra fish. Mdm4 and p53 having the good high score in *Danio rerio*, *Oryzias latipes* and *Tetraodon miurus* which indicates the strong association. Its function is to inhibit p53 and p73 mediated cell cycle arrest and apoptosis by binding its transcriptional activation domain. We have demarcated the best top ten protein-protein interaction network of p53(Lu et al., 2009; Oren, 1999; Wang et al., 2004).

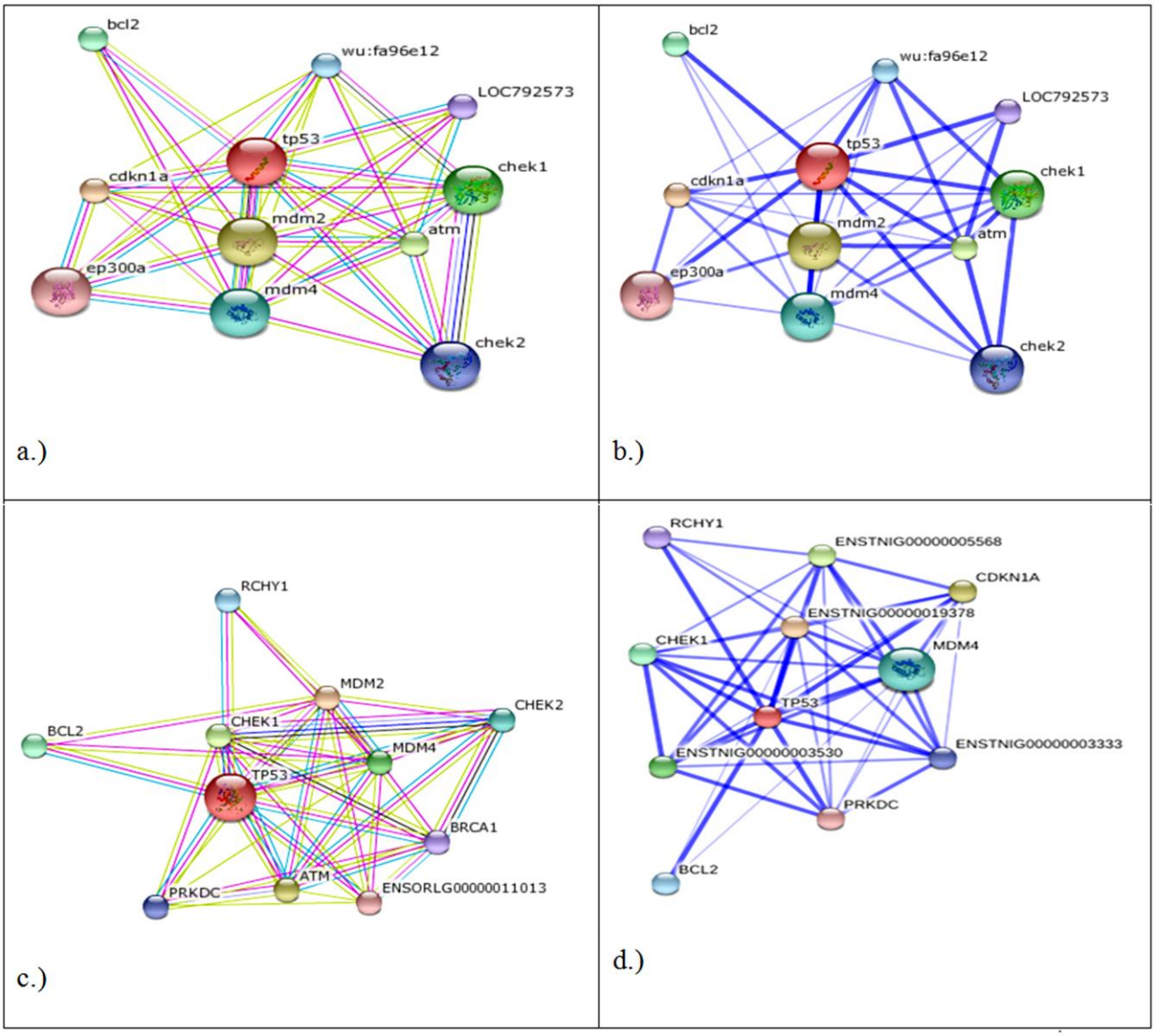

Figure 3 Protein interaction network. a) Evidence view of p53 protein network showing functional association with 10 proteins (Zebra fish). Here, a node represents proteins; an edge represents the predicted functional associations. Different line colors represent the types of evidence for the association. Red line indicates the presence of fusion evidence; yellow line text miming evidence; Light blue line indicates database evidence; Black line indicates the co-expression evidence. b.) Confidence view of p53 network (Zebra fish). In this fig stronger associations are represented by thicker lines. c.) Evidence view of p53 protein network showing functional association with 10 proteins (*Oryzias latipes*) d.) Confidence view of p53 network (*Teraodan miurus*)

Table 4 Interaction of p53 (zebra fish) with functional nodes

SI.No.	Functional node	Actions view	Score	Types of evidence for the association.
1.	Cdkn1a (Novel protein Fragment)	Binding, activation, expression	0.997	Experiments, databases, text mining
2.	Mdm2 (E3 ubiquitin-protein ligase Mdm2)	Binding, activation, post translation modification, expression	0.996	Coexpression, Experiments, databases, text mining
3.	Atm (Ataxia telangiectasia mutated Fragment)	Binding, catalysis, post translation modification, expression	0.993	Experiments, databases, text mining
4.	Chek1(checkpoint kinase 1)	Binding, post translation modification, expression	0.991	Experiments, databases, text mining
5.	bcl2 (Bcl2)	Binding, reaction	0.988	Experiments, databases, text mining
6.	Mdm4 (Protein Mdm4)	Binding, activation	0.987	Experiments, databases, text mining
7.	Wu:fa96e12 (wu-fa96e12)	Binding, post translation modification	0.985	Experiments, databases, text mining
8.	Chek2 (CHK2 checkpoint homolog)	Binding, post translation modification, activation	0.983	Experiments, databases, text mining
9.	LOC792573 (ring finger and WD repeat domain 2)	Binding	0.980	Experiments, databases, text mining
10.	Ep300a (P300-A Fragment)	Binding, post translation modification	0.980	Experiments, databases, text mining

Table 5 Interaction of p53 (*Oryzias latipes*) with functional nodes

SI.No.	Functional node	Actions view	Score	Types of evidence for the association.
1.	MDM2 (Mdm2 p53 binding protein homolog)	Activation, binding, translation modification , expression	0.991	Coexpression, Experiments, databases, text mining
2.	ATM (ataxia telangiectasia mutated)	Binding, catalysis, translation modification , expression	0.982	Experiments, databases, text mining
3.	CHEK1 (CHK1 checkpoint homolog)	binding, translation modification , expression	0.974	Experiments, databases, text mining
4.	MDM4 (Mdm4 p53 binding protein homolog)	Activation, binding	0.974	Experiments, databases, text mining
5.	BCL2 (B-cell CLL/lymphoma 2)	Binding, reaction, expression	0.970	Experiments, databases, text mining
6.	CHEK2 (CHK2 checkpoint homolog)	Activation, binding, translation modification	0.968	Experiments, databases, text mining
7.	RCHY1 (ring finger and CHY zinc finger domain containing 1)	Binding, expression	0.966	Experiments, databases, text mining
8.	PRKDC (protein kinase, DNA-activated, catalytic polypeptide)	Binding, translation modification	0.961	Experiments, databases, text mining
9.	BRCA1 (breast cancer 1, early onset)	binding	0.959	Experiments, databases, text mining
10.	ENSORLG00000011013	Activation, binding, expression	0.954	Experiments, databases, text mining

Table 6 Interaction of p53 (*Teraodan miurus*) with functional nodes

SL.No.	Functional node	Actions view	Score	Types of evidence for the association.
1.	ENSTNIG00000019378 (Mdm2 p53 binding protein homolog)	Activation, binding, translation modification, expression	0.986	Coexpression, Experiments, databases,text mining
2.	CDKN1A (cyclin-dependent kinase inhibitor 1A)	Activation, binding, expression	0.983	Experiments, databases, text mining
3.	ENSTNIG00000005568 (Mdm2 p53 binding protein homolog)	Activation, binding, translation modification, expression	0.982	Coexpression, Experiments, databases, text mining
4.	ENSTNIG00000003530 (ataxia telangiectasia mutated)	binding, catalysis, translation modification, expression	0.980	Experiments, databases, text mining
5.	CHEK1 (CHK1 checkpoint homolog)	Binding, translation modification, expression	0.976	Experiments, databases, text mining
6.	MDM4 (Mdm4 p53 binding protein homolog)	Binding	0.975	Experiments, databases, text mining
7.	BCL2 (B-cell CLL/lymphoma 2)	Binding, reaction, expression	0.971	Experiments, databases, text mining
8.	ENSTNIG00000003333 (ataxia telangiectasia mutated)	Binding, reaction, expression, catalysis, translation modification	0.967	Experiments, databases, text mining
9.	RCHY1 (ring finger and CHY zinc finger domain containing 1)	Binding, expression	0.964	Experiments, databases, text mining
10.	PRKDC (protein kinase, DNA-activated, catalytic polypeptide)	Binding, translation modification	0.963	Experiments, databases, text mining

Mdm2 protein has shown the strong association with p53 protein in Zebra fish using STRING tool. So, to study the interaction at the structure level we have done docking. Firstly, the 3D structure of p53 of zebrafish obtained from Swiss-Model by performing homology modeling using retrived homologous strucures such as PDB ID; 3Q05_A, 3Q01_A, 3Q06_A, 4MZR_A with identity 58, 57, 58, 57 percent respectively. The obtained 3D structure was verirified with SAVES server (Figure 4). The molecular docking was performed between p53 and Mdm2 using PatchDock followed by refine the structures using FireDock. Docking score and atomic contact energy (ACE) of the both complexes were calculated using Patch DockBoth PDB structures were used for docking analysis (Figure 4). The docking between p53 and Mdm2 revealed that requires global energy 10.57 and ACE 0.18 respectively.

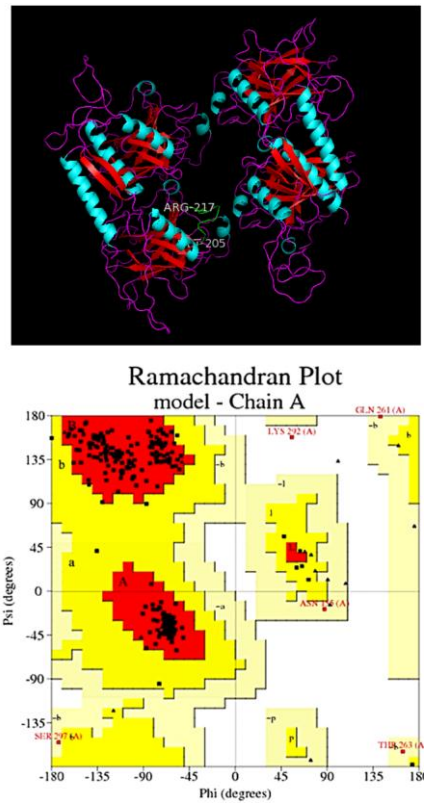

Figure 4 p53 model of zebrafish with conserved region in green color obtained through homology modeling. Ramachndran plot of model p53 showing, residues in most favoured regions 91.9%, Residues in additional allowed regions 5.9%, Residues in generously allowed regions 1.7% and Residues in disallowed regions 0.4% etc

3 Conclusion

This is first comprehensive study on comparative study of tumor suppressor antigen p53 among fishes. In this study, we have investigated that most of physico-chemical properties were almost same in Q92143 (*Xiphophorus maculates*) and O57538 (*Xiphophorus helleri*). After performing alignment we have seen that "MCNSSCMGGMNRR" is the conserved (identical) motif which present in all nine protein sequences of p53 and predicted overall 42 hit from the database which indicated the importance of this region. From protein-protein interactions network study, we have seen the functional node -CHEK1, BCL2, MDM4 were common in p53 protein network along with high confidence score in species *Danio rerio*, *Oryzias latipes*, *Tetraodon miurus.* Moreover, protein-protein interaction pathway of this tumor suppressor p53 has helped us to understand the roles and associated proteins in various cellular pathways. The docking study also confirmed that p53 having interaction with mdm2 with global energy. Thus finally, present work will support to understand more about tp53 proteins of different species including fish (Figure 5).

Authors' contributions

SK and KDR designed the study and procedure of for work plan. SK, KDR, PJ, JKS and SN analyzed the data and prepared the manuscript. All authors read and approved the final manuscript

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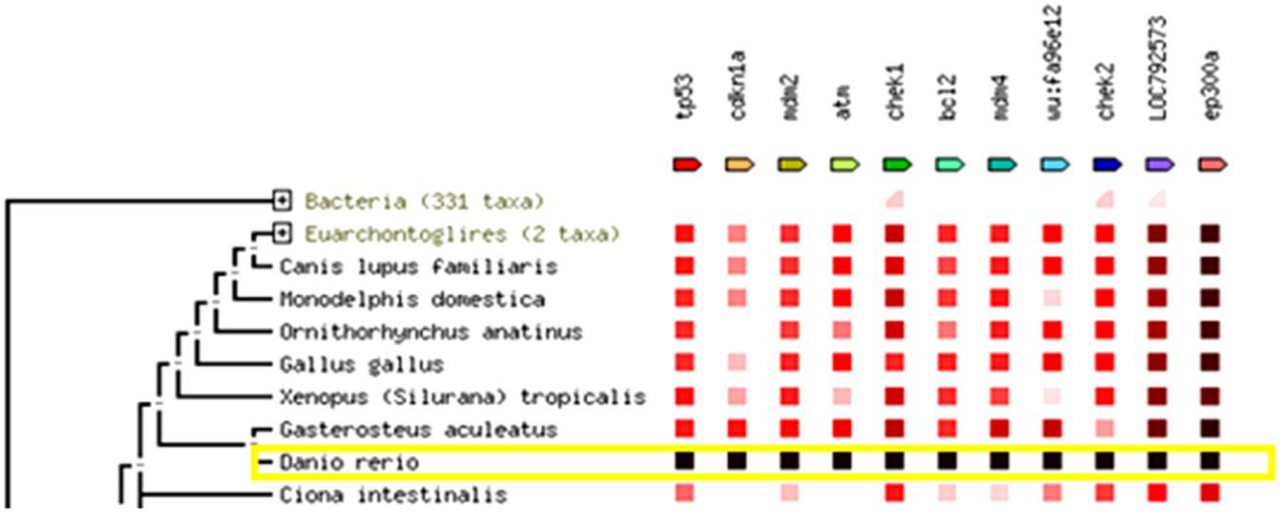

Figure 5 Snapshot of occurrence result. Here black color indicates the 100% sequence conservation in Zebra fish

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