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# In silico Characterization of *Hspb1* Mutant Protein in *Canine* and *Feline* Cancers

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**Abstract:** Cancer is one of the topmost cause of deaths all over the world after some contagious and heart diseases. *Hspb1* provides protective mechanism against intrinsic and extrinsic stress conditions like inflammation, exposure to toxins, infection and elevated temperature, disease and injury respectively. It also plays anti-apoptotic role in cancer. Effect of *Hspb1* mutants in various *feline* and *canine* tumors and a comparison of its wild and mutant types were conducted using online bioinformatics tools. Physiochemical properties, secondary structures, conserved domains, transmembrane structures and post-translational modifications were analyzed. One mutant of cat *Hspb1* at c.34 was observed in Siamese mammary tumor case which does not change the amino acid sequence but its protein characteristics were determined, similarly one mutant was observed at c.148 in dog granuloma and mammary tumor each while a 5 bp insertion was observed at c.220 in canine lymphoma. The results indicated that more obvious changes are found in this protein in lymphoma sample because of insertion of 5 bp whereas the mutations in mammary tumor and granuloma cause slight changes in this protein as compare to wild type Hspb1 protein in *Canis familiaris*. Our findings about the *Hspb1* protein and introduction to basic bioinformatics tools in this communication helped in better understanding the role and dynamics of this protein in cancerous tissues before delving into real bench work, purification and further analysis of this protein.

**Key words:** Hspb27, Cat cancer, Dog cancer, Bioinformatics tools, Hspb1 mutants
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## Introduction

Regardless of massive research endeavors in cancer, it is still a fatal and deadly disease worldwide. It is one of the major issues in human and as well as in small pet animals. Cancer is the major cause of deaths in the west as well as in eastern societies [1]. Many successful and analytical techniques are being used for its diagnosis and prognosis but still few cancers are able to defy the diagnosis and are being detected but at the last stages of disease. Early prognosis and diagnosis of cancer is a wide area of research in which applied genomic experts and molecular oncologists are working together to get affirmative results, in which different biomarkers are being used for the detection and can be considered as a potential targets.

Hsp27 is the protein that is encoded by *Hspb1* gene which belongs to most conserved protein family and has its role in the homeostasis and regulation of heat stress pathways [2]. The expression of this protein prevails in the stress conditions during anticancer drug therapy, high temperature and cellular intrinsic and extrinsic stresses. The caspase interaction is interfered by the Hsp27 and thus by the various protein interaction the apoptosis is inhibited. Hsp27 has a major role in controlling the apoptosis which is important for the cellular repair. For other cellular proteins it also acts as a chaperon and has a cytoprotective role [3]. During the chronic stress inappropriate signals can occur because of the misfolding of the protein. Throughout the stress condition it can either stabilize or cause the degradation of the protein by proteasome for the existence of the cell [2]. All the event provides the cellular recovery to the cell so it is beneficial for the organism but it can also be disadvantageous on the other hand as the survival of carcinogen cell increases by the over expression of the anti-apoptotic proteins and due to its over expression it can be used as a target to inactive while performing cancer therapy. The regression of

tumor was observed by the depletion of protein [4]. It is found that the over expression of the protein is been detected which causes the resistance to the apoptotic activity in the cancerous cells. Hsp27 is an ATP independent protein that has a conserved structure. The functions of this protein are regulated by the equilibrium of oligomer and dimer. The activity of Hsp27 is controlled by site specific phosphorylation [5].

*Hspb1* mutations were observed in this study and their mutant proteins were analyzed through online bioinformatics tools which may be served as biomarker for different malignancies of dog and cats. These two species may be used as a model to study the comparative molecular oncology. Canine *Hspb1* is 96% identical with its human counterpart, similarly feline *Hspb1* is 86% identical to human[5]. So the observed mutations in these two species may be helpful in diagnosis of different malignancies. The *Hspb1 CDS* was sequenced in different tumors such as TVT, mammary tumor, squamous cell carcinoma (SCC), granuloma, lymphoma and melanoma in *Canis familiaris*, while mammary tumor of *Felis catus*. Mammary carcinomas are more common in carnivorous animals than herbivores [7]. In *Felis catus* the most common are mammary tumor, about 17% after skin tumors [8]. In *Canis lupus* the most common type of malignant neoplasms are the mammary tumors and show similarity to human disease.

Observed mutation in this gene may be helpful in the diagnosis of cancer in these animal species and a better understanding of the protein can be made. Then an exhaustive analysis was conducted to ascertain the effect of these mutations on the translation of protein, how physiochemical, transmembrane structure, conserved domains and post-translational modifications characteristics of these proteins are different from its wild type counterpart.

## MATERIALS AND METHODS

### *Sample Collection*

Tumor samples of cat and dog were collected from the pet center, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, which includes six mammary tumors of Siamese cat breed and twenty samples of dog including mammary tumor and TVT, five from each, PAC and SCC three from each, granuloma and lymphoma, two from each. Protein sequence of H*spb1* of wild type *Felis catus* and *Canis lupis familiaris* were taken from Ensemble genome browser (http://www.ensembl.org/index.html), (ENSFCAT00000026034 and ENSCAFT00000043541) respectively.

### *DNA Extraction, PCR & Sequencing*

DNA from the core tissues of tumorous mass was extracted and amplified through *Hspb1* gene specific primers. PCR products were then sequenced from the commercial facility available, later on these sequences were edited and multiple sequence alignment was conducted through "Sequencher" software [6] (www.genecodes.com/).

### *Primer Designing*

Primers were designed using NetPrimer (http://www.premierbiosoft.com/netprimer/) and Primer 3 (http://primer3.ut.ee/) software using wild type cat *Hspb1* sequence taken from Ensemble Genome Browser ID ENSFCAG00000031543 in cat and ENSCAFT00000043541 in *Canis familiaris*.
One primer set in each species was designed to perform long range PCR. Forward and reverse primers in cat are 5'CAGTTAGTGGCAGAACCAGGATGT3', 5'TGTAGAAGGTATGGGAGGTGTAGCA3' and in dog 5'CGTCATTGCCTTTAATAGAGACCTG3', 5'AAAGTAAACAGACTTGCCGAGGTC3'.
Furthermore four and six new internal sequencing primers were also designed for cat and dog *Hspb1* respectively.

### *Bioinformatics Tools*

The protein sequence of the wild and mutant types of *Hspb1* in *Felis catus* and *Canis lupus familiaris* were characterized using online bioinformatics tools to ascertain physiochemical properties, secondary structures (α –helices and β pleated sheets ), conserved domains, transmembrane structures and the post-translational modifications in this protein. But before applying these tools, wild and mutant types of *Hspb1* protein sequences were BLAST through NCBI (http://blast.ncbi.nlm.nih.gov/blast/Blast) to check the similarity index in these wild and mutant protein sequences.

*i)**"ProtParam"*** tool (http://web.expasy.org/protparam) was applied on

the wild and mutant type protein sequences of *Hspb1* in both species to analyze the physiochemical properties, molecular weight, composition of amino acids, theoretical PI, atomic composition, instability and aliphatic index, estimated half-life, GRAVY and the extinction co-efficient were analyzed (Table 1).

*ii)* Secondary structure of the protein sequences was analyzed using the ***"Psipred"*** (http://bioinf.cs.ucl.ac.uk/psipred/) tool. Wild and mutant type protein sequences of the cat and dog were analyzed respectively.

*iii)* Conserved regions in the protein sequences of the wild and mutant types of *Felis catus* and *Canis familiaris* can be determined using motif scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan), NCBI CD Server (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and Interproscan 5 (http://www.ebi.ac.uk/Tools/pfa/iprscan5/). Here ***"Motif Scan"*** tool was used to find out the conserved regions. Match scores and details of each of the protein sequence were also analyzed.

*iv)* ***"Protscale"*** **(**http://web.expasy.org/protscale/) tool was used to find out the transmembrane structures of the protein. Kyte & Doolittle scale was used in this study, having the threshold value above 1.6. Hydrophobicity and hydrophilicity of the amino acids of the protein sequences and the trans-membrane α- helices of the wild and mutant types of cat and dog *Hspb1* protein were studied.

*v)* Nascent protein is always nonfunctional until and unless it undergoes post-translational modifications. So ***"ScanProsite"*** (http://prosite.expasy.org/scanprosite/) tool was used to find-out post-translational changes in the *Hspb1* protein sequences of cat and dog. Phosphorylation, n-myristoylation, ATP/GTP binding sites motif A etc. are the changes which were observed in these proteins.

## RESULTS AND DISCUSSION

### *Canine Hspb1 Protein Analysis*

Three mutations were observed in the CDS of *Hspb1* through MSA using "Sequencher" software[7](Fatima *et al.*, 2011). After translation and performing protein BLAST, three mutant proteins were observed and named as M1, M2 and M3 in this study in dog mammary tumor, granuloma and lymphoma cases respectively. M1 was found changed at position 148A>T which replaced Serine residue to Cysteine at 50 a.a sequence in mammary tumor, similarly M2 has 148A>G, in which Serine is replaced by Glycine in dog granuloma and M3 has a major 5 bp insertion c.220Ins5 in its CDS sequence which proved as frame shift mutation and produced a premature protein in canine lymphoma case.

#### *i) Physiochemical properties*

The bioinformatics tool **"*Protparam*"** was applied to determine the physiochemical properties of the wild type and its three mutants found in mammary, granuloma and lymphoma cases. As M1 and M2 don't differ extensively so their results are only slightly changed. Major differences were observed in M3. The molecular weight is calculated by the addition of water molecule and also the mass of isotopes, all the three mutants have different molecular weights. By using amino acid pKa value which depends on the side chain of the amino acid theoretical PI is calculated it describes the pH characteristic of the protein. The wild type and M2 has the same theoretical PI. The total no. positive and negative residues in M1 and M2 are similar to the wild type, mutant 3 shows major difference. The light that is absorbed by the protein at a certain wavelength is explained by extinction coefficient the results indicate that it is same for wild type and M2. Estimated half-life is same for wild type and mutants; it is the time when half of the protein disappears after its synthesis. The instability index is same for wild type and M1. It indicates the stability of a protein in the test tube if the value is greater than 40 which specify that protein is unstable however in M3 half part of the protein is stable. The aliphatic index of wild type and M1, M2 is same but M3 shows difference because of frameshift, it is the volume covered by the aliphatic side chains of the amino acids. GRAVY value of all three mutants is different, the more positive the score is, the greater is the hydrophobicity. The second fragment of the M3 does not have a negative value (Table 1).

| **Physiochemical Properties** | **Wild type *Hspb1*** | **M1** | **M2** | **M3** | |
|---|---|---|---|---|---|
| No. of amino acid | 206 | 206 | 206 | 111 | 79 |
| Molecular weight | 22765.5 | 22781.6 | 22735.5 | 12039.5 | 7910.1 |
| Theoretical PI | 6.23 | 6.22 | 6.23 | 11.01 | 6.90 |
| Total no. of +ve charge residue | 25 (Asp + Glu) | 25 | 25 | 7 | 6 |
| Total no. of- ve charged residue | 23( Arg + lys) | 23 | 23 | 13 | 6 |
| Formula | $C_{1019}H_{1564}N_{286}O_{303}S_3$ | $C_{1019}H_{1564}N_{286}O_{302}S_4$ | $C_{1018}H_{1562}N_{286}O_{302}S_3$ | $C_{542}H_{806}N_{162}O_{147}S_3$ | $C_{351}H_{575}N_{107}O_{99}S_1$ |
| No. of atoms | 3175 | 3175 | 3171 | 1660 | 1133 |
| Extinction coefficient | 40450 | 40575 | 40450 | 36105 | 1490 |
| Estimated half-life | 30 hours in mammals | 30 hours in mammals | 30 hours in mammals | 30 hours in mammals | 30 hours in mammals |
| Instability index | 64.47 Unstable | 64.47 Unstable | 64.57 Unstable | 94.75 Unstable | 33.86 Stable |
| Aliphatic index | 65.87 | 65.87 | 65.87 | 45.14 | 116.33 |
| GRAVY | -0.541 | -0.525 | -0.539 | -0.544 | 0.441 |

**Table 1.** Comparison of Physiochemical Properties of Wild and Mutant type *Hspb1* in Dog

### *ii) Protein secondary structure prediction*

A second tool named **"*PsiPred*"** was applied to study the secondary structure of the protein and results showed coiled, helical and extended form of the protein structure in the wild and all mutant protein. Along with the secondary structure of the protein the confidence value is also given for the amino acids. This confidence value has a range of 0-9; the higher the score is, it denotes the exact prediction of the secondary structure. The target sequence of amino acids is also represented in the results along with the secondary structures. The results indicate that M1 and M2 have similarity with the wild type. The major difference in the secondary structure of the protein is present in M2 in which there is insertion of base pairs. The first and second fragment of M3 has entirely different secondary structure than the wild type. According to the key, the helical structure is denoted by a cylinder shape, the coil is represented by a straight line and the strand is represented by an arrow. Results specify that M3 has more helical structure than the M1 and M2. Whereas mutant 1, 2 and the wild type has more stranded and coiled secondary structure.

In wild type, M1 and M2 the confidence score is higher as compared to confidence score in M3 (Figure 1).

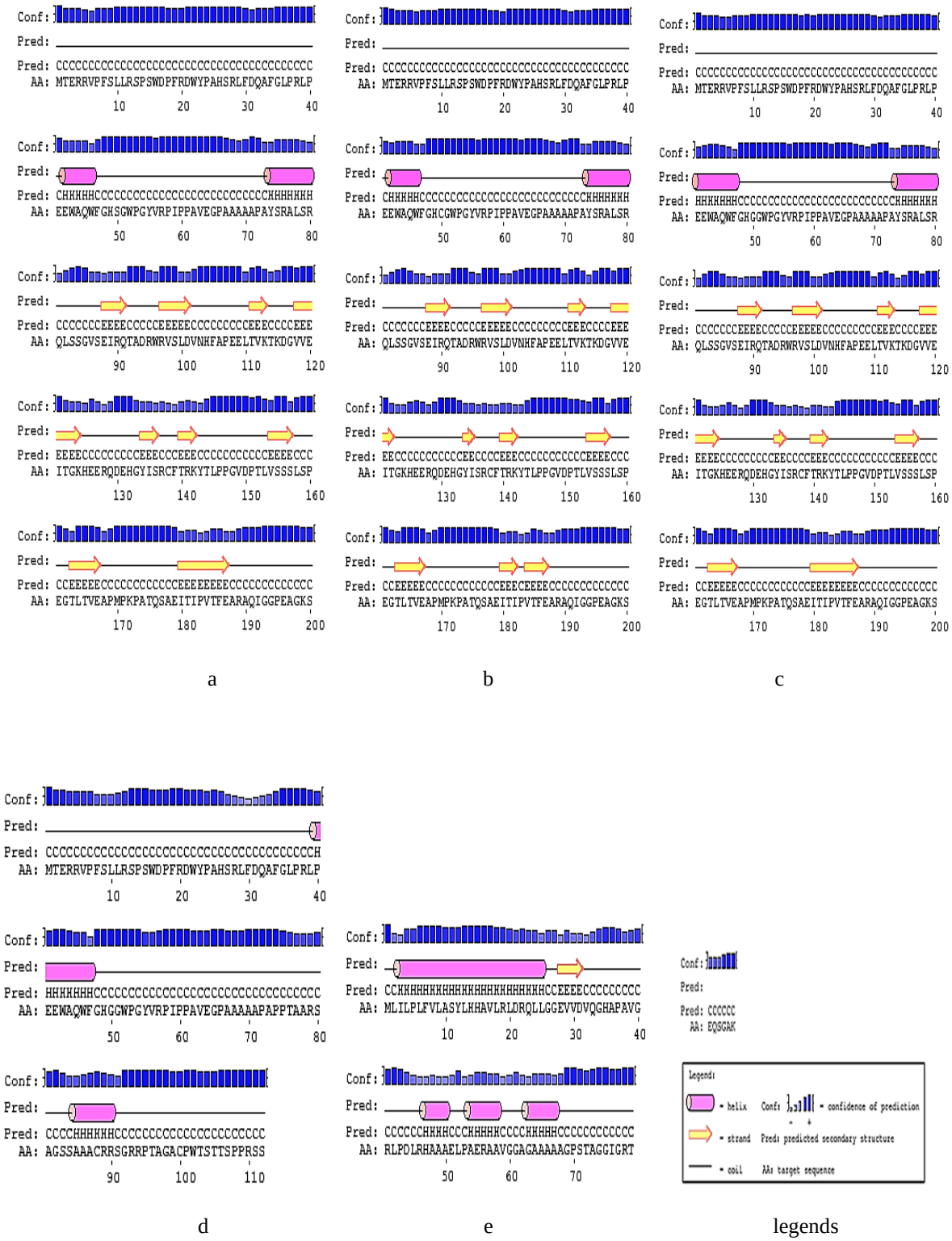
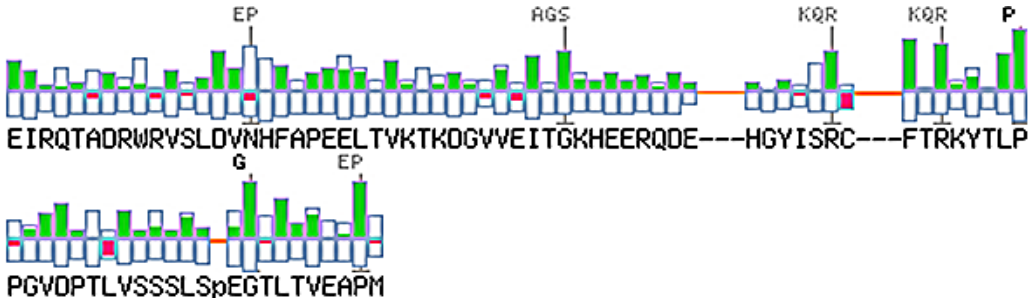



**Figure 1.** (a) Wild type *Hspb1*, (b) M1 protein in mammary tumors, (c) M2 in granuloma, and (d) (e) M3 in lymphoma appeared in two fragments because premature protein was formed due to stop codon after a.a 111. Straight black line indicates coil structure; pink cylinders indicate helical and yellow arrows show extended structure. M1 and M2 predicted almost similar results because they have difference of only one a.a as compare to wild type. Whereas M3 is obvious with two fragments and produce a premature protein due to frame shift mutation and created the stop codon.

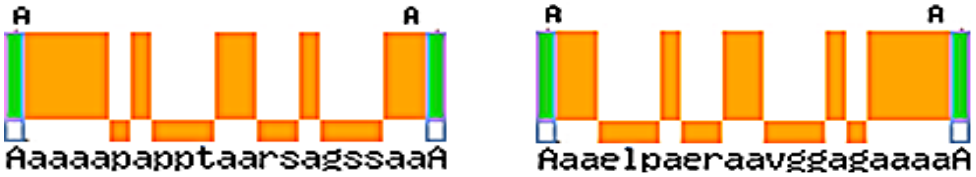
### *iii) Finding conserved domain*

Conserved domains are present in protein structures which execute distinct functions and they act as building blocks of amino acid residues

which can recombine to form various proteins and perform different functions. They were analyzed using "***Motif Scan***", in which length of the bars tells us about how conserved this particular amino acid is, on a particular site. White regions in the rectangular bars show that the amino acid is conserved but not in its correct position, whereas green regions show that the amino acid is highly conserved in all proteins and is present at its accurate place. Waterman Smith algorithm score was observed 122 in this protein, higher the score the more conserved domain it has and vice versa. Negatively scored amino acids in red color are present below the central-line which is aligned. E-value of wild type, M1 and M2 is 1.7e-12 and M3 is 0.35. Wild type, M1 and M2 have same match details (Figure 2a) as they underwent single nucleotide replacement in their CDS which shows the major vault protein region repeats profile that contains vaults involved in signal transduction and nucleo cytoplasmic transport. In M3 with five bp insertion in dog lymphoma match details are different. Proteins have specific regions called biased sequence regions which are low complexity regions as they contain any one or few amino acids in access. M3 contains alanine rich region. The alanine rich region profile of both fragments of M3 and the difference in the sequence of profile of both the fragments is clearly visible in (Figure 2b & 2c)




(a)




(b) (c)

**Figure 2.** (a) Match details (Heat shock hsp20 proteins family profile) of wild type, M1 in mammary tumor, M2 in granuloma of hspb1 in Dog, (b) Match details of fragment 1of M3 in lymphoma (Alanine- rich region profile). (c) Alanine-rich region profile of fragment 2 of M3.

***CD- server:*** *NCBI CD server* was also used along with motif scan so a comparison can be made. Alpha crystalline domain (ACD) and IbpA (Figure 3) were two conserved domains found in the protein sequence. Similar domains were also observed in other species by performing BLAST.

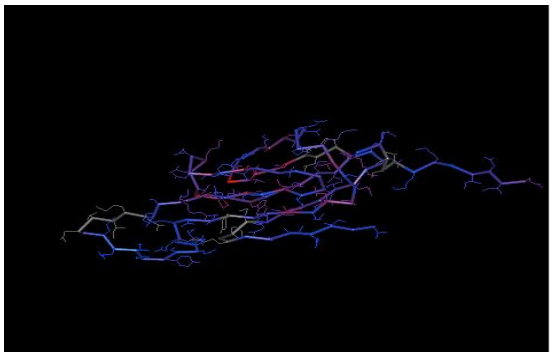


**Figure 3.** The structure IbpA domain viewed by Cn3D software

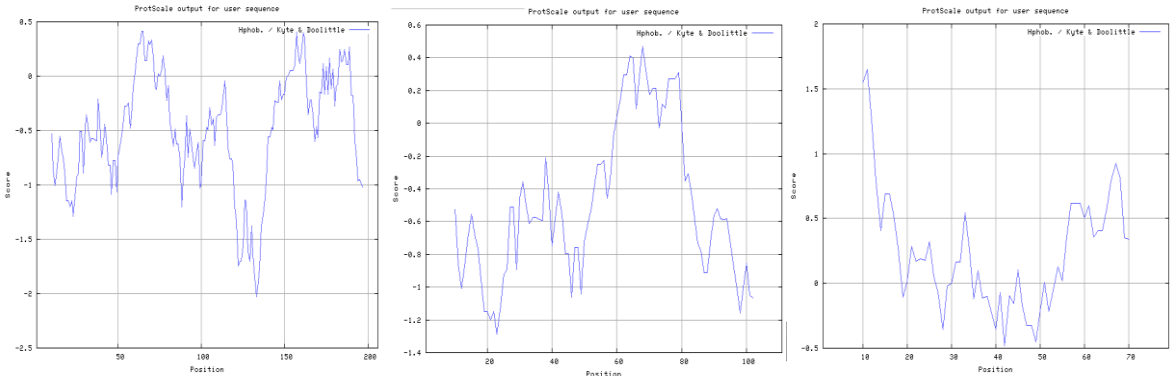
### *iv) Transmembrane structure*

***Protscale:*** This tool was applied to find-out the transmembrane structures using Kyte & Doolittle algorithm. Results of wild type, M1 and M2 were similar (Figure 4a). Protein sequence of M3 was fragmented due to frame shift mutation and two graphs of the M3 shows its transmembrane structures **(**Fig. 4b, 4c**)**. Protscale tool can compute polarity, hydrophobicity, and

hydrophilicity and transmembrane structures using 55 different algorithms having different threshold values and the amino acids present above threshold values show transmembrane structures. Each algorithm attributes different values to the amino acids and those given by Kyte & Doolittle algorithm are shown in (Figure 4a)

```
Ala:  1.800  Arg: -4.500  Asn: -3.500  Asp: -3.500  Cys:  2.500  Gln: -3.500
Glu: -3.500  Gly: -0.400  His: -3.200  Ile:  4.500  Leu:  3.800  Lys: -3.900
Met:  1.900  Phe:  2.800  Pro: -1.600  Ser: -0.800  Thr: -0.700  Trp: -0.900
Tyr: -1.300  Val:  4.200  : -3.500  : -3.500  : -0.490
```

(a)




(b) (c) (d)

**Figure 4.** (a) Individual values of 20 a.a using Kyte & Doolittle algorithm. Kyte & Doolittle plot for transmembrane structures- (b): plot showing transmembrane structures of *Hspb1* protein of wild type and mutant 1 and 2. (c) Plot showing transmembrane structures of fragment 1 of mutant 3. (d) Plot showing transmembrane structures of fragment 2 of mutant 3 *hspb1* protein sequences.

### *v) Post-translational modification*

***ScanProsite:*** M1 and M2 have same post-translational modification sites as in wild type, a single myristoylation site difference was observed. i.e., there are 2 n-myristoylation sites in wild type and M1, whereas 3 n-myristoylation sites in M2. M3 has different post-translational sites as shown in the (Table 2). "[ ]" shows any one of the amino acid (a.a) mentioned in this brackets may be present at this position, "x" shows any of the a.a, while a.a within"{}"shows any a.a except within braces similarly parenthesis "( )" shows, how many a.a should be there at particular site. $V_{max}$ and $K_m$ of the phosphorylation reaction is enhanced by the presence of basic residues at the C or N terminal of the particular target amino acid. C terminal residue has serine or threonine a.a present very close to it which is phosphorylated by protein kinase C. In tyrosine kinase phosphorylation reactions, substrates of the tyrosine kinase are either lysine or arginine present near N terminal of phosphorylated tyrosine. At N terminal certain acidic residues like Asp and Glu are also present in tyrosine. cAMP and cGMP dependent protein kinases are involved in the phosphorylation of the serine and threonine residues which are present near two N terminal consecutive basic residues [16]. Glycine should be the N terminal residue in case of myristoylation reaction. Glycine provides basic residue or lysine and arginine as two consecutive basic residues can act as precursor cleavage site in amidation reaction.

**Table 2.** Post-translational modifications in *Hspb1* protein sequence of wild type and in all mutants in Dog.

| Sr. No | Post Translational Modification | Amino Acid position | Wild Type & M1 | M2 | M3 | Consensus Sequence |
|---|---|---|---|---|---|---|
| 1 | **Protein kinase C phosphorylation site** | 2-4<br>91-93<br>111-113<br>122-124<br>140-142 | ✓<br>-<br>✓<br>✓<br>✓ | ✓<br>-<br>✓<br>✓<br>✓ | ✓ Fragment 1<br>✓ Fragment 1<br>-<br>-<br>- | [ST]-x-[RK] |
| 2 | **Tyrosine kinase phosphorylation site** | 128-134 | ✓ | ✓ | - | [RK]-x(2)-[DE]-x(3)-Y or[RK]-x(3)-[DE]-x(2)-Y |
| 3 | **cAMP and cGMP dependent protein kinase** | 93-96<br>141-144 | -<br>✓ | -<br>✓ | ✓ Fragment 1<br>- | [RK](2)-x-[ST] |
| 4 | **N- myristoylation site** | 50-55<br>60-65<br>61-66<br>63-68<br>82-87<br>148-153<br>193-198 | -<br>-<br>-<br>-<br>-<br>✓<br>✓ | ✓<br>-<br>-<br>-<br>-<br>✓<br>✓ | ✓ Fragment 1<br>✓ Fragment 2<br>✓ Fragment 2<br>✓ Fragment 2<br>✓ Fragment 1<br>-<br>- | G-{EDRKHPFYW}-x(2)-[STAGCN]-{P}[GistheN-myristoylationsite] |
| 5 | **ATP/GTP binding site motif A (P-loop)** | 193-200 | ✓ | ✓ | - | [AG]-x(4)-GK-[ST] |
| 6 | **Amidation site** | 91-94 | - | - | ✓ Fragment 1 | x-G-[RK]-[RK] |

### *Feline Hspb1 Protein Analysis*

Same tools were applied on this protein as on *canine Hspb1* and here a single mutation was observed in its c.34C>A position, which appeared as synonymous mutation so similar results were observed as there was no change in protein sequence in wild and mutant type.

#### *i) Protparam*

The physiochemical properties of wild and mutant type protein showed the similar results. The properties indicated that there are 205 amino acids which have molecular weight of 22720.5, theoratical PI was 6.23 which indicated the pH characteristics of the protein, no of +ve residues were 25 and no. of –ve residues were 23, its chemical formula : $C_{1019}H_{1565}N_{285}O_{301}S_{3,}$ total no. of atoms are 3173, the extintion coefficient is 40450 that indicated the light absorbed by the protein, estimated half life is 30 hours in the mammalian cell which is the time in which the protein is half degraded after the synthesis, the instablity index is 65 which indicated that the protein is unstable in the test tube, aliphatic index is 67.61 which specifies the volume occupied by the aliphatic amino acids such as alanine, valine, isoleucine and grand average of hydropathecity is observed -0.524 the value is negative which means it is less hydrophobic, the more positive value indicates more hydrophobicity.

#### *ii) Psipred*

The secondary structure of the wild and mutant type was same as there is no change in the amino acid sequence. The coiled, helical and extended structure is given by psipred. The strand is denoted by an arrow, a straight line indicated coiled confirmation and cylinder shape indicted helical structure. According to the results, the secondary structure is more coiled and extended rather that helical confirmation. Only two helical structures are present one at 41-46 amino acid position and a second helix is at 72-79 position. The confidence value is also high from a.a 1-40 which means the higher confidence value more

accurate sturcture is predicted. A low confidence score can be seen from a.a 135-143 and then from 179-183 **(Fig. 5).**

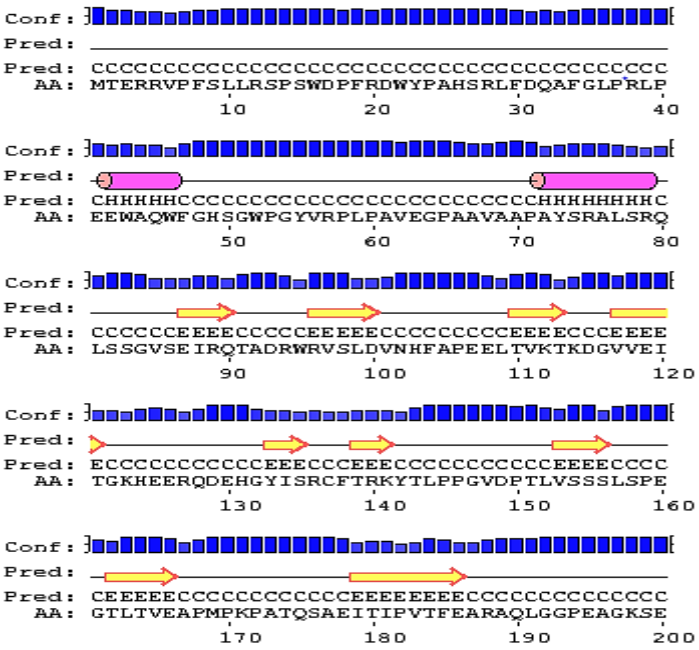
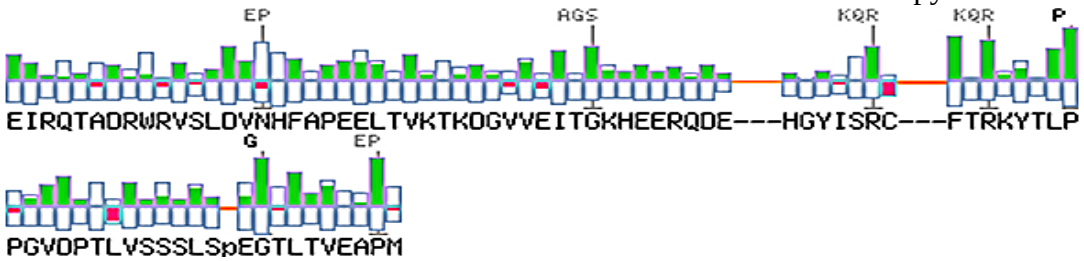


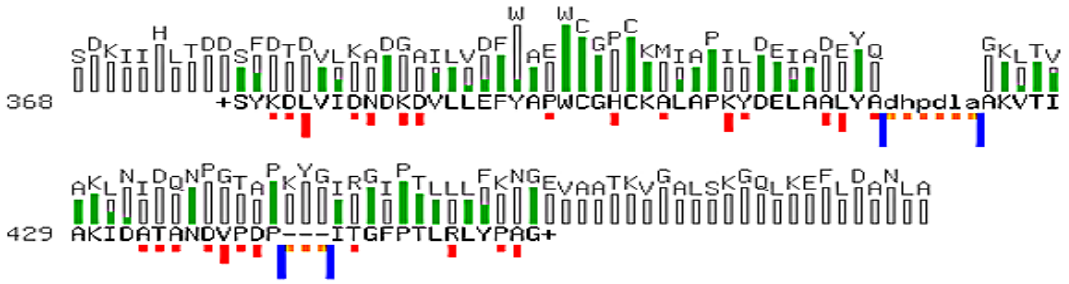

**Figure 5.** Secondary structure of Feline Hspb1 protein by Psipred tool, top most blue line shows the confidence of predicted structure, Straight black line indicates coil structure, pink cylinders indicate helical and yellow arrows show extended structure

*iii)* ***Motif Scan***

**(**Figure 6b) shows the local alignment of the two sequences. Waterman Smith algorithm score is 122, which is high in case of more conserved domain. Negatively scored amino acids are shown in red color. Match details of the wild and mutant type *Hspb1* protein sequence in cat were same (Figure 6a). Length of the bars tells that particular domain is highly conserved. Green bars show that amino acids in that particular domain are highly conserved in all species and are present at correct position whereas blank bars indicate that the highly conserved amino acid lacks its correct position. Conserved sequence motifs or patterns are present in conserved domains which help in detection in the polypeptide protein sequences. The MVP repeats region profile of the protein sequences of wild type and mutants of cat is same as shown in the (Figure 6a). This region contains vaults which are outsized ribonucleoprotein units, having 3 distinct proteins. Higher eukaryotes also contain few small RNA vaults which are packed to form tub-shaped molecules. The main component of the vault is MVP which is major vault protein as shown in (Figure 6a) which correlates with poor diagnosis and is concerned to resistance of cancer therapy.




(a)




(b)

**Figure 6.** (a) Match Details of wild and mutant type *Feline Hspb1* protein. (b) Graphical representation of a local alignment of two sequences

***NCBI-CD server*** was also used and two conserved domains were found, alpha crystalline domain and IbpA similar to *Canis familiaris*.

*iv)* ***Protscale***

The proteins which are embedded in the membrane of the cell are transmembrane structures which are integral proteins. Transmembrane proteins have three domains which include the one inside the cell, one outside the cell and one is embedded in the membrane. These domains can be hydrophobic or hydrophilic and this nature can be found out using protscale tool. Hydropathy of the transmembrane structures was found using Kyte & Doolittle plot. Similar results of wild and mutant protein sequences of cat were found. Seven transmembrane structures were observed (Figure 7).

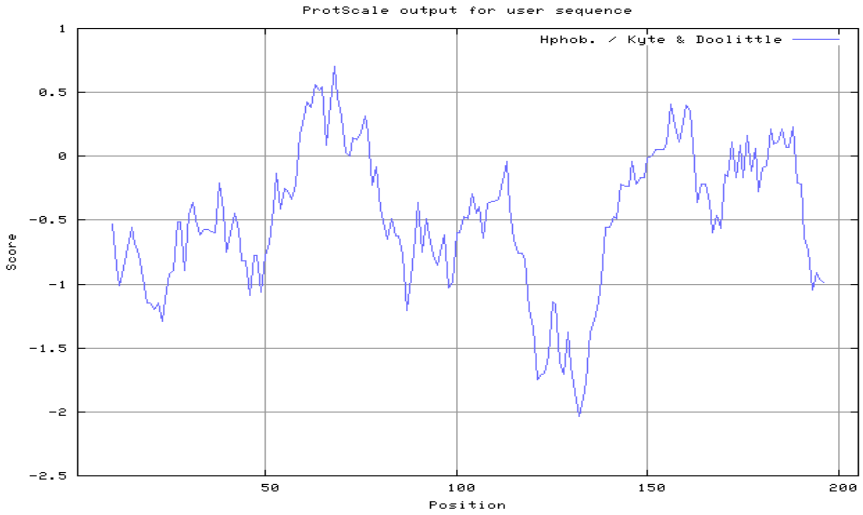



**Figure 7.** Kyte & Doolittle Plot showing transmembrane structures in hspb1 protein sequence of cat.

*v)* ***ScanProsite***

Wild and mutant *Hspb1* protein sequences of cat have same post translational modifications as shown in the (Table 3). "[ ]" shows any one of the a.a mentioned in this brackets may be present at this position, "x" shows any of the a.a, while a.a within"{}"shows any a.a except within braces and parenthesis "( )" shows, how many a.a should be at particular site. Serine or threonine is the protein kinase C phosphorylation site. Apart from aspartate and glutamate, enolase or lipocortin II residues can also act as tyrosine kinase phosphorylation site. Crystallographic results show that the proteins which bind with ATP and GTP contain less or more conserved sequence patterns [8]. A flexible loop of glycine rich region is formed between alpha helix and beta sheets which interact with nucleotide phosphate groups in P-loop site modification.

**Table 3:** Post-translational modifications in *Feline Hspb1*

| Sr. No | Post Translational Modification | Amino Acid position | Consensus Sequence |
|---|---|---|---|
| 1 | Protein kinase C phosphorylation site | 2-4<br>110-112<br>121-123<br>139-141 | [ST]-x-[RK] |
| 2 | Tyrosine kinase phosphorylation site | 127-133 | [RK]-x(2)-[DE]-x(3)-Y or[RK]-x(3)-[DE]-x(2)-Y |
| 3 | cAMP and cGMP dependent protein kinase | 140-143 | [RK](2)-x-[ST] |
| 4 | N- myristoylation site | 147-152<br>192-197 | G-{EDRKHPFYW}-x(2)-[STAGCN]-{P}[GistheN-myristoylationsite] |
| 5 | ATP/GTP binding site motif A (P-loop) | 192-199 | [AG]-x(4)-GK-[ST] |

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