Computational based Characterization of Heat shock protein Hsp27 from Humans and Canines

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Abstract

Heat shock protein functions as molecular chaperones with an imperative role in diverse cellular processes including protein folding, actin organization and stress resistance. Heat shock protein 27 (Hsp27), a member of the small Hsp (sHsp) has been linked to tumor progression as well as a therapeutic target for cancer treatment in humans as well as canines. A detailed in silico computational investigation of the Hsp27 protein from humans and canines was analyzed with respect to its structural, functional and phylogenetic properties. Although less extensively characterized in canines, attempts were made to compare the human protein with that of canines using the computational tools available to help the researchers get acquainted with the protein structure. Hsps are the most evolutionary conserved class of proteins.

Nevertheless, the Hsp27 proteins of humans and canines are clearly related. Though divergent in sequence, Hsp27 proteins are conserved in their structural properties. Hsp27 protein also appears to share other functional properties as well. In this study, a flexible, unstable, hydrophillic protein with an average molecular weight of 27KDa was reported for humans as well as canines. Specifically, the phosophorylation of protein supports the development of anti-Hsp27 agents for treatment of cancer. Moreover, this theoretical in silico analysis of Hsp27 protein will be substantial for cancer research and health care.

Keywords: Heat shock protein, hsp27, protein, cancer, analysis, canine.

Introduction

Heat shock proteins or Hsps are a group of proteins that are produced in response to heat. This response mechanism is the most highly conserved genetic system known, existing in all the known organisms from archaebacteria to eubacteria, from animals to plants, even though the response might vary in different organisms. Hsps were first initially studied in response to heat shock at elevated temperatures[31] but are now acknowledged to express during stresses like cold exposure[24], tissue remodeling or wound healing[22] and UV light[4]. Hsps play a vital role in normal cell functioning as well as functioning as intra cellular chaperones by ensuring correct folding of the proteins[9].

Based on their molecular weights, Hsp proteins are classified as Hsp70, Hsp90 where 70 stands for size of the protein in kilodaltons[23]. Ubiquitin is a small 8-Kda protein when induced by heat, it also produces the heat shock response[30]. Small heat shock protein (sHSP) is a 80 amino-acid alpha crystallin conserved protein binding domain[21]. Proteins are ubiquitously the most abundant proteins induced by heat shock as well as in normal development. Besides their role in thermotolerance, they also play an important part in cell proliferation, cancer vaccines and therapeutics, drug resistance and agriculture which make them proteins of special clinical interest.

Heat shock protein 27 (Hsp27) is a member of the small Hsp (sHsp) family among α-crystallin, Hsp20 and others and is also known as heat shock protein beta-1 (HSPB1)[5]. The common functions of sHSPs include thermotolerance, actin organization, inhibition of apoptosis, cell differentiation and regulation of cell development[40]. The sHps are reported as highly pleiotropic molecules both in terms of cell biology reports and molecular function. High expression levels of Hsp 27 are found in various cancers[7] including the sera of breast cancer patients indicative of metastasis and resistance to chemotherapy[33]. Hsp27 levels are triggered in stress by transcriptional activation and post-translational modification (phosphorylation) subsequential of the p38 MAPK stress kinase pathway[3].

Hsp27 has also been shown to be a mediator in cancer development and progression being a vital player of angiogensis[39]. A recent study reported the elevated serum Hsp27 in dogs with mammary tumors as compared to healthy dogs irrespective of the mammary tumor histotypes suggesting expoitation of Hsp27 as neoplastic signature of canine mammary tumors[1]. Hsp27 also appears to serve a significant role in various muscle/neurodegenerative diseases[34]. Notably, heritable mutations in Hsp27 central Pro residue have been reported to cause the hereditary motor neuropathy Charcot-Marie-Tooth disease[10].

The goal of this manuscript is to analyze the structural and functional properties of the Hsp27 protein in humans and canines. Although less extensively characterized in canines, attempts were made to compare the human protein with that of canines using the computational tools available to help the researchers get acquainted with the protein structure.

Material and Methods

Sequence retrieval and Multiple sequence alignment (MSA): The protein sequences of the human and the canine heat shock protein Hsp27 were retrieved from the NCBI protein database. Basic Local Alignment Search Tool (BLASTp) was used to get the similar sequences of other organisms (cattle, buffalo, goat, pig). Accession numbers of the following proteins are presented in table I. The sequences once retrieved in FASTA format were aligned using Clustal omega online tool using default parameters.

Table I
Accession numbers of the Hsp27 protein

Species name	Accession number
Homo Sapiens (Human)	CAA38016.1
Canis lupus (Dog)	AXQ88113.1
Bos Taurus (Cattle)	NP_001020740
Bubalus bubalus (Buffalo)	AIU47315.1
Capra hircus (Goat)	AFK93550.1
Sus scrofa (Pig)	AAV54182.1

Primary structural analysis of Hsp27 protein: Primary structural analysis of the Hsp27 protein of humans and canines was determined using ProtParam tool from Expert Protein Analysis System (ExPASy) which is the proteomic server of Swiss Institute of Bioinformatics (SIB) (https://www.expasy.org/)[12]. The biophysical and biochemical properties include molecular weight (Mw), number of positive and negative residues, isoelectric point(pI), extinction coefficients (EC-quantitative study of protein-protein and protein-ligand interactions)[13], instability index (II-stability of proteins)[15], aliphatic index (AI-relative volume of protein occupied by aliphatic side chains)[16], grand average hydropathicity (GRAVY-sum of all hydropathicity values of all amino acids divided by number of residues in a sequence)[17] and half life[14].

Secondary and tertiary structure prediction of Hsp27 protein: The amino acid sequence of the human and canine Hsp27 was subjected to secondary protein structure prediction using PSIPRED 4.0 server (http://bioinf.cs.ucl.ac.uk/psipred/)[17]. The tertiary structure prediction of the Hsp27 protein was modelled using *ab initio* approach with online available tool Swiss model software (https://swissmodel.expasy.org/)[42]. The model thus obtained, was further validated by ramachandran's plot (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php)

Functional analysis: Functional characterization of the human and canine Hsp27 for identifying protein families, functional sites and domains was done using Inter-ProScan (https://www.ebi.ac.uk/interpro/)[18]. ProtComp 9.0 identified the sub-cellular localization of proteins (ProtComp - Version 9). Motifs in the Hsp27 protein sequences were predicted by Psite online tool which is a protein domain database for functional annotation and description[36]. Potential phosphorylation sites of the protein sequences were predicted using NetPhos3.1[2] (http://www.cbs.dtu.dk/services/NetPhosK) server that is provided by Centre for Biological Sequence Analysis, Technical University of Denmark (CBS DTU).

The server predicts serine, threonine or tyrosine phosphorylation sites in eukaryotic proteins using ensembles of neural networks. The SS bonding of the cysteine residues in the Hsp27 protein was predicted using CYC_REC tool (CYS_REC). Location of signal peptide cleavage sites was predicted using Signal P-4.1[27].

Phylogenetic analysis: Phylogenetic analysis helps to understand the evolutionary relationship among the diverse species. The phylogenetic tree was constructed using unweighted pair group method (UPGMA) of the Molecular Evolutionary Genetics Analysis version 6.0 (MEGA)[19]. The consistency of the inferred phylogenetic tree was checked by bootstrap analysis of 1000 replications. The gaps and missing data were eliminated.

Protein-protein interaction study: STRINGv10.0 web server (http://string-db.org) was used to predict the interaction of human and canine Hsp27 protein with their other closely allied proteins respectively. A critical assessment of the protein-protein interaction network based on the direct and indirect associations for both the proteins was generated[38].

Ethical approval: Since the study does not involve any animal study, animal ethics committee approval was not required. The study utilizes the available online computational tools and the online gene sequences.

Results

Sequence retrieval and Multiple sequence alignment (MSA): % similarity of the human and canine Hsp27 protein with other species was determined. Human hsp27 was found to be 88, 87, 86, 89 and 88 percent identical to dog, cattle, buffalo, goat and pig respectively. Similarly, canine hsp27 was also 88, 90, 90, 89 and 91 percent identical to human, cattle, buffalo, goat and pig respectively. Human protein had the highest percent identity with goat while the canine protein resembled with pig. Multiple sequence alignment using Clustal omega software suggests variability among different species (Figure I).

A remarkable feature of sHsps to note was that it showed greater homology within organisms than between organisms. A report of soybean small hsp family revealed 90% amino-acid identity with each other whereas 20% amino-acid identity with the proteins of D. melanogaster, C. elegans and X. laevis was reported[25].

Primary structural analysis of Hsp27 protein: Table II shows the amino acid composition of the human and canine Hsp27 protein as evaluated by the ProtParam online server. Human Hsp27 has 205 amino acids while the canine Hsp27

has 206 amino acids. Proline and serine are the most abundant amino acids in humans followed by alanine. In canines, proline was the most abundant amino acid in canines followed by alanine and serine. Both the proteins have a homologus and highly conserved amino acid sequence with similar structural features.

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Human       MTERRVPFSLLRGPSWDPFRDWYP-HSRLFDQAFGLPRLPEEWSQWLGGSSWPGYVRPLP   59
Canine      MTERRVPFSLLRSPSWDPFRDWYPAHSRLFDQAFGLPRLPEEWAQWFGHSGWPGYVRPIP   60
Pig         MTERRVPFSLLRSPSWDPFRDWYPAHSRLFDQAFGLPRLPEEWSQWLSHSGWPGYVRPLP   60
Cattle      MAERRVPFSLLRGPSWDPFRDWYPAHSRLFDQAFGLPRLPEEWSQWLSHSGWPGYVRALP   60
Buffalo     MAERRVPFSLLRGPSWDPFRDWYPAHSRLFDQAFGLPRLPEEWSQWLSHSGWPGYVRALP   60
Goat        MAERRVPFSLLRGPSWDPFRDWYPAHSRLFDQAFGLPRLPEEWSQRLSHSGWPGYVRPLP   60
            *:**********.********** *****************:* :. *.****** :*

Human       PAAIESPA-VAAPAYSRALSRQLSSGVSEIRHTADRWRVSLDVNHFAPDELTVKTKDGVV   118
Canine      P-AVEGPAAAAAPAYSRALSRQLSSGVSEIRQTADRWRVSLDVNHFAPEELTVKTKDGVV   119
Pig         PPAIEGPAAVAAPAYSRLLSRQLSSGVSEIQQTADRWRVSLDVNHFAPEELTVKTKDGVV   120
Cattle      AAAIEGP------AYNRALSRQLSSGVSEIQQTADRWRVSLDVNHFAPEELTVKTKDGVV   114
Buffalo     AAAIEGP------AYNRALSRQLSSGVSEIQQTADRWRVSLDVNHFAPEELTVKTKDGVV   114
Goat        AAAIEGP------AYSRALSRQLSSGVSEIQQTADRWRVSLDVNHFAPEELTVKTKDGVV   114
              *:*.*      **.* ***********::***************:**********

Human       EITGKHEERQDEHGYISRCFTRKYTLPPGVDPTQVSSSLSPEGTLTVEAPMPKLATQSNE   178
Canine      EITGKHEERQDEHGYISRCFTRKYTLPPGVDPTLVSSSLSPEGTLTVEAPMPKPATQSAE   179
Pig         EITGKHEERQDEHGFISRCFTRKYTLPPGVDPTQVSSSLSPEGTLSVEAPLPKPATQSAE   180
Cattle      EITGKHEERQDEHGYISRCFTRKYTLPPGVDPTLVSSSLSPEGTLTVEAPLPKSATQSAE   174
Buffalo     EITGKHEERQDEHGYISRCFTRKYTLPPGVDPTLVSSSLSPEGTLTVEAPLPKSATQSAE   174
Goat        EITGKHEERQDEHGYISRCFTRKYTLPPGVDPTQVSSSLSPEGTLTVEAPLPKSATQSAE   174
            *************:***************** **********:****:** **** *

Human       ITIPVTFESRAQLGGPEAAKSDETAAK---     205
Canine      ITIPVTFEARAQIGGPEAGKSEQSGAK---     206
Pig         ITIPVTFEARAQLGGTEAGKSEKPGTK---     207
Cattle      ITIPVTFQARAQLGGPEAGKSEQPETSKDP     204
Buffalo     ITIPVTFQARAQLGGPEAGKSEQPENK---     201
Goat        ITIPVTFQA----------------------     183
            *******::
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Figure I: Multiple sequence alignment of Hsp27 protein

Table II
Amino acid composition of Hsp27

	Human Hsp27		Canine Hsp27	
Amino acid	**No. of amino acid**	**% of amino acid**	**No. of amino acid**	**% of amino acid**
Ala (A)	17	8.3%	20	9.7%
Arg (R)	16	7.8%	16	7.8%
Asn (N)	2	1.0%	1	0.5%
Asp(D)	10	4.9%	8	3.9%
Cys (C)	1	0.5%	1	0.5%
Gln (Q)	7	3.4%	8	3.9%
Glu (E)	16	7.8%	17	8.3%
Gly (G)	13	6.3%	15	7.3%
His (H)	5	2.4%	5	2.4%
Ile (I)	6	2.9%	7	3.4%
Leu (L)	16	7.8%	13	6.3%
Lys (K)	7	3.4%	7	3.4%
Met (M)	2	1.0%	2	1.0%
Phe (F)	7	3.4%	8	3.9%
Pro (P)	21	10.2%	22	10.7%
Ser (S)	21	10.2%	19	9.2%
Thr (T)	14	6.8%	13	6.3%
Trp (W)	6	2.9%	6	2.9%
Tyr (Y)	5	2.4%	5	2.4%
Val (V)	13	6.3%	13	6.3%

Table III shows the different physicochemical properties of the human and canine Hsp27 protein. Isoelectric point (pI) of a molecule is the pH at which it carries no net charge and is electrically neutral. The computed pI will help in choosing a buffer for protein purification and crystallization. pI of human Hsp27 protein was computed to be 5.98 signifying its acidic nature while the pI for canine Hsp27 was 6.23 suggestive of its mild acidic nature as compared to the human hsp27. As the value of instability index for both the Hsp27 protein was higher than 40, it signifies its unstable nature[41].

Aliphatic index stands for the relative volume of protein occupied by its aliphatic side chains (Alanine, isoleucine, leucine and valine). Aliphatic index values are directly propotional to the structural stability of a protein[16]. Both the proteins have a higher aliphatic index which suggests that the proteins are thermostable. The grand average of hydropathy (GRAVY) value indicates the solubility of proteins wherein the hydrophobic and hydrophilic properties of each amino acid chain are considered thoroughly. A positive value indicates a hydrophobic protein while a negative value indicates hydrophillic protein[20].

A negative GRAVY score for both the Hsp27 proteins indicate its hydrophilic character and a better interaction of protein and water. The estimated half life was around 30 hrs. Positively charged residues are calculated by the total number of arginine (Arg) and lysine (Lys) while the negatively charged residues are calculated by the total number of aspartic acid (Asp) and glutamic acid (Glu). These are supportive in resolving the topology of protein[26]. Though divergent in sequence, the Hsp27 proteins of humans and canines are conserved in their physicochemical properties. Both the proteins are flexible with an average molecular weight of 27Kda, unstable and similar hydropathy profiles.

Secondary and tertiary structure prediction: The secondary structure prediction of the amino acid sequence of human Hsp27 and the canine Hsp27 was determined elaborately using the PSIPRED online server. Upon comparative structure analysis, it was observed that both the human and canine Hsp27 protein structure had dominant coil structural content followed by strand and then helix. 57% of total amino acids contributed to coils, 26% to strand and 15% to helix in human Hsp27 protein (Figure IIa) whereas, 59% of total amino acids contributed to coils, 24% to strand and 16% to helix in canine Hsp27 protein (Figure IIb).

The dominance of coil structural content in the protein might be attributed to the presence of proline, as being the most abundant amino acid found in both humans as well as canine amino acid sequence. Proline has an exceptional property of creating kinks in the polypeptide chains, thus disrupting the secondary structure and consequential of coiling.

The tertiary structures of the Hsp27 proteins were predicted through the SWISS-MODEL. A template sequence with a significant similarity with the query sequence is required to predict the three dimensional structure of the protein. In our study, the selected template sequence was 6dv5.1, a crystal structure of Heat shock protein beta-1. The sequence identity of the template sequence with the query sequence was 100% for human model whileas it was 88.29% for canine model.

The oligo state of both the predicted protein models was homo-24-mer. Based on QMEAN and Z score, a good quality model for human (Figure IIIa) and canine was selected (Figure IIIb). Analysis of Ramachandran plot revealed 73.72% of the amino acids were in favoured and 9.61% were outliers for human Hsp27 model (Figure IVa). 73.69% of the amino acids were in favoured and 9.23% were outliers for canine Hsp27 model (Figure IVb). Similar *in silico* evaluation of human small heat shock protein Hsp27 by homology modeling has been reported[11] whereas the canine protein has been poorly characterized.

Table III
Physicochemical properties of lysyl oxidase protein

S.N.	Biophysical and biochemical Properties	Human Hsp27 protein	Canine Hsp27 protein
1.	No of amino acids	205	206
2.	Molecular weight	22782.52	22765.54
3.	Isoelectric point	5.98	6.23
4.	Negatively charged residues (Asp + Glu)	26	25
5.	Positively charged residues (Arg + Lys)	23	23
6.	Extinction coefficients	40450	40450
7.	Abs 0.1%	1.775	1.777
8.	Instability index:	62.82 (Unstable)	64.47 (Unstable)
9.	Aliphatic index:	68.54	65.87
10.	(GRAVY)	-0.567	-0.541
11.	Half life	30 hrs	30hrs

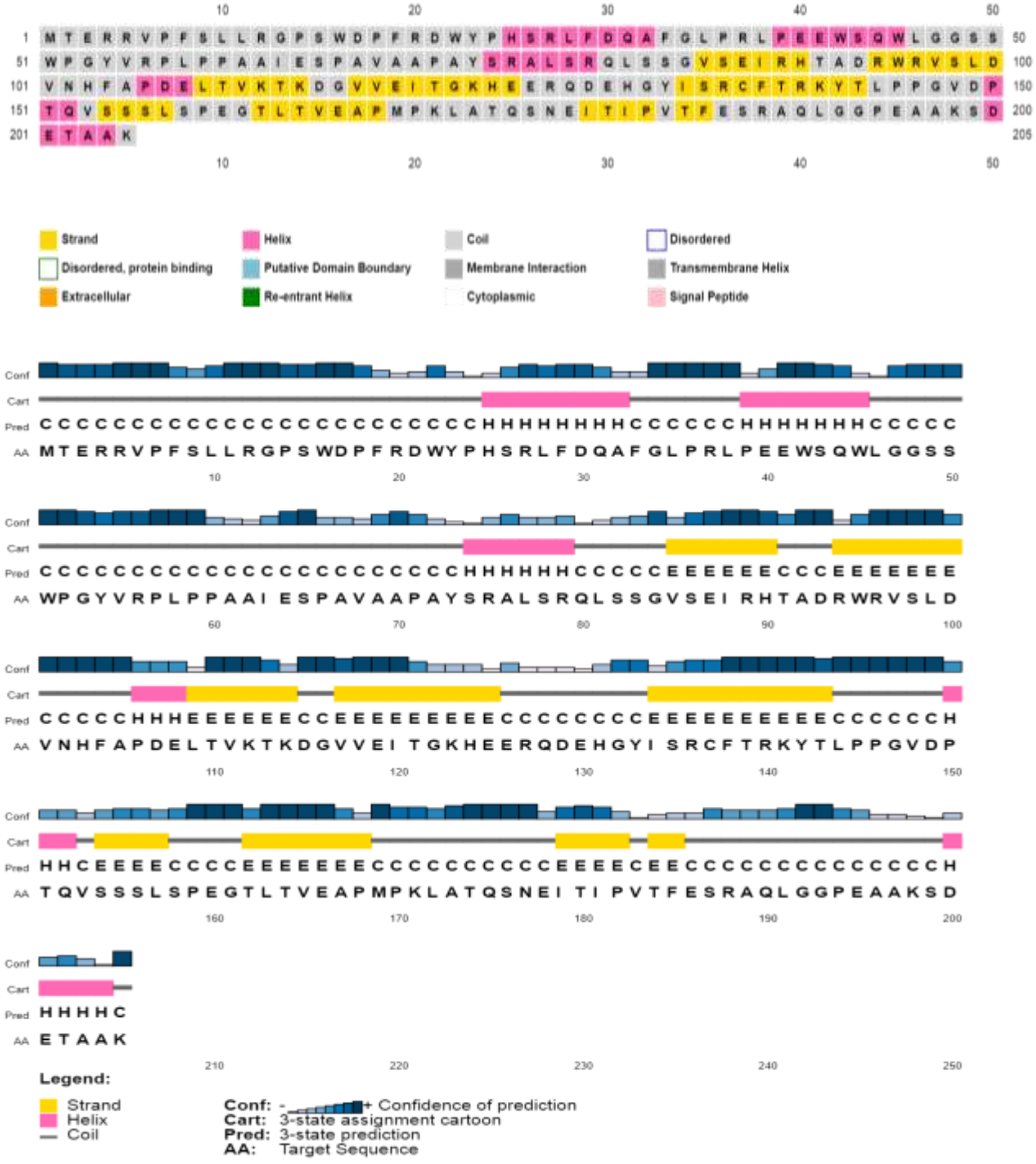
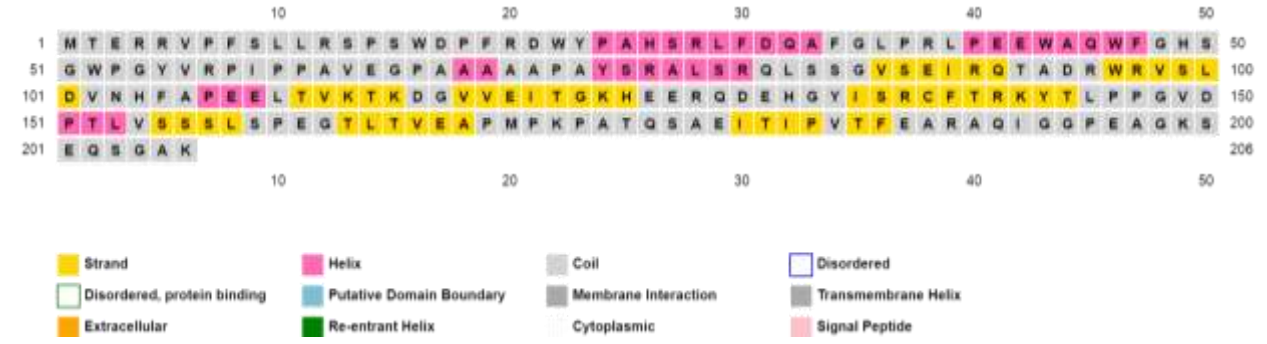


Figure IIa: Secondary structure prediction of human Hsp27 protein using PSIPRED
Coil dominates the secondary structure elements followed by strand



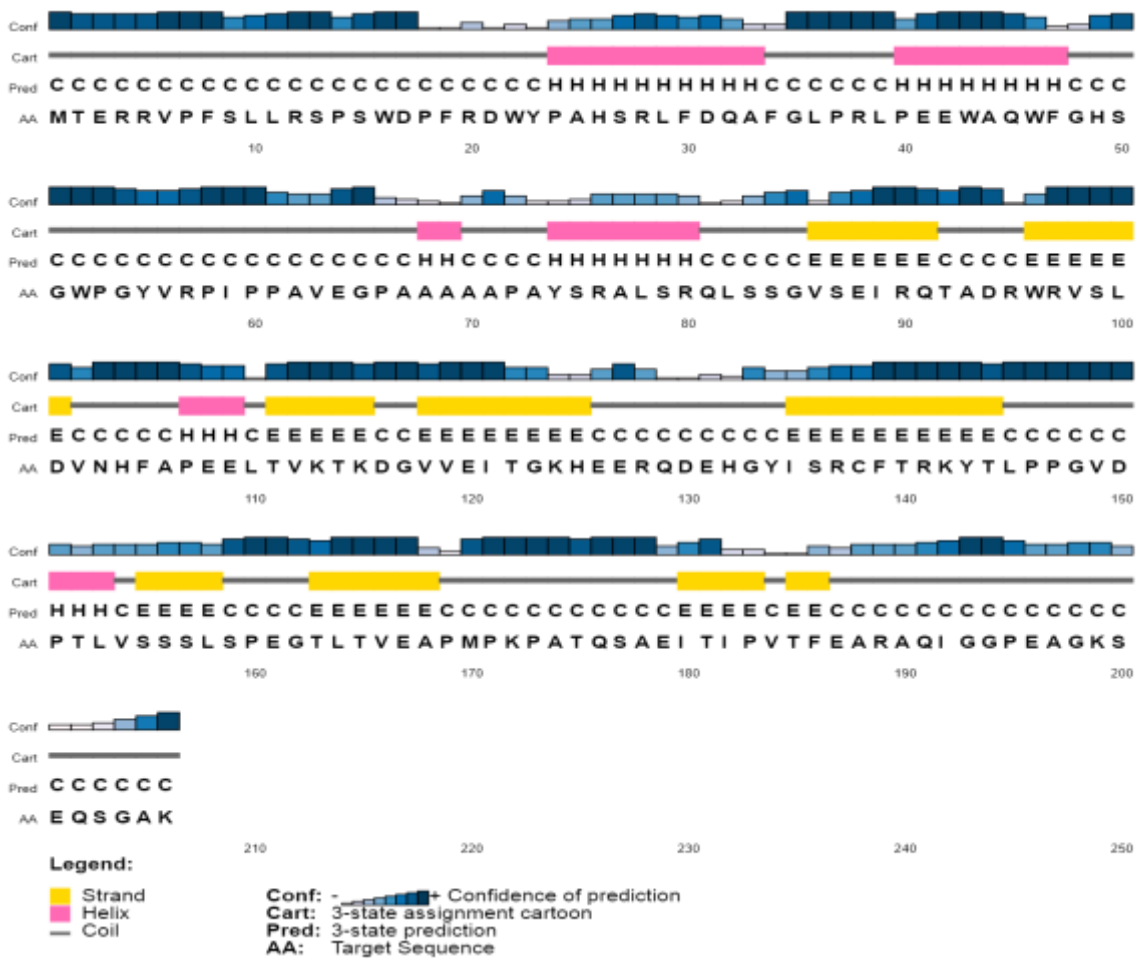


Figure IIb: Secondary structure prediction of canine Hsp27 protein using PSIPRED
Coil dominates the secondary structure elements followed by strand

Functional analysis: Both the mammalian proteins appear to share the functional properties as well. InterproScan predicted protein's family membership with small heat shock protein Hsp20 and alpha crystallin for both proteins. ProtComp 9.0 revealed that the sub-cellular localization of the protein in humans as well as canines was possibly multilocated – cytoplasm and nucleus.

Hsp27 has the property of being induced during different stages in development suggestive of its significant role in cell differentiation. Using the Psite software, it was found that the human protein sequence had somewhat similar motifs when compared with the canine protein except for ATP/GTP-binding site motif A (P-loop) found only in canine sequence at 193-200 amino acid residue.

Protein kinase C phosphorylation in both the mammalian proteins protects against a non apoptotic cell death, thus supporting the development of anti-Hsp27 agents for treatment of cancer. Motif regions are summarized in table IVa and IVb. Serine (21), threonine(14) and tyrosine (5) are predicted as potential phosphorylation sites in human protein while serine (19), threonine(13) and tyrosine (5) are predicted as potential phosphorylation sites in canine protein. Phosphorylation is closely associated with protein activity and indicative of protein function regulation.

Hsp27 phosphorylation has been reported to resist TNF-α-induced apoptosis and increase in the formation of oligomers[29]. A single cysteine residue was found in both the protein sequence at position 137 in humans and position 138 in canines using the CYC_REC tool and is probably not SS-bounded. Cysteine residues play a vital role in protein's thermostability. The SignalP 5.0 server predicts the incidence of signal peptides and the position of their cleavage sites. The likelihood of the signal peptide was around 0.0019 for human protein and 0.0021 for canine protein.

Phylogenetic analysis: The phylogenetic tree constructed using MEGA software showed that the human Hsp27 was phylogenetically more similar to goat and cattle sequence while the canine Hsp27 was more similar to buffalo and pig sequence (Figure V). Human Hsp27 is forming an independent clad together with cattle and goat whilst it clusters away from the canine Hsp27 sequence which is forming another independent clad with buffalo and pig sequence. To infer the evolutionary history of sHSPs, a small

eukaryotic sHSP was isolated from the mycobacterium *M. leprae*[43] suggesting that the small HSP family might have existed for over a billion years.

Protein-protein interaction study: Proteins generally function by interaction forming protein complexes. This network of protein illuminates the important clues as to the functioning of novel proteins. Based on various network parameters like gene fusion, text mining, co-expression, co-occurence, neighborhood and databases, a protein interaction network with 10 potential interacting protein associates was depicted for human (figure VIa) and canine Hsp27 protein (figure VIb). For human Hsp27, the closest interacting protein was found MAP kinase-activated protein kinase 2 (MAPKAPK2) and death domain-associated protein 6 (DAXX) while the distant interacting protein was MAP kinase-activated protein kinase 3 (MAPKAPK3) and estrogen receptor. The closest interacting protein for canine Hsp27 was mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2) while the distant interacting protein was polyadenylate-binding protein (PABPC1). Potential interacting protein associates of HSP27 protein are listed in figure VII a and b.

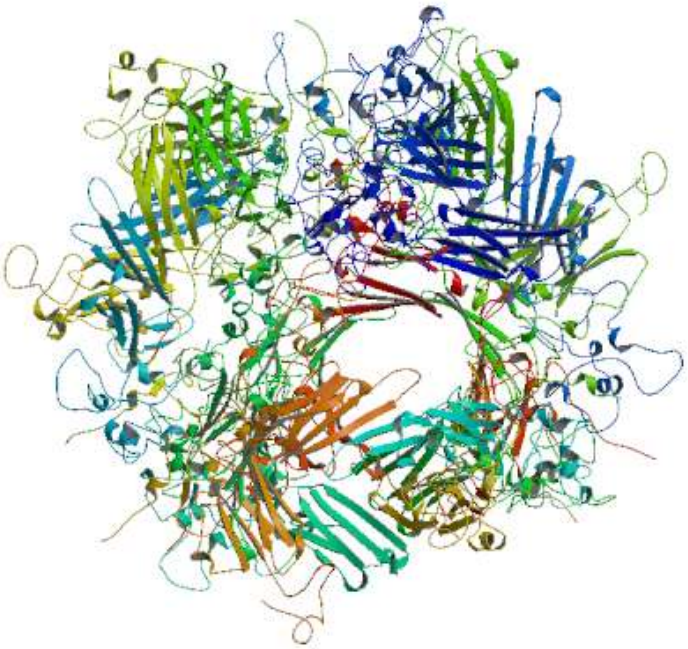


Figure IIIa: Tertiary structure prediction of human Hsp27 protein

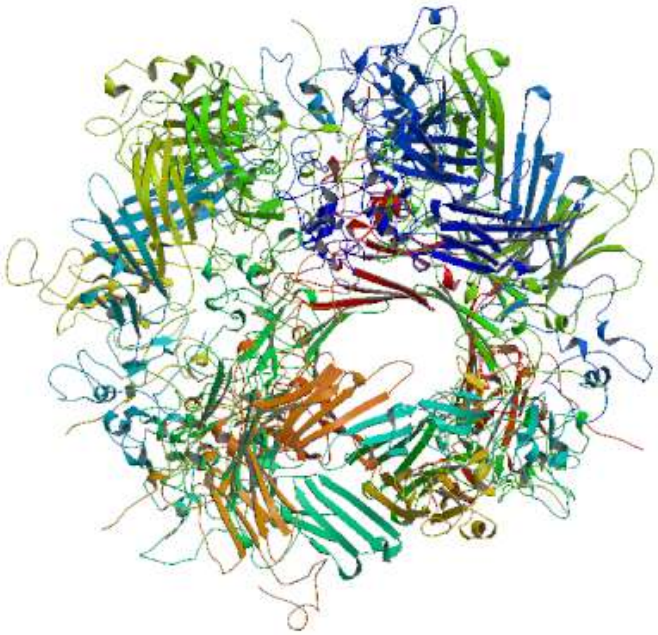


Figure IIIb: Tertiary structure prediction of canine Hsp27 protein

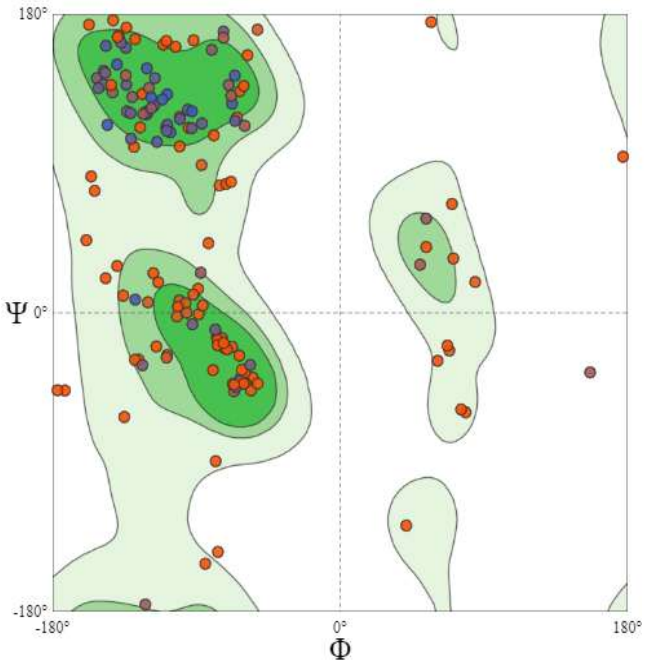


Figure IVa: Structure Validation by Ramachnadran Plot (Human Hsp27 protein)

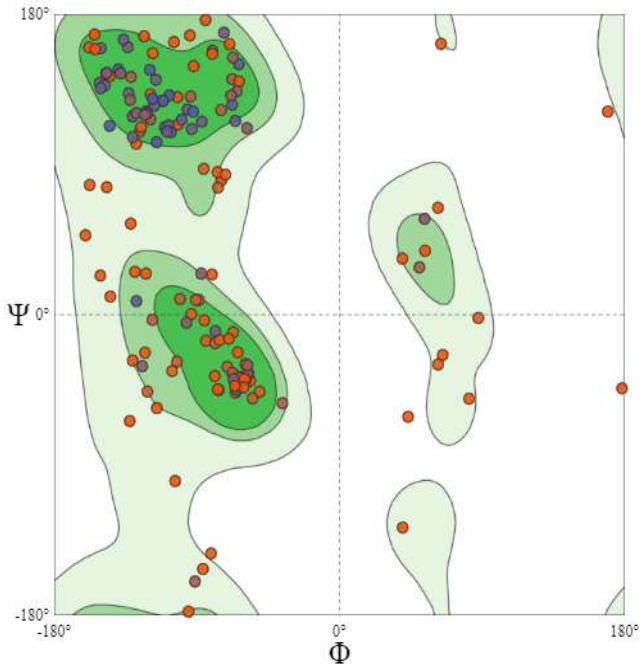


Figure IVb: Structure Validation by Ramachnadran Plot (Canine Hsp27 protein)

p38 MAP kinase when stimulated by different agents or mitogens or inflammatory cytokines like IL-1β and TNFα, leads to the activation of MAPKAP kinases 2 and 3 phosphorylating the mammalian sHSPs[32]. Phosphorylation is the key mode of activation of Hsp27 which facilitates the chaperone process under cellular stress[32]. Phosphorylated Hsp27 block the apoptotic pathways by their interaction with other pro-apoptotic proteins such as DAXX and ASK1[6].

Conclusion

Heat shock proteins are a group of proteins which not just regulate the normal physiology but are also important mediators in diseases including cardiovascular, wound healing and cancer. A long standing assumption about the heat shock response is their protective action against the toxic effects of heat and other stresses. These are the most evolutionary conserved class of molecules playing an imperative role in cellular homeostasis. However, the most baffling of them would be the small Hsps which are not just abundant but are universally disseminated proteins for normal development as well as the for the heat shock response. Hsp27 has been implicated in tumor invasiveness and appears to be a promising therapeutic target for cancer in humans as well as canines.

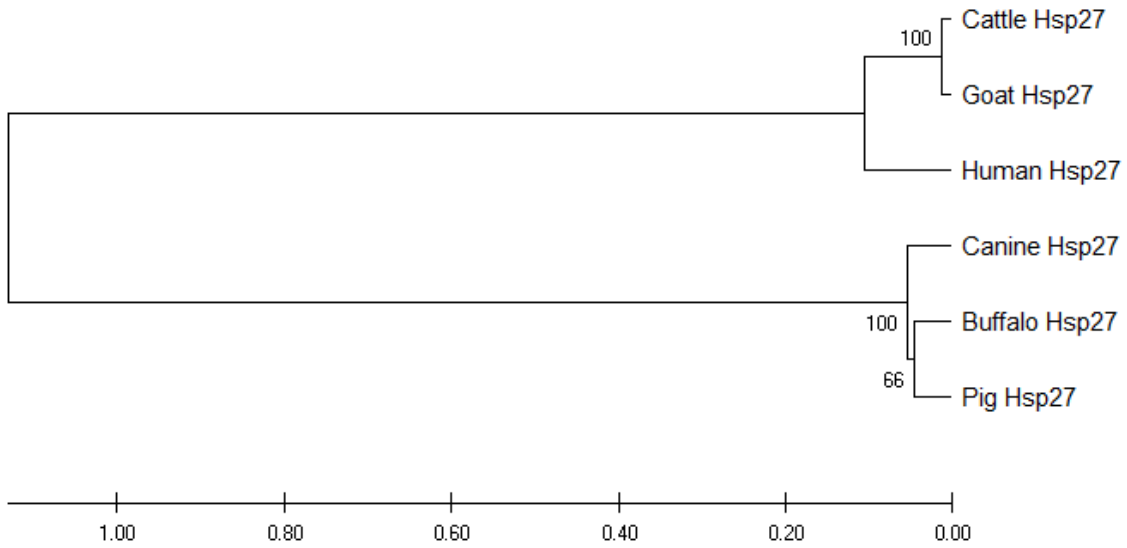


Figure V: Phylogenetic tree showing evolutionary relationship among different organisms using MEGA 6.0 software (Boot-straps: 1000 replicates)

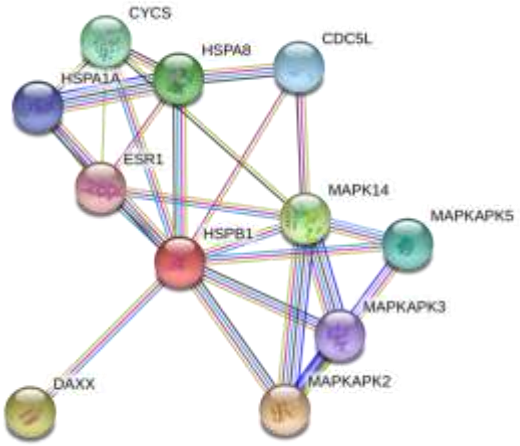


Figure VIa: Protein interaction map of human Hsp27 protein

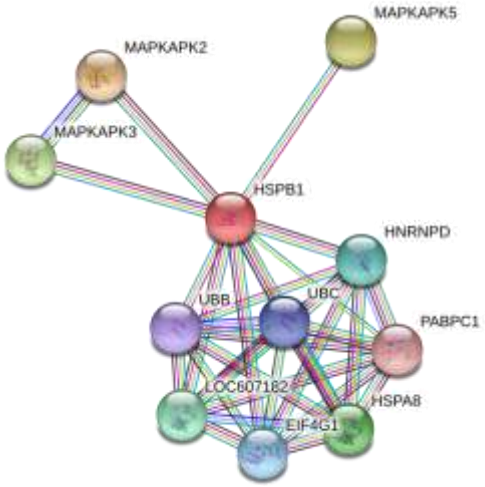


Figure VIb: Protein interaction map of canine Hsp27 protein

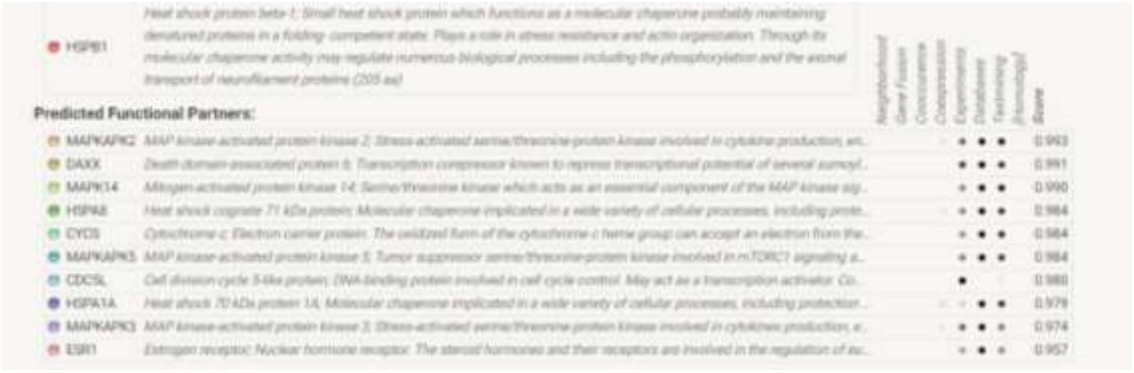


Figure VIIa: Screenshot from STRING server of predicting interacting proteins with the query sequence

Figure VIIb: Screenshot from STRING server of predicting interacting proteins with the query sequence

Table IVa
Motif regions present in the human Hsp27 protein

Motif information	No. of sites	Amino acid residues
cAMP- and cGMP-dependent protein kinase phosphorylation site	1	140-143
Protein kinase C phosphorylation site	4	2-4, 110-112,121-123, 139-141
Tyrosine kinase phosphorylation site	1	127-133
N-myristoylation site	2	147-152, 192-197
Microbodies C-terminal targeting signal	4	26-28, 74-76, 102-104, 187-189

Table IVb
Motif regions present in the canine Hsp27 protein

Motif information	No. of sites	Amino acid residues
cAMP- and cGMP-dependent protein kinase phosphorylation site	1	141-144
Protein kinase C phosphorylation site	4	2-4, 110-112,122-124, 140-142
Tyrosine kinase phosphorylation site	1	128-134
N-myristoylation site	2	148-153, 193-198
Microbodies C-terminal targeting signal	4	27-29, 75-77, 103-105, 188-190
ATP/GTP-binding site motif A (P-loop)	1	193-200

Thus, understanding the structural and functional properties of the protein will further facilitate a better understanding of mechanism of enzyme action. A comparative analysis of the human and canine Hsp27 protein signifies that the protein is more conserved in its structural properties. A flexible, unstable and a hydrophilic protein with a molecular weight of 27KDa was found in both the species. Further, the homology in their functional property would be sufficient to suggest a common function. The predicted secondary and tertiary structures will aid in shedding light on the biological functions of the protein. The study of Hsp27 protein can be further explored and utilized for beneficial therapeutic and diagnostic purposes aiding medical as well as animal experts.

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