INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 5, p. 265-272, 2017

RESEARCH PAPER OPEN ACCESS

Exploration of novel somatic *Hspb1* mutations in widespread neoplasms of *Canis familiaris*

Rashid Saif*[1], Ali Raza Awan[2], Leslie A. Lyons[3], Barbara Gandolfi[3], Muhammad Wasim[2]

[1]*Department of Biotechnology, Virtual University of Pakistan, Lahore, Pakistan*

[2]*Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan*

[3]*Department of Veterinary Medicine & Surgery, University of Missouri, Columbia, USA*

Key words: *Hspb1* mutations, Dog cancer, Tumor variations

http://dx.doi.org/10.12692/ijb/10.5.265-272 Article published on May 29, 2017

Abstract

Genotype-phenotype relationship is the matter of great concern in modern genetics, and exploring potential molecular markers through case control studies is considered to be a powerful diagnostic tool in field of comparative oncology. Eighteen cancers samples of *Canis familiaris* were genotyped through direct sequencing to ascertain the genetic alteration within *Hspb1* gene. Total 17 polymorphic sites were observed in this gene, and most of the cancers are observed heterozygous on these altered loci of exon 1 and exon 3 region. *Hspb1* gene, CDS locus 1349, 1407 and 1411 are homozygous, while CDS positions 148 and 1050 are mixed loci, and both are missense mutations. It is also observed that most of the cancer samples are heterozygous in intronic regions. These cancer-risk SNP markers may have significant role in diagnostic, prognostic and may be helpful hotspots in case-control association studies.

* **Corresponding Author:** Rashid Saif ✉ rashid.saif@vu.edu.pk

Introduction

Cancer may arises due to many reasons including chemical and radiation exposure as well as genetic causes by defecting allelic copies of the genes involved in cancer pathways. It may also occur due to sporadic or random causes due to spontaneously mutations. Seven types of cancers were analyzed including mammary tumors is one of the wide spread neoplasm of the aged female dogs. Mostly intact animals are more prone in this ailment due to hormonal imbalances (Withrow and Vail, 2007). Second one is canine transmissible venereal tumor (CTVT) or sticker's sarcoma which is another horizontally sexually transmitting tumor of the genital organs in dogs (Eze *et al.*, 2008; Ganguly *et al.*, 2013; Vermooten, 1987).

Third one is perianal adenocarcinoma which is cancer of hairless skin around anus. (North and Banks, 2009). Fourth one is canine lymphoma which involves the lymphocytes and lymphocytes storage organs (lymphoid tissues) like spleen, bone marrow and lymph nodes, especially in the head and neck region(Withrow and Vail, 2007). Oral Squamous cell carcinomas are also being address and analyzed in this study. Canine oral cancers are mostly malignant melanoma, squamous cell carcinoma or fibrosarcoma. Other includes osteosarcoma, chondrosarcoma, anaplastic sarcoma, hemengiosarcoma and mast cell sarcomas.

Somatic mutations in the cancerous tissue cannot pass through the offspring because they only accumulate in the neoplastic tissues, That's why, these somatic mutations are being considered potential markers for cancer diagnosis. Different molecular biomarkers are being studied, which have great implication in diagnosis and prognosis of different cancer. *Hsps* are highly significant and attention seeking in human and animal species (Paoloni and Khanna, 2007; Zoubeidi and Gleave, 2012). Therefore among these, *Hspb1* in molecular oncology is interesting biomarker for diagnostic and prognostic purpose, which has significant role as molecular chaperones and overexpressed in a wide range of cancers and implicated in tumor cell proliferation, differentiation, invasion, metastasis, apoptosis and recognition by the immune system(Garrido *et al.*, 2006).

Materials and methods

Sample collection

Total twenty one tumor samples and peripheral blood were collected from the Pet Center, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan and other private pet clinics through standard techniques. Out of total 21 samples, 18were successfully amplified and screened. Five CTVT cancers, 4 samples were mammary carcinoma, 3 from each perianal adenocarcinoma and SCC, 2 from each lymphocytic lymphoma (mostly necropsy sample of neck region lymph node) and granuloma, 01 from each melanoma and pelvic-warts. All samples were obtained through excisional biopsies resected tissues and stored immediately at -86C^{o}. Five normal tissues of dog were also collected (Table 1).

DNA extraction

TaiGen genomic DNA tissue kit (TaiGen Biotechnology Co., Ltd, Neihu Dist., Taipe, Taiwan) was used to extract the DNA from the tumorous tissues according to manufacture guidelines (Vogelstein, 1979). DNA concentrations and integrity was measured by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and gel electrophoresis methods respectively. High quality DNA of 50ng/uL concentration was used for downstream PCR amplifications.

Primer designing

One long-range primer set was designed for each gene from the DNA sequence ID ENSCAFT00000043541 for *Hspb1* using primer3 and Net Primer software (PREMIER Biosoft International, Palo Atlo, CA) (Rozen and Skaletsky, 1999). These primer sets amplified the total genes of 2998 bp of *Hspb1*. Then further internal primers were designed for Sanger sequencing of the complete gene (Table 2). *Hspb1* has two transcripts with 732bp and 2277bp mRNA in length and encodes 206 amino acids.

PCR amplification, protocol and reagent concentrations

Long-range polymerase chain reaction were conducted using Applied Biosystem thermo cycler with 94C° temperature for 2 minutes as initial denaturation, then 10 cycles of (94C° as cyclic denaturation for 10 sec, annealing at 61C° for 30 sec and extension at 68C° was adopted for 1 minute/kb).

Then remaining 30 cycles were conducted at (94C° as cyclic denaturation for 10 sec, annealing at 59C° for 30 sec and extension at 68C° for 1 minute/kb with an increment of 20 sec per cycle), at the end final extension was given at 72C° for 5 min and finally hold at 4C°. Optimized long-range PCR kit with dNTPs was used containing high-fidelity long polymerase, 5 U/µL with final concentration of 1.8 U, PCR 10X enhancer A with final concentration of 1x and PCR additive dimethyl sulfuoxide (DMSO) for the amplification of GC-rich region, 10X reaction buffer with final concentration of 1x was used to amplify the required gene of interest (Innis *et al.*, 1990).

Gel electrophoresis, PCR amplicon sequencing and data analysis

PCR product were separated through 1.5% agarose gel for 50 minuts (Fig. 1), then specific product were obtained and cleaned with ExoSAP for downstream sequencing of one strand. Sequencing was done with ABI BigDye terminator sequencing Kit (Applied Biosystems, Foster City, CA, USA). "Sequencher software" 5.2.3 was used for sequence analysis to ascertain the sequence variants and its other statistical attributes (Gene Codes Corporation, Ann Arbor, MI, USA).

Results

Hspb1 mutational landscape

As a potential marker, heat shock proteins are the most attention seeking and widely studies in medicine.

Table 1. Gist of the samples collection procedure, mode of obtaining biopsies of malignant tissues and representation of disease prevalence data in relation to their age, sex and breed in dogpopulation.

Sample ID	Breed	Tumor type/type of tissue	Age years	Sex	Tissue collection method
DP1	Non-descriptive	Canine Lymphoma	7	Male	Necropsy lymph node
DP3	English Springer	CTVT	6	Female	Chunk of tissues
DP5	German Shepherd	Mammary adenocarcinoma	8	Female	Surgical excisional
DP8	Labrador	Perianal adenocarcinoma	7	Female	Surgical excisional
DP9	Non-descriptive	Oral tumor/SCC	2	Male	Surgical excisional
DP10	Non-descriptive	CTVT	7	Female	Surgical excisional
DP11	German shepherd	Mammary adenocarcinoma	10	Female	Surgical excisional
DP12	German shepherd	Mammary adenocarcinoma	11	Female	Surgical excisional
DP14	Non-descriptive	Perianal adenocarcinoma	10.5	Male	Surgical excisional
DP15	German shepherd	Perianal adenocarcinoma	3.5	Female	Surgical excisional
DP17	Non-descriptive	Oral tumor/SCC	5	Male	Surgical excisional
DP18	Non-descriptive	Canine lymphoma	12	Male	Necropsy lymph node
DP19	Non-descriptive	Melanoma	10	Male	Chunk of tissues
DP20	Non-descriptive	Mammary adenocarcinoma	11	Female	Surgical excisional
DP21	Labrador	CTVT	2.5	Female	Chunk of tissues
DP22	Rottweiler	Granuloma	2	Female	Chunk of tissues
DP23	Non-descriptive	Granuloma	3	Male	Chunk of tissues
DP24	Sheep dog	CTVT	2.5	Male	Chunk of tissues
DP25	German shepherd	CTVT	2.5	Female	Chunk of tissues
DP26	German shepherd	Head&Neck SCC	2.5	Male	Surgical excisional
DP27	Labrador	Pelvic Warts	4 Month	Male	Chunk of tissues
Normal 1	Random bred	Lungs	5	Male	Necropsy samples
Normal 2	Random bred	Liver	6	Male	Necropsy samples
Normal 3	Random bred	Testes	8	Male	Necropsy samples
Normal 4	Random bred	Kidney	9	Male	Necropsy samples
Normal 5	Random bred	Endometrial	5	Male	Necropsy samples

It is a highly conserved group of genes. Among these, *Hsp27 Beta 1* is being analyzed in current study and found that exon 1 gained heterozygosity in two cases at CDS c.148 position, (DP2) is heterozygous (A/T) which is mammary lesion, and it is non-synonymous mutation, which changed the serine residue to cysteine in the protein sequence.

Similarly one of the granuloma case (DP22) was also found heterozygous (A/G) at the same position and it appeared as non-synonymous mutation, which changed the serine amino acid to glycine. A significant and exciting insertion of 5 bp (CCGCC) was also observed in one of the lymphoma sample (DP1) in exon 1.

Table 2. Primer sequences of *Hsp1* gene in *Canis familiaris*.

Primer name & label	5' to 3' sequence	Total (bp)	Type	Tm (C°)	GC %	Rating (%)	(hairpin, self & cross diming)	Product size (bp)
HSPB1-Cf-LRF	CGTCATTGCCTTTAATAGAGACCTG	25	Long-range (Forward)	61	44		91	2998
HSPB1-Cf-LRR	GACCTCGGCAAGTCTGTTTACTTT	24	Long-range (Reverse)	61.26	45.85		90	
HSPB1-Cf-E2F	CTGGGGCGTGTGTAGGTTTG	20	Internal (Forward)	61.13	60		100	
HSPB1-Cf-2SF	CCGCTGCTTCACTCGAAAATAC	22	Internal (Forward)	61.8	50		81	
HSPB1_Cf_3SF	TGCTGAGAGCCTACTCTGTGTGTG	24	Internal (Forward)	62.11	54.17		91	
HSPB1-Cf-4SF	GTGGAAGAGAAGCAAAGACCCTG	23	Internal (Forward)	62.13	52.17		100	
HSPB1-Cf-5SF	AGCAGGTCCCAGCCTTCAGA	20	Internal (Forward)	61.92	60		99	
HSPB1-Cf-6SR	AGCACACCCCTTGTTCCATGT	21	Internal (Reverse)	61.48	52.38		88	

Exon 3 has smaller coding region and its 3'UTR region found more variable, but all these position are non-coding. Our results showed that, out of total eight altered positions in this exon, seven position gain heterozygosity in one or more cases. Gene position 1050 is heterozygous (G/A) in three mammary tumor (DP4, DP5, DP12), two of the perianal adenocarcinoma (DP14, DP15) and one CTVT sample (DP21). It is also homozygous (A) in one of the TVT (DP10), lymphoma (DP18) and granuloma cases (DP23). Gene position 1214 is heterozygous (T/G) in one of the CTVT case (DP3) (Fig. 2a), similarly the same case found homozygous (A) rather than the homozygous (G) and (C) at position 1349, 1411 and homozygous (T) at position 1407 of 3'UTR respectively. It is observed that mammary tumor is comparatively more altered than the other types of cancers. Three mammary tumors (DP2, DP11, DP12) (Fig. 2b) and one perianal adenocarcinoma case (DP15) were found heterozygous (A/G) at position 1837. Another mammary tumor (DP20) was found heterozygous (G/A) and (G/C) at position 1839 and 2179 respectively (Table 3).

Table 3. Signature exonic mutations of *Hspb1*gene in different tumor in *Canis familiaris*.

Animal ID	Phenotype/ Tissue Type	Exon 1	Exon3, 3' UTR							
		c.148 A>T>G	220-224 CCGCC Ins	1050 G>A	1214 T>G	1349 G>A	1407 C>T	1411 C>A	1837 A>G	1839 G>A	2179 G>C
Reference	Normal	A	-	G	T	G	C	C	A	G	G
Control	Normal/Blood	A	-	G	T	G	C	C	A	G	G
DP1	Case/Lymphoma	A	Insertion	G	T	G	C	C	A	G	G
DP2	Case/Mammary T.	A/T	-	G	T	G	C	C	A/G	G	G
DP3	Case/CTVT	A	-	G	G/T	A	T	A	A	G	G
DP4	Case/Mammary T.	A	-	G/A	T	G	C	C	A	G	G
DP5	Case/Mammary T.	A	-	G/A	T	G	C	C	A	G	G
	Case/Blood	A	-	-	-	-	-	-	-	-	-
DP6	Case/CTVT	A	-	G	T	G	C	C	A	G	G
DP10	Case/CTVT	A	-	A	T	G	C	C	A	G	G
DP11	Case/Mammary T.	A	-	G	T	G	C	C	A/G	G	G
DP12	Case/Mammary T.	A	-	G/A	T	G	C	C	A/G	G	G

DP14	Case/Perianal adenocarcinoma	A	-	G/A	T	G	C	C	A	G	G
DP15	Case/Perianal adenocarcinoma	A	-	G/A	T	G	C	C	A/G	G	G
DP17	Case/Oral SCC	A	-	G	T	G	C	C	A	G	G
DP18	Case/Lymphoma	A	-	A	T	G	C	C	A	G	G
DP20	Case/Mammary T.	A	-	G	T	G	C	C	A	G/A	G/C
DP21	Case/CTVT	A	-	G/A	T	G	C	C	A	G	G
DP22	Case/Granuloma	A/G	-	G	T	G	C	C	A	G	G
DP23	Case/Granuloma	A	-	A	T	G	C	C	A	G	G
DP26	Case/Head & Neck T	A	-	G/A	T	G	C	C	A	G	G
Amino acid change		Serine to Cysteine/Glycine		-	-	-	-	-	-	-	-

As far as the intronic regions of *Hspb1* gene are concerned, 396C>T homozygous position was found in normal control (domestic short hair randomly bred). Actually this locus might be more informative as only the control is different from all of diseased cases. Position 518 in intron 1 was found heterozygous (T/C) in two of the mammary tumor (DP2, DP11) and one head & neck SCC (DP26) in German shepherd dogs, while the same position is homozygous (T) in one of the granuloma case (DP22) instead of homozygous (C) in control reference (Fig. 2c). One of the lymphoma sample (DP1) is heterozygous (A/G) at position 537 (Fig. 2d), while all other cases including control and reference sequence are homozygous (A) at this locus.

Table 4. Signature Intronic Mutations of *Hspb1* Gene in Different Tumor in *Canis familiaris*

Animal ID	Phenotype/ Tissue type	Intron 1				
		396 C>T	518 T>C	537 A>G	563 Ins A>C	736 A>G
Reference	Normal	C	T	A	A	A
Control	Normal/Blood	T	C	A	A	G
DP1	Case/Lymphoma	C	C	A/G	C/CC	G
DP2	Case/Mammary T.	C	T/C	A	A	G
DP3	Case/TVT	C	C	A	A	G
DP4	Case/Mammary T.	C	C	A	A	G
DP5	Case/Mammary T.	C	C	A	A	G
	Case/blood	C	C	A	A	G
DP6	Case/CTVT	C	C	A	A	G
DP10	Case/CTVT	C	C	A	A	G
DP11	Case/Mammary T.	C	T/C	A	A	G
DP12	Case/Mammary T.	C	C	A	A	G
DP14	Case/Perianal adenocarcinoma	C	C	A	A	G
DP15	Case/Perianal adenocarcinoma	C	C	A	A	G
DP18	Case/Lymphoma	C	C	A	A	G
DP20	Case/Mammary T.	T	C	A	A	G
DP21	Case/CTVT	C	C	A	A	G
DP22	Case/Granuloma	C	T	A	A	G
DP23	Case/Granuloma	C	C	A	A	G
DP26	Case/Head & Neck T.	C	T/C	A	A	G

The lymphoma case (DP1), which is the tumor of lympho reticular cells storage gland (lymph nodes) is heterozygous at 563 position, sense strand found with one (C) insertion, while anti-sense with two (C). The gene position 736A>G is homozygous (G) in all tumor cases, while homozygous (A) in reference control. This locus might be very informative regarding the disease particular mutation in all seven types of neoplasm (Table 4). Out of total 15 altered site in this gene, 11 mutations were observed transitional changes, while 4 loci were noticed transversion changes. Exon 1 locus c.148 appeared both in transition and transversion change in one of the mammary and granuloma samples.

Discussion

Several scenarios might be envisioned concerning possible causes of cancer development. Cancer development is micro-evolutionary process, in which not a single point mutation is responsible for such a drastic outbreak however a single mutation can start the process of chaos in genome. *Hspb1*was selected in this study, which has tumorigenic and anti-apoptotic function.

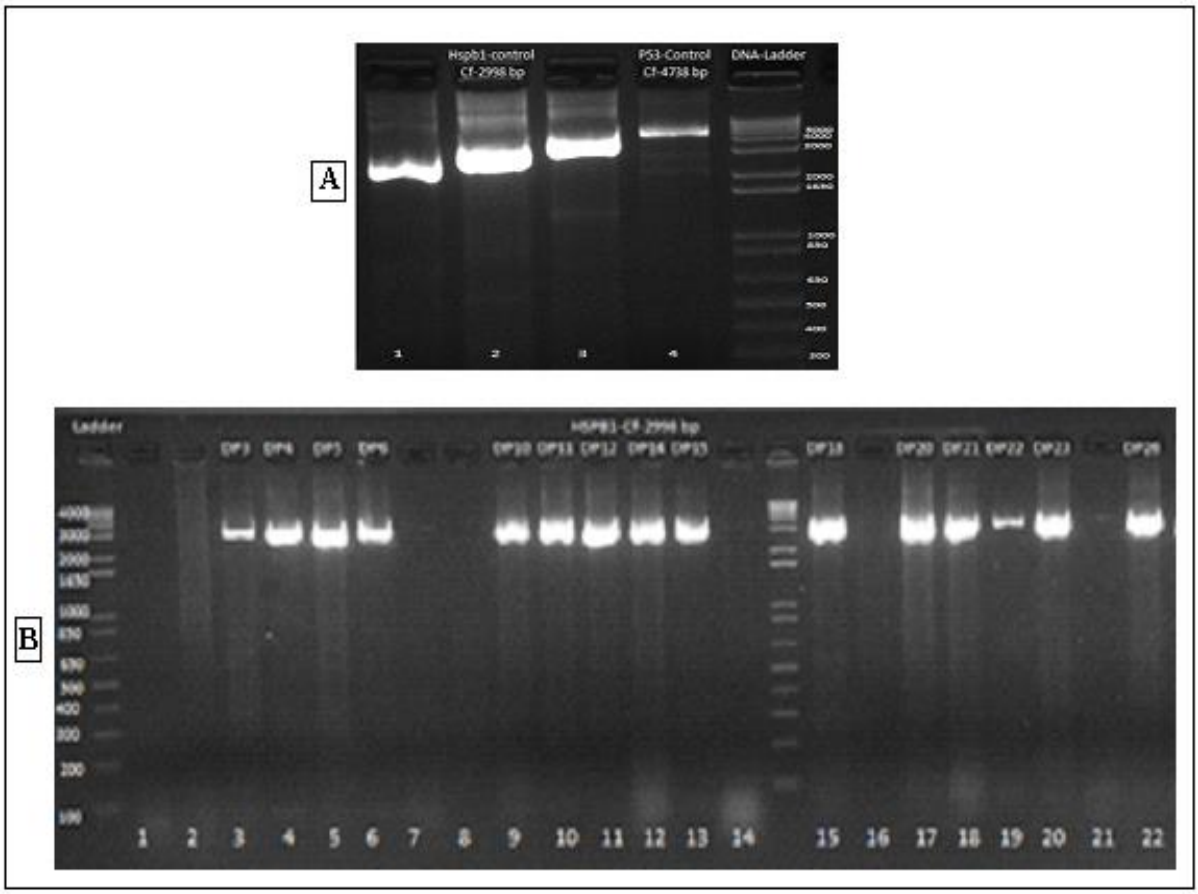

Fig. 1. Long-range PCR amplified product of *Hspb1*gene in control samples shown in well # 2 (A) and in tumor samples with 1Kb Gene Ruler DNA ladder (B).

This phenomenon is very interesting that *Hspb1* gene does not comes under the major categories of cancer related genes but instead it was found mutated, which explains the complex nature of cancer cascades, which are involved in tumorigenesis. Normally, mutations damage the proto-oncogene and trigger them to become oncogene and keep the acceleration-on all the time, which has to stop in fully functional proto-oncogenes. On the other hand these mutations alter the tumor suppressing function and these genes lose their function of cell growth brakes, which usually tells the cell to stop growing as their life span has ended (Robinson, 2005).

As far as the *Hspb1* is concerned, in few of the human lungs cancer studies CG/GG has lower risk at rs2868371 than CC at rs2868370, promoter region of *Hspb1* at 1271G>C found polymorphic (Guo *et al.*, 2010; Xu *et al.*, 2012). For more fidelity in the field of diagnostic, current study can be extended by gene expression profiling in different heat shock genes.

Two of such type of study were conducted on *Tp53* and *Hsps* markers in CTVT cases, which augment the diagnostic confidence on the basis of these polymorphism and expression based techniques (Chu *et al.*, 2001; Stockmann *et al.*, 2011). Similarly, treatment of the cancer gaining acceptance by inhibiting *Hspb27* gene to combat the cancer in any species (Gleave *et al.*, 2009).

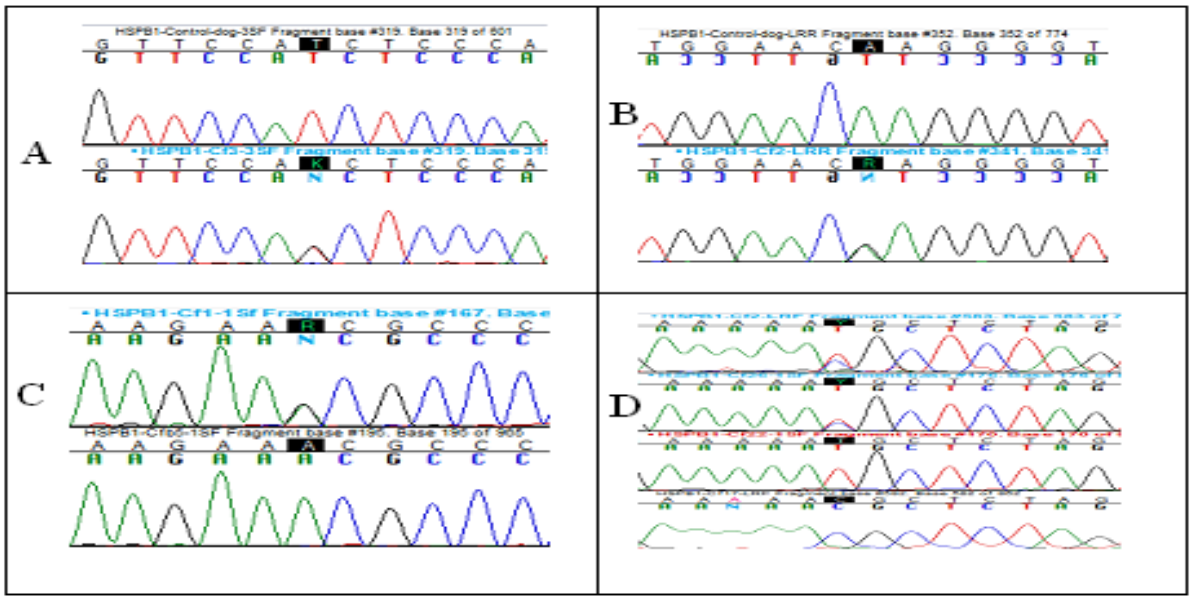

Fig. 2. A) Chromatogram of position 1214 in 3'UTR region of *Hspb1* gene in *Canis familiaris* showing (T/G) heterozygosity in DP3 (CTVT) sample instead of (T) in control dog, mentioned in (Table 3), B) Chromatogram of position 1837 in 3'UTR region of *Hspb1* gene in *Canis familiaris* showing (A/G) heterozygosity in DP2 (mammary tumor) sample instead of (A) in control dog, mentioned in (Table 3). C) Chromatograms of position 518 in intron 1 of *Hspb1* gene in *Canis familiaris* showing (T/C) heterozygosity in DP2 and DP26 (mammary tumor and H&N SCC respectively) samples and homozygous (T) in DP22 (granuloma) case instead of (C) in control and DP17 (oral SCC), mentioned in (Table 4). D) Chromatograms of position 537 in intron 1 of *Hspb1* gene in *Canis familiaris* showing (A/G) heterozygosity in lymphoma sample (DP1) instead of homozygous (C) in control and DP (mammary tumor), mentioned in (Table 4).

Cross-tissue comparison

As an ancillary finding, comparison of neoplastic tissues and blood of the same diseased animal were also compared, in which*Hspb1*gene was amplified in one of the mammary tumor (DP5) in *Canis familiaris* to ascertain the comparison of mutational variations in blood and tumorous tissues of the animal. As acquired somatic cell mutations in the neoplastic tissues are those which are due to cancer outbreak, while germ line mutation in the blood comes through generations. As for as the *Hspb1* gene exonic position c.1050 is concerned, this locus acquired somatic mutation of (G/A) in cancerous tissues instead of germ line mutation of (G) in blood of DP5 sample of dog mammary tumor In nutshell, regarding the variations in *Hspb1* gene in different tumors, mammary tumors indicated more alterations as comparison to other tumor types. Furthermore, this study revealed that 11 mutation sites in *Hspb1* gene were observed due to the supplanting of purine base with a purine or pyrimidine with a pyrimidine base, while 4 loci out of 15 mutant sites were observed transversion.

It was deduced that, mostly heterozygous changes were predominantly observed in intronic regions in this gene rather than exonic part.

Acknowledgement

Authors would like to thank the whole staff at Lyons Feline Genetics lab at University of Missouri-Columbia USA. Pet Center-UVAS team is also appreciated for helping in sample collection. We pay our gratitude to HEC for funding this project.

References

Chu R, Sun T, Yang H, Wang D, Liao K, Chuang T, Lin C, Lee W. 2001. Heat shock proteins in canine transmissible venereal tumor. Veterinary Immunology and Immunopathology **82(1),** 9-21. http://dx.doi.org/10.1016/s0165-2427(01)00327-0.

Eze C, Anyanwu H, Kene R. 2008. Review of canine transmissible venereal tumour (tvt) in dogs. Nigerian Veterinary Journal **28(1),** 54-70. http://dx.doi.org/10.4314/nvj.v28i1.3544.

Ganguly B, Das U, Das A. 2013. Canine transmissible venereal tumour: A review. Veterinary and comparative oncology.10.1111/vco.12060.

Garrido, C, Brunet M, Didelot C, Zermati Y, Schmitt E, Kroemer G. 2006. Heat shock proteins 27 and 70: Anti-apoptotic proteins with tumorigenic properties. Cell Cycle **5(22)**, 2592-2601. http://dx.doi.org/10.4161/cc.5.22.3448.

Gleave M, Ong C, Hayashi N. 2009. Treatment of cancer by inhibition of hsp27. Google Patents.

Guo H, Bai Y, Xu P, Hu Z, Liu L, Wang F, Jin G, Wang F, Deng Q, Tu Y. 2010. Functional promoter-1271g> c variant of hspb1 predicts lung cancer risk and survival. Journal of Clinical Oncology**28(11),** 1928-1935. http://dx.doi.org/10.1200/jco.2009.24.4954.

Innis MA, Gelfand DH, Sninsky JJ, White TJ. 1990. PCR protocols: A guide to methods and applications. Access Online via Elsevier. http://dx.doi.org/10.1002/mrd.1080280418.

North SM, Banks TA. 2009. Small animal oncology: An introduction. Saunders/Elsevier.

Paoloni MC, Khanna C. 2007. Comparative oncology today. Veterinary Clinics of North America: Small Animal Practice **37(6),** 1023- 1032 http://dx.doi.org/1032.10.1016/j.cvsm.2007.08.003.

Robinson TR. 2005. Genetics for dummies. Wiley. com.

Rozen S, Skaletsky H. 1999. Primer3 on the www for general users and for biologist programmers. In: Bioinformatics methods and protocols. Springer: 365-386 P.

Stockmann D, Ferrari H, Andrade A, Cardoso T, Luvizotto M. 2011. Detection of the tumour suppressor gene tp53 and expression of p53, bcl-2 and p63 proteins in canine transmissible venereal tumour. Veterinary and Comparative Oncology **9(4),** 251-259. http://dx.doi.org/10.1111/j.1476-5829.2010.00260.x.

Vermooten M. 1987. Canine transmissible venereal tumor (tvt): A review. Journal of the South African Veterinary Association **58(3),** 147.

Vogelstein B, Gillespie D. 1979. Genomic DNA mini kit (Tissue). Proceedinds National Academy of Science, USA.

Withrow SJ, Vail D.M. 2007. Withrow and macewen's small animal clinical oncology. Saunders Elsevier.

Xu T, Wei Q, Lopez Guerra J. Wang L, Liu Z, Gomez D, O'Reilly M, Lin S, Zhuang Y, Levy L, Mohan R, Liao Z. 2012. Hspb1gene polymorphisms predict risk of mortality for us patients after radio (chemo) therapy for non-small cell lung cancer. International Journal of Radiation Oncology Biology Physics **84(2),** 229-235. http://dx.doi.org/10.1016/s0169-5002(05)80664-6.

Zoubeidi A, Gleave M. 2012. Small heat shock proteins in cancer therapy and prognosis. Int J Biochem Cell Biol **44(10),** 1646-1656. http://dx.doi.org/10.1016/j.biocel.2012.04.010.