Heat shock proteins: A Review

[1]Dr.Babitha GA, [2]Dr.Rahath Firdose, [3]Dr.K Shashanka Holla, [4]Dr.Shobha Prakash

[1]Professor, [2,3]Postgraduates, [4]Professor and Head
Department of Periodontics, College of Dental Sciences, Davangere, India

***Abstract*: Heat shock proteins (HSPs) are a large class of proteins that have been conserved throughout evolution and exist in prokaryote and eukaryote organisms. HSP plays an important role in protein homeostasis. They can be found in all major cellular compartments. The HSP90 family is important in the formation of the steroid receptor complex. The HSP70 family is necessary for protein synthesis, translocation, and folding. The HSP60 family is important in protein stability. Many factors, e.g. heavy metals and organic toxic substances, and elevated temperature in all cells are responsible for the formation of these proteins and hence are called stress proteins.**

***Keywords*: Heat shock protein, periodontitis, chaperone, stress, temperature, vaccine.**

INTRODUCTION

Heat Shock Proteins (HSP), also known as Stress-induced Proteins or Stress Proteins, are one such class of proteins that are produced in the body in response to stress, under the control of Heat Shock Factors (HSF), although some are constitutively expressed. This was first seen in Drosophila in the year in 1974. [1]

The term "heat shock protein" is a misnomer because many agents other than heat induce the expression of the heat shock protein gene. Consequently, "stress protein" is the preferred term. [2]

Heat shock protein (HSP), or stress proteins, are a highly conserved class of protective cellular proteins that are produced under various conditions of environmental challenges, such as temperature changes, certain drugs, viral infections, radiation, heavy metal ions, ethanol, oxidants, nutrient and growth factor deprivation, anoxia and malignant transformation. [2-5]

The normal counterparts of HSP are constitutively synthesized by the cell under non-stressful conditions, depending on the cell cycle, hormone status and differentiation stage. [6] HSPs are believed to have a number of protective functions such as assembly/disassembly of protein, interaction with surface receptors, and antigen presentation. [2,7]

Overexpression of HSPs have been observed in certain cancers (breast, uterine cervix, lung, pancreas, ovary, liver, leukaemia and oral cavity). [2-4,6,8]

HISTORY

It all started with a curious incident in a laboratory in Italy sometimes described as serendipity (Ritossa 1962).[9] The heat shock reaction was discovered by Feruccio Ritossa, who observed an enlargement of special sections of Drosophila melanogaster chromosomes (heat shock puffs) after heat treatment of the flies.[10]

Ritossa subjected these flies to temperature shock-induced specific gene activation; the first product of these genes was identified in 1974 and the term "heat shock protein" was adopted.[10] Heat-shock proteins (HSP), or stress proteins, are present in all organisms and in all cells of all organisms (Lindquist, 1986).[6]

HSP60 was first identified in E.coli by Hendrix in 1979 and has been termed GroEL.[11]

CLASSIFICATION AND LOCATION OF HEAT SHOCK PROTEIN:

HSP member	Location	Description
Small HSP		
Ubiquitin	Cytoplasm/nucleus	Facilitates targeting and removal of denatured proteins
Hsp10	Mitochondria	Cofactor for HSP60
Hsp27	Cytoplasm/nucleus	Involved in intracellular actin dynamics
β-crystallin	Cytoplasm	Involved in cytoskeletal stabilisation
HSP40		
Hsp40	Cytoplasm/nucleus	Regulates activity of HSP70, binds non-native protein
Hsp47	Endoplasmic reticulum	Processing of pro-collagen
HSP60		
Hsp60	Mitochondria	Molecular chaperone
HSP70		
Hsp72	Cytoplasm/nucleus	Highly stress inducible, protects against ischemia
Hsp73	Cytoplasm/nucleus	Constitutively expressed molecular chaperone
Hsp75	Mitochondria	Induced by stress including hypoxia
HSP90		
Hsp90	Cytoplasm (migrates to nucleus)	Part of the steroid receptor complex
HSP110		
Hsp110	Nucleolus/cytoplasm	Thermal tolerance
Hsp105	Cytoplasm	Protein refolding

Table 1. Key members of the heat shock protein family in humans [12,13,14]

FUNCTIONS OF HEAT SHOCK PROTEINS:

1. As new polypeptide chains (proteins) are being produced by ribosome within the cell, heat shock proteins assist in correct folding of polypeptide chain into functional protein.
   Presence of heat shock protein (purple) assures that the new protein will assume its functional three-dimensional configuration (Figure 1).

2. After stress events, heat shock proteins also assist in refolding or degradation of damaged or denatured proteins (Figure 1).

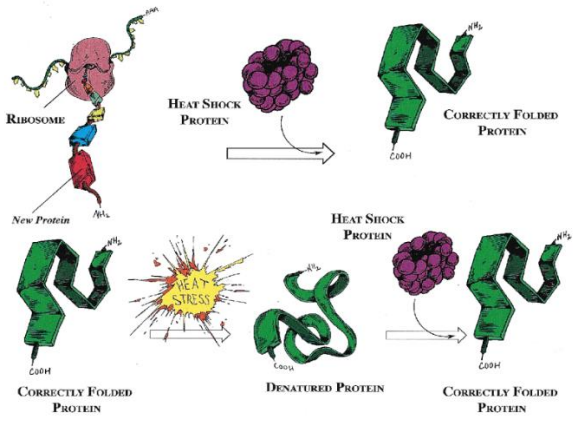

Figure 1: The presence of heat shock protein (purple) assures that the new protein will assume its functional three-dimensional configuration [15]

BENEFITS OF HEAT SHOCK PROTEIN

- Reparation of misfolded and damaged proteins.
- Increased immune response.
- Reduction of free radicals.
- Faster muscle recovery and repair.

- Heart protection.
- Higher insulin production.

THE ACTIVATION OF HEAT SHOCK GENE

Heat shock factor (HSF) is present in the cytoplasm as a latent monomeric molecule that is unable to bind to DNA. Under stressful conditions, the flux of non-native proteins (which are non-functional, prone to aggregation, protease-sensitive, and bind to chaperones) leads to phosphorylation (P) and trimerisation of HSF.

The trimmers translocate to the nucleus, bind the promoter regions of heat shock protein (HSP) genes and mediate HSP gene transcription. The activity of HSF trimers is downregulated by HSPs (e.g. HSP 70) and the heat shock binding protein 1 (HSBP1) that is found in the nucleus.[12](Figure 2).

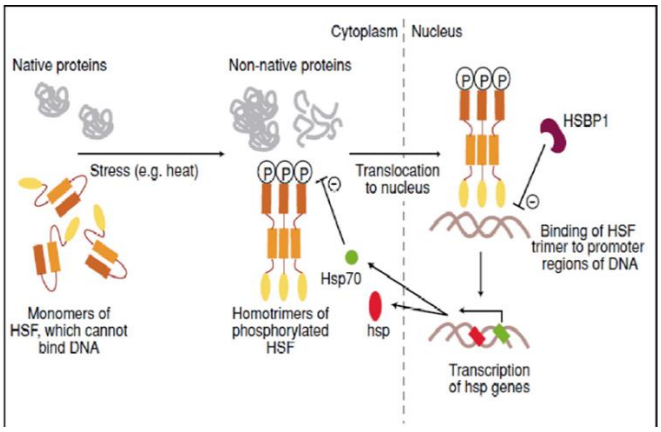

Figure 2. Regulation of Transcription of heat shock protein genes by heat shock factor. [12]

HEAT SHOCK PROTEIN IN DENTISTRY

In recent years, several studies have reported the characterization of HSPs produced by oral micro-organisms. Like the HSPs involved in other diseases, these stress-induced proteins may contribute to the pathogenic process of oral infections. HSPs are now considered key elements in the pathogenesis of several infections, emerging as important diagnostic markers (Macario, 1995).

PRESENCE OF HEAT SHOCK PROTEIN:

HSP production has been reported in periodontopathic bacteria such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum Eikenella corrodens, and Treponema denticola*. [16]

HSP 60,70,90 are present in saliva due to passive transport from blood serum. The source of HSP in the saliva is largely from the salivary glands, mucosal cells, periodontal tissues either from exudate or direct blood contamination and other sources from the bloodstream [17].In periodontitis cases, HSP60 is increased in P.gingivalis.[18].The 65 kilodaltons HSP is found in the four species of Streptococci, S. pyogenes, S. sanguis, S. faecalis and S.salivarius.[19]

HSP 70:

HSP70 is the best-known heat shock protein reported in the brain, heart and dental pulp. These proteins have been reported as present in the pulp during several stressful conditions, including development, reparative dentin formation, cavity preparation, and after replantation and orthodontic tooth movement.[20]

HSP70 in PDL fibroblasts is significantly increased compared with fibroblasts, and this occurred in a time-dependent manner. HSP70 has also been reported to induce osteoblast differentiation. The periodontal ligament, which overexpresses HSPs, may function actively as a biomaterial with high regenerative capacity.[21]

HSP70 has also been expressed in the pulp due to tooth trauma and pulpitis,[22] it has also been reported that trauma resulting from pulp extirpation increases HSP70 expression which compromises the cell walls of the pulp tissue.[23] HSP70 has been proven to modulate the production of inflammatory cytokines, chemokines, and other mediators via regulating the intracellular signaling pathways, such as MAPKs and NF-κB, and inflammatory mediators play critical roles in pain sensitization.[24] Extracellular or exosome-bound HSP70 can bind to TLRR2 or TLR4 and cause the activation of NF-κB and the production of TNF-α, IL-1β, and IL-6.[25]

In the oral cavity, HSP70 has a role of mucosal defense including entrapping, agglutinating

and opsonizing bacteria, and inhibiting pathogenic adhesion to the mucosal surface **(Fabian et al. 2012).**

Frank Tavassol et al. (2011) evaluated the prognostic significance of Heat shock protein 70 in oral cancer and showed that the survival of patients suffering from T2 tumors with positive HSP70 expression was 8 times higher.

HSP 60:

The basal layer of periodontal pockets exhibits positive expression of (HSPs) and increased infiltration of mononuclear inflammatory cells in periodontal pockets beneath the basal layer. As a result, periodontal bacteria induce the development of HSPs in the periodontal cells, which in turn triggers the production of pro-inflammatory cytokines by macrophages and other inflammatory cells.

Lundqvist et al. (1994)[28] found the expression of HSP60 to be higher in gingival epithelial cells of inflamed tissue samples from periodontitis patients compared with samples from periodontally healthy individuals.

Petit et al. (1999)[29] suggested that the higher responsiveness to HSP60 and HSP70 observed in gingivitis subjects may prevent the conversion from gingivitis to periodontitis.

Tabeta et al. (2000)[30] reported that gingival tissue extracts from healthy or periodontitis patients contain antibodies to the Porphyromonas gingivalis GroEL protein (Heat shock protein).

Ueki et al (2002)[31] demonstrated that Human HSP60 is expressed abundantly in periodontitis lesions and, also stimulates tumor necrosis factor (TNF) - α production from macrophages.

Yamazaki et al (2002)[32] demonstrated that HSP60-specific T cells accumulated in the gingival lesions of periodontitis patients but not in gingivitis patients and that the T cell clones with an identical specificity to those in peripheral blood existed in periodontitis lesions.

Choi et al (2004)[33] showed that Porphyromonas gingivalis HSP reactive T cell immune response might be involved in the immunopathogenesis of periodontal disease. They suggested that T cells in the circulating peripheral blood may be home to periodontal lesions where P.gingivalis have infiltrated potentially leading to T cell response cross-reactive to mammalian HSP of gingival fibroblasts.

HSP90:

The HSP90 molecular chaperone family is highly conserved and expressed in various organisms ranging from prokaryotes to eukaryotes and even under normal conditions. This protein accounts for 1-2% of all cellular proteins in most cells.[34]

Many reports have indicated that HSP90 plays an important role in the progression of malignant disease and HSP90 expression is 2 to 10-fold higher in tumor cells than in normal cells. [35-37]

As HSP 90 is a molecular chaperone with essential functions for cell growth and differentiation, cell communication, signal transduction, and apoptosis.

HSP90 consists of three domains: the N-terminal domain, the middle segment or M domain and the C-terminal domain. The N-terminal domain was found to be the binding site for nucleotides such as ATP, whereas the M domain was the major site for HSP90 client protein interactions. The C-terminal domain is important for HSP90 dimerization. [38]

REVIEW IN GENERAL

All organisms respond to heat by inducing the synthesis of a group of proteins called heat shock proteins or HSPs. The response is the most highly conserved genetic system known, existing in every organism in which it has been sought, from archaebacteria to eubacteria, from plants to animals. Although certain features of the response vary from organism to organism, many are universal, or nearly so.

In the case of exposure to heat, the phenomenon has been called “thermotolerance” and has launched many experiments in which an association has been found between the heat shock response and protection against other stresses, such as hypoxia or ischemia

This thermotolerance treatment strategy has proved successful in experimental models of cardiac ischemia, arterial injury, endotoxic shock, renal and hepatic ischemia, ethanol-induced gastric ulcerations, and skeletal muscle.[15]

Abnormal levels of stress proteins have been found in a number of disorders, including atherosclerosis, fever, aging, infection Alzheimer’s disease, malignant diseases, and autoimmunity. There is growing evidence that some stress proteins may be associated with atherosclerosis.[39]

HSP70 is a danger signal of cellular stress or cellular death. The binding of HSP70 to HSP70-receptors on antigen-presenting cells (i.e., macrophages) and T lymphocytes induces the secretion of proinflammatory cytokines. Extracellular HSP70 also induces inducible nitric oxide (NO) synthases and NO released from macrophages. It is a dangerous signal inducing the secretion of proinflammatory cytokines such as factor TNF-α, interleukin (IL)-1β, and IL-6 from monocytes and macrophages.[40]

REVIEW ON HSP IN SYSTEMIC CONDITIONS:

HSP and its associations	**Clinical Implications**	**Source**
HSP and cardiovascular diseases	Antibodies to HSP60 have been associated with carotid stiffness, hypertension and atherosclerosis	Zhu et al 2001
HSP and acute conditions	Several studies have observed a decrease in intracellular HSP 70 and HSP 90 levels in monocytes and polymorphonuclear cells, along with a pattern of early extracellular induction in acute conditions like sepsis	Kaiser et al 2018
HSP and Rheumatoid Arthritis (RA)	HSP 96 is Elevated in RA Elevated HSP levels are noted in the synovium of smokers with RA.	Huang et al 2009 Furose et al 2020
HSP and Pregnancy	HSPs have been found to be associated with decidualization, implantation and placentation, with their dysregulation associated with implantation failure, pregnancy loss and other feto-maternal complications	Jee et al 2021
HSP in diabetes and wound healing	The numerous defects in the function of HSPs associated with diabetes could contribute to the commonly observed complications and delayed wound healing in diabetics	Atalay et al 2009
HSP and liver regeneration	HSP70 is required for optimal liver regeneration	Wolf et al 2014
HSP and cancer	HSP are overexpressed in a wide range of human cancers and are implicated in tumor cell proliferation, differentiation, invasion and metastasis. HSP27 and HSP70 are implicated in resistance to chemotherapy in breast cancer, HSP27 predicts a poor response to chemotherapy in leukemia patients, whereas HSP70 expression predicts a better response to chemotherapy in osteosarcomas. HSP could be targeted as pharmacological modification of HSP expression or molecular chaperone activity and using the immunological role of HSPs as anticancer vaccines. HSP70 is associated with poor differentiation, lymph node metastasis, increased cell proliferation, block of apoptosis, and higher clinical stage, which are markers of poor clinical outcome	Ciocca and Calderwood 2005
Host HSP 90 and Human coronavirus	HSP90 inhibitors can be repurposed as a potent and broad-spectrum antiviral against human coronaviruses	Li et al 2020
HSP and renal ischemia reperfusion	The concept that HSPs are cytoprotective after IRI	Zhang et al 2008

ROLE OF HEAT SHOCK PROTEINS IN HORMONAL CHANGES:

HSP and PCOS	The elevated serum HSP70 level has been used to denote ovarian damage and also plays a role in the activation and maturation of dendritic cells.	Narayan Singh RM et al 2004.
HSP and menstruation	HSP70 and HSP90, has shown to be increased significantly during the mid-secretory phase. This suggests that these proteins may play an important role in preparing the endometrium for menstruation and the subsequent regeneration of the lining.	S.Tabibzadeh et al 1996.

REVIEW IN ORAL CONDITION:

Table 2. Evidence for the involvement of HSP/Chaperonin in Oral disease [41]

HSP	Disease
HSP 10	Acts as a Growth factor of osteoclast.
HSP 27	Causes Osteoclastic resorption – calcium release Ameloblastoma, Salivary gland cancer, dysplastic lesion (oral cancer) , squamous cell carcinoma
HSP 60	Associated with Ameloblastomas Periodontitis Chronic periodontitis
HSP 70	Observed in Dental caries Ameloblastoma, salivary gland cancer, squamous cell carcinoma Oral squamous cell carcinoma Dysplastic lesion and oral squamous cell carcinoma Cancer of gingivo-buccal and tongue
HSP 90	Seen in Salivary gland cancer
HSP 110	Seen in Salivary gland cancer

VACCINE OF HSP

Heat shock protein (Hsp) can be possibly explored as a candidate for vaccination against periodontitis

Choi et al (2005)[42] found that in P.gingivalis HSP60 could potentially be developed as a vaccine against multiple periodontopathic bacteria

Lee et al (2006) [43]found that there was a very strong inverse relationship between post immune anti-P. gingivalis HSP immunoglobulin G (IgG) levels and the amount of alveolar bone loss produced by bacterial infections.

Leonel Ampie (2015)[44] found that Vaccines formulated from HSP-peptide complexes, derived from an autologous tumor, have been applied to the field of immunotherapy for glioblastoma. They concluded that HSP vaccines are safe and well-tolerated in human patients with glioblastoma.

Jinho Kang(2022)[45] described a novel peptide-based vaccine targeting (HSP90) that induces effective antitumor immunity in a HER2+ breast cancer murine model. The vaccine also induced a significant reduction in tumor growth and improved survival in the treated mice compared to control mice.

CONCLUSION:

The role of HSPs and related proteins in normal growth, demonstrates a vital role for at least some of these proteins. HSPs are involved in protein folding and assembly (or disassembly) of protein complexes. The heat-inducible proteins may be involved in reassembling structures damaged by heat shock or other stresses. The diverse processes in which HSPs function have been implicated indicate that these proteins are involved in many cellular processes. In dentistry, Heat Shock Proteins (HSPs) have been shown to play a crucial role in protecting oral tissues from various stressors, including heat, oxidative stress, and inflammation. HSPs are present at high levels in the oral tissues and are involved in maintaining the proper functioning of the cells and tissues. Studies have suggested that HSPs may play a role in the development and progression of various dental diseases, including periodontal disease and oral cancer. HSPs have been shown to protect cells from the damaging effects of these diseases and may help to prevent their development or progression. Further studies are needed to fully understand the mechanisms by which HSPs function in the oral tissues and how they can be harnessed for therapeutic purposes.

REFERENCES:

1. Whitley D, Goldberg SP, Jordan WD. Heat shock proteins: a review of the molecular chaperones. Journal of vascular surgery. 1999 Apr 1;29(4):748-51.
2. Kaur J, Das SN, Srivastava A, Ralhan R. Cell surface expression of 70 kDa heat shock protein in human oral dysplasia and squamous cell carcinoma: correlation with clinicopathological features. Oral oncology. 1998 Mar 1;34(2):93-8.
3. Bramanti TE, Dekker NP, Lozada-Nur F, Sauk JJ, Regezi JA. Heat shock (stress) proteins and γδ T lymphocytes in oral lichen planus. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1995 Dec 1;80(6):698-704.
4. Sugerman PB, Savage NW, Xu LJ, Walsh LJ, Seymour GJ. Heat shock protein expression in oral lichen planus. Journal of oral pathology & medicine. 1995 Jan;24(1):1-8.
5. Ito T, Kawabe R, Kurasono Y, Hara M, Kitamura H, Fujita K, Kanisawa M. Expression of heat shock proteins in squamous cell carcinoma of the tongue: an immunohistochemical study. Journal of oral pathology & medicine. 1998 Jan;27(1):18-22.
6. LINDQUIST S, SCHIRMER EC. Howard Hughes Medical Institute and the Department of Molecular Genetics and Cell Biology, The University of Chicago, 5841 S. Maryland Avenue, MC 1028, Chicago, IL 60637, USA. Molecular Chaperones and Folding Catalysts: Regulation, Cellular Functions and Mechanisms. 2003 Sep 2:384.
7. Ramírez JR, Ortiz S, Valenzuela M, Durán V, Cabello G, Lobato S, Botella S, Botella35 LM. Expression and localization of Hsp70 and Hsp27 in human breast tumors. Rev Esp Patol. 2001;34:9-
8. Chaiyarit P, Kafrawy AH, Miles DA, Zunt SL, Van Dis ML, Gregory RL. Oral lichen planus: an immunohistochemical study of heat shock proteins (HSPs) and cytokeratins (CKs) and a unifying hypothesis of pathogenesis. Journal of oral pathology & medicine. 1999 May;28(5):210-5.
9. Evgen'ev MB. Heat shock proteins: a history of study in Russia. Cell Stress and Chaperones. 2021 Jul;26(4):617-27.
10. Tissiéres A, Mitchell HK, Tracy UM. Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. Journal of molecular biology. 1974 Apr 15;84(3):389-98.
11. Hendrix RW. Purification and properties of groE, a host protein involved in bacteriophage assembly. Journal of molecular biology. 1979 Apr 15;129(3):375-92.
12. Pockley AG. Heat shock proteins in health and disease: therapeutic targets or therapeutic agents?. Expert reviews in molecular medicine. 2001 Sep;3(23):1-21.
13. Ranford JC, Coates AR, Henderson B. Chaperonins are cell-signalling proteins: the unfolding biology of molecular chaperones. Expert reviews in molecular medicine. 2000 Sep;2(8):1-7.
14. Tsan MF, Gao B. Cytokine function of heat shock proteins. American Journal of Physiology-Cell Physiology. 2004 Apr;286(4):C739-44.
15. Whitley D, Goldberg SP, Jordan WD. Heat shock proteins: a review of the molecular . Journal of vascular surgery. 1999 Apr 1;29(4):748-51.
16. Ando T, Kato T, Ishihara K, Ogiuchi H, Okuda K. Heat shock proteins in the human periodontal disease process. Microbiology and immunology. 1995;39(5):321-7.
17. Fábián TK, Fejerdy P, Csermely P. Salivary genomics, transcriptomics and proteomics: the emerging concept of the oral ecosystem and their use in the early diagnosis of cancer and other diseases. Current genomics. 2008 Mar 1;9(1):11-21.

18. Yamazaki K, Ueki-Maruayama K, Honda T, Nakajima T, Seymour GJ. Effect of periodontal treatment on the serum antibody levels to heat shock proteins. Clinical & Experimental Immunology. 2004 Mar;135(3):478-82.
19. Ando T, Kato T, Ishihara K, Ogiuchi H, Okuda K. Heat shock proteins in the human periodontal disease process. Microbiology and immunology. 1995;39(5):321-7.
20. Pileggi R, Holland GR. The expression of heat shock protein 70 in the dental pulp following trauma. Dental Traumatology. 2009 Aug;25(4):426-8.
21. Kaneko-Tanaka K, Kurokawa A, Mitsuhashi M, Fujita S, Goseki T, Yamaguchi M, Kasai K. Expression of heat shock protein 70 in the periodontal ligament during orthodontic tooth movement. International Journal of Oral-Medical Sciences. 2010;9(2):115-21.
22. Kanno K, Shimizu K, Shinoda M, Hayashi M, Takeichi O, Iwata K. Role of macrophage-mediated Toll-like receptor 4–interleukin-1R signaling in ectopic tongue pain associated with tooth pulp inflammation. Journal of Neuroinflammation. 2020 Dec;17(1):1-5.
23. Sampoerno G, Sunariani J. Expression of NaV-1.7, TNF-α and HSP-70 in experimental flare-up post-extirpated dental pulp tissue through a neuroimmunological approach. The Saudi Dental Journal. 2020 May 1;32(4):206-12.
24. Chen X, Smith A, Plummer C, Lei W. Heat shock proteins and pain. Heat Shock Proteins in Human Diseases. 2021:211-35.
25. Galler KM, Weber M, Korkmaz Y, Widbiller M, Feuerer M. Inflammatory response mechanisms of the dentine–pulp complex and the periapical tissues. International journal of molecular sciences. 2021 Feb 2;22(3):1480.
26. Fábián TK, Hermann P, Beck A, Fejérdy P, Fábián G. Salivary defense proteins: their network and role in innate and acquired oral immunity. International journal of molecular sciences. 2012 Apr 2;13(4):4295-320.
27. Tavassol F, Starke OF, Kokemüller H, Wegener G, Müller-Tavassol CC, Gellrich NC, Eckardt A. Prognostic significance of heat shock protein 70 (HSP70) in patients with oral cancer. Head & neck oncology. 2011 Dec;3:1-6.
28. Lundqvist C, Baranov V, Teglund S, Hammarström S, Hammarström ML. Cytokine profile and ultrastructure of intraepithelial gamma delta T cells in chronically inflamed human gingiva suggest a cytotoxic effector function. Journal of immunology (Baltimore, Md.: 1950). 1994 Sep 1;153(5):2302-12.
29. Petit MD, Wassenaar A, Van der Velden U, Van Eden W, Loos BG. Depressed responsiveness of peripheral blood mononuclear cells to heat-shock proteins in periodontitis patients. Journal of dental research. 1999 Aug;78(8):1393-400.
30. Tabeta K, Yoshie H, Yamazaki K. Characterization of serum antibody to Actinobacillus actinomycetemcomitans GroEL-like protein in periodontitis patients and healthy subjects. Oral microbiology and immunology. 2001 Oct;16(5):290-5.
31. Ueki K, Tabeta K, Yoshie H, Yamazaki K. Self-heat shock protein 60 induces tumour necrosis factor-α in monocyte-derived macrophage: possible role in chronic inflammatory periodontal disease. Clinical & Experimental Immunology. 2002 Jan;127(1):72-7.
32. Yamazaki K, Ohsawa Y, Tabeta K, Ito H, Ueki K, Oda T, Yoshie H, Seymour GJ. Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. Infection and immunity. 2002 May;70(5):2492-501.
33. Choi JI, Chung SW, Kang HS, Rhim BY, Park YM, Kim US, Kim SJ. Epitope mapping of Porphyromonas gingivalis heat-shock protein and human heat-shock protein in human atherosclerosis. Journal of dental research. 2004 Dec;83(12):936-40.
34. Csermely P, Schnaider T, So C, Prohászka Z, Nardai G. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacology & therapeutics. 1998 Aug 1;79(2):129-68.
35. Clarke PA, Hostein I, Banerji U, Stefano FD, Maloney A, Walton M, Judson I, Workman P. Gene expression profiling of human colon cancer cells following inhibition of signal transduction by 17-allylamino-17-demethoxygeldanamycin, an inhibitor of the hsp90 molecular chaperone. Oncogene. 2000 Aug;19(36):4125-33.
36. Burrows F, Zhang H, Kamal A. Hsp90 activation and cell cycle regulation. Cell cycle. 2004 Dec 1;3(12):1530-6.
37. Ferrarini M, Heltai S, Zocchi MR, Rugarli C. Unusual expression and localization of heat-shock proteins in human tumor cells. International Journal of Cancer. 1992 Jun 19;51(4):613-9.
38. Elhassan A, Ramalingam K, Peeran SW, Ganesh R, Abdalla KA. Role Of Heat Shock Proteins in Various Diseases with Special Emphasis on Periodontal Inflammation-A Review. Journal of Pharmaceutical Negative Results. 2022 Sep 29:934-8.
39. Whitley D, Goldberg SP, Jordan WD. Heat shock proteins: a review of the molecular chaperones. Journal of vascular surgery. 1999 Apr 1;29(4):748-51.

40. Fábián TK, Fejérdy P, Nguyen MT, Sti C, Csermely P. Potential immunological functions of salivary Hsp70 in mucosal and periodontal defense mechanisms. Archivum immunologiae et therapiae experimentalis. 2007 Apr 1;55(2):91.
41. Amin MN. The prospect of heat shock protein (HSP) as biomarker of oral disease. Insisiva Dental Journal: Majalah Kedokteran Gigi Insisiva. 2012 Jul 15;1(2).
42. Choi JI, Choi KS, Yi NN, Kim US, Choi JS, Kim SJ. Recognition and phagocytosis of multiple periodontopathogenic bacteria by anti-Porphyromonas gingivalis heat-shock protein 60 antisera. Oral microbiology and immunology. 2005 Feb;20(1):51-5.
43. Lee JY, Yi NN, Kim US, Choi JS, Kim SJ, Choi JI. Porphyromonas gingivalis heat shock protein vaccine reduces the alveolar bone loss induced by multiple periodontopathogenic bacteria. Journal of periodontal research. 2006 Feb;41(1):10-4.
44. Ampie L, Choy W, Lamano JB, Fakurnejad S, Bloch O, Parsa AT. Heat shock protein vaccines against glioblastoma: from bench to bedside. Journal of neuro-oncology. 2015 Jul;123:441-8.
45. Kang J, Lee HJ, Lee J, Hong J, Kim YH, Disis ML, Gim JA, Park KH. Novel peptide-based vaccine targeting heat shock protein 90 induces effective antitumor immunity in a HER2+ breast cancer murine model. Journal for ImmunoTherapy of Cancer. 2022 Sep 1;10(9):e004702.