Research Article

Role of HSP 90 alpha in oral lichen planus: An immunohistochemical evaluation

A Soumya[1*], N Malathi[2], D Prathiba[3], S Anandan[4], Satha Sivasubramaniam[5]

[1]Senior Research fellow, [2,5]HOD, [3]Professor, [4]Dean, Department of Oral Pathology and Microbiology, Faculty of Dental sciences, Sri Ramachandra University, Porur, Chennai-600116, Tamil Nadu, INDIA.
Email: dimplesoumya@gmail.com, narasimhan.malathi@gmail.com, anandan_subbu@yahoo.co.in

Abstract

Background: Intracellular homeostasis is maintained by the molecular chaperones through protein folding and stabilization of other proteins. The proteome against the misfolded protein and aggregation are guarded by the molecular chaperones. They are also critical for the survival of cells that undergo stress due to these misfolded proteins. The current immunohistochemical study was conducted to unravel the expression pattern of Heat shock protein 90. **Aim**: To evaluate and compare the immunohistochemical pattern of expression of Heat shock protein 90 alpha with various clinical and histological parameters of Oral lichen planus and Oral squamous cell carcinoma. **Materials and Methods**: Archival tissues from the department of oral pathology were retrieved. The cases based on histopathological diagnosis were chosen. The immunohistochemistry kit containing monoclonal anti HSP 90 alpha was purchased and the procedure was performed in the department lab. The samples distribution was 8/31 normal and 23/31 Oral lichen planus. Heat shock protein 90 alpha was assessed on basis of percentage of cells and intensity of staining. **Results**: Overall, our study indicates that Heat shock protein 90 alpha expression in normal oral mucosa was mild, with increase in expression in Oral lichen planus. The results were statistically significant with a p- value of 0.000 **Conclusion**: This study brings out the importance of Heat shock protein 90 in oral tumorigenesis and malignant transformation. These finding defines Heat shock protein 90 as a marker protein for risk assessment in malignant transformation of oral lichen planus.

Keywords: Molecular chaperones, oral lichen planus, Heat shock protein 90 alpha, malignant transformation, risk assessment

[*]**Address for Correspondence:**
Dr. A. Soumya, Senior Research fellow, Department of Oral Pathology and Microbiology, Faculty of Dental sciences, Sri Ramachandra University, Porur, Chennai-600116, Tamil Nadu, INDIA.
Email: dimplesoumya@gmail.com

Received Date: 12/06/2015 Revised Date: 18/06/2015 Accepted Date: 22/06/2015

Access this article online

Quick Response Code:

Website: www.statperson.com

DOI: 03 July 2015

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease. This potential malignant disorder has an overall malignant transformation rate of 1.09 %.[1] Percentages of patients who have OLP with skin lesions is 50%. Lichen planus of skin resolves by 2 years whereas the OLP persists for 20 years or more. The male to female ratio been 1.4:1 and all races are affected.[2,3] Intracellular homeostasis is maintained by the molecular chaperones through protein folding and stabilization of other proteins.. They are also critical for the survival of cells that undergo stress due to these misfolded proteins[4] Heat shock proteins are a group of molecular chaperones having a significant role in malignant cell transformation. When the chaperones undergo stress due to hypoxia, this in turn increases the activity of the heat shock proteins in cancer cells their by producing more mutated and unstable proteins.[5,6] The most abundant protein in human cells are the HSP 90 comprising 1-2% during non-stress conditions. Its role is proven in various cellular events including signal transduction, protein folding, protein degradation and morphological evolution.[6,4] Heat shock protein 90 has two major forms of isoforms the HSP 90 alpha (Major) and HSP 90 Beta (Minor). A recent report added another isoform to the Heat shock protein 90family, Hsp90N, which is associated with cellular transformation. Additional Heat shock protein 90 analogues include Grp94 in the endoplasmic reticulum and Hsp75/ TRAP1 in the mitochondrial matrix. Heat

How to site this article: A Soumya, N Malathi, D Prathiba, S Anandan, Satha Sivasubramaniam. Role of HSP 90 alpha in oral lichen planus: An immunohistochemical evaluation. *International Journal of Recent Trends in Science and Technology*. July 2015; 15(3): 510-514 http://www.statperson.com (accessed 04 July 2015).

shock protein 90 isoforms have five highly conserved regions, called 'signature sequences', of which three are in the N-terminal domain[8, 9, 10] The current information on the role of the Heat Shock Protein 90 in Oral lichen planus is very limited. This study was undertaken with the following aims and objectives:

1. To analyse the expression of Heat shock protein 90 alpha in Oral lichen planus.
2. To evaluate and compare the immunohistochemical pattern of expression of Heat shock protein 90 alpha with various clinical and histological parameters of Oral lichen planus.

MATERIALS AND METHODS

This laboratory based study involved the use of 31 archival tissues which were 10% formalin fixed paraffin embedded. The samples were categorised into 2 groups, Group I: Normal tissues (8/31), Group II: Oral Lichen Planus (23/31). The Study proposal was approved by the Institutional ethical committee, Sri Ramachandra University, Porur, Chennai (IEC-NI/11/OCT/25/64). The cases were then retrieved from the Department of Oral pathology and Microbiology, Faculty of Dental sciences, Sri Ramachandra University, Chennai. The clinical and histological parameters included in the study were also obtained from the records of the patients which were documented in the Department of Oral pathology and Microbiology, Faculty of Dental sciences, Sri Ramachandra University, Chennai. The age group of study sample ranged from 14years to 80 years. The maximum number of patients ranged from 25-50 years followed by patients above 50 years. The mean age of the patients in Group I and Group II were 22.87 years, 36.95 years respectively.

Immunohistochemical study

Sections of 5µm thickness were taken from the paraffin blocks and mounted on a glass slide. Indirect two step method of Immunohistochemical staining was followed. Antihuman Hsp90 and the Secondary antibody kit monoclonal antibodies (Scytech laboratories, Germany) were used. Sections were deparaffinised in xylene, hydrated through graded alcohol and washed with phosphate-buffered saline (PBS). Endogenous peroxidase activity was quenched using peroxidase block for 15 min. The sections were then washed in PBS. The antigen retrieval was pressure cooker based (3 whistle time).Cooker was rapidly cooled to room temperature by plunging it into the sink with water for 15minutes.Primary antibody of Anti HSP 90 alpha was added following a 15minute power block. The slides were incubated for a period of 90minutes without drying. Secondary antibody was added to slides and kept incubated for 30min at room temperature. The slides were then washed 3 times in TBS at pH 7.6. DAB was prepared prior to step by mixing 1drop of DAB chromogen in 1ml of substrate. DAB was added to slides and kept for 5minutes following which a counter stain with Elrirch's hematoxylin was done for 1 minute. The slides were then dried and mounted with DPX for normal viewing. Breast carcinoma was used as controls. The immunostained slides were evaluated semi quantitatively. The percentage of cells in hot spot areas and intensity of staining was evaluated in 40x view. The positivity was graded under 2 parameters, (Table 1 and 2)

Table 1: The percentage of HSP 90 alpha in Hot spot field

Scoring system	Grading
0	Nil
<25% of cells	Score 1
25%-50% of cells	Score 2
50%-75% of cells	Score 3
>75% of cells	Score 4

Table 2: Intensity of staining of HSP 90 alpha

Scoring system
Negative
Mild
Moderate
High

Oral lichen planus and Normal tissue specimens were compared for differences in Hsp90 alpha immunostaining scores. According to the statistical characteristics of our investigated data, Kruskal- Wallis multiple comparison Z value test was selected to compare the immunostaining scores between these three groups. Significance was established at a value of p value- 0.000 for both the parameters.

RESULTS

Assessment of the immunohistochemical staining of Hsp90 alpha

Expression of Hsp90 with relation to the percentage of cells

In normal tissue samples, out of the 8 cases, 2 cases (25%) of Group I had negative expression and 5 cases (62.5%) had Score 1 and 1 case (12.5%) showed Score 2. All cases of OLP showed positivity for expression of Heat shock protein 90 of which 4 cases (17.39%) had Score 1, 18 cases (78.26%) had Score 2, and 1 case (4.34%) had Score 3.In the study the percentage of heat shock proteins 90 alpha expression was significantly high in OLP with a mean of 1.86 when compared to Normal (Group I) with a mean of 0.8750 Statistical significance was seen using Kruskal Wallis multiple comparison z-value test with a p value- 0.000 (Table III, V)

Expression of Hsp90 with relation to the intensity of staining

In regard to intensity of Heat shock protein 90 positivity, the following observations were made. Out of 8 cases of normal 2 cases (25%) had negative expression and 5 cases (62.5%) had Mild expression and 1 case (12.5%) showed Moderate expression. All cases of OLP showed positivity for expression of Heat shock protein 90 of which 19 cases (82.6%) had Mild expression, 1 case (4.34%) had Moderate expression, and 3 cases had intense expression (13.04%) In the study the Intensity of heat shock proteins 90 alpha expression was inconclusive in normal with a mean of 1.50 when compared to OLP with a mean of 1.34 and OLP with a mean of 1.34.No statistical significance was obtained. (Table IV, V)

Table 3: Inter group comparison of Percentage of cells of heat shock protein 90 alpha study groups using Kruskal Wallis multiple comparison z-value test

HSP 90 Alpha Percentage	Group I	Group II
GROUP I	0.0000	2.1101
GROUP II	2.1101	0.0000

Z-value above the value of 1.96000 was considered significant

Table 4: Inter group comparison of intensity of staining of Heat shock protein 90 alpha study groups using Kruskal Wallis multiple comparison z-value test

HSP 90 alpha intensity	Group I	Group II
Group I	0.0000	0.5710
Group II	0.5710	0.0000

Z-value above the value of 1.96000 was considered significant

Table 5: Expression of heat shock protein 90 alpha in the oral epithelia from biopsy specimens of control subjects and patients with oral lichen planus

Immunostaining Score	Percentage of cells		Immunostaining Score	Intensity of staining	
	Controls	OLP		Controls	OLP
0	2	0	Negative	2	0
1	5	4	Mild	5	19
2	1	18	Moderate	1	1
3		1	Intense		3
4					
Total	**8**	**23**	**Total**	**8**	**23**

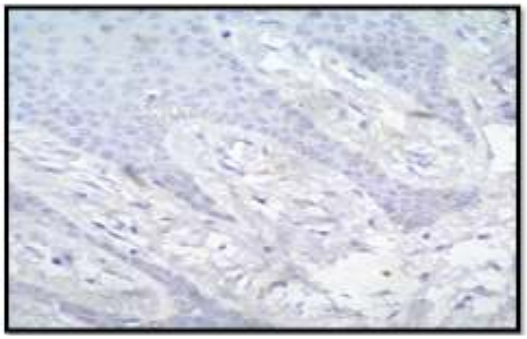

Figure 1: Normal section showing negative expression (40x view:h and e ihc)

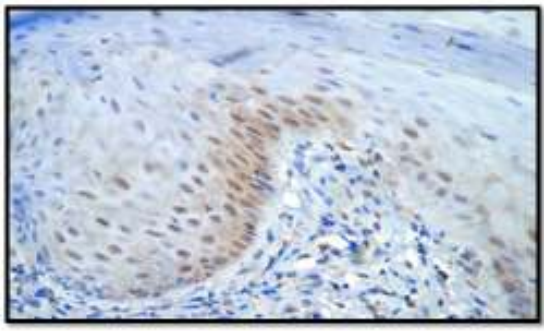

Figure 2: Normal section shows less than 25% of cells showing positivity with mild intensity pattern (40x view: ihc)

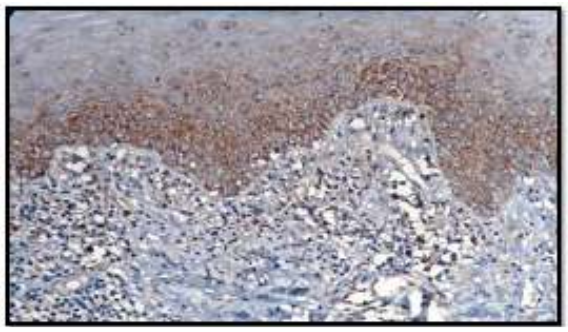

Figure 3: Olp section shows 25% - 50% of cells showing positivity with mild intensity pattern (40x view: ihc)

Figure 4: Olp section shows 50%- 75% of cells showing positivity with intense intensity pattern (10x view: ihc)

DISCUSSION

Heat shock protein 90 is over expressed in a wide range of human cancers and is implicated in tumour cell proliferation, differentiation, invasion, metastasis, death, and recognition by the immune system.[80]Regulation of Heat shock protein 90 in oral lichen planus and oral squamous cell carcinoma is not well understood. This study aims at understanding the pattern of expression in the study groups comprising of normal oral mucosa and Oral lichen planus by immunohistochemical method. It is an established fact that heat shock proteins are normally expressed 1-2% of cellular proteins. Heat shock protein 90 family is the most abundant proteins in mammalian cells and increases in folds after stress. HSP90 is a regulatory protein and acts by inhibiting or stimulating the activity of its target protein. HSP90 is ubiquitous in all tissues of the human cells.[10].Ponlatham *et al* (2009) found low expression of HSP 90 in OLP compared to the normal controls. On the contrary our study found the expression higher in OLP. Heat shock proteins expressed by oral keratinocytes may be auto antigenic in Oral lichen planus. Susceptibility to Oral lichen planus may result from dysregulated heat shock protein gene expression by stressed oral keratinocytes or from an inability to suppress an immune response following self-Heat shock protein recognition.[12]Over expression of Heat shock protein 90 in Oral lichen Planus has been associated to the persistence or chronicity of the disease, or they could have simply reflected cellular injury.[13]The findings were substantiated with the study done by Bramanti T.E. *et al* (1995),[13] and Yin Cao Shen Li Jia *et al* (2006) [14] In addition, infiltrating lymphocytes in the lamina propria were positive for Heat shock protein 90 in our study. This finding also was concurrent with the results of the study done by Ponlatham *et al* (2009)[11], Bramanti T.E *et al* (1995).[13]In our study we found that the Heat shock protein was up regulated in Oral lichen planus when compared to the normal mucosa. Indeed these finding define Heat shock protein 90 as a marker protein for risk assessment in malignant transformation of oral lichen planus. In our study group, all the patients had burning sensation. We also found 3 cases of Oral Lichen planus having high intensity pattern, out of which one patient even had Score 3 (percentage of heat shock protein 90 expression). Taking into consideration the Clinical history in these patients, we found that all these three patients had a previous history of Skin lichen planus lesions and all the 3 patients were male patients. Another finding was the duration of the lesion in all the three patients were more when compared to the overall samples of the Oral lichen planus group (Group II).The Intensity of staining and percentage of cells showing positivity were more in the basal and para basal layers. Only 1 case out of the 23 cases showed over expression up to Spinous layer. This finding was attributed to the chronicity of the disease. The study was aimed to assess the expression of Heat shock protein 90.An overexpression of the HSP 90 protein was noted In OLP when compared to normal mucosa. Further studies with larger sample size and other molecular methods should be carried out to substantiate the findings. Based on the samples analysed the same has been achieved. We suggest further studies with larger sample size and other molecular methods should be carried out in this field, which will provide the insight and passable foundation to establish the role of Heat shock protein 90 in carcinogenesis.

CONCLUSION

The results of the study support the role of Heat shock protein 90 in oral tumorigenesis and malignant transformation. Indeed these finding pave the way for using Heat shock protein 90 as a marker protein for risk assessment in malignant transformation of oral lichen planus.

ACKNOWLEDGEMENTS

We are grateful to Dr.Suresh Varadarajan, Associate professor, Community Medicine from my university. We

extend the deep sense of gratitude to the ICMR, Delhi for providing an opportunity to work on this TSS talent research programme grant.

REFERENCES

1. Fitzpatrick, SG Hirsch SA, Gordon SC, The malignant transformation of Oral Lichen Planus: A systemic Review, J Am, Dental Assoc, 2014 Jan; 145C
2. Mona Soliman, Ahmed El Kharbotly, Ali Saafan. Management of Oral Lichen Planus Using Diode Laser (980nm). A Clinical Study; Egyptian Dermatology Online Journal, June 2005, Vol. 1 No 1:3.
3. Ponlatham Chaiyarit, Abdel H. Kafrawy, Dale A.Miles, Susan l. Zunt, Margot L. Van Dis and Richard L. Greogory,. Oral Lichen Planus: An Immunohistochemical Study of Heat Shock Proteins (Hsps) and Cytokeratins (Cks), And a Unifying Hypothesis of Pathogenesis; J Oral Pathol Med 1999; 28: 210-5.
4. S. Lindquist, E. A. Craig. The Heat-Shock Proteins, Annual. Rev. Genet. 1988. 22:631-77.
5. Jason C. Young, Ismail Moarefi, and F. Ulrich Hartl. Hsp90: A Specialized but Essential Protein-Folding Tool, The Journal Of Cell Biology, Volume 154, Number 2, July 23, 2001 267–273.
6. Péter Csermely, Tamás Schnaider, Csaba S Ti, Zoltán Prohászka and Gábor Nardai.The 90-Kda Molecular Chaperone Family: Structure, Function, and Clinical Applications. A Comprehensive Review, Pharmacol. Ther. 1998, Vol. 79, No. 2, Pp. 129–168.
7. Vladimir L. Gabai and Michael Y. Sherman. Invited Review: Interplay between Molecular, Chaperones and Signaling Pathways In Survival of Heat Shock, J ApplePhysiology92:1743-1748, 2002.
8. Amere Subbarao Sreedhara, Eł Va Kalma.Ra, Pe.Ter Csermelya, Yu-Fei Shen. Hsp90 Isoforms: Functions, Expression and Clinical Importance, Febs Letters 562 (2004) 11-15.
9. Jana Tkáčová, Mária Angelovičová. Heat Shock Proteins (Hsps): A Review, Animal Science and Biotechnologies, 2012, 45 (1), 349-353.
10. Péter Csermely and Ichiro Yahara.Heat Shock Proteins, Chapter 6, Page 67-75.
11. Ponlatham Chaiyarit, Darunee Jintakanon, Poramaporn Klanrit, Mookhda Siritapetawee, Kobkan Thongprasom. Immunohistochemical Analyses of Survivin and Heat Shock Protein 90 Expression in Patients with Oral Lichen Planus, J Oral Pathol Med (2009) 38: 55–62
12. P.B. Sugerman, N.W. Savage, L.J. Walsh, Z.Z. Zhao, X.J. Zhou, A. Khan, G.J. Seymour, M. Bigby. The Pathogenesis of Oral Lichen Planus, Crit Rev Oral Biol Med, 2002, 13(4):350-365.
13. Bramanti TE, Dekker NP, Lozada-Nur F, Sauk JJ, Regezi JA. Heat shock (stress) proteins and gamma delta T lymphocytes in oral lichen planus, Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995 Dec; 80(6):698-704.
14. Yin Cao, Shen Li-Jia, Xie Si Ming, Xie Li Qun. expression and significance of heat shock protein 79, 90 in oral squamous cell carcinoma and oral pre-cancerous lesions, J 4thMil med univ, 2006, 27 (19)5-10.

Source of Support: None Declared
Conflict of Interest: None Declared