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The Role of Heat Shock Proteins in Pathogenesis of Oral Squamous Cell Carcinoma

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Abstract

Cell in the distress situation, denaturation of proteins may occur, and may also respond by expressing stress proteins. However, such homeostasis effort does not always succeed and even may lead to disease, including cancer. In distress situation also ensue much protein misfolding. **Objective;** This research were to explain the role of heat shock protein 40 (Hsp40) and Hsp70 in pathogenesis of occurred oral squamous cell carcinoma (OSSC) patient which realized human papilloma virus (HPV) infection. **Material and Method;** Tissue biopsy frozen section were taken from BOSC and OSCC patients was cut into three part. Parrafin blocks were made from cutting I, which was subsequently stains with HE to ascertain the type of neoplasm. Cutting II was subjected to DNA isolation. The DNA isolation results were subjected to PCR to amplify L1-HPV gene for fixed the HPV stressor. Protein isolation was treated from Cutting III, followed with Blottdot test by using antibody monoclonal anti Hsp40 and Hsp70 and continued with measurement using densitometer to find the concentration of Hsp40 and Hsp70. The collected data were analyzed with F Test (Manova) and discriminant analysis. **Result;** This experiment showed the differences in concentration of Hsp40 ($p<=0,070$) and Hsp70 ($p<=0,006$) between benign oral squamous cell (BOSC) and OSCC patients which realized HPV infection. **Conclusion;** This experiment proved that OSCC patients which realized HPV infection indicated an up regulated of Hsp70 concentration, so that there was occurs misfolding of the proteins cell. The misfolding was ensue obstacle of apoptosis and to raise cell proliferation which to storm carcinogenesis. An up regulated of Hsp40 was role as co-chaperone.

Key Words : Hsp40; Hsp70; Pathogenesis; OSCC.

Introduction

Cancer may be defined as uncontrolled tissue growth in susceptible patient, with result from imbalance between apoptosis and cell proliferation. Damage to cells can engage one of two opposing responses: apoptosis, a form of cell death that removes damaged cells to prevent inflammation and the heat shock or stress response that prevents damage or facilitates recovery to maintain cell survival. Interactions between these two pathways determine the fate of a cell and, as such, have a profound effect on the biological consequences of stress. [1,2]

Factor that known implicated as a potential cock and or promoter cancer were tobacco, alcohol, radiation of sunrise, ionization radiation, carcinogen related work, environment pollutant, medicines, nutrition and infectious agent. Another factor is life in village, social-economic factor, age, gender and response immune mechanism. Information about another factor was little. The followed factor is periodontal disease chronic, bed oral hygiene, diseases of tooth, sharp of set teeth, elektrogalvanism and edentulism. Another researcher found that *HPV*, especially 16 and 18 type, implicated in OSCC pathogenesis. [1,3]

Stress to the cell causes protein denaturation: the protein molecule loses its native functional conformation when it unfolds. Chaperones assist the damaged molecule to regain its functional conformation. If cellular stress proceeds unchecked by such anti-stress mechanisms as the protein-refolding action of chaperones, intracellular proteins become denatured and insoluble. These denatured proteins tend to stick to one another, precipitate, and form inclusion bodies. The development of inclusion bodies is a common pathologic process in Parkinson's, Alzheimer's, Huntington's diseases and cancer, even in the absence of cellular stress. Denatured and aggregated proteins cannot function and must either be rescued or eliminated with the help of chaperones. The biological function of a protein depends on its tri-dimensional structure, which is determined by its amino acid sequence during the process of protein folding. In the last few years, diverse diseases have been shown to arise from protein misfolding and are now grouped together under the name of protein conformational disorders (PCDs) including cancer. [4,5,6,7]

The research that to investigate the concentration of Hsp aims to explain the role of Hsp40 and Hsp70 in pathogenesis of occurred OSSC patient which realized HPV infection.

Material and Method

Kind of this research is observasional – analytic cross sectional and the design of this research is post test only control group design. Ethical clearance was done by dr Muwardi Distric Hospital Surakarta team and sign at August 5, 2008. Eighty tissue biopsy frozen section were taken from nine samples BOSC and nine samples OSCC patients collected from Oral and Dental Clinic of dr Muwardi Distric Hospital in Surakarta was cut into three part.

Parrafin blocks were made from cutting I, which was subsequently stains with Haematoxyline Eosine (HE) to ascertain the type of neoplasm.

Cutting II was stibjected to DNA isolation. The DNA isolation results were subjected to PCR to amplify L1-HPV for fixed the HPV stressor. Diagnose related with HPV infections are made by Henk Schmits and/or Nigel McMillan and Nina Fowler PCR-method with some modifications. [8,9] Twenty five µl microfuge tube Ready To Go PCR Bead (Amersham Pharmacia Biotech) mixed with 2 µl HPV consensus primers (MY09 and MY11) (CYBERGENE AB) PCR protocol for both amplifications are 94^0 C for 50 seconds, 59^0 C for 50 seconds, 72^0 C for 50 seconds and 4^0 C soak. The Amplification of L1-HPV gene produced 450 bp long.

Table 1. Densitometer reading data of Hsp40 and Hsp70 concentration in BOSC and OSCC which realized HPV infection from Blottdot test.

No.	Hsp40 *(area)*	Hsp70 *(area)*	Characteristic
1	3	2	4
1	520,55	429,52	Benign
2	575,92	520,48	Benign
3	100,04	100,01	Benign
4	316,59	356,70	Benign
5	100,05	352,30	Benign
6	1653,15	100,02	Benign
7	326,37	1230,39	Benign
8	1728,55	1017,54	Benign
9	873,57	661,42	Benign
Mean	688,31	529,82	
SE	242,71	181,10	
Coefisien Discriminant		0,002	
Mean (Anova)		1,0596	
Sd		0,77042	
SE		0,25681	
10	605,21	293,56	Malignant
11	975,83	1771,15	Malignant
12	323,20	2287,43	Malignant
13	2132,96	879,63	Malignant
14	965,02	1622,84	Malignant
15	1249,96	619,05	Malignant
16	2994,47	1968,23	Malignant
17	1173,16	1062,77	Malignant
18	1771,46	1612,16	Malignant
Mean	1354,59	1346,32	
SE	242,71	181,10	
Sig. Wulk,s L	p=0,070	p=0,006	
Coefisien Discriminant		0,005	
Mean (Anova)		6,7316	
Sd		3,32393	
SE		1,10798	
F (Anova)		24,870	
Sig		,000	

Protein isolation was treated from Cutting III, [8] followed with Dot Blott Test by using antibody monoclonal anti Hsp40 and Hsp70 (Stressgen bioreagents) and continued with

measurement using densitometer (*Shimadzu CS 930, Dual Wave Length Cromato Scanner*) to find the concentration of Hsp40 and Hsp70.[10]

The collected data were analyzed with F Test (Manova) to find the differences between BOSC and OSCC patients with HPV and discriminant analysis to identify the role of Hsp40 and Hsp70 at the pathogenesis of OSCC patient with HPV by SPSS for Window 16.

Result

The result of the experiment showed in figure 1-2., table 1. and graphic 1. (enclosure).

This experiment showed the differences in concentration of Hsp40 ($p<=0,070$) and Hsp70 ($p<=0,006$) between BOSC and OSCC patients which realized HPV infection (Table 1 and Graphic 1, SPSS for Window 16). The pattern of the role of Hsp70 in OSCC patients which realized HPV infection indicates in ratio 6.7: 1

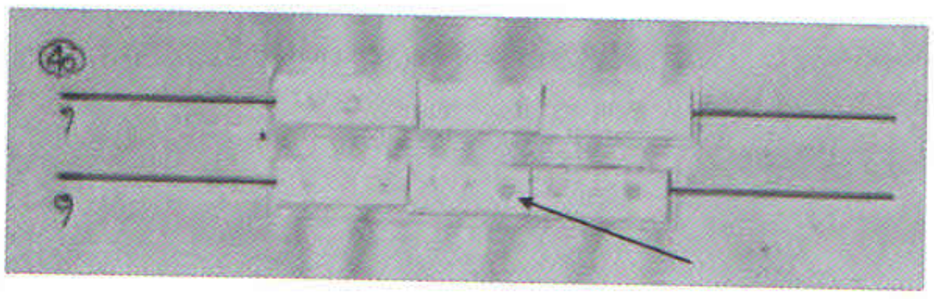


Figure 1. High concentration of Hsp40 showed with dark-brown dot (arrow) from Blottdot test (up : j : benign oral squamous cell : BOSC; lower : g : oral squamous cell carcinoma : OSCC).

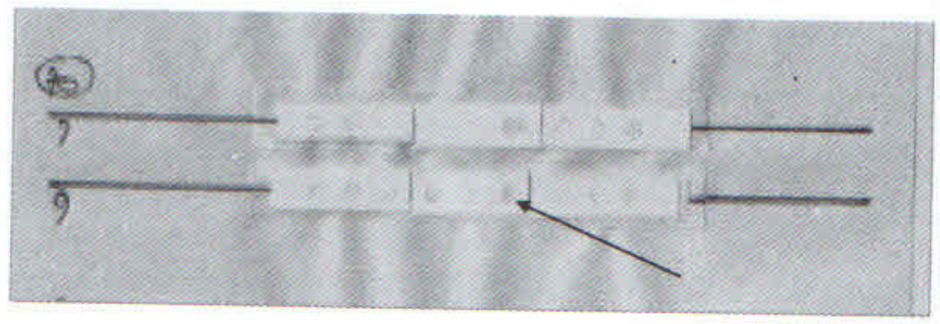


Figure 2. High concentration of Hsp70 showed with dark-brown dot (arrow) from Blottdot test (up : j : benign oral squamous cell : BOSC; lower : g : oral squamous cell carcinoma : OSCC).

as compare that occurred in BOSC patients which realized HPV infection.

Discussion

In the face of injury or stress with the use of various mechanisms for anticipated, including systems of proteins called molecular chaperones. The typical function of a chaperone is to assist a nascent polypeptide chain to attain a functional conformation as a new protein and then to assist the protein's arrival at the site in the cell where the protein carries out its functions. It has become increasingly clear that disruption of chaperoning mechanisms contributes to aging and disease. This review outlines the involvement of defective chaperones in senescence and in several diseases. Since chaperones are ubiquitous, their deficiencies and defects are bound to affect diverse tissues and, hence, to be of interest to those in internal medicine, ophthalmology,

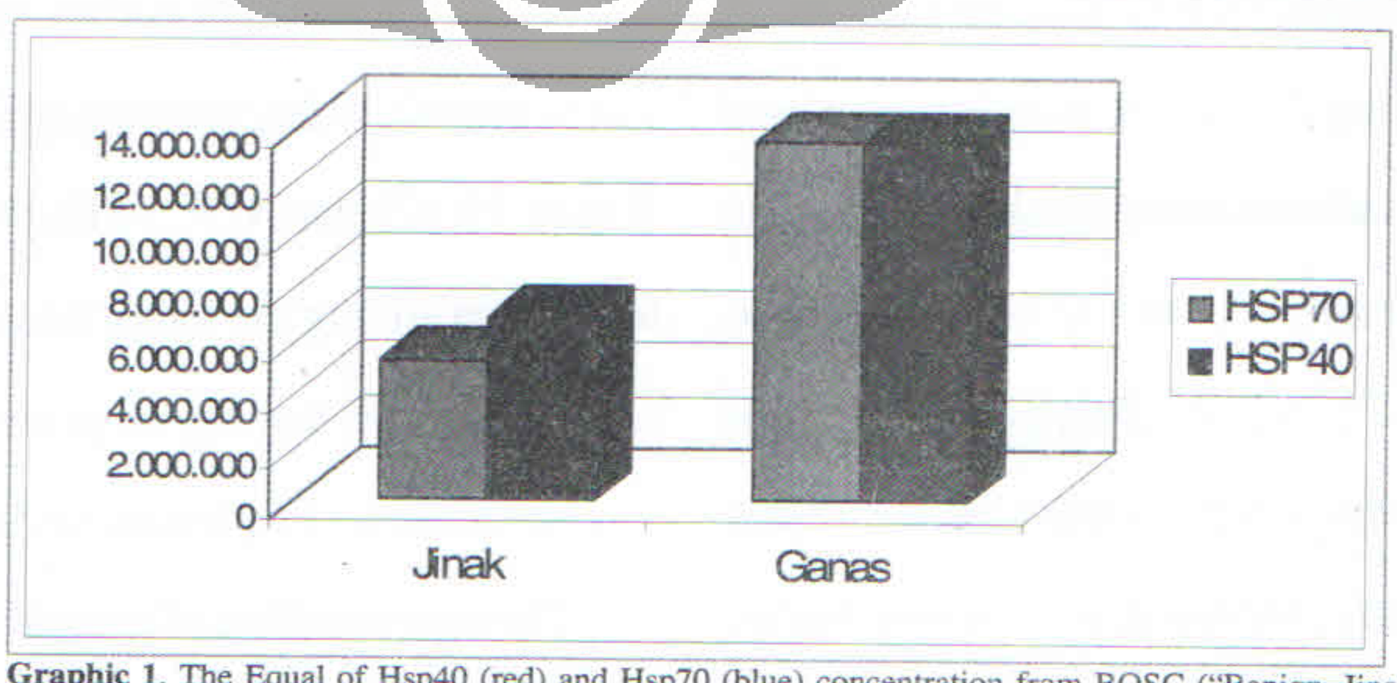
neurology, immunology, endocrinology, pediatrics, and gerontology. Only a fraction of chaperones are encoded in genes that are inducible by stressors and thus belong to the large class of stress proteins. If the stressor is heat shock, the induced chaperones are named heat-shock proteins (Hsps). For historical reasons, the term Hsp is used even if the parent gene is not induced by heat shock. Conversely, many Hsps are not chaperones. Therefore, these terms have to be used carefully to avoid misunderstandings. Chaperones and Hsps are classified into groups according to phylogeny and structure or molecular mass in kilodaltons (a classifier useful for clinical laboratory analyses). Heat shock proteins (Hsp) are a family of molecules that are highly conserved during evolution and involved in many cellular functions, such as protein folding. Consequently, their alteration may have multiple pathophysiologic effects and the number of papers studying their expression in normal and pathologic conditions is constantly increasing. In particular, the role of a number of Hsps, such as Hsp27, -70, -72 and -90, during carcinogenesis has already been widely investigated, *in vivo* and *in vitro*, in many conditions, such as lung, breast, esophageal and ovarian cancer, as well as osteosarcoma, and lymphoblastic leukemia. Despite the obvious importance of stress responses, only recently has scrutiny focused on the role of heat shock proteins in the control of disease pathology and in the survival and virulence of pathogens. [7, 11, 12]

Most of the proposed reaction cycles of Hsp70 fall into two broad classes: 1) those which propose that it is only Hsp70 with bound ATP which interacts with the unfolded substrate, and that the interaction of this ternary complex with Hsp40 leads to rapid ATP hydrolysis, and 2) those which postulate that Hsp40 first interacts with an unfolded (nascent) polypeptide, targeting it for binding to Hsp70. Although there have been several reports that Hsp40 binds to some unfolded proteins, unambiguous evidence that Hsp40 or its homologs will bind to unfolded proteins in general is currently lacking. [13, 14, 15, 16, 17, 18, 19, 20, 21, 22] Explanation above can conclude that in protein folding have many factor, namely Hsp40, Hsp70 dan *ATP*. So that happened the unbalance among the factor caused in protein misfolding and leading on protein abnormal function and direct to disease including cancer.

The accumulation of protein misfolded in distress condition to be the result of an increase

of toxic functions, which are often accompanied by Hsp70 and other chaperones. The association of aggregates or misfolded proteins with Hsp70 implies that the chaperone system including those involving Hsp70 acts as a defense mechanism for the prevention of misfolded protein accumulation, although the attempt seems to have ended without success. The exact mechanism by which protein misfolding and aggregation are linked to disease is still unclear, but accumulating evidence suggests that the main toxic agents are unfolded, but still soluble species of the disease proteins, rather than their end products, the insoluble fibrils. Misfolded proteins may sequestrate components of the chaperone, reducing the ability of the cell to manage unfolded proteins. Reduction in the chaperone activity would be very harmful to cells because it affects both the folding system and the intracellular degradation systems. Note that Hsp70 promotes folding when associated with Hsp40, but it could promote proteasomal degradation of substrates when associated with Bag-1 and CHIP. No matter how toxicity is generated, either by soluble forms or insoluble fibrils of the disease proteins, the identification of protein aggregates, including Hsp70, inside or around dead cells has tempted many researchers to manipulate the level of Hsp70 to examine whether over-expression of chaperones would reduce the extent of aberrant aggregation, thereby suppressing disease phenotypes or delaying the onset of the disease. [23, 24, 25, 26, 27, 28, 29, 30, 31]

Stress also caused in down regulation of ATP production by mitochondria. Research in *Intestinal ischemia-reperfusion* is a common clinical event and as distress disease confirm possible disturbance in protein folding process. [32, 33, 34, 35]



Graphic 1. The Equal of Hsp40 (red) and Hsp70 (blue) concentration from BOSC ("Benign=Jinak") and OSCC ("Malignant=Ganas").

Conclusion

The conclusion of this research were confirmed that OSCC patients which realized HPV infection indicated an up regulated of Hsp70 concentration and down regulated of ATP production, so that there was occurs misfolding of the proteins cell. The misfolding make proteins does not normal in function, with be result in present a new homeostasis or there was ensue obstacle of apoptosis and to raise cell proliferation which to storm carcinogenesis. An up regulated of Hsp40 was role as co-chaperone. To leads this theory, therefore Hsp40 and Hsp70 can be used as pathogenesis indicator of OSCC patients which realized HPVinfection.

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