

THE EXPRESSION OF MITOCHONDRIAL HEAT SHOCK PROTEIN 10 IN ORAL LESIONS WITH HPV POSITIVE

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ABSTRACT

Hundreds of studies over the last 25 years have shown that stress contributes to many illnesses including cardiovascular disease, endocrine disease and cancer. Persistent infection with human papilloma-virus may play a central role in cervical carcinogenesis. Cancer may be defined as uncontrolled tissue growth in susceptible patient, with result from imbalance between cell division and program cell death (apoptosis). Psychosocial factors may influence cervical carcinogenesis via a psychoneuroimmunology pathway. Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer worldwide. Alcohol/tobacco consumption is the most important risk factor for this neoplasia; nevertheless, since 1983 it has been suggested that human papilloma viruses (HPV) have a role in HNSCC. The aim of this study is to know the protein expression especially heat shock protein 10 in oral lesions with HPV positive. Seventeen samples from many kind oral cavity lesions were collected from 1 January 2007 to 1 November 2007. DNA isolation and diagnose related HPV infections are made by Henk Schmits and/or Nigel McMillan and Nina Fowler method with some modifications. Protein isolation was doing with Vincent and Philippe method with some modifications and the SDS-PAGE protein electrophoresis was doing with 30mA for 6 hour. The result of this experiment was seen the band as 10kDa protein. And the conclusion is the expression oh heat shock protein 10 was up regulated in oral lesion with HPV positive. Certainly the apoptosis process was on

Keywords: Stress Cells; HPV; Heat Shock Proteins 10; Apoptosis; Psychoneuroimmunology

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INTRODUCTION

Hundreds of studies over the last 25 years have shown that stress contributes to many illnesses including cardiovascular disease, endocrine disease and cancer. It has been calculated that 70-80 % of all visits to the doctor are for stress-related and stress-induced illnesses and that stress contributes to 50% of all illness in the U.S. Many animal models indicate a link between stress and infectious disease. For example, psychological stress such as crowding prior to and following tuberculosis infection affects disease outcome. Psychological stress can inhibit natural killer cell lysis, T-cell responses and antibody production both in vivo and in-vitro. However, the degree of suppression of the immune system necessary to allow for severe infections is not fully understood (Colin, 2005).

Cervical intra-epithelial neoplasia (CIN) is a premalignant lesion of the cervix uteri. CIN lesions can progress to higher CIN grades (1-3) and cervical cancer,

persist or regress to lower CIN grades normal cervical epithelium. Persistent infection with human papilloma-virus may play a central role in cervical carcinogenesis. Psychosocial factors may influence cervical carcinogenesis via a psychoneuroimmunology pathway (Colin 2005). Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer worldwide. In 2005, 400,000 cases of HNSCC were diagnosed worldwide. The most frequently affected site is the oral cavity. Alcohol/tobacco consumption is the most important risk factor for this neoplasia; nevertheless, since 1983 it has been suggested that human papilloma viruses (HPV) have a role in HNSCC, mainly in the oropharynx (Gallegos-Hernández JF et al 2007).

Twenty-two proteins were identified as differentially expressed between the human normal oral keratinocytes (HNOKs) and oral squamous cell carcinomas (OSCC)-derived cell lines. Of these, 9 spots were up-regulated and 13 were down-regulated in OSCC-derived cell lines compared to the HNOKs. These spots included the

cancer-related proteins; annexin A1, lamin A/C, interleukin 1 receptor antagonist, serine proteinase inhibitor clade B5, stathmin 1, and superoxide dismutase 2. Those results are a first step toward identifying a protein profile of HNOKs and OSCC-derived cell lines. The identified proteins in this experiment may be used in future studies of carcinogenesis or as diagnostic markers and therapeutic targets for OSCC (Koike et al 2005). The highly conserved heat shock proteins (hsps) accumulate in cells exposed to heat and a variety of other stressful stimuli. Hsps, which function mainly as molecular chaperones, allow cells to adapt to gradual changes in their environment and to survive in otherwise lethal conditions. The events of cell stress and cell death are linked and hsps induced in response to stress appear to function at key regulatory points in the control of apoptosis. Hsps include anti-apoptotic and pro-apoptotic proteins that interact with a variety of cellular proteins involved in apoptosis. Their expression level can determine the fate of the cell in response to a death stimulus, and apoptosis-inhibitory hsps, in particular hsp27 and hsp70, may participate in carcinogenesis (C Garrido, et. al., 2001).

The mitochondrial heat-shock proteins hsp60 and hsp10 form a mitochondrial chaperonin complex, and previous studies have shown that their increased expression exerts a protective effect against ischemic injury when cardiac myocytes are submitted to simulated ischemia. The more detailed mechanisms by which such a protective effect occurs are currently unclear. They wanted to determine whether hsp60 and hsp10 could exert a protection against simulated ischemia and reoxygenation (SI/RO)-induced apoptotic cell death and whether such protection results from decreased mitochondrial cytochrome *c* release and caspase-3 activation and from the preservation of ATP levels by preservation of the electron transport chain complexes. In addition, they explored whether increased expression of hsp60 or hsp10 by itself exerts a protective effect (Kurt M. Lin 2001).

MATERIALS AND METHODS

Seventeen frozen section tissues are collected from oral and dental part of doctor Muwardi Hospital Surakarta patient with oral lesion from January 2007 to Oktober 2007. DNA isolation was made by Henk Schmits and/or Nigel McMillan and Nina Fowler method with some modifications. Cut up to 25 mgr of tissue into small pieces, place in 1.5 ml a microfuge tube volume, and add 200 µl of DNA extraction buffer. Add 20 µl of Proteinase K stock solution, mix by vortexing, and incubate at 55°C overnight.

Diagnose related with HPV infections are made by Henk Schmits and/or Nigel McMillan and Nina Fowler method with some modifications. 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) and 2 µl DNA template. PCR protocol for both amplifications are 94°C for 50 seconds, 59°C for 50 seconds, 72°C for 50 seconds and 4°C soak. The Amplification of HPV-L-1 gene produced 450 bp long.

Protein isolation was made by Vincent and Philippe method with some modifications. Cut up to 25 mgr of tissue into small pieces, place in 1.5 ml a microfuge tube volume, and add 1 ml Phosphate Buffer Saline (PBS). Suspension and sonication 4 times (each 30 second) on ice. Centrifuge at 3000 rpm (20 minute at 40C). Getting supernatant and count the concentration by spectrophotometer (Vincent and Philippe, 1997). Load samples into the bottom of wells SDS-PAGE 15% with a long narrow tip. Start eletrophoresis at 40V for 18h 0r 30mA for 5h.

RESULTS

The result of this experiment was shown below,



Figure 1. The expression 450bp of DNA L1 gene HPV oral lesion. M:Marker; N:Normal; Ep:Epulis; Gi:Gingivitis; GE:GingivalEnlargment; Po:Polyps.

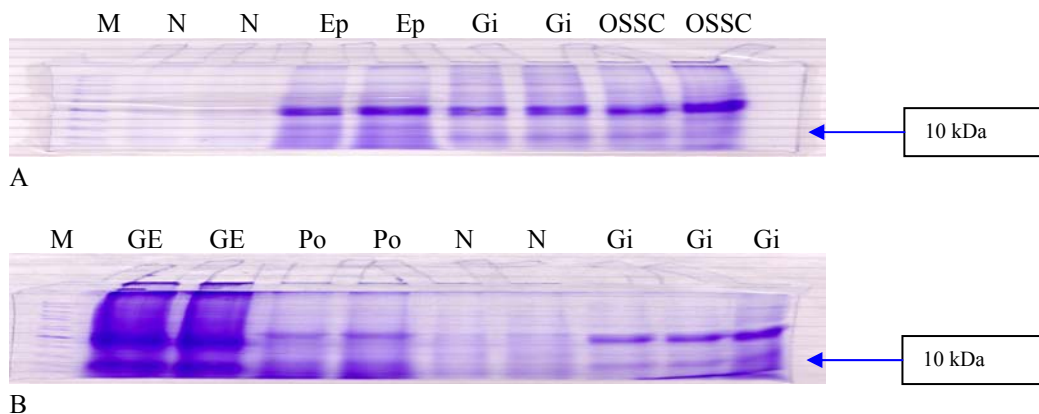


Figure 2A dan 2B. The expression of protein in many kind of oral lesion. M:Marker; N:Normal; Ep:Epulis; Gi:Gingivitis; OSSC:OralSquamousCellCarcinoma; GE:GingivalEnlargment; Po:Polyps.

DISCUSSION

Stress is a familiar aspect of modern life, being a stimulator for some, but having a negative impact for many others. Stress can be defined as a constellation of events resulting from a stimulus (the stressor), and precipitating a series of events activating physiologic response are generally adaptive in the body (the stress response). The physiologic response results in the release of neurotransmitters to the body. The results of the physiologic response are generally adaptive in the short-term, but can be detrimental when the stress is chronic and prolonged. There are wide variations in behavioral and biological reactions to stressful situations depending on genetic factors, gender, physiologic and psychological history. A great deal research remains to be carried out concerning these individual differences and how they can be recognized (Colin 2005; Suhartono 2005; Alberto 2005).

Stress to the cell also causes protein de-naturation: 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, and Huntington's diseases, 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. (Claudio Soto, 2001; Alberto, 2005). 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) (Claudio Soto 2001). Cancer may be defined as uncontrolled tissue growth in susceptible patient, with result from imbalance between cell division and program cell death (apoptosis) (Samer, 2005). Paradoxically, 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 (Helen M. Beere 2004)

Cells maintain a complete set of functionally competent proteins normally and in the face of injury or stress with the use of various mechanisms, 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 (Alberto, 2005).

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). For HSPs, the groups are as follows: high-molecular-mass HSPs (≥ 100 kD), HSP90 (81 to 99 kD), HSP70 (65 to 80 kD), HSP60 (55 to 64 kD), HSP40 (35 to 54 kD), and small HSPs (≤ 34 kD) (Jesús Villar, 2000; Alberto J.L., 2005).

Apoptosis is mediated by the activity of the aspartate-specific cysteine proteases – caspases (cysteiny, aspartate-specific proteases) – which cleave either to inactivate or activate target substrates. Caspases form a cascade in which 'initiator' caspases interact with specific adaptor molecules to facilitate their own autocatalytic processing. These, in turn, cleave and activate the downstream 'executioner' caspases that orchestrate the proteolytic dismantling of the cell. The sequence of events culminating in the activation of caspases can be broadly categorized into two pathways: the 'intrinsic' pathway the 'extrinsic' pathway. The intrinsic pathway is characterized by the permeabilization of the outer mitochondrial membrane and the release of several pro-apoptotic factors into the cytosol. These include cytochrome *c*, Smac/Diablo, EndoG and HtrA2/Omi. The precise mechanism of cytochrome *c* release remains unclear but is regulated by the antagonistic activities of the Bcl-2 family. Once released into the cytosol, cytochrome *c* binds to an adaptor protein, Apaf-1, which self-oligomerizes and recruits pro-caspase-9 to form the apoptosome complex. This promotes the autoprocessing of pro-caspase-9, which in turn recruits and cleaves pro-caspase-3 that is then released into the cytosol to degrade target substrates proteolytically (Helen M. Beere 2004)

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 pathophysiological 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. The data obtained in these studies seems to suggest that this group of HSPs may be useful as tools in the

management of primitive neoplasms. Some articles have also suggested a possible relationship between HSP expression and lymph node metastasis formation (Cappello 2005; Daniel 2005). HSP60 and HSP10 are two chaperones that interact in a two-step folding mechanism in the mitochondria of prokaryotic and eukaryotic cells. In addition, these proteins may be involved in other cellular functions, such as mediating specific tumour signals, but these roles are not yet well understood. In the last few years, the research group has evaluated the presence and expression of HSP60 and HSP10 in a series of carcinogenetic models, such as the "dysplasia-carcinoma" sequences of uterine exocervix, large bowel and prostate. These data have highlighted that these chaperones are overexpressed during the carcinogenetic steps; in particular, they accumulate in the cytoplasm of dysplastic and neoplastic cells, and their levels of expression increases in the sequence leading from dysplasia towards carcinoma.

This study has hypothesised that HSP60 and HSP10 might be considered as new diagnostic and prognostic tools for these cancers, being involved in the molecular steps of carcinogenesis, analogously to what has already been demonstrated with other tumours (Afshin Samali, 1999; Kurt M. Lin, 2001; Francesco Cappello, 2005). HSP10 was recently shown to be selectively expressed by myelocyte and megakaryocyte precursors in normal human bone marrow. This feature disappears during lineage maturation, and it was hypothesised that HSP10 might have another role during differentiation and/or proliferation of those normal cellular lineages apart from the co-chaperonin one, although the obtained results could not explain this selective expression (Cappello 2005).

CONCLUSIONS

The result of this experiment was seen the band as 10kDa protein in all oral lesion. And our conclusion is the expression of heat shock protein 10 was up regulated. Certainly the apoptosis process was on

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