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Comparative proteomic analysis of oral squamous cell carcinoma and adjacent non-tumour tissue from Thailand

Pitak Chanthammachat[a], *Waraporn Promwikorn*[a], *Kowit Pruegsanusak*[b], *Sittiruk Roytrakul*[c], *Chantragan Srisomsap*[d], *Daranee Chokchaichamnankit*[d], *Jisnuson Svasti*[d], *Pleumjit Boonyaphiphat*[e], *Singkhamanan K*[f], *Paramee Thongsuksai*[e,*]

[a] *Department of Anatomy, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*
[b] *Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*
[c] *Genome Institute, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathumthani 12120, Thailand*
[d] *Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok 10210, Thailand*
[e] *Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*
[f] *Department of Biomedical Science, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*

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SUMMARY

Objective: The study was aimed at analysing and identifying the proteins that are differentially expressed in oral squamous cell carcinoma (OSCC) compared to adjacent non-tumour tissue.

Materials and methods: Two-dimensional (2D) sodium dodecyl sulphate–polyacrylamide gel electrophoresis accompanied by mass spectrometry (matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry and liquid chromatography–tandem mass spectrometry) was used to analyse and identify the differentially expressed proteins in 10 pairs of tumours and adjacent non-tumour tissues from five cases of early-stage and five cases of late-stage OSCC. The statistical differences of the protein spots were analysed by the Wilcoxon signed-rank test. A validation study using immunohistochemistry and quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was performed.

Results: A total of 68 proteins (63 up-regulated, five down-regulated) were differentially expressed in early-stage disease, and 39 proteins (37 up-regulated, two down-regulated) were significantly altered in late-stage disease. Among these, 14 proteins were altered in both groups.

A total of 44 proteins were identified, including heat shock proteins (HSPs: Hsp90, HSPA5 and HSPA8), keratins (K1, K6A and K17), tubulin, cofilin 1, 14-3-3σ and metabolic enzymes. These proteins are involved in various cellular processes essential for cell growth, survival and cell migration. The validation study on α-tubulin and 14-3-3σ using

* *Corresponding author*. Tel.: +66 74451591; fax: +66 74212908.
E-mail addresses: coolmilk222@gmail.com (P. Chanthammachat), pwarapor@yahoo.com (W. Promwikorn), pkowit1964@yahoo.com (K. Pruegsanusak), sittiruk@biotec.or.th (S. Roytrakul), chantragan@cri.or.th (C. Srisomsap), daranee@cri.or.th (D. Chokchaichamnankit), scjv@mahidol.ac.th (J. Svasti), pleamjit.b@psu.ac.th (P. Boonyaphiphat), skamonnu@medicine.psu.ac.th (S. K), tparamee@gmail.com, paramee.t@psu.ac.th (P. Thongsuksai).
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immunohistochemistry and KIAA1199 expression using real-time RT-PCR confirmed the results in proteomics analysis.

Conclusions: The study identified many proteins, both known and unknown, for cancer cell processes. At least two proteins, KIAA1199 and Horf6, are novel for oral cancer.

1. Introduction

Oral cancer is a significant cancer worldwide, as it ranks as the eighth most common cancer in males with an average global incidence of 6.3 per 100,000.[1] It is more prevalent in developing countries than in developed countries. The incidence of oral cancer in the southern province of Songkhla is the highest in Thailand, with an age-standardised incidence rate of 9.8 per 100,000 in males.[2] Mortality from oral cancer averages less than half the incidence.[1] The 5-year survival rate has only been increasing subtly during the past two decades, in contrast with the advances in treatment modality[3]; this figure is largely due to the advanced stages of the disease at diagnosis which, in turn, limits or causes suffering from treatment. The identification of the molecular mechanisms underlying cancer initiation and progression could aid in the development of new diagnostic and treatment options for the disease.

The processes of cancer cell transformation and progression are extremely complex events involving the deregulation of a variety of genes controlling cell proliferation, differentiation and cell death.[4,5] Different proteins, probably in the hundreds or thousands, may be up-regulated or down-regulated simultaneously and may conduct distinct cell functions. Therefore, the analysis of hundreds of proteins simultaneously holds great promise for accurately predicting the function of marker proteins. Recent advances in mass spectrometry (MS) technology, combined with the rapid growth in genomic databases, allows for the identification of thousands of proteins in a given cell at a time. This approach, called proteomics, is therefore, a promising technique for the identification of new biomarkers for early cancer detection or as a new target for therapeutic intervention.[6]

In this study, we identified proteins that were altered during tumour development and progression of oral squamous cell carcinoma (OSCC). Two MS schemes, matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry (MALDI-TOF MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS), were used to maximise protein identification efficiency. The proteins that change in early-stage tumour may be implicated as potential markers of early diagnosis or detection of disease reappearance. Likewise, the proteins that change in late-stage tumour may be candidate prognostic or predictive markers.

2. Materials and methods

2.1. Tissue samples

Ten cases of OSCCs from seven women and three men aged 45–82 years were included in the study. Five cases were stage I and II (early-stage group) and five cases were stage III and IV (late-stage group). The tumours of all cases were well-differentiated OSCCs.

Fresh tissue samples of cancer and their adjacent normal mucosa were obtained at the time of surgical resection from 10 patients with OSCC at Songklanagarind Hospital, Faculty of Medicine, Prince of Songkla University, located in Songkhla, Thailand. Each tissue was sharply bisected: one half was frozen-sectioned for histopathological confirmation and the other half was stored at −80 °C until analysis. The histopathological evaluations of all cases were reviewed and confirmed by an experienced pathologist. Cancer staging was defined by the extent of the lesion (Tumour, Node, Metastasis (TNM) system) according to the *AJCC Staging Manual* 6th edition. The research protocol was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University (Reference Number SUB.EC 49/400-008).

2.2. Protein extraction

Each sample tissue, approximately $0.8 \times 0.8 \times 0.4\ cm^3$, was ground with a 300-μl lysis buffer (7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS), 1% dithiothreitol (DTT) and 2% immobilised pH gradient (IPG) buffer), frozen in liquid nitrogen, then thawed. The homogenate was centrifuged at 14,000 rpm at 4 °C for 15 min and the supernatants were transferred into a new microcentrifuge tube for protein purification. The protein concentration was measured with a Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Hercules, CA, USA) based on the Bradford method, using bovine serum albumin (BSA) as a standard.

2.3. 2D electrophoresis

Fifty micrograms of extracted protein was cleaned up using a 2-D Clean-Up Kit (GE Healthcare, Little Chalfont, UK) and then mixed with a rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 60 mM DTT and 0.5% IPG buffer). The sample was subjected to first-dimension electrophoresis by being rehydrated and focussed on a 13-cm IPG strip (pH 3–10) for a total of 18.8 kVh in Ettan IPGphore II (GE Healthcare, Little Chalfont, UK) at 20 °C. The focussed IPG strips were immediately continued to second-dimension electrophoresis using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). The IPG strip was equilibrated with an equilibration solution (75 mM Tris–HCl pH 8.8, 6 M urea, 2% SDS, 34.5% glycerol, a trace of bromophenol blue and 65 mM DTT) for 15 min, followed by a second equilibration for 15 min in the same solution containing 135 mM iodoacetamide instead of DTT. Then, the equilibrated strip was transferred to the top of a 12.5% polyacrylamide gel and held in position with molten