CYCLIN D1 AMPLIFICATION IN TONGUE AND BUCCAL MUCOSA SQUAMOUS CELL CARCINOMA

DR HAYDAR M. MAHDEY

BDS (BGHD)

This research thesis is submitted in total fulfillment

of the requirements for the degree of

Master of Dental Science (MDSc)

Department of ORAL AND MAXILLOFACIAL SURGERY

FACULTY OF DENTISTRY

UNIVERSITY OF MALAYA

KUALA LUMPUR

2010

UNIVERSITY MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: (I.C./Passport No):

Registration/Metric No:

Name of Degree:

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

Field of Study:

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this work;
(2) This work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purpose and any excerpt or exact from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this work;
(4) I do not have any actual knowledge nor do I ought reasonable to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as any be determined by UM.

Candidature's Signature Date

Subscribed and solemnly declared before,

Witness's Signature Date

Name:

Designation:

ABSTRACT

Introduction

Oral cancer is a significant health problem worldwide with almost 300,000 new cases are diagnosed each year. Despite the numerous studies done, and even with the best treatment option utilized, more than 50% of patients with oral cancer will experience relapse. In search for better options for prognostication, researches are now focusing on the molecular biology of cancer, for instance in search of reliable tumor markers. Among the markers reported in the literatures, Cyclin D1 is actively studied protein. Cyclin D1 regulates the cell cycle progression by forming a complex with different cyclin dependant kinase. Dysregulation of cyclin D1 can result in loss of normal cell growth and tumor development. The aim of this study is to determine and compare the amplification of Cyclin D1 in buccal mucosa and tongue oral squamous cell carcinoma(OSCC) and to associate its amplification in buccal mucosa and tongue OSCC with tumor depth, tumor front, histopathological grading, pathological tumor size, lymph node status, TNM staging and survival rate.

Materials and methods

The study samples were paraffin-embedded OSCC surgical specimens obtained from the archives of the Department of Oral Pathology, Oral Medicin and periodontolgy and Oral Pathology Diagnostic Laboratory. Fifty samples of patients with primary OSCC of buccal mucosa and tongue were included in the study. The sociodemographic and clinical data were obtained from the Malaysian Oral Cancer Tumor and Database System coordinated by the Oral Cancer Research and Coordinating Centre (OCRCC), University of Malaya. There were 31(62%) female and 19(38%) male with the overall age ranging from 26 to 94 years with a mean age of 60years.

The OSCC samples were from 44(68%) Indians, 10(20%) Malays and 6(12%) Chinese. The fluorescent-in-situ hybridization (FISH) technique was used to detect the amplification of Cyclin D1 using the Vysis protocol. Fluorescence evaluation of Cyclin D1 was performed using the image analyzer where the Cyclin D1 amplification signal appears as a small spot. At least 200 nuclei were scored using a 100X objective in each defined histological area, and each nucleus was assessed for the chromosome copy number. Statistical correlations of Cyclin D1 and certain clinicopathological parameters of OSCC were analyzed using the chi-square method or Fisher's exact test

Results

The present study found positive amplification of cyclin D1 in 72% (36) of OSCC. Detection of positive amplification for cyclin D1 was observed in 88% (22) and 56% (14) of the tongue and buccal mucosa OSCC respectively where the difference was statistically significant($p=0.012$). There was a significant correlation between Cyclin D1 positivity and ethnicity for the OSCC of the buccal mucosa ($p=0.037$); larger pathological tumor greatest dimension (pT) ($p = 0.019$), higher pTNM stages ($p=0.014$), tumor depth $\geq$ 5mm in tongue cases ($p<0.001$) and survival rate ($p=0.009$) for overall SCC cases and ($p<001$) for buccal mucosa SCC cases.

Conclusion

There is a significant correlation between amplification of Cyclin D1 with tumor depth and size of the tumor for tongue SCC; ethnicity and survival rate for buccal mucosa SCC .

ACKNOWLEDGEMENTS

First of all, I thank Allah the Almighty, for granting me the will and strength to accomplish this modest research. I pray that Allah's blessing upon me continue throughout my life, and Allah's blessing and peace be upon the messenger Mohammad.

I would like to express my sincere appreciation and deepest gratitude to my first supervisor Dr. Siti and second supervisor Prof. Dr. Rosnah Binti Mohd Zain for Their inspiration and continuous scientific suggestions throughout the preparation of this thesis. Their efforts are deeply appreciated.

My great thanks and praise goes to the Head of Oral and Maxillofacial Department Prof. Zainal Arrif Bin Abdul Rahman whose inspiration and unconditional will to teach enabled me and my fellow trainees to reach this stage.

I also seize this opportunity to thank and appreciate the efforts and moral support presented by the lecturers, my colleagues and the staff of the Department of Oral and Maxillofacial Surgery.

All the staff in Division of Pathology lab was an invaluable asset to my work and I would like to specify Dr Thomas Abraham, Dr. Thomas G. Kallarakkal, Dr. Anand R, Mrs Khoo Guat Sim, Mrs Rusnani Kamal and Mr. Siew Koi Kheong for all their assistance.

Last but not least, my parents for believing in me and for their continuous support. I dedicate this thesis to them.

CONTENTS **PAGE**

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVATIONS

CCND1	Cyclin D1
CDK4\6	Cyclin dependent kinases 4 and 6
DAPI	4',6'-diamidino-2-phenylindole
EBV	Epstein-Barr virus
FISH	Florescence in Situ Hybridization
H&E	Hematoxylin and Eosin
FITC	Fluorescein Isothiocyanate
HIV	Human immunodeficiency virus
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human papilloma virus
IHC	Immunohistochmistry
MOCTDBS	Malaysian Oral Cancer Tumor and Database
OCRCC	Oral Cancer Research Coordinating Center
OSCC	Oral squamous cell carcinoma
pN	Pathological lymph node metastasis
pT	Pathological tumor greatest dimension
pTNM	Pathological TNM stage
Rb	Retinoblastoma
SPSS	Computer program used for statistical analysis
TNF	Tumor necrosis factor
WHO	World Health Organization
XP	Xeroderma Pigmentosum