UNIVERSITI PUTRA MALAYSIA

CHROMOSOMAL REARRANGEMENT AND LOSS OF HETEROZYGOSITY IN CERVICAL CANCER AMONGST PATIENTS IN HOSPITAL KUALA LUMPUR

SITI NORLASIAH BINTI ISMAIL.

IB 2004 2

CHROMOSOMAL REARRANGEMENT AND LOSS OF HETEROZYGOSITY STUDIES IN CERVICAL CANCER AMONGST PATIENTS IN HOSPITAL KUALA LUMPUR

By

SITI NORLASIAH BINTI ISMAIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

January 2004

For my beloved husband, Mat, my children, Nina, Niza, Amirah and Muneer, my mother, Siti Rokiah, my late father, Ismail, my brothers and sisters.

.....Allah will raise up, to (suitable) ranks (and degrees), those of you who believe and who have been granted knowledge.....

(Surah 58 Al - Mujaadalah : 11)

Abstract of thesis submitted to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CHROMOSOMAL REARRANGEMENT AND LOSS OF HETEROZYGOSITY STUDIES IN CERVICAL CANCER AMONGST PATIENTS IN HOSPITAL KUALA LUMPUR

By

SITI NORLASIAH BINTI ISMAIL

January 2004

Chairman: Professor Abdul Manaf Ali, Ph.D

Institute: Bioscience

Cervical carcinoma is the second most common malignancy among women worldwide. The highest incidence rates are observed in developing countries. The susceptibility to cervical carcinoma in high incidence populations may result from several factors including human papillomavirus (HPV) exposure and both inherited and acquired genes. HPV infection does not always led to cervical cancer. In cervical carcinoma the other common genetic characteristic of cancer is the presence of several recurrent genetic alterations, not related to HPV. The type of recurrent genetic damage might take different forms such as gene amplification, chromosomal translocation, deletions, loss of heterozygosity, point mutation, transcriptional silencing, and in some tumors viral DNA integration.

To determine the possible genetic alterations amongst the Malaysian women with cervical cancer, this study was conducted on 50 cervical

tumor biopsies received from the Department of Obstetrics and Gynecology of the Hospital Kuala Lumpur. The objectives of the study were; i) the establishment of short-term primary cell culture of human cervical epithelial cells derived from cervical tumors for the determination of the prevalence and the type of chromosomal aberrations, ii) characterization of the allelic losses of the chromosomes 3p, 5p, 11 and 17p (p53), subsequently identification of a possible site of candidate tumor suppressor gene(s) and iii) to determine the HPV status of the cervical cancers. Primary cell culture and cytogenetic techniques were performed on the cervical tumor biopsies. G-banding was employed for the identification of the chromosomes. To define the localization of the tumor suppressor genes, loss of heterozygosity study was performed on 37 cervical cancer cells. Twenty-four microsatellite polymorphic markers for the chromosomes 3p, 5p, 11 and p53 were chosen, the normal and tumor DNAs from each patient were analyzed for the allelic loss using PCR-based microsatellite analysis. The status for HPV 16 E6 and HPV 18 E6 was detected by PCR method.

Twenty-five cervical cancer biopsies were successfully karyotyped and near-diploid was the modal number, with a majority of them being hypodiploid (35-45). About 50% of the metaphases obtained in the 25 tumors were hypodiploids, 12.1% were hyperdiploids, and 36.7% were diploids. Numerical abnormalities were predominantly observed in the patients, with monosomies of chromosomes 17, 22, X, 11, 18, 19, 13, and 6. Fluorescence *in situ* hybridization using centromeric

probes 11, 17 and 18 confirmed the presence of monosomies 11, 17, 18 in a low percentage, 12.0%, 16.2% and 26.4% respectively. Several clones of cells were observed, with possibility of 45,XX,-22; 45,XX,-11; 45,XX,-19 and 45,XX,-18. Twenty-six of the 36 informative individuals exhibited LOH at one locus or more. The highest incidence was observed in chromosome 3p with the frequency of 48.6%, while a low frequency of 2.7% was detected in the short-arm of chromosome 17 at position 17p13.1, whereby lies the p53 tumor suppressor. LOH was confined to four prominent regions, 11q23.3, 3p14.2-3p14.1, 3p21.32 and 3p25.3-3p25.1. No significant correlation was found between the LOH and the grade of cancer differentiation. The difference between the LOH frequency in cervical carcinomas with early stage and those with advanced stage was not statistically significant. Only 51.4% of the 35 tumors were positive for HPV 16 E6 and 17.2% was HPV 18 E6 positive. HPV 16 was found to be positive in 64.7% of the Chinese, 41.7% in Malays and 50% in the Indians. Both the Malays and the Indians were observed to harbor the HPV 18 at a higher frequency (40 and 33.3% respectively) than the Chinese. In conclusion, besides HPV infection, other genetic abnormalities play a role in cervical carcinogenesis. LOH is a better method than chromosomal analysis in searching for possible tumor suppressor gene(s) that is responsible for cervical tumorigenesis. Mapping of the smallest region of LOH in these tumors and analysis of candidate genes present in the region of LOH will be continued.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan bagi mendapatkan Ijazah Doktor Falsafah

PENYUSUNAN SEMULA KROMOSOM DAN KAJIAN KEHILANGAN HETEROZIGOSITI DALAM BARAH SERVIKS DI KALANGAN PESAKIT DI HOSPITAL KUALA LUMPUR

Oleh

SITI NORLASIAH BINTI ISMAIL

Januari 2004

Pengerusi: Professor Abdul Manaf Ali, Ph.D

Institut: Biosains

Karsinoma serviks adalah malignan kedua yang kerap berlaku di kalangan wanita di seluruh dunia. Insiden yang paling tinggi kerap dilihat di negara yang sedang membangun. Populasi yang mempunyai insiden tinggi mungkin mudah mendapat karsinoma serviks akibat pendedahan terhadap papilomavirus manusia (HPV) dan gen yang diwarisi atau diperolehi. Infeksi HPV bukan selalu menyebabkan kanser serviks. Bagi kanser serviks, ciri genetik yang kerap dilihat adalah beberapa pengubahan genetik yang berulang-ulang, yang tak berkaitan dengan HPV. Jenis kerosakan genetik yang berulang-ulang adalah seperti amplifikasi gen, translokasi kromosom, delesi, kehilangan heterozigositi, mutasi titik, *transcriptional silencing* dan integrasi virus. Kajian ini dijalan untuk mengetahui pengubahan gen di kalangan wanita yang mempunyai kanser serviks.

Kajian ini melibatkan 50 biopsi barah serviks dari wanita yang

menjalani rawatan barah serviks di Jabatan Obstetrik dan Ginekologi, Hospital Kuala Lumpur. Tujuan kajian ini adalah; i) membangunkan kultur sel primer daripada sel barah serviks bagi menentukan prevalen dan jenis aberasi kromosom, ii) pencirian kehilangan alel (*allelic loss*) pada kromosom 3p, 5p, 11 dan 17p (p53), seterusnya pengenalpastian lokasi gen penumpas barah (*tumor suppressor genes*) dan iii) untuk menentukan status HPV dalam kanser serviks. Kaedah kultur sel primer dan sitogenetik telah diperlakukan ke atas sampel biopsi barah serviks. Penjaluran-G telah digunakan untuk mengenalpasti kromosom. Untuk menentukan lokasi gen penumpas barah, kajian kehilangan heterozigositi telah dilakukan ke atas 37 sampel barah serviks. Dua puluh empat penanda polimorfik mikrosatelit pada kromosom 3p, 5p, 11 dan p53 telah dipilih. Pelet DNA normal dan barah dari setiap 37 sampel telah dianalisis menggunakan kaedah analisis mikrosatelit berasaskan PCR (*PCR-based microsatellite analysis*) untuk mengesan kehilangan alel.

Dua puluh lima biopsi barah serviks telah berjaya dikariotip dan *near-diploid* telah dilihat sebagai *modal number* dengan majoriti adalah hipoploid (35-45). Lebih kurang 50% daripada taburan metafasa adalah hipoploid, 12.1% hiperploid dan 36.7% adalah diploid. Keabnormalan pada jumlah kromosom dilihat lebih utama di kalangan pesakit, terutamanya monosomi kromosom 17, 22, X, 11, 18, 19, 13, dan 6. Penghibridan pendarfluor *in situ* (*fluorescence in situ hybridization*) menggunakan prob sentromerik bagi kromosom 11, 17 dan 18 telah

mengesahkan kehadiran monosomi kromosom tersebut tetapi pada kadar yang rendah, iaitu, 12.0%, 16.2% dn 26.4% masing-masing. Beberapa klon sel telah dijumpai, berkemungkinan 45,XX,-22; 45,XX,-11; 45,XX,-19 dan 45,XX,-18. Dua puluh enam daripada 36 individu yang informatif menunjukkan LOH pada satu lokus atau lebih. Insiden LOH yang tertinggi dikesan pada kromosom 3p dengan kekerapan pada kadar 48.6%, manakala LOH yang terendah adalah pada kekerapan 2.7% yang dilihat pada kromosom 17p di posisi 17p13.1. LOH telah dikesan pada empat kawasan yang utama, 11q23.3, 3p14.2-3p14.1, 3p21.32 dan 3p25.3-3p25.1. Tiada korelasi yang signifikan ditemui di antara LOH dan gred pembezaan kanser. Perbezaan di antara kekerapan LOH dalam karsinoma serviks di peringkat awal dan karsinoma di peringkat akhir adalah tidak signifikan. Hanya 51.4% daripada 35 barah serviks positif bagi HPV 16 E6 dan 17.2% positif untuk HPV 18 E6. HPV 16 didapati positif di dalam 64.7% kaum Cina, 41.7% Melayu dan 50% di kalangan kaum India. Peratusan positif HPV 18 didapati lebih tinggi di kalangan kaum Melayu dan India berbanding kaum Cina. Sebagai rumusan, selain infeksi HPV, keabnormalan genetik juga berperanan dalam proses karsinogenesis serviks. LOH adalah kaedah yang lebih baik daripada kaedah analisis kromosom untuk mencari gen penumpas barah yang bertanggungjawab bagi proses barah serviks. Kehadiran LOH dalam barah serviks mungkin boleh digunakan sebagai indikator terhadap prognosis penyakit tersebut. Pemetaan kawasan terkecil bagi LOH dalam barah ini serta analisis calon gen dalam kawasan LOH akan diteruskan.

بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيمِ

ACKNOWLEDGEMENTS

First of all praise to the Almighty Allah for giving me the ability to learn and strength to complete this research. This study would never have materialized without the contribution and support of many people to whom I have the pleasure of expressing my appreciation and gratitude. I would like to express my sincere gratitude to Professor Dr. Abdul Manaf Ali for his guidance, understanding, patience and encouragement that lead to the completion of this thesis, and my supervisory committee members: Professor Datin Dr. Khatijah Mohd. Yusoff, for providing the facility for molecular work, monitoring the progress of research, helpful comments and editing of the thesis; Associate Professor Dr. Sabariah Abdul Rahman and Associate Professor Dr. Siti Aishah Mohd. Ali, for their invaluable comments and Dr. Noorjahan Banu Mohd. Ali-Theen for proofreading and editing this thesis.

I am greatly indebted to the full cooperation and support of many individuals in the Department of Obstetrics and Gynecology, Hospital Kuala Lumpur, for providing the samples, without them, this research would have not materialized. Thanks are extended to the ex-Director Dr. Alex Mathews and Dr. Wong Sum Keong and his medical and nursing team.

My sincere gratitude to the Director of Institute for Medical Research (IMR) and the staff of the Genetics Laboratory for allowing the use of their laboratory for the Interphase *in situ* hybridization technique. Special thanks to Dr. Puteri JamilatulNoor Megat Baharuddin for sharing her experience and knowledge in this technique and her continuous encouragement towards the completion of this postgraduate program, Ms. Roshidah Ishak for the valuable tips on PCR technique, Mrs. Wang Lily for her technical assistance in FISH technique, Mr. Roslaini Majid for the simplified Hematoxylin and Eosin (H & E) technique and Ms. Ten Sew Keoh for her input. I am grateful to Ms Subha Bhassu of the Department of Biochemistry and Microbiology, UPM for the microsatellite analysis technique. My sincere thanks to Dr. Norhayati Zainal Abidin of Malaya University for providing the CaSki cell line.

I would like to extend my sincere thanks to the staff of the Genetics Laboratory of the National Population and Family Development Board (NPFDB) for their technical assistance during the study period, including taking messages from the doctors for specimen collection. My gratitude to my organization, NPFDB, for allowing me to pursue the postgraduate program, and the Public Service Department, as on behalf for the Government of Malaysia, for sponsoring me throughout this program.

I greatly appreciated my colleagues from the Animal Cell Culture

Laboratory and Biochemistry Laboratory for their unduly support and encouragement during the hard times. Lastly, but not the least, to my husband and children for their patience, sacrifices, support and understanding throughout this postgraduate program, my beloved mother and my siblings for their unconditional love and encouragement throughout the trying period.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CIN	Cervical intraepithelial neoplasia
CGH	Comparative genomic hybridization
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DMSO	Dimethylsulfoxide
dTTP	Deoxythymidine triphosphate
FISH	Fluorescence *in situ* hybridization
G-band	Giemsa band
HCl	Hydrogen chloride
H & E	Hematoxylin and Eosin
HPV	Human papillomavirus
ISCN	International System for Human Cytogenetic Nomenclature
ISH	*In situ* hybridization
KCl	Potassium chloride
LOH	Loss of heterozygosity
mar	Marker chromosome
MOH	Ministry of Health
N/C	Nucleus and cytoplasmic ratio
NPFDB	National Population and Family Development Board
PBS	Phosphate buffered-saline
PCR	Polymerase chain reaction