MOLECULAR GENETIC STUDY OF SELECTED RIBOSOMAL PROTEIN GENES IN NASOPHARYNGEAL CARCINOMA CASES

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A thesis submitted
in fulfillment of the requirements for the degree of
Ph.D (Molecular Biology)

Faculty of Resource Science and Technology
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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text.

______________
Name : MA XIANG RU
Date  : 9th June 2015
DEDICATION

A dream that will need
All the love you can give,
Every day of your life,
For as long as you live.

Climb every mountain,
Ford every stream,
Follow every rainbow,
Till you find your dream.

~ Climb Every Mountain, from the movie ‘The Sound of Music’ ~

For grandpa, who wished to see me graduate but lost his life to cancer during the later stage of my thesis writing.
ACKNOWLEDGEMENTS

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Last but not least, I give thanks to my parents and sister, who have always believed in me, and to my beloved husband for his unfailing support and love throughout this journey.
Nasopharyngeal carcinoma (NPC) is a cancer of the head and neck that is highly associated with Epstein - Barr virus (EBV) infection and shows strong ethnic and geographical clustering. In Malaysia, NPC is the fourth most common cancer on overall and the third most common cancer among males. The disease is often diagnosed at relatively later stages due to the signs and symptoms that are not obvious and which could often be mistaken as common illness. It is thus important to identify the molecular pathway(s) and genes involved in NPC carcinogenesis in order to have better prognosis of the disease. Ribosomal protein (RP) genes have recently been implicated in many human disorders and diseases. Apart from their roles in the canonical protein biosynthesis pathway, studies have shown that RP genes could also have extra ribosomal functions. Herein, the potential involvements and role(s) of nine selected ribosomal protein (RP) genes were examined at transcript level in NPC-derived cell lines and paired biopsies using real-time PCR, microarray and DNA sequencing techniques. Western-blotting was performed on NPC-derived cell lines to study the expression of RPs at protein level. Student’s- $t$ test, correlation test and multiple linear regression test were used to determine the statistical significance of result obtained. Both $RPS15$ and $L14$ were underexpressed at transcript level in cases of NPC whereas $RPS3$, $S7$, $S15$, $S26$, $S27$, $L32$ and $L34$ were not differentially expressed. Protein-protein dock models built via bioinformatics approach showed potential interactions between $RPS15$ with the Agenet-like motif 1 of FMRP. This motif is located in the NDF domain that has been reported to be involved in protein-RNA and protein-protein interactions. No nucleotide aberrancy was detected in the coding regions of all nine RPs examined. There was no association established between the expression of each RP with $p53$, as well as with NPC related clinicopathologic factors studied.
p53 which normally acts as the genome guardian of cells was also not differentially expressed at transcript and protein levels; and no mutation was detected in its entire coding region. Current findings suggested possible involvement of RPS15 and L14 in NPC pathogenesis. RPS15 protein could possibly regulate translation by interacting with FMRP – a predicted function that warrants further experimental investigation. Evidence showed that RPS3, S7, S15, S26, S27, L32 and L34 were unlikely to be directly involved in NPC pathogenesis. All these further strengthen the view that NPC is a unique and distinct type of head and neck cancers.
ABSTRAK

Kajian Molekul Genetik Ke Atas Gen-Gen Protein Ribosom Yang Terpilih Dalam Kes-Kes Karsinoma Nasofarinks

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ABBREVIATIONS

NPC  Nasopharyngeal carcinoma
EBV  Epstein-Barr virus
RP   Ribosomal protein
FOR  Fossa of Rossmuller
ENT  Ear, nose and throat
FNA  Fine needle aspiration
WHO  World health organization
AJCC American JointCommittee on Cancer
TNM  Tumor, node, metastasis
HLA  Human leukocyte antigen
CGH  Comparative genomic hybridization
TSG  Tumor suppressor gene
DBA  Diamond Blackfan Anemia
SSH  Suppression subtractive hybridization
MPS-1 Metallopanstimulin-1
PXN  Paxillin
NP   Nasopharyngeal
MMLV-RT Moloney Murine Leukemia Virus reverse transcriptase
PCR  Polymerase chain reaction
AGE  Agarose gel electrophoresis
LB   Luria broth
DMSO Dimethyl sulfoxide
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<td>PMSF</td>
<td>Phenylmethylsulfonyl fluoride</td>
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<td>MLR</td>
<td>Multiple linear regression</td>
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<tr>
<td>SLR</td>
<td>Single linear regression</td>
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<td>HNSCC</td>
<td>Head and neck squamous cell carcinoma</td>
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CHAPTER ONE
INTRODUCTION

Nasopharyngeal carcinoma (NPC) refers to cancer that arises from the nasopharynx tissue. According to the year 2007 report of the National Cancer Registry, NPC ranked fourth on overall and third for males of the ten leading cancers in Malaysia (Ariffin and Nor Saleha, 2011). Globally, the Cantonese-Chinese were reported to be genetically most susceptible to NPC whereas locally the native Bidayuh population had been reported as high risk group (Devi et al., 2004, Tao and Chan, 2007). Studies have identified environmental factors, genetic susceptibility and Epstein - Barr virus (EBV) infection as etiological factors of NPC (Lo et al., 2004b, Tao and Chan, 2007). However, NPC cases are often diagnosed at a relatively later stage due to the nature of the disease itself – the signs and symptoms of NPC are not obvious and are often mistaken as common illness. It is therefore crucial to identify and understand the driving signaling pathways of NPC tumorigenesis in order to better diagnose and control this disease.

Ribosomal protein (RP) genes encode proteins that form the integral part of ribosomes and traditionally these RPs are thought to be involved mainly in protein biosynthesis. Recent studies however revealed extra ribosomal roles of RP genes in cell cycle control and apoptosis, as well as in causing various human disorders and diseases (Kasai et al., 2003, Gazda et al., 2006, Warner and McIntosh, 2009). RP genes had also been reported to be possible cancer causing genes in several studies that employed zebrafish as model (Amsterdam et al., 2004, Uechi et al., 2006, Chakraborty et al., 2009). Interestingly, previous studies also showed underexpression of \textit{RPS26} and \textit{RPS27} in local NPC biopsies; as well as \textit{RPL27}, \textit{RPL37a} and \textit{RPL41} in NPC cell lines (Sim et al., 2008, Sim et al., 2009). To date, there are not many
reports on RP genes in NPC cancer model. Less is known about the extra ribosomal roles of RPs in NPC tumorigenesis (if any); and also the underlying mechanism(s) involving RPs that contributes to the progression of this cancer.

In this study, we aimed to address the aforementioned questions. We hypothesized that: (1) anomalies in the expression of RPs contribute to NPC tumorigenesis; and (2) the expression aberrancy is caused by variation/mutation(s) in the nucleotide sequence. The investigation was then carried out by focusing on these main objectives:

1. To identify and characterize nucleotide variation in RP genes previously proven to be associated with cases of NPC;
2. To isolate new RP and RP related genes that may influence tumorigenesis of NPC.
3. To evaluate expression patterns of RP and RP related genes associated with NPC tumorigenesis.
4. To delineate possible role(s) of RP genes in the pathogenesis pathway of NPC.