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Expression Analysis of *uS19* and its Association with Nasopharyngeal Carcinoma

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Nasopharyngeal carcinoma (NPC) is currently the fourth most common cancer in Malaysia. Ribosomal protein genes are crucial in producing functional ribosomes that assist in cell functions and have shown to be associated in pathways that link to the formation of cancer. Previous studies have shown that *uS19* gene is involved in the Mdm2-p53-MdmX pathway, where it inhibits the p53, a tumor suppressor. Furthermore, expression of ribosomal proteins have been studied to observe its expression levels, in which potential biomarkers could be discovered that assist in the early diagnosis of cancers. Notably, uS19 is currently understudied, though studies have been done on the expression of *uS19* gene in NPC, where it was found to be upregulated in poorly differentiated NPC tissue samples and HK1 cell line. However, further validation is required. Studies on the post-translational effect of uS19 in NPC carcinogenesis are yet to be done. In this study, mRNA and protein expression of uS19 data obtained revealed a differential expression at both gene and protein levels in NPC cell lines compared to normal nasopharyngeal epithelial (NPE) cells. The gene expression of *uS19* was found to be down-regulated following a trend of cell type and differentiation, while protein expression of uS19 was found to be upregulated in all NPC cell lines, with C666-1 cell line being the highest. The data attained showed that uS19 might be involved in the NPC tumorigenesis based on the difference in transcription and translational level. Protein-protein interaction via *in silico* approach revealed uS19 could possibly bind with 10 proteins, which includes LMP2 and EBNA1, a known Epstein-Barr virus (EBV) protein. Our findings strengthen the potential role of uS19 in NPC tumorigenesis based on the expression data obtained and calculated interactions through bioinformatics analysis that

revealed a high possible chance of uS19 protein interacting with EBV proteins involved in the carcinogenesis of NPC.

Keywords: Nasopharyngeal carcinoma, uS19, expression, protein-protein interaction

Ekspresi Analisa uS19 dan Hubungannya dengan Karsinoma Nasofarink

ABSTRAK

Karsinoma nasofarinks (NPC) merupakan kanser keempat di Malaysia. Gen protein ribosom adalah penting dalam menghasilkan ribosom berfungsi untuk membantu dengan fungsi sel dan laluan yang membentukkan tumor. Kajian terdahulu telah menunjukkan bahawa gen uS19 terlibat dalam laluan Mdm2-p53-MdmX, di mana ia menghalangkan p53, penindas tumor. Tambahan pula, ekspresi protein ribosom telah dikaji untuk memperolehi potensi biomarker yang dapat membantu dalam prognosis awal kanser. Walaubagaimanapun, uS19 di NPC pada masa kini belum lagi difahami, tetapi kajian telah dilakukan pada ekspresi gen uS19 dalam sampel tisu dan sel NPC, di mana ia menunjukkan ekspresi yang tinggi, bagaimanapun pengesahan selanjutnya diperlukan. Kajian mengenai kesan pasca translasi dari uS19 dalam perbentukkan kanser NPC masih belum dilakukan. Dalam kajian ini, ekspresi gen dan protein telah dilakukan di mana perbezaan dalam segi ekspresi di antara gen dan protein dalam sel NPC berbanding dengan sel normal berbeza antara satu sama lain. Ekspresi gen uS19 didapati mengikuti trend menurun dalam perbezaan ciri-ciri sel NPC, sementara ekspresi protein uS19 menunjukkan ekspresi yang tinggi dalam semua sel NPC dibandingkan dengan sel normal dengan ekspresi uS19, di mana sel C666-1 mempunyai ekspresi paling tinggi. Data yang diperoleh menunjukkan kemungkinan penglibatan uS19 dalam perbentukkan kanser NPC berdasarkan perbezaan transkripsi dan tahap translasi. Interaksi protein-protein melalui kaedah in-silico mendedahkan bahawa uS19 didapati untuk mungkin berinteraksi dengan 10 protein, termasuk LMP2 and EBNA1, protein Epstein-Barr virus (EBV). Penemuan kami

menguatkan potensi peranan uS19 dalam perbentukkan kanser NPC berdasarkan data eksperimen yang diperolehi dan analisa bioinformatic, yang menunjukkan peluang yang tinggi untuk uS19 berinteraksi dengan protein EBV yang terlibat dalam karsinogenesis NPC.

Kata kunci: *karsinoma nasofarinks, uS19, interaksi protein-protein*

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LIST OF ABBREVIATION

AJCC	American Joint Committee on Cancer
APS	Ammonium persulfate
ASR(W)	Age-world standardized incidence rate
BLAST	Basic local alignment search tool
BCL-2	B-cell lymphoma 2
CLL	Chronic lymphocytic leukemia
CRT	Chemoradiation therapy
DARS	Decoys as the Reference State
D-KSFM	Defined-keratinocyte serum-free medium
DNA	Deoxyribonucleic acid
EBNA1	Epstein-Barr nuclear antigen 1
EBNA-LP	Epstein-Barr nuclear antigen leader protein
EBV	Epstein-Barr Virus
ECL	Enhanced chemiluminescent
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
FBS	Fetal bovine serum
FFT	Fast fourier transform
FMRP1	Fragile X Mental Retardation Protein 1
HRP	Horse-radish peroxide
IMRT	Intensive-modulated radiation therapy

LMP1	Latent membrane protein 1
LMP2	Latent membrane protein 2
LRRK2	Leucine-rich repeat kinase 2
M-MLV-RT	Moloney Murine Leukemia Virus Reverse Transcriptase
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NaCl	Sodium Chloride
NCBI	National Centre for Biotechnology Information
NPC	Nasopharyngeal carcinoma
NPE	Nasopharyngeal epithelial
PML	Promyelocytic leukemia
PMSF	Phenylmethylsulfonyl fluoride
PPI	Protein-protein interaction
PSI-BLAST	Position-Specific Iterated BLAST
PVDF	Polyvinylidene difluoride
QMEAN	Qualitative model energy analysis
RIPA	Radioimmunoprecipitation assay
RMSD	Root-mean-square deviation
RNA	Ribonucleic acid
RP	Ribosomal proteins
RPS	Ribosomal protein subunit
RT-PCR	Reverse-transcription Polymerase Chain Reaction
rRNA	Ribosomal ribonucleic acid
SCC	Squamous cell carcinoma

SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
TBS	Tris-Buffered Saline
TBST	Tris-Buffered Saline Tween 20
TEMED	Tetramethylethylenediamine
UV	Ultraviolet
V	Voltage
VAST	Vector alignment search tool
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Cancers are progressive diseases that worsen and spread due to the accumulation of defected genes (Devereux et al., 1998). Early diagnosis on cancer diseases is crucial thus research regarding the possible causes of oncogenesis is looked into to provide an insight into the development of cancers, which could reduce the fatality of this disease. Recent studies have associated ribosomal protein (RP) genes with oncogenesis and tumour progression in humans through the activation or silencing of tumour suppressors, though the exact molecular mechanism remains a challenge. Genes in the ribosomal protein subunit (RPS) family gives instructions for producing ribosomal proteins used in protein synthesis and possesses extra-ribosomal functions such as cell proliferation and apoptosis. Studies revealed that changes in the RP expression levels are involved and regulates an extensive variety of activities correlating with cell growth and death and has been known to be implicated in carcinogenesis such as colon cancer, breast cancer, and nasopharyngeal carcinoma. In Malaysia, nasopharyngeal carcinoma (NPC) is currently the fourth most common cancer and is known to have an unbalanced geographical distribution, which makes it hard to diagnose especially due to its subtle symptoms. To this date, early diagnosis of NPC cases is hard to be detected causing the mortality rate for NPC patients to be high. Nasopharyngeal cancer is a type of cancer that starts at the nasopharynx, which is the upper part of the throat behind the nose, near the base of the skull. It is a malignant tumour that begins in the epithelial nasopharyngeal cells. Poorly differentiated squamous cell carcinoma (SCC) cell line is common in NPC patients in South China and has a high level of NPC incidence and mortality reported, according to Wei et al. (2014). The factors known to cause

NPC incidence are mainly Epstein-Barr virus infection (EBV), gender, age, race, geographical, environment and diet.

Though EBV is one of the main suspects in NPC carcinogenesis, current studies have suggested that RP genes are candidates to cancer-causing genes as it could be involved in certain pathways that coincides with EBV leading to NPC. Multiple studies have looked into the expression patterns of RP genes to obtain a further understanding regarding the molecular mechanism of how RP genes can be linked to NPC formation. Findings by Fang et al. (2008) through microarray analysis revealed an up-regulation of *uS19* gene, which was previously known as *RPS15*, in the poorly differentiated SCC of the NPC primary tumour when compared to normal nasopharyngeal tissue. Expression of *uS19* gene was also found to be upregulated in HK1 cell line in one of our previous studies (Sim et al., 2018). Furthermore, the *uS19* gene is known to be involved in regulating the Mdm2-p53-Mdmx network that inhibits the p53, which is a tumour suppressor in cells, from allowing the cell to undergo cell arrest or apoptosis, therefore, allowing the proliferation of the cells (Daftuar et al., 2013). This gene also exhibits expression patterns across the liver cancer samples (Wong et al., 2014) illustrating that *uS19* gene among many other genes has extra-ribosomal functions and is vital in the biological processes in humans but also can be highly active in cancerous and tumour cells. Furthermore, p53 has been known to be involved in the apoptotic pathway of NPC (Tulalamba & Janvilisri, 2012).

Although the *uS19* gene possesses a substantial case to be further looked into past transcription, however, no studies have been done on the expression pattern of uS19 protein in NPC cell lines compared to normal nasopharyngeal epithelial (NPE) cell lines. Hence,

whether the expression level of protein and mRNA are similar to one another or different is unknown. This could play a role in understanding whether the expression level of *uS19* gene during the transcription could affect the translation level of uS19 protein and lead to tumorigenesis. Moreover, identifying protein partners that associate with uS19 protein could lead to an understanding of the molecular pathway involved in the formation of NPC. Hence, we hypothesised that (1) *uS19* will be differentially expressed in NPC compared to NPE in both transcript and translation levels, (2) uS19 could play a role in NPC progression, and (3) uS19 might be interacting with EBV proteins and possibly pathways in cancer survival. The present study aimed to compare the transcription and translation level of *uS19* gene from both normal nasopharyngeal cell line, which is NP69, and six different NPC cell lines, which are HK1, HONE-1, SUNE-1, TW 01, TW 04, and C666-1, and to identify the protein-protein interaction between uS19 and its candidate protein partners through structural neighbouring and docking simulation.

The objectives of this study were:

1) To detect and quantify the transcription level of *uS19* gene in NPC cell lines compared to normal nasopharyngeal cell line
2) To detect and quantify the translation level of uS19 protein in NPC cell lines compared to normal nasopharyngeal cell line
3) To identify candidate protein partners associated with uS19 in NPC formation via structural neighbouring approach and docking simulation.

CHAPTER 2

LITERATURE REVIEW

2.1 Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is a rare malignant tumour that begins in the epithelial nasopharyngeal cells. It occurs in the nasopharynx located in the upper part of the throat behind the nose and near the base of the skull (Figure 2.1). Early diagnosis of NPC is rarely obtained due to its hidden location and subtle signs and symptoms. Common diagnosis to test NPC is physical examination using nasal endoscopy, blood testing for Epstein-Barr virus (EBV) and biopsy to observe the histopathology of the cells.

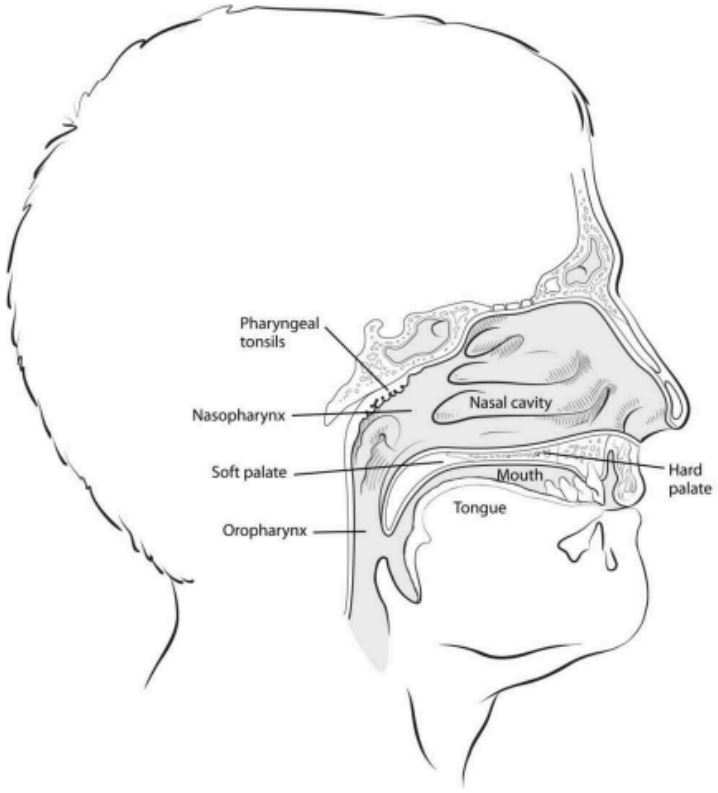

Figure 2.1: Anatomy of the pharynx. [Adapted from Nasopharyngeal cancer. (2013). *American Cancer Society*. Retrieved from http://www.cancer.org/acs/groups/cid/documents/webcontent/003124-pdf.pdf]

2.1.1 Histopathology of Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is a non-lymphomatous, squamous cell carcinoma (SCC) of an undifferentiated type. Histopathology studies on NPC are crucial in distinguishing prognosis and treatment. According to the World Health Organization (WHO), NPC is subcategorized into keratinizing carcinoma (Type I) and non-keratinizing carcinoma (Type II). Type 1, which is a keratinizing, well-differentiated SCC, is seen in 25% of NPC cases (Gullo et al., 2008). Type 1 NPC shows keratinization of the cell which is differentiated and possesses intracellular bridges. In this study, TW 01 cell line is used as a representative of Type 1 NPC (Gullo et al., 2008).

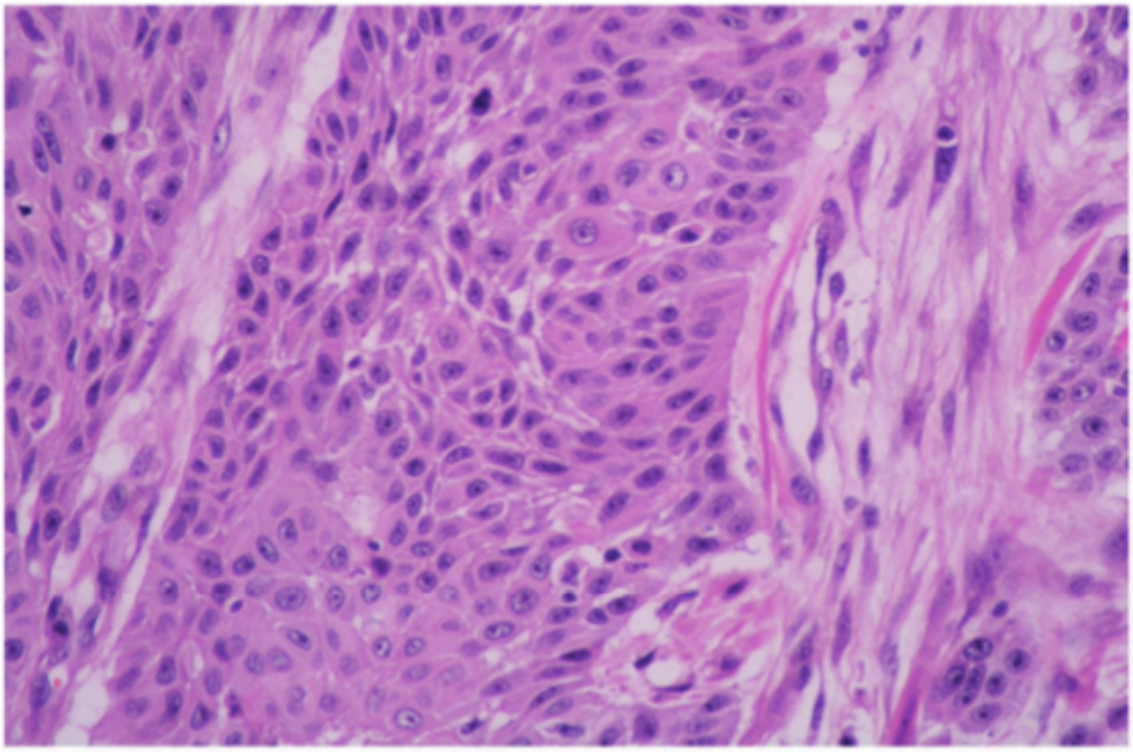

Figure 2.2: Histopathology of Type I NPC. Cells display individual cell keratinization and intracellular bridges Adopted from https://ars.els-cdn.com/content/image/1-s2.0-S2090074015000055-gr2.jpg (Lahuri et al., 2015).