MOLECULAR STUDY OF SELECTED DIFFERENTIALLY EXPRESSED GENES IN NASOPHARYNGEAL CARCINOMA

Chua Suk Ngo

Master of Science 2011
MOLECULAR STUDY OF SELECTED DIFFERENTIALLY EXPRESSED GENES IN NASOPHARYNGEAL CARCINOMA

CHUA SUK NGO

A thesis submitted in fulfillment of the requirements for the degree of Master of Science

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2011
ACKNOWLEDGEMENTS

I would like to acknowledge the advice and guidance of my supervisor, Assoc. Prof. Dr Edmund Sim Ui Hang who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I also thank the members of HMGL (Human Molecular Genetics Lab) for their guidance and suggestions, namely Ma Xiang Ru, Chia Sze Wooi, Johnson Chong, Nur Diana, Ang Chow Hiang and Tiong Wen ni for all their advice, encouragement, and help in retrieving journals articles.

I also thank the lab assistants especially Kak Lim, who has helped us a lot in lab purchasing. I acknowledge Unimas Postgraduate Scholarship for provision of fund to sustain my postgraduate pursuit. Thanks also to the Faculty for the equipment I have needed to produce and complete my thesis.

The major biopsy samples for this study were provided by Sarawak General Hospital and Hospital Serian Sarawak. Thanks also to Dr Selva, Dr Tiong Thung Sing, Mr Lai Wei Han and Mr Tan Sia Hong for sample collection and storage. Without these specimens, this study would have not been possible.

I would like to thank God for my family members and friends, who have always been supportive and encouraging.
ABSTRACT

Nasopharyngeal Carcinoma (NPC) occurs as a consequence of multiple molecular events induced by environmental factors and Epstein–Barr virus (EBV) infections. The neoplastic processes may involve alterations of the tumor suppressor genes and oncogenes by genetic damage and interference with the normal cellular functions by the EBV latent gene products. Overexpression of TNF receptor-associated factor 6 (TRAF6) has been reported to suppress oriP activity and loss of EBV from Burkitt's lymphoma cell line. The role of vigilin in cytoplasmic mRNA metabolism has been suggested whereby alterations in mRNA regulation by RNA binding protein have been reported in diverse cancer types. BCL2/adenovirus E1B 19kD interacting protein like (BNIPL2) has been reported to have tumor suppressor function. Human growth factor, augmenter of liver regeneration (GFER) is thought to be one of the factors responsible for the extraordinary regenerative capacity of the mammalian liver but its increased expression has also been reported in hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC). There have been several studies reporting the increased expression of macrophage migration inhibitory factor (MIF) in pre-cancerous, cancerous and metastatic tumours. Our earlier preliminary findings that revealed differential expression of these genes in NPC have led us to further investigate their expression. In this study, it aimed to examine the expression pattern (transcript and protein level) of these genes in local nasopharyngeal biopsy samples and cell lines and the existence of mutations (if any) in an effort to evaluate role(s) of these genes in NPC progression. The expression analysis study demonstrated that TRAF6 mRNA was underexpressed in tumor in 3 out of 8 biopsy samples screened via conventional PCR approach. This trend of underexpression was further confirmed via quantitative PCR in 5 out of 9 samples. Mutational analysis found no mutation
in coding sequence of *TRAF6*. TRAF6 protein expression however failed to be detected via Western blot. The results suggested tumor suppressive role for *TRAF6* in NPC. Our results also revealed underexpression of vigilin in 1 out of 8 samples via conventional PCR and 1 out of 9 samples with real time PCR. Underexpression of vigilin protein was found not to be significant in NP cell lines (3 NPC cell lines and 1 normal NP cell line) via Western blot. Mutational analysis revealed no mutation in vigilin coding region. *BNIPL2* was observed to be underexpressed in 3 out of 8 samples. However, further studies are needed to confirm the expression. No significant deregulation of *MIF* expression pattern was observed between tumor and normal samples via conventional PCR. Results also revealed underexpression of *GFER* mRNA in 1 out of 8 samples. Vigilin, *MIF* and *GFER* were suggested not directly associated with development of NPC.
Kajian molekul gen-gen terpilih yang menunjukkan pengekspresan yang berbeza dalam Karsinoma Nasofaringeal.

ABSTRAK

Karsinoma Nasofaringeal (NPC) terjadi akibat penggabungan antara pengaruh infeksi dengan virus Epstsein Barr (EBV), genetik dan faktor lingkungan lain. Proses neoplastik mungkin melibatkan perubahan gen supresor tumor dan onkogen melalui kerosakan genetik dan gangguan fungsi sel normal oleh produk gen laten EBV. Ekspresi dari TNF receptor-associated factor 6 (TRAF6) telah dilaporkan untuk menekan aktiviti oriP dan kehilangan EBV dari sel temurun limfoma Burkitt. Peranan vigilin dalam metabolisme mRNA sitoplasmik dicadangkan berdasarkan perubahan dalam regulasi mRNA yang disebabkan oleh protein pengikat RNA telah dilaporkan dalam pelbagai jenis kanser. BCL2/adenovirus E1B 19kD interacting protein like (BNIPL?) telah dilaporkan mempunyai fungsi supresor tumor. Gen human growth factor, augmenter of liver regeneration (GFER) dianggap salah satu faktor yang bertanggung jawab ke atas keupayaan regenerasi hati mamalia yang luar biasa. Pengekspresan GFER yang meningkat juga telah dilaporkan pada karsinoma hepatoselular dan karsinoma cholangiocarcinomas (cholangiocellular carcinoma). Terdapat beberapa kajian yang melaporkan peningkatan ekspresi macrophage migration inhibitory factor (MIF) pada tumor pra-kanser, kanser dan metastasis. Penemuan awal yang menunjukkan perbezaan ekspresi gen-gen tersebut dalam NPC telah mendorong penyiasatan lebih lanjut pengekspresannya dari sampel biopsi nasofarinks dan sel temurun dan kehadiran mutasi (jika ada) dalam usaha untuk menilai peranan gen-gen tersebut dalam NPC.
Kajian analisa ekspresi kami menunjukkan bahawa mRNA TRAF6 mempunyai kadar pengekspresan yang rendah dalam tumor dalam 3 daripada 8 sampel melalui pendekatan PCR konvensional. Trend pengekspresan yang rendah ini disahkan selanjutnya melalui PCR kuantitatif dalam 5 daripada 9 sampel. Analisis mutasi ke atas TRAF6 tidak menemui sebarang mutasi dalam jujukan gen tersebut. Namun, ekspresi protein TRAF6 gagal dikeaskan melalui kaedah blot Western. Siasatan mencadangkan peranan penekan tumor bagi TRAF6 dalam NPC. Keputusan juga mendedahkan pengekspresan rendah transkrip vigilin dalam tumor melalui kaedah konvensional PCR (1 daripada 8 sampel) dan PCR kuantitatif (1 daripada 9 sampel). Pola pengekspresan rendah protein vigilin didapati tidak signifikan melalui kaedah blot Western yang menggunakan tiga jenis sel temurun kanser nasofarinks (HONE1, HK1 dan SUNE1) dan 1 sel temurun nasofarinks normal (NP69). Analisis mutasi mendedahkan tidak ada mutasi pada jujukan gen vigilin. BNIPL2 juga didapati menunjukkan pengekspresan rendah dalam 3 daripada 8 sampel. Walau bagaimanapun, kajian lanjut diperlukan diperlukan untuk mengesahkan pola gen ekspresi ini. Tiada deregulasi ekspresan gen MIF yang signifikan diperhatikan antara sampel tumor dan normal melalui PCR konvensional. Keputusan juga mendedahkan pengekspresan rendah mRNA GFER dalam 1 daripada 8 sampel. Vigilin, MIF dan GFER dicadangkan tidak berperanan secara langsung dalam patogenesis NPC.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTERS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER ONE

**Introduction**

## CHAPTER TWO

**Literature Review**

2.1 Definition 5

2.2 Fossa of Rosenmuller 6

2.3 Normal histology 8

2.4 Histopathology 8

2.5 Epidemiology and Incidence 9

2.6 Etiologies 10

2.6.1 Genetic susceptibility factors 10

2.6.2 Environmental factors 11

2.6.3 Epstein-Barr Virus (EBV) 12

2.7 Molecular alteration and pathogenesis 12
2.7.1 Genetic and Epigenetic events 12
2.7.2 Alterations of subcellular mechanisms 13
2.7.3 Roles of EBV latent infection 17
2.7.4 Carcinogenesis of NPC 18

2.8 Genes of study 20
2.8.1 TNF RECEPTOR-ASSOCIATED FACTOR 6 (TRAF6) 20
2.8.2 HIGH DENSITY LIPOPROTEIN-BINDING PROTEIN (HDLBP) /Vigilin 22
2.8.3 BCL2/ADENOVIRUS E1B-KD PROTEIN-INTERACTING PROTEIN 2-LIKE (BNIPL) 24
2.8.4 MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) 26
2.8.5 GROWTH FACTOR, ERV1-LIKE (GFER) 28

2.9 Rationale 30
2.10 Research Objectives 31

CHAPTER THREE Materials and Methods 32
3.1 Tissue Specimens 32
3.1.1 Sources of total RNA samples 32
3.1.2 Isolation of total RNA 34
3.1.3 Total RNA analysis and quantification 34
3.2 Oligonucleotides 35
3.3 Semi-quantitative RT-PCR 38
3.3.1 Preparation of first strand cDNA 38
3.3.2 PCR 38
3.3.3 PCR products verification 40
3.3.4 Statistical analysis 40

3.4 Quantitative PCR 40
3.4.1 Dnase treatment 40
3.4.2 Purification 41
3.4.3 Preparation of first strand cDNA 41
3.4.4 Quantitative PCR amplification 41
3.4.5 Efficiency test 42
3.4.6 Delta Delta C_T Relative Quantitation 42

3.5 Cloning, screening and sequencing for mutational analysis 43

3.6 Protein analysis 43
3.6.1 Protein isolation using Nonidet P-40 lysis buffer 43
3.6.2 Protein quantification using Bradford assay 44

3.6.2.1 Reagents and standard curve preparation 44
3.6.2.2 Protein quantification 45

3.6.3 Western blot 45
3.6.3.1 Preparing SDS-PAGE 45
3.6.3.2 Preparing samples, gel-loading and running the gel 46
CHAPTER FOUR  Expression Analysis \textit{TRAF6}, vigilin, \textit{BNIPL2}, \textit{MIF} and \textit{GFER} in Nasopharyngeal Carcinoma via conventional RT-PCR

4.1 Introduction 50
4.2 General methodology 52
4.3 Results 53
  4.3.1 Total RNA samples assessment 53
  4.3.2 Internal control 55
  4.3.3 \textit{TRAF6} 57
  4.3.4 Vigilin 61
  4.3.5 \textit{BNIPL2} 64
  4.3.6 \textit{MIF} 67
  4.3.7 \textit{GFER} 70
4.4 Discussion 73

CHAPTER FIVE  Expression Analysis \textit{TRAF6} and vigilin in Nasopharyngeal Carcinoma via Quantitative PCR

5.1 Introduction 77
5.2 General methodology 79
CHAPTER SIX

PROTEIN EXPRESSION OF TRAF6 AND VIGILIN IN NASOPHARYNGEAL CELL LINES

6.1 Introduction 107
6.2 General Methodology 107
6.3 Results 109
  6.3.1 Protein sample concentration determination 109
  6.3.2 Vigilin and TRAF6 protein expression 112
6.4 Discussion 115
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Major gene alterations in nasopharyngeal carcinoma</td>
<td>15</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Preliminary findings of down-regulated genes in nasopharyngeal</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>carcinoma</td>
<td></td>
</tr>
<tr>
<td>Table 3.1</td>
<td>List of total RNA from biopsy tissues and their source information</td>
<td>33</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>List of total RNA from NPC cell lines (HONE, HK1 and SUNE1) and normal</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>NP 69 cell line</td>
<td></td>
</tr>
<tr>
<td>Table 3.3</td>
<td>List of synthetic oligonucleotide primers designed for amplification</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>of vigilin, TRAF6, BNIPL2, MIF, GFER, GAPDH, and ACTB in semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)</td>
<td></td>
</tr>
<tr>
<td>Table 3.4</td>
<td>List of synthetic oligonucleotide primers designed for amplification</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>of vigilin and TRAF6 in mutational analysis</td>
<td></td>
</tr>
<tr>
<td>Table 3.5</td>
<td>List of synthetic oligonucleotide primers designed for amplification</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>of vigilin, TRAF6 and GAPDH in real time PCR</td>
<td></td>
</tr>
<tr>
<td>Table 3.6</td>
<td>Conventional PCR reaction setup</td>
<td>39</td>
</tr>
<tr>
<td>Table 3.7</td>
<td>Conventional PCR cycling conditions</td>
<td>39</td>
</tr>
<tr>
<td>Table 3.8</td>
<td>Quantitative PCR Cycling conditions</td>
<td>42</td>
</tr>
<tr>
<td>Table 3.9</td>
<td>Recipe for resolving and stacking gels for SDS-PAGE</td>
<td>46</td>
</tr>
<tr>
<td>Table 3.10</td>
<td>Primary and secondary antibody dilutions.</td>
<td>49</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Expression (fold change) of TRAF6 in biopsy samples screened</td>
<td>60</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Expression (fold change) of TRAF6 in nasopharyngeal cell lines.</td>
<td>60</td>
</tr>
</tbody>
</table>
Table 4.3 Expression (fold change) of vigilin in biopsy samples screened 63
Table 4.4 Expression (fold change) of BNIP2 in biopsy samples screened 66
Table 4.5 Expression (fold change) of MIF in biopsy samples screened 69
Table 4.6 Expression (fold change) of GFER in biopsy samples screened 72
Table 5.1 GAPDH standard curve involving five-fold dilutions: 50, 10, 2, 0.4, 0.08 (ng) with two replicates at each point. 84
Table 5.2 TRAF6 standard curve involving five-fold dilutions: 50, 10, 2, 0.4, 0.08 (ng) with two replicates at each point. 84
Table 5.3 Vigilin standard curve involving five-fold dilutions: 50, 10, 2, 0.4, 0.08 (ng) with two replicates at each point 84
Table 5.4 Slope, amplification and reaction efficiency for GAPDH, TRAF6 and vigilin 86
Table 5.5 Negative RT control amplification C_T using GAPDH 91
Table 5.6 TRAF6 and GAPDH amplification C_T of replicate 1 93
Table 5.7 Expression (fold change) of TRAF6 in 9 nasopharyngeal samples 96
Table 5.8 Expression (fold change) of vigilin in 9 nasopharyngeal samples 99
Table 5.9 Summary of expression analysis of target gene expression (after normalization) in paired tumor and normal nasopharyngeal biopsy samples via conventional and quantitative PCR approach. 101
Table 6.1 Standard curve preparation 109
Table 6.2 Quantification of protein extract from cell lines. 111
Table 6.3 Expression (fold change) of vigilin protein in 3 NPC cell lines relative to one normal cell line (NP69). 114
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Lateral relations of the fossa of Rosenmuller</td>
<td>7</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Multistep carcinogenesis of nasopharyngeal carcinoma</td>
<td>19</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Total RNA isolated from paired biopsy samples using Trizol method.</td>
<td>54</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Total RNA isolated from nasopharyngeal cell lines using Trizol method.</td>
<td>54</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>Expression of \textit{GAPDH} and \textit{ACTB} in all nasopharyngeal biopsy samples screened.</td>
<td>55</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Expression of \textit{GAPDH} and \textit{ACTB} in three NPC cell lines (HONE1, HK1, SUNE1) and 1 normal NP (NP69) cell line</td>
<td>56</td>
</tr>
<tr>
<td>Figure 4.5</td>
<td>Expressions of \textit{TRAF6} in eight paired samples from local biopsies on 2.0% (w/v) agarose gel</td>
<td>59</td>
</tr>
<tr>
<td>Figure 4.6</td>
<td>Expressions of \textit{TRAF6} in three NPC cell lines (HONE1, HK1, SUNE1) and one normal cell line (NP69) on 2.0% (w/v) agarose gel.</td>
<td>59</td>
</tr>
<tr>
<td>Figure 4.7</td>
<td>Expressions of vigilin in eight paired samples from local biopsies on 2.0% (w/v) agarose gel.</td>
<td>62</td>
</tr>
<tr>
<td>Figure 4.8</td>
<td>Expressions of \textit{BNIPL2} in eight paired samples from local biopsies on 2.0% (w/v) agarose gel.</td>
<td>65</td>
</tr>
<tr>
<td>Figure 4.9</td>
<td>Expressions of \textit{MIF} in eight paired samples from local biopsies on 2.0% (w/v) agarose gel</td>
<td>68</td>
</tr>
</tbody>
</table>
Figure 4.10  Expressions of GFER in eight paired samples from local biopsies on 2.0% (w/v) agarose gel. 71

Figure 5.1  Total RNA isolated from paired biopsy sample 55 using Trizol method. 81

Figure 5.2  Standard curve plots: log of input cDNA (HONE1, NPC cell line) from a 5-fold serial dilution 83

Figure 5.3  Standard curve (a) GAPDH (b) TRAF6 (c) vigilin 85

Figure 5.4  Melting curve for samples used in quantitative study for (a) GAPDH (b) TRAF6 (c) vigilin 88

Figure 5.5  GAPDH (a) NAC amplification curve and (b) NAC melting curve for nine paired biopsy samples 90

Figure 5.6  A bar chart showing relative concentration of TRAF6. 95

Figure 5.7  A bar chart showing relative concentration of vigilin. 98

Figure 6.1  Protein quantification using Bradford assay. 110

Figure 6.2  Vigilin and GAPDH protein expression from 10µg total protein extracted from nasopharyngeal cell lines. 113
ABBREVIATIONS

% percent
°C degree Celsius
µl microlitre
$A_{260}$ absorbance at wavelength 260
$A_{280}$ absorbance at wavelength 280
$BNIPL2$ BCL2/adenovirus E1B 19 kDa protein interacting protein-like-2
bp base pairs
BSA bovine serum albumin
cDNA complementary DNA
DEPC diethyl pyrocarbonate
dH2O distilled water
DNA deoxyribonucleic acid
dNTP dinucleotide triphosphate
EBV Epstein–Barr virus
EDTA ethylenediamene tetra-acetic acid
EtBr ethidium bromide
$GFER$ growth factor, augmenter of liver regeneration
kb kilobases
MgCl$_2$ magnesium chloride
$MIF$ macrophage migration inhibitory factor
M-MLV Moloney Murine Leukemia Virus
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>ng</td>
<td>nano gram</td>
</tr>
<tr>
<td>NP</td>
<td>nasopharyngeal</td>
</tr>
<tr>
<td>NPC</td>
<td>nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PMSF</td>
<td>phenylmethylsulfonyl fluoride</td>
</tr>
<tr>
<td>PVDF</td>
<td>polyvinylidene difluoride</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcription</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription-polymerase chain reaction</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>TAE</td>
<td>tris-acetate EDTA</td>
</tr>
<tr>
<td>TRAF6</td>
<td>TNF receptor associated factor 6</td>
</tr>
</tbody>
</table>
CHAPTER ONE

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a cancer originating in the nasopharynx, the uppermost region of the pharynx or "throat", where the nasal passages and auditory tubes join the remainder of the upper respiratory tract. It has a remarkably distinctive ethnic and geographical distribution (Kwok et al., 2004). In 2005, 85,248 new cases were registered worldwide, and more than 68% of those were reported from China and Southeast Asia (Parkin et al., 2005). Yu and Yuan in 2002 (cited in Kwok et al., 2004) stated that regardless of race/ethnicity, men are 2-3 fold more frequently affected than women.

According to Kwok and Dolly (2002), there is strong evidence linking NPC to three well defined factors; a close association with the ubiquitous Epstein-Barr virus, certain traditional Southern Chinese diets containing chemical carcinogens, and an inherited genetic predisposition/susceptibility to the disease in certain patients. It is thought that these factors (in isolation or in combination) cause multiple genetic alterations that result in the disruption of various cellular mechanisms including cell cycle regulation, apoptosis, signal transduction, cell adhesion and other novel pathways (Kwok and Dolly, 2002).

By the comprehensive genome-wide studies, multiple genetic defects have been identified in NPC (Lo et al., 2004). Consistently high frequencies of genetic losses are observed on chromosomes 3p, 9, 11q, 13q, 14q, and 16q, while recurrent chromosomal gains were identified on chromosome 12 (Hui et al., 1999; Lo et al., 2000). Genes located on
chromosomes 9p21 \((p14, p16)\) and 3p21.3 \((RASSF1A)\) were found to be defective due to deletion or promoter hypermethylation \(\text{Lo et al., 1996, 2001; Kwong et al., 2002}\). The tumor suppressor properties of \(p16\) and \(RASSF1A\) have also been demonstrated in NPC cells \(\text{Wang et al., 1999; Chow et al., 2004}\).

Previous preliminary data \(\text{Sim et al., 2008}\) has detected differential expression of vigilin and \(TRAF6\) via the GeneFishing\textsuperscript{TM} Differential Expressed Genes (DEG) analysis techniques. In accordance with previous unpublished microarray-based transcriptional profiling data by our research group, \(BNIPL2\) showed down expression \(0.176\) fold change in NPC tumor sample. \(\text{Shen et al. (2003)}\) reported interaction of \(BNIPL2\) with MIF and GFER tested via yeast two-hybrid system. These observations suggested that many more genes may be involved in the multistep progression of NPC as the cause and mechanism of nasopharyngeal carcinoma (NPC) progression are multigenic in nature. Therefore, we aimed to study the existence of mutations \(\text{if any}\) and the expression pattern of these genes in local NPC biopsy samples using reverse transcription-polymerase chain reaction (RT-PCR) and Western blot approach, in an effort to evaluate the role(s) of these genes in NPC progression.

Vigilin, also known as high density lipoprotein-binding protein, is a 110-kD and 1268 amino acids long protein encoded by \textit{HDLBP} gene located at chromosome 2q37.3 \(\text{UniProtKB, 2007}\). Vigilin is a ubiquitous and highly conserved protein containing 14 related, but non-identical, K-homology (KH) nucleic acid binding domains \(\text{Goolsby and Shapiro, 2003}\). Vigilin is identified as important in the regulation of mRNA stability \(\text{Dodson and Shapiro, 2002}\). Many clinically relevant mRNAs including several encoding cytokines, growth factors and oncoproteins are regulated by differential RNA stability \(\text{Wilkinson, 2007}\).
Dysregulation of mRNA stability has been associated with human diseases including cancer, inflammatory disease, and Alzheimer's disease (Hollams et al., 2002).

TNF receptor-associated factor 6 is a member of the TNF receptor associated factor (TRAF) protein family encoded by TRAF6 located at chromosome 11p12 (NCBI, 2007b). TRAF6 that mediates activation of nuclear factor kappa-B (NF-κB) and the subsequent production of cytokines, chemokines, growth factors, and antiapoptotic proteins have been found to be involved in cancer progression and chemoresistance (Inoue, 2007). Toll-like receptors are thought possibly the signal initiators for NF-κB activation and inflammation-induced carcinogenesis (Chen et al., 2007).

BCL2/adenovirus E1B 19kD interacting protein like (BNIPL2) is a gene located at chromosome 1q21.1. Overexpression of BNIPL-2 has been reported to increase cell migration and invasion in vitro and promoted the metastasis of hepatocellular carcinoma (HCC) cells in vivo. Overexpression of BNIPL-2 has also been shown to inhibit colony formation and cell proliferation in BEL-7402 cells. Their results indicated that BNIPL-2 might inhibit cell growth and promote apoptosis (Shen et al., 2003).

Paralkar and Wistow (1994) showed that the MIF gene is remarkably small; it has only 3 exons and covers less than 1 kb. The gene is localized to human chromosome 22q11.2 Intense MIF protein was observed in the metastatic prostatic adenocarcinoma and the human prostatic adenocarcinoma cell line, LNCaP. Higher expression of MIF in tumor tissues was detected to be significantly higher than that of normal lung tissue (Tomiyasu et al., 2002).
Growth factor, augmenter of liver regeneration (GFER) gene resides on chromosome 16, at 16p13.3-p13.12. Increased GFER serum levels were detected for various types of acute liver disease (Tanigawa et al., 2000); in addition, increased expression of GFER in livers from patients with cirrhosis and hepatocellular and cholangiocellular carcinoma was reported (Thasler et al., 2006).

There is increasing knowledge about the genetic basis of NPC. Tumor suppressor genes (e.g. p16 and ARF) and oncogenes (e.g. Bcl 2) have been shown to be involved in the development of NPC (Kwok et al., 2004). Despite the increasing knowledge about the genetic basis of NPC, proposed tumorigenesis model for NPC is not yet complete.
CHAPTER TWO

LITERATURE REVIEW

2.1 Definition

The nasopharynx is defined as that portion of the pharynx which lies behind the nasal fossae and extends inferiorly as far as the level of the soft palate (Prasad, 2000). Its role is solely respiratory, probably functioning as a collecting space where the inspired air is filtered of impurities by the lymphoid tissue (Hasselt and Gibb, 1999).

Nasopharynx comprises of two distinct components, an upper anterior segment which developmentally, morphologically and histologically has all the features of the nasal cavity and a lower portion which is developed from foregut and has similarities to the alimentary tract (Hasselt and Gibb, 1999).

The close proximity to the nose and pharynx, plus its connection with the middle ear via eutachian tube, identify the nasopharynx as the central hub around which the otorhinolaryngology revolves (Hasselt and Gibb, 1999).