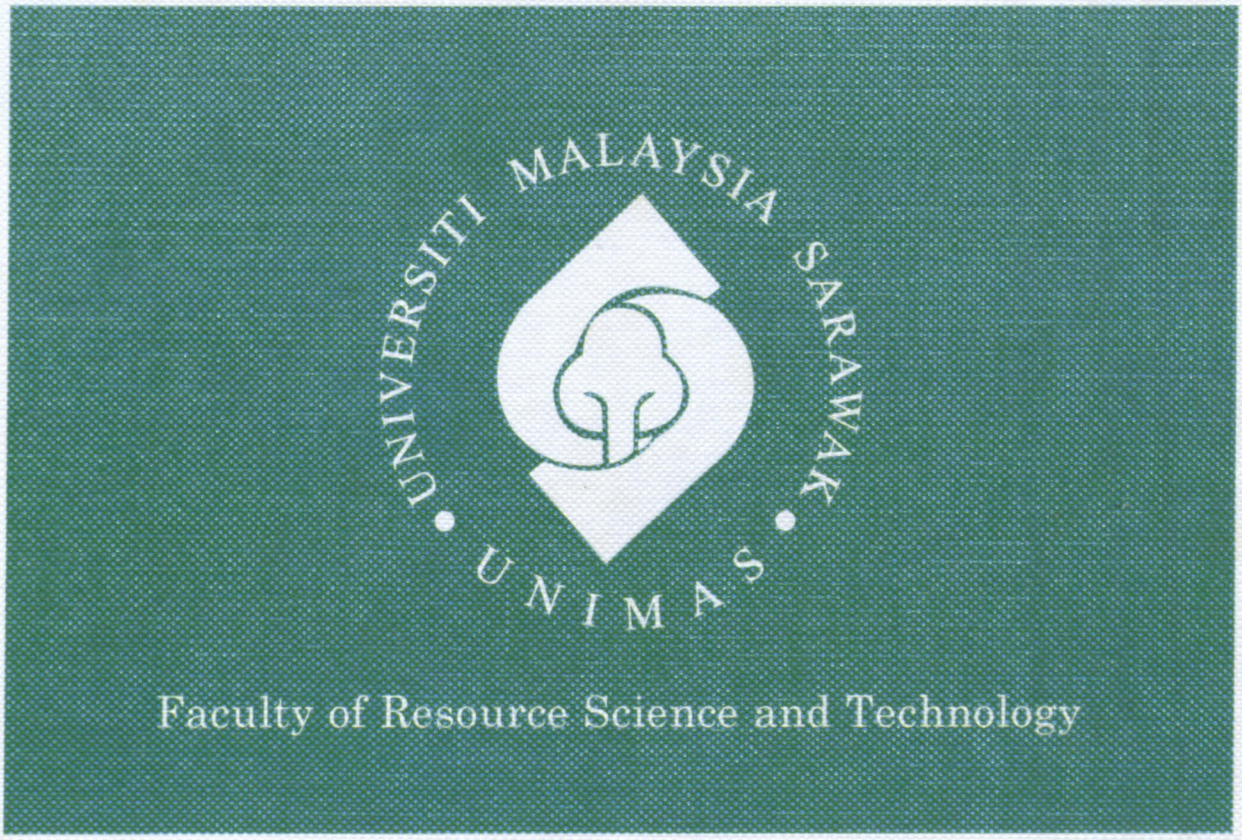

CHARACTERIZATION OF EXPRESSION PATTERN *RPS3*, *BolA1* AND *MIF* GENES IN NORMAL AND TUMOUR HUMAN CELL LINE

Liew Joe Yee
(33841)

Bachelor of Science with Honours
(Resource Biotechnology)
2014

Borang PTA4

[redacted]

Saya LIEW JOE YEE (nama) no. pelajar 33841 mengaku telah membuat perubahan yang perlu* / tidak ada perubahan terhadap Laporan Projek Tahun Akhir yang bertajuk:

Characterization of expression pattern of RPS3, BOLA1, and MIF genes in normal and tumour human cell line.

Bersama ini saya kemukakan 3 salinan Laporan Projek Tahun Akhir dan 1 salinan 'softcopy' Laporan berkenaan.

Tandatangan Pelajar

(LIEW JOE YEE)

Tandatangan Penyelia

Assoc. Prof. Dr. Edmund Sim Ui Hang
Department of Molecular Biology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

(Nama & Cop rasmi)

Pengesahan
Tandatangan Penyelaras Program

(Nama & Cop Rasmi)

Senior Lecturer
Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
94300 Kota Samarahan

* - potong yang tidak berkaitan

Characterization of Expression Pattern *RPS3*, *BolA1* and *MIF* Genes in Normal and Tumour Human Cell Line

Liew Joe Yee (33841)

A Thesis Submitted In Partial Fulfilment of the Requirement of
The Degree of Bachelor of Science with Honours (Resource Biotechnology)

Supervisor: Associate Professor Dr. Edmund Sim Ui Hang

Resource Biotechnology
Department of Molecular Biology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2014

ACKNOWLEDGEMENTS

First and foremost, I would like to express my highest appreciation to The Faculty of Science and Technology Resources, Universiti Malaysia Sarawak (UNIMAS) that had given me this opportunity to go through my final year project which is a prerequisite for undergraduates study. Second, my deepest gratitude would be conveyed to my supervisor, Associate Professor Dr. Edmund Sim Ui Hang whom has enlightened me in the direction of study associated with the significance of findings by sharing his profound knowledge and guidance with me.

Apart from that, my gratitude goes to the group of postgraduate seniors of the Immunoglobulin Human Molecular Genomic Laboratory who are Ms. Stella Chan Li Li, Ms. Ng Kher Lee and Ms. Shruti Talwar. They have given me supports and suggestion throughout this project. They have shared their experiences and valuable information besides helping to troubleshoot problems that had been encountered in this project. We were actively engaged in a fortnight meeting with the laboratory members where we shared our findings; discuss our progress and re-orientate the direction of this project from time to time.

Last but not least, I felt grateful to the supports that came from the laboratory mates whom have mutually encouraging each other. We were staying united in most of ups and downs, reminding each other on the mistakes and suggesting improvements to be made. In addition, I felt thankful to my family members, my course mates as well as my religious supports which had assisted me throughout this project.

UNIVERSITI MALAYSIA SARAWAK

Grade: _____

Please tick (√)

Final Year Project Report ☑

Masters ☐

PhD ☐

DECLARATION OF ORIGINAL WORK

This declaration is made on the 23rd day of...June.....2014.

Student's Declaration:

I , **LIEW JOE YEE, 33841, From Faculty of Resource Science and Technology**, hereby declare that the work entitled **Characterization of Expression Pattern *RPS3*, *BolA1* and *MIF* Genes in Normal and Tumour Human Cell Line** is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

23rd June 2014 — Date submitted

LIEW JOE YEE, 33841 — Name of the student (Matric No.)

Supervisor's Declaration:

I Associate Professor Dr Edmund Sim Ui Hang (SUPERVISOR'S NAME) hereby certifies that the work entitled Characterization of Expression Patterns RPS3, BolA1 and MIF Genes in Normal and Tumour Human Cell Line (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment of The Degree of Bachelor of Science with Honour (Resource Biotechnology) (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

Received for examination by: _____ Date: 23/6/14

Associate Professor Dr. Edmund Sim Ui Hang
(Name of the supervisor)

I declare that Project/Thesis is classified as (Please tick (√)):

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*
☐ **RESTRICTED** (Contains restricted information as specified by the organisation where research was done)*
☑ **OPEN ACCESS**

Validation of Project/Thesis

I therefore duly affirm with free consent and willingly declare that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abiding interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitalise the content for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes the sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student signature _____ 23/6/14 (Date)

Supervisor signature: _____ 23/6/14 (Date)

Current Address:
301, Block G3, Section 3, Bandar Baru Wangsa Maju, Setapak, 53300 Kuala Lumpur, Malaysia.

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument is duly prepared by The Centre for Academic Information Services]

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AGE	Agarose gel electrophoresis
BolA1	BolA homolog 1
cDNA	Complementary DNA
cm	Centimeter
c-myc	Myc proto-oncogene protein
CXCL-8	Chemokine (C-X-C motif) ligand 8
df	Degree of freedom
DJ-1	Parkinson disease protein 1
DNA	Deoxyribonucleic acid
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
IL-8	Interleukin 8
KH	K homolog
MDM2	Murine double minute 2
MIF	Macrophage migration inhibitory factor
miR-451	MicroRNA-451
ml	Millimeter
M-MLV	Moloney Murine Leukemia Viruses
MMP2	Matrix metalloproteinase 2
NF-KB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NPC	Nasopharyngeal carcinoma
OGG1	Oxoguanineglycosylase 1
p53	Tumour suppressor gene
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
Ref1	Retrovirus resistant factor 1
RNA	Ribonucleic acid
RP	Ribosomal protein
RPS3	Ribosomal protein small subunit 3
RT-PCR	Reverse transcriptase polymerase chain reaction
SAGE	Serial analysis of gene expression
TAE	Tris-acetate-EDTA
Th17	T helper 17 cell
Tm	Annealing temperature
µg	Microgram
µl	Microlitre
µM	Micromole
UV	Ultraviolet

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

Characterization of Expression Pattern *RPS3*, *BolA1* and *MIF* Genes in Normal and Tumour Human Cell Line

Liew Joe Yee

Resource Biotechnology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Protein-coding genes such as ribosomal protein S3 (*RPS3*), BolA homolog 1 (*BolA1*) and macrophage migration inhibitory factor (*MIF*) are the genes that regulate protein synthesis, redox potential and immunological function respectively. Bioinformatics study has postulated the possible interaction of these proteins *in silico* and the possible association in carcinogenesis. Literature reviews have reported the up-regulation of *RPS3* and *MIF* in different types of carcinoma cell lines. However, expression and interaction study involving these genes have not been conducted. The objectives of this study were to compare and characterize the expression patterns of these genes in human nasopharyngeal non-malignant cell line and nasopharyngeal malignant cell lines. Reverse transcription polymerase chain reaction (RT-PCR) had been employed to examine the expression level of gene transcripts. PCR products of *RPS3* and *MIF* with the estimated size of 428bp and 221bp were targeted in this study, whereas expression of *BolA1* gene was undetectable due to unspecific primer. At the end of this study, *RPS3* and *MIF* have manifested up-regulation in tumour cell lines as compared to the normal cell line however there was no significant difference between expression of *RPS3* and *MIF* ($p>0.05$).

Keywords: *BolA1*, Characterization, Gene Expression, *MIF*, *RPS3*

ABSTRAK

Gen pengekodan protein seperti protein ribosom S3 (RPS3), BolA homolog 1 (BolA1), dan gen pelarang macrophage penghijrahan faktor (MIF) adalah gen yang mengawal fungsi protein sintesis, potensi redoks dan imunologi. Kaijian bioinformatik mengandaikan interaksi protein dalam silico serta penglibatan gene ini dalam karsinogenesis. Ulasan sastera telah mengaitkan corak kenaikan bagi protein ribosom S3 dan MIF dalam pelbagai jenis sel karsinoma. Di sini, corak ungkapan gen ini telah dibandingkan dalam experimen yang menggunakan sel nasofarinks bukan malignan dan sel-sel nasofarinks karsinoma. Transcripsi songsang reaksi rantaian polimerase (RT-PCR) telah diaplikasikan untuk mengesan ungkapan gen transkrip. Produk PCR RPS3 dan MIF dengan saiz anggaran 428bp dan 221bp disasarkan dalam kajian ini tetapi BolA1 tidak disasarkan disebabkan primer yang lemah. Pada akhir kajian, corak kenaikan dikesan bagi RPS3 dan MIF dalam sel tumor berbanding dengan sel normal. Bagaimanapun tiada perbezaan yang signifikasi wujud di antara ungkapan gene RPS3 dan MIF ($p>0.05$).

Kata kunci: BolA1, MIF, Pencirian, RPS3, Ungkapan Gen

1.0 INTRODUCTION

Ribosomal protein *RPS3*, mitochondria protein *BolA1*, and cytokine protein, *MIF* are protein-coding genes that play significant roles in regulating physiological and metabolic processes in normal homeostatic condition. However genes manifest remarkably different characteristics in tumour cells where the homeostatic condition and deoxyribonucleic acid (DNA) constituents are altered. Under oxidative stress condition, *RPS3* induces apoptosis (Jang, Lee & Kim, 2004); *BolA1*affects morphology of mitochondria (Pelicarno, Carney & Huang, 2004); *MIF* promotes malignant cell transformation (Mitchell, 2004) in tumour cells. In relating the possible roles of these three genes in tumorigenesis, *RPS3* and *MIF* genes shared a pathway that involved nuclear factor kappa-light-chain-enhancer of activated B cells ($NF\text{-}_{K}B$). This implies that *RPS3* and *MIF* might work in collaboration which affects tumour formation if transcriptional environment is uncontrolled. In addition, knockdown of *BolA1* which induce oxidation in mitochondria and generate oxidative stress is likely to affect the expression patterns of *RPS3* and *MIF* (Rofi et al., 2013). Apart from the possible roles in tumorigenesis, bioinformatics study has further supported the protein interactions by postulating the protein structures of these genes and hence suspecting the correlation in expression patterns in tumour cell lines. Herein, an interest to investigate the differential gene expression in normal and tumour cell lines has been developed.

A considerable experiment had been designed to study the characteristics of the expression patterns and possible association of these genes in the human nasopharyngeal cell lines. In this project, nasopharyngeal cell lines were selected as the *in vitro* model system for gene expression study. Two types of cell lines which are normal and carcinoma cell lines were feasible for comparative and differential expression analyses. Three tumour carcinoma cell

lines were prepared as a measure to provide variety and reliability of the collected data, which are the well differentiated cell line, HK1 and the poorly differentiated cell lines, HONE1 and SUNE1. Duplicate reactions were performed to minimize variation of the results. A reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was selected to normalize the expression level of the genes. Reverse transcription polymerase chain reaction (RT-PCR) had been employed to synthesize the complementary DNA (cDNA) from ribonucleic acid (RNA) followed by amplification of cDNA. Agarose gel electrophoresis (AGE) was carried out to detect PCR products. Genes that have been detected on gel had to be confirmed prior proceed with comparative expression study. Further analyses such as relative quantification and statistical test were performed to analyze the band intensities which would generate the expression values. This finding perhaps laid a platform to encourage more research in transcriptomic expression and hence support proteomic expression studies such as biomarkers development for nasopharyngeal carcinoma (NPC) progression could be developed and be beneficial in the therapeutic industry.

Objectives of this research:

1. To observe and compare the gene expression patterns of *RPS3*, *BolA1*, and *MIF* genes in non-malignant nasopharyngeal epithelial cell line NP69 and nasopharyngeal carcinoma cell lines SUNE1, HONE1 and HK1.
2. To postulate the possible relationship based on the expression patterns of these genes in nasopharyngeal carcinoma cell lines.

2.0 LITERATURE REVIEW

2.1 Nasopharyngeal Epithelial

Human nasopharyngeal epithelial had been chosen to facilitate the differential expression for *in vitro* study. Human nasopharyngeal epithelial is derived from nasopharynx. Nasopharynx is located at upper part of the throat and lies behind the nose. This type of nasopharyngeal cancer is one of the endemic cancers that gained significant incidences in the population in South East Asia. This type of cancer is predominantly caused by Epstein-Barr viruses and possible factors such as genetics, nitrosamine-rich diets and alcoholic lifestyle (Wolters Kluwer Health Inc., 2013).

Nasopharyngeal carcinoma is a cancer derived from nasopharynx mucosa epithelium. In histopathological context, World Health Organisation has recognised three types of nasopharyngeal carcinoma cell lines (NPC), there are type I well differentiated squamous keratinized carcinoma, type II non-keratinized squamous carcinoma and type III non-keratinized undifferentiated carcinoma (Chan, Teo & Johnson, 2001). In cytological context, the keratinised cell presents as a squamous cell with the presence of intracellular bridges and the absence of cellular infiltrate. The non-keratinized cell present as a stratified and well-defined edged cell. Type III cell has a distinct nucleolus and less-defined cell boundaries (Wei & Sham, 2005).

In this project, normal cell line which is NP69, and tumorous cell lines HK1, SUNE1 and HONE1, had been chosen. Among the tumorous cell lines, HK1 represents the well differentiated cell line, whereas HONE1 and SUNE1 represent the poorly differentiated cell

lines. Consideration of using three different carcinoma cell lines was of intention to provide a representative data besides giving variability in cell lines for the genes expression study.

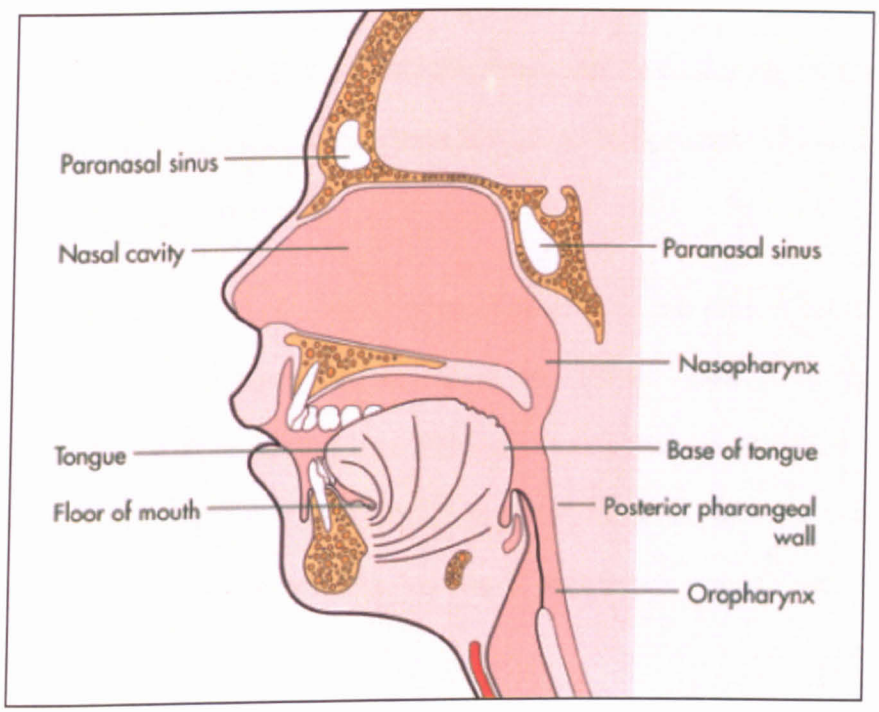

Figure 2.1: Side view of the nasopharynx structure.

(Adopted from Macmillan Cancer Support, 2013)

2.2 Ribosomal Protein S3 (*RPS3*)

In this study, the ribosomal protein S3 (*RPS3*) gene [GenBank: NM_001260506.1] was selected with the region targeted from 363th to 790th nucleotide. *RPS3* is a ribosomal protein located at chromosome 11, between loci of 11q13.3 and 11q13.5. This gene has 2187 base pairs with seven exons. The gene codes for *RPS3* protein and the coding region spans from 58th to 837th nucleotide, generating the products with 259 amino acids, weight 33 kilo Dalton (Santa Cruz Biotechnology, Inc., 2014c).

RPS3 regulates translation and transcription of proteins as this protein constitutes part of the translational initiation domain (Kenmochi et al., 1998). Apart from this, *RPS3* is responsible for mediating extra-ribosomal activities such as ribosomal maturation, DNA repair, inflammation regulation and apoptosis. *RPS3* involves in DNA damage recognition by interacting with genes that have prominent function in base excision repair such as *Ref-1* and *OGG1* (Itsara et al., 2014).

RPS3 interacts with nuclear transcription factor, oncogenes and tumour suppressor genes via K Homology (KH) domain. The transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is found to be associated with *RPS3* in structural context where *RPS3* constitutes the subunit of nuclear factor NF-κB complexes which eventually guide the transcription factor to bind to the promoter site. Selective recruitment of transcription factors by *RPS3* to promoter sites would result in differential gene expression (Gao & Hardwidge, 2011). The evidence of knocking down *RPS3* gene had seen to impose a failure of the transcriptional factor NF-κB in mediating p65 gene (Wan et al., 2007).

Apart from the transcription factor, KH domain also allows *RPS3* to exhibit a high degree of interaction with oncogene murine double minute 2 (*MDM2*) and tumour suppressor gene (*p53*). Relationship of these two genes is initiated by murine double minute 2 (*MDM2*) which plays as an agent that mediates ubiquitination and eventually proteasomal degradation of the *p53* (Iwakuma & Lozano, 2013). Similar discovery on the *MDM2* binding activities has been reported with the cell arrestment and apoptosis (Ma et al., 2012).

RPS3 had been studied of the expression patterns over various cancerous cells. Overexpression of *RPS3* is prevalent under stress condition, describing the role in inducing apoptosis (Gao & Hardwidge, 2011). *RPS3* has manifested increased expression in colorectal tumours, colon adenocarcinoma and adenomatous polyps. Thus, overexpression of *RPS3* has gained importance as a biomarker for cancerous occurrence (Pogue-Geile et al., 1991).

Figure 2.2: Gene transcript of *RPS3* with NM_001260506.1.

(Adopted from GenBank, updated by 2014)

2.3 *BolA* Homolog 1 (*BolA1*)

In this study, the *BolA1*gene [GenBank:NM_016074.3] was selected with the region targeted from 60th to 490th nucleotide. *BolA1* is an aerobic protein that located at chromosome 1q21.2. This gene has 872 base pairs with two exons. This gene codes for *BolA*-like protein 1, and the coding region spans from 122th to 535th nucleotide, generating the products with 137 amino acids, weight 14 kilo Dalton. *BolA1* is one of the members of *BolA* protein family which is homolog to the *Escherichia coli* protein (Santa Cruz Biotechnology, Inc., 2014a).

BolA1 is an aerobic mitochondrial protein which is highly conserved from prokaryotes to eukaryotes. This protein participates in the cell cycles regulation, cells proliferation and nucleic acid binding (Santa Cruz Biotechnology, Inc., 2014a). Human *BolA1* protein has capacity to fuse with myc proto-oncogene protein (*c-Myc)* (Zhou et al., 2008). This *c-Myc* is an oncogene that code for transcription factor and regulates cellular proliferation. Malfunction of *c-Myc* results in constitutive expression in cancerous cells that subsequently induces cancer formation (Miller, Thomas, Islam, Muench, & Sedoris, 2012).

BolA1 is yet to be studied of the expression level in cancerous cells however overexpression is significant during oxidative stress. Mitochondrial morphology experienced aberration caused by depletion of reduced glutathione during oxidative stress. Overexpression of *BolA1* has subsequently exhibits inhibitory effects by reducing the oxidative shift of the thiol redox potential of mitochondria as a preventive measure for the disturbance of cells components such as proteins, nucleic acids and lipids. The function of maintaining the redox potential is further supported by the evidence of knocking down *BolA1* gene which had observed with the increased oxidation of mitochondrial thiol group (Willem et al., 2013).

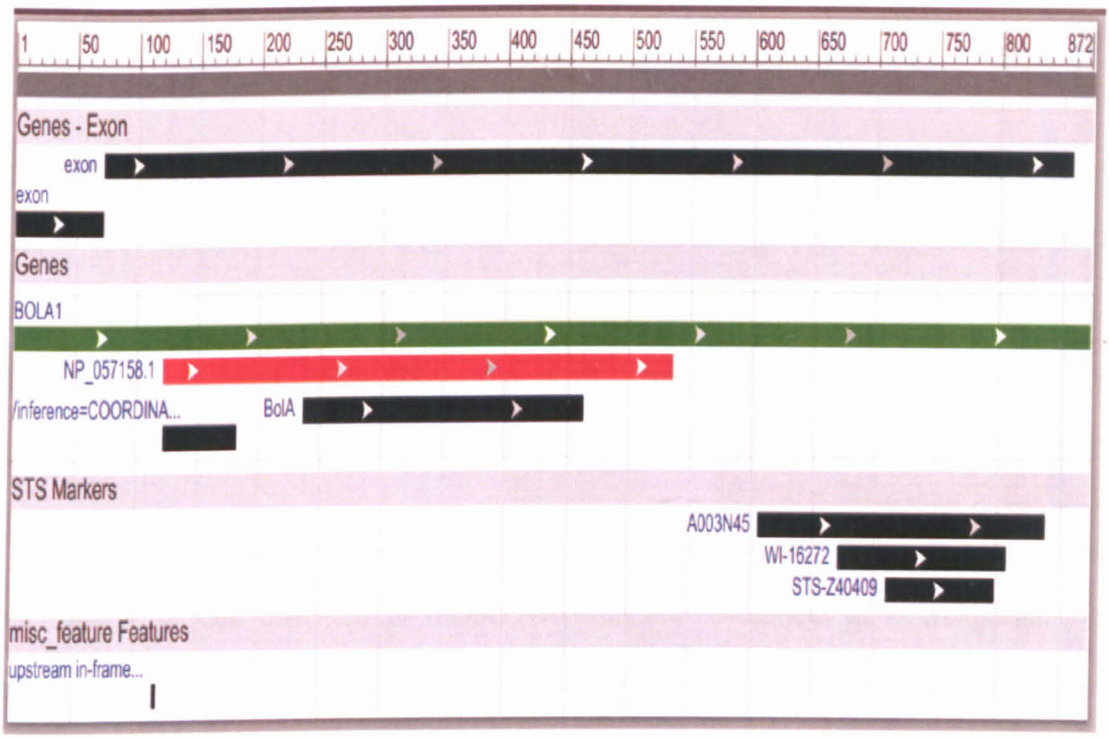

Figure 2.3: Gene transcript of *BolA1* with NM_016074.3.

(Adopted from GenBank, updated by 2014)

2.4 Macrophage migration inhibitory factor (*MIF*)

In this study, the *MIF* gene [GenBank: NM_002415.1] was selected with the region targeted from 110th to 330th nucleotide. *MIF* is an abbreviation of macrophage migration inhibitory factor which also known as glycosylation-inhibiting factor. This gene is located at chromosome 22q11.23. The total length of *MIF* gene is 561 base pairs with three exons. This gene codes for *MIF* protein and the coding region spans from 98th to 445th nucleotide, generating the products with 125 amino acids, weight 12.5 kilo Dalton (Santa Cruz Biotechnology, Inc., 2014b).

MIF protein regulates immunological function and inflammation. Overexpression of *MIF* has consistently been observed in tumour environment. Evidences of overexpression had shown in different types of carcinoma cells such as hepatocellular cancers (Wilson et al., 2005), lung cancers (Rendon et al., 2007), pancreatic cancers (Tan et al., 2014), breast cancers (Verjans et al., 2009) and prostate cancers (Chen et al., 2008) suggesting association of *MIF* in chronic inflammation and carcinogenesis. Extensive expression and interaction study of *MIF* gene in NPC cell lines has been conducted which noticed with an up-regulation of *MIF* gene, besides revealed the association of *MIF* gene with other molecules to constitute the regulatory functions such as cell invasiveness, metastasis and angiogenesis.

In normal circumstances, *MIF* works to suppress p53-dependent apoptosis while allowing survival of macrophage for the defense mechanism (Mitchell et al., 2004). *MIF* is also known to regulate protein molecules via trans-activation or suppression. *MIF* actively promotes cell migration by gathering the Th17 cells. Evidence of such function was supported by a knock down experiment on *MIF* gene (Li et al., 2012).

In addition, *MIF* recruits interleukin *CXCL8* in inflammatory stimulation in NPC cell lines. *MIF* and *CXCL8* were shown to be highly expressed in poorly differentiated carcinoma cell lines such as HONE1 and SUNE1. However, the elevated expression of *CXCL8* was suppressed by specific small interfering RNA which results in suppressed inflammatory function and subsequently reduced tumour sphere formation (Lo et al., 2013).

MIF protein was reported to participate in AKT pathway, also known as protein kinase B pathway which promotes cell invasion and metastasis by targeting the Parkinson DJ-1 protein (Pei et al., 2014). *MIF* enhances cell migration by regulating the expression of matrix metalloproteinase 2 (MMP2) and IL-8 in poorly differentiated carcinoma cell lines CNE-1 and CNE-2 (Li, Lin & Liang, 2004).

Despite of the regulatory roles played by *MIF* gene towards other factors, *MIF* could be targeted and suppressed by micro-RNA such as miR-451. In general, up-regulation of *MIF* is prevalent in poorly differentiated cell lines such as SUNE1, SUNE2 and CNE2. The miR-451 has been reported to suppress *MIF* and to reduce cell invasion, cell migration and metastasis potential of the *MIF* gene in NPC cell lines (Liu et al., 2013).

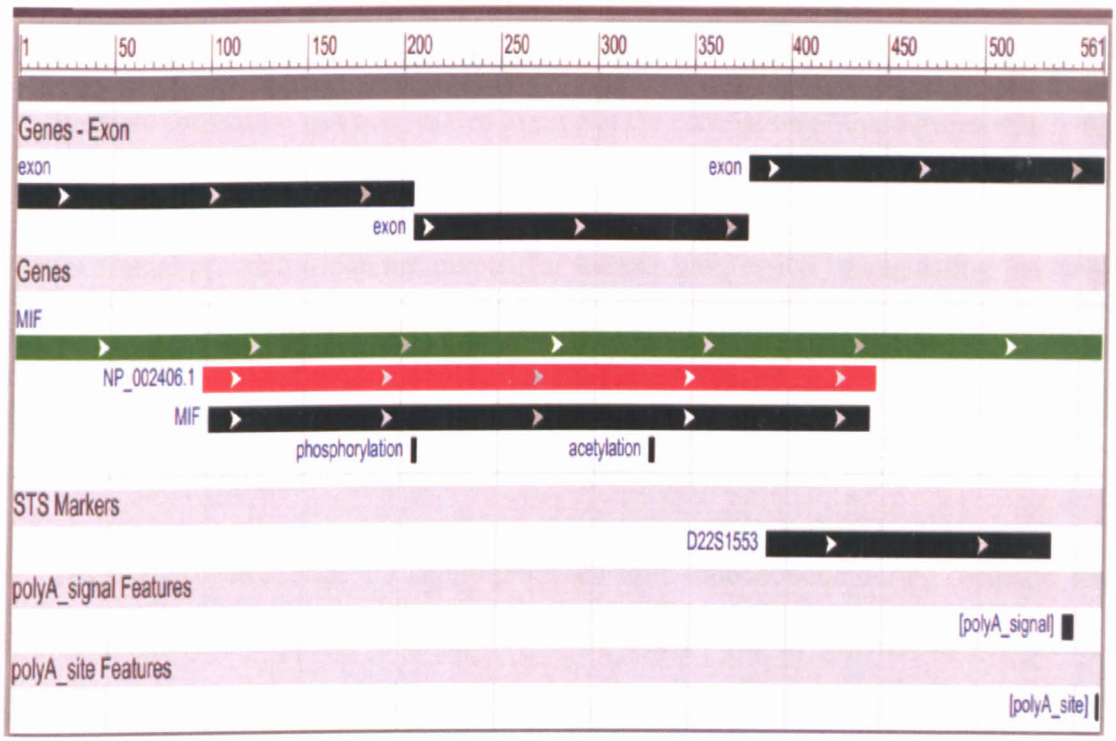

Figure 2.4: Gene transcript of *MIF* with NM_002415.1.

(Adopted from GenBank, updated by 2014)