IDENTIFICATION OF GENETIC LESIONS IN THE RIBOSOMAL PROTEIN GENE, RPL41 FROM HUMAN NASOPHARYNGEAL DERIVED CELL LINES

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The project proposal is submitted in partial fulfilment of the requirements for the degree of Bachelor of Science with Honours
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LIST OF ABBREVIATIONS

A,C,G,T  Adenine, Cytosine, Guanine, Thymine
bp  Base pair (s)
CDS  Coding sequence
cDNA  Complementary deoxyribonucleic acid
CKII  Casein kinase II
cm²  Square
Da  Dalton
ddH₂O  Double distilled water
DEPC  Diethyl pyrocarbonate
DNA  Deoxyribonucleic acid
dNTP  Deoxy-nucleotide-triphosphate
kb  Kilobase
MgCl₂  Magnesium chloride
mg  Milligram
ml  Milliliters
Mm  Millimolar
M-MLV RT  Moloney Murine Leukemia Virus Reverse Transcriptase
mRNA  Messenger ribonucleic acid
NIH3T3  National Institutes of Health "3-day transfer" mouse embryonic fibroblasts cell line
NPC  Nasopharyngeal carcinoma
OD  Optimal density
PBS  Phosphate-buffered saline
PCR  Polymerase Chain Reaction
RNA  Ribonucleic acid
RNPs  Ribonucleoprotein particles
RP  Ribosomal protein
RPL41  Ribosomal Protein, family of L41
RPL27  Ribosomal Protein, family of L27
RPL37a  Ribosomal Protein, family of L37a
rRNA  Ribosomal ribonucleic acid
rpm  Rotation/revolution per minute
RT-PCR
Tris-HCl
UICC
WHO
μl

Reverse transcriptase-polymerase chain reaction
Tris-hydrochloric acid
Union for International Cancer Control
World Health Organization
microlitres
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ABSTRACT

Nasopharyngeal carcinoma (NPC) is associated with three RP genes, namely RPL27, RPL37a and RPL41 that were significantly downregulated in all cell lines derived from NPC tissues when compared with nonmalignant nasopharyngeal epithelial cell lines. However, there is no report on RPL41 genes in human nasopharyngeal tissue. The objective of this study is to investigate the correlation between NPC and RPL41 gene sequence using normal and tumour cell lines derived from nasopharyngeal tissues. Total RNA of cell lines were used as the starting material and were transcribed into cDNA using M-MLV transcriptase. The 227 bp RPL41 gene was successfully amplified by polymerase chain reaction and was subsequently purified and sent for sequencing. The result shows that there is no nucleotide variation been observed in the coding sequences of RPL41 among these cell lines.

Key words: Nasopharyngeal carcinoma, RT-PCR, cDNA, RPL41

ABSTRAK


Kata kunci: Penyakit kanser hidung, RT-PCR, cDNA, RPL41
CHAPTER 1

1.0 INTRODUCTION

In all organisms, the ribosome plays the role as the pivotal catalyst of protein synthesis (Nakao, Yoshihama & Kenmochi, 2004). It consists of four RNA molecules (rRNAs) and around 80 various ribosomal proteins (RP) (Wool, Chan, & GluÈck, as cited in Nakao et al., 2004). Generally, the genes that encodes for rRNAs are clustered at a few regions in the eukaryotic genome, while those genes that encodes for RP are broadly dispersed (Kenmochi et al., as cites in Nakao et al., 2004). Among those RP genes, the ribosomal protein RPL41 will be investigated in this project, in terms of the possible genetic lesions that can occur in this gene in the human nasopharyngeal derived cell lines.

According to Gofman and O'Connor (1996), a genetic lesion is an injury or loss of function that normally occurs in the genetic molecules at any age of the cells. The genetic lesions are one of the implications due to mutational alteration (Glass & Glass, 1982). They mentioned that the genetic lesions can cause the inactivation of the gene product, or modify the normal activity or function of the gene. However, formation of new product due to the genetic lesions is reported as an uncommon scenario (Glass & Glass, 1982). The genetic lesion of RPL41 is still yet to be investigate as only little studies has been done so far.

The RP genes commonly associated with their functions in the regulation of cellular growth and differentiation (Sim, Ang, Ng, Lee, & Narayanan, 2010). However, the studies suggest that they also may be associated to congenital disorders and cancer as a result of deregulation in expression of those genes (Sim et al., 2010). Several researches have been
carried out to study the RPL41 gene's functions and behaviours in different part of the tissues, particularly using human tissue. For instance, RPL27, RPL37a and RPL41 were found to be significantly downregulated in all cell lines derived from NPC (nasopharyngeal carcinoma) tissues when compared with nonmalignant nasopharyngeal epithelial cell lines (Sim et al., 2010). With the current findings, the existence of NPC-associated RP genes is proven, and also shows their roles in human nasopharyngeal organogenesis.

Throughout this study, it is hoped that RPL41 gene can be isolated and identified, particularly for its genetic lesion from both normal and malignant human nasopharyngeal derived cell lines. Subsequently, comparison of the genes from both types of samples will enable detailed studies of the characteristics of genetic lesions in RPL41 in human nasopharyngeal derived cell lines.

1.1 OBJECTIVES

The aims of this study are to isolate the RPL41 cDNA from the total RNA of both normal and tumour nasopharyngeal derived cell lines and to obtain the sequence information of purified plasmid DNA for mutational analysis in the nasopharyngeal derived cell lines. This is believed to help in the investigation of the correlation between genetic lesion in RPL41 and NPC.
CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Ribosome

Ribosomes are large ribonucleoprotein particles (RNPs) with diameter of 20 nm and comprises of two subunits of different size (Wintermeyer & Rodnina, 2005), where for mammals, the two subunits are small 40S subunit and a large 60S subunit (Refseq, 2008). The eukaryotic ribosome is a complex structure made up of four rRNAs and about 80 ribosomal proteins (r-proteins) (Barakat et al., 2001). The mature ribosomes that competent to translate cellular mRNA are produced through series of steps which is highly coordinated (Ruggero & Pandolfi, 2003).

It is often recognized as the pioneer of molecular machinery that evolved in biological systems (Warner & Nierras, as cited in Bortoluzzi, d'Alessi et al., 2001). Barakat et al. (2001) stated that it is really an essential organelle in the cell mechanism, which responsible for protein synthesis and plays a pivotal role in controlling cell growth, division and development.
2.1.1 Ribosomal Protein (RP)

The eukaryotic ribosome is composed of four ribosomal RNA (rRNA) molecules and about 80 ribosomal proteins (RPs) genes (Wool, as cited in Hu & Li, 2007). It has been suggested that primordial ribosome comprises only rRNA and RPs adapted from a later evolutionary process in order to aid in protein synthesis (Wool et al., as cited in Naora, Takai, Adachi & Naora, 1998). There are two possibilities of the RP evolution, which are; 1) It may have been taken among a set of preexisting proteins that originally possessed and may have maintain other cellular functions, 2) It may have been designed for the ribosome and then employed for extraribosomal function (as cited in Naora et al., 1998). The ribosomal protein (RP) genes are believed to give a better view in understanding gene regulation and for assembling gene regulatory systems, since it is small sized and the high conservation of RP genes in the aspects of sequences, expression, and functions (Hu & Li, 2007).

As mentioned by Sim, et al. (2010), cellular protein biosynthesis requires the products of ribosomal protein (RP) genes. In addition, the study of this RP gene is related to human congenital disorders and cancers (Sim et al., 2010). Until now, about 80 RP have been discovered (Wang et al., 2010). Some of these proteins have been identified to be downregulated in tumors, suggesting that they are more pivotal to cell proliferation and/or transformation, which contradicting with the original function of the proteins in translation process (Kasai et al.; Choi & Chen, as cited in Wang et al., 2010). RPL41 is one of the examples of these proteins.
2.2 Ribosomal Protein L41 (RPL41)

Ribosomal protein L41 (RPL41) is one of RP genes with high specificity (Ishii et al., 2006). In human, the coding sequence (CDS) size of RPL41 is the shortest (78 bp) compared to other RP genes, which have the average size around 521 bp (Ishii et al., 2006). It is a small peptide of 3456 Dalton made up from 25 amino acids, where 17 of them are basic arginines and lysines (Odintsova et al., 2003). This gene is located on the chromosome 12q13, (Wang et al., 2010), which is illustrated in Figure 1. In eukaryotes, RPL41 is highly conserved, and it exists in several archaea, but not in eubacteria (Bult et al., as cited in Wang et al., 2010). However, in mature RPL41, the posttranslational modifications, including N-terminal loss of methionine, acetylation, internal methylation, or hydroxylation, has not been found (Odintsova et al., 2003). It is understood that RPL41 is the smallest and most fundamental protein in eukaryotes (Yu & Warner, as cited in Wang et al., 2010).

Figure 1: Location of gene RPL41 on chromosome (Adapted from GeneCards, 2011)
2.2.1 Structure and Function of RPL41

The genomic structure of the human RPL41 gene was investigated by Go, Miyado and Taniguchi (1998) with aid of Polymerase Chain Reaction (PCR) technique. From that, it is proven that subsequent sequencing revealed the structure of the gene, in which it constituted with 4 exons and 3 introns (Go et al., 1998). The structure of the RPL41 gene is shown in Figure 2. In addition, the amplified gene contained introns and open reading frame that conclude the fragment correspond to the functional gene (Go et al., 1998).

One of the role of this gene can be observed in the interaction with the beta subunit of protein kinase CKII and subsequently stimulate the DNA topoisomerase II-alpha to phosphorylates by CKII (Lee, Kim, Kim, Lee, Marshak, & Bae, 1997). In addition, Lee et al. (1997) also reported that RPL41 helps to enhance the autophosphorylation of CKII alpha. The data obtained from the study shows that RPL41 correlates with CKII and able to modulate its activity towards a specific substrate or substrates. This supports that CKII beta or CKII holoenzyme may participate in ribosome assembly or translational control (Lee et al., 1997). Besides that, RPL41 also essential as microtubule-associated protein for functional spindles and integrity of centrosome, as well as in stabilizing the microtubule (Wang et al., 2010).

Figure 2: Schematic representation of RPL41 (Go, Miyado and Taniguchi, 1998)
2.2.2 Behaviour and possible genetic lesions that have been identified in RPL41

The RPL41 from various parts of tissues, especially from human shows different types of behaviours. One of them was found in the research done for endometriosis which associated to the dysregulation of 14 genes (Hu, Tay, & Zhao, 2005). Among these 14 genes, RPL41 was proven to play a pivotal role in the establishment and evolution of endometriosis. Besides that, in certain human tissue, there are thirteen RP genes were found to be differentially expressed (Bortoluzzi, d’Alessi, Romualdi, & Danieli, 2001). Eight of them were detected highly expressed in the skeletal muscle, and RPL41 is one of them (Bortoluzzi et al., 2001).

Studies by Wang et al. (2010) showed that RPL41 depletion with gene-specific small interfering RNA causes the NIH3T3 cells to undergo malignant transformation, together with increased tumor growth in mice. Besides that, another type of genetic lesion in RPL41 that been detected by Wang et al. (2010), which was the RPL41 deletion in 59% of tumor cell lines by fluorescence in situ hybridization analyses and also RPL41 down-regulation in 75% of primary breast cancers by real-time quantitative reverse transcription-polymerase chain reaction. They concluded that all these are the evidences to support the tumor suppression role for RPL41. RPL41 also proven to play a important role in stabilizing the microtubule, as cells with RPL41 knock-down are observed to be abnormal spindles, frequent failure of cytokinesis, formation of polynuclear cells, and in interphase cells, these cells had premature splitting of centrosome (Wang et al., 2010).

A preliminary study was done by Sim et al. (2006), in order to examine the differential expression of the genes in tumours of Malaysian colorectal carcinoma. The study shows that 819 and 98 genes were found to be up-regulated and down-regulated respectively, in
tumours when compared to their normal cells. In 121 genes, the differential expression patterns were constant in all tumours. Among those genes, 33 are ribosomal proteins (RPs) genes, which include the RPL41 (Sim et al., 2006).

In hepatocellular carcinoma, the stable expression of RPL41 was been observed by Waxman and Wurmbach (as cited in Sim et al., 2010). However, this findings contradict with studies that previously done by others (Liu et al. & Yoo et al., as cited in Sim et al., 2010). Moreover, findings by Sim et al. (2010), suggest its deregulation in cancer cells, particularly in NPC-derived cell lines. However, the instability of RPL41 in cancer cells of nasopharyngeal carcinoma (NPC) remains to be explored (Sim et al., 2010).
2.3 Nasopharynx

The nasopharynx is a trapezoid chamber, which is situated posterior to the nasal choanae and extends inferiorly to the lower border of the soft palate (Anita, Brickman, Alwin, & Doerr, 2006). In the nasopharynx, more than 95% of nasopharyngeal malignancies occur in adults, while 20 to 35% of nasopharyngeal malignancies occur in children (Paulino, as cited in Anita et al., 2006). The anatomy of the nasopharynx is illustrated in the Figure 3.

![Anatomy of nasopharynx](http://www.painneck.com/nasopharynx)

Figure 3: Anatomy of nasopharynx

(Adapted from http://www.painneck.com/nasopharynx)

There are three types of epithelial cells found on the nasopharynx, which are pseudostratified ciliated columnar, stratified squamous, and transitional (Tock & Tan, 1969). According to UICC (1963) the nasopharynx is comprised of three walls, namely anterior, postero-superior, and lateral (Tock & Tan, 1969). The anterior wall of the nasopharynx is covered 60% by squamous epithelial cells, the rest are ciliated (Tock & Tan, 1969). In the lateral wall, nearly half of the area is lined by ciliated epithelium, which is scattered in irregular patches alternating with islets of squamous and transitional epithelium. While, the posterior wall is largely consists of squamous epithelium, with a little amount of the ciliated type (Tock & Tan, 1969).
2.3.1 Nasopharyngeal carcinoma (NPC)

Nasopharyngeal carcinoma (NPC) is a malignancy of the head and neck region that initiated from epithelial cells that wrap the surface and line of the nasopharynx (Cao et al., 2010). NPC can be initiated from any part if nasopharynx, however it is more frequently seen at the fossa of Rosenmuller, the recess located to the medial crura of the Eustachian tube (Bailey, Johnson, & Newlands, 2006). It was firstly reported in 1901, but only clinically tested in 1922 (Wei & Sham, as cited in Cao et al., 2010). The squamous cell carcinoma, lymphoma, salivary gland malignancy, and sarcoma are the types of malignancy that can exist in the nasopharynx (Weber, 2007). Cao et al. also reported that NPC is endemic in many geographical regions, including Southern China and Southeast Asia. The NPC is associated with multiple factors, such as living condition, genetics, viral infection, and environment (Wang et al., 2007). The main factor that causes NPC is by the consumption of salted fish, where it may associate to the carcinogenic compound, nitrosamine (Bailey, Johnson, & Newlands, 2006).

According to World Health Organization (WHO), NPC can be classified into three types (I, II, and III) and the organization is done according to the degree of differentiation (Shanmugaratnam & Sobin, as cited in Chang et al., 1989.). Type I is a keratinizing form, Type II is nonkeratinizing and poorly differentiated and Type III is nonkeratinizing and undifferentiated (Weber, 2007). As explained by Sham et al., type I is characterized by the presence of intracellular bridges and prominent keratin formation, type II comprises of squamous cell carcinoma with no keratin formation, and type III is varies in terms of morphology, frequently known as lymphoepithelioma, which consists of clumps of benign T cells intermixed within the tumor mass (as cited in Anita et al., 2006). The statistics showed that observed incidence rates range from 15 to 50 per 100,000 persons (Cao et al.,
2010). For instance, the nasopharyngeal carcinoma (NPC) has been investigated and well documented over past two decades, however the genetic basis for the tumorigenesis of this cancer is still poorly understood (Hasselt & Gibb, 1999).

In a study done by Burgos (2005), the results shows that EBV has the potential in the mechanism of pathogenesis in NPC Burgos (2005) reported that about 90% of infections worldwide are caused by Epstein-Barr virus (EBV). It is a prototype of gamma herpes virus According to Chan et al., Epstein Barr virus (EBV) may acts as an important infectious agent which consequently leads to the development to severe dysplasia of the nasopharynx (as cited in Sumitsawan & Srisukho, 2003). In all geographic regions, nonkeratinizing and keratinizing squamous carcinoma are usually associated (Pathmanathan, Prasad, Chandrika, Sadler, Flynn, & Raab-Traub, 1995). However, in regions with low cases of NPC, only a small proportion of keratinizing squamous carcinoma are found positive for EBV (Zhang et al.; Niedobitek et al., as cited in Perezordonez, 2007). Eventhough there is no evidence that showing the association of EBV and NPC, but EBV can play a pivotal role in the etiology of the NPC, especially in activations of oncogenes or the inactivation of tumor suppressor genes (Burgos, 2005).

The study using squamous epithelial type was done by retrieving from a Chinese male 17 1/2 years upon radiation therapy (Huang et al., 1980). To confirm the cell is squamous epithelial type, light and electron microscopes were used. Huang et al. reported that after transplant the cell into the back of athymic nude BALB/c (nu/nu) mice, development of tumor was observed at the sites of inoculation. This observation proves the similarities in morphology between the well-differentiated squamous carcinomas and recurrent human tumor from which they derived (Huang et al., 1980).
Another studies conducted by Glaser et al. (1989), involves two epithelial tumor cell lines were obtained from biopsy specimens of poorly differentiated squamous cell of the nasopharynx. The epithelial cell lines were designated as HONE-I and HNE-I and been passages for more than 90 and 100 times respectively. The results shows that EBV genome-positive HNE-1 and HONE-1 cells were lost as the cells were cultivated in vitro and by cloning the cells at the beginning passage level might be essential in maintaining EBV genome-positive epithelial NPC cells. Thus, these cells can provide useful information for studies on EBV and NPC (Glaser et al., 1989).
2.3.2 RPL41 Gene and Nasopharyngeal Carcinoma

Sim, Toh, & Tiong (2008) conducted a study to investigate on genetic factors that might be responsible in the correlation with tumourigenesis of nasopharyngeal carcinoma (NPC). Comparative gene analysis was carried out between normal and tumour nasopharyngeal biopsy samples, and the result obtained shows that two RP genes, which are RPS27 and RPS26, are downregulated in nasopharyngeal carcinoma (NPC) tumors (Sim, Toh, & Tiong, 2008). Recently, three RP genes, namely RPL27, RPL37a and RPL41 were significantly downregulated in all cell lines derived from NPC tissues when compared with nonmalignant nasopharyngeal epithelial cell lines (Sim et al., 2010). In their previous studies, there is no report on RPL37a and RPL41 genes in human nasopharyngeal tissue. With the current findings, the existence of NPC-associated RP genes is proven; subsequently show their roles in human nasopharyngeal organogenesis (Sim et al., 2010).