Bibliographic Analysis of Ribosomal Protein Genes for Small Ribosomal Subunit and Gene Expression Analysis of *RPs11* in Nasopharyngeal Carcinoma Cells

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours
(Resource Biotechnology)

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Programme of Resource Biotechnology
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2022

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ABSTRACT

The bibliography analysis considered many aspects including the author's research area and article content. Nasopharyngeal carcinoma (NPC) is the fourth most frequent cancer in Malaysia. Previously, there is research about overexpression of ribosomal protein S11 (*RPs11*) correlates with tumor recurrence in diverse malignancies. However, there is a lack of research on the bibliographic analysis of ribosomal protein genes for small ribosomal subunits in nasopharyngeal carcinoma cells and research on *RPs11* on NPC. Since the previous study found that high expression of *RPs11* in colorectal cancer. Thus, a study on the gene expression analysis of *RPs11* in nasopharyngeal carcinoma cells are carry out to understand the relationship between the *RPs11* and NPC. The objective of this study was to identify the most country scientific publication, corresponding author's country, and top 10 globally cited documents of ribosomal protein genes for small ribosomal subunit in NPC as well as to observe the *RPs11* expression between NPC cell lines EBV infected NP460 and C666-1. Scopus was used to obtain data, selected the better literature with the PRISMA checklist, and then analyse with RStudio. In gene expression, M-MLVRT converted RNA to cDNA, PCR was performed and was observed. Malaysia has the highest frequency of scientific production among the country, which was 19, and the country led with 7 manuscripts and a frequency of 0.5833, SCP was 2, MCP was 5, and MCP ratio was 0.714. A total of 103 citations were made by LIU SC's publications in 2013, equating to 10.300 citations per year and 1.573 normalized total citations. For gene expression result, there was no observable differential expression detected.

Key Words: Bibliographic Analysis, Nasopharyngeal Carcinoma (NPC), *RPs11*, Small Ribosomal Subunit, Gene Expression

ABSTRAK

Analisis bibliografi mempertimbangkan banyak aspek termasuk kawasan penyelidikan pengarang dan kandungan artikel. Karsinoma nasofaring (NPC) adalah kanser keempat paling kerap di Malaysia. Sebelum ini, terdapat penyelidikan mengenai ekspresi berlebihan protein ribosom S11 (RPs11) *berkorelasi dengan kekambuhan tumor dalam pelbagai keganasan. Walau bagaimanapun, terdapat kekurangan penyelidikan mengenai analisis bibliografi gen protein ribosom untuk subunit ribosom kecil dalam sel karsinoma nasofaring dan penyelidikan tentang* RPs11 *pada NPC. Sejak kajian terdahulu mendapati bahawa ekspresi tinggi* RPs11 *dalam kanser kolorektal. Oleh itu, kajian mengenai analisis ekspresi gen* RPs11 *dalam sel karsinoma nasofaring dijalankan untuk memahami hubungan antara* RPs11 *dan NPC. Objektif kajian ini adalah untuk mengenal pasti negara yang paling banyak mengeluarkan penerbitan saintifik, negara pengarang yang sepadan dan 10 dokumen teratas global yang dipetik gen protein ribosom untuk subunit ribosom kecil dalam NPC serta untuk memerhatikan ekspresi* RPs11 *antara NPC talian sel NP460 dan C666-1 dijangkiti EBV. Scopus digunakan untuk mendapatkan data, memilih literatur yang lebih baik dengan senarai semak PRISMA dan kemudian menganalisis dengan RStudio. Dalam ekspresi gen, M-MLVRT menukar RNA kepada cDNA, PCR dilakukan dan diperhatikan. Malaysia mempunyai kekerapan pengeluaran saintifik tertinggi antara negara, iaitu 19, dan negara mendahului dengan 7 manuskrip dan kekerapan 0.5833, SCP ialah 2, MCP ialah 5, dan nisbah MCP ialah 0.714. Sebanyak 103 petikan telah dibuat oleh penerbitan LIU SC pada tahun 2013, bersamaan dengan 10.300 petikan setahun dan 1.573 jumlah petikan ternormal. Untuk hasil ekspresi gen, tiada ekspresi pembezaan yang boleh diperhatikan.*

***Kata Kunci**: Analisis bibliografi, karsinoma nasofaring (NPC),* RPs11*, subunit ribosom kecil, ekspresi gen*

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LIST OF ABBREVIATIONS

AGE	Agarose Gel Electrophoresis
CRC	Colorectal Cancer
EBV	Epstein-Barr Virus
EtBr	Ethidium Bromide
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GBM	Glioblastoma
HCC	Hepatocellular Carcinoma
IHC	Immunohistochemistry
MCP	Multiple Country Publication
MMLV-RT	Moloney Murine Leukemia Virus Reverse Transcriptase
NCBI	National Centre for Biotechnology Information
NK	Natural Killer
NPC	Nasopharyngeal Carcinoma
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta Analysis

RPs11	Ribosomal Protein S11
rRNA	Ribosomal RNA
SCP	Simple Country Publication
TAE	Tris-acetate EDTA
tRNA	Transfer RNA
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

The bibliography analysis takes into account many factors, including the research areas of the author, the content of the article, and the citation network (Lim & Buntine, 2016). The Web of Science and Scopus are two platforms that have excellent analytical tools when doing bibliographic analysis. However, Web of Science offers highly effective cited reference searches, with the ability to trace a given author's work in order to analyse the scope of the academic network on a certain topic. Besides that, the h-index is used by Scopus to rank journals. Scopus includes several fantastic analysis and visualization capabilities (Henninger, 2012).

According to Kun, Liang, Guo, and Li (2014) found that the ribosomal protein S11 (*RPs11*) gene encodes a ribosomal protein of the family S17p, which is a part of the small ribosomal subunit 40S. It is mainly found in eukaryotes. A study has found overexpression of ribosomal protein S11 (*RPs11*) correlates with tumour recurrence in diverse malignancies (Zhou et al., 2020). Lai and Xu (2007) stated that the immunohistochemistry (IHC) studies of 18 colorectal cancer (CRC) and matched normal mucosa revealed that *RPs11* and *RPL7* were substantially expressed in colorectal cancer (CRC), particularly in immature mucosal cells situated in the crypt base, while they are barely detected in normal mucosa.

Nasopharyngeal carcinoma (NPC) is a kind of cancer that affects the epithelial cells that line the nasopharynx (Linton, Daker, Soo, Chung, Viljoen, & Neilsen, 2021). Nasopharyngeal carcinoma (NPC) is Malaysia's fourth most frequent cancer (Wong, Tan, Dompok, Mohamad Ishak, & Loong, 2021). Nasopharyngeal cancer (NPC) is quite common among Malaysian men (Linton et al., 2021). The global death toll for nasopharyngeal cancer

was 72987 in 2018, with 54,280 male deaths and 18,707 female deaths. The Sex Ratio is 2.90 (Kumar & Mydin, 2019).

Furthermore, since there is a lack of research on bibliographic analysis of ribosomal protein genes for small ribosomal subunit in nasopharyngeal carcinoma cells. Hence, this study is done in order to study bibliographic analysis of ribosomal protein genes for small ribosomal subunit in nasopharyngeal carcinoma cells. Yong et al. (2015) stated that patients with newly diagnosed primary glioblastoma (GBM) with overexpression of *RPs11* and *RPs20* show decreased survival at the transcriptional as well as the protein level. Hepatocellular carcinoma (HCC) patients with elevated *RPs11* levels may be at risk for poor outcomes because of pathways linked to cancer recurrence, metastasis, and drug resistance highlighted in the *RPs11*-high group (Zhou et al., 2020). However, the research on *RPs11* on nasopharyngeal carcinoma (NPC) is still limit. Thus, further study on the gene expression analysis of *RPs11* in nasopharyngeal carcinoma cells (EBV infected NP460 and C666-1) need to carry out to understand the relationship between the *RPs11* and nasopharyngeal carcinoma (NPC).

The objective of this study is:

1. To identify the country with the highest number of scientific publications on ribosomal protein genes for small ribosomal subunit in the field of nasopharyngeal carcinoma (NPC).
2. To investigate the corresponding author's country of origin that had published in topic related to ribosomal protein genes for small ribosomal subunit in nasopharyngeal carcinoma (NPC).
3. To analyses top 10 global cited documents of the ribosomal protein genes for small ribosomal subunit in nasopharyngeal carcinoma (NPC).

4. To observe the *RPs11* expression between nasopharyngeal carcinoma (NPC) cell lines EBV infected NP460 and C666-1.

The hypothesis of this study is:

1. In this research, the null hypothesis is there is no significant difference in the expression of *RPs11* in both EBV infected NP460 and C666-1 cell lines.

CHAPTER 2

LITERATURE REVIEW

2.1 Bibliographic analysis

There are many types of information to be considered when modeling bibliographic data. Metadata including authors, categories, time, and publishing data are typically attached to articles. It is also possible to view cited papers. To model the topic preferences of authors, we have to link topic information on documents with the authors (Lim & Buntine, 2016). Scopus is created by Elsevier in 2004 scientific bibliographic database that has been widely analyzed and utilized in scientometric investigations (Guerrero-Bote, Chinchilla-Rodríguez, Mendoza, & de Moya-Anegón, 2021). There are currently 18,500 titles available in Scopus, plus over 5,000 international publishers and 14.4 million records. Its scientific foundation is built on citations from its publications, such as book series, conference proceedings, and peer-reviewed journals. (Henninger, 2012). Web of Science was the first broad scope of international bibliographic databases (Pranckutė, 2021). There are nearly 9,300 most influential publications worldwide covered by the Web of Science, making it one of the largest citation databases (Andrés, 2009). Bibliographic databases frequently include bibliometric research questions with author affiliations that often do not include conventional institution names (Guerrero-Bote et al, 2021).

2.2 Small Ribosomal Subunit

Ribosomes are made up of two distinct subunits which are the 40S and 60S, both of which are essential for translation (Gregory et al., 2019). Pecoraro, Pagano, Russo, and Russo (2021) stated that the 40S subunit decodes mRNAs via aminoacyl–transfer RNA (tRNA) and is made up of the 33 cytosolic ribosomal proteins (CRPs) as well as 18S

ribosomal RNA (rRNA). The small (40S) subunit acts as a decoding site, connecting with the anticodon-containing ends of complementary tRNAs to transform mRNA codon information into its matching amino acid sequence (Kang et al, 2021).

2.2.1 Ribosome Protein subunit 11 (*RPs11*)

A human gene encoding 40S ribosomal protein S11 is known as *RPs11* (Feo, Davies, & Fried, 1992). The ribosomal protein S11 (*RPs11*) gene encodes a ribosomal protein of the family S17p, which is a part of the small ribosomal subunit 40S (Kun, Liang, Guo, & Li, 2014). The ribosomal protein S11 (*RPs11*) gene is co-transcribed with U35B, a small nucleolar RNA gene located in the third intron. RPs11 is tandem in both genomes and is spaced only 4.6 kb apart in the human gene. At 19q13.3, RPs11 occupies a 0.6 Mb region and is less than 5 kb from a single cosmid (Higa, Yoshihama, Tanaka, & Kenmochi, 1999). For its secondary structure in domains, *RPs11* features a conventional fold of α-helix packed against a β-sheet and a long N-terminal tail (Wang, Duo, Bai, & Liang, 2017).

2.3 Nasopharyngeal Carcinoma (NPC)

Nasopharyngeal carcinoma (NPC) is a tumour that develops from the epithelial cells that cover and line the nasopharynx (Brennan, 2006). The nasopharynx is an anatomical component of the upper airway system that connects the nasal cavities to the larynx and trachea via the oropharynx. In the pharynx, the nasopharynx is the uppermost part, with the skull base as a superior anatomical boundary and the soft palate as an inferior one (Jicman (Stan) et al., 2021). Nasopharyngeal carcinoma (NPC) is classified by the World Health Organization (WHO) into three different histological subtypes which are basaloid squamous cell carcinoma, non-keratinizing carcinoma (differentiated and undifferentiated) as well as keratinizing squamous cell carcinoma (Argirion et al., 2020). According to WHO classification, 73.58 percent of tumours were non-keratinizing undifferentiated carcinoma,

23.58 percent were non-keratinizing differentiated carcinoma, and 2.83 percent were keratinizing squamous cell carcinoma (Ding et al, 2021).

These are several factors that contribute to the development of nasopharyngeal carcinoma (NPC) are alcohol, nitrosamine-containing food consumption, and tobacco (Jicman (Stan) et al., 2021). Tobacco nitrosamines are active carcinogenic metabolites that induce DNA damage and persistent inflammation in the nasopharyngeal mucosa. Nasopharyngeal carcinoma (NPC) has also been associated with risk factors such as occupational fumes and dust exposure, and genetic variations (Yong et al., 2017). Trismus, otitis media, pain, nasal regurgitation owing to soft palate paresis, cranial nerve palsies, and hearing loss, are all symptoms of a primary tumour in nasopharyngeal carcinoma (NPC). Nasal blockage or bleeding as well as a "nasal twang" may be caused by larger growths of the tumour (Brennan, 2006). At all stages of the Nasopharyngeal carcinoma (NPC), radiotherapy is the major therapeutic method. Surgical therapy is used as a second resort for individuals who have remaining cervical lymphadenopathy following radiation therapy or who acquire cervical metastases after radiation therapy. Chemotherapy and radiation are used to treat individuals with late locoregional illnesses which are stages III and IV (Abdullah, Alias, & Hassan, 2009).

2.3.1 *RPs11* and Cancer

Kasai et al. (2003) found that there was high expression of *RPL7* and *RPs11* in colorectal cancer, particularly in the immature mucosal cell in the crypt base. However, these genes are difficult to identify in normal mucosa. The overexpression of *RPL7, RPs11, RPL15, RPs15a, RPL18, RPs19, RPL19, RPs27,* and *RPL31* in human colon cancer has been verified in several studies in comparison with normal colon tissues (Khoury & Nasr, 2021). Nadano, Aoki, Yoshinaka, Irie, and Sato (2001) stated that apoptotic MCF-7 cells and other apoptotic

breast carcinoma cells showed downregulation of *RPs11*. Apoptosis inhibition is linked to colorectal carcinogenesis (Tsujitani et al, 1996). *RPL7* and *RPs11* are significant in the suppression of apoptosis in colorectal cancer (Kasai et al., 2003).

2.4 EBV

The Epstein-Barr virus (EBV) is a Herpes family virus with double-stranded DNA (Tian et al, 2019). Epstein-Barr virus (EBV) is a common human virus that infects almost all individuals during their lifespan and lingers beyond the acute phase for the rest of the individual's life (Kerr, 2019). Epstein-Barr virus (EBV) will spread via kissing, coughing, and food sharing because this virus is usually present in the saliva. Infected people may have tiny amounts of Epstein-Barr virus (EBV) in their saliva for the rest of their lives (Fugl & Andersen, 2019). There are many types of cells that can be infected by Epstein-Barr virus (EBV), which are mesenchymal cells (like smooth muscle cells), epithelial cells, B, T, as well as natural killer (NK) lymphocytes. It is widely recognised that EBV infection is linked to tumorigenesis, which are lymphoepithelial carcinoma, NK/T and Burkitt lymphomas, and certain sarcomas (Tian et al, 2019).

Globally, Epstein-Barr virus (EBV) affects 90 to 95 percent of all adults and is responsible for approximately 1 percent of all cancers (Bakkalci et al., 2020). Nasopharyngeal carcinoma (NPC) is among the uncommon Epstein–Barr virus (EBV) positive cancers, regardless of the fact that the virus causes no severe symptoms in the majority of lifetime carriers (Liu, Zhang, & Pang, 2020). Molecular and serological methods can be combined to detect viruses early and accurately diagnose infections. In the case of Epstein-Barr virus (EBV) infection, serological testing is widely employed, with the detection of VCA-IgG antibodies being the best serological test to suggest past exposure. Molecular testing is a useful diagnostic technique, particularly in immunocompromised

individuals where serology results might be misleading and imprecise due to insufficient humoral response (Smatti et al., 2018).

2.5 NP460 Cell Line

NP460 is a newly immortalised nasopharyngeal epithelial (NP) cell line from primary non-malignant nasopharyngeal biopsies (Tsang, Takada, Seto, & Tsao, 2008). On the platforms of two-dimensional as well as three-dimensional, cell migratory behaviours of an immortalised nasopharyngeal epithelial cell line (NP460) and a nasopharyngeal cancer cell line (NPC43) were explored. The migration of NP460 and NPC43 cells through porous membranes and into trenches below was studied using time-lapse imaging. Before passing through the pores, NP460 and NPC43 cells moved around them, but NPC43 cells migrated slower and disseminated their lamellipodia less. NPC43 cells travelled quicker with an alternating elongated morphology (mesenchymal migration mode) and round morphology (amoeboid migration mode) after travelling to trenches below than NP460 cells with solely mesenchymal migration mode (Liu, Zhang, & Pang, 2020). According to To and Chan (2009), NP69 and NP460 are normal nasopharyngeal epithelial cells capable of surviving infection with SARS coronavirus (SARS-CoV).

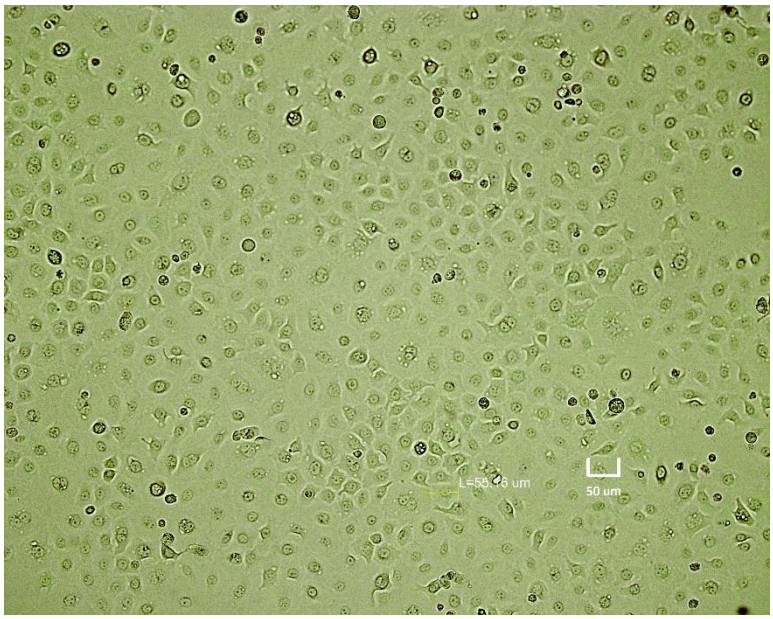

Figure 1: Images of the NP460 that infected by EBV from the microscope inventor.

2.6 C666-1 Cell Line

In long-term cultures, the Epstein-Barr virus (EBV) is constantly present in the C666-1 cell line. This type of cell line develops as an adherent culture with rare contact inhibition (Cheung et al., 1999). C666-1 was derived from nasopharyngeal carcinoma (NPC) xenograft (X666) that had been propagated for a long time (Lin et al., 2018). Tso et al. (2013) found that C666-1 includes a typical NPC-associated EBV genome and might be used to examine the roles or functions of viral proteins in nasopharyngeal carcinoma carcinogenesis. C666-1 induces tumours in athymic nude mice. Cytokeratin, an epithelial marker, is positively stained in cells continuously expressing EBV-encoded RNAs (Cheung et al., 1999).

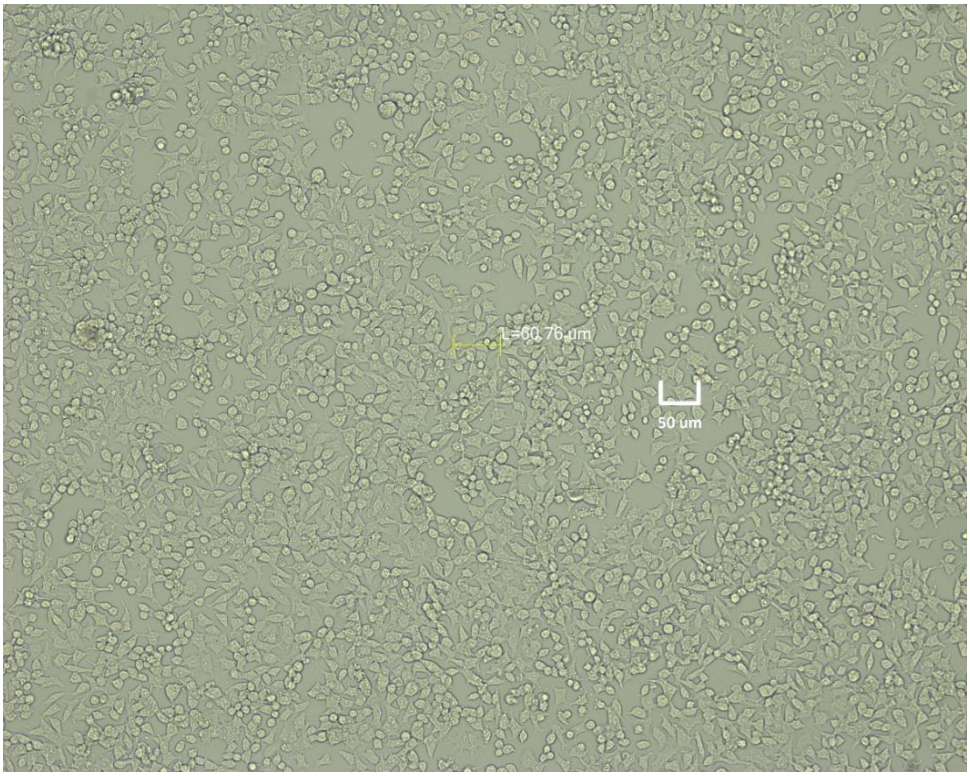

Figure 2: Images of the C666-1 that infected by EBV from the microscope inventor.

2.7 Housekeeping gene

Housekeeping genes are necessary for the preservation of baseline cellular activities that are important for the survival of a cell, independent of its specialised function in the tissue or organism (Eisenberg & Levanon, 2013). Housekeeping genes are also known as control genes which are widely used to normalise mRNA levels among different samples (Silver,

Best, Jiang, & Thein, 2006). In gene expression experiments and computational biology studies, housekeeping genes are widely used as internal controls because they are considered a minimum gene set for normal cellular physiology (Wei, Zhang, & Ma, 2018).

2.7.1 *GADPH*

Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is a significant housekeeping protein with around 2,000,000 molecules per cell as well as approximately 0.4μM concentrations. *GAPDH* triggers the glyceraldehyde-3-phosphate phosphorylation and oxidation to produce 1,3-biphosphoglycerate while employing NAD^+ as an electron acceptor, leading to the creation of NADH (Lazarev, Guzhova, & Margulis, 2020). The sixth enzyme in glycolysis is Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (Ramzan, Weber, Linne, & Vogt, 2013). *GAPDH* serves a variety of tasks aside from its role in energy metabolism. Cell proliferation and tumourigenesis are associated with *GAPDH* gene expression and enzymatic function, yet oxidative stress impairs *GAPDH* catalytic activity and causes cellular aging and apoptosis (Nicholls, Li, & Liu, 2011). Tarze et al. (2007) found that apoptosis and several chronic human diseases are associated with an overexpressed enzyme called glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

2.7.2 *β-actin*

Aside from *GADPH*, which was the most commonly utilised housekeeping gene, *β-actin* was also one of the housekeeping genes. Among the most widely expressed housekeeping genes, *β-actin* and *GAPDH*, a glycolysis enzyme, appear to be the two most consistent (Glare, Divijak, Bailey & Walters, 2002). *β-actin* is a protein that forms filaments in the cytoskeleton, it is one of the isoforms in the actin family (Zhang et al., 2019). *β-actin* is present in approximately all eukaryotic cells and is engaged in essential housekeeping function as cell shape formation as well as maintenance, signalling, cell division, and growth.

In addition to the role of transcriptional control, it is also involved in transport mRNA, remodels chromatin and processes mRNA (Khan et al., 2014). Sikand, Singh, Ebron and Shukla (2012) stated that numerous carcinomas have varying expression levels of β-actin and *GAPDH*. Tumor metastatic potential and invasiveness are positively correlated with *β-actin* expression.