

UNIVERSITI TEKNOLOGI MARA

**IDENTIFICATION AND ANALYSIS OF
NOVEL LUNG CANCER RNAs BY
USING *ALU*-PCR**

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ABSTRACT

Lung cancer is the leading cause of cancer-related death worldwide where tobacco smoking is considered as the main cause of the disease. However, the carcinogenesis of lung cancer is yet to be fully elucidated. Thus, our study proposed that *Alu* elements can be used to identify novel genomic alterations without the knowledge of the sequences. As the *Alu* elements are widely dispersed in human genome, they can be used as universal primer for the detection of genomic alteration in lung cancer. The aim of this study is to clone novel RNA transcripts, validate these genes by using real-time PCR and obtain the full length sequence of the selected gene. This study also aims to validate the expression of the selected gene by using MTT assay. Total of 4 fragments named as C11, C12, C13 and C20 which are 791 bp, 601 bp, 719 bp and 583 bp in length, respectively, were successfully isolated using *Alu*-PCR. BLAST search revealed no significant match to Ref_Seq mRNA database, indicating they are novel transcripts. The expression of C11, C12 and C20 was identified to be downregulated by 0.32, 0.022 and 0.611 fold, respectively in lung cancer. Meanwhile C13 was upregulated by 10.25 fold in lung cancer. Full-length amplification of C13 sequence resulted in 2176 bp with open reading frame of 302 amino acids. Based on the full length sequence of C13 transcript, it could be classified as a novel gene or non coding RNA. Meanwhile, *in-vitro* assessment showed that the C13 transcript act as an oncogenic. Therefore, this study concluded that the C13 transcript can be a potential biomarker for lung cancer.

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CHAPTER ONE

BACKGROUND OF THE STUDY

1.1 INTRODUCTION

Lung cancer remains as the most common cause of cancer-related death with nearly 1.6 million deaths worldwide in 2012 (Chan & Hughes, 2015). Meanwhile, in Malaysia, lung cancer had been reported as the commonest cancer in males (Loh et al., 2006). In 2003, 1758 lung cancer cases were reported in Peninsular Malaysia (Hashim, Omar, Abdullah, & Ismail, 2010). Further, National Cancer Registry 2007 reported that there were 1,865 cases of lung cancer cases that had been diagnosed and registered (Omar & Tamin, 2011). The number of lung cancer cases and consequent death from this disease is anticipated to increase over the next decade due to high rates of smoking (Siang & John, 2016).

Lung cancer can be divided into two histological subtypes which are small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC). NSCLC can be further subdivided into three histological subtypes which are squamous cell carcinoma, large cell carcinoma and adenocarcinoma (Bayzadeoglu, Ozyigit, & Ebruli, 2012).

To date, there is still no cure to the lung cancer disease and the disease usually is detected at the last stage. Available treatment for lung cancer is chemotherapy which has shown only 4% to 5% improvements in 5-year survival rates for stage I-III and prolongation only one month for patients with stage IV (D. H. Johnson, Schiller, & Jr, 2014). Most recent is the emergence of oncogene-directed targeted therapies which target specific molecular oncogene driver such as gefitinib, erlotinib and afatinib that target the epidermal growth factor receptor (EGFR); and crizotinib which targets anaplastic lymphoma kinase (ALK) (Chan & Hughes, 2015; Kim-Son H Nguyen, 2014). In future, there will be many more identification of specific molecular target that involved in lung cancer development which will lead to the development of many effective targeted therapies.

Transposable elements were discovered in 1940 which was led to an increase in knowledge of the distribution and regulation of transposable elements within the genome