UNIVERSITI TEKNOLOGI MARA

CLONING AND SEQUENCING OF microRNA-17 AND -18a GENES FROM HEPG2 CANCER CELL LINE

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ABSTRACT

miRNA is a small non-coding RNA region that is not translated to protein. This region usually involved in the regulation and expression the cell. Many studies were done on the miRNA and showed high correlation with certain cancer disease such as liver cancer. The aim of the study is to clone the miRNA -17 -18a of HepG2 cancer cell line in a vector. Initially, the region was amplified using pair of primer designed based on the sequences of the miRNA -17 -18a region fron GenBank. Optimum annealing temperature obtained from the PCR optimization was 51^0C. The PCR product was then ligated into the plasmid based on TA cloning method. The presence of insert and plasmid into the competent cell was confirmed using blue white screening method. Besides that, PCR cloning using M13 universal primer was done to further prove the presence of insert in the plasmid vector. Nevertheless, based on the result of sequencing, the inserted region was not the desired region as it did not aligned with the sequence of miRNA17 and 18a from NCBI. This might due to the unspecific binding of the primer pair designed earlier.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Cancer is a well known dreaded disease that cause agony and misery to human being. It gives big impact on patient's psychology and quality of life. Cancer is not a new disease as it was described from the ancient Chinese and Egyptian civilizations. the *Huang-ti Nei Ching*, or "Yellow Emperor's Book of Internal Medicine," is attributed from China to an era some 4,500 years ago.(Phoon, 2000). According to the oxford dictionary, cancer is a disease caused by an uncontrolled division of cell. This happen due to the mutation occurs at the gene E2Fs which control the proliferation of the cell(Helin, 1998). Based on the statistic made by Ministry of Health of Malaysia, up to 18, 219 cancer cases (appendix 2) were reported on year 2007 and cancer is the third common cause of death in government hospitals after cardiovascular and pulmonary disease and septicemia (appendix 3).

Generally there are three types of cell that are differs from their ability to proliferate which are stable, labile and permanent cells. Stable cells are the cells

that divided only when needed. Examples of stable cells are liver and kidney. Labile cells are the cell that continuously divided throughout life such as skin cells whereas permanent cells do not have ability to multiply such as neuron cells.(McConnell, 2007). The division of the cells undergo four stages which are G1 (presynthetic), S (synthetic), G2 (premetotic), and M (mitosis). In tumor, there is sometimes a shortening in cell cycle compared with normal cells or there is an increase in fraction of proliferating cell or a decrease in the rate of cell loss. These three factors determine aggressiveness of the tumor.(van den Heuvel, 2005).

Based on National Cancer Institute, there are three main treatments used to treat the cancer which are surgery, radiation therapy, and chemotherapy.(Prise *et al.*, 1994). Surgery is used to treat localized cancer tumor by removed it from the body. Whereas, the other two therapies is used to prevent the invasion of cancer cells to other parts of the body by destroy the mutated cells(Skeel *et al,* 2011). The mechanism of chemotherapy treatment involved the destruction on DNA materials of the cancer cells, prevent the synthesis of new DNA of the cancer cells and inhibit the mitosis of the cells. Nevertheless, the action of the therapy is not specific. It tends to reduce the growth rate of cancer cells and at the same time damaged the normal healthy cells.(Ophardt, 2003). The unspecific properties lead to numerous unwanted effects such as nausea, vomiting, severe fatigue and loss of hair. Hence these treatment leave a significant emotional distress on the patients and reduce their quality of life.(Love *et al*, 1989). Interestingly the new strategy by using microRNA (miRNA) or targeting endogenous miRNA would have