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DETECTION OF MET GENE COPY NUMBER IN A549 CELL LINE BY POLYMERASE CHAIN REACTION (PCR)

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ABSTRACT

MET gene copy number changes in lung cancer have been recognized as one of the contributors in the pathogenesis of lung cancer and lead to activation of MET. As that is the case, potential therapeutic therapy that specifically target MET gene for the treatment of lung cancer in the future can be developed. To determine the fold change of MET gene in cancer DNA versus normal human DNA, this study aims to find the relative changes in MET gene copy number using delta-delta Ct method in PCR reaction. The cancer DNA and normal human DNA was isolated each from cultured A549 cell line and normal human blood using the DNA isolation kit. Cancer DNA and normal human DNA were subjected to nucleic acid concentration measurement using a spectrophotometer. As described in a previous study done by Ishii et al., reverse and forward MET primers were designated. A housekeeping gene, RPLP0 was used as reference gene. The cancer DNA and normal human DNA was amplified using PCR. The amplification plot and melt curve report were analyzed and threshold cycle (Ct) was determined for each samples. The PCR data was normalized using RPLP0 gene. The fold change of MET gene copy number in cancer DNA relative to normal human DNA was calculated using delta-delta method by utilizing the Ct values of the amplification plot. The fold change of MET gene copy number was 0.08 fold. It was found that there are no differences in MET gene copy number in A549 cell line compared to normal human cell. This showed that MET gene copy number in A549 cell line did not undergo amplification where it commonly did in lung cancer in previous study. There are few possibilities behind this finding. Passage number of cell culture may alter how the cells behave after the passaging procedure. The cancer cells copy number may not amplify at passage 18 and has almost the same copy number with normal human DNA. Medium used for the cell culture also may exert effect on the mitosis of the cells that lead to reduce copy number of the cancer cell. The finding also suggest that MET role in the carcinogenesis in A549 is not significant. Further validation could enhance our understanding on the passage number and medium effects on cell lines growth and propagation in relation to carcinogenesis of lung cancer.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Cancer is a worldwide killer. It is a disease where cells undergo uncontrolled growth. Dysregulation of genomic activity leads to uncontrolled cell regulation and oncogenesis. One of the most cancer worldwide is lung cancer (Ravinder Singh & Kathiresan, 2014). It is uncontrolled growth of cells that reside in one or both of the lungs. Abnormal mass of tissue will be produced during the growth. The cells will turn to cancerous tissue and affect the normal function of lungs' tissues. Primary lung cancer indicates that the cancerous cell is originated in the lung while secondary lung cancer is cancer cells that spread to lungs after arising in other part of body such as breast. The most important risk factor is cigarette smoking. More than 80% of cases are associated to cigarette smoking (Ravinder Singh & Kathiresan, 2014). Other factors that contribute to lung cancer are radon gas exposure, air pollution and asbestos exposure.

Lung cancer has two main types; 1) non-small cell lung cancer (NSCLC) and 2) small cell lung cancer (SCLC). NSCLC is the most common type of lung cancer with high