MicroRNA16 Inhibit Lung Cancer Cell Proliferation

石文昭、蔡孟

E-mail: 9806537@mail.dyu.edu.tw

ABSTRACT

MicroRNAs (miRNAs) are small endogenous RNAs, approximately 18-24 nucleotides that can down-regulate various target gene products by translational repression or by directing mRNA degradation. miRNAs exhibit diverse biological functions in metabolism, differentiation, proliferation, and cell cycle. Recent studies have shown that aberrant expression of miRNAs in various human cancers, suggesting that they may play an important role in cancer development. In this study, we investigated and identified the miRNA candidates related with non-small cell lung carcinoma (NSCLC) by using homemade miRNA microarray and cell line model (normal human bronchial epithelial cell: BEAS-2B; lung cancer cell: CL1-0 and CL1-5). The results revealed that 13 out of 354 human miRNAs tested are more than 3-fold changed between BEAS-2B and lung cancer cells, such as miR16, miR23a, miR127, miR494, miR638, and so on. Furthermore, we detected the miRNA expression in different lung cancer cell lines by miRNA stem loop RT-PCR. We also found that the low expression level of miR16 in CL1-0, CL1-5, A549, CRL-5802, CRL-5806, H2981 and HTB54 lung cancer cells compared with that of BEAS-2B cells. To study the biological functions of miR16 in lung cancer cells, we constructed a miR16 over-expression system in CL1-5 cells and analyzed the cell proliferation. We demonstrated that over-expression of miR16 can inhibit lung cancer cell proliferation. Through 2-D electrophoresis analysis, we find out the proteins NYGGF4 and ALDOA will be reduced after miR16 over-expression. Finally, we also suggest that miR16 might regulate cell proliferation through down-regulated NYGGF4 and ALDOA gene expression.

Keywords : MicroRNA ; miR16 ; lung cancer ; invasion ; migration

Table of Contents

目錄 封面內頁 簽名頁 授權書

ABSTRACT

目錄 封面內頁 簽名頁 授權書

中文摘要

英文摘要

誌謝

目錄

目錄 頁目錄 表目錄

1. 前言

1.1 癌症

1.2 肺癌之分類與臨床症狀

1.3 致癌基因與腫瘤抑制基因

1.4 小片段RNA之起源

1.5 MicroRNA及其作用機制

1.6 MicroRNA與癌症之間的探討

2. 研究動機

3. 材料方法

3.1 細胞株

3.2 細胞培養

3.2.1 培養液的準備

3.2.2 細胞培養之生長條件

3.3 RNA之萃取

3.4 cDNA的製備

3.5 即時定量PCR

3.6 小量質體萃取

3.7 hsa-miR-16表現載體之構築

3.7.1 hsa-miR-16的片段取得

3.7.2 質體與miR-16片段之酵素切與黏合

3.7.3 限制酵素切處理後DNA之純化

3.7.4 大量生產表現載體

3.8 CL1-5細胞轉染miR-16表現載體

3.9 在CL1-5細胞中建立hsa-miR-16穩定表現系統

3.10 細胞生長速率測試

3.11 細胞群落形成分析

3.12 細胞基質侵襲力分析

3.13 傷口癒合分析

3.14 GIEMSA染色

3.15 二維電泳之蛋白質分析

4. 結果與討論

4.1 肺癌相關microRNA之篩選

4.2 在CL1-5肺腺癌細胞株中miR-16大量表現系統的建立與細胞功能之探討

4.3 miR-16目標基因之鑑定

5. 結論

參考文獻

附錄