

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.....	iii
中文摘要.....	iv
英文摘要.....	v
誌謝.....	vi
目錄.....	viii
圖目錄.....	x
表目錄.....	xii
1. 前言.....	1
1.1 癌症.....	1
1.2 肺癌之分類與臨床症狀.....	2
1.3 致癌基因與腫瘤抑制基因.....	3
1.4 小片段RNA之起源.....	4
1.5 MicroRNA及其作用機制.....	6
1.6 MicroRNA與癌症之間的探討.....	7
2. 研究動機.....	10
3. 材料方法.....	11
3.1 細胞株.....	11
3.2 細胞培養.....	11
3.2.1 培養液的準備.....	11
3.2.2 細胞培養之生長條件.....	12
3.3 RNA之萃取.....	12
3.4 cDNA的製備.....	13
3.5 即時定量PCR.....	14
3.6 小量質體萃取.....	14
3.7 hsa-miR-16的片段取得.....	15
3.7.2 質體與miR-16片段之酵素切與黏合.....	16
3.7.3 限制酵素切處理後DNA之純化.....	18
3.7.4 大量生產表現載體.....	19
3.8 CL1-5細胞轉染miR-16表現載體.....	19
3.9 在CL1-5細胞中建立hsa-miR-16穩定表現系統.....	20
3.10 細胞生長速率測試.....	21
3.11 細胞群落形成分析.....	21
3.12 細胞基質侵襲力分析.....	22
3.13 傷口癒合分析.....	22
3.14 GEMSA染色.....	23
3.15 二維電泳之蛋白質分析.....	23
3.15.1 蛋白質樣品的備製.....	23
3.15.2 等電點電泳分離.....	24
3.15.3 聚丙烯胺膠體分析.....	24
3.15.4 銀染.....	25
4. 結果與討論.....	26
4.1 肺癌相關microRNA之篩選.....	28
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.....	30
4.3 miR-16目標基因之鑑定.....	30
5. 結論.....	35
參考文獻.....	38
附錄.....	57
	61

REFERENCES

1. 衛生署, 97年死因統計結果分析。
2. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297.
3. Boffetta P (2006) Human cancer from environmental pollutants: the epidemiological evidence. *Mutat Res* 608: 157-162.
4. Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, et al. (2005) miR-15a and miR-16-1 down-regulation in pituitary adenomas. *J Cell Physiol* 204: 280-285.
5. Bushati N, Cohen SM (2008) MicroRNAs in neurodegeneration. *Curr Opin Neurobiol* 18: 292-296.
6. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. *Nat Rev Cancer* 6: 857-866.
7. Carthew RW, Sontheimer EJ (2009) Origins and Mechanisms of miRNAs and siRNAs. *Cell* 136: 642-655.
8. Chan JA, Krichevsky AM, Kosik KS (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 65: 6029-6033.
9. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, et al. (2005) Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res* 33: e179.
10. Chu CY, Rana TM (2007) Small RNAs: regulators and guardians of the genome. *J Cell Physiol* 213: 412-419.
11. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, et al. (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* 102: 13944-13949.
12. Collins LG, Haines C, Perkel R, Enck RE (2007) Lung cancer: diagnosis and management. *Am Fam Physician* 75: 56-63.
13. Deng CX (2009) SIRT1, is it a tumor promoter or tumor suppressor? *Int J Biol Sci* 5: 147-152.
14. Doench JG, Sharp PA (2004) Specificity of microRNA target selection in translational repression. *Genes Dev* 18: 504-511.
15. Du J, Yang S, An D, Hu F, Yuan W, et al. (2009) BMP-6 inhibits microRNA-21 expression in breast cancer through repressing deltaEF1 and AP-1. *Cell Res* 19: 487-496.
16. Eulalio A, Huntzinger E, Izaurralde E (2008) Getting to the root of miRNA-mediated gene silencing. *Cell* 132: 9-14.
17. Fan D, Ma C, Zhang H (2009) The molecular mechanisms that underlie the tumor suppressor function of LKB1. *Acta Biochim Biophys Sin (Shanghai)* 41: 97-107.
18. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, et al. (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391: 806-811.
19. Gillies JK, Lorimer IA (2007) Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. *Cell Cycle* 6: 2005-2009.
20. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2008) miRBase: tools for microRNA genomics. *Nucleic Acids Res* 36: D154-158.
21. Hamaguchi T, Iizuka N, Tsunedomi R, Hamamoto Y, Miyamoto T, et al. (2008) Glycolysis module activated by hypoxia-inducible factor 1alpha is related to the aggressive phenotype of hepatocellular carcinoma. *Int J Oncol* 33: 725-731.
22. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, et al. (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 65: 9628-9632.
23. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2008) Cancer statistics, 2008. *CA Cancer J Clin* 58: 71-96.
24. Jemal A, Thomas A, Murray T, Thun M (2002) Cancer statistics, 2002. *CA Cancer J Clin* 52: 23-47.
25. Kefas B, Godlewski J, Comeau L, Li Y, Abounader R, et al. (2008) microRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. *Cancer Res* 68: 3566-3572.
26. Ko YC, Huang YL, Lee CH, Chen MJ, Lin LM, et al. (1995) Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. *J Oral Pathol Med* 24: 450-453.
27. Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75: 843-854.
28. Lee S, Gang J, Jeon SB, Choo SH, Lee B, et al. (2007) Molecular cloning and functional analysis of a novel oncogene, cancer-upregulated gene 2 (CUG2). *Biochem Biophys Res Commun* 360: 633-639.
29. Lu M, Zhang Q, Deng M, Miao J, Guo Y, et al. (2008) An analysis of human microRNA and disease associations. *PLoS One* 3: e3420.
30. Mountain CF, Lukeman JM, Hammar SP, Chamberlain DW, Coulson WF, et al. (1987) Lung cancer classification: the relationship of disease extent and cell type to survival in a clinical trials population. *J Surg Oncol* 35: 147-156.
31. Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA (2009) MicroRNAs--the micro steering wheel of tumour metastases. *Nat Rev Cancer* 9: 293-302.
32. Novakova J, Slaby O, Vyzula R, Michalek J (2009) MicroRNA involvement in glioblastoma pathogenesis. *Biochem Biophys Res Commun*.
33. Parkin DM, Bray FI, Devesa SS (2001) Cancer burden in the year 2000. The global picture. *Eur J Cancer* 37 Suppl 8: S4-66.
34. Rintala-Maki ND, Goard CA, Langdon CE, Wall VE, Traulsen KE, et al. (2007) Expression of RBM5-related factors in primary breast tissue. *J Cell Biochem* 100: 1440-1458.
35. Rosti G, Bevilacqua G, Bidoli P, Portalone L, Santo A, et al. (2006) Small cell lung cancer. *Ann Oncol* 17 Suppl 2: ii5-10.
36. Selaru FM, Oлару AV, Kan T, David S, Cheng Y, et al. (2009) MicroRNA-21 is overexpressed in human cholangiocarcinoma and regulates programmed cell death 4 and tissue inhibitor of metalloproteinase 3. *Hepatology* 49: 1595-1601.
37. Sleeman J, Schmid A, Thiele W (2009) Tumor lymphatics. *Semin Cancer Biol*.
38. Travis WD, Travis LB, Devesa SS (1995) Lung cancer. *Cancer* 75: 191-202.
39. Urbich C, Kuehnbacher A, Dimmeler S (2008) Role of microRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc Res* 79: 581-588.
40. Venturini L, Battmer K, Castoldi M, Schultheis B, Hochhaus A, et al. (2007) Expression of the miR-17-92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. *Blood* 109: 4399-4405.
41. Wang B, Zhang M, Ni YH, Liu F, Fan HQ, et al. (2006) Identification and characterization of NYGGF4, a novel gene containing a phosphotyrosine-binding (PTB) domain that stimulates 3T3-L1 preadipocytes proliferation. *Gene* 379: 132-140.