

MIR-142-5p and miR-9 may be involved in squamous lung cancer by regulating cell cycle related genes

Y.-H. SU¹, Z. ZHOU², K.-P. YANG¹, X.-G. WANG¹, Y. ZHU³, X.-E. FA⁴

¹Department of Thoracic Surgery, ²Department of Respiratory Medicine, ³Department of Orthopedics, ⁴Department of Cardiovascular Surgery; the Second Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Abstract. – OBJECTIVE: To identify key genes associated with squamous lung cancer (SLC) through analyzing gene expression data with bioinformatic tools, which could be potential biomarkers for diagnosis and treatment.

MATERIALS AND METHODS: Gene expression data set GSE3268 was downloaded from Gene Expression Omnibus, including 5 SLC samples and 5 healthy controls. Data pre-treatment and differential analysis were performed with packages of R. Cluster analysis was done based on gene expression values to globally present the difference between the two states. Differentially expressed genes (DEGs) were divided into up-regulated and down-regulated genes, and then underwent functional enrichment analysis with DAVID tools. WebGestalt was used to retrieve microRNAs for the DEGs and then a regulatory network was constructed. GENECODIS was selected for functional annotation for all the genes in the network.

RESULTS: A total of 537 DEGs were obtained. Functional enrichment analysis revealed that cell cycle was significantly enriched in up-regulated genes. Besides, two microRNAs (miRNAs), MIR-142-5p and miR-9, were retrieved, which were potential tools to regulate the expression of key genes.

CONCLUSION: These DEGs may be involved in pathogenesis of SLC and some of them could be potential biomarkers. Besides, MIR-142-5p and miR-9 may be utilized to treat SLC as they could modulate cell cycle.

Key Words:

Squamous lung cancer, Differentially expressed gene, Function enrichment analysis, MicroRNA, Regulatory network.

based Gene Set Analysis Toolkit; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; NEK2 = NIMA-related kinase 2; FOXM1 = Forkhead box M1; PTTG1 = pituitary tumor-transforming 1; SMAD7 = SMAD family member 7; FOXF1 = Forkhead box F1.

Introduction

Squamous lung cancer (SLC) is a common form of lung cancer, which is the main cause of cancer-related death in the world. Accounting for 25%-30% of all lung cancers, SLC can spread to bones, adrenal glands, the liver, small intestine, or brain that introduce great threat to human life. SLC patients with clinical stage IA disease have a 5-year survival rate of about 60%, while the 5-year survival rate for clinical stage II to IV disease ranges from 40% to less than 5%¹. The poor outcome of SLC patients could be explained, in part, by the difficulty of early diagnosis and the poor prognosis.

Understanding the pathogenesis of SLC can improve the current diagnosis, treatment and prognosis of SLC. Both genetic and environmental factors contribute to the development of SLC. It is widely recognized that tobacco smoking is the main risk factor for lung cancer, especially for SLC and SCLC (small cell lung cancer)². Now there is considerable information concerning molecular abnormalities involved in the pathogenesis of SLC. Focal fibroblast growth factor receptor 1 (FGFR1) amplification which was associated with tumor growth and survival was identified in SLC³. EGFR mutations in lung cancer are associated with clinical response to gefitinib therapy⁴. Besides, roles of ubiquitin and ubiquitin-like proteins⁵ as well as cytokines⁶ in development of lung cancer have also been indicated. Although many molecules have been identified, it is still need to explore new genes related to SLC.

MicroRNAs (miRNAs) are approximately 22-nucleotide non-coding RNAs that are known to

Abbreviations

SLC = squamous lung cancer; DEGs = differentially expressed genes; EGFR = epidermal growth factor receptor; miRNAs = microRNAs; GEO = Gene Expression Omnibus; BH = Benjamini and Hochberg; FDR = False discovery rate; DAVID = Database for Annotation, Visualization and Integrated Discovery; WebGestalt = Web

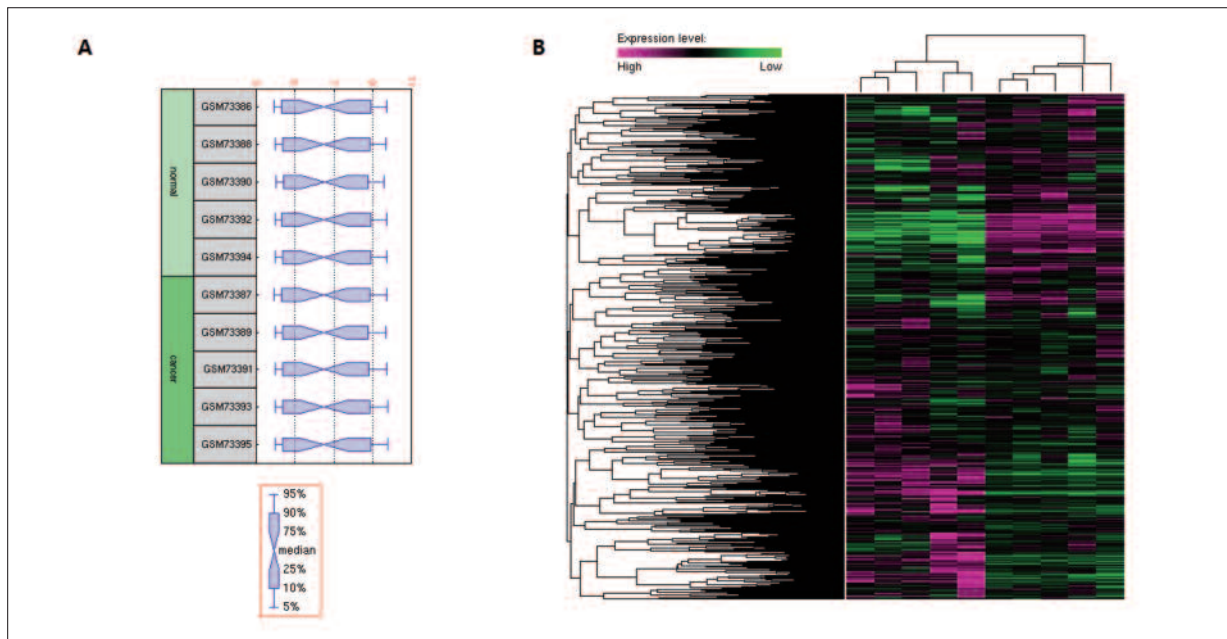


Figure 1. Box plot for normalized gene expression data and cluster analysis result. **A**, Box plot for gene expression data. The medians are almost at the same level, indicating a high performance of normalization. **B**, Cluster analysis result for gene expression data. The expression values Clustering Fig. Red indicates over-expression while green for under-expression.

regulate gene expression by targeting mRNAs for cleavage or translational repression⁷. It has been estimated that miRNAs can control the activity of about 30% of human genes⁸. Through this function they participate in many physiological processes including stem cells development, cell differentiation, regulation of cell cycle, apoptosis and transformation⁹. Thus, deregulation of miRNAs can induce a variety of human diseases. Indeed, the abnormal expression of miRNAs is closely related with development of cancers^{10,11}, so they can be good makers for diagnosis, treatment or prognosis for cancers¹². Therefore, miRNAs associated with potential key genes in SLC were also investigated in present study.

Microarray technology is a powerful tool to explore the global changes in the incidence and development of cancer¹³. Therefore, gene expression profiles for SLC samples were compared with those for healthy controls to identify differentially expressed genes (DEGs). Combined with functional enrichment analysis and regulatory network analysis, our study would not only provide insights into the pathogenesis of SLC, but also discover potential biomarkers that may provide contribution to diagnosis, treatment or prognosis of SLC.

Materials and Methods

Microarray Data

Microarray data set GSE3268¹⁴ was downloaded from Gene Expression Omnibus (GEO), including 5 SLC samples and 5 corresponding healthy controls. Each pair of samples represent a single patient with squamous lung cancer. One is derived from the cancer cells, and the other is from the normal cells. There are five patients, with two arrays for each patient. The annotation information of chip was downloaded from GPL96 [HG-U133A] Affymetrix Human Genome U133A Array.

Screening of Differentially Expressed Genes (DEGs)

Raw data was converted into recognizable format and missing values were imputed¹⁵. After data normalization¹⁶, multtest package¹⁷ in R was chosen for differential analysis. Multiple testing correction was applied with Benjamini-Hochberg method¹⁸. FDR < 0.05 and $|\log_{2}FC| > 1$ were set as the cut-offs to screen out DEGs.

Cluster Analysis

To globally present the difference in gene expression pattern between SLC and healthy control, cluster analysis was conducted for all the samples¹⁹.

Figure 2. Functional enrichment analysis results for up-regulated genes (top) and down-regulated genes (bottom).

Table I. Relevant miRNAs for up- (A) and down-regulated genes.

microRNA	Parameter
A	
hsa_ACTGCCT, miR-34B	O = 7; rawP = 4.30e-05; adjP = 0.0007
hsa_TTTGCAG, miR-518A-2	O = 6; rawP = 0.0003; adjP = 0.0021
hsa_ACTTTAT, miR-142-5p	O = 6; rawP = 0.0015; adjP = 0.0048
hsa_TAGCTTT, miR-9	O = 5; rawP = 0.0035; adjP = 0.0070
B	
hsa_ACTTTAT, miR-142-5p	O = 14; rawP = 9.39e-08; adjP = 9.70e-07
hsa_ACCAAAG, miR-9	O = 11; rawP = 0.0022; adjP = 0.0023

O: number of genes in the pathways; adjP: *p* values after multiple testing correction.

Deregulation of cell cycle and blood vessel development was closely associated with tumorigenesis, indicating potential roles of these DEGs in SLC.

Relevant miRNAs and Regulatory Networks

Four miRNAs were retrieved for up-regulated genes and 2 ones for down-regulated genes (Table I). In these miRNAs, miR-142-5p and miR-9 were found to interact with both up- and down-regulated genes, and thus corresponding regulatory networks were constructed for the two miRNAs to further look into their biological functions (Figure 3).

Gene Functional Annotation Result

Eight functional terms were significantly over-represented for miR-142-5p regulatory network

and 5 for miR-9 regulatory network (Table II). Positive regulation of cellular process and cell differentiation were the most significant term in each network.

Discussion

In recent years, with progress in researches of gene function and signal transduction, molecular targeted therapy becomes available and provides a new way for the treatment of tumors²⁵. In present study, by comparison of gene expression data between SLC and healthy control a range of DEGs were screened out, which not only offered insights into the molecular mechanisms of SLC, but also provided potential biomarkers for this tumor. Ac-

Table II. Functional annotation results for genes in the networks.

GO-ID	corr <i>p</i> -value	x	Description
(A) miR-142-5p			
48522	0.0009995	12	positive regulation of cellular process
48518	0.0009995	12	positive regulation of biological process
48731	0.0017834	12	system development
23033	0.002004	11	signaling pathway
48856	0.0023442	12	anatomical structure development
7275	0.0047838	12	multicellular organismal development
32501	0.0061241	14	multicellular organismal process
32502	0.0062943	12	developmental process
(B) miR-9			
65008	0.039728	6	regulation of biological quality
30154	0.046665	6	cell differentiation
48869	0.046665	6	cellular developmental process
48731	0.035755	8	system development
48856	0.037195	8	anatomical structure development
7275	0.046665	8	multicellular organismal development

x: the total number of genes in each GO category.

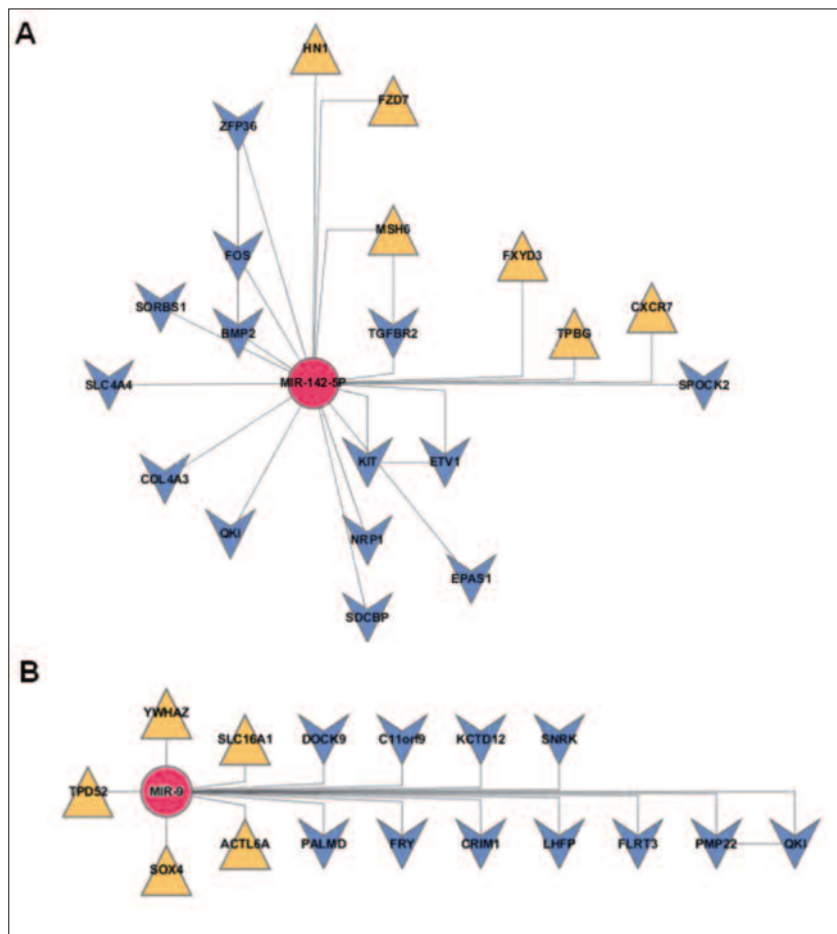


Figure 3. The regulatory networks for miR-142-5p (**A**) and miR-9 (**B**). Yellow triangles represent up-regulated genes and blue inverted triangles for down-regulated genes.

cording to the functional enrichment analysis result, cell cycle and other functions were significantly over-represented. Considering the close relationship between cell cycle and cancers²⁶, it is possible that these DEGs may be involved in cancers in a certain degree. In fact, many DEGs have been reported to take parts in pathogenesis of cancers. NIMA-related kinase 2 (NEK2) is a serine/threonine-protein kinase that is involved in mitotic regulation. Hayward et al²⁷ indicated that it is up-regulated in breast cancer and may contribute to chromosome instability²⁸. Forkhead box M1 (FOX M1) is a transcriptional activator involved in cell proliferation and it regulates the expression of several cell cycle genes, such as cyclin B1 and cyclin D1. It has been found to be involved in recurrence of non-small cell lung cancer with predictive potential²⁹. It promotes tumor metastasis³⁰ and thus inhibition of FOXM1 can suppress cell proliferation and tumor growth³¹. Pituitary tumor-transforming 1 (PTTG1), highly expressed in various tumors^{32,33}, has transforming activity *in vitro* and tumorigenic activity *in vivo*. Huang et

al³⁴ reported that knockdown of PTTG1 with RNAi would suppress the proliferation and invasive potential of PC3 human prostate cancer cells. SMAD family member 7 (SMAD7) presents a protective role in colitis-associated cancer³⁵ and low-level expression of Smad7 correlates with lymph node metastasis and poor prognosis in patients with pancreatic cancer³⁶. Forkhead box F1 (FOXF1) is a potential tumor suppressor gene that epigenetically silenced in breast cancer³⁷. Taken together, all above genes which are DEGs between SLC samples and controls have been identified to participate in the development of different cancers, so we deduced these DEGs may serve as potential biomarkers of SLC. However, more studies are needed to validate our result.

In addition, many investigations have indicated that miRNAs play important roles in development of cancers, such as miRNA-296, which can inhibit the growth of esophageal cancer cells via regulating cyclinD1 and p27³⁸. Takeshita et al³⁹ report that miRNA-16 can suppress the

growth of metastatic prostate tumors via down-regulation of multiple cell cycle genes. We retrieved relevant miRNAs for DEGs, among which miR-142-5p and miR-9 showed more potential relationship with SLC as they regulated both up- and down-regulated genes. Functional annotation further revealed their involvement in regulation of cellular process and cell differentiation. Many cellular processes, such as cell growth², division⁴⁰, differentiation⁴¹ and signal transduction⁴², are all involved in development of cancers.

In addition, many studies have reported the relationship of miR-142-5p or miR-9 with cancers. Zhang et al⁴³ propose that hsa-miR-142-5p combined with hsa-miR-375 can act as a predictor for recurrence risk in gastric cancer patients following surgical resection. Liu et al^{44,45} have reported that miR-142-5p is repressed in transgenic lung cancers and overexpression of this miRNA would significantly inhibit lung cancer cell growth. These works suggest that miR-142-5p may be a potential target to treat SLC. When referred to miR-9, it can be activated by MYC/MYCN and regulates E-cadherin as well as cancer metastasis⁴⁶ and thus regulates the development of cancers. A study have reported that it can be used to differentiate primary from metastatic brain tumors⁴⁷. Besides, it's also regarded as a potential biomarker for recurrent ovarian cancer⁴⁸. Hildebrandt et al⁴⁹ further point out that its methylation status is associated with cancer development and metastatic recurrence. The important roles of these two miRNAs in development of different cancers suggest that they may take a place in regulating the pathogenesis of SLC and also suggest the key positions of corresponding DEGs in the whole regulatory mechanisms.

Conclusions

Overall, a range of DEGs were obtained through comparing gene expression profiles of SLC with those of healthy controls. These genes may play important roles in the pathogenesis of SLC according to the functional enrichment analysis. Besides, miR-142-5p and miR-9 that regulated both up- and down-regulated genes were identified and they may act as potential biomarkers for SLC. Of course, more researches are needed to demonstrate this result and develop their potentials in clinical applications.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

Reference

- 1) TANQUE LT, DETTERBECK FC. New TNM classification for non-small-cell lung cancer. *Expert Rev Anti-cancer Ther* 2009; 9: 413-423.
- 2) TOKUMO M, TOYOOKA S, KIURA K, SHIGEMATSU H, TOMII K, AOE M, ICHIMURA K, TSUDA T, YANO M, TSUKUDA K, TABATA M, UEOKA H, TANIMOTO M, DATE H, GAZDAR AF, SHIMIZU N. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. *Clin Cancer Res* 2005; 11: 1167-1173.
- 3) WEISS J, SOS ML, SEIDEL D, PEIFER M, ZANDER T, HEUCKMANN JM, ULLRICH RT, MENON R, MAIER S, SOLTERMANN A, MOCH H, WAGENER P, FISCHER F, HEYNCK S, KOKER M, SCHÖTTE J, LEENDERS F, GABLER F, DABOW I, QUERINGS S, HEUKAMP LC, BALKE-WANT H, ANSEN S, RAUH D, BAESSMANN I, ALTMÜLLER J, WAINER Z, CONRON M, WRIGHT G, RUSSELL P, SOLOMON B, BRAMBILLA E, BRAMBILLA C, LORIMIER P, SOLLBERG S, BRUSTUGUN OT, ENGEL-RIEDEL W, LUDWIG C, PETERSEN I, SÄNGER J, CLEMENT J, GROEN H, TIMENS W, SIETSMA H, THUNNISSEN E, SMIT E, HEIDEMAN D, CAPPUZZO F, LIGORIO C, DAMIANI S, HALLEK M, BEROUKHIM R, PAO W, KLEBL B, BAUMANN M, BUETTNER R, ERNESTUS K, STOELBEN E, WOLF J, NÜRNBERG P, PERNER S, THOMAS RK. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med* 2010; 2: 62ra93-62ra93.
- 4) PAEZ JG, JANNE PA, LEE JC, TRACY S, GREULICH H, GABRIEL S, HERMAN P, KAYE FJ, LINDEMAN N, BOGGON TJ, NAKI K, SASAKI H, FUJI Y, ECK MJ, SELLERS WR, JOHNSON BE, MEYERSON M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304: 1497-1500.
- 5) HOELLER D, HECKER C-M, DIKIC I. Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat Rev Cancer* 2006; 6: 776-788.
- 6) DRANOFF G. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer* 2004; 4: 11-22.
- 7) BARTEL DP. Micronas: target recognition and regulatory functions. *Cell* 2009; 136: 215-233.
- 8) BARTEL DP. Micronas: genomics, biogenesis, mechanism, and function. *Cell* 2004; 116: 281-297.
- 9) BEREZIKOV E, GURYEV V, VAN DE BELT J, WIENHOLDS E, PLASTERK RH, CUPPEN E. Phylogenetic shadowing and computational identification of human microRNA genes. *Cell* 2005; 120:21-24
- 10) YANAIHARA N, CAPLEN N, BOWMAN E, SEIKE M, KUMAMOTO K, YI M, STEPHENS RM, OKAMOTO A, YOKOTA J, TANAKA T, CALIN GA, LIU CG, CROCE CM, HARRIS CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006; 9: 189-198.
- 11) ESQUELA-KERSCHER A, SLACK FJ. Oncomirs—microRNAs with a role in cancer. *Nat Rev Cancer* 2006; 6: 259-269.

- 12) PUYOL M, MARTÍN A, DUBUS P, MULERO F, PIZCUETA P, KHAN G, GUERRA C, SANTAMARÍA D, BARBACID M. A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. *Cancer Cell* 2010; 18: 63-73.
- 13) DEIRISI J, PENLAND L, BROWN PO, BITTNER ML, MELTZER PS, RAY M, CHEN Y, SU YA, TRENT JM. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* 1996; 14: 457-460.
- 14) WACHI S, YONEDA K, WU R. Interactome-transcriptome analysis reveals the high centrality of genes differentially expressed in lung cancer tissues. *Bioinformatics* 2005; 21:4205-4208
- 15) TROYANSKAYA O, CANTOR M, SHERLOCK G, BROWN P, HASTIE T, TIBSHIRANI R, BOTSTEIN D, ALTMAN RB. Missing value estimation methods for DNA microarrays. *Bioinformatics* 2001; 17: 520-525.
- 16) FUJITA A, SATO JR, RODRIGUES LDE O, FERREIRA CE, SOGAYAR MC. Evaluating different methods of microarray data normalization. *BMC Bioinformatics* 2006; 7: 469.
- 17) POLLARD KS, DUDOIT S, VAN DER LAAN MJ. Multiple testing procedures: R multtest package and applications to genomics. In: *Bioinformatics and Computational Biology Solutio???* Using R and Bioconductor. Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S, Editors. Springer Statistics for Biology and Health Series, 2005; 251-272.
- 18) BENJAMINI Y, HOCHBERG Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Statist Society. Series B (Methodological)* 1995: 289-300.
- 19) EISEN MB, SPELLMAN PT, BROWN PO, BOTSTEIN D. Cluster analysis and display of genome-wide expression patterns. *Proc Nat Acad Sci* 1998; 95: 14863-14868.
- 20) NAM D, KIM S-Y. Gene-set approach for expression pattern analysis. *Briefings Bioinform* 2008; 9: 189-197.
- 21) DA WEI HUANG BTS, LEMPICKI RA. Systematic and integrative analysis of large gene lists using david bioinformatics resources. *Nat Prot* 2008; 4: 44-57.
- 22) ZHANG B, KIROV S, SNODDY J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Res* 2005; 33: W741-W748.
- 23) DUNCAN D, PRODDUTURI N, ZHANG B. WebGestalt2: an updated and expanded version of the web-based gene set analysis toolkit. *BMC Bioinformatics* 2010; 11: P10.
- 24) LODISH HB, MATSUDAIRA A, KAISER P, KRIEGER C, SCOTT M, ZIPURSKY M. SL & DARNELL, J. (2004). *Mol Cell Biol* 5th ed. WH Freeman: New York, NY.
- 25) BLAGOSKLONNY MV, PARDEE AB. Exploiting cancer cell cycling for selective protection of normal cells. *Cancer Res* 2001; 61: 4301-4305.
- 26) MISHINA T, DOSAKA-AKITA H, HOMMURA F, NISHI M, KOJIMA T, OGURA S, SHIMIZU M, KATO H, KAWAKAMI Y. Cyclin E expression, a potential prognostic marker for non-small cell lung cancers. *Clin Cancer Res* 2000; 6: 11-16.
- 27) HAYWARD DG, CLARKE RB, FARAGHER AJ, PILLAI MR, HAGAN IM, FRY AM. The centrosomal kinase Nek2 displays elevated levels of protein expression in human breast cancer. *Cancer Res* 2004; 64: 7370-7376.
- 28) HAYWARD DG, FRY AM. Nek2 kinase in chromosome instability and cancer. *Cancer Lett* 2006; 237: 155-166.
- 29) XU N, WU SD, WANG H, WANG Q, BAI CX. Involvement of FOXM1 in non-small cell lung cancer recurrence. *Asian Pac J Cancer Prev* 2012; 13: 4739-4743.
- 30) XU N, JIA D, CHEN W, WANG H, LIU F, GE H, ZHU X, SONG Y, ZHANG X, ZHANG D, GE D, BAI C. FOXM1 is associated with poor prognosis of non-small cell lung cancer patients through promoting tumor metastasis. *PLoS One* 2013; 8: e59412.
- 31) YANG C, CHEN H, YU L, SHAN L, XIE L, HU J, CHEN T, TAN Y. Inhibition of FOXM1 transcription factor suppresses cell proliferation and tumor growth of breast cancer. *Cancer Gene Ther* 2013; 20:117-124.
- 32) YOON CH, KIM MJ, LEE H, KIM RK, LIM EJ, YOO KC, LEE GH, CUI YH, OH YS, GYE MC, LEE YY, PARK IC, AN S, HWANG SG, PARK MJ, SUH Y, LEE SJ. PTTG1 oncogene promotes tumor malignancy via epithelial to mesenchymal transition and expansion of cancer stem cell population. *J Biol Chem* 2012; 287: 19516-19527.
- 33) ZHU X, MAO Z, NA Y, GUO Y, WANG X, XIN D. Significance of pituitary tumor transforming gene 1 (PTTG1) in prostate cancer. *Anticancer Res* 2006; 26: 1253-1259.
- 34) HUANG SQ, LIAO QJ, WANG XW, XIN DQ, CHEN SX, WU QJ, YE G. RNAi-mediated knockdown of pituitary tumor-transforming gene-1 (PTTG1) suppresses the proliferation and invasive potential of PC3 human prostate cancer cells. *Braz J Med Biol Res* 2012; 45: 995-1001.
- 35) RIZZO A, WALDNER MJ, STOLFI C, SARRA M, FINA D, BECKER C, NEURATH MF, MACDONALD TT, PALLONE F, MONTELEONE G, FANTINI MC. Smad7 expression in T cells prevents colitis-associated cancer. *Cancer Res* 2011; 71: 7423-7432.
- 36) WANG P, FAN J, CHEN Z, MENG ZQ, LUO JM, LIN JH, ZHOU ZH, CHEN H, WANG K, XU ZD, LIU LM. Low-level expression of Smad7 correlates with lymph node metastasis and poor prognosis in patients with pancreatic cancer. *Ann Surg Oncol* 2009; 16: 826-835.
- 37) LO PK, LEE JS, LIANG X, HAN L, MORI T, FACKLER MJ, SADIK H, ARGANI P, PANDITA TK, SUKUMAR S. Epigenetic inactivation of the potential tumor suppressor gene FOXF1 in breast cancer. *Cancer Res* 2010; 70: 6047-6058.
- 38) HONG L, HAN Y, ZHANG H, LI M, GONG T, SUN L, WU K, ZHAO Q, FAN D. The prognostic and chemotherapeutic value of miR-296 in esophageal squamous cell carcinoma. *Ann Surg* 2010; 251: 1056.
- 39) TAKESHITA F, PATRAWALA L, OSAKI M, TAKAHASHI R-U, YAMAMOTO Y, KOSAKA N, KAWAMATA M, KELNAR K, BADER

- AG, BROWN D, OCHIYA T. Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. *Mol The* 2009; 18: 181-187.
- 40) TAKEICHI M. Cadherins in cancer: implications for invasion and metastasis. *Curr Opin Cell Biol* 1993; 5: 806-811.
- 41) ALMAND B, RESSER JR, LINDMAN B, NADAF S, CLARK JI, KWON ED, CARBONE DP, GABRILOVICH DI. Clinical significance of defective dendritic cell differentiation in cancer. *Clin Cancer Res* 2000; 6: 1755-1766.
- 42) GUPTA AK, MCKENNA WG, WEBER CN, FELDMAN MD, GOLDSMITH JD, MICK R, MACHTAY M, ROSENTHAL DI, BAKANAUSKAS VJ, CERNIGLIA GJ, BERNHARD EJ, WEBER RS, MUSCHEL RJ. Local recurrence in head and neck cancer relationship to radiation resistance and signal transduction. *Clin Cancer Res* 2002; 8: 885-892.
- 43) ZHANG X, YAN Z, ZHANG J, GONG L, LI W, CUI J, LIU Y, GAO Z, LI J, SHEN L, LU Y. Combination of hsa-miR-375 and hsa-miR-142-5p as a predictor for recurrence risk in gastric cancer patients following surgical resection. *Ann Oncol* 2011; 22: 2257-2266.
- 44) LIU X, SEMPERE LF, GALIMBERTI F, FREEMANTLE SJ, BLACK C, DRAGNEV KH, MA Y, FIERING S, MEMOLI V, LI H, DiRENZO J, KORC M, COLE CN, BAK M, KAUPPINEN S, DMITROVSKY E. Uncovering growth-suppressive micrornas in lung cancer. *Clin Cancer Res* 2009; 15: 1177-1183.
- 45) SEMPERE LF, LIU X, DMITROVSKY E. Tumor-suppressive micrornas in lung cancer: diagnostic and therapeutic opportunities. *Sci World J* 2009; 9: 626-628.
- 46) MA L, YOUNG J, PRABHALA H, PAN E, MESTDAGH P, MUTH D, TERUYA-FELDSTEIN J, REINHARDT F, ONDER TT, VALASTYAN S, WESTERMANN F, SPELEMAN F, VANDESOMPELE J, WEINBERG RA. miR-9, a MYC/ MYCN-activated microrna, regulates E-cadherin and cancer metastasis. *Nat Cell Biol* 2010; 12: 247-256.
- 47) NASS D, ROSENWALD S, MEIRI E, GILAD S, TABIBIAN-KEISSAR H, SCHLOSBERG A, KUKER H, SION-VARDY N, TOBAR A, KHARENKO O, SITBON E, LITHWICK YANAI G, ELYAKIM E, CHOLAKH H, GIBORI H, SPECTOR Y, BENTWICH Z, BARSHACK I, ROSENFELD N. MiR-92b and miR-9/9* are specifically expressed in brain primary tumors and can be used to differentiate primary from metastatic brain tumors. *Brain Pathol* 2009; 19: 375-383.
- 48) LAIOS A, O'TOOLE S, FLAVIN R, MARTIN C, KELLY L, RING M, FINN SP, BARRETT C, LODA M, GLEESON N, D'ARCY T, MCGUINNESS E, SHEILS O, SHEPPARD B, O'LEARY J. Potential role of miR-9 and miR-223 in recurrent ovarian cancer. *Mol Cancer* 2008; 7: 35.
- 49) HILDEBRANDT M, GU J, LIN J, YE Y, TAN W, TAMBOLI P, WOOD C, WU X. Hsa-miR-9 methylation status is associated with cancer development and metastatic recurrence in patients with clear cell renal cell carcinoma. *Oncogene* 2010; 29: 5724-5728.