A polymorphism at the miR-502 binding site in the 3' untranslated region of the *SET8* gene is associated with the outcome of small-cell lung cancer

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Abstract. microRNAs (miRNAs) bind to the 3' untranslated regions (UTRs) of messenger RNAs, where they interfere with translation of genes that regulate cell differentiation, apoptosis and tumourigenesis. The histone methyltransferase *SET8* has been reported to methylate TP53 and regulate genomic stability. We analysed a single nucleotide polymorphism (rs16917496) within the miR-502 miRNA seed region at the 3' UTR of *SET8* in small-cell lung cancer (SCLC) patients. The *SET8* CC+CT genotype was identified to be independently associated with longer survival in SCLC patients by multivariate analysis (relative risk, 0.453; 95% CI 0.217-0.944; p=0.035). The analysis of genetic polymorphisms in miRNA binding sites may help to identify patient subgroups at high risk of poor outcome.

Introduction

Small-cell lung cancer (SCLC) patients are commonly classified into limited stage and extensive stage groups by the Veterans Administration Lung Cancer Study Group (VALSG) and SCLC makes up approximately 15% of all lung cancer cases and is markedly associated with cigarette smoking (1-3). The prognosis of SCLC, which has been linked to the extent of disease as well as other factors, is poor. The life expectancy of those with untreated SCLC is approximately 3.5 months for the limited group and 6 weeks for the extensive group (4-6). Certain genetic factors have been identified as prognostic factors for SCLC; however, the underlying mechanism of this cancer remains unknown (7,8).

microRNAs (miRNAs) are RNA molecules that are approximately 22 nucleotides in length that are implicated in a number of biological processes, such as embryonic development, cellular differentiation, proliferation, apoptosis, cancer development and insulin secretion (9,10). More than 700 miRNAs have been identified in humans, and these miRNAs are responsible for regulating at least 30% of protein-coding gene expression (11). Specifically, miRNAs target nucleotides 2-8 at the 5' end, which is known as the 'seed region' of the 3' UTR of the target messenger RNA (mRNA). Perfect complementarity between the miRNA and its target mRNA sequence reduces protein levels due to RNA silencing (12,13). There is increasing evidence indicating that single nucleotide polymorphisms (SNPs) in the 3' UTR region, that is targeted by miRNAs, alter the expression of target genes and thereby affect an individual's cancer risk, and miRNA-binding SNPs have been extensively examined in recent genotyping studies (14-19).

PR-Set7/Set8/KMT5a (SET8), which is regulated by miR-502 via the binding site in the SET8 3' UTR, encodes a histone H4 lysine 20 monomethyltransferase that is implicated in normal cell cycle progression (20-22). Previous studies have suggested that the SNP rs16917496, which is located within the miR-502 binding site in the SET8 3' UTR, modulates SET8 expression and contributes to risk and age-at-onset of cancer (16,23). We also found that this SNP modifies hepatocellular carcinoma (HCC) outcome by altering SET8 expression, which depends, at least in part, on its binding affinity with miR-502 (24). In this study, we genotyped this SNP in SCLC patients to assess its association with cancer risk and disease outcome.

Materials and methods

DNA extraction. Blood samples were collected at the Fourth Hospital of Hebei University from 44 SCLC patients who received treatment at the Department of Respiratory Medicine between 2005 and 2009. Blood samples were also collected from 44 healthy female controls. The genomic DNA was immediately extracted using the Wizard Genomic DNA extraction kit (Promega, Madison, WI, USA). All of the patients received and signed consent forms, and all procedures were supervised and approved by the hospital's Human Tissue Research Committee.

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Genotyping of rs16917496. The SNP rs16917496 was genotyped using the ligation detection reaction (LDR) method with the forward and reverse primers, 5'-CCTGGTCAGTGGTCAGCAAAT-3' and 5'-CTGGGAAACACGCTCAAAATC-3', respectively, to amplify the DNA fragments flanking rs16917496 in the *SET8* 3' UTR using the sequence in the NCBI database (http://www.ncbi.nlm.nih.gov/snp/). PCR was performed using a PCR Master Mix kit according to the manufacturer's instructions (Promega). The ligation was performed using the probes S1 (5'-TTGTGGTTTAGCTTTGTATTTAAAC-3'), S2 (5'-TTTTTGTGGTTTAGCTTTGTATTTAAAT-3') and S3 (5'-AAGGAAATAAACTTGAAAATTATTT-3'), and the ligated products were separated using the ABI PRISM Genetic Analyzer 3730XL (Applied Biosystems, Foster City, CA, USA). Polymorphisms were confirmed based on the 3-bp difference in length for different alleles of rs16917496.

Statistical analysis. The χ^2 test was used to analyze dichotomous values, such as the presence or absence of an individual SNP in the SCLC patients and healthy controls. Survival curves were calculated using the Kaplan-Meier method, and comparisons between the curves were carried out using the log-rank test. Multivariate survival analysis was performed using a Cox proportional hazards model. All of the the statistical analyses were performed using the SPSS 18.0 software package (SPSS Company, Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical characteristics of the SCLC patients. A total of 44 SCLC patients, including 13 limited stage and 31 extensive stage cases, were enrolled in this study. A review of the patients was performed every 3 months for 2 years. No patients were lost during follow-up. The correlation between the data collected during the 2-year follow-up and patient clinical characteristics was analysed using the Kaplan-Meier method. As shown in Table I, treatment was associated with overall survival of the SCLC patients, while gender, age, smoking and VALSG classification were not statistically significant predictors of post-operative survival time (Table I). The treatment included platinum-based chemotherapy and chemotherapy combined with radiotherapy, but no survival difference existed between these two treatments, therefore we treated the two groups together for comparison with no-treatment patients.

Association of SET8 polymorphisms with SCLC outcome. A total of 44 SCLC patients and healthy controls were genotyped for the SNP analysis of the rs16917496 polymorphism. The *SET8* CC, CT and TT genotype frequencies in the control samples were 6, 12 and 24, which were comparable to the genotype frequencies in the SCLC patients (8, 12 and 22 for CC, CT and TT). No statistically significant association with cancer risk was detected for the distribution of the rs16917496 polymorphism between the 44 SCLC cases and healthy controls (data not shown). We subsequently assessed the correlation between rs16917496 and overall survival of these SCLC patients.

Table I. Univariate analysis of clinical characteristics associated with the overall survival of the SCLC patients.

Characteristics	No. of cases	2-year survival rate (%)	P-value
Treatment			<0.001
Yes	41	26.8	
No	3	0.0	
Gender			0.135
Male	28	17.9	
Female	16	37.5	
Age (years)			0.157
≤55	22	31.8	
>55	22	18.2	
VALSG classification			0.936
Limited disease	13	23.1	
Extensive disease	31	25.8	
Smoking			0.243
Yes	23	17.4	
No	21	33.3	
SET8 genotype			0.088
CC+CT	22	36.4	
TT	22	13.6	

SCLC, small-cell lung cancer; VALSG, Veterans Administration Lung Cancer Study Group.

Table II. Multivariate analysis of prognostic factors associated with overall survival in SCLC patients with Cox proportional hazards model.

Factors	Relative risk	95% CI	P-value
Treatment	0.050	0.011-0.231	<0.001
SET8 genotype	0.453	0.217-0.944	0.035

SCLC, small-cell lung cancer; CI, confidence interval.

Since only 8 SCLC patients carried C/C alleles, we combined the C/C and C/T carriers together for further analysis. The survival curves of the SCLC patients were plotted using the Kaplan-Meier method and analysed by log-rank test. The 2-year survival rate of the C/C+C/T and T/T patients were 36.4 and 13.6%, respectively. A borderline difference with a p-value of 0.088 for the survival rate of the two genotypes was found, with the T allele linking with shorter survival time (Fig. 1).

Multivariate analysis with the Cox proportional hazards model was performed for these survival predictive factors. As shown in Table II, a statistical difference in survival rate appeared for the rs16917496 SNP, and following adjustment for the predictive factor of treatment, this SNP was identified as an independent predictor of SCLC outcome (relative risk, 0.453; 95% CI 0.217-0.944; p=0.035).

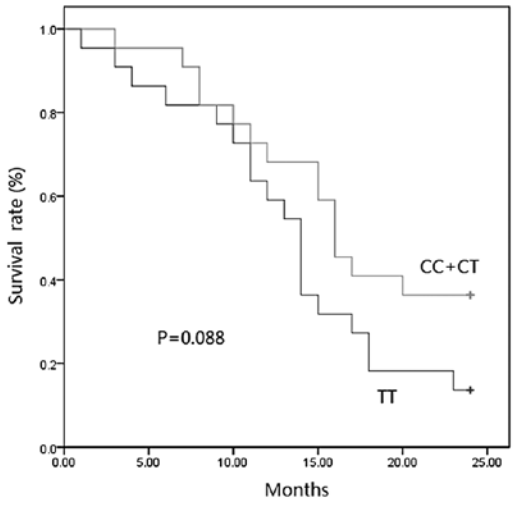

Figure 1. Genotypes of rs16917496 and their association with SCLC survival. SCLC, small-cell lung cancer.

Discussion

Yu *et al* found that 12 miRNA binding site SNPs displayed an aberrant allelic frequency in human cancers using case-control association studies (16). The presently studied SNP has also been proven to be associated with the early onset of breast cancer (23). In this study, we elucidated its association with SCLC cancer risk and outcome, and revealed that the T/T genotype of rs16917496 is associated with shorter survival time. These data are consistent with our previous study, which revealed that the T/T allele was associated with shorter survival time in post-operative HCC patients (24). No statistical differences were detected for SCLC cancer risk and age-at-onset among the genotypes of this SNP (data not shown).

The limited stage SCLC patients displayed a longer survival rate than that of extensive stage patients in previous reports (4,5), but the lack of statistical difference for overall survival between these two groups may be due to the small sample size that we used.

As a methyltransferase, *SET8* modulates p53 expression by specifically methylating lysine 382 of histones that are associated with the p53 genomic sequence (25). The correlation between the methylation status of p53 in response to SET8 and their correlation with SCLC carcinogenesis requires further study.

Although SNP studies in miRNA binding sites are at an early stage, our results indicate that the alleles of SNPs in miRNA binding sites have an effect on cancer outcome. However, the results from this study require validation in other populations and laboratory-based functional studies. miRNAs are key factors associated with the susceptibility of a patient to therapeutic responses in a number of complex diseases, including cancer (26). The analysis of genetic polymorphisms in miRNA binding sites may help to identify patient subgroups with a high risk of poor outcome.

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References

1. Adjei AA, Marks RS and Bonner JA: Current guidelines for the management of small cell lung cancer. Mayo Clin Proc 74: 809-816, 1999
2. Simon GR and Wagner H: For the American College of Chest Physicians. Small cell lung cancer. Chest 123 (Suppl): 259S-271S, 2003.
3. National Comprehensive Cancer Network. National Comprehensive Cancer Network practice guidelines in oncology v. 1.2007: small cell lung cancer. Available at: http://www.nccn.org/professionals/physician_gls/PDF/sclc.pdf. Accessed January 9, 2007.
4. Hyde L, Yee J, Wilson R and Panto ME: Cell type and the natural history of lung cancer. JAMA 193: 52-54, 1965
5. Lassen U, Osterlind K, Hansen M, Dombernowsky P, Bergman B and Hansen HH: Long term survival in small-cell lung cancer: posttreatment characteristics in patients surviving 5 to 18+ years – an analysis of 1,714 consecutive patients. J Clin Oncol 13: 1215-1220, 1995.
6. Rawson NS and Peto J: An overview of prognostic factors in small cell lung cancer. A report from the Subcommittee for the Management of Lung Cancer of the United Kingdom Coordinating Committee on Cancer Research. Br J Cancer 61: 597-604, 1990.
7. Knez L, Sodja E, Kern I, Košnik M and Cufer T: Predictive value of multidrug resistance protein, topoisomerases II and ERCC1 in small cell lung cancer: A systematic review. Lung Cancer 72: 271-279, 2011.
8. Kin YH, Ishii G, Goto K, Ota S, Kubota K, Murata Y, Mishima M, Saijo N, Nishiwaki Y and Ochiai A: Expression of the breast cancer resistance protein is associated with a poor clinical outcome in patients with small-cell lung cancer. Lung Cancer 65: 105-111, 2009.
9. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281-297, 2004.
10. Ambros V: The functions of animal microRNAs. Nature 431: 350-355, 2004.
11. Lewis BP, Burge CB and Bartel DP: Conserved seed pairing, often flanked by adenosines, indicated that thousands of human genes are microRNA targets. Cell 120: 15-20, 2005.
12. Zeng Y, Yi R and Cullen BR: MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc Natl Acad Sci USA 100: 9779-9784, 2003.
13. Zeng Y, Wagner EJ and Cullen BR: Both natural and designed microRNAs can inhibit the expression of cognate mRNAs when expressed in human cells. Mol Cell 9: 1327-1333, 2002.
14. Chin LJ, Ratner E, Leng S, *et al*: A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. Cancer Res 68: 8535-8540, 2008.
15. Brendle A, Lei H, Brandt A, Johansson R, Enquist K, Henriksson R, Hemminki K, Lenner P and Försti A: Polymorphisms in predicted microRNA-binding sites in integrin genes and breast cancer: ITGB4 as prognostic marker. Carcinogenesis 29: 1394-1399, 2008.
16. Yu Z, Li Z, Jolicoeur N, Zhang L, Fortin Y, Wang E, Wu M and Shen SH: Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. Nucleic Acids Res 35: 4535-4541, 2007.
17. Landi D, Gemignani F, Naccarati A, Pardini B, Vodicka P, Vodickova L, Novotny J, Forsti A, Hemminki K, Canzian F and Landi S: Polymorphisms within microRNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis 29: 579-584, 2008.
18. Saunders MA, Liang H and Li W-H. Human polymorphism at microRNA and microRNA target sites. Proc Natl Acad Sci USA 9: 3300-3305, 2007.
19. Gao Y, He Y, Ding J, *et al*: An insertion/deletion polymorphism at miRNA-122-binding site in the interleukin-1alpha 3' untranslated region confers risk for hepatocellular carcinoma. Carcinogenesis 30: 2064-2069, 2009.
20. Fang J, Feng Q, Ketel CS, Wang H, Cao R, Xia L, Erdjument-Bromage H, Tempst P, Simon JA and Zhang Y: Purification and functional characterization of SET8, a nucleosomal histone H4-lysine 20-specific methyltransferase. Curr Biol 12: 1086-1099, 2002.
21. Nishioka K, Rice JC, Sarma K, Erdjument-Bromage H, Werner J, Wang Y, Chuikov S, Valenzuela P, Tempst P, Steward R, Lis JT, Allis CD and Reinberg D: PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. Mol Cell 9: 1201-1213, 2002.

22. Wu S, Wang W, Kong X, Congdon LM, Yokomori K, Kirschner MW and Rice JC. Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. Genes Dev 24: 2531-2542, 2010.
23. Song F, Zheng H, Liu B, Wei S, Dai H, Zhang L, Calin GA, Hao X, Wei Q, Zhang W and Chen K: An miR-502-binding site single-nucleotide polymorphism in the 3'-untranslated region of the SET8 gene is associated with early age of breast cancer onset. Clin Cancer Res 19: 6292-6300, 2009.
24. Guo Z, Wu C, Wang X, Wang C, Zhang R and Shan B: A polymorphism at the miR-502 binding site in the 3'-untranslated region of the histone methyltransferase SET8 is associated with hepatocellular carcinoma outcome. Int J Cancer: Nov 18, 2011. doi: 10.1002/ijc.27352. (E-pub ahead of print).
25. Shi X, Kachirskaia I, Yamaguchi H, West LE, Wen H, Wang EW, Dutta S, Appella E and Gozani O: Modulation of p53 function by SET8-mediated methylation at lysine 382. Mol Cell 27: 636-46, 2007.
26. Merritt WM, Lin YG, Han LY, *et al*: Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med 359: 2641-2650, 2008.