Int J Clin Exp Pathol 2015;8(5):5457-5463
www.ijcep.com /ISSN:1936-2625/IJCEP0006457

Original Article

DNA methylation of hMLH1 correlates with the clinical response to cisplatin after a surgical resection in Non-small cell lung cancer

Fang Wu[1], Min Lu[1], Lu Qu[1], Dai-Qiang Li[2], Chun-Hong Hu[1]

[1]*Department of Oncology, The Second Xiangya Hospital of Central South University, 139 Middle Renmin Road, Changsha 410011, China;* [2]*Department of Pathology, The Second Xiangya Hospital of Central South University, 139 Middle Renmin Road, Changsha 410011, China*

Received January 29, 2015; Accepted March 23, 2015; Epub May 1, 2015; Published May 15, 2015

Abstract: Our previous study demonstrated that promoter methylation of human mutL homolog 1 (hMLH1) is involved in determining sensitivity to cisplatin in NSCLC A549/DDP cell line, The present study was designed to determine whether DNA methylation of hMLH1 affects the prognosis of non-small cell lung cancer patients who received cisplatin-based adjuvant chemotherapy. Methylation status of hMLH1 was examined by nested methylation-specific PCR (nested MSP) in 84 archived NSCLC surgically resected tissue specimens from patients receiving cisplatin-based adjuvant chemotherapy. Univariate and multivariate analysis were used to investigate the relationship between hMLH1 methylation status and the clinical prognosis of the patients mentioned above. In the cohort of 84 NSCLC cases, 80 tissue samples were successfully amplified by nested MSP. Among them, 36 samples (45%) were identified to be methylated. Moreover, hMLH1 methylation was not associated with age, gender, smoking status, T stage, histology and differentiation, but correlated with lymphatic metastasis (P=0.021). Multivariate logistic regression analysis showed that hMLH1 methylation may function as a significant independent prognostic factor for tumor recurrence in NSCLC patients treated with adjuvant cisplatin (HR 3.114, 95% CI 1.032-9.399; P=0.044). However, Kaplan-Meier method (P=0.093) and multivariate Cox regression analysis (P=0.598) revealed that hMLH1 methylation was not associated with the survival of these patients. To conclude, the cisplatin-based adjuvant chemotherapy is more beneficial for NSCLC patients without hMLH1 methylation. hMLH1 methylation may have a potential to become a biomarker of individualized therapy for NSCLC patients.

Keywords: NSCLC, hMLH1, hypermethylation, cisplatin, prognosis

Introduction

Non-small cell lung cancer (NSCLC) is the most common malignancy and the leading cause of cancer death in Asian and Western countries [1, 2]. Although surgical resection is usually the most effective therapeutic strategy for NSCLC, adjuvant (postoperative) chemotherapy also plays an important role in achieving a lower cancer recurrence rate. Cisplatin (DDP) has been widely used for adjuvant chemotherapy for NSCLC, nevertheless, lots of patients still develop tumor recurrence within 5 years. So, enhanced prognostication power is becoming more desirable.

Aberrant promoter hypermethylation is noted in most solid tumors [3-5], including NSCLC. In addition, epigenetic alterations have been reported to be associated with the prognosis and chemosensitivity of NSCLC [4, 6]. Human mutL homolog 1 (hMLH1) is the most important member of Mismatch repair (MMR) genes encoding a number of DNA repair enzymes and thus cooperating to recognize and repair DNA mismatches [7]. hMLH1 methylation has been found in ovarian and colorectal cancer cell lines for resistance to cisplatin and restoration of MMR activity in these cells are sufficient to reestablish susceptibility to chemotherapy [5, 8]. Our previous study also demonstrated that promoter methylation of hMLH1 is involved in determining sensitivity to cisplatin in NSCLC A549/DDP cell line, and cisplatin resistance could be reversed by the demethylating agent 5-zaz-2'-deoxycytidine (5-Aza-dc) in vitro [9].

Table 1. The clinical characteristics of the 80 patients

Item	Number	Item	Number
Gender		Age	
Male	58	<59	38
female	22	≥59	42
Smoking		Histology	
Yes	56	squamous	36
No	24	Others	44
N stage		Differentiation	
N0	32	I	8
N1	19	II	49
N2	29	III	23
T stage			
T1	2	T3	17
T2	59	T4	2

The current study aims to determine whether promoter methylation in hMLH1 affects the prognosis of non-small cell lung cancer patients who received cisplatin-based adjuvant chemotherapy.

We analyzed hMLH1 methylation status in archived specimens from 84 patients receiving cisplatin-based chemotherapy following surgical resection, examined the possibility of hMLH1 methylation "signature" affecting clinic pathological features and prognosis, and investigated for the first time whether hMLH1 methylation is essential for predicting the drug effect of adjuvant chemotherapy with cisplatin in NSCLC patients.

Materials and methods

Patients and tissue samples

The Formalin-Fixed, Paraffin-Embedded tumor samples (FFPE) used in this study were derived from 84 patients with NSCLC who underwent curative resection at the Department of Cardiothoracic Surgery in the 2nd Xiangya Hospital (Changsha, China) from March 1, 2001 to March 1, 2006 and then accepted cisplatin-based chemotherapy at the Department of Clinical Oncology in this hospital. The patients were followed up at least 5 years. All available FFPE blocks were carefully reviewed by a pathologist. To be included in the cohort, an eligible patient must have a confirmed diagnosis of NSCLC and a sufficient amount of archived tumor material to allow for DNA extraction (tissue is preserved in sectioned blocks; >50% of cells are malignant). Cases of small-cell lung cancers, mixed histology, metastatic tumors to the lung, and indeterminate clinical stage were excluded. Demographic and clinical information including survival were obtained from the computerized tumor registry at the Department of Clinical Oncology in the 2nd Xiangya Hospital. 80 successfully amplified cases were eligible for the follow-up study; the clinical characteristics of the patients were summarized in **Table 1**. This study followed the ethical guidelines of the Internal Review Board of the Second Xiangya Hospital, Central South University.

DNA extraction from FFPE blocks

DNA was extracted from 4 deparaffinized, 10 μm-thick tissue sections. Sections of each block were collected in 1.5 ml Eppendorf tubes, deparaffinized with xylene, and digested overnight at 50°C with proteinase K buffered in 1% SDS (pH=8.0). DNA was isolated by phenol-chloroform extraction and ethanol precipitation.

Bisulfite modification of DNA for nested MSP

Nested methylation-specific PCR was carried out in the DNA samples from NSCLC patients, negative and positive controls. Prior to nested MSP, bisulfite modification was performed using the EZ DNA Methylation Kit (Zymo Research, orange, CA, USA) as the manufacturer's instructions. The bisulfite-modified DNA was stored at -20°C~-80°C until subsequent nested MSP.

Nested MSP amplification and primers

To facilitate MSP analysis on DNA retrieved from the archived FFPE tissues, the hMLH1 methylation was determined by the method of MSP further modified as a nested two-step approach. Briefly, 2 μl of modified DNA were amplified in stage 1 MSP (40 cycles) using primers which recognize the bisulfite-modified template but do not discriminate between methylated and unmethylated alleles. PCR products of step one were subjected to step 2 MSP (35 cycles) using primers selective for the methylated or the unmethylated genotype [10]. All PCRs were performed with controls for unmethylated alleles (DNA from Normal human blood),

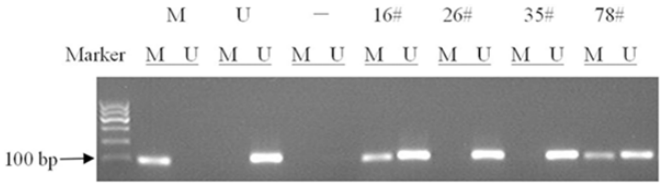

Figure 1. Representative results of nested MSP analysis from 4 NSCLC patients. Lanes U, nMSP product with primers recognizing unmethylated hMLH1 promoter; Lanes M, nMSP product with primers recognizing methylated hMLH1 premotor; the numbers shown are sample identification numbers. PC represents positive control for methylated (M) allele; NC represents positive control for unmethylated (M) allele; blank means blank control.

methylated alleles [normal human blood DNA treated in vitro with SssI methyltransferase (New England Biolabs)], and a control without DNA. In some cases, no fragments could be amplified after bisulfite treatment, either with primers specific for the methylated or the unmethylated genotype. These samples were designated "non-informative".

Cloning and sequencing of nMSP product

3 species of nMSP products were randomly selected from both the methylation and unmethylation groups, the cloning and sequencing were accomplished by Sangon Biotech (Shanhai, China).

Statistical analysis

A statistical analysis was performed using the SPSS 13.0. Overall survival (OS) was calculated from the date of initial diagnosis until either death or the date of last follow-up (censored). Disease-free survival (DFS) was calculated from the date of initial diagnosis to the date of tumor recurrence. The correlation between the hMLH1 methylation status and clinical characteristics was analyzed by chi-square test. OS was estimated with Kaplan-Meier method (log-rank test) and multivariate Cox regression analysis; and DFS was calculated using Multivariate logistic regression analysis. *P* value <0.05 was considered as statistically significant.

Results

Frequency of hMLH1 methylation

In 80 patients with successful amplification of nested MSP, 36 individuals (45%) carry methylated hMLH1 gene, and 44 (55%) carry unmethylated hMLH1 gene. The representative results of 4 tumor samples are shown in **Figure 1**. 26# and 35# are unmethylation cases, 16# and 78# are methylation cases.

Cloning and sequencing of nMSP product

The data of sequencing showed in **Figure 2** that there was no change about "C" in methylated products, but in unmethylated products, the "C" converted to "T", suggesting nMSP results are reliable in this study.

Comparison between hMLH1 methylation and patients' clinicpathological features

We analyzed the correlation between hMLH1 methylation status and clinical characteristics including age, gender, smoking status, T stage, N stage, histology and differentiation (**Table 2**). Of 80 patients, median age is 59-years-old (range 35 to 75), 72.5% are men, 70% are smokers, and 45% are squamous carcinoma. 36 individuals (45%) carry methylated hMLH1 gene. There was a significant relationship between hMLH1 methylation and N stage (P= 0.021). However, the hMLH1 methylation status does not correlate with age, gender, smoking status, T stage, histology, differentiation of patients in the cohort of 80 NSCLC cases.

Correlations between hMLH1 methylation status and survival

We used Kaplan-Meier method (log-rank test) and multivariate Cox regression analysis to evaluate whether the hMLH1 methylation status affects 5-year survival (**Table 3**). 78 patients were followed for at least 5 years or till their death, while 2 cases lost to follow up. Survival curves are shown in **Figure 3**, the 5-year OS of hMLH1 methylation group was worse than unmethylation group. Mean survival time was 46.2 months for the methylation group and 66.0 months for the unmethylation group, the survival curve also obviously separated, but this difference was not statistically significant (*P*=0.093). The multivariate Cox regression analysis identified only N stage as independent prognostic factors for survival (*P*=0.003). The methylation status did not significantly correlated with 5-year overall survival (*P*=0.598).

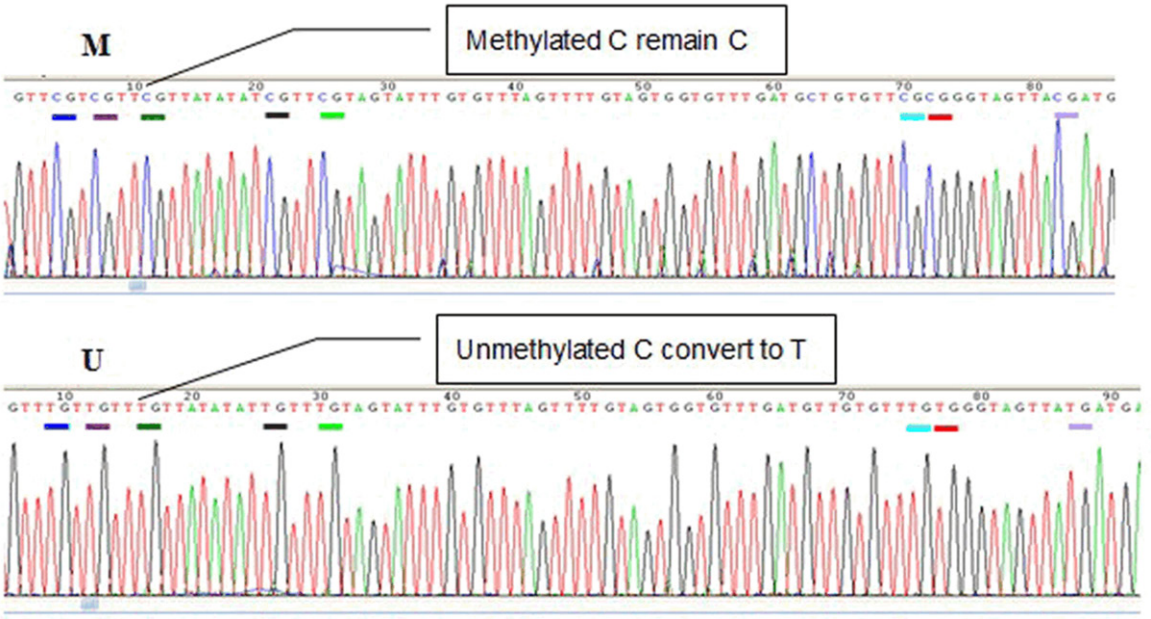

Figure 2. Representative results of Cloning and Sequencing of nMSP products. M. methylated product; U. unmethylated product; CpG sites were underlined. In methylated products, methylated "C" remains "C", but in unmethylated products, the "C" converted to "T".

Table 2. Comparison between hMLH1 methylation status and clinicpathological features

Clinicpathological features	n	hMLH1 methylation status		*P*
		+	-	
Age (ys)				
≥59	42 (52.5%)	20 (47.6%)	22 (52.4%)	0.621
<59	38 (47.5%)	16 (42.1%)	22 (57.9%)	
Gender				
male	58 (72.5%)	28 (48.3%)	30 (51.7%)	0.339
female	22 (27.5%)	8 (36.4%)	14 (63.6%)	
Smoking status				
yes	56 (70%)	27 (48.2%)	29 (51.8%)	0.377
no	24 (30%)	9 (37.5%)	15 (62.5%)	
T stage				
T_{1-2}	61 (76.25%)	27 (44.3%)	37 (60.7%)	0.812
T_{3-4}	19 (23.75%)	9 (47.4%)	10 (52.6%)	
N stage				0.021*
N_{0-1}	51 (63.75%)	18 (35.3%)	33 (64.7%)	
N_2	29 (36.25%)	18 (62.1%)	11 (37.9%)	
Histology				
Squamous	36 (45%)	17 (47.2%)	19 (52.8%)	0.718
others	44 (55%)	19 (43.2%)	25 (56.8%)	
Differentiation				
I-II	57 (71.25%)	22 (38.6%)	35 (61.4%)	0.070
III	23 (28.75%)	14 (60.9%)	9 (39.1%)	

*P values of less than 0.05 were considered to indicate statistical significance.

Associations between hMLH1 methylation status and the disease free survival

Multivariate logistic regression analysis was done to control for the potential confounding effects of variables, such as age, gender, T stage, N stage, hitology, smoking, differentiation, histology and hMLH1 methylation status, and then calculate the odds ratios. Of all pa-tients, 28 patients (80%) recurred in hMLH1 methylation group, and only 21 patients (48.8-3%) recurred in unmethylation group. The result (**Table 4**) indicated that N stage and hMLH1 methylation status were independent factors for 5-year DFS. The risk of recurrence and metastasis for the co-hort with hMLH1 methylation was determined to be 3.114 times as

Table 3. Multivariate cox regression analysis of prognostic factors associated with 5-year OS

Variables	Hazard ratio	*p* value
Age	0.673 (0.360-1.259)	0.215
Smoking status	9766.207 (0.000-6.608E+066)	0.901
Gender	0.000 (0.000-9.409E+058)	0.904
T stage	0.988 (0.539-1.812)	0.970
N stage		0.003*
N_{0-1}	3.300 (1.396-7.802)	0.007*
N_2	4.054 (1.771-9.280)	0.001*
Differentiation	1.408 (0.837-2.370)	0.198
Histology	0.700 (0.330-1.485)	0.352
hMLH1 methylation	1.199 (0.610-2.356)	0.598

*P values of less than 0.05 were considered to indicate statistical signi-ficance.

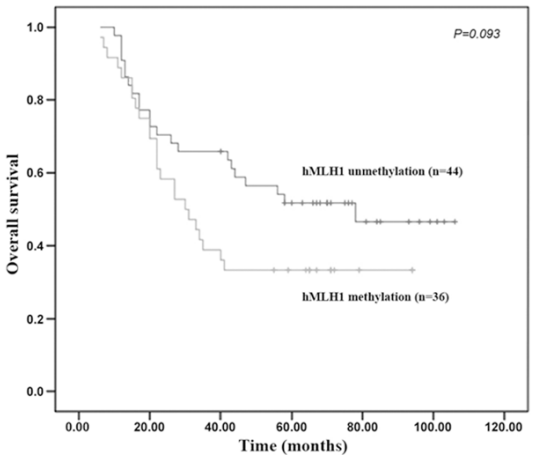

Figure 3. Statistical analysis of hMLH1 methylation and 5-year OS using the Kaplan-Meier method.

high as that of the reference group (HR 3.114, 95% CI 1.032-9.399; *P*=0.044).

Discussion

Lung cancer is the most common cause of cancer related death in the world. Surgical resection is a major modality to cure this malignancy in early stage, but often failed as a result of recurrence and metastasis. Cisplatin has been widely used for adjuvant chemotherapy for non-small cell lung cancers, and the sensitivity of cisplatin is a very hot research focus. hMLH1 methylation status has been reported to be correlated with cisplatin resistance in several ma-lignancies, including colorectal and ovarian ca-ncer [5, 8, 11, 12]. Our previous study found that hMLH1 methylation is involved in determining sensitivity to cisplatin in NSCLC A549/DDP cell line, and cisplatin resistance could be reversed by the demethylating agent 5-zaz-2'-deoxycytidine (5-Aza-dc) in vitro [9], however, its functional role in NSCLC is still to be defined. In the present study, hMLH1 methylation was analyzed in 84 archived non-small cell lung cancer surgically resected tissue specimens from patients receiving cisplatin-based adjuvant chemotherapy by nested methylation-specific PCR (MSP). Our data showed that the frequency of hMLH1 methylation in 80 patients with successful amplification of nested MSP was 45% in agreement with previous reports (12%-80%) [13-15], suggesting that hMLH1 methylation is a common event in NSCLC. In 80 patients, the methylation status was not correlated with the age, gender, smoking, TNM stage or histology. However, when the T stage and N stage were separated from TNM as independent factors, we found a significant relationship between hMLH1 methylation and the N stage, indicating that the hMLH1 methylation phenotype may influence the lymphatic metastasis of the local lesion. However, some previous studies indicate that hMLH1 methylation was not associated with N stage. Safar et al showed hMLH1 methylation status was not correlated with the clinical characteristics of the patients only in the N0 stage [16]. Hsu et al did not identify the N stage as an independent factor. Song et al analyzed N stage independently, and showed hMLH1 methylation did not significantly correlated with N stage, a possible limitation is that only six cases with lymphatic metastasis participated in the study [14]. However, whether hMLH1 methylation is associated with lymphatic metastasis remains to be confirmed by more researches.

In the prognostic analysis, multivariate logistic regression analysis was carried out to identify N stage and hMLH1 methylation status as independent factors for 5-year DFS. Our previous study has demonstrated that hMLH1 methylation status is involved in determining sensitivity to cisplatin in NSCLC A549/DDP cell line [9]. Therefore, we speculated that the patients carrying methylated hMLH1 gene would be easier to acquire cisplatin-resistance and be more likely to recurrent after accepting cisplatin-

Table 4. Multivariate logistic regression analyses of prognostic factors associated with 5-year DFS

variables	Hazard ratio	*p* value
Age	0.440 (0.137-1.412)	0.168
Smoking status	1.243 (0.023-66.511)	0.915
Gender	0.737 (0.013-42.949)	0.883
T stage	1.200 (0.379-3.794)	0.756
N stage		0.004*
N_{0-1}	5.010 (1.296-19.369)	0.019
N_2	8.908 (2.226-35.650)	0.002
Differentiation	1.090 (0.431-2.758)	0.856
Histology	0.918 (0.248-3.399)	0.898
hMLH1 methylation	3.114 (1.032-9.399)	0.044*

*P values of less than 0.05 were considered to indicate statistical significance.

based adjuvant chemotherapy. However, Multivariate Cox regression analysis indicated that hMLH1 methylation status was not associated with 5-year survival. The various secondary treatments such as non-platinum chemotherapy (gemcitabine, docetaxel and pemetrexed), EGFR-TKI (gefitinib, Erlotinib), VEGFR inhibitor (endostar) and radiotherapy may prolong the survival time, thus leading to no statistically significance of the two groups. The bias may result from the secondary treatments, so PFS may be more appropriate to represent the effect of hMLH1 methylation status on the prognosis, as compared with os. In addition, the survival curve obviously separated, the hMLH1 methylation group had a tendency towards poorer prognosis than the unmethylation group, although there was no statistic difference. These results still need to be confirmed in a larger cohort of patients.

As the development of individualized therapy, the researchers found that many therapies have dominant crowd, who can be identified by biomarkers and get appropriate therapy [17]. For example, the patients with EGFR mutations can benefit from EGFR-TKI [18], high expression of ERCC1 protein predicts no benefit more from cisplatin chemotherapy [19], and the strong expression of RRMI mRNA implies patients cannot benefit from Gemzar-based chemotherapy [20]. However, DNA biomarkers are more stable and reliable than protein and mRNA, which are influenced by multifactor, such as environmental, preservation condition and measurement techniques. Thus, the hMLH1 promoter methylation might be a new DNA biomarker for NSCLC patients receiving cisplatin-based chemotherapy.

In summary, our results identify hMLH1 methylation as a common event in NSCLC, it could be an important factor to influence the lymphatic metastasis. hMLH1 methylation may have a potential to predict recurrence and metastasis of NSCLC patients who accepted post-operative adjuvant cisplatin-based chemotherapy, and may be expected to become a biomarker of individualized therapy for NSCLC.

Acknowledgements

This work was supported by Hunan Provincial Natural Science Foundation of China (No. 11-JJ4069) and The Fundamental Research Funds for the Central Universities (No. 2012QNZT139).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chun-Hong Hu, Department of Oncology, The Second Xiangya Hospital of Central South University, 139 Middle Renmin Road, Changsha 410011, China. Tel: 00861350-8486908; Fax: 0086-731-85295929; E-mail: huchunh0606@163.com

References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.

[2] Zeng H, Zheng R, Guo Y, Zhang S, Zou X, Wang N, Zhang L, Tang J, Chen J, Wei K, Huang S, Wang J, Yu L, Zhao D, Song G, Chen J, Shen Y, Yang X, Gu X, Jin F, Li Q, Li Y, Ge H, Zhu F, Dong J, Guo G, Wu M, Du L, Sun X, He Y, Coleman MP, Baade P, Chen W and Yu XQ. Cancer survival in China, 2003-2005: A population-based study. Int J Cancer 2015; 136: 1921-30.

[3] Moskalev EA, Jandaghi P, Fallah M, Manoochehri M, Botla SK, Kolychev OV, Nikitin EA, Bubnov VV, von Knebel Doeberitz M, Strobel O, Hackert T, Buchler MW, Giese N, Bauer A, Muley T, Warth A, Schirmacher P, Haller F, Hoheisel JD and Riazalhosseini Y. GHSR DNA hypermethylation is a common epigenetic alteration of high diagnostic value in a broad spectrum of cancers. Oncotarget 2015; 6: 4418-27.

[4] Miao Y, Wang L, Zhang X, Xu X, Jiang G, Fan C, Liu Y, Lin X, Yu J, Zhang Y and Wang E. Promoter methylation-mediated silencing of beta-catenin enhances invasiveness of non-small cell lung cancer and predicts adverse prognosis. PLoS One 2014; 9: e112258.

[5] Wang J, Yu L, Cai J, Jia J, Gao Y, Liang M and Wang Z. The role of EZH2 and DNA methylation in hMLH1 silencing in epithelial ovarian cancer. Biochem Biophys Res Commun 2013; 433: 470-476.

[6] Zhang YW, Zheng Y, Wang JZ, Lu XX, Wang Z, Chen LB, Guan XX and Tong JD. Integrated analysis of DNA methylation and mRNA expression profiling reveals candidate genes associated with cisplatin resistance in non-small cell lung cancer. Epigenetics 2014; 9: 896-909.

[7] Vageli D, Daniil Z, Dahabreh J, Karagianni E, Vamvakopoulou DN, Ioannou MG, Scarpinato K, Vamvakopoulos NC, Gourgoulianis KI and Koukoulis GK. Phenotypic mismatch repair hMSH2 and hMLH1 gene expression profiles in primary non-small cell lung carcinomas. Lung Cancer 2009; 64: 282-288.

[8] Steele N, Finn P, Brown R and Plumb JA. Combined inhibition of DNA methylation and histone acetylation enhances gene re-expression and drug sensitivity in vivo. Br J Cancer 2009; 100: 758-763.

[9] Wu F and Hu CH. [Reversal effect of 5-Aza-dc on cisplatin-resistance in human NSCLC cells in vitro]. Zhonghua Zhong Liu Za Zhi 2011; 33: 349-353.

[10] van Engeland M, Weijenberg MP, Roemen GM, Brink M, de Bruine AP, Goldbohm RA, van den Brandt PA, Baylin SB, de Goeij AF and Herman JG. Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer. Cancer Res 2003; 63: 3133-3137.

[11] Arnold CN, Goel A and Boland CR. Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. Int J Cancer 2003; 106: 66-73.

[12] Plumb JA, Strathdee G, Sludden J, Kaye SB and Brown R. Reversal of drug resistance in human tumor xenografts by 2'-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. Cancer Res 2000; 60: 6039-6044.

[13] Liu Z, Zhao J, Chen XF, Li W, Liu R, Lei Z, Liu X, Peng X, Xu K, Chen J, Liu H, Zhou QH and Zhang HT. CpG island methylator phenotype involving tumor suppressor genes located on chromosome 3p in non-small cell lung cancer. Lung Cancer 2008; 62: 15-22.

[14] Song H, Yi J, Zhang Y, Wang R and Chen L. [DNA methylation of tumor suppressor genes located on chromosome 3p in non-small cell lung cancer]. Zhongguo Fei Ai Za Zhi 2011; 14: 233-238.

[15] Geng X, Wang F, Zhang L and Zhang WM. Loss of heterozygosity combined with promoter hypermethylation, the main mechanism of human MutL Homolog (hMLH1) gene inactivation in non-small cell lung cancer in a Chinese population. Tumori 2009; 95: 488-494.

[16] Safar AM, Spencer H, Su X, Cooney CA, Shwaiki A and Fan CY. Promoter hypermethylation for molecular nodal staging in non-small cell lung cancer. Arch Pathol Lab Med 2007; 131: 936-941.

[17] Gentzler RD, Yentz SE, Johnson ML, Rademaker AW and Patel JD. The changing landscape of phase II/III metastatic NSCLC clinical trials and the importance of biomarker selection criteria. Cancer 2014; 120: 3853-3858.

[18] Castanon E, Martin P, Rolfo C, Fusco JP, Ceniceros L, Legaspi J, Santisteban M and Gil-Bazo I. Epidermal growth factor receptor targeting in non-small cell lung cancer: revisiting different strategies against the same target. Curr Drug Targets 2014; 15: 1273-1283.

[19] Wang S, Pan H, Liu D, Mao N, Zuo C, Li L, Xie T, Huang D, Huang Y, Pan Q, Yang L and Wu J. Excision repair cross complementation group 1 is a chemotherapy-tolerating gene in cisplatin-based treatment for non-small cell lung cancer. Int J Oncol 2015; 46: 809-817.

[20] Meng X, Wang G, Guan R, Jia X, Gao W, Wu J, Yu J, Liu P, Yu Y, Sun W, Dong H and Fu S. Predicting chemosensitivity to gemcitabine and cisplatin based on gene polymorphisms and mRNA expression in non-small-cell lung cancer cells. Pharmacogenomics 2015; 16: 23-34.