CDX2 methylation may predict the prognosis of patients with lung cancer

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Abstract

Introduction: *CDX2* methylation predicts poor prognosis in gastric cancer, squamous esophageal cancer and colorectal cancer. The present study was performed to investigate the roles of *CDX2* methylation in lung cancer.
Material and methods: One hundred and sixty-seven patients with lung cancer were enrolled. Methylation-specific PCR (MSP) was performed to investigate the methylation status of *CDX2*. Sequencing of the *CDX2* 5′ CpG island was conducted as well. A 5-year follow-up was performed by a research nurse or dedicated physician. A Kaplan-Meier curve was used to analyze the survival situation of patients. Univariate and multivariate Cox analysis was performed to investigate the potential predictors for prognosis of patients with lung cancer.
Results: The patients were classified into two groups according to *CDX2* status: methylation (n = 75) and unmethylation (n = 92). After the 5-year follow-up, we found that the survival rate of patients with methylation of *CDX2* was much lower than in those with unmethylation of *CDX2* (56% vs. 84.8%, p = 0.000). Among the smoking patients, methylation of *CDX2* was associated with poorer prognosis of patients with lung cancer (p = 0.000). DFS of patients with *CDX2* methylation was lower than in those without *CDX2* methylation (56.0% vs. 73.9%, p = 0.009). Univariate and multivariate Cox analysis demonstrated that *CDX2* methylation served as an independent prognostic predictor of patients with lung cancer (univariate: HR = 3.705, 95% CI: 1.922–7.139; multivariate: HR = 3.418, 95% CI: 1.826–6.397).
Conclusions: *CDX2* methylation may serve as an independent prognostic predictor for patients with lung cancer.
Key words: *CDX2*, methylation, prognosis, lung cancer.

Introduction

Lung cancer is regarded as the most common cause of cancer-related death with 1.3 million deaths worldwide each year. Smoking accounts for 50% and 80% of deaths from lung cancer for females and males, respectively [1]. The 5-year survival rate of lung cancer patients is merely 17% [2], largely due to the fact that most cases are already metastatic at diagnosis or recur after radiotherapy or initial surgery. Metastatic cases are incurable due to intrinsic resistance to chemotherapy or acquired resistance after an initial response [3]. The prognosis of cancer patients is commonly affected by multiple factors [4–7]. To improve the clinical outcomes, a better understanding on the molecular pathogenesis of lung cancer is needed to identify novel therapeutic targets.

Genetic and epigenetic factors exhibit key roles in the initiation and progression of lung cancer. Genetic abnormalities in key components are closely related to the pathogenesis of lung cancer [8, 9]. CDX2 and CK20 are proteins related to intestinal development and differentiation, which are also useful markers to identify adenocarcinomas and normal intestinal epithelium of colorectal origin [10–12]. *CDX2*, an intestinal transcription factor, contributes to regulating the expression of many intestine-specific genes responsible for intestinal proliferation and differentiation [13–15]. It is regarded as a tumor suppressor gene [16–18] that is involved in the Wnt/β-catenin signaling pathway [19–21]. Promoter CpG island methylation is a crucial mechanism of silencing tumor suppressor genes during the carcinogenic process. DNA methylation could result in silencing of *CDX2* in gastric cancer and squamous esophageal cancer [22, 23]. Grimminger *et al*. reported that up-regulation of *CDX2* mRNA expression appeared to be associated with the pathogenesis of non-small cell lung cancer [24]. In another study by Liu *et al*., the researchers found that *CDX2* is frequently methylated in lung cancer and expression of *CDX2* is regulated by promoter region hypermethylation [25]. In addition, enhanced *CDX2* methylation was reported to be associated with shorter survival of CRC patients [26]. However, the prognostic role of *CDX2* methylation in lung cancer was not explored.

In the present study, methylation status of *CDX2* was determined with the methylation-specific PCR (MSP) method and then the prognostic role of *CDX2* methylation in lung cancer was analyzed after a 5-year follow-up. Univariate and multivariate Cox analyses were performed to evaluate whether *CDX2* methylation could serves as an independent predictor for the prognosis of lung cancer patients.

Material and methods

Subjects

A total of 167 patients with lung cancer were enrolled between April 2012 and October 2013 from the Affiliated Hospital of Weifang Medical University. The patients comprised 98 men and 69 women. With biopsy, 167 tissue samples were collected from all patients. These patients had not undergone any treatment before surgery. The patients underwent surgery or chemotherapy according to disease status.

Current smoking status was defined as an individual who had smoked continuously for 6 months (at least one cigarette per day). Former smoking status was defined as an individual who smoked but did not meet the standard of current smoking. All patients signed written informed consent and the study was approved by the review committee of the Affiliated Hospital of Weifang Medical University.

Methylation-specific PCR (MSP)

Bisulfate modification of DNA was performed with a Bisul-Flash DNA Modification Kit (EpiGentek) following the manufacturer's instructions. The methylation of *CDX2* was assessed by methylation-specific PCR (MSP). The DNA treated by bisulfite was amplified with methylation-specific primers of *CDX2*. The methylation-specific primers were: 5′-TTTTCGTGTTTTTCGGTAGTTTTTAGC-3′ (methylation forward primer, MF) and 5′-ACTCACGTACATAATAACGAAAATCCG-3′ (methylation reverse primer, MR). The unmethylation-specific primers were: 5′-TTTTTTGTGTTTTTTGGTAGTTTTTAGT-3′ (unmethylation forward primer, UF) and 5′-TAACTCACATACATAATAACAAAAATCCA-3′ (unmethylation reverse primer, UR). The MSP products were analyzed with 3% agarose gels. All the MSP procedures were performed more than twice.

Endpoints and follow-up

The primary endpoint was death from any cause. A 5-year follow-up on all participants was performed by a research nurse or dedicated physician. Information about any clinical events was obtained by telephone contact and/or direct interview and/or medical records. Disease-free survival (DFS) was also analyzed to explain the prognostic role of *CDX2* methylation.

Statistical analysis

All data were analyzed with SPSS 18.0 software. The difference in categorical variables between methylation and unmethylation status was compared by the χ^2 test. The Kaplan-Meier method was adopted to analyze cumulative probability of survival and statistical significance was determined with the log-rank test. Univariate and multivariate Cox analysis was performed to investigate whether the participants' characteristics (age, gender, smoking status, TNM, histology and *CDX2*) could predict the prognosis of overall survival (OS). *P*-value less than 0.05 indicated statistically significant difference.

Results

Basic information of patients with lung cancer

The MSP method was used to detect the methylation status of *CDX2* of all patients. The patients were classified into two groups according to *CDX2* status: methylation (n = 75) and unmethylation (n = 92) groups. The results indicated that there

Table I. Clinicopathological characteristics of all participants

Characteristic	*CDX2* status		*P*-value
	Methylation, *n* (%)	Unmethylation, *n* (%)	
No.	75 (44.91)	92 (55.09)	
Age [years]			
< 60	44 (58.67)	52 (56.52)	0.780[1]
≥ 60	31 (41.33)	40 (43.48)	
Gender			
Male	40 (53.33)	58 (63.04)	0.205[1]
Female	35 (46.67)	34 (36.96)	
Smoking status			
Never	21 (28.00)	27 (29.35)	0.434[1]
Current	7 (9.33)	4 (4.35)	
Former	47 (62.67)	61 (66.30)	
TNM			
I	19 (25.33)	25 (27.17)	0.825[1]
II	24 (32.00)	30 (32.61)	
III	31 (41.33)	34 (36.96)	
IV	1 (1.33)	3 (3.26)	
Histology			
Squamous cell carcinoma	38 (50.67)	45 (48.91)	0.897[1]
Adenocarcinoma	22 (29.33)	30 (32.61)	
Small-cell lung cancer	15 (20.00)	17 (18.48)	

[1]χ^2 *test, TNM – tumor, nodes, metastases, p < 0.05 indicates a significant difference.*

were no significant differences in age, gender, smoking status, TNM, and histology between two groups ($p > 0.05$, Table I).

Kaplan-Meier analysis

The Kaplan-Meier curve is shown in Figure 1. There were 33 deaths among patients with methylation of *CDX2* and 14 deaths among patients with unmethylation. After the 5-year follow-up, the survival rate of patients with methylation of *CDX2* was 56%, while the survival rate of patients with unmethylation of *CDX2* was 84.8%. The outcome indicated that the survival rate of patients with unmethylation of *CDX2* was significantly higher than in those with methylation of *CDX2* (log-rank: $p = 0.000$). In addition, we plotted the Kaplan-Meier curve for patients who smoke currently or had ever smoked (Figure 2). Among the smoking patients, methylation of *CDX2* resulted in a much lower survival rate (50.9%) compared to unmethylation of *CDX2* (84.6%) (log-rank: $p = 0.000$). As shown in Figure 3, DFS of patients with *CDX2* methylation was lower than in those without *CDX2* methylation (56.0% vs. 73.9%, $p = 0.009$).

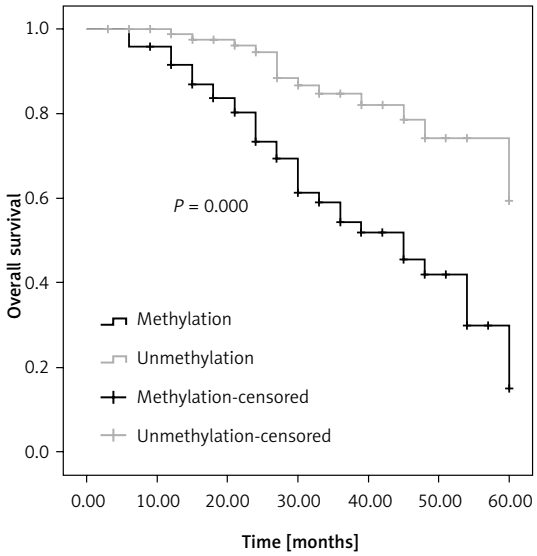

Figure 1. Overall survival rate of patients grouped by methylation status of *CDX2*

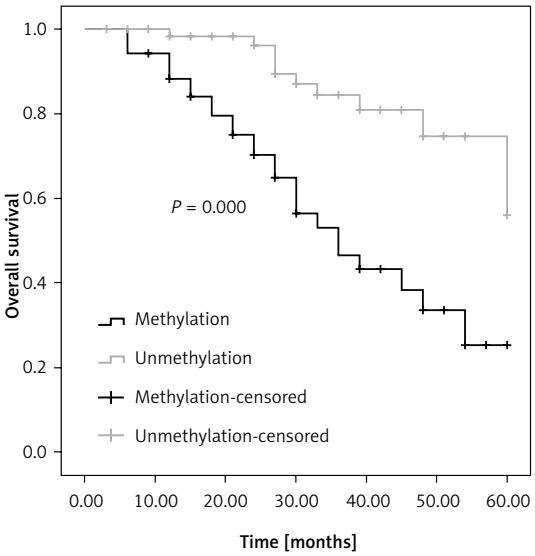

Figure 2. Overall survival rate of smoking patients grouped by methylation status of *CDX2*

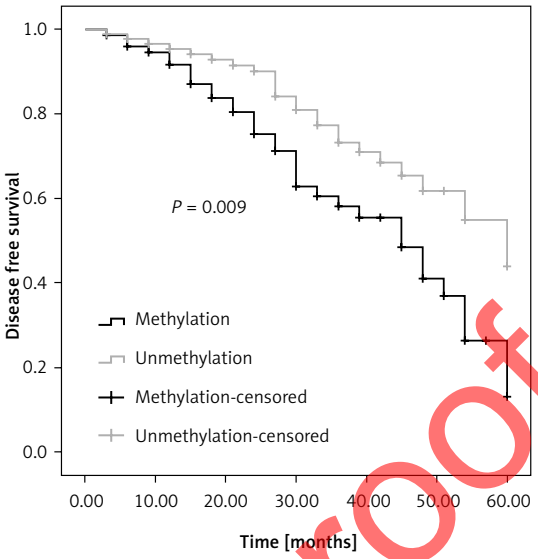

Figure 3. Disease-free survival of patients grouped by methylation status of *CDX2*

Table II. Univariate Cox analysis

Variables	HR	95% CI	P-value
Age (≥ 60 vs. < 60)	0.707	0.382–1.307	0.268
Gender (female vs. male)	0.835	0.432–1.617	
Smoking			
Never	Reference	Reference	
Current	1.678	0.361–7.789	0.509
Former	1.345	0.644–2.810	0.430
TNM			
I	Reference	Reference	
II	1.566	0.710–3.452	0.266
III	1.181	0.532–2.621	0.683
IV	2.612	0.533–12.797	0.236
Histology			
Squamous cell carcinoma	Reference	Reference	
Adenocarcinoma	1.103	0.546–2.230	0.784
Small-cell lung cancer	0.792	0.334–1.880	0.597
CDX2 (methylation vs. unmethylation)	3.705	1.922–7.139	< 0.001

TNM – tumor, nodes, metastases, HR – hazard ratio, CI – confidence interval. P < 0.05 indicates a significant difference.

Table III. Multivariate Cox analysis (logistic regression)

Variables	HR	95% CI	*P*-value
CDX2 (methylation vs. unmethylation)	3.418	1.826–6.397	< 0.001

HR – hazard ratio, CI – confidence interval. P < 0.05 indicates a significant difference.

Univariate and multivariate Cox analysis

Univariate and multivariate Cox analyses were performed to analyze the potential predictors for prognosis of patients with lung cancer. In univariate Cox analysis, we found that age, gender, smoking, TNM, and histology were all not independent predictors for prognosis of patients with lung cancer (Table II).

Further analysis by the multivariate Cox method indicated that *CDX2* methylation served as an independent prognostic predictor (HR = 3.418, 95% CI = 1.826–6.397) (Table III).

Discussion

CDX2 expression is a marker of intestinal differentiation, which is expressed in any gastrointestinal cancer [27]. According to accumulated evidence, *CDX2* is generally used as a specific marker for adenocarcinoma of the lower digestive tract [28–31]. It plays an important role in some relevant digestive system cancers, including gastric and colon cancer. Recent studies adopted the methods of real-time PCR and immunohistochemistry and found that *CDX2* expression was detectable in primary lung adenocarcinomas except for the metastasis of colorectal origin [32, 33]. In addition, *CDX2* mRNA expression could be detected in normal lung tissues of patients with non-small-cell lung cancer (NSCLC), the level of which is much lower compared to the matched tumor tissues. Downregulation of the *CDX2* gene may result in the loss of relevant differentiation function and it may interact with other genes in the pathogenesis of relevant tumors.

It is well known that increased DNA methylation within the promoter regions of tumor suppressor genes has been associated with gene silencing in various cancers. It was reported that *CDX2* methylation is frequently present in colorectal cancers and may play a key role in inactivating *CDX2* expression [34]. The study by Wang *et al.* found that the methylation rate of the promoter region of the *CDX2* gene in normal colorectal tissue was 43.5%, whereas that in the lesion tissue of CRC was 78.5% [35]. Liu *et al.* reported that *CDX2* is frequently methylated in lung cancer, and expression of *CDX2* is regulated by promoter region hypermethylation [25].

A previous study by Jiang *et al.* reported that enhanced *CDX2* promoter methylation is associated with enhanced lymph node metastases and shorter survival time in colorectal cancer [26]. Our study detected the methylation status of *CDX2* in patients with lung cancer with the MSP method. The results showed that the methylated rate was 44.9%. The Kaplan-Meier curve showed that methylation of *CDX2* was related to poor prognosis of patients with lung cancer. Among the smoking patients, methylation of *CDX2* resulted in a much lower survival rate (50.9%) compared to unmethylation of *CDX2* (84.6%). DFS of patients with *CDX2* methylation was lower than in those without *CDX2* methylation (56.0% vs. 73.9%, $p = 0.009$). Univariate and multivariate Cox analyses indicated that *CDX2* methylation was an independent predictor for prognosis of patients with lung cancer. The results were similar to those of a study by Bae *et al.* on colorectal cancer [36].

The study analyzed the prognostic role of *CDX2* methylation in lung cancer among a Chinese population. The results were credible and reliable, but there were several limitations in the study. The functional mechanism of *CDX2* in lung cancer, which contributes to revealing the pathogenesis of lung cancer, was not explored. In addition, the related factors of *CDX2* methylation, which might provide potential therapeutic targets for lung cancer, were not analyzed.

In conclusion, *CDX2* methylation may serve as an independent predictor for poor prognosis of patients with lung cancer.

Conflict of interest

The authors declare no conflict of interest.

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