The relationship of EZH2 and HOXA5 with non-small cell lung carcinoma patient survival rate

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Background: The primary cause of cancer-related death globally is non-small cell lung cancer (NSCLC). Our objectives were to show the expression and distribution of EZH2 and homeobox A5 (HOXA5) in 194 human NSCLC tissues, as well as to evaluate the expression of EZH2 and HOXA5 with patients' survival rate. We also aimed to determine the statistical correlations of GST-ϖ and survivin expression with chemotherapy and survival rate.

Method: A total of 194 patients with NSCLC and 18 standard controls were included. Immunohistochemistry was used to detect the EZH2, HOXA5, GST-ϖ, and survivin expression. The expression of EZH2, HOXA5, GST-ϖ and Survivin with clinicopathological features in NSCLC patients was assessed and the prognostic value of EZH2, HOXA5, GST-ϖ and Survivin expression was evaluated using a Kaplan-Meier curve and log-rank analysis.

Results: The results showed that EZH2 and Survivin were not detected in normal lung tissues, but for the HOXA5, the ratio of expression in adenocarcinoma reached up to 80%, and up to 75% in squamous carcinoma. NSCLC patients with late stage TNM (III, IV) had a relatively high EZH2 expression compared to those with early stage (I, II) (P<0.01), and patients with lymphatic metastasis had a higher EZH2 expression compared to those with no metastasis (P<0.01). Cox analysis revealed that an independent biomarker for forecasting poor overall survival (OS) in NSCLC patients was a strong HOXA5 and Survivin combination.

Conclusions: EZH2 improves NSCLC cells' metastatic ability and that HOXA5 and Survivin could be a potential biomarker for diagnostic and prognostic use in patients with NSCLC.

Keywords: Non-small cell lung cancer (NSCLC); EZH2; homeobox A5 (HOXA5); survivin

Introduction

Including adenocarcinoma and squamous cell carcinoma, non-small cell lung cancer (NSCLC) is the primary type of lung cancer responsible for most cancer deaths worldwide (1). Despite recent advancements in surgical and experimental oncology, lung cancer prognosis remains poor, with an average 5-year survival rate of about 11 percent (2). Clinically, NSCLC shows a wide range of behavioral habits that vary from slowly advancing to quickly advancing, and may be extremely metastatic or only invasive locally. The molecular origin of these behavioral differences, however, is not well understood. In 194 cases of NSCLC tumors, the sequences of 4 proteins were analyzed immunohistochemically to identify a series of prognostic factors that can be readily tested with high effectiveness in clinical practice, and then compared with clinical outcomes.

EZH2 is a polycomb repressor complex 2 (PRC2), which catalyzes lysine 27 (H3K27me) methylation of histone H3 and mediates target gene silencing via local chromatin reorganization. Numerous pieces of evidence indicate that EZH2 plays a critical role in the development, growth, and metastasis of cancer (3-5). Recent studies have shown that EZH2 leads to the development of NSCLC (6) and accompanies poor survival (7,8). It has also been shown that EZH2 controls the intrinsic expression of multidrug resistance gene 1 (MDR1), indicating its contribution to drug resistance (9,10). The fundamental molecular events associated with increased expression of EZH2 with cancer metastasis and poor prognosis, however, remain unclear.

Homeobox genes constitute a family of regulatory genes comprising a standard sequence of 183 nucleotides (homeobox) and encoding unique nuclear proteins (homeoproteins) acting as transcription factors (11). HOX genes are called the clustered group of homeobox genes in humans. Homeobox A5 (HOXA5) is a master morphogenesis and cell differentiation regulator that is active in breast cancer as a tumor suppressor gene (12). It is shown that the deregulated expression(s) of a specific HOX gene(s) is associated with cancer growth and progression, such as invasion and metastasis (13). Several studies have recently reported that HOXA5 plays an important role in tumorigenesis in NSCLC by directly controlling the expression of p21 (14). Nevertheless, the biological function and therapeutic importance of HOXA5 is still not well known in the development and advancement of NSCLC and should be further studied.

Some studies report that the expression of Gst-π and survivin in NSCLC promotes multidrug-resistance and inhibits apoptosis.

However, little is known about the role of EZH2, HOXA5, GST-π, and survivin expression in NSCLC tissues, especially in the survival of lung cancer patients at present. This study is likely to aid in clarifying the correlations of EZH2, HOXA5, GST-π, and survivin expression, and clinical features with the overall survival (OS) and prognosis in patients with lung cancer.

Methods

Patients and tumor specimens

A total of 194 patients who underwent complete tumor resection from 2010 to 2013 were chosen for this research at the Harbin Medical University, China. Written informed consent was received from each patient and tissue specimens were prepared according to the Harbin Medical University Ethics Committee guidelines. After tumor removal in 2018, the OS rate was calculated. Specimens from these patients were collected from the University of Harbin Medical University's Department of Pathology and the Department of Thoracic Surgery. The study also included 18 matched normal lung tissues (10 cm from the tumor) from the 194 specimens. As defined in *Table 1*, the clinical pathological

Table 1 Expression of EZH2, HOXA5, survivin, and GST-π in lung tissues (cases)

Gene	Normal tissues			Adenocarcinoma			Squamous carcinoma		
	n	+	Positive (%)	n	+	Positive (%)	n	+	Positive (%)
EZH2	5	0	0	37	22	59.5	37	23	62.2
HOXA5	5	4	80.0	37	14	37.8	42	15	35.7
Survivin	4	0	0	10	3	30.0	11	3	27.3
GST-π	4	3	75.0	8	5	62.5	12	12	100.0

HOXA5, homeobox A5.

characteristics of patients is based on the requirements of the World Health Organization (WHO) (14).

Patients aged 28–82, with a median age of 58 years, and complete clinical pathological data with follow-up to December 2, 2013, were included.

Immunohistochemical analysis

Paraffin-embedded, formalin-fixed tissues were immunostained for EZH2, HOXA5, survivin, and GST-π to determine the expression levels of EZH2, HOXA5, survivin and GST-π in NSCLC tissues. Immunohistochemical staining was conducted overnight at 4 ℃ with a rabbit anti-HOXA5 antibody (1:100, Bioss, Beijing) on an automated staining system (TechMate 500, DakoCytomation). For immunohistochemical measurement of EZH2, HOXA5, survivin, and GST-π expression, the signal was amplified and visualized with diaminobenzidine chromogen, followed by counterstaining with hematoxylin. For EZH2, HOXA5, survivin and GST-π, an immunohistochemistry (IHC) score of 2+ or more was defined as positive, and IHC scores of 0 and 1+ were defined as negative.

Statistical processing SPSS 19.0 statistical software for data processing was used. The same index expression in different organizations and relationships with different clinical pathological characteristics were analyzed using a chi-square test. Using EZH2, HOXA5 multivariable logistic regression analysis, the influence factors of survivin and GST-PI-positive expression and the correlation of protein expression was calculated using the Spearman correlation analysis. Inspection level of alpha =0.05.

Results

EZH2, HOXA5, and survivin expression in normal lung tissues and NSCLC tissues

EZH2, HOXA5, survivin, and GST-π were expressed in NSCLC tissues and normal lung tissue, as shown in *Table 1*. Using χ^2 test two comparison, EZH2 and HOXA5 showed significant difference (P<0.05), but there were obvious differences between lung adenocarcinoma and squamous carcinomas (P>0.05); survivin showed significant difference in normal lung tissue and NSCLC tissues (P>0.05), while GST-π showed no obvious difference between normal lung and NSCLC tissues (P>0.05), but there was a significant difference between lung adenocarcinoma and squamous carcinoma (P<0.05).

The relationship of EZH2, HOXA5, GST-π, and survivin expression with patient survival

The association between EZH2, HOXA5, GST-π, and survivin expression with patient survival was assessed. Kaplan-Meier survival analysis was conducted to investigate the correlation between EZH2, HOXA5, GST-π, and survivin expression and NSCLC patient prognosis. The EZH2 positive expression of lung cancer patients survival time was 59.0 months on average, and the negative expression in lung cancer patients with an average survival time was 66.5 months; HOXA5 positive expression of lung cancer patients survival time was 61.0 months on average, and negative expression in lung cancer patients with an average survival time was 43.0 months; survivin positive expression of lung cancer patients survival time was 40.5 months on average, and the negative expression in lung cancer patients with an average survival time was 21.3 months; GST-PI-positive expression in lung cancer patients with an average survival time was 69.0 months, and the average negative expression in lung cancer patients survival time was 66.5 months. HOXA5 and survivin expression was associated with the disease-free survival (DFS) and the OS of lung cancer patients (P<0.05, *Figure 1*).

EZH2, HOXA5, survivin, and GST-PI relationship expression and clinicopathological characteristics of lung cancer

EZH2 expression and patients' gender, age, smoking history, gross tumor type, and differentiation were not significantly different (P>0.05), and were associated with lymph node metastasis and TNM stages (P<0.01); HOXA5, GST-PI expression and patients' gender, age, smoking history, gross tumor type, degree of differentiation, lymph node metastasis and TNM stages were not significantly different (P>0.05); survivin expression was related to tumor TNM stage (P<0.05), while patients' gender, age, smoking history, gross tumor type, degree of differentiation, lymph node metastasis, and other factors were not significantly different (P>0.05, *Table 2*).

The results of cox model analysis

Cox model single-factor and multiple-factor regression analyses of gender, age, smoking history gross tumor type, degree of tumor differentiation, lymph node metastasis, and TNM stage of patients with lung cancer were performed.
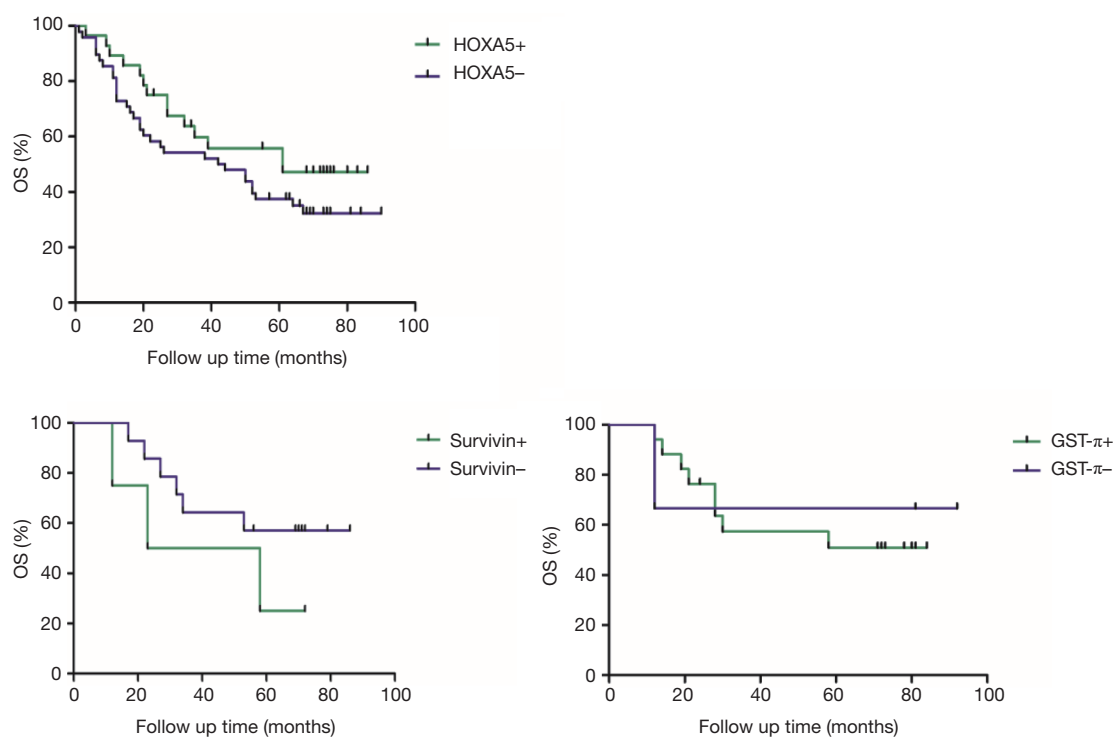
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Figure 1 Patient survival. Kaplan-Meier survival analysis (P<0.05). HOXA5, homeobox A5.

The results show that gross tumor type and gender were independent factors for survival in patients with lung cancer (*Table 3*).

Discussion

We detected the expression of EZH2, HOXA5, and SURVIVIN in NSCLC by IHC, and we found that there were significant differences in the expression of EZH2 and HOXA5 in normal lung tissue, adenocarcinoma, and squamous cell carcinoma (P<0.05). However, there was no significant difference in the expression between adenocarcinoma and squamous cell carcinoma (P>0.05). There were significant differences in expression of survivin in normal lung tissue, adenocarcinoma, and squamous cell carcinoma (P<0.05); however, the expression of adenocarcinoma and squamous cell carcinoma (P>0.05) was not significantly different. Studies have shown that abnormal expression of HOXA5 plays an important role in the development of NSCLC, and is differently expressed in different tissues. The expression of HOXA5 is down-regulated by DNA methylation in cancer, which is related to tumor stage, tumor size, and lymph node metastasis (8).

EZH2 is carcinogenic, even though it is overexpressed in many human cancers (9), the regulation of expression and the role in NSCLC is still unclear. Our experiments further identified these results and found that the expression of HOXA5 in normal tissues was significantly higher than that in lung cancer; conversely, the expression of EZH2 in lung cancer was significantly higher than that in normal lung tissues.

We analyzed the relationship between the expression of EZH2, HOXA5, and survivin, with the clinicopathological characteristics of NSCLC. The expression of EZH2 was related to lymph node metastasis and TNM stage (P<0.01); the expression of HOXA5 was not related to gender, age, smoking history, general classification, degree of differentiation, lymph node metastasis, or TNM stage (P>0.05); the expression of survivin was related to lymph node metastasis, TNM stage (P<0.05), but not to gender, age, smoking history, general classification, or degree of differentiation (P>0.05, *Table 2*). Similarly, it is (9) found that EZH2 was related to tumor stage and grade, tumor size, and lymph node metastasis in renal clear cell carcinoma. EZH2 can thus be used as a clinical factor to predict the prognosis and progress of renal clear cell

Table 2 Correlation between the expression of EZH2, HOXA5, survivin, and GST-π and clinical significance in lung cancer

Parameter	EZH2					HOXA5					Survivin					GSTP1				
	n	+	−	χ^2	P	n	+	−	χ^2	P	n	+	−	χ^2	P	n	+	−	χ^2	P
Gender																				
Male	58	34	24	0.575	0.448	55	18	37	1.235	0.266	14	3	11	0.263	0.608	16	15	1	1.985	0.159
Female	16	13	3			24	11	13			7	3	4			4	2	2		
Age																				
≤50	17	12	5	0.885	0.347	18	6	12	0.114	0.735	3	0	3	0.243	0.622	3	2	1	0.008	0.930
>50	57	33	24			61	23	38			18	6	12			17	15	2		
Smoking use																				
Yes	50	31	19	0.002	0.967	46	13	33	3.383	0.066	13	3	10	0.045	0.831	13	12	1	0.349	0.555
No	24	15	9			33	16	17			8	3	5			7	5	2		
Tumor differentiation																				
Well	11	9	2	2.122	0.145	11	4	7	0.001	0.980	1	0	1	0.420	0.517	8	8	0	0.801	0.371
Moderate poor	63	37	26			68	25	43			20	6	14			12	9	3		
Lymph node metastasis																				
Yes	31	27	4	12.804	0.000**	33	10	23	1.001	0.317	11	5	6	3.226	0.072	8	7	1	0.067	0.796
No	43	20	23			46	19	27			10	1	9			12	10	2		
TNM stage																				
I–II	51	25	26	12.050	0.001**	58	22	36	0.140	0.708	18	3	15	5.143	0.023*	17	14	3	1.064	0.302
III–IV	23	21				21	7	14			3	3	0			3	3	0		

*, P<0.05; **, P<0.01.

Table 3 Cox model analysis

Factors	Categories	Multivariate	
		OR (95% CI)	P
Gender	Male/female	1.094 (0.513–2.331)	0.816
Age	≤50/>50	0.611 (0.324–1.151)	0.127
Smoking use	Yes/no	0.785 (0.393–1.568)	0.492
Tumor differentiation	Well/moderate-poor	0.847 (0.344–2.087)	0.718
Lymph node metastasis	No/yes	0.410 (0.187–0.902)	0.027*
TNM stage	I–II/III–IV	2.665 (0.854–5.681)	0.197
EZH2 expression	Negative/positive	0.248 (0.066–0.935)	0.040*
HOXA5 expression	Negative/positive	0.274 (0.086–0.870)	0.028*
Survivin expression	Negative/positive	0.460 (0.153–1.379)	0.166

*, P<0.05.

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carcinoma. This result was consistent with our own.

Using the Kaplan-Meier method, the survival of patients with EZH2 expression was shown to be significantly shorter than that of patients without EZH2 expression, whereas the survival of patients with HOXA5 expression was significantly longer than that of patients without HOXA5 expression. The survival of patients with a high expression of survivin was significantly shortened. Tang *et al.* (10) found that HOXA5 gene methylation indicated the shortened survival of patients and a reduced survival rate, but it was not as a single risk factor of prognosis. Geng *et al.* (6) found that high expression of EZH2 suggested poor prognosis and low survival of patients with lung cancer and was closely related to tumor size, high expression of VEGF-A, and activation of AKE pathway.

Using the Cox regression model, we analyzed patients' gender, age, smoking status, tumor differentiation, lymph node metastasis, and TNM stage.

Some studies found that HOXA5 was a tumor suppressor gene, and that the abnormal expression of HOXA5 gene inhibited cell invasion, cell metastasis, and pseudopodia formation in highly invasive cancer. HOXA5 impacts metastasis in a variety of cancer cells. For instance, there is an HOXA5 binding site in the P53 promoter region, while transient transfection of HOX/HOXA5 was found to activate the P53 promoter; therefore, the expression of HOXA5 in epithelial cell carcinoma promotes the expression of wild-type P53 (11). Evidence shows that the absence of P53 in breast cancer might be due to the loss of expression HOXA5. The expression of HOXA5 can also be used as a marker for acute myeloid leukemia (AML), and it may have some effects on the prognosis of AML patients. HOXA5 also had an impact on multi-drug resistance in small cell lung cancer and may be a therapeutic target for SCLC (12). While there is a low expression of HOXA5 in stationary endothelial cells, it is highly expressed in an active reproductive vascular endothelial cell. In one study, sustaining the expression of HOXA5 regulated the expression of effector molecules of neovascularization, significantly increased the expression of TSP-2, reduced the expression of VEGF, and inhibited tumor neovascularization. Active HOXA5 in endothelial cells decreased the incidence of tumor inflammation, reduced neovascularization, and inhibited tumor growth (13).

In summary, in the study of NSCLC, the expression of EZH2, HOXA5, and survivin may be useful for predicting the prognosis and progression of NSCLC, and may form a theoretical basis for the further study of anti-NSCLC targeted therapy.

Footnote

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