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Research

VHL expression level in the pathological tissue is significantly associated with clinical outcomes of platinum-based chemotherapy in non-small cell lung cancer patients.

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Abstract

Objective: To investigate the association between tissue expression level of the Von Hippel-Lindau (VHL) suppressor gene and outcome of platinum-based chemotherapy in non-small cell lung cancer (NSCLC) patients.

Material and Methods: Pathological samples of NSCLC patients were investigated for immunohistochemical analysis of VHL expression levels. The clinical data of patients were taken from the medical records. The prognosis was traced by phone. Chi-square, logistic regression and log-rank tests served for correlation analysis between gene expression levels and clinical data.

Results: A total of 110 NSCLC patients were enrolled in this study, with a median follow-up time of 27.5 months and a median survival time of 24.3 months. 31 patients died during the follow up period (January 2016 – November 2020), 59 patients (53.6%, cohort I) presented with negative or weak and 51 patients (46.4%, cohort II) with moderate or high VHL expression levels. Neutropenia after platinum-based chemotherapy occurred more frequently in cohort I patients in comparison to cohort II

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patients (OR=0.264, 95% CI=0.085-0.818, *P*-value=0.021). VHL expression level was significantly correlated with the overall survival OS (Logrank test : *P*-value=0.007, HR= 4.219, 95% CI: 1.75-10.174, *P*-value=0.001); however not correlated with the disease free survival DFS (Logrank test : *P*-value=0.256, HR= 1.334, 95% CI: 0.642-2.769, *P*-value=0.440).

Conclusion: VHL expression level of NCLC is related with granulocytopenia and leukocytotoxicity after platinum-based chemotherapy. It might be used as a biomarker to predict the risk of neutropenia and the prognosis of NSCLC patients.

Keywords: VHL, NSCLC, platinum-based chemotherapy, survival.

Introduction

Lung cancer is still a leader in cancer related morbidity and mortality world-wide. Non-small cell lung cancer (NSCLC) remains the dominating lung cancer cell type and accounts for about 85% of total lung cancer cases [1, 2].

Limited tumor stages are rarely diagnosed in NSCLC patients and potential curative surgery is often impossible. Therefore, chemotherapy is required to prolong the patients' survival time. Recently, adjuvant cancer therapy based upon programmed cellular death molecule-1 (PD-1) and its ligand (PD-L1) has significantly improved the survival time of NSCLC patients [3].

However, the promotion of immunological therapy is restricted by its low objective response rate and its high expenses in China. Furthermore, platinum-based chemotherapy is often combined with immunotherapy, and platinum-based chemotherapy is also the first-line in treatment of lung cancer. Therefore, the investigations on the platinum-based chemotherapy in cancer treatment are still urgently needed. [1, 4, 5]

Chemotherapeutic platinum-based substances of NSCLC mainly kill tumor cells by DNA damage, which, in addition inevitably damages normal cells. [4, 5, 12]. The most common side effects in clinical are hematotoxicity and gastrointestinal reactions. Hematotoxicity, including neutropenia and leukopenia is the most common toxicity in platinum chemotherapy. It affects the quality of NSCLC patients' life and possesses great impact on the performance of treatment.

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VHL is a classic tumor suppressor gene, located on the short arm of chromosome 3 (3p25–26) [7]. Several investigations report that VHL has the ability to influence the origin and metastasis of carcinomas by regulating the HIF pathway.[6-9]. They demonstrated that lack of VHL might induce cellular resistance against chemotherapy of different carcinomas, including clear-cell renal cell carcinoma (ccRCC) and other solid tumors [7, 10]. However, little is known about the correlation between NSCLC chemotherapy and the expression of the VHL gene.[7-10]

The current study aims to investigate the association between Von Hippel-Lindau (VHL) gene expression and the impact of platinum-based chemotherapy in NSCLC patients. In detail, we evaluated the VHL gene expression in cancer tissue of NSCLC patients by IHC analysis. Taking into account that biomarkers, which predict the risk of hematotoxicity after platinum-based chemotherapy are important in clinical application the results of our study might display with significant implications on the treatment of NSCLC patients.

Materials and methods

Ethical approval

Our study procedures are in agreement with the ethical standards of the Research Committee of the Shanghai East Hospital and are consistent with the ethical standards of the 1964 Helsinki Declaration and its revisions. All study patients were notified and approved its performance. Animals were used in this research. The laboratory experiments were conducted in accordance with approved standard guidelines and regulations.

Study cohorts

In this study, 110 patients who accepted the first-line platinum-based double blind chemotherapy protocol were recruited from January 2016 to July 2020. All patients underwent surgery in the Shanghai East Hospital from 2016 to 2020. The NSCLC were microscopically diagnosed and approved prior to the chemotherapy.

Treatment

All 110 NSCLC patients were treated with platinum-based chemotherapy which targets damage of double-DNA string (cisplatin or nedaplatin in combination with gemcitabine or pemetrexed) and platinum-tubulin structures (cisplatin or nedaplatin in combination with docetaxel). The substances were applied every three weeks for two

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to four cycles. The dosages of cisplatin and nedaplatin were 75 mg/m^2 on day 2, and 100mg/m^2 on day 2. The dosages of gemcitabine and pemetrexed were 1000mg/m^2 on day 1 and day 8 and 500mg/m^2 on day 1. The dosages of docetaxel were 75mg/m^2 on day 2.

Specimen collection and immunohistochemistry assay

The tissue samples in our study were taken from NSCLC patients who underwent potential curative surgery in the Shanghai East Hospital from 2016 to 2020. Immunohistochemistry using the streptavidin-peroxidase（SP）visualization technique was used to detect pVHL the carcinoma sections (Figure 1).

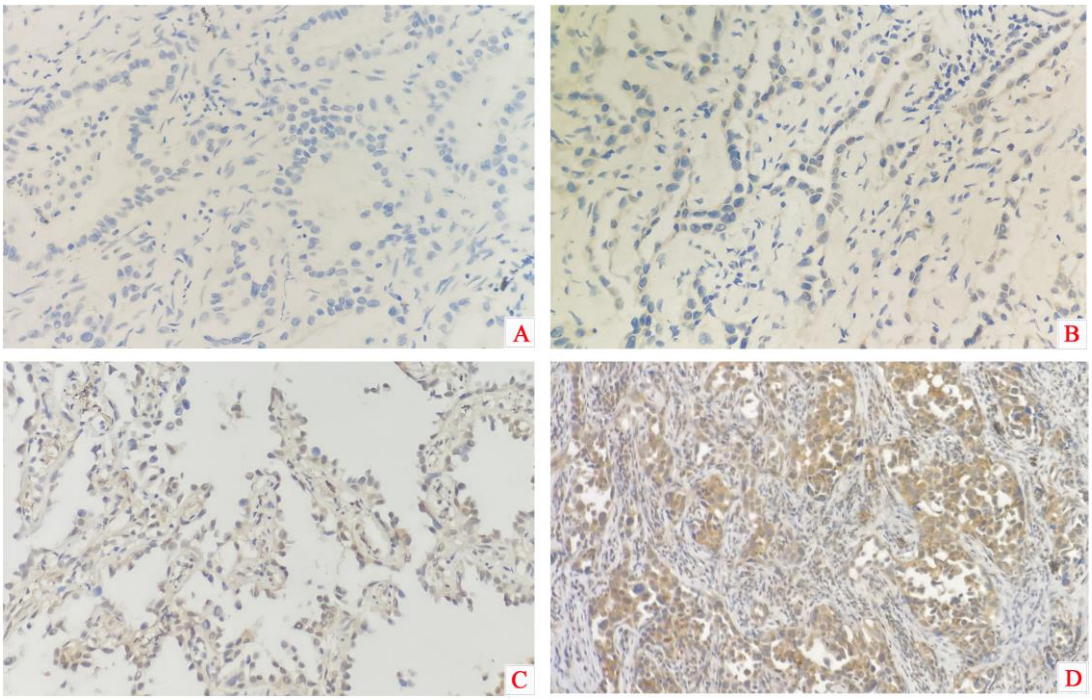

Figure1: Representative IHC staining for VHL expression in non-small cell lung cancer (NSCLC). A, negative expression of VHL in tumor tissue. B, Weak expression of VHL. C, Moderate expression of VHL D, High expression of VHL., ×200

The paraffin sections were dewaxed and dehydrated, repaired under high pressure, sealed with hydrogen peroxide, incubated with the primary antibody in a refrigerator at 4°C, incubated with secondary antibody and the 3,3-diaminobenzidine (DAB) was used as a chromogen. The specimen were restained with hematoxylin, differentiated

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with hydrochloric acid alcohol, and sealed with neutral gum. Pathologists of the Shanghai East Hospital evaluated the slides by semi-quantitative interactive counting.

Toxicity

Chemotherapeutic toxicities were evaluated every third week and classified in accordance to the guidelines of the National Cancer Institute (NCI) [19]. No side effects and Grade 1 were classified negative and weakly positive respectively. The grades 2, 3 and 4 were summarized into severe. Grade 5 (death) did not occur in our study.

Statistical analysis

We used the SPSS version 26.0 software to analyze the data. Chi-square tests served for illustrating the significance between the VHL expression level and the hematotoxicity of platinum-based chemotherapy. Logistic regression, Kaplan-Meier curves and Cox regression analysis were applied to visualize the relationship between the VHL expression level and the patients' OS/PFS. Probabilities $P<0.05$ were considered statistically significant.

Results

Clinical characteristics of patients

The clinical characteristics of the 110 NSCLC patients are summarized in Table 1. 59 patients (53.6%) had negative or weak VHL expression levels in their carcinomas, and 51 patients (46.4%) presented moderate or high expression. Only the age ($P=0.024$) and none of the other clinical parameters was associated with the of VHL expression level at a statistically significant level.

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Table1.The clinical characteristics of 110 NSCLC patients and its relationship with the expression of VHL

Variables	N=110（%）	The expression of VHL		Chi-square	
		None or low expression,n(%)	Mid or high expression,n(%)	X^2	*P*-value
Age(years)				5,616	0,024
<60	35	13（11.8）	22（20）		
≥60	75	46（41.8）	29（26.4）		
Gender				0,117	0,840
Male	73	40（36.4）	33（30）		
Female	37	19（17.3）	18（16.3）		
Smoking status				0,058	0,831
Nonsmoker	81	44（40）	37（33.7）		
Smoker	29	15（13.6）	14（12.7）		
TNM				1,007	0,810
Ⅰ	33	19（17.3）	14（12.7）		
Ⅱ	20	12（10.8）	8（7.3）		
Ⅲ	39	19（17.3）	20（18.2）		
Ⅳ	T	9（8.2）	9（8.2）		
Tumor Histology				3,060	0,238
Squamous carcinoma	28	19（17.3）	9（8.2）		
adenocarcinoma	74	36（32.7）	38（34.6）		
Others[a]	8	4（3.6）	4（3.6）		
The size of tumor(cm)				2,961	0,126
<3	55	25（22.7）	30（27.3）		
≥3	55	34（30.9）	21（19.1）		

[a] Other carcinomas include neuroendocrine carcinoma and mixed carcinoma.
Abbreviations: TNM, tumor node metastasis.
All patients received racial surgery of lung cancer in Shanghai East Hospital.

Correlation of neutropenia with VHL expression levels

Occurrence of neutropenia and leukopenia in NSCLC patients who received platinum-b ased chemotherapy was statistically significantly correlated with lacking or weak VHL e xpression levels OR=0.264, 95% CI=0.085-0.818, *P*=0.021; and X2=5.044, *P*=0.025). The noted correlation of low VHL expression levels with the patients' gender (X2=4.632, *P*= 0.031) could not be confirmed by multivariate statistical analysis. The details are listed in Table 2.

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Association with progression-free survival (PFS) or overall survival (OS)

The relationship between the VHL expression levels and PFS or OS is depicted in (Figure 2). In total, 31 patients died during the observation period. The median survival time was calculated 24.3 months.

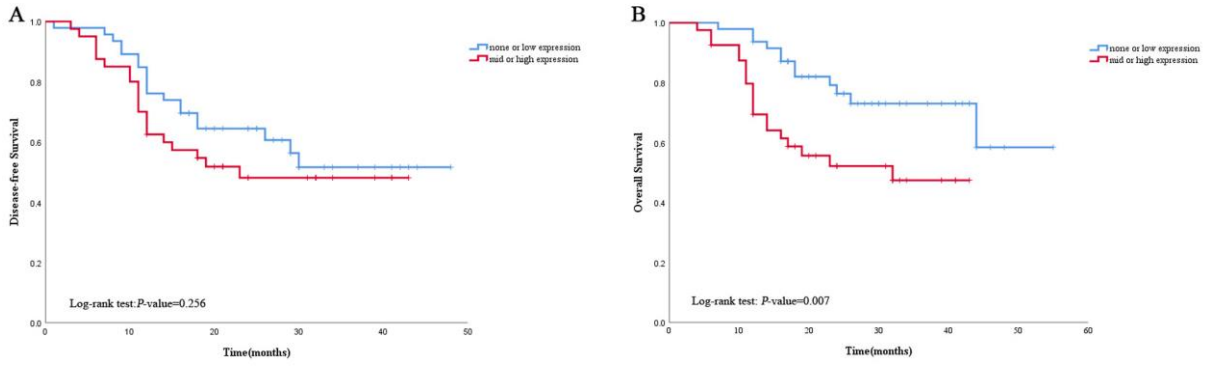

Figure2: Kaplan-Meier survival curves for patients with non-small cell lung cancer (NSCLC) according to VHL expression level. A, Disease-free Survival (DFS) of patients with non-small cell lung cancer (NSCLC) receiving platinum-based chemotherapy. B, Overall survival(OS) of patients with non-small cell lung cancer (NSCLC) receiving platinum-based chemotherapy.

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The VHL expression levels were statistically significantly correlated with OS (P=0.007), and not with PFS (P=0.256). Patients with high VHL expression levels presented with a poorer prognosis than those with negative or weak VHL expression levels (HR=4.219, 95% CI: 1.75-10.174, P=0.001).

As expected, patients with extended tumors (TNM stages) had a worse prognosis and a shorter PFS too (HR=4.96,95%, CI: 1.902-12.932, P=0.001; and HR=5.072, 95% CI: 2.101 -12.244, P<0.001).). The results are summarized in (Table 3).

Table3.Association Between VHL Expression and OS in Patients With Platinum-Based Chemotherapy.

		Univariate analysis			Multivariate analysis		
Variables	N=87	HR	95%CI	*P*- value	HR	95%CI	*P*- value
Age(years)							
<60	30	0,894	0.425-1.879	0,767	1,548	0.658-3.644	0,317
≥60	57						
Gender							
Male	54	0,630	0.296-1.342	0,231	0,748	0.299-1.869	0,534
Female	33						
Smoking status							
Nonsmoker	63	1,233	0.567-2.684	0,597	1,436	0.482-4.28	0,516
Smoker	24						
Tumor Histology							
Squamous carcinoma	19	0,510	0.255-1.019	0,057	0,503	0.232-1.089	0,081
Adenocarcinoma	63						
Others[a]	5						
TNM							
Ⅰ - Ⅱ	43	3,222	1.478-7.020	0.003*	4,96	1.902-12.932	0.001*
Ⅲ -Ⅳ	44						
The size of tumor(cm)							
<3	48	1,562	0.772-3.162	0,215	1,558	0.678-3.578	0,296
≥3	39						
The expression level of VHL							
none or low expression	47	2,631	1.250-5.537	0.011*	4,219	1.75-10.174	0.001*
mid or high expression	40						

a Other carcinomas include neuroendocrine carcinoma and mixed carcinoma.
*p<0.05. Abbreviations: HR, hazards ratio; CI, confidence interval; TNM, tumor node metastasis.
Tests of Model Coefficients,*P* -value=0.001
All patients received racial surgery of lung cancer in Shanghai East Hospital.

Discussion

The risk of granulocytosis for NSCLC patients who are treated with platinum-based chemotherapy is high and might become life threatening. The correlation of VHL expression levels in NSCLC tissue with leukopenia and neutropenia is an interesting result. It might be used to calculate the risk and forecast the occurrence of granulocytosis in patients treated with platinum – based chemotherapy. Granulocytosis is more likely to occur among NSCLC patients whose carcinomas display with lack of or weak VHL expression levels.

This phenomenon may be related to the regulation of VHL-mediated HIF pathway and its apoptosis downstream genes. The pathway is analogue to the mechanism of MMP2 gene mediating NSCLC granulocyte toxicity. [21, 22] and should be explored more in detail by application of digitized tissue sections (virtual slides) [23]. The observed correlation of VHL expression and gender with leukopenia should be explored more in detail by additional studies and increased cohorts of patients.

Yejun Cao, Lingwei Wang, Qiying Zhang, Yang Han, Guangxue Wang, Liang Ma, Jie Liu, Jinyi Wang, Zhengliang Sun, Tian Zhao, Guohan Chen,Xuan Hong
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Previous investigations reported that VHL is a classic tumor suppressor gene, which plays a significant role in the occurrence and progression of different cancer cell types. They include clear-cell renal cell carcinoma, and carcinomas of the colon and pancreas. [7-10, 13-18].

Analysis of the cellular pathways indicates that absence of VHL leads to a continuous accumulation of HIF-1α, which in return induces a continuous opening of the HIF pathway. [6-8]. It results in a continuous overexpression of downstream factors such as vascular endothelial growth factor (VEGF), which promotes increased vascularization of the normal tumor surrounding tissues and the production of erythropoietin (EPO). These factors ultimately evoke growth and proliferation and hinder the degradation of carcinoma cells [8].

In addition, the HIF pathway might become mediated by VHL and increase the resistance against multiple chemotherapeutic protocols and radiotherapy. [7, 16].

VHL is known to regulate the HIF pathway, and to induce an overexpression of the MDR (multi-drug resistance) gene, which increase the resistance against cisplatin, vinblastine, anthracycline, taxane and additional chemotherapies which address hypoxic carcinomas. The herein described correlation between the VHL gene expression, applied NSCLC chemo- radiotherapy and prognosis has rarely been reported. until today [10-13, 18].

Our findings that absent or poorly expressed VHL gene expression is more likely in NSCLC tissues of aged patients may support the findings of previous studies which report negative or weak VHL expression in tumor cells.

We also discovered that the expression of VHL was related to the neutropenia after platinum-based therapy (P=0.005), further multivariate regression analysis showed that lack or low expression of VHL increased the risk of neutropenia after platinum-based chemotherapy.

VHL expression levels are significantly correlated with DFS and OS. Patients who displayed with negative or weak expression in their tumor tissue lived longer than those who had moderate or high expression in the tissue. Therefore, the analysis of VHL expression in NSCLC tissue may be a useful tool to forecast response and survival of NSCLC patients. This is in agreement of a previous trial which report that the loss of VHL expression is correlated with the poor survival of lung adenocarcinoma patients [24]. This study includes a considerably larger cohort of patients who display with different pathological pattern when compared to the adenocarcinoma study.

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It could be speculated that the measurement of VHL polymorphisms in the serum might accurately predict hemotoxicity of platinum-based chemotherapy, because the VHL expression level could forecast the risk of neutropenia in platinum-based chemotherapy. Further studies are recommended to confirm the predictive results of this investigation.

In summary, severe neutropenia caused by platinum-based chemotherapy often result in life threatening infections affecting the patient's quality of life and subsequent treatment. In this study, we found that the biomarker VHL gene expression level in cancer tissues could be used to forecast the risk of neutropenia. The result is of great significance for individual platinum cancer patient therapies if a clinical transformation could be achieved. In addition, the analysis of VHL expression levels could forecast the prognosis of NSCLC patients who are treated with chemotherapy.

Authors' Note

Yejun Cao, Qiying Zhang, Liang Ma, Jie Liu, Jinyi Wan g,Zhengliang Sun,Guangxue Wang and Tian Zhao prepared the sample, Yejun Cao and Lingwei Wang carryed out the data analysis. Yang Han evaluated the result of immunohistochemistry. Yejun Cao, Lingwei Wang and Xuan Hong collected and checked the data of patients. Yejun Cao, Guohan Chen and Xuan Hong designed the project and wrote the manuscript.

Declaration of Conflicting Interests

The authors declare no conflict of interest.

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