Low Expression of SCN4B Correlated with DNA Hypermethylation and Poor Prognosis in Non-small Cell Lung Cancer

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Research Article

Keywords: Voltage-gated sodium channels β subunits 4, Non-small cell lung cancer, prognosis, methylation

Posted Date: March 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-250714/v1

Abstract

Background:Voltage-gated sodium channels β subunits 4 (SCN4B), a tumor suppressor, was previously reported to be associated with DNA methylation and poor prognosis in multiple cancers except lung cancer. This study aimed to explore whether the low expression of SCN4B was correlated with DNA methylation and clinical prognosis in non-small cell lung cancer (NSCLC) .

Methods:The gene expression profiles (GDS3837and GSE50081) were extracted from Gene Expression Omnibus (GEO). The differentially expressed genes (DEGs) analysis was performed to explore the expression of SCN4B in NSCLC tissue compared with normal tissue, with the cut-off value $p < 0.05$ and the absolute value of the log2 fold change ≥ 1.5. Immunohistochemistry staining was used to validated its expression using The Human Protein Atlas database. And MPRESS was used to analysis the relations of SCN4B expression between DNA methylation. Then, the Fisher exact and Wilcoxon rank-sum tests were used to calculate the associations of SCN4B expression with NSCLC clinicopathological features such as clinical grade and tumor node metastasis (TNM) stage, while Kaplan–Meier survival analysis and cox regression analysis were performed to estimate the prognostic value of SCN4B expression in NSCLC.

Results: Our DEGS analysis results showed a significantly decreased expression of SCN4B ($p=6.5e-22$) in NSCLC, which were validated by immunohistochemistry staining. Besides, this decreasing trend continued as the clinical grade and T stage advanced ($p<0.05$). There was a negative correlation between the SCN4B expression and DNA promoter methylation ($p<0.01$). Kaplan–Meier survival analysis indicated that NSCLC patients with low expression of SCN4B had a worse prognosis than those with high expression ($p < 0.004$). Meanwhile, univariate and multivariate analysis indicated SCN4B expression was an independent unfavorable prognostic factor for OS in NSCLC (Hazard Ratio= 0.236, $p = 0.009$; Hazard Ratio=0.219, $p = 0.003$, respectively).

Conclusions: SCN4B expression was significantly downregulated in NSCLC, which might be attributed to DNA promoter hypermethylation. The low expression of SCN4B indicated a potential unfavorable prognostic factor for NSCLC patients.

Introduction

Lung cancer remains the most common malignancy resulting in 1.59 million deaths globally per year estimated by the World Health Organization (WHO), exceeding those from any other malignancy worldwide [1]. As a heterogeneous group of tumors, lung cancer consists of more than 50 histomorphological subtypes, while non-small cell lung cancer (NSCLC) counts for approximately 80-90% of all lung cancers [2].

Clinically, only 16% of NSCLC patients are diagnosed at an early stage (grade I or II) and best cured by surgical resection [3]. Other patients with advanced NSCLC usually have poor prognosis with a 5- year overall survival (OS) rate at approximately 15-20% and a median survival of 17- 28 months even within

standard concurrent chemoradiation approaches using a platinum-based doublet regimen [4]. However, certain patients with metastatic NSCLC who are eligible for newer immunotherapies or targeted therapies such as anti-epidermal growth factor receptor (EGFR)/anaplastic lymphoma kinase (ALK) [5], are now have improved clinical outcomes with the 5-year survival rates increasing to 15% -50% , depending on biological markers [6]. It is obviously that the development of targeted therapies has advantage than the conventional chemo-and radiation-based therapy [7,8]. Therefore, to find new biological markers of NSCLC that can improve prognosis and serve as individualized targeted therapies in clinical practice is urgently needed.

Voltage-gated sodium channels β subunits 4 (SCN4B) was a subunit of voltage-gated sodium channels expressed in a variety type of cells [9,10]. Usually, the voltage-gated sodium channels β subunits are identified as auxiliary subunits to modulate the gating, kinetics, and to localized the ion channel pore, There are emerging studies found they are dysregulated in oncogenic processes [11]. Especially the expression of SCN4B was reported to be decreased in a variety of cancer cells, including (cervical, colorectal and prostate cancer cells[12-14]. In addition, the preserved SCN4B expression was regarded as an independent favorable prognosis in papillary thyroid cancer, while its expression might be suppressed by DNA hypermethylation [15]. These studies indicated SCN4B was a potential tumor suppressor [13,14]. Regarding that aberrant DNA methylation is well reported in NSCLC, however, the correlation of SCN4B expression with DNA methylation and clinical prognosis in NSCLC has not yet been reported.

Here, we explored the relations between the expression of SCN4B in NSCLC and DNA methylation, as well as NSCLC progression such as clinical grade, TNM stage and OS respectively based on the gene expression data of 181 NSCLC patient samples. It was demonstrated that SCN4B expression can serve as a biomarker for improving prognosis and targeted therapy of NSCLC.

Materials And Methods

Microarray data information

High-throughput gene expression data of patients with NSCLC were obtained from microarray dataset (GSE50081 and GDS3837) in Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) [16] . The GEO database is an international public repository that archives and freely distributes high-throughput gene expression and other functional genomics data sets [17].

In GSE50081 datasets, the expression profiling was performed on RNA from frozen, resected tumor tissues corresponding to 181 samples of patients in NSCLC. All the gene expression data derived from the Affymetrix Human Genome U133 Plus 2.0 Array platforms. All designs and quality control of the microarray experiment and data normalization were in line with the standard Affymetrix protocols. Clinical classification of these NSCLC patients was staged according to the American Joint Committee on Cancer (AJCC) clinical grade or Union for International Cancer Control (UICC) TNM system (8th edition) [18].

In GDS3837 datasets, there were 60 paired primary NSCLC tumor and adjacent normal lung tissue specimens obtained from nonsmoking female NSCLC patients.

Analysis the expression of *SCN4B in* NSCLC patients

The main differentially expressed genes (DEGs) of 60 paired NSCLC patients were analyzed by comparing the primary NSCLC tumor with adjacent normal lung tissue specimens extracted from dataset GDS3837. The Cut-off values was $p < 0.05$ and the absolute value of the log2 fold change ≥ 1.5 [19].

Immunohistochemistry staining validation of the *SCN4B* expression in lung cancer using The Human Protein Atlas database

Immunohistochemistry staining of the expression of SCN4B in normal lung tissue and lung cancer tissue was explored using The Human Protein Atlas (HPA, http://www.proteinatlas.org) and the key word for search strategy was “SCN4B” [20]. The HPA was a valuable tool constituting for researchers studying protein localization and expression in human tissues and cells [21].

Analysis the correlations of SCN4B expression between DNA promoter methylation

The analysis to confirm the relationship between SCN4B expression and DNA promoter methylation was performed using of MEXPRESS [20]. MEXPRESS was an online database for the integration and visualization of gene expression, DNA methylation and clinical data [22]. By default, the SCN4B expression value was selected in order of samples which contain lung tumor and normal tissue samples. The Pearson correlation analysis was then used to calculate the difference of SCN4B expression value between DNA promoter methylation data [20].

Analysis the expression of SCN4B at different clinical classification and characteristics

In GSE50081 dataset, 181 NSCLC patients were divided into different groups according their AJCC clinical grade, TNM stage, gender and age, respectively. We then compared the different expression level of SCN4B within these groups respectively using the Fisher exact or Wilcoxon rank-sum test [23]. Statistical significance was set at 0.05.

In addition, we validated the expression of SCN4B at different stage in lung cancer via Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/), which was a a web-based tool provides

key interactive and customizable functions including correlation analysis and patient survival analysis [24].

The Kaplan–Meier survival analysis of $SCN4B_{high}$ and $SCN4B_{low}$ group in NSCLC

The Kaplan–Meier survival analysis was used to estimate the effects of SCN4B expression on the OS of NSCLC. Patients with SCN4B expression values above the median for all NSCLC patients were classified as $SCN4B_{high}$ group, and the others were considered to be $SCN4B_{low}$ group. The difference of OS and survival status between low or high SCN4B expression group was assessed by log-rank test with R package “survival” [25]. A P value less than 0.05 was identified as significant.

Multivariate analysis and univariate analysis of the prognostic value of *SCN4B* expression in NSCLC

The Cox regression model was used to conduct multivariable and univariate survival analyses. Multivariate Cox analysis was used to compare the influence of SCN4B expression, along with clinical characteristics including clinical grade, TNM stage, OS, survival status, age and gender. All these clinical characteristics were entered in the multivariate Cox regression analysis as categorical variables, which set p value ≤ 0.05. The cut-off value of SCN4B expression was set based on the best separation [25]. Statistical significance for a two-tailed test was set at 0.05. Univariate Cox analysis was conducted to compare the influence of SCN4B expression and above clinical characteristics on OS and survival status, respectively.

Results

Characteristics in the GSE24080 dataset of 181 patients with NSCLC

In GSE50081 dataset, there were 83 female patients (age, 68.0561 ± 10.030), and 98 male ones (age, 69.393 ± 8.824). T stage ranged from 1 to 3, N stage ranged from 1 to 2, M stage were all 0, and clinical grade ranged from IA to IIB. See Table 1.

Table 1
non-small cell lung cancer patient characteristics from GEO data

Clinical characteristics		Total (181)	%
Age at diagnosis(y)	≦ 65	59	32.6
	> 65	122	67.4
gender	Male	98	54.1
	Female	83	45.9
histology	adenocarcinoma	127	70.2
	adenosquamous carcinoma	2	1.1
	large cell carcinoma	7	3.9
	squamous cell carcinoma	47	26.0
	Others	3	1.7
Stage	A	48	26.5
	B	79	43.6
	A	9	5
	B	45	24.9
Tumor	1	57	31.5
	2	122	67.4
	3	2	1.1
Node	0	129	71.2
	1	52	28.7
Metastasis	0	181	100

Significantly decreased expression of SCN4B in NSCLC

There were 315 main DEGs of NSCLC by comparing the primary NSCLC tumor with adjacent normal lung tissue specimens, including 79 upregulated genes and 236 downregulated genes. See Fig. 1. We found a downexpression of SCN4B in NSCLC patients with log2 FC = -1.990 and $p = 6.5^{e-22}$.

Lower SCN4B expression in lung cancer validated by HPA database

Immunohistochemistry staining got from the HPA was also verified the decreased SCN4B protein expression in lung cancer, as shown in Fig. 2. Using HPA017293 antibody, SCN4B protein was identified in 3 out 3 normal liver tissue samples. However, in lung cancer tissues, 9 out 12 (75%) samples were not stained.

Significant negative correlation between the SCN4B expression and DNA promoter methylation in lung cancer

Our MEXPRESS plot showed a significant negative correlation between the expression of SCN4B and DNA promoter methylation, with the Pearson correlation coefficients range from − 0.166 to − 0.244 ($p < 0.001$). See Fig. 3. The default MEXPRESS plot for SCN4B were sorted based on the its expression value in each sample. It was obviously clear that the normal samples tended to have higher SCN4B expression. Which also verified our DEGs and immunohistochemistry staining results.

Significant correlation with SCN4B2 expression and clinicalpathological characters in NSCLC

Our results in Fig. 4 revealed that SCN4B expression was significantly associated with clinical grade and T stage ($P < 0.05$). In addition, SCN4B expression was continuously decreased as the cancer progressed from grade 1A to 2B ($P < 0.0048$). This SCN4B lower expression trend could also be seen in T1 stage, compared with those with T0 stage respectively ($P = 0.018$). This trend was consistent with the results provided by GEPIA database. While SCN4B expression was not connected with age and gender in NSCLC ($P > 0.05$).

Decreased expression of SCN4B indicated poor prognosis in NSCLC

Our kaplan-Meier survival curves demonstrated that SCN4B expression was associated with OS significantly. NSCLC patients with low SCN4B expression had unfavorable OS ($p < 0.004$) (Fig. 5). Furthermore, in multivariate analysis for OS, the hazard ratio of SCN4B expression was 0.236 (P = 0.009). While in univariate analysis, the hazard ratio of SCN4B expression was 0.219 ($p = 0.003$), see Fig. 6.

Discussion

The voltage-gated sodium channels β subunits have been well reported to express in lung, prostate, breast, and cervical cancers [26]. Considering its multifunction in both excitable and nonexcitable cell types, this protein family becomes an emerging therapeutic target [10]. While SCN4B has been shown to play crucial roles as multifunctional signaling molecules involved in cell adhesion, cell migration, neuronal pathfifinding, fasciculation, and neurite outgrowth [27]. Moreover, one recently study have

shown that SCN4B expression is decreased in cervical cancer biopsies compared with non-cancer samples [28]. As the research goes on, SCN4B was identified as a metastasis suppressor and a new biomarker of aggressive cancers [29]. However, there was no information regarding its potential involvement in the NSCLC process.

In our study, the significantly decreased SCN4B expression was found in 60 paired NSCLC patients by comparing the primary tumor tissue with adjacent normal lung tissue specimens. This result was verified by immunohistochemistry staining got from the HPA. Furthermore, SCN4B expression was highly correlated with clinicalpathological characters of NSCLC, which shown a continuous decreased trend when NSCLC tumor progressing($P<0.05$) . In addition, our kaplan-Meier survival curves also verified low SCN4B expression was associated with unfavorable OS significantly($P<0.004$) (**Figure 5**). And our multivariate analysis and univariate analysis both showed the SCN4B expression was the high hazard ratio of OS ($p = 0.009$, $p = 0.003$ respectively).

Dysregulated SCN4B has been reported in multiple types of cancer by emerging studies. In cervical and prostate cancer cells, SCN4B expression levels were lower in comparison to noncancerous cells [12,30]**. This results supported what we observed by** NSCLC DEGS analysis and immunohistochemistry staining analysis: SCN4B was significantly downregulated in NSCLC tissue compared to normal lung tissue.

What's more, in breast cancer, reduced SCN4B expression was demonstrated specifically when tumour gained invasive properties (transition from grade I to grade II). SCN4B was almost absent in high-grade tumour and metastase. More specially, its expression was associated with increased RhoA activity, enhanced cell migration and invasiveness, primary tumor growth and metastatic spreading [31]. This tumor suppressor potential utility of SCN4B was also verified in colorectal and prostate cancer [12,13]. In our study, SCN4B expression was also significantly correlated with clinical characteristics of NSCLC, such as AJCC grade, TNM stage and OS. Taken the following univariate and multivariate analysis results together, we inferred that low SCN4B expression was an independent unfavorable indicator in patients with NSCLC and might be served as a promising prognostic biomarker of NSCLC.

To explore the mechanisms that could be contributed to the decreased SCN4B in NSCLC, we performed the genetic analysis. We found the significantly negative correlation between SCN4B expression and DNA promoter hypermethylation in lung cancer ($P<0.01$). Aberrant DNA methylation is a common feature of human cancers and its utility is already recognized in cancer management [32]. Promoter methylation status of specific gene, such as ASC/TMS1/PYCARD, can affect NSCLC tumor behavior and therefore modulate clinical outcome [33]. Study has reported DNA methylation are important mechanisms leading to suppressed transcription of some important tumor suppressors in cancers, including NSCLC [34,35]. Besides, DNA hypermethylation has been reported to suppress SCN4B expression in papillary thyroid cancer [15]. Taken together, these findings suggested that suppressed SCN4B expression in NSCLC might be attributed to DNA hypermethylation.

Conclusion

In summary, our study showed that SCN4B expression was downregulated in NSCLC, and downregulating continuously with NSCLC progressed. And its decreasing might be attributed to DNA promoter hypermethylation. Besides, low SCN4B expression was significantly correlated with poor NSCLC prognosis, which might served as a potential unfavorable prognostic factor for NSCLC patients.

Abbreviations

SCN4B: Voltage-gated sodium channels β subunits 4; NSCLC: non-small cell lung cancer; GEO: Gene expression omnibus; DEGs: differentially expressed genes; TNM: Tumor node metastasis; GEPIA: Gene Expression Profiling Interactive Analysis; OS: Overall survival; GEPIA: Gene Expression Profiling Interactive Analysis.

Declarations

Ethics approval

The study was approved by the Ethics Committee of Zhongshan Hospital Affiliated to Shanghai Fudan University (Shanghai, 200025, China) and Ruijin Hospital Affiliated to Shanghai Jiaotong University (Shanghai, 200025, China).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data collections and processions were performed according to policies of GEO (accession number: GDS3837and GSE50081). The public access to the databases is open.

Competing interests

The authors declare that they have no conflicts of interest.

Funding

This study was founded by the Project of Shanghai Municipal health planning commission (NO.2016ZB0301).

Authors' contributions

Qz L. and Wh Y. conceived and designed the study; Mx Y. and Z W. performed the data analysis and wrote the manuscript; all authors revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank GEO databases for providing high-quality clinical data on NSCLC diseases.

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Figures

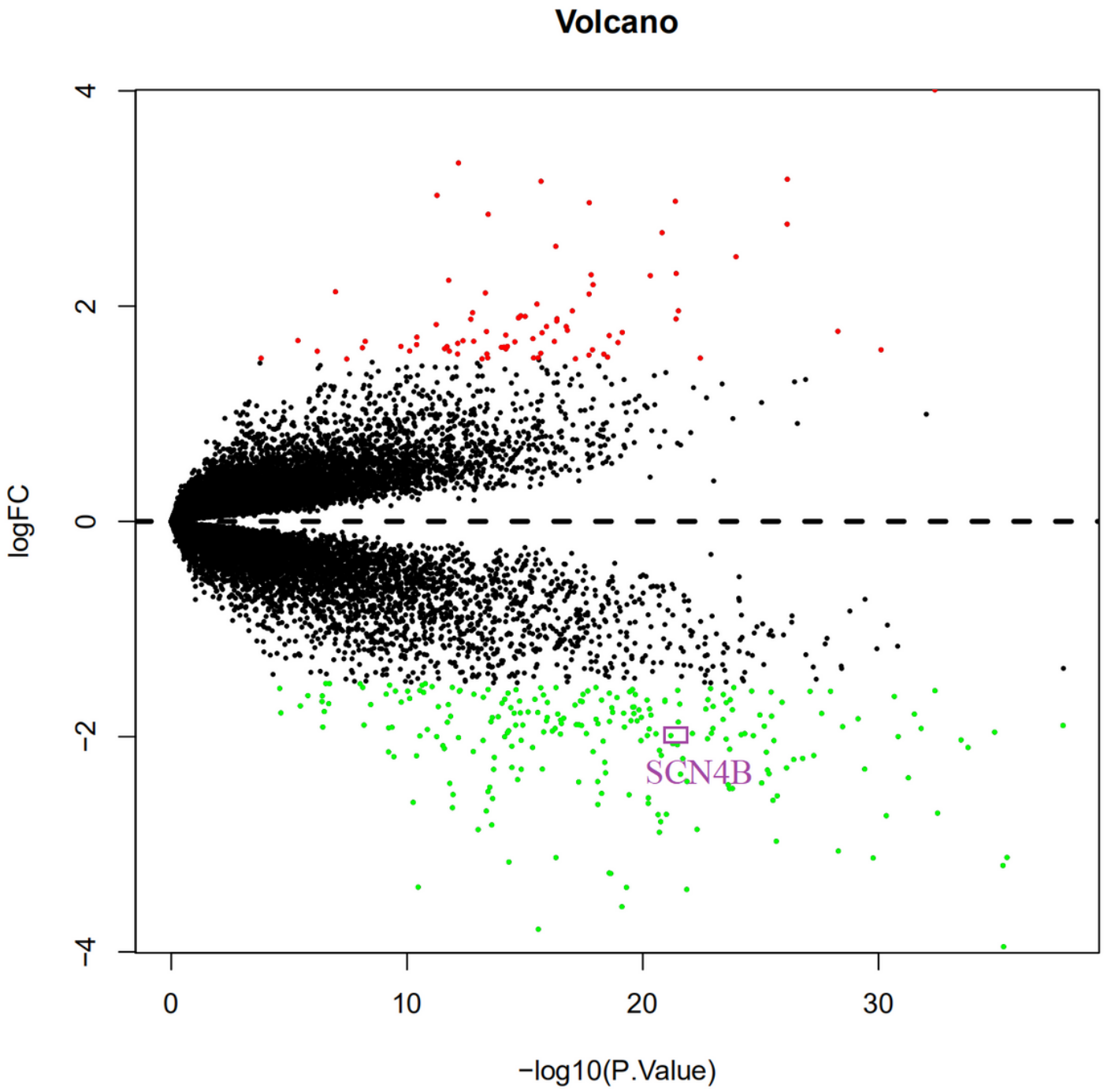

Figure 1

Volcano map of 315 DEGs of NSCLC. Red nodes represent up-regulated genes and green nodes represent down-regulated genes.

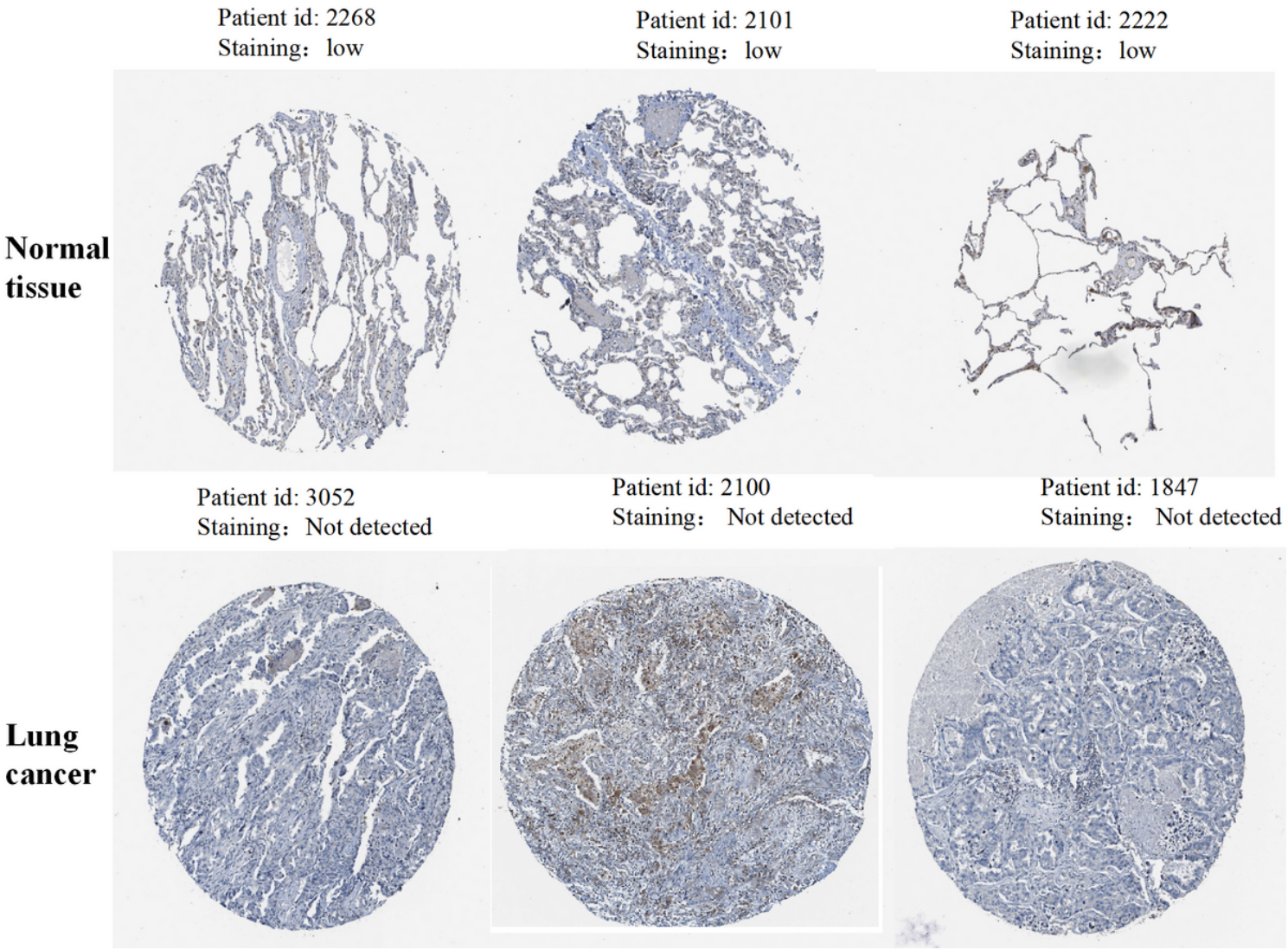

Figure 2

Immunohistochemistry staining validation of the SCN4B protein expression in lung cancer by HPA database.

Figure 3

MEXPRESS plot visualization of the SCN4B in lung cancer and the correlation between its expression and DNA promoter methylation. The negative correlation between SCN4B expression and promoter methylation is highlighted in red box with the Pearson correlation coefficients on the right. The height of the gray line represent the SCN4B expression value.

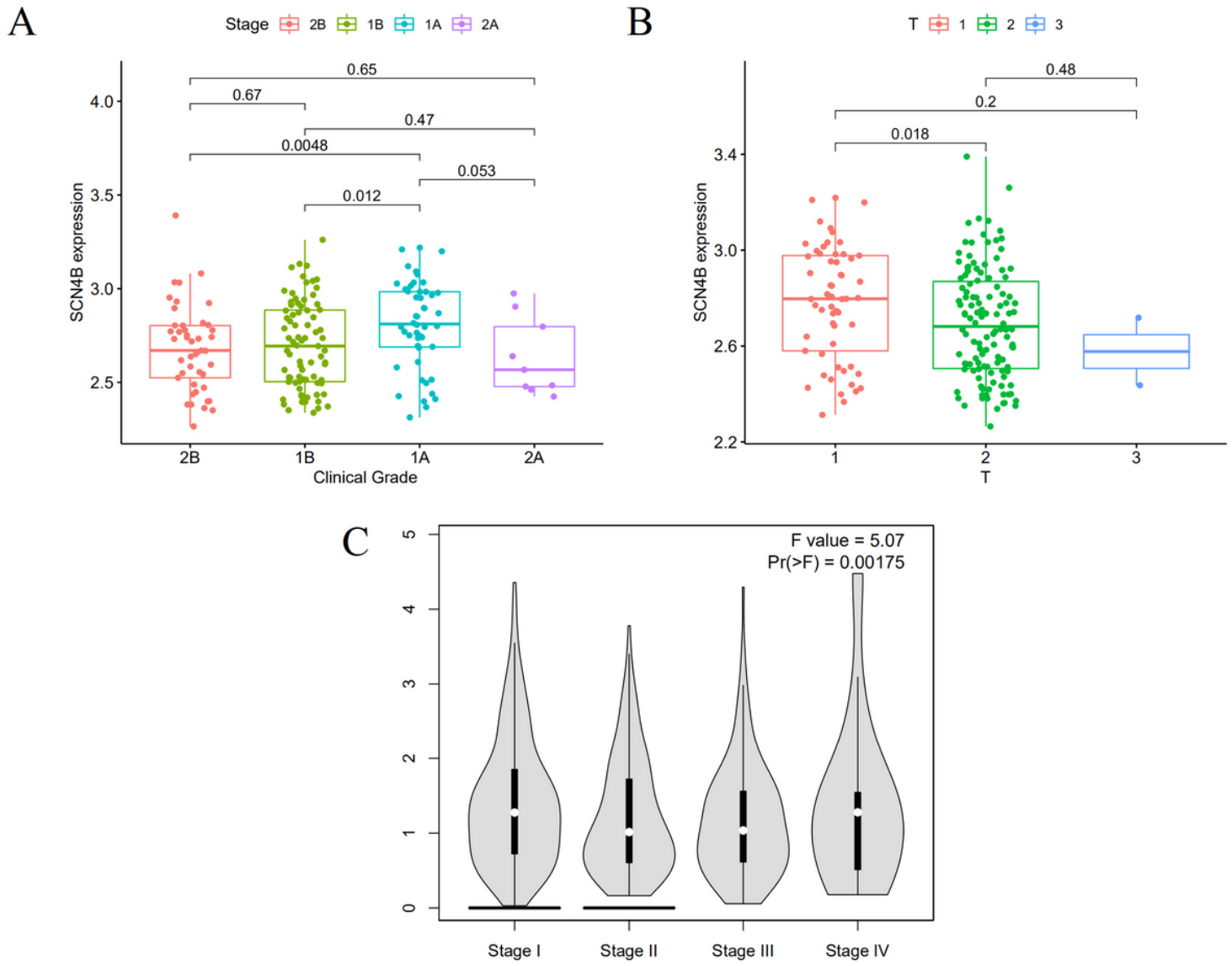

Figure 4

Association of SCN4B expression with the clinicalpathological characters. A and B is analysis of AJCC stage and T stage, respectively; C is analysis from GEPIA database.

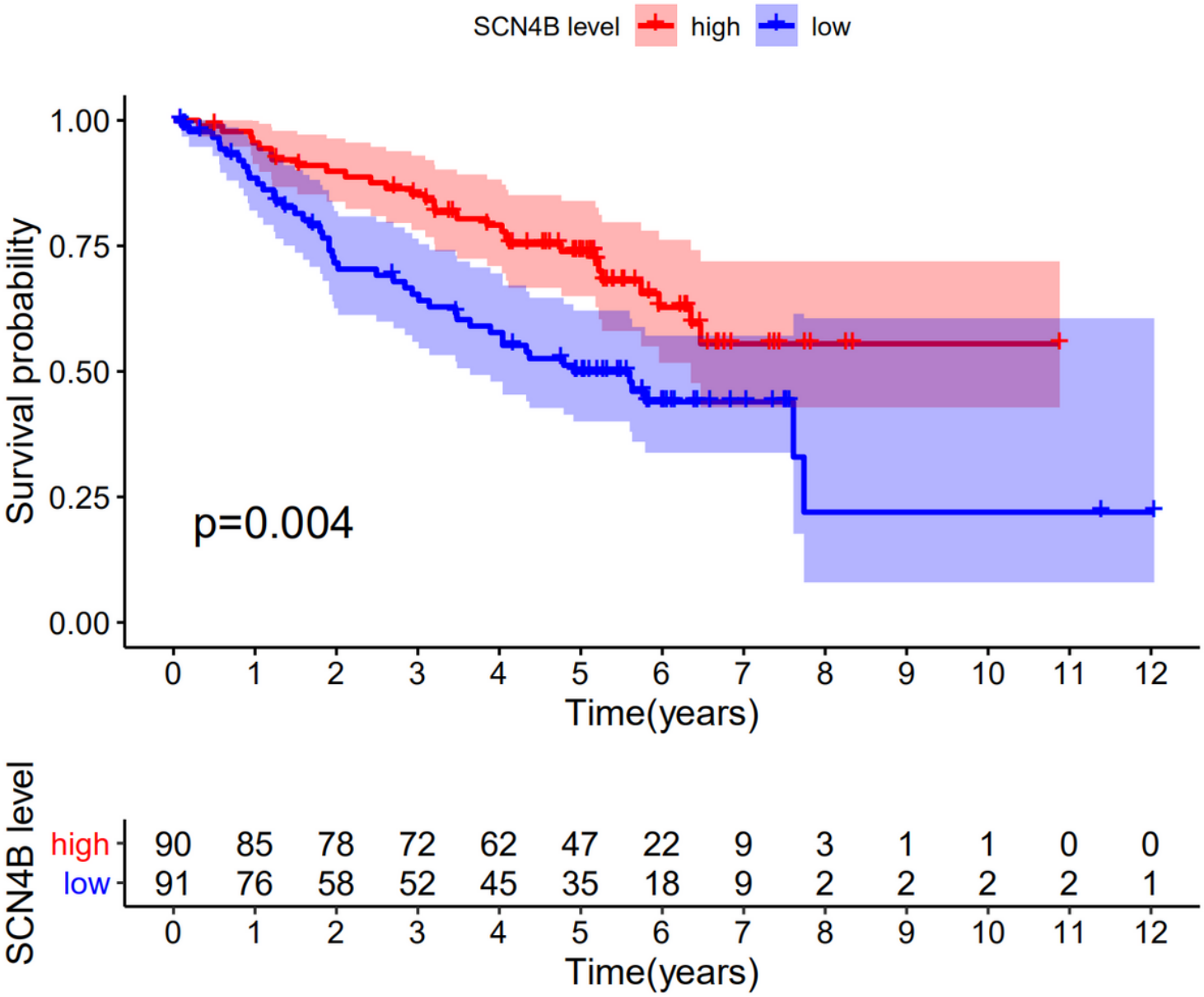

Figure 5

Survival analysis of SCN4Bhigh and SCN4Blow group. The X-axis represents the OS time(year) and the Y-axis represents survival probability. Kaplan-Meier survival curves show that SCN4B low expression predicts poor prognosis of NSCLC.

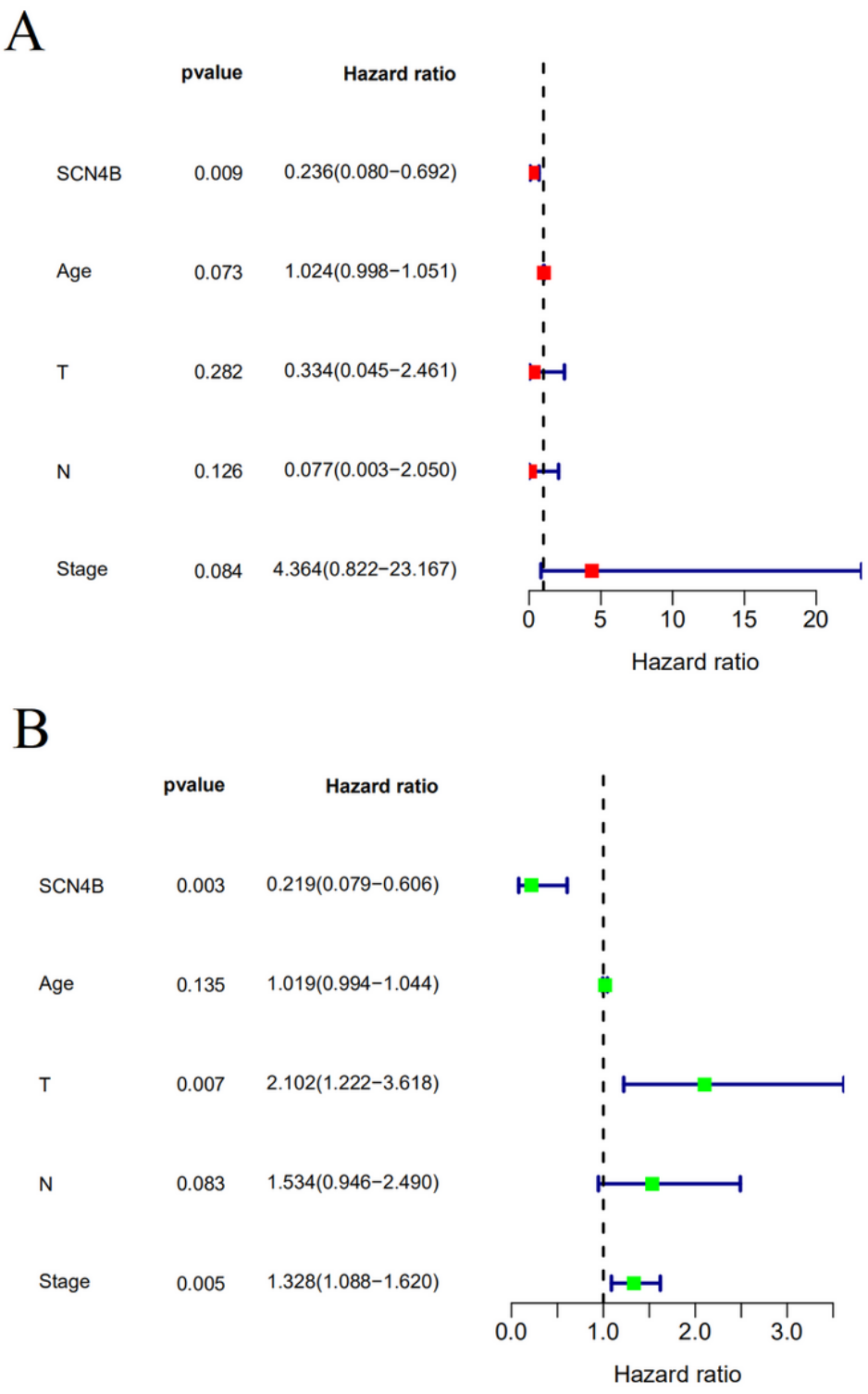

Figure 6

The prognostic value of SCN4B expression in NSCLC. A. The result of multivariate analysis, B. the result of univariate analysis.