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# Expression and Prognostic Significance of the Nicotinic Receptor Cluster on 15q25 about CHRNA5 and PSMA4 mRNA in Lung Adenocarcinoma Utilizing TCGA Datasets

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## Abstract

**Background:** Genome-wide association studies of lung cancer have shown a common variation at 15q24-25.1 as a determinant of risk, but the role of specific genes has not been proven. This study aims to explore the expression of mutations and the prognostic significance of 15q25 (*CHRNA5* and *PSMA4*) mRNA in lung adenocarcinoma (LAC) based on immunohistochemistry, TCGA and bioinformatics.

**Methods:** The expression of mutations on chromosome 15q25 of 576 primary LAC patients was selected and survival and gene expression data were extracted from TCGA. The relationship between expression of genes on 15q25 and clinical and prognostic Significance of LAC. An experiment with Beas-2b, A549 and H1299 cell lines was performed to further prove the difference in *CHRNA5* and *PSMA4* expression between lung cancer and normal cells. Immunohistochemistry data of *CHRNA5* and *PSMA4* were detected in LAC and normal tissues from 122 patients. Finally, Gene enrichment analysis (GSEA) was conducted to predict the regulatory genes of *CHRNA5* and *PSMA4*.

**Results:** *CHRNA5* and *PSMA4* are frequently mutated in TCGA (*CHRNA5*, 1.7%; *PSMA4*, 1.3%). Besides, the expression of *CHRNA5* and *PSMA4* was obviously higher in A549 and H1299 cells. And the immunohistochemical staining revealed that the levels of *CHRNA5* and *PSMA4* were considerably higher in the LAC group than in the normal group. Meanwhile, there was a significant association between high *CHRNA5* expression and smoking history (P=0.011), smoking history pack year value (P=0.010). Furthermore, there was a significant correlation between *CHRNA5* and *PSMA4* expression levels and prognosis (P=0.003; P=0.008), and between higher expression and worse prognosis. GSEA results suggested that between samples with high *CHRNA5* and *PSMA4* expression were respectively enriched to cell cycle, base excision repair, oxidative phosphorylation, protein export, and aminoacyl tRNA biosynthesis, among others.

**Conclusions:** *CHRNA5* and *PSMA4* mRNA expression has a significant impact on the clinical and survival of LAC, and they may be a potential target for treating patients with lung adenocarcinoma.


## Background

Lung cancer is the most common form of malignant tumor, with the highest morbidity and mortality rates worldwide, 80% of which are Non-Small Cell Lung Cancer (NSCLC). Lung adenocarcinomas (LAC) are the most common pathology subtype in lung cancer [1]. Increasing evidence has revealed that multigene mutations also contribute to the risk of carcinogenesis and may play a detrimental role in an individual's susceptibility to LAC [2]. Although LAC therapy has made little progress during the early stages, most patients are at a later stage, and there is no effective treatment for them - the 5-year overall survival rate is only 10% to 15% [3]. Currently, genetics has achieved favorable curative effects as a novel therapy for advanced LAC patients, and has significantly prolonged their survival time [4]. Effective prognostic biomarkers for LAC must be found urgently.

Epidemiological data show that there is a close connection between the occurrence and development of lung cancer and multiple environmental factors, such as smoking [5], air pollution [6], and occupation [7], among others. Nicotine is one of the main psychoactive ingredients in tobacco that contributes to the harmfulness of smoking tobacco [8]. A common feature of addictive drugs, including nicotine, is that they increase dopamine (DA) release in the nucleus accumbens (NAc). The principal dopaminergic projections to NAc arise from the neurons in the ventral tegmental area (VTA). Furthermore, nicotine acts as an agonist in the VTA to activate and desensitize the nicotinic acetylcholine receptors (nAChRs) that facilitate the reinforcing effects of nicotine [9]. More recently, several large genome-wide association studies identified an association between single nucleotide polymorphism (SNP) variation at 15q24-15q25.1 and susceptibility to lung cancer [10]. Iron-responsive element-binding protein 2 (*IREB2*), Neuronal acetylcholine receptor subunit alpha-5 (*CHRNA5*), Neuronal acetylcholine receptor subunit alpha-3 (*CHRNA3*), Neuronal acetylcholine receptor subunit beta-4 (*CHRNB4*), Hydroxylysine kinase (*HYKK*), and Proteasome subunit alpha type-4 (*PSMA4*) were located on chromosome 15 25.1 gene cluster with signaling pathways involved in nicotine metabolism and addiction. Smoking for a while there's relativity between peripheral blood nicotine levels, and gene polymorphism gene cluster in nicotine metabolism existed obvious correlation, and case fatality rate of obvious correlation with smoking related diseases, including lung cancer [11], chronic obstructive pulmonary disease (COPD) [12], etc. In recent ten years, the 15q25 region has been associated with lung-cancer risk and might also be associated with the prognosis of lung cancer. In particular, multiple functional polymorphism were found at this site that were significantly correlated with the prognosis of lung cancer. Guang Jin et al [13]had studied that the CHRNA3 rs6495309C > T polymorphism may affect survival in patients with early-stage NSCLC. Moreover, there were four SNPs (rs2036534C>T, rs667282C>T,rs12910984G>A, and rs6495309T>C) that map to a region of strong linkage disequilibrium in the CHRNA5-CHRNA3-CHRNB4 gene cluster have been also reported to be strongly associated with the risk of lung cancer in Chinese populations[14].In addition to genetic polymorphisms in chromosome 15q25, other mechanism may also exist to explain the importations of abnormal gene expression in chromosome 15q25 on lung cancer. At first, some studies had showed that the nAChR subunit were expressed at varying levels in normal and malignant cells[15]; secondly, it has become apparent that nAChRs in lung cells act as central mediators in the activation of cancer signaling pathways, for example cell proliferation, NF-kB, PI3K and ERK signaling pathways[16, 17].

Recently, genome-wide association studies of lung cancer have shown a common variation at 15q24-25.1 as a determinant of risk. Results of genetic association studies for nicotine dependence, smoking behavior, and smoking related diseases have converged to implicate chromosome 15q25.1 region. Therefore, this study explored the effect of *IREB2*, *CHRNA5*, *CHRNA3*, *CHRNB4*, *HYKK*, and *PSMA4* gene cluster expression in LAC on the prognosis for survival utilizing The Cancer Genome Atlas (TCGA) datasets, and sought to identify a highly sensitive molecular marker. It can provide clues and ideas for early diagnosis and prognosis of LAC.

## Methods

*TCGA data collection*

Using Bioconductor packages/TCGA biolinks in the TCGA Research Network (http://cancer genome.nih.gov/) for downloading and preprocessing data, a total of seven cohorts with lung adenocarcinoma-related research were found. They include the Broad, Memorial Sloan Kettering Cancer Center (MSKCC), Nature, PanCancer, Provisional, and MSK-IMPACT cohorts respectively, with a total of 3,232 samples. According to the integrity of the gene sequence, copy number variation, and smoking history, the provisional cohort finally met our research criteria. Mutation in the *IREB2*, *CHRNA5*, *CHRNA3*, *CHRNB4*, *HYKK*, and *PSMA4* genes in LAC were generated from cBioPortal (www.cbioportal.org) datasets. There was total of 576 samples of LAC for which the data included whole exome sequencing, total exon sequencing, RNA-seq, microRNA-seq, protein expression, gene expression, and Copy number variations (CNV) data. Furthermore, we also downloaded the clinical data, such as patient age, gender, race, smoking history, tumor staging, metastasis, and survival for clinicopathological correlation analysis and prognosis analysis.

*Cell culture*

Cell lines Beas-2b, A549, and H1299 were purchased from the Shanghai Institute of Life Sciences. The culture conditions included 1,640 constant temperature incubator culture cells containing 10% fetal bovine serum training based on wet 37°C and 5% $CO_2$.

*Tissue sample collection*

A total of 60 FFPE samples with normal and carcinoma tissues samples were obtained from patients with LAC at the Biobank of Northern Jiangsu People's Hospital between July 2015 and December 2017. All tumor tissues were histologically reviewed by two pathologists. Patients signed an informed consent, and the study protocols for the use of human tissues were approved by and performed in accordance with the ethical standards of the Ethics Committee of Northern Jiangsu People's Hospital.

*Immunohistochemical detection*

Four-micron-thick sequential histological tumor sections were obtained from a representative formalin-fixed, paraffin-embedded tumor block and used for immunohistochemistry (IHC) testing. Polyclonal rabbit anti-*CHRNA5* antibody (Abcam, UK) was used as the primary antibody at 1:50 dilution and incubated overnight at 4°C. *HYKK* used polyclonal rabbit anti-*HYKK* antibody (Abgent, USA) at a dilution of 1:50 and *PSMA4* used polyclonal rabbit anti-*PSMA4* antibody (Origenes, USA) at a dilution of 1:25. Polyclonal goat anti-HRP antibody (ZSGB, China) was used as the second antibody. To measure the IHC expression of the different markers and quantify inflammatory cell expression, the slides containing whole tumor sections were digitally scanned at ×200 magnification. The images were visualized and analyzed as described in the Supplementary Methods.

*Western blotting*

Total protein concentration was measured with the bicinchoninic acid assay. Protein (60 μg) was separated with 10% SDS-PAGE and subsequently transferred onto polyvinylidene difluoride membranes. After blocking with 5% bovine serum albumin, the membrane was incubated overnight with the appropriate primary antibody at 4˚C, followed by washing and incubation with horseradish peroxidase goat-anti-rabbit Immunoglobulin G secondary antibody for 1h at room temperature and detection using an enhanced chemiluminescence kit. Western blots were performed using polyclonal rabbit anti-CHRNA5 antibody (Abcam, 1:500). *HYKK* used polyclonal rabbit anti-*HYKK* antibody (Abgent, 1:250) and *PSMA4* used polyclonal rabbit anti-*PSMA4* antibody (Origenes, 1:50) and GAPDH specific polyclonal antibody (KC-5G4; Kangcheng Shanghai, China). The images were captured using the Gel Dox XR system (Bio-Rad, Philadelphia, PA).

*Gene set enrichment analysis (GSEA)*

GSEA 2.2.1 software was used for enrichment analysis. According to *CHRNA5* and *PSMA4* expression levels, they were divided into a high-expression group and a low expression group. In this study, we obtained c2.cp.cp. kegg.v5.1 Symbols GMT datasets from the MsigDB database on the GSEA website. Then, the number of random combinations was set to 1,000 times by default, according to the weighted enrichment statistics method for enrichment analysis.

*Statistical analyses*

All statistical analyses were performed using the SPSS 22.0 software package. The association between *CHRNA5* and *PSMA4* expression and clinicopathological features was analyzed using the $\chi 2$ test. Kaplan-Meier curves and log-rank test were used to analyze survival data, $P<0.05$ was considered statistically significant, and all values were reported as mean ± SE.

## Results

*IREB2, CHRNA5, CHRNA3, CHRNB4, HYKK, and PSMA4 mutations in LAC based on the TCGA database*

A total of 576 patients with a histologically confirmed diagnosis of LAC were collected by TCGA in the present study, the main data of which contained whole exome sequencing, RNA-seq, copy number, gene expression, and CNV data. The individual mutation frequencies of the Provisional cohort are illustrated in Figure 1. *IREB2* was mutated in 7% of tumors and 41 of the 576 sequenced patients had *IREB2* mutations; *CHRNA5* was mutated in 5% of tumors and 30 of the 576 sequenced patients had *CHRNA5* mutations; *CHRNA3* was mutated in 5% of tumors and 30 of the 576 sequenced patients had *CHRNA3* mutations; *CHRNB4* was mutated in 7% of tumors and 41 of the 576 sequenced patients had *CHRNB4* mutations. Also, *HYKK* was mutated in 6% and *PSMA4* had a mutation rate of 9%, with mutations in 35 and 53 of the 576 sequenced patients, respectively.

*Association between IREB2, CHRNA5, CHRNA3, CHRNB4, HYKK, and PSMA4 expression and clinical pathological features based on the TCGA database*

To understand the role of *IREB2*, *CHRNA5*, *CHRNA3*, *CHRNB4*, *HYKK*, and *PSMA4* in the tumorigenesis and progression of LAC, this study analyzed the association between *IREB2*, *CHRNA5*, *CHRNA3*, *CHRNB4*, *HYKK*, and *PSMA4* expression and the clinicopathological features. LAC patients were divided into two groups according to *IREB2*, *CHRNA5*, *CHRNA3*, *CHRNB4*, *HYKK*, and *PSMA4* expression, based on median value, namely, a low expression group and a high-expression group. In this investigation, there was a significant association between high *CHRNA5* and *PSMA4* expression and gender (*P=0.000, P=0.005*), overall survival status (*P=0.050, P=0.006*), and recurrence-free survival status *(P=0.019, P=0.049*). There was also a significant association between high *CHRNA5* expression and smoking (*P=0.002*), as shown in Table 1. However, *CHRNA5* and *PSMA4* expression was not associated with age (*P=0.855*), race (*P=0.752*), TNM stage (*P=0.725*), or disease stage (*P=0.971*) in the cohort (*P>0.05*).

***Assessment of IREB2, CHRNA5, CHRNA3, CHRNB4, HYKK, and PSMA4 gene expression in LAC tissues based on the TCGA database***

We examined lung expression of *IREB2*, *CHRNA5*, *CHRNA3*, *CHRNB4*, *HYKK*, and *PSMA4* in normal and carcinoma tissues, in two experiment groups, based on the TCGA database. The results showed that *CHRNA5*, *CHRNB4*, *HYKK*, and *PSMA4* were highly expressed in lung adenocarcinoma tissues, and *IREB2* and *CHRNA3* showed low expression in cancerous tissues (Figure 2). Furthermore, *IREB2*, *CHRNA5*, *CHRNA3*, *CHRNB4*, *HYKK*, and *PSMA4* expression were significantly different between the normal and lung adenocarcinoma tissues (*P<0.001, P<0.001, P=0.021, P<0.001, P<0.001, P<0.001*) (Table 2).

***Association between CHRNA5 and PSMA4 expression and survival based on the TCGA database***

This investigation used TCGA datasets and Kaplan-Meier Plotter online data respectively for survival analysis. The results showed that the *CHRNA5* and *PSMA4* expression levels were significantly correlated with the prognosis. There was a significant correlation between *CHRNA5* and *PSMA4* expression level and prognosis. There were significant differences in overall survival (OS)/recurrence-free survival (RFS) between the high and low *CHRNA5* and *PSMA4* expression groups (*P<0.05*) and the higher the expression, the worse the prognosis. Further utilization of Kaplan-Meier Plotter online data analysis is shown in Fig. 3. However, there were no differences in OS/RFS between the high and low *IREB2*, *CHRNA3*, *CHRNB4*, and *HYKK* expression groups (shown in Supplemental Figure 2). The above results suggest that *CHRNA5* and *PSMA4* are adverse prognostic factors and high expression was significantly associated with shorter survival.

***CHRNA5 and PSMA4 expression in Beas-2b, A549, and H1299 cells***

To further demonstrate the role of *CHRNA5* and *PSMA4* expression in the development and progression of lung adenocarcinoma, we conducted a cell experiment to compare *CHRNA5* and *PSMA4* expression in normal and cancerous cells. The results showed that *CHRNA5* and *PSMA4* expression in *A54*9 and H1299 cells was obviously higher than in Beas-2b cells (Figure 4).

***CHRNA5 and PSMA4 expression in a tissue microarray of lung adenocarcinoma***

Immunohistochemical staining revealed that *CHRNA5* and *PSMA4* levels in the lung tissues of the carcinoma group were considerably higher than those in the normal group. The positive expression products were brown-yellow granules, which were located in the nucleus and cytoplasm, respectively, as shown in Figure 5.

***Relationship between CHRNA5 and PSMA4 expression levels and the clinical characteristics of patients with lung adenocarcinoma.***

To further explore the effects of *CHRNA5* and *PSMA4* on the clinical pathology of lung adenocarcinoma, this study analyzed the association between *CHRNA5* and *PSMA4* expression and clinicopathological features. The LAC patients were divided into two groups according to *CHRNA5* and *PSMA4* expression, based on the median value, namely a low expression group and a high-expression group. In this investigation, there was a significant association between high *CHRNA5* expression and T stage (*P=0.026*), OS status (*P=0.007*), and progression-free survival status (*P=0.013*). There was also a significant association between high *PSMA4* expression and OS status (*P=0.006*) and progression-free survival status (*P=0.007*), as shown in Table 3. However, *CHRNA5* and *PSMA4* expression was not associated with age, race, TM stage, or disease stage within the cohort (*P>0.05*).

***Correlation between the expression levels of seven PSMAs and OS/RFS***

We used the Kaplan-Meier curve for survival analysis of our clinical data. The results showed that *CHRNA5* and *PSMA4* expression levels were significantly correlated with prognosis. There was a significant correlation between *CHRNA5* and *PSMA4* expression levels and prognosis. There were significant differences in OS/RFS between the high and low *CHRNA5* and *PSMA4* expression groups (*P<0.05*). It was determined that the higher the expression, the worse the prognosis (Figure 6).

***Functional enriched analysis of CHRNA5, HYKK, and PSMA4***

The results of the GSEA method suggested that the *CHRNA5*, *HYKK*, and *PSMA4* high-expression samples were respectively enriched to cell cycle, base excision repair, oxidative phosphorylation, protein export, and aminoacyl tRNA biosynthesis, among others (see Figure 7).

## Discussion

Over the past decades, the prognosis of LAC has been poor, and still lacks a highly sensitive and specified method for early diagnosis of LAC. Among all the therapies for LAC, surgery is the main treatment, but if LAC can be diagnosed at an early stage, its will greatly improve. Previous studies have found many tumor markers, but many of them resulted in limited practical clinical value. Considering these circumstances, it is urgent to find effective prognostic biomarkers for LAC. Recent studies have shown that gene polymorphism and mRNA expression located on chromosome 15q25.1 was significantly correlated with human smoking behavior, extent of smoking, and even smoking times. It was also significantly correlated with several studies. For example, the high-risk

loci for lung cancer among the African-American population on chromosome 15q25 and the genome-wide significant 15q25.1 marked by rs2036527 near CHRNA5 [13]. Ji et al reported that genetic variants on the 15q25.1 lung cancer susceptibility locus may influence susceptibility to NPC, particularly for smoking-associated nasopharyngeal carcinoma [14]. Single nucleotide polymorphisms (SNPs) in the cholinergic nicotinic receptor subunit genes on chromosome 15q25.1, including *CHRNA3*, *CHRNB4*, and *CHRNA5*, are well-established biomarkers of chronic obstructive pulmonary disease (COPD) and lung cancer [15]. Several polymorphisms and their haplotypes in *CHRNA5/ CHRNA3* genes may have functional effects on (i) *CHRNA5* mRNA levels, (ii) polycyclic aromatic hydrocarbon-DNA adduct levels, (iii) TP53 mutations, and (iv) susceptibility to lung cancer [16]. However, the function of the nicotinic receptor cluster on chromosome 15q25 in LAC is still unclear.

In this study, we used the TCGA databases to analyze the association of 15q25 locus genes with LAC. The results showed that mRNA expression levels of *CHRNA5* and *PSMA4* were significantly upregulated in LAC compared to normal tissues. Furthermore, we also analyzed the relationship between the mRNA expression levels of *CHRNA5* and *PSMA4* and various clinicopathological features and survival outcomes of LAC, based on the TCGA database. The results show that there was a significant association between high expression of *CHRNA5* and *PSMA4* and OS status and recurrence-free survival status. However, *CHRNA5* and *PSMA4* expression was not associated with age, race, TNM stage, or disease stage in the cohort (*P>0.05*). Moreover, survival curve analysis was also conducted, and the results showed that *CHRNA5* and *PSMA4* expression was significantly higher in patients with reduced OS(*P < 0.05*) and reduced recurrence-free survival (*P < 0.05*). We believe that there is an obvious correlation between *CHRNA5* and *PSMA4* expression and the pathologic features and prognosis of LAC. All TCGA analysis results support the hypothesis that a change in *CHRNA5* and *PSMA4* expression may participate in the development of lung cancer, especially LAC.

In order to further demonstrate our hypothesis, we also used the cell experiments and clinical data to verify it. The results show that *CHRNA5* and *PSMA4* expression in A549 and H1299 cells were obviously higher than in Beas-2b cells, and they were also widely expressed in the cells of LAC tissue samples, especially in the cytoplasm. Our clinical data analysis also showed that there was a significant association between high expression of *CHRNA5* and T stage (*P=0.026*), OS status (*P=0.007*), and progression-free survival status (*P=0.013*). Furthermore, there was a significant association between high expression of *PSMA4* and OS status (*P=0.006*) and progression-free survival status (*P=0.007*). Moreover, there was a significant correlation between *CHRNA5* and *PSMA4* expression level and prognosis. There were significant differences in OS/RFS between the high and low *CHRNA5* and *PSMA4* expression groups (*P<0.05*). The results above further support our hypothesis.

CHRNA proteins are expressed in lung epithelial cells and bind to nicotine, an addictive compound in cigarettes, and nitrosamines, potential lung carcinogens in cigarettes and food [17, 18]. Signal transduction through CHRNA proteins was suggested to cause cell proliferation, and to facilitate neoplastic transformation. Recently, the locus containing two genes encoding nicotine acetylcholine receptor (nAChR) subunits, *CHRNA3* and *CHRNA5*, was shown to be associated with lung cancer risk in Caucasians by three genome-wide association studies [17, 19, 20]. A decreased survival correlation with lung cancer has been shown because the reduced treatment efficacy and worse survival may be well explained by the nAChRs pathway through CHRNA proteins on tumor cell proliferation, apoptosis, epithelial-mesenchymal transition, and proinvasive and angiogenic effects [21, 22]. Our results showed that *CHRNA5* was higher expressed in lung adenocarcinoma tissues, and that there is a significant association between the expression and clinical characteristics and patient prognosis. As for the upper result, *CHRNA5* could be used as a potential prognostic marker and a therapeutic target in lung adenocarcinoma.

*PSMA4* was also located at the 15q24-25.1 locus and encodes a structural protein of the 20S proteasome core and was required for proteasomal activities. The proteasome is responsible for the regulation of many cellular processes including transcription, cell cycle progression, and apoptosis [23]. Proteasome dysfunction can lead to many diseases, including cancer, and drugs inhibit the proteasome activity that directly affects lung cancer susceptibility through its modulation of cell proliferation and apoptosis [24]. Aberrations and abnormal expression of proteasome subunits have been demonstrated in many tumors including breast cancer [25-27], lung cancer [28], hepatocellular carcinoma [29], and colorectal cancer [30]. PSMA4 is a component of the Adenosine Triphosphate- and ubiquitin-dependent nonlysosomal pathway, and it is involved in the processing of class I major histocompatibility complex peptides. It has been reported that *PSMA4* [31] mRNA levels were increased in lung tumors. In our results, we also found that *PSMA4* were highly expressed in lung adenocarcinoma tissues and showed a significant association between the expression and clinical characteristics and prognosis of patients. This further confirmed that *PSMA4* plays an important role in the occurrence and development of lung adenocarcinoma.

However, the onset of lung cancer is a multi-step process involving multiple genes. How does *CHRNA5* and *PSMA4* expression regulate the development of lung adenocarcinoma? What processes underlie this regulation, and how do they relate to one other? We used the GSEA method to predict the signal pathways in which they may participate. The results suggest that the *CHRNA5* and *PSMA4* high-expression samples were respectively enriched to cell cycle, base excision repair, oxidative phosphorylation, protein export, and aminoacyl tRNA biosynthesis. Its specific mechanism requires further study. Furthermore, methods for detecting the expression of the *CHRNA5*, *HYKK*, and *PSMA4* in the sera of patients, which would be of great value for clinical diagnosis, must be developed.

## Conclusions

In summary, our study systematically analyzed the mRNA expression levels and prognostic significance of *CHRNA5* and *PSMA4* in lung adenocarcinoma. *CHRNA5* and *PSMA4* exhibited significant expression differences between tumor and normal tissues. In addition, we further confirmed that *CHRNA5* and *PSMA4* were widely expressed in cancer cells such as A549 and H1299 cells. Moreover, the expression of *CHRNA5* and *PSMA4* could significantly affect the prognosis of LAD patients. However, the occurrence of lung cancer is a multi-step process involving multiple genes. How does the *CHRNA5* and *PSMA4* expression regulate the development of lung adenocarcinoma? What processes underlie this regulation, and how do they relate to one other? In future studies, we will increase sample volume to explore the detailed roles of the *CHRNA5* and *PSMA4* in tumor initiation and development, which may strengthen the evidence that the *CHRNA5* and *PSMA4* could be promising therapeutic targets and novel prognostic biomarkers for lung adenocarcinoma.

## Declarations

### Ethics approval and consent to participate

All participants were voluntary and provided written informed consent before taking part in this research. This study was approved by the Ethics Committee of the Northern Jiangsu People's Hospital, and in compliance with the Declaration of Helsinki (2015KY-148). The design and performance of this study involving human subjects were obviously described in a research protocol.**Consent to publish**No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.**Availability of data and materials**All the data regarding the findings are available within the manuscript. Anyone who is interested in the information should contact the corresponding author.**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**YW performed the histological examination of the lung tissues, and was a major contributor in writing the manuscript. XW, LW and JJ analyzed and interpreted the patient data. JG, DG and SH mainly performed the cell examination. LM, XX and SY mainly implement the modification after writing of this article. All authors read and approved the final manuscript.

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## Tables

**Table 1: The association between the expression of IREB2, CHRNA5, CHRNA3, CHRNB4, HYKK and PSMA4 and clinical pathological features based on the TCGA database.**

| Characteristics | n | IREB 2(%) | | $X^2$ | P | CHRNA5(%) | | $X^2$ | P | CHRNA3(%) | | $X^2$ | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Low[288] | High[288] | | | Low[281] | High[286] | | | Low[275] | High[282] | | |
| Gender | | | | | | | | | | | | | |
| Female | 310 | 163(28.3) | 147(25.5) | 1.788 | 0.`105 | 176(30.4) | 112(19.8) | 12.322 | 0.000 | 156(27.1) | 132(23.0) | 0.028 | 0.46 |
| Male | 266 | 125(21.7) | 141(24.5) | | | 134(23.6) | 154(27.2) | | | 154(26.7) | 134(23.3) | | |
| Age | | | | | | | | | | | | | |
| <40 | 177 | 98(17.0) | 79(13.7) | 3.622 | 0.164 | 95(16.8) | 82(14.5) | 1.797 | 0.407 | 93(16.1) | 84(14.6) | 1.054 | 0.59 |
| 40-60 | 346 | 163(28.3) | 183(31.8) | | | 167(29.5) | 179(31.6) | | | 166(28.8) | 180(31.3) | | |
| >60 | 34 | 15(2.6) | 19(3.3) | | | 19(3.4) | 15(2.6) | | | 16(2.8) | 18(3.1) | | |
| smoking | | | | | | | | | | | | | |
| Yes | 383 | 191(33.2) | 192(33.3) | 0.004 | 0.519 | 65(11.5) | 177(31.2) | 9.265 | 0.002 | 54(9.4) | 189(32.8) | 0.308 | 0.32 |
| No | 103 | 51(8.9) | 52(9.0) | | | 38(6.7) | 206(36.3) | | | 49(8.5) | 194(33.7) | | |
| T stage | | | | | | | | | | | | | |
| 1 | 189 | 98(17.0) | 91(15.8) | 0.493 | 0.921 | 106(18.7) | 83(14.6) | 5.764 | 0.124 | 99(17.2) | 90(15.6) | 2.994 | 0.39 |
| 2 | 315 | 156(27.1) | 159(27.6) | | | 148(26.1) | 167(29.5) | | | 150(26.0) | 165(28.6) | | |
| 3 | 49 | 25(4.3) | 24(4.2) | | | 25(4.4) | 24(4.2) | | | 29(5.0) | 20(3.5) | | |
| 4 | 20 | 9(1.6) | 11(1.9) | | | 7(1.2) | 13(2.3) | | | 9(1.6) | 11(1.9) | | |
| N stage | | | | | | | | | | | | | |
| 1 | 364 | 177(30.7) | 187(32.5) | 5.822 | 0.121 | 179(31.6) | 185(32.6) | 2.004 | 0.572 | 180(31.3) | 184(31.9) | 1.057 | 0.78 |
| 2 | 108 | 63(10.9) | 45(7.8) | | | 53(9.3) | 55(9.7) | | | 59(10.2) | 49(8.5) | | |
| 3 | 87 | 40(6.9) | 47(8.2) | | | 44(7.8) | 43(7.6) | | | 42(7.3) | 45(7.8) | | |
| 4 | 2 | 2(0.3) | 0(0) | | | 0(0) | 2(0.4) | | | 1(0.2) | 1(0.2) | | |
| M stage | | | | | | | | | | | | | |
| 0 | 387 | 195(33.9) | 192(33.3) | 1.501 | 0.155 | 187(33.0) | 10(1.8) | 0.401 | 0.337 | 193(33.5) | 12(2.1) | 0.000 | 0,57 |
| 1 | 24 | 9(1.6) | 15(2.6) | | | 200(35.3) | 14(2.5) | | | 194(33.7) | 12(2.1) | | |
| Disease stage | | | | | | | | | | | | | |
| I | 308 | 151(26.2) | 157(27.3) | 2.956 | 0.398 | 156(27.5) | 152(26.8) | 0.843 | 0.839 | 152(26.4) | 156(27.1) | 1.176 | 0.75 |
| II | 134 | 74(12.8) | 60(10.4) | | | 67(11.8) | 67(11.8) | | | 71(12.3) | 63(10.9) | | |
| III | 97 | 47(8.2) | 50(8.7) | | | 44(7.8) | 53(9.3) | | | 45(7.8) | 52(9.0) | | |
| IV | 28 | 11(1.9) | 17(3.0) | | | 14(2.5) | 14(2.5) | | | 15(2.6) | 13(2.3) | | |
| Overall Survival Status | | | | | | | | | | | | | |
| 0 | 355 | 180(31.3) | 175(30.4) | 0.430 | 0.258 | 188(33.2) | 95(16.8) | 2.962 | 0.050 | 182(31.6) | 99(17.2) | 0.800 | 0.21 |
| 1 | 209 | 100(17.4) | 109(18.9) | | | 167(29.5) | 114(20.1) | | | 173(30.0) | 110(19.1) | | |
| Recurrence Free Survival Status | | | | | | | | | | | | | |
| 0 | 312 | 156(27.1) | 75(13.0) | 1.669 | 0.116 | 169(29.8) | 75(13.2) | 4.694 | 0.019 | 164(28.5) | 82(14.2) | 0.940 | 0.19 |
| 1 | 171 | 156(27.1) | 96(16.7) | | | 143(25.2) | 96(16.9) | | | 148(25.7) | 89(15.5) | | |

***n:*** *the numbers of patients;* ***Low:*** *the low expression of genes;* ***High:*** *the high expression of genes;* ***X2:*** *Chi-square****; P:****P value****; IREB2:****Iron-responsive element-binding protein 2;* ***CHRNA5:*** *Neuronal acetylcholine receptor subunit alpha-5;* ***CHRNA3:*** *Neuronal acetylcholine receptor subunit alpha-3;* ***CHRNB4:*** *Neuronal acetylcholine receptor subunit beta-4;* ***HYKK:*** *Hydroxylysine kinase;* ***PSMA4:*** *Proteasome subunit alpha type-4;*

**Table 2: The expression difference of IREB2, CHRNA5, CHRNA3, CHRNB4, HYKK,and PSMA4 genes between normal and lung adenocarcinoma tissues based on the TCGA database.**

| | | IREB2 | | CHRNA5 | | CHRNA3 | | CHRNB4 | | HYKK | | PSMA4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Normal | LAD | Normal | LAD | Normal | LAD | Normal | LAD | Normal | LAD | Normal | LAD |
| Mean value | | 10.211 | 10.055 | 3.653 | 6.383 | 3.315 | 2.989 | 0.699 | 2.427 | 10.789 | 11.132 | 4.716 | 5.120 |
| 95% confidence interval of the difference | Lower | 0.087 | | -3.029 | | 0.051 | | -1.942 | | -0.451 | | -0.540 | |
| | Upper | 0.225 | | -2.431 | | 0.600 | | -1.514 | | -0.235 | | -0.268 | |
| T | | 4.446 | | -18.106 | | 2.359 | | -15.968 | | -6.312 | | -5.871 | |
| P | | 0.000 | | 0.000 | | 0.021 | | 0.000 | | 0.000 | | 0.000 | |

***LAD:*** *Lung adenocarcinoma****;T:*** *T value****; P:****P value****; IREB2:****Iron-responsive element-binding protein 2;* ***CHRNA5:*** *Neuronal acetylcholine receptor subunit alpha-5;* ***CHRNA3:*** *Neuronal acetylcholine receptor subunit alpha-3;* ***CHRNB4:*** *Neuronal acetylcholine receptor subunit beta-4;* ***HYKK:*** *Hydroxylysine kinase;* ***PSMA4:*** *Proteasome subunit alpha type-4;*

**Table 3: The association between the expression of CHRNA5 and PSMA4 and clinical pathological features.**

| | n | CHRNA5(%) | | $X^2$ | P | PSMA4(%) | | $X^2$ | P |
|---|---|---|---|---|---|---|---|---|---|
| | | Low（53） | High（69） | | | Low（50） | High（72） | | |
| Gender | | | | | | | | | |
| Female | 65 | 32(26.2) | 33(27.0) | 1.897 | 0.116 | 27(24.1) | 38(33.9) | 0.018 | 0.521 |
| Male | 57 | 21(17.2) | 36(29.5) | | | 23(20.5) | 34(30.4) | | |
| Age | | | | | | | | | |
| <60 | 44 | 21(17.2) | 23(18.9) | 0.589 | 0.745 | 18(16.1) | 26(23.2) | 0.848 | 0.654 |
| 60-80 | 75 | 31(25.4) | 44(36.1) | | | 30(26.8) | 45(40.2) | | |
| >80 | 3 | 1(0.8) | 2(1.6) | | | 2(1.6) | 1(0.9) | | |
| smoking | | | | | | | | | |
| Yes | 40 | 18(14.8) | 22(18.0) | 0.030 | 0.510 | 15(13.4) | 25(22.3) | 0.346 | 0.351 |
| No | 67 | 29(23.8) | 38(31.1) | | | 29(25.9) | 38(33.9) | | |
| T stage | | | | | | | | | |
| 1 | 62 | 31(27.7) | 31(27.7) | 9.293 | 0.026 | 26(23.2) | 36(32.1) | 0.923 | 0.820 |
| 2 | 40 | 10(8.9) | 30926.8） | | | 16(14.3) | 24(21.4) | | |
| 3 | 11 | 4(3.6) | 7(6.3) | | | 3(2.7) | 8(7.1) | | |
| 4 | 2 | 2(1.8) | 0(0) | | | 1(0.9) | 1(0.9) | | |
| N stage | | | | | | | | | |
| 1 | 81 | 35(31.3) | 46(41.1) | 2.282 | 0.320 | 32(28.6) | 49(43.8) | 0.671 | 0.715 |
| 2 | 22 | 7(6.3) | 15(13.4) | | | 9(8.0) | 13(11.6) | | |
| 3 | 18 | 10(8.9) | 8(7.1) | | | 9(8.0) | 9(8.0) | | |
| 4 | 0 | 0(0) | 0(0) | | | 0(0) | 0(0) | | |
| M stage | | | | | | | | | |
| 0 | 118 | 52(46.4) | 66(58.9) | 2.318 | 0.182 | 50(44.6) | 68(60.7) | 2.166 | 0.198 |
| 1 | 3 | 0(0) | 3(2.7) | | | 0(0) | 3(2.7) | | |
| Disease stage | | | | | | | | | |
| I | 1 | 1(0.9) | 0(0) | 3.817 | 0.421 | 0(0) | 1(0.9) | 1.360 | 0.715 |
| II | 71 | 28(25.0) | 43(38.4) | | | 27(24.1) | 44(39.3) | | |
| III | 25 | 9(8.0) | 16(14.3) | | | 11(9.8) | 14(12.5) | | |
| IV | 19 | 10(8.9) | 9(8.0) | | | 9(8.0) | 10(8.9) | | |
| Overall Survival Status | | | | | | | | | |
| 0 | 90 | 44(39.3) | 46(41.1) | 6.807 | 0.007 | 40(35.7) | 50(44.6) | 7.094 | 0.006 |
| 1 | 22 | 4(3.6) | 18(16.1) | | | 3(2.7) | 19(17.0) | | |
| Progress Free Survival Status | | | | | | | | | |
| 0 | 78 | 38(33.9) | 40(35.7) | 5.744 | 0.013 | 34(30.4) | 44(39.3) | 6.807 | 0.007 |
| 1 | 30 | 7(6.3) | 23(20.5) | | | 5(4.5) | 25(22.3) | | |

***n:*** *the numbers of patients;* ***Low:*** *the low expression of genes;* ***High:*** *the high expression of genes;* ***X2:*** *Chi-square****; P:****P value****; CHRNA5:*** *Neuronal acetylcholine receptor subunit alpha-5;* ***PSMA4:*** *Proteasome subunit alpha type-4;*

## Figures

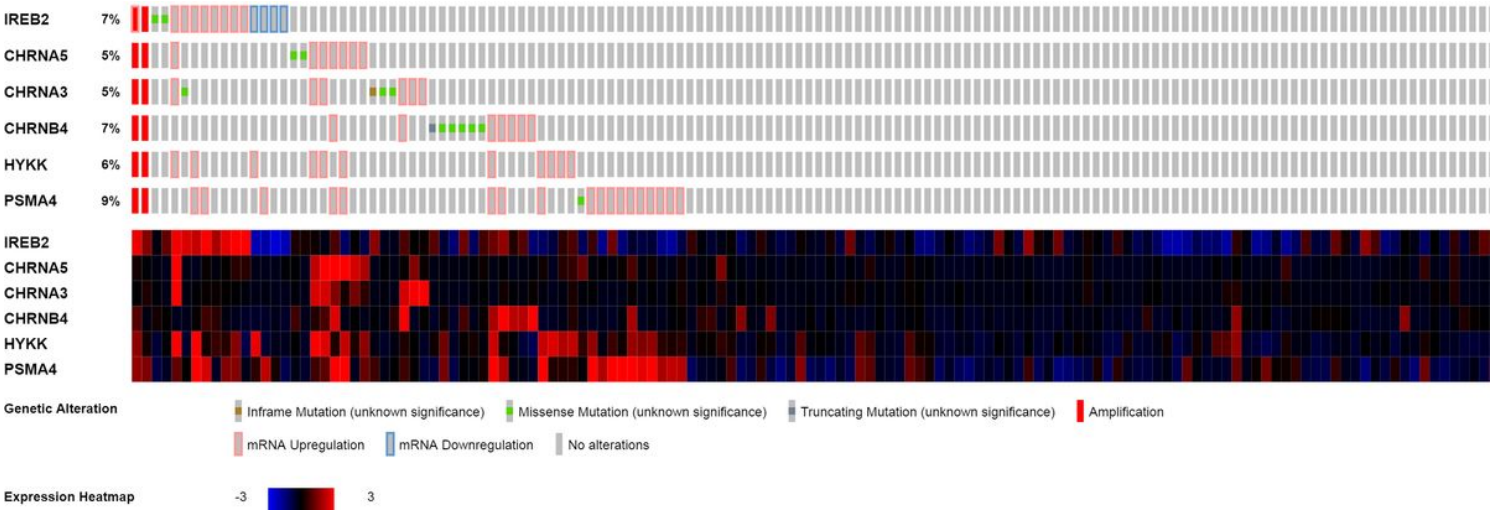


Figure 1

The mutation of IREB2, CHRNA5, CHRNA3, CHRNB4, HYKK,and PSMA4 genes in lung adenocarcinoma based on the TCGA database.

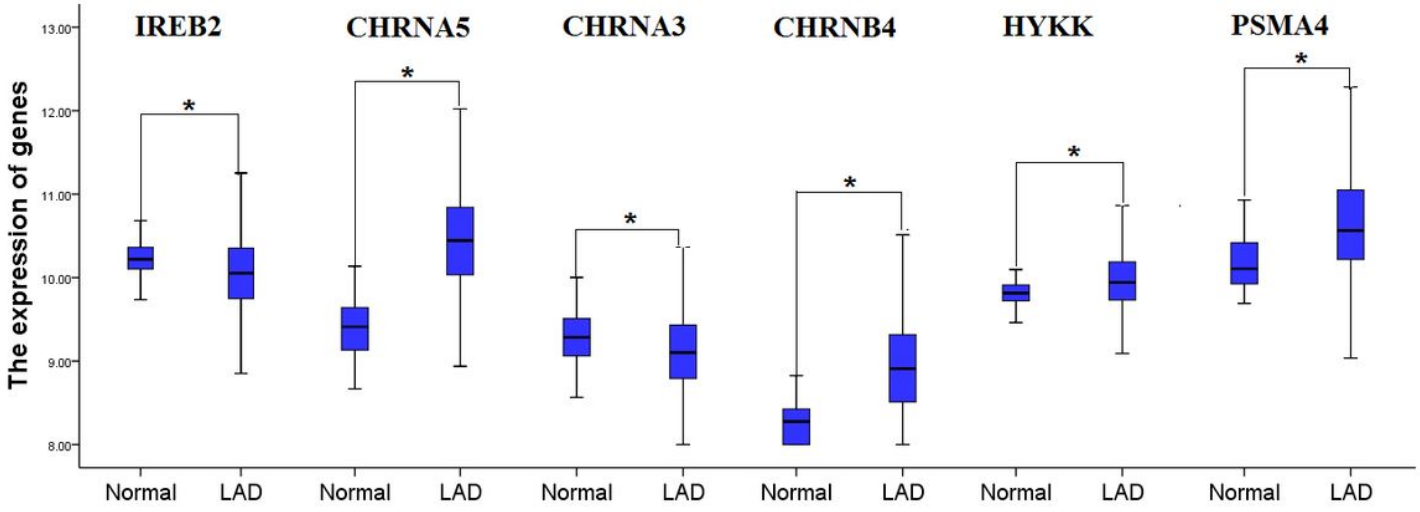


Figure 2

The expression of IREB2, CHRNA5, CHRNA3, CHRNB4, HYKK,and PSMA4 genes between normal and lung adenocarcinoma tissues based on the TCGA database.

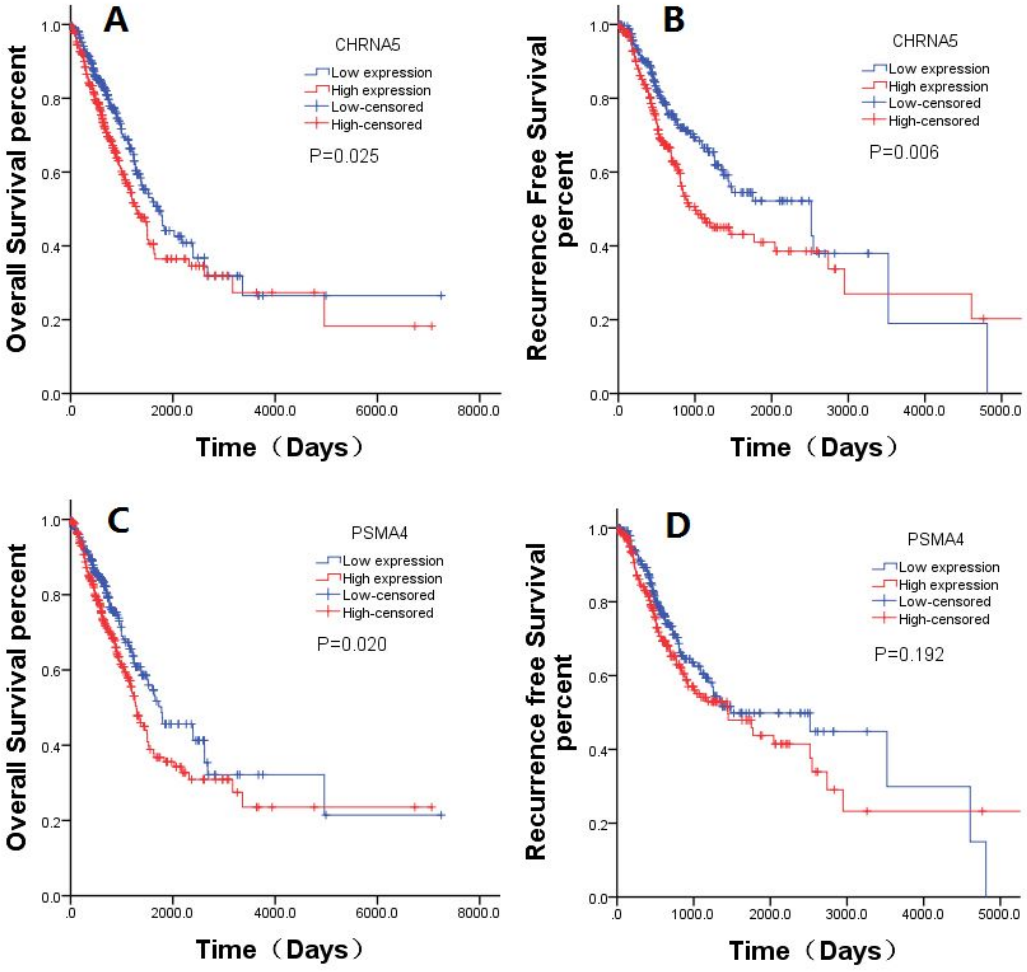


Figure 3

Kaplan–Meier analysis of the correlation between CHRNA5, PSMA4 expression level and overall survival (OS) / recurrence free survival (RFS) based on the TCGA database.

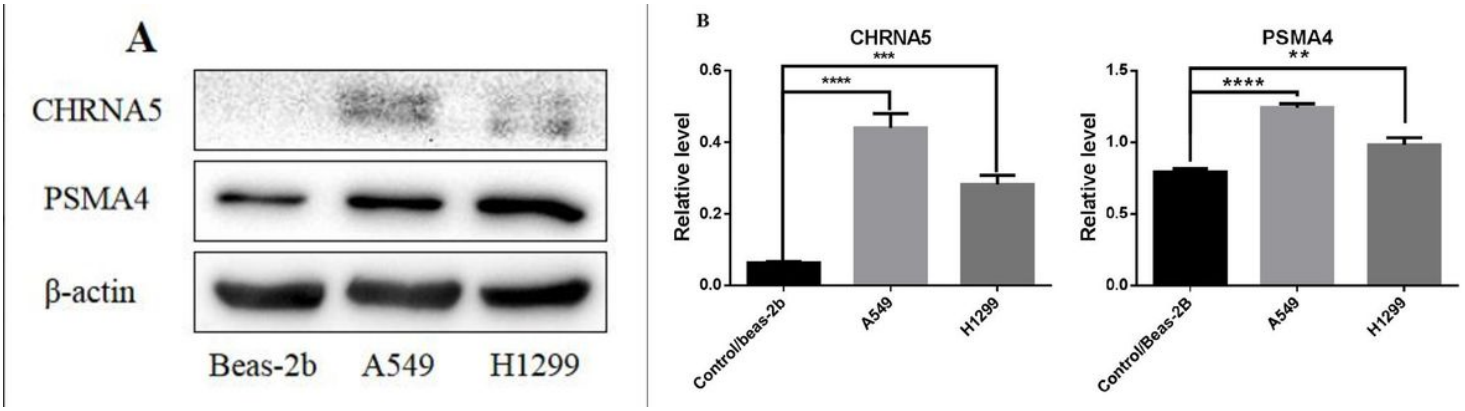


Figure 4

The expression of CHRNA5 and PSMA4 in Beas-2b, A549 and H1299 cells. The expression of CHRNA5 and PSMA4 in A549 and H1299 cells were obvious higher than Beas-2b cell.A. The Western blotting result of the expression of CHRNA5 and PSMA4; B. The relative levels of the expression of CHRNA5 and PSMA4 based on The Western blotting.*** means that the P<0.001;**** means that the P<0.0001.

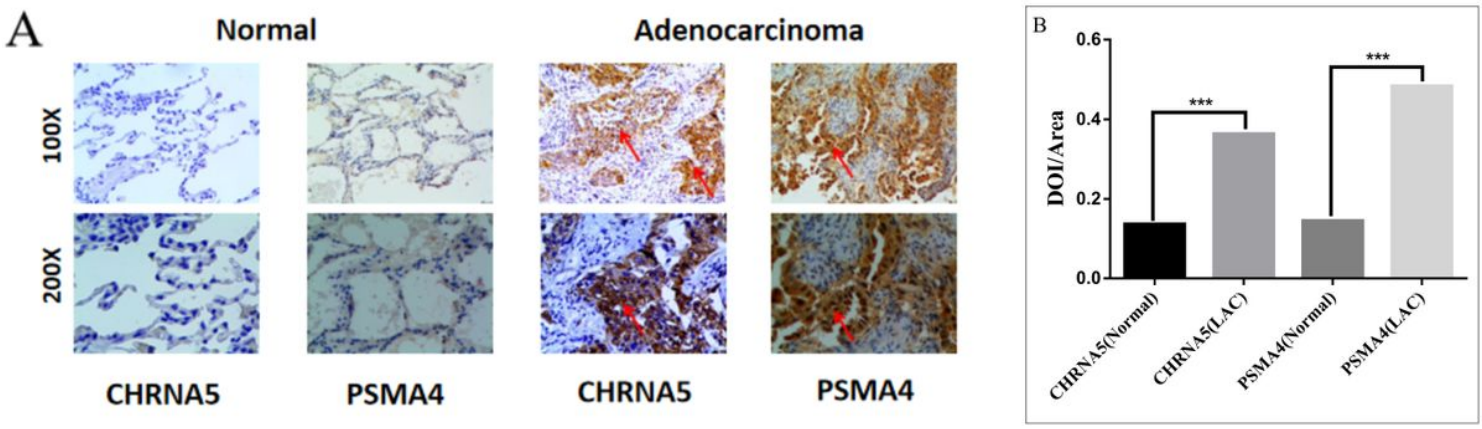


Figure 5

Expression of CHRNA5 and PSMA4 in lung adenocarcinoma The expression of CHRNA5 and PSMA4 in the lung tissue were located in the cytoplasm, and the expression of CHRNA5 and PSMA4 were higher in lung adenocarcinoma tissues, and lower in normal tissues. The results showed that the figure was upper 100 * and below 200 *.

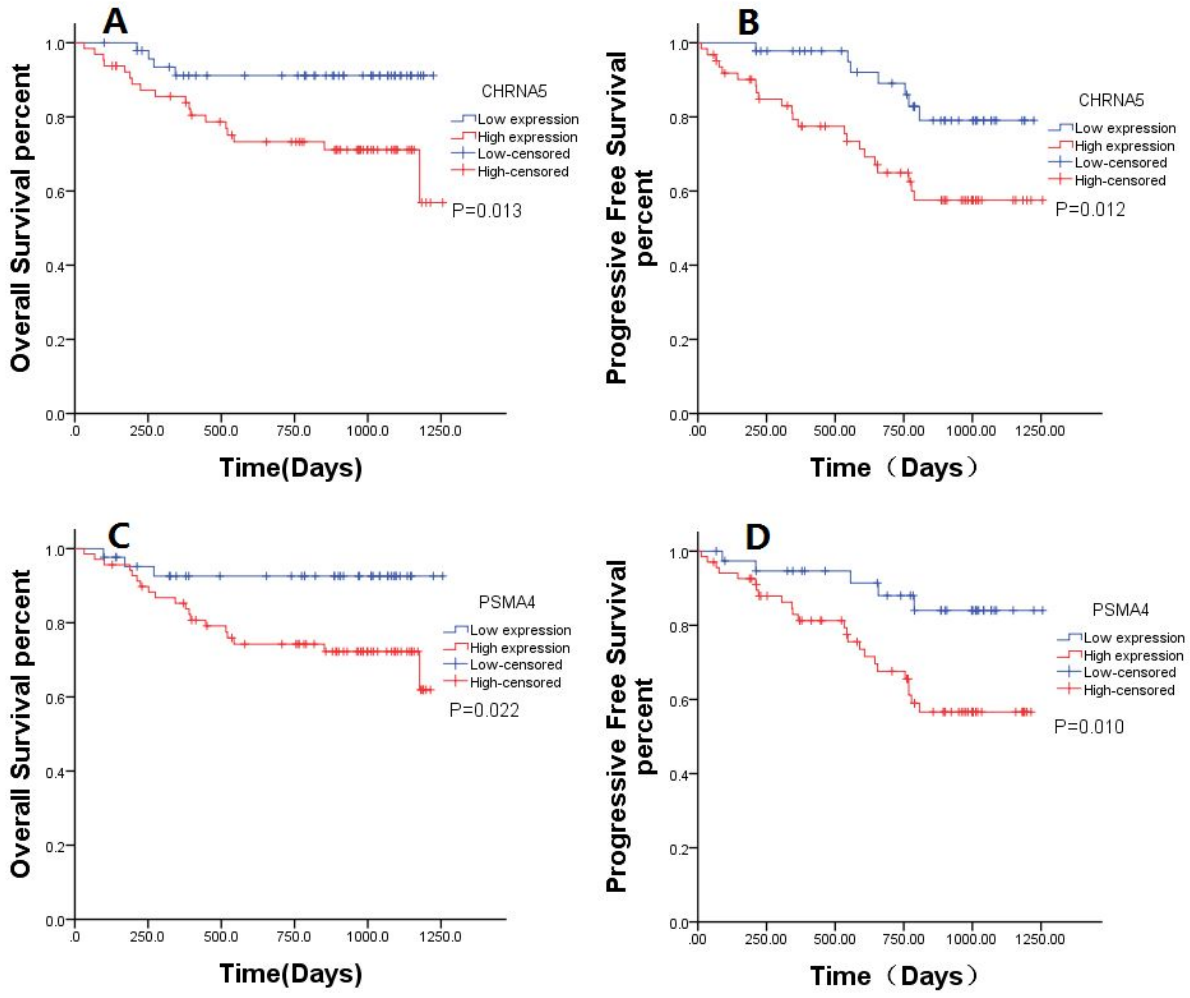


Figure 6

Kaplan–Meier analysis of the correlation between CHRNA5, PSMA4 expression level and overall survival (OS) / recurrence free survival (RFS) based on the clinical data.

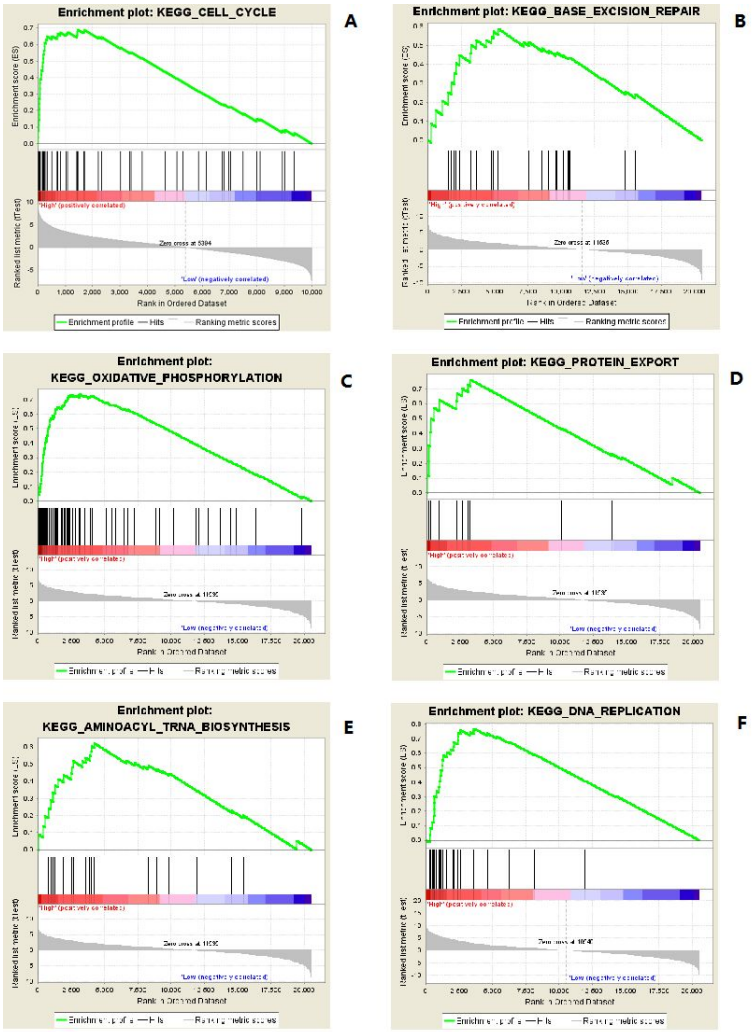


**Figure 7**

GSEA with high expression of CHRNA5, HYKK and PSMA4. The results of GSEA method suggested that CHRNA5 high expression samples were enriched to cell cycle, base excision repair, oxidative phosphorylation, protein export, aminoacyl tRNA biosynthesis

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementFigure1.png
- Supplement.docx