Original Article

MS4A15 gene expression as a prognostic marker for clinical outcomes in lung adenocarcinoma

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Background: Lung adenocarcinoma (LUAD) is the predominant histological subtype of lung cancer. In the past decade, various targeted drugs have prolonged the survival of LUAD patients. Unfortunately, not all LUAD patients can benefit from the current target agents. Although the membrane spanning 4-domains A15 (*MS4A15*) gene has been implicated in the progression of various cancers, its role in LUAD remains understudied. This study aimed to evaluate the role and potential mechanism of MS4A15 in the progression of LUAD.

Methods: The pan-cancer RNA sequencing and clinical data of LUAD patients, originally comprising data from The Cancer Genome Atlas and Genotypic Tissue Expression, were acquired from University of California Santa Cruz XENA (UCSC XENA). Additionally, the GSE116959 and GSE130779 data sets were retrieved from the Gene Expression Omnibus database. The Differential Expression analysis of Sequencing data version 2 (DESeq2) package was used to identify the differentially expressed genes. The ClusteProfiler package was used to perform the Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and gene set enrichment analyses. An immune cell infiltration analysis was conducted using the gene set variation analysis (GSVA) package. The expression level of *MS4A15* was analyzed by the Wilcoxon rank-sum test. A logistic regression analysis was conducted to examine the correlation between the clinical pathological factors of LUAD patients and the high-low dichotomy of *MS4A15*. A receiver operating characteristic (ROC) curve analysis was employed to evaluate the effectiveness of *MS4A15* as a biomarker for distinguishing LUAD patients from healthy individuals. A Kaplan-Meier analysis was conducted to examine the overall survival of LUAD patients based on *MS4A15*. All the bioinformatic results were obtained using R (version 3.6.2) package. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed to validate the messenger RNA transcription level *in vitro*.

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Results: *MS4A15* expression was significantly more decreased in the tumor tissues from the LUAD patients than the normal adjacent samples. *MS4A15* expression was positively correlated with various immune cell types, notably including mast cells (MCs), dendritic cells, and macrophages. Specifically, *MS4A15* was most positively associated with MCs. Lower expression levels of *MS4A15* in LUAD patients were correlated with a poorer pathologic stage and poorer primary therapy outcomes. The area under the curve of the ROC curve for *MS4A15* effectiveness was 0.863. *MS4A15* was validated to be more lowly expressed in the tumor tissues samples than the normal tissues samples in both the GSE116959 and GSE130779 data sets. The expression of *MS4A15* was also significantly lower in the in A549 cells than the normal human bronchial epithelia cells.
Conclusions: Overall, *MS4A15* emerged as a promising prognostic biomarker for LUAD and could serve as a potential target for the development of novel therapeutic interventions.

Keywords: Lung adenocarcinoma (LUAD); The Cancer Genome Atlas (TCGA); Genotypic Tissue Expression (GTEx); Gene Expression Omnibus (GEO); membrane spanning 4-domains A15 (*MS4A15*)

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Introduction

Lung cancer is one of the most common cancers worldwide, and its incidence continues to increase each year (1). The high mortality rate of lung cancer has caused serious global health problems and economic burdens (2). Lung adenocarcinoma (LUAD) is the most common histological type in lung cancer, accounts for over 60% of newly diagnosed lung cancer cases (3,4). The widely accepted classic prognostic biomarkers for LUAD include epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), and c-ros 1 oncogene (*ROS1*) (2). These biomarkers play an important role in predicting treatment response and survival time in patients with LUAD. However, existing prognostic markers do not fully meet clinical needs as they have limitations in predicting disease progression and guiding personalized treatment. For example, although EGFR and ALK targeted therapies have achieved significant results in some patients, not all patients can benefit from these treatments, and the issue of post-treatment drug resistance also urgently needs to be addressed (5,6).

In recent years, some studies have attempted to identify early and novel prognostic markers for LUAD. Hu *et al.* showed that tsRNA-5001a can increase the risk of postoperative recurrence in LUAD patients (7). Xu *et al.* revealed a correlation between KLRB1 and both prognosis and immune infiltration in LUAD patients (8). However, the molecular heterogeneity of LUAD is high (2), which means that more biomarkers need to be explored to comprehensively predict the prognosis of LUAD patients.

A newly identified four transmembrane protein, the membrane spanning 4-domains A15 (*MS4A15*), has been reported to mediate the occurrence and development of some tumors by regulating various biological processes (BPs), such as iron removal, cell metabolism, and immune cell infiltration (9-13). For example, in ovarian cancer, higher *MS4A15* expression was found to be associated with a poor prognosis (9). Lower mRNA expressions of *MS4A15* correlated with better overall survival (OS) in gastric cancer patients (13).

Highlight box

Key findings

- This study identified a novel prognostic marker for lung adenocarcinoma (LUAD) to improve the personalized management of LUAD patients.

What is known, and what is new?

- Previous research has shown that LUAD is characterized by heterogeneity. Not every LUAD patient can benefit from current targeted drugs.
- This study showed that lower expression levels of membrane spanning 4-domains A15 (*MS4A15*) in LUAD patients was correlated with a poorer pathologic stage and poorer primary therapy outcomes. The low expression of *MS4A15* was also associated with poor overall survival in LUAD patients.

What is the implication, and what should change now?

- *MS4A15* may serve as a prognostic biomarker and a potential treatment target for LUAD.

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Considering the established correlation between *MS4A15* and overall survival in certain malignancies, yet the limited researches on lung cancer, this study is designed to focuses on the prognostic implications of *MS4A15* expression in patients with LUAD. We also examined the biological functions of *MS4A15* in regulating LUAD cells. Additionally, the relationship between *MS4A15* and immune cell infiltration in the tumor microenvironment (TME) was investigated. This study aimed to evaluate the role and potential mechanism of *MS4A15* in the progression of LUAD and identified a novel prognostic marker for LUAD, which could improve the personalized management of LUAD patients. We present this article in accordance with the REMARK reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-623/rc).

Methods

Obtaining RNA sequencing (RNA-seq) data and data preprocessing

The RNA-seq data for pan-cancer from The Cancer Genome Atlas (TCGA) and Genotypic Tissue Expression (GTEx) databases were downloaded and processed uniformly by University of California Santa Cruz XENA (UCSC XENA) (https://xenabrowser.net/datapages/?host=https%3A%2F%2Ftoil.xenahubs.net). The GSE116959 data set and GSE130779 data set were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Differentially expressed genes (DEGs) analysis

The (low or high) expression level of *MS4A15* in the LUAD samples was analyzed by Differential Expression analysis of Sequencing data version 2 (DESeq2) package in R Project for Statistical Computing (R) software to identify the DEGs based on a cut-off threshold of 50%. The top 10 DEGs were illustrated in a heat map.

Functional enrichment analysis

A functional enrichment analysis of the DEGs was conducted based on the following criteria: $|\log_2$ fold change (FC)$|$ >2 and adjusted P value (P_{adj}) <0.05. Meanwhile, ClusteProfiler package in R software was used to analyze Gene Ontology (GO) function of DEGs, including the cellular components (CCs), molecular functions (MFs), BPs. A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was also conducted using ClusteProfiler package.

Gene set enrichment analysis (GSEA)

A GSEA is a meaningful tool that can identify gene sets associated with specific BPs. In this study, a GSEA of *MS4A15* was conducted using ClusteProfiler in R software. A P_{adj}<0.05 and a false discovery rate q value <0.25 were considered statistically significant.

Immune infiltration analysis by single-sample gene set enrichment analysis (ssGSEA)

Using the gene set variation analysis (GSVA) package, immune infiltration analysis of *MS4A15* was conducted through ssGSEA. A total of 24 types of infiltrating immune cells were obtained. Divide the samples into two groups based on the expression level of *MS4A15*: a high-expression group and a low-expression group, using the median expression value of *MS4A15* as the cutoff. Based on the above analysis, the correlation scores between *MS4A15* and the enrichment of 24 types of immune cells were calculated by Spearman correlation. In addition, the enrichment scores of the high and low expression groups were obtained by the Wilcoxon rank-sum test.

Prognostic prediction

The clinical data of the LUAD patients were obtained from TCGA and GTEx databases. All the LUAD patients were included in the data analysis. The survival package in R software were used to perform survival analyse. The visualization of data was conducted utilizing the survminer package in R software. All statistical tests were double-tailed with 0.05 as the statistical significance level.

In vitro MS4A15 expression

The A549 cell line was purchased from Procell Life Science & Technology Co., Ltd., and the normal human bronchial epithelial (NHBE) cell line was obtained from the Chinese Academy of Sciences Cell Bank. Both cell lines were authenticated through Short Tandem Repeats (STR) profiling and tested for mycoplasma contamination. Both A549 and NHBE cells were cultured in Dulbecco's

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Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. A549 cells were initially plated in 25 cm^2 culture flasks at a concentration of 2×10^4 cells per square centimeter, ensuring ample space for their attachment and proliferation. NHBE cells were inoculated at a higher density of 2×10^5 cells per square centimeter, tailored to their growth requirements. All cultures were nurtured in a controlled environment at 37 ℃ with 5% CO_2 to maintain humidity, simulating physiological conditions. The growth medium was exchanged every two days to replenish nutrients and clear metabolic byproducts. Upon reaching a confluence of 80% to 90%, the cells underwent subculturing. RNA extraction was performed using the RNeasy mini kit (74106; Qiagen, Hilden, Germany). TB Green® Premix Ex Taq™ II (RR820A; Takara, Otsu, Japan) was used to reverse transcribe the RNA into complementary DNA. The real-time quantitative polymerase chain reaction (RT-qPCR) experiment was conducted using the PrimeScript™ RT reagent kit with genomic DNA (gDNA) eraser (RR047A; Takara). Calculation of the relative gene expression levels between the target gene and the reference gene in RT-qPCR is performed using the $2^{-\Delta\Delta\text{cycle threshold (Ct)}}$ method.

Statistical analysis

Statistical analyses were also used to validate the potential association between the clinical characteristics and *MS4A15* expression. The Wilcoxon rank-sum test was used to analyze the expression levels of *MS4A15* in the unpaired samples with the median expression of *MS4A15* as the cut-off value. To distinguish the LUAD samples from healthy samples, a receiver operating characteristic (ROC) curve analysis was used to assess the effectiveness of the transcriptional expression of *MS4A15*. A Kaplan-Meier analysis was performed to predict OS. $P<0.05$ is considered statistically significant. All the analysis result and graphs were obtained using R software (version 3.6.2).

Results

Expression of MS4A15 in pan-cancers and LUAD

RNA-seq data for multiple tumors and normal tissue samples were downloaded from TCGA and GTEx databases. We first assessed the expression of *MS4A15* in various cancers. In our analysis, the expression of *MS4A15* was found to be significantly decreased in seven types of cancers, including LUAD (*Figure 1A,1B*).

DEGs in LUAD samples with low and high expression of MS4A15

The DEGs between the low and high *MS4A15* expression groups were identified in the LUAD samples. The following criteria were used to define the DEGs: $|\log_2 FC|>2$ and $P_{adj}<0.05$. A total of 1,605 DEGs were identified, of which 23 were upregulated and 1,582 were downregulated (*Figure 2A*). The heat map shows the top five upregulated DEGs and the top five downregulated DEGs between the high and low *MS4A15* expression groups (*Figure 2B*).

Functional enrichment analysis of the DEGs

A total of 1,605 DEGs in LUAD were obtained between the low and high *MS4A15* expression samples. Subsequently, GO and KEGG enrichment analyses were conducted to summarize the potential functional of the 1,605 DEGs. The associations among the BPs, CCs, MFs, and KEGG pathways are summarized in the table available at https://cdn.amegroups.cn/static/public/tlcr-24-623-1.xlsx. The BP enrichment results included nucleosome assembly, protein-DNA complex assembly, and the positive regulation of heterotypic cell-cell adhesion (*Figure 3A*). The CC enrichment results included the DNA packaging complex, and protein-DNA complex (*Figure 3B*). The MF enrichment results included messenger RNA base-pairing post-transcriptional repressor activity, protein heterodimerization activity, and translation repressor activity (*Figure 3C*). The KEGG pathway enrichment results included neutrophil extracellular trap formation, viral carcinogenesis, and neuroactive ligand-receptor interaction (*Figure 3D*).

The GSEA revealed the biological pathways involved in LUAD between the low and high *MS4A15* expression groups. A total of 82 biological pathways were identified ($P_{adj}<0.05$) (table available at https://cdn.amegroups.cn/static/public/tlcr-24-623-2.xlsx). The main enriched biological pathways included reactome M phase, reactome DNA repair, reactome RHO GTPase effectors, reactome translation, reactome cell cycle checkpoints, reactome mitotic metaphase and anaphase, reactome mitotic prometaphase, reactome separation of sister chromatids, and reactome S phase (*Figure 4A-4I*).

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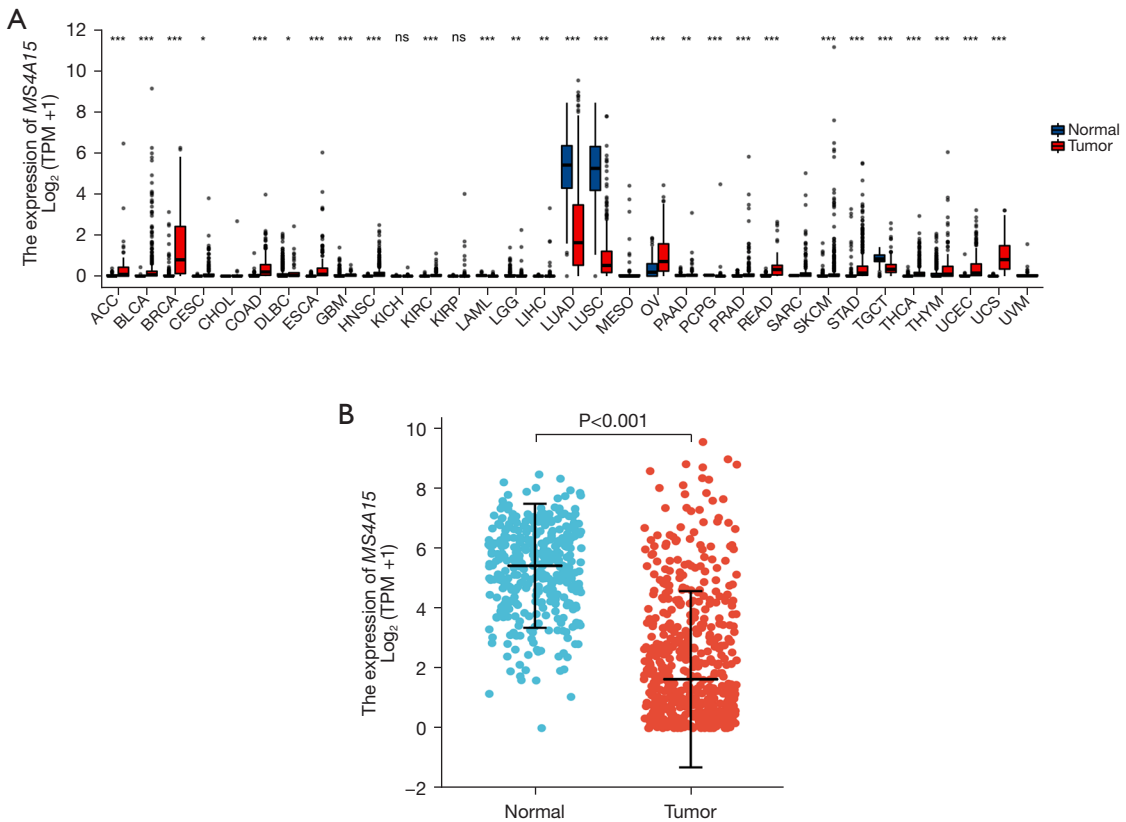

Figure 1 The expression levels of *MS4A15* between the tumor and normal samples. (A) The expression levels of *MS4A15* in the pan-cancer and normal samples. (B) The expression levels of *MS4A15* between the LUAD and normal tissues. Analysis between two groups: Wilcoxon rank-sum test. ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001. *MS4A15*, membrane spanning 4-domains A15; LUAD, lung adenocarcinoma; TPM, transcript per million; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Immune cell infiltration analysis in LUAD

The Spearman correlation analysis results revealed a relationship between *MS4A15* and various immune cell infiltration levels (*Figure 5*, and Table S1). The forest plots illustrated a positive association between *MS4A15* and immune cells, including mast cells (MCs), dendritic cells (DCs), macrophage cells, and natural killer cells, and a negative relationship between the type 2 helper T (Th2) cell subset and *MS4A15*. Notably, *MS4A15* was most positively associated with MCs (*Figure 5B*,*5C*).

Association between MS4A15 expression and clinical features

Table 1 summarizes the main clinical characteristics of the LUAD patients. All the LUAD patients recorded in TCGA and GTEx database were included in this research. The data set comprised 535 patients (of whom 249 were male and 286 were female). The LUAD patients were divided into groups based on the median expression level of *MS4A15*. *MS4A15* expression was high in 268 (50.1%) and low in 267 (49.9%) LUAD patients.

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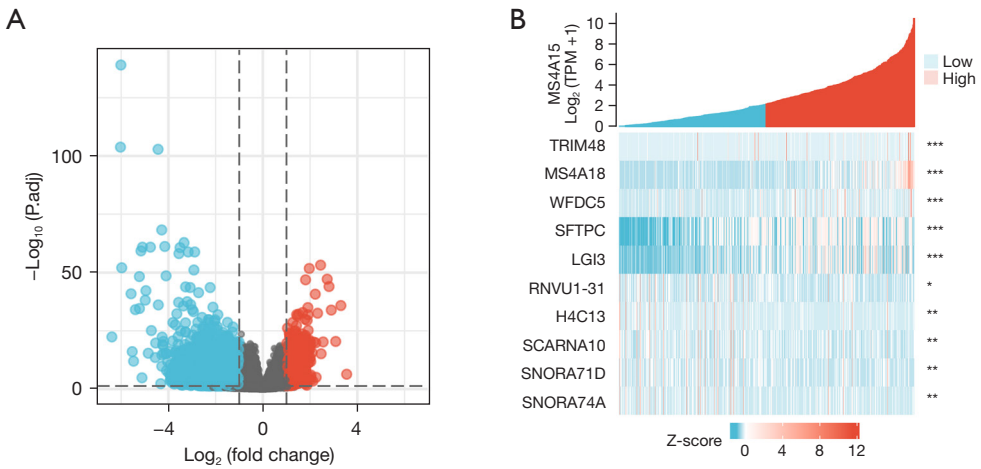

Figure 2 DEGs between the low and high *MS4A15* expression groups in LUAD. (A) Volcano plot showing the DEGs between the low and high *MS4A15* expression groups. The red dots represent the upregulated genes, the blue dots represent the downregulated genes. Genes that do not show a statistically significant differences in expression are represented by gray dots. (B) Heat map showing 10 DEGs, comprising the top five upregulated and downregulated genes, respectively. The X-axis represents the samples, and the Y-axis represents genes. The blue squares represent the downregulated genes, while the red squares represent the upregulated genes. *, P<0.05; **, P<0.01; ***, P<0.001. DEGs, differentially expressed genes; *MS4A15*, membrane spanning 4-domains A15; LUAD, lung adenocarcinoma; TPM, transcript per million.

A logistic regression analysis was performed to verify the correlation between the clinical pathological factors of LUAD patients and the *MS4A15* high-low dichotomy. The results indicated that the low expression of *MS4A15* was significantly positively associated with smoking [odds ratio (OR), 2.043; P=0.006] and age (≤65 years old) (OR, 1.523; P=0.02), and negatively correlated with pathologic stage (stage III–IV *vs.* stage I–II) (OR, 0.543; P=0.005) and the primary therapy outcome [progressive disease (PD) *vs.* partial response (PR) & complete response (CR)] (OR, 0.443; P=0.003) (*Table 2*). The discriminatory potential of *MS4A15* as a biomarker for LUAD was evaluated by comparing tumor tissues with adjacent normal lung tissues using ROC curve analysis. The area under the curve was calculated as 0.863, which suggests that *MS4A15* holds promise as a biomarker (*Figure 6*).

Low MS4A15 expression affected the prognosis of LUAD patients

A Kaplan-Meier analysis was conducted to assess the relationship between *MS4A15* expression and OS in all the included 535 LUAD patients. As shown in *Figure 7*, the prognosis of patients with low expression of *MS4A15* was significantly worse than that of patients with high expression of *MS4A15* [OS hazard ratio, 0.64; 95% confidence interval (CI): 0.48–0.86; P=0.003].

Verifying the expression of MS4A15 in other transcriptional datasets

To gain a further understanding of the expression of *MS4A15* in LUAD, we retrieved the LUAD transcriptome data set from the GEO database. The GEO dataset with the accession number GSE116959 and GSE130779 were utilized for our analysis. The GSE116959 dataset comprises tumor specimens from 57 LUAD patients and 11 normal lung tissue specimens. The GSE130779 dataset included samples from eight lung cancers and adjacent non-tumor tissues that were excised from a cohort of 8 patients with LUAD. After analyzing these two data sets, we again found that *MS4A15* expression was lower in the LUAD tissues than in the normal tissues in the two data sets (*Figure 8*).

RT-qPCR results validated the expression of MS4A15 in A549 cells

The A549 and NHBE cells were cultured to verify the expression of *MS4A15* in the LUAD cells *in vitro*. The results showed that *MS4A15* expression was significantly

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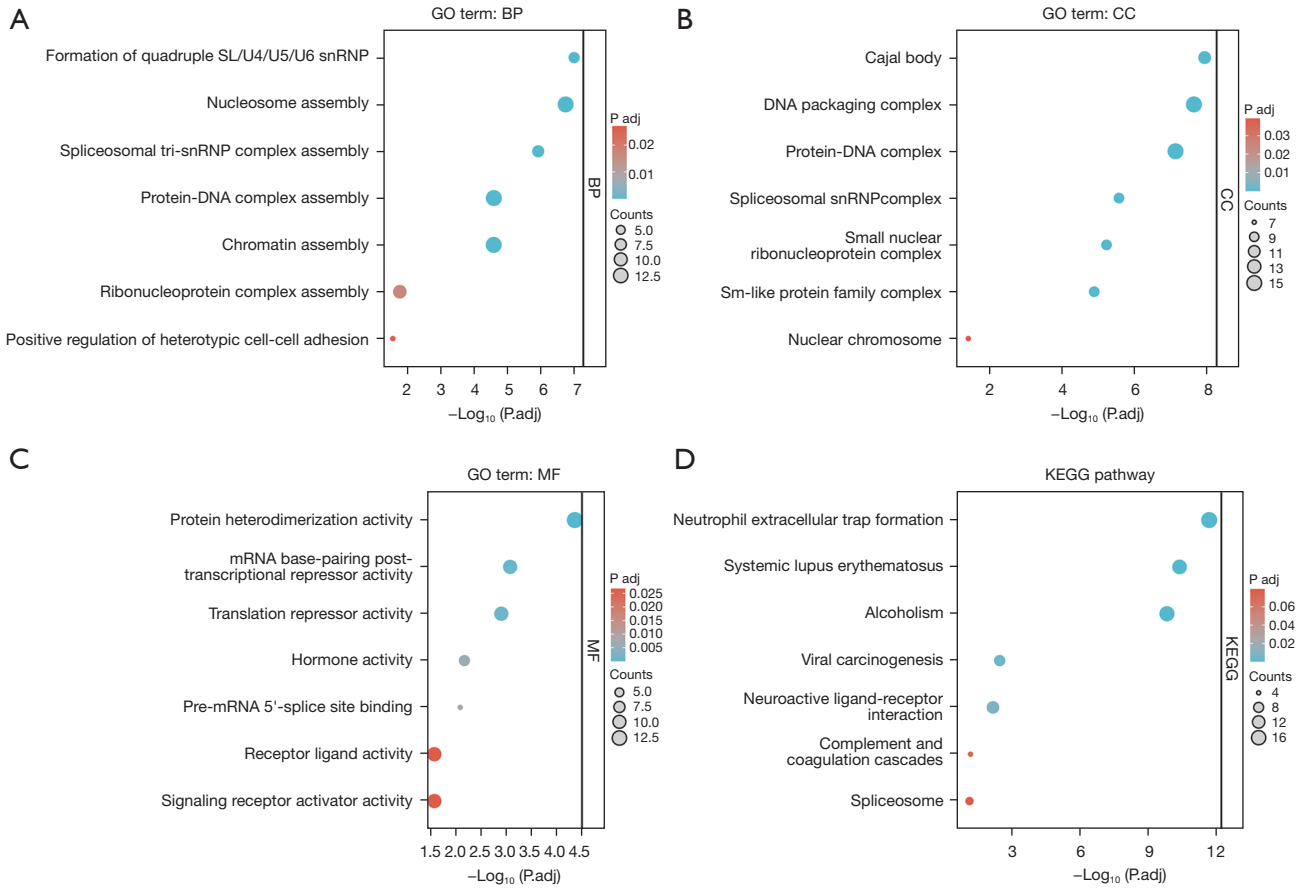

Figure 3 The DEG results of the GO and KEGG enrichment analysis results between the high and low *MS4A15* expression groups in the LUAD patients. (A) BP terms. (B) CC terms. (C) MF terms; (D) KEGG pathway annotations. DEG, differentially expressed gene; *MS4A15*, membrane spanning 4-domains A15; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; LUAD, lung adenocarcinoma; BP, biological process; CC, cellular component; MF, molecular function.

lower in the A549 cells than in the NHBE cells (*Figure 9*).

Discussion

A study highlighted that the 5-year relative survival rate of lung cancer has improved in the last few years (14). Additionally, data from the 2021 World Health Organization's Classification of Thoracic Tumors has shown that annual decline in lung cancer mortality more than doubled from 2.4% to 5.5% between the two 5-year periods ending in 2013 and 2018. There are numerous factors that could explain this improvement. First, the emergence of more potent targeted therapies and immunotherapies has enhanced OS rates for specific patient populations (15). Second, advancements in precision medicine now allow for the tailoring of personalized treatment plans for LUAD patients (16). In this context, the identification of new biomarkers could help researchers gain a deeper understanding of the molecular basis of LUAD, thus resulting in a crucial role in the diagnosis, prognostic stratification, and treatment response prediction of LUAD, and, ultimately, accelerating the development of new targeted drugs.

In recent years, the study of MS4A family members has revealed abnormal expression patterns and diverse functions in various solid tumor tissues (9,11,13). MS4A proteins have been found to be differentially regulated in LUAD (11). Specifically, *MS4A15*, a multi-pass membrane protein, has been shown to activate cell surface receptor signaling pathways (17). Normally, MS4A15 expression is restricted to the lung, which suggests that *MS4A15* may play a certain role in assisting in regulating the normal physiological

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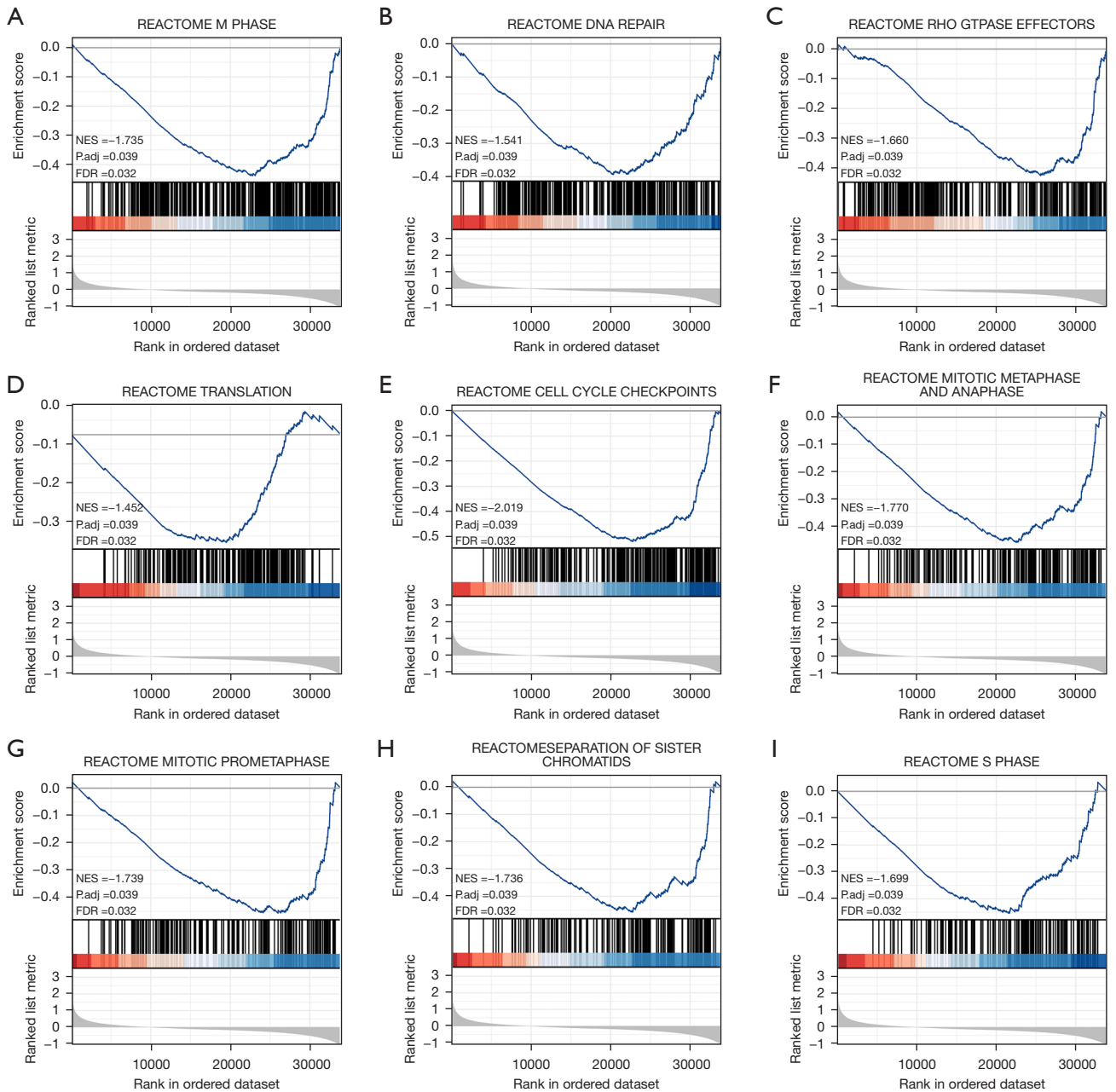

Figure 4 Enrichment plots from the GSEA. NES, normalized enrichment score; FDR, false discovery rate; P_{adj}, adjusted P value; GSEA, gene set enrichment analysis.

function of the lungs (18). In view of this, further research on the relationship between *MS4A15* and LUAD is required.

This present study sought to explore effective treatment and prognostic biomarkers for lung cancer. The main results of this study are summarized as follows. First, the low expression of *MS4A15* in LUAD was found to be associated with low MCs, low DCs, high Th2 cells, poor T staging, poor pathological staging, poor primary treatment outcomes, and a poor prognosis. Second, the GSEA results revealed a significant association between the low expression of *MS4A15* and genes associated with cell cycle (S phase, mitotic metaphase, mitotic anaphase, cell cycle checkpoints), DNA repair, and translation. Third,

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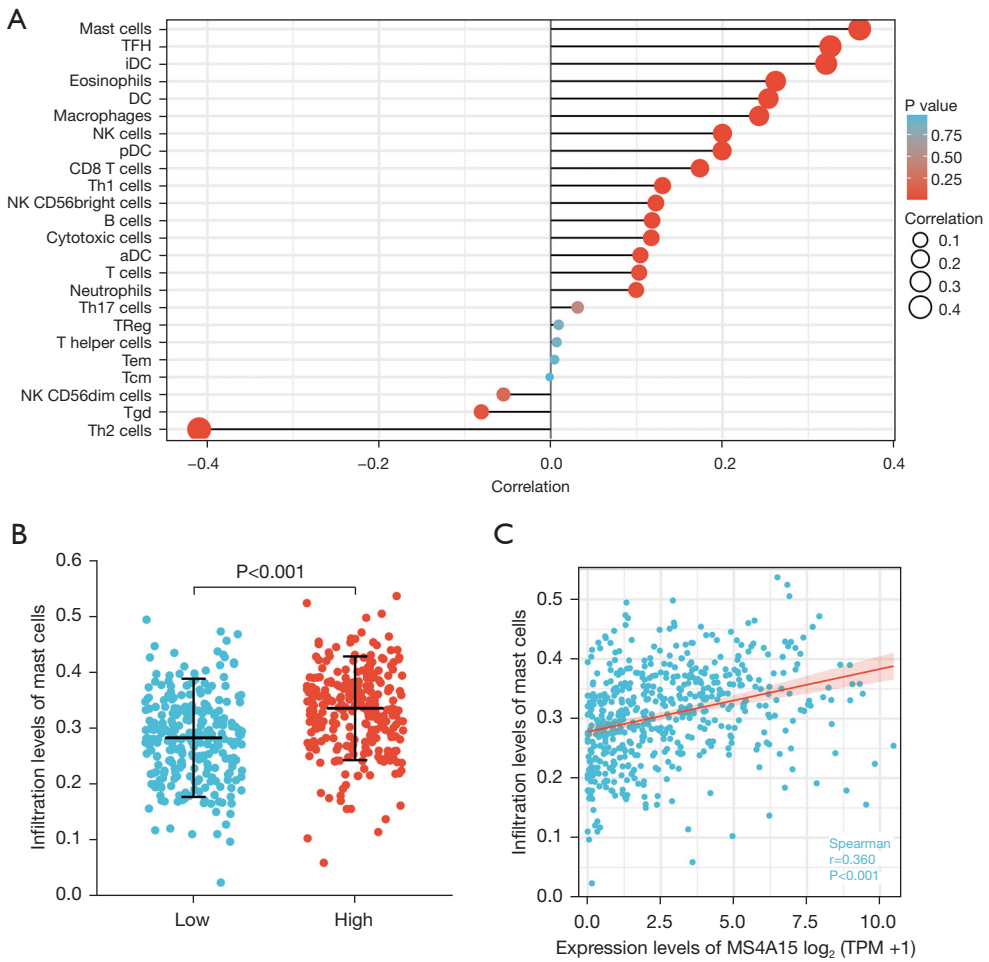

Figure 5 Relationship between immune cell infiltration and the expression of *MS4A15* in the LUAD microenvironment. (A) Expression level of *MS4A15* in relation to the 24 immune cells displayed by the forest plots. (B) Differences in mast cell infiltration were observed between the low and high *MS4A15* expression groups. (C) The correlation between the relative enrichment score of the mast cells and the expression level [$\log_2$(TPM+1)] of *MS4A15*. *MS4A15*, membrane spanning 4-domains A15; LUAD, lung adenocarcinoma; TPM, transcript per million; TFH, T follicular helper cells; iDC, immature dendritic cells, DC, dendritic cells; NK, natural killer cells; pDC, plasmacytoid dendritic cells; aDC, activated dendritic cells; Treg, regulatory T cells; Tem, effector memory T cells; Tcm, central memory T cells; Tgd, gamma delta T cells; Th2, type 2 helper T.

and this appears the most clinically relevant finding of this study, a correlation was found between the low expression of *MS4A15* in LUAD tissues and the low survival rate of LUAD patients. Finally, the RT-qPCR results validated the expression of *MS4A15* in the A549 cells compared to NHBE cells, which shows the consistency between the *in vitro* experiment and bioinformatics analysis results.

The TME refers to the local environment associated with tumor development and metastasis (19-21). The interaction between the tumor cells and TME determines the progression of tumors. MCs are immune cells with multiple functions that influence tumor biology, including cell cycle, proliferation, invasion, angiogenesis, and metastasis. In some cancers, MCs are linked to a poor prognosis (22-24). The relationship between MCs and prognosis in non-small cell lung cancer (NSCLC) is complex and not yet fully understood. Different studies have reported conflicting findings on the effects of MCs on NSCLC prognosis. Carlini *et al.* showed that low MC density in the peritumoral zone of stage-I NSCLC is associated with a poor prognosis (25). Conversely, Imada *et al.* showed that in resected stage-I LUAD patients, higher MCs counts were associated with

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Table 1 Association between *MS4A15* expression and the clinicopathologic features of LUAD patients

Characteristic	Low expression of *MS4A15* (n=267)	High expression of *MS4A15* (n=268)	P
Gender, n (%)			0.002
Female	124 (23.2)	162 (30.3)	
Male	143 (26.7)	106 (19.8)	
Age, n (%)			0.02
≤65 years	142 (27.5)	113 (21.9)	
>65 years	118 (22.9)	143 (27.7)	
Age, years, median [IQR]	64 [58.75, 72]	67 [59, 73]	0.06
Smoker, n (%)			0.008
No	26 (5.0)	49 (9.4)	
Yes	232 (44.5)	214 (41.1)	
T stage, n (%)			0.005
T1	68 (12.8)	107 (20.1)	
T2	160 (30.1)	129 (24.2)	
T3	26 (4.9)	23 (4.3)	
T4	11 (2.1)	8 (1.5)	
N stage, n (%)			0.09
N0	166 (32.0)	182 (35.1)	
N1	48 (9.2)	47 (9.1)	
N2	45 (8.7)	29 (5.6)	
N3	2 (0.4)	0	
M stage, n (%)			0.61
M0	190 (49.2)	171 (44.3)	
M1	15 (3.9)	10 (2.6)	
Pathologic stage, n (%)			0.04
Stage I	137 (26.0)	157 (29.8)	
Stage II	58 (11.0)	65 (12.3)	
Stage III	52 (9.9)	32 (6.1)	
Stage IV	16 (3.0)	10 (1.9)	
Primary therapy outcome, n (%)			0.005
PD	47 (10.5)	24 (5.4)	
SD	15 (3.4)	22 (4.9)	
PR	1 (0.2)	5 (1.1)	
CR	156 (35.0)	176 (39.5)	

MS4A15, membrane spanning 4-domains A15; LUAD, lung adenocarcinoma; IQR, interquartile range; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

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Table 2 The relationship between the clinicopathological factors of LUAD patients and *MS4A15* expression using a logistic analysis

Characteristics	Total (N)	Odds ratio (95% CI)	P value
Gender (male *vs*. female)	535	0.567 (0.402–0.799)	0.001
Age (>65 *vs*. ≤65 years)	516	1.523 (1.077–2.158)	0.02
Smoker (no *vs*. yes)	521	2.043 (1.236–3.446)	0.006
Race (Black or African American *vs*. White)	461	0.809 (0.457–1.422)	0.46
Pathologic stage (stage III–IV *vs*. stage I–II)	527	0.543 (0.351–0.831)	0.005
T stage (T3–4 *vs*. T1–2)	532	0.809 (0.483–1.347)	0.42
N stage (N2–3 *vs*. N0–1)	519	0.577 (0.347–0.944)	0.03
M stage (M1 *vs*. M0)	386	0.741 (0.314–1.675)	0.48
Primary therapy outcome (PD *vs*. PR & CR)	409	0.443 (0.256–0.750)	0.003

LUAD, lung adenocarcinoma; *MS4A15*, membrane spanning 4-domains A15; CI, confidence interval; PD, progressive disease; PR, partial response; CR, complete response.

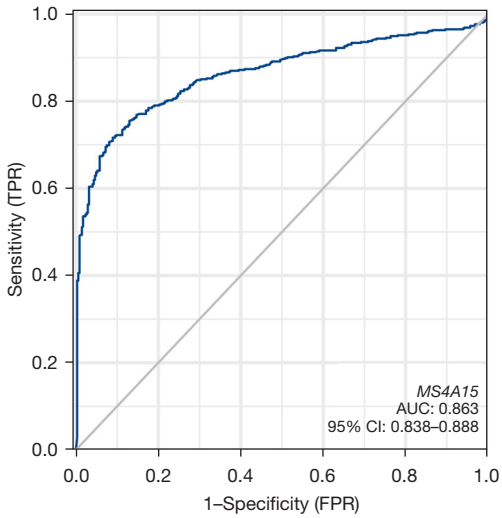

Figure 6 The diagnostic efficacy of *MS4A15* in LUAD analyzed by the ROC curve. *MS4A15*, membrane spanning 4-domains A15; LUAD, lung adenocarcinoma; ROC, receiver operating characteristic; TPR, true positive rate; FPR, false positive rate; AUC, area under the curve; CI, confidence interval.

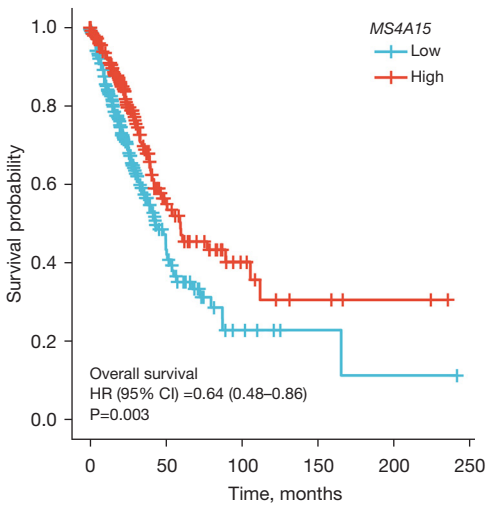

Figure 7 Low expression of *MS4A15* was associated with poor overall survival in LUAD patients. *MS4A15*, membrane spanning 4-domains A15; LUAD, lung adenocarcinoma; CI, confidence interval; HR, hazard ratio.

poor prognosis (26). Meanwhile, Qu *et al.* reported that MCs are recruited into NSCLC microenvironment to enhance the epithelial-to-mesenchymal transition and migration of cancer cells, thereby accelerating the growth of NSCLC (27). Conversely, Tataroğlu *et al.* reported higher concentrations of MCs in early NSCLC, but without any prognostic impact (28).

It is perhaps unsurprising that there are contradictory reports of the correlation between MC tumor infiltration and prognosis in lung cancer. To date, few studies have explored adenocarcinoma and squamous cancer separately. Recently, Tamminga *et al.* separately compared MCs in adenocarcinoma and squamous cell carcinoma, and reported a higher MC concentration in adenocarcinoma, which was also associated with longer OS (29). In our study, we found downregulated *MS4A15* implied a poor prognosis, and the

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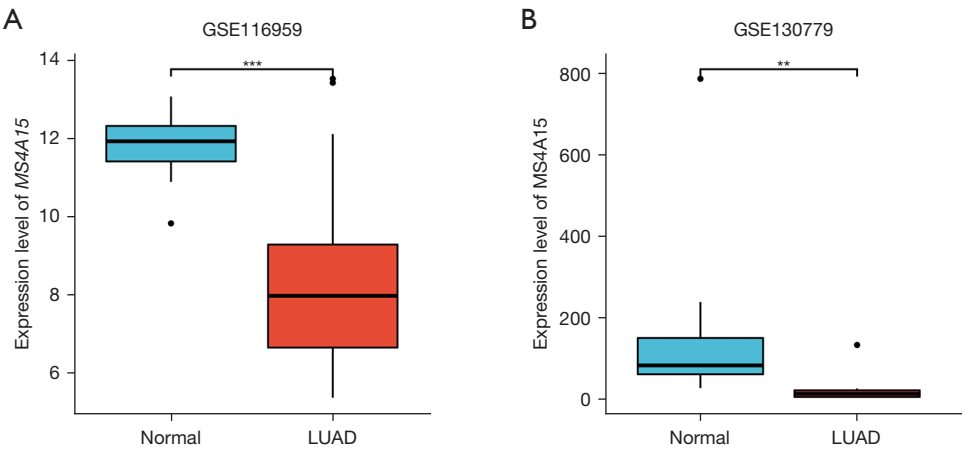

Figure 8 *MS4A15* was significantly downregulated in the LUAD group compared to the healthy control group. (A) GSE116959 data set. (B) GSE130779 data set. **, P<0.01; ***, P<0.001. *MS4A15*, membrane spanning 4-domains A15; LUAD, lung adenocarcinoma.

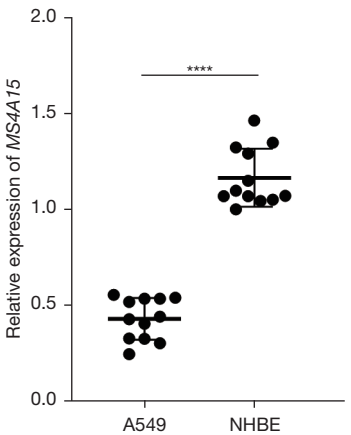

Figure 9 RT-qPCR validation results of *MS4A15* between the A549 and NHBE cells. The unpaired *t*-test was used to compare the differences between the two groups. ****, P<0.001. Each plot represents a sample. The middle horizontal line marks the mean, while the top and bottom horizontal lines mark the positive and negative standard deviations, respectively. RT-qPCR, real-time quantitative polymerase chain reaction; *MS4A15*, membrane spanning 4-domains A15; NHBE, normal human bronchial epithelial.

low expression of *MS4A15* in LUAD cells was associated with lower MC density infiltration. Thus, we speculate that low-density MCs in the tumor matrix could mediate the low expression of *MS4A15* in LUAD cells.

Aberrant cell cycle progression is one of the fundamental mechanisms leading to tumor development (30). Dysregulation of the cell cycle machinery has been observed in a wide range of tumor types, which represents the driving force behind tumorigenesis and makes cell cycle regulatory factors a reasonable target for anti-cancer treatment (31-33). In addition, cell cycle checkpoint inhibitors are critical in combating drug resistance in tumors (34). The past decade has witnessed the emergence of a variety of cell-cycle targeted cancer treatments. Palbociclib, a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor targeting the cell cycle in breast cancer, has been shown to prolong progression-free survival in postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced, or metastatic breast cancer (35). Cetuximab has been shown to enhance the radiosensitivity of esophageal squamous cell carcinoma cells by inducing G2/M phase of the cell cycle arrest and delaying DNA repair processes (36). The targeted therapeutic drug osimertinib for LUAD has also been shown to inhibit cell growth and cycle progression by regulating the phosphorylation of p53 and p21, and reducing the expression of cyclin D1 (37). A study by Xie *et al.* revealed that apatinib induces cell cycle arrest at the G1 phase and suppresses the expression of cyclin D1 and CDK4 (38). Erlotinib and crizotinib are linked to G2/M phase arrest, resulting in the decreased phosphorylation of downstream targets of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathways, leading to enhanced cell death (39). In the present study, we found that *MS4A15* was significantly associated with the primary therapy outcome. A GSEA was performed between the low and high *MS4A15* expression

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groups, which revealed that *MS4A15* may mediate these cellular biological functions, such as M phase, S phase, cell cycle checkpoints, mitotic metaphase, mitotic anaphase, and mitotic prometaphase. Therefore, we speculate that drugs based on *MS4A15* development may inhibit tumor growth by arresting the cell cycle.

In this study, we evaluated the expressions and prognostic values of *MS4A15* using data from TCGA, GTEx, and GEO databases. We found that the expression level of *MS4A15* was significantly lower in the tumor tissues of LUAD patients than the normal adjacent samples. The LUAD patients with lower *MS4A15* expression levels exhibited poorer overall survival rates. Overall, our research provided novel insights into *MS4A15* as a prognostic marker and drug target for LUAD.

Despite this, our study had certain limitations. In the future, more *in vivo* and *in vitro* experiments are needed to validate the potential mechanisms of *MS4A15* and the cell cycle of lung cancer cells, as well as the regulatory mechanisms between *MS4A15* and MC infiltration.

Conclusions

In conclusion, our research identified *MS4A15* as a novel prognostic marker for LUAD that could improve the personalized management of LUAD patients. Further, this finding may provide novel insights into target drugs for LUAD.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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