TREM1 is involved in the mechanism between asthma and lung cancer by regulating the Toll-like receptor signaling pathway

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Abstract. Lung cancer and asthma are both global health problems with significant economic consequences. Recent studies have demonstrated that asthma may be a risk factor for lung cancer. The present study aimed to explore the pathogenesis between these two diseases through a comprehensive analysis. Differentially expressed genes (DEGs) screened in the asthma-related GSE165934 dataset were analyzed to find relevant inflammatory pathways. Overlapping genes regulated by inflammatory pathways and lung cancer-DEGs from The Cancer Genome Atlas (TCGA) were obtained and subjected to survival and gene-wide mutation analyses, and nomogram construction to determine the hub gene. The hub gene was further analyzed through expression validation, immunoassays and functional experiments to investigate its role and mechanism in lung cancer. Functional enrichment analysis showed that 1,275 DEGs from GSE165934 were closely associated with the Toll-like receptor signaling pathway, and 8 overlapping genes were identified from 12 genes regulated by the Toll-like receptor signaling pathway and 3,134 TCGA-DEGs. After a series of bioinformatics analyses, it was found that triggering receptor expressed on myeloid cells 1 (TREM1) was the hub gene involved in the mechanism of asthma and lung cancer. TREM1 was also found to be a suppressor gene in lung cancer correlated with immune cells, immune checkpoint-related genes and tumor mutational burden score. Additionally, the results of Cell Counting Kit-8 and Transwell experiments demonstrated that overexpression of TREM1 could significantly inhibit the invasion, proliferation and migration of lung cancer cells. Reverse transcription-quantitative PCR and western blotting demonstrated that the overexpression of TREM1 could also significantly reduce the level of Toll-like receptor signaling pathway proteins. The present findings suggest that TREM1 is associated with the mechanism of asthma and lung cancer through its regulation of the Toll-like receptor signaling pathway. Furthermore, TREM1 may serve as a potential treatment target and prognostic indicator for patients with lung cancer.

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Introduction

Lung cancer, including small cell lung cancer (SCLC) and non-SCLC (NSCLC), is the leading cause of cancer-related deaths worldwide (1,2). Among the risk factors associated with lung cancer, the role of asthma, a chronic inflammatory disease affecting the airways, has become a topic of marked interest in recent years. Certain evidence points to a positive correlation between an asthma diagnosis and an elevated risk of lung cancer (3,4). The chronic inflammation induced by asthma could potentially promote carcinogenic processes within lung tissues, which lead to malignant transformation and subsequent cancer development (5). This emerging perspective offers novel insights into the role of chronic inflammatory respiratory diseases in lung cancer etiology. Nevertheless, some studies provide a contrasting viewpoint. In a study on contemporaneous chronic obstructive pulmonary disease, Rosenberger *et al* (6) discovered a negative correlation between asthma and lung cancer. Additionally, studies that account for co-occurring allergic diseases found a weakened positive connection between asthma and lung cancer (7,8).

Asthma is a chronic inflammatory disorder impacting the respiratory airways, driven by a complex interplay between inflammatory and structural airway cells, and cytokines (9,10). Asthma is primarily triggered by allergic reactions, often caused by environmental and dietary factors, which culminate in bronchial asthma (11). Although there are various treatments, such as nebulized therapy and topical corticosteroids, to treat asthma (12), its molecular mechanism is still unclear due to its complexity. A previous study reported that asthma is a risk factor for lung cancer (4), shedding light on a possible intersection between these respiratory conditions. However, exploring the underlying mechanisms of lung cancer and asthma remains a challenge.

To explore the specific mechanisms underlying the pathogenesis of asthma and lung cancer, the present study conducted a comprehensive bioinformatics analysis and identified triggering receptor expressed on myeloid cells 1 (TREM1) as a key

gene associated with both diseases. The results of expression validation, immunoassays and *in vitro* cell assays suggest that TREM1 may serve as a novel and effective biomarker for lung cancer. This finding could potentially inform more targeted strategies for the prevention and treatment of asthma in patients who are at a heightened risk of developing lung cancer.

Materials and methods

Gene expression files. The Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) is the world's biggest and most comprehensive repository of gene expression data (13). The keyword was set as 'asthma', the organism as '*Homo sapiens*', the experiment type as 'expression analysis by array', and the data set GSE165934 (14) was selected, which included 10 patients with asthma and 9 healthy controls. Differentially expressed genes (DEGs) were screened for using the 'limma' software package, with $P<0.05$, $\log_2$(fold change)>1 for upregulation and $\log_2$(fold change)<-1 for downregulation. The STRING database (https://string-db.org/) was used to build a protein-protein interaction (PPI) network of aggregated DEGs (15), and Cytoscape software (v3.8.2) was used for visualization (16). Gene Ontology (GO) analysis (http://geneontology.org/) was performed, and terms with $P<0.01$ were selected. Pathway analysis of node genes was also performed in WebGestalt (http://www.webgestalt.org/) using the WikiPathway functional database (https://www.wikipathways.org/).

Immune Cell Abundance Identifier (ImmuCellAI). ImmuCellAI was used to predict the abundance of 24 immune cell types in a sample. Differences in immune cell infiltration in different groups were analyzed by examining immune cell abundance in the groups. The abundance of 24 immune cells in 19 samples from the GSE165934 database was first analyzed. Next, the abundance of 24 immune cells in asthma and normal groups were investigated.

The Cancer Genome Atlas (TCGA) dataset. RNAseq and relevant clinical data of NSCLC were obtained from the TCGA dataset (https://portal.gdc.com) to screen TCGA-DEGs. The common genes of regulation of Toll-like receptor signaling pathway genes and TCGA-DEGs were targeted using the online Venn tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Kaplan-Meier (KM) curves were generated by Kaplan-Meier Plotter (https://kmplot.com/analysis/). Cut-off values and other parameters were chosen as default to assess differences in overall survival (OS) across overlapping genes in NSCLC. Survival results were visualized using KM plots and statistically significance was assessed using the log-rank test.

Gene Set Cancer Analysis (GSCA) database. The transcriptomic data, gene mutation data and clinical data of the TCGA-Lung Adenocarcinoma (LUAD) and TCGA-Lung Squamous Cell Carcinoma (LUSC) datasets were obtained from the TCGA database. The GSCA database (http://bioinfo.life.hust.edu.cn/GSCA/#/expression) was used to assess the copy number variation (CNV) and single nucleotide variation (SNV) of the survival-associated mutant genes (17). Furthermore, to comprehensively study somatic mutations in patients with LUAD and LUSC, mutation data were acquired and processed by the 'Maftools' R package (version 4.10) (18).

Prognostic nomogram construction using independent parameters. Univariate Cox analysis was used to evaluate the prognostic power of 6 survival-associated mutant genes and a number of clinical parameters, including patient age, tumor grade, and pT, pN and pTNM stages (19). Subsequently, multivariate Cox analysis was used to determine whether these genes and clinical parameters could serve as independent indicators for patients. According to the results of multivariate Cox analysis, a composite nomogram was designed by the 'rms' R software package (version 4.3.1; https://www.r-project.org/) to evaluate the impact of independent indicators on the probability of 1-, 3- and 5-year OS. The 45˚ line represents a perfect match between predictions and observations, and the closer the nomogram model to the calibration curve, the better the prediction result of the model.

University of Alabama at Birmingham cancer data analysis portal (UALCAN). UALCAN is a smart web application for the deep analysis of TCGA and the retrieval of cancer data (20); it allows users to find potential genes of interest between biomarker or computer approvals and assess gene expression across different clinical factors such as sex, ethnicity and tumor grade (21). In the present study, TREM1 expression in patients with LUSC and LUAD with different sample types, tumor grades, smoking and lymph node metastasis statuses were assessed.

Tumor Immune Estimation Resource (TIMER). TIMER (https://cistrome.shinyapps.io/timer/) enables systematic analysis of immune infiltrate abundance in different cancer types (22). Correlations between the hub gene (TREM1) and immune cells ($CD4^+$ T cells, B cells, $CD8^+$ T cells, macrophages, neutrophils, and dendritic cells) in the TCGA-LUAD dataset and the TCGA-LUSC dataset were analyzed by Spearman correlation analysis. $P<0.05$ was selected as the cut-off value. For reliable immune score evaluation, the 'immunedeconv' TIMER algorithm in the R software package was adopted to evaluate the immune scores. The samples were divided into TREM1 high-expression and low-expression groups based on the median expression value of TREM1. Those with TREM1 expression above the median were classified as the high-expression group, while those with expression below the median were classified as the low-expression group. Subsequently, the expression of 8 immune checkpoints in the TREM1 high-expression group and low-expression group was analyzed by the R software package. The relationship between TREM1 expression and the tumor mutational burden (TMB) was examined. $P<0.05$ was regarded as statistically significant when using Spearman's correlation analysis.

Cell culture. Human bronchial epithelioid cells (16HBE) and lung cancer cell lines (H292, A549 and H1299) were purchased from the American Type Culture Collection . Cells were cultured in DMEM (Thermo Fisher Scientific, Inc.) at 37˚C in a humidified atmosphere with 5% CO_2, supplemented with 10% FBS (Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin.

Reverse transcription-quantitative PCR (RT-qPCR). TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract RNA from lysed cells of the lung cancer cell lines, and Prime script RT Master mix (Takara Biotechnology Co., Ltd.) used to reverse transcribe the extracted RNA into cDNA according to the manufacturer's instructions. Reactions were performed in duplicate using an ABI 7500 Fast Real-Time PCR system (Applied Biosystems). The reaction conditions were 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 35 sec. Subsequently, qPCR detection was performed using the SYBR-Green I (cat. no. S7563; Thermo Fisher Scientific, Inc.) fluorescence technique computed using the $2^{-\Delta\Delta Cq}$ method (23). The primer sequences were as follows: β-actin forward, 5'-TGGATCAGCAAGCAGGAGTATG-3' and reverse, 5'-GCATTTGCGGTGGACGAT-3'; TREM1 forward, 5'-TCCGAATGGTCAACCTTCAAGTGG-3' and reverse, 5'-GAACAGCATGTGAGGCTCCTTGG-3'; MyD88 forward, 5'-GGCTGCTCTCAACATGCGA-3' and reverse, 5'-CTGTGTCCGCACGTTCAAGA-3'; TLR2 forward, 5'-CTTCACTCAGGAGCAGCAAGCA-3' and reverse, 5'-ACACCAGTGCTGTCCTGTGACA-3'; and TLR4 forward, 5'-CCAGCCTCCTCAGAAACA-3'and reverse, 5'-TCCAGCAGTGAAGAAGGG-3'.

Cell transfection. The lung cancer cells were cultured in 6-well plates at 37˚C overnight to reach 70% confluence, and the overexpression plasmid (pcDNA3.1-TREM1; cat. no. V79020; Invitrogen; Thermo Fisher Scientific, Inc.) and negative control (pcDNA3.1 empty vector; cat. no. V79020; Invitrogen; Thermo Fisher Scientific, Inc.) were constructed. When the cells were at ~90% confluency, 1 μg plasmid was transfected into the cells using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C in 5% CO_2 for 4-6 h, according to the manufacturer's instructions. After 48 h, subsequent experiments were performed.

Cell Counting Kit-8 (CCK-8). A total of $2x10^3$ lung cancer cells were added to a 96-well plate, and then treated with 10 μl CCK-8 solution (Dojindo Molecular Technologies, Inc.), and incubated at 37˚C for 2 h. The optical density (OD) value at 450 nm was recorded at 1, 2, 3 and 4 days using a microplate reader to generate a proliferation curve. The analysis was performed in triplicate.

Cell invasion and migration assay. In the migration experiment, $4x10^4$ lung cancer cells, in serum-free DMEM, were seeded into the upper chamber of a Transwell insert and a medium with 20% FBS was added to the lower chamber as a chemoattractant. For the invasion experiment, Matrigel (BD Biosciences) was coated on the upper chamber at 37˚C for 2 h prior to being seeded with $9x10^4$ cells, and the lower chamber contained medium with 20% FBS. After incubation at 37˚C, in 5% CO_2, for 48 h, the Transwell chamber was removed and the medium in the well was discarded and washed with calcium-free PBS. The cells were fixed with methanol for 15 min at room temperature and then stained with DAPI for 10 min at room temperature. The upper unmigrated cells were gently removed with a cotton swab, and cells in the lower chamber were counted under a fluorescence microscope (x200 magnification).

Western blotting assay. The lung cancer cells were lysed by RIPA lysis buffer (Thermo Fisher Scientific, Inc.) with 1% PMSF. The cell lysates were then centrifuged at 14,000 x g for 15 min at 4˚C to separate the soluble proteins. Proteins were extracted from the cell's lysates and supernatants. The concentration of proteins was determined using a Pierce® BCA Protein Assay Kit (Thermo Fisher Scientific, Inc.). The quantified proteins (50 μg/lane) were separated by SDS-PAGE on 10% gels and then transferred onto a PVDF membrane. The membrane was blocked with 5% non-fat dry milk at room temperature for 3 h. Membranes were probed at 4˚C overnight with primary antibodies against TREM1 (1:5,000; cat. no. ab90808; Abcam), MyD88 (1:5,000; cat. no. ab133739; Abcam), TLR2 (1:5,000; cat. no. ab9100; Abcam), TLR4 (1:5,000; cat. no. ab13556; Abcam) and GAPDH (1:5,000; cat. no. ab9485; Abcam). The following day, the blot was probed using an HRP-conjugated secondary antibody (1:5,000; cat. no. ab205718; Abcam) and a Goat Anti-Mouse IgG H&L (HRP) secondary antibody (1:5,000; cat. no. ab97023; Abcam) for 1 h at room temperature. Finally, protein bands were visualized using an ECL Plus kit (Cytiva) and the band density was semi-quantified using ImageJ software (version 1.52; National Institutes of Health).

Statistical analysis. All study data were processed by SPSS 22.0 software (IBM Corp), and each experiment was performed in triplicate. All quantitative data are expressed as the mean ± SD. Comparison between groups was performed using one-way ANOVA followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification and analysis of GSE165934-DEGs. Through analysis of the dataset and comparison between asthmatic and control groups, 235 upregulated DEGs and 1,040 downregulated DEGs from GSE165934 were acquired and presented on a volcano plot and heat map (Fig. 1A and B). Subsequently, the PPI network of all DEGs was constructed, and the interconnectedness between genes was shown (Fig. 1C), with 460 nodes and 1,590 edges. The 460 node genes were used for functional enrichment analysis. Node genes were enriched in 'neutrophil mediated immunity', 'intracellular membrane-bounded organelle', 'RNA binding' and others in the GO analysis (Fig. 1D). WikiPathway analysis showed that node genes were mainly associated with Toll-like receptor signaling related to MyD88, regulation of the Toll-like receptor signaling pathway and the glucocorticoid receptor pathway (Fig. 1E).

Genes in GSE165934 are associated with immune cells in asthma. The present study explored immune cells in 19 samples of GSE165934 by ImmuCellAI. Fig. 2A shows the proportion of immune cells in control samples (GSM5058526-GSM5058534) and asthma samples (GSM5058535-GSM5058544) marked with different colors, and the length of the bars in the bar graph represent the level of immune cell populations. The percentages of NK cells and Tc cells in the samples were significantly reduced. As shown in Fig. 2B, only nTreg, Th17, CD8 naive, NKT and Tex expression was significantly reduced in the

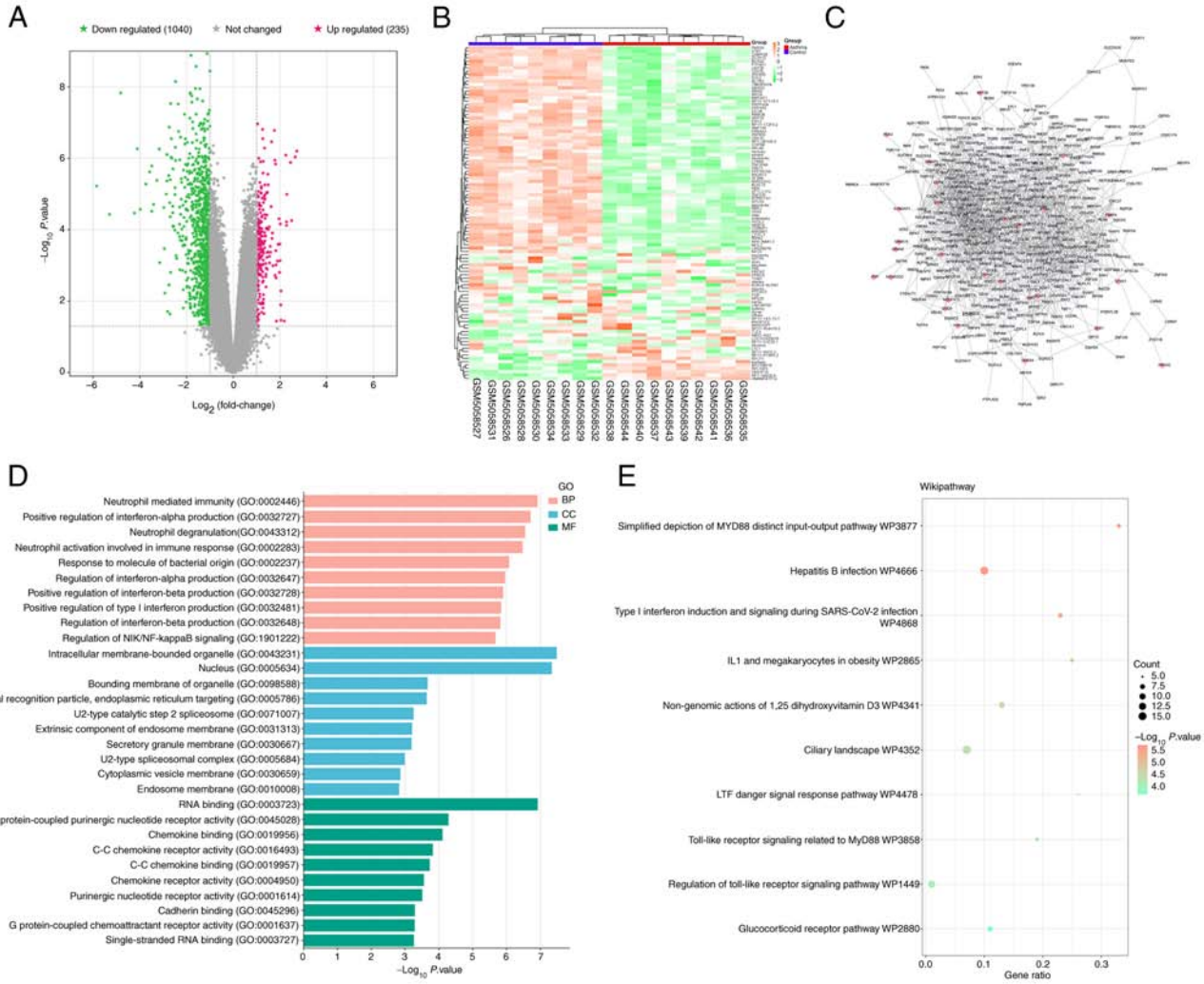


Figure 1. Identification and bioinformatics analysis of DEGs. (A) Volcano plot of 235 upregulated DEGs and 1,040 downregulated DEGs, with the gray area in the middle representing unchanged genes. (B) Cluster heatmap of DEGs in GSE165934. In the heatmap, the color gradient from green to orange represents the expression levels of DEGs, with green indicating lower expression levels and orange indicating higher expression levels. Blue denotes the control group and red denotes the asthmatic group. (C) Protein-protein interaction network of DEGs, where nodes represent genes (pink diamonds represent upregulated DEGs, gray arrowheads represent downregulated DEGs) and edges represent interconnectedness between genes. (D) Bar graph of GO analysis. BP enrichment result (pink), CC enrichment result (blue), and MF enrichment result (green). (E) WikiPathway analysis on 460 node genes. The larger the node, the more genes are enriched on this pathway. DEGs, differentially expressed genes; GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

asthma group. While other cells, such as B cells, macrophages and neutrophils, increased significantly.

Survival analysis of gene overlap in the Toll-like receptor signaling pathway and TCGA-DEG. Fig. 3A shows a Venn diagram of 8 overlapping genes from genes regulated by 'regulation of Toll-like receptor signaling pathway' and TCGA-DEGs. Using batch survival analysis, 6 genes with significant P-values for OS analysis were retained (Fig. 3B-G; $P<0.05$). High expression of IL1B resulted in poor OS probability, while low expression of JUN, TLR4, TREM1, TLR2 and TLR8 indicated poor OS.

Mutational landscape analysis of CNVs, SNVs and cellular mutations for 6 identified genes. A genetic variation analysis for 6 genes with significant P-values in survival analysis, including CNVs and SNVs, was performed using the GSCA database in LUAD and LUSC. In LUAD, TLR4 had the highest percentage of CNVs whereas in LUSC, TLR2 had the highest percentage of CNVs (Fig. 4A). The percentage of SNVs for the 6 genes in LUAD and LUSC were also explored, and it was found that in LUAD, TLR4 exhibited the most SNVs, followed by TLR8, TLR2, TREM1, IL1B and JUN. In LUSC, TLR4 exhibited the most SNVs, followed by TLR8, TREM1, IL1B, TLR2 and JUN (Fig. 4B). The most common type of mutation in patients with LUAD and LUSC was a missense mutation. Single nucleotide polymorphisms (SNPs) were the main type of mutational variation, with C>A being dominant over other SNV categories. As shown in Fig. 4C, the median mutation variation per sample was 1, and each color box represented a mutation. Fig. 4C displays the six most frequently mutated genes in the present study, including TLR4 (68%), TLR8 (19%), TLR2 (9%), TREM1 (6%), JUN (4%) and IL1B (4%). Histograms in Fig. 4D show the mutation frequencies of the 6 genes in the patients with LUAD and LUSC (n=145).

TREM1 is a potential prognostic indicator of lung cancer. After univariate and multivariate Cox analyses were performed, it

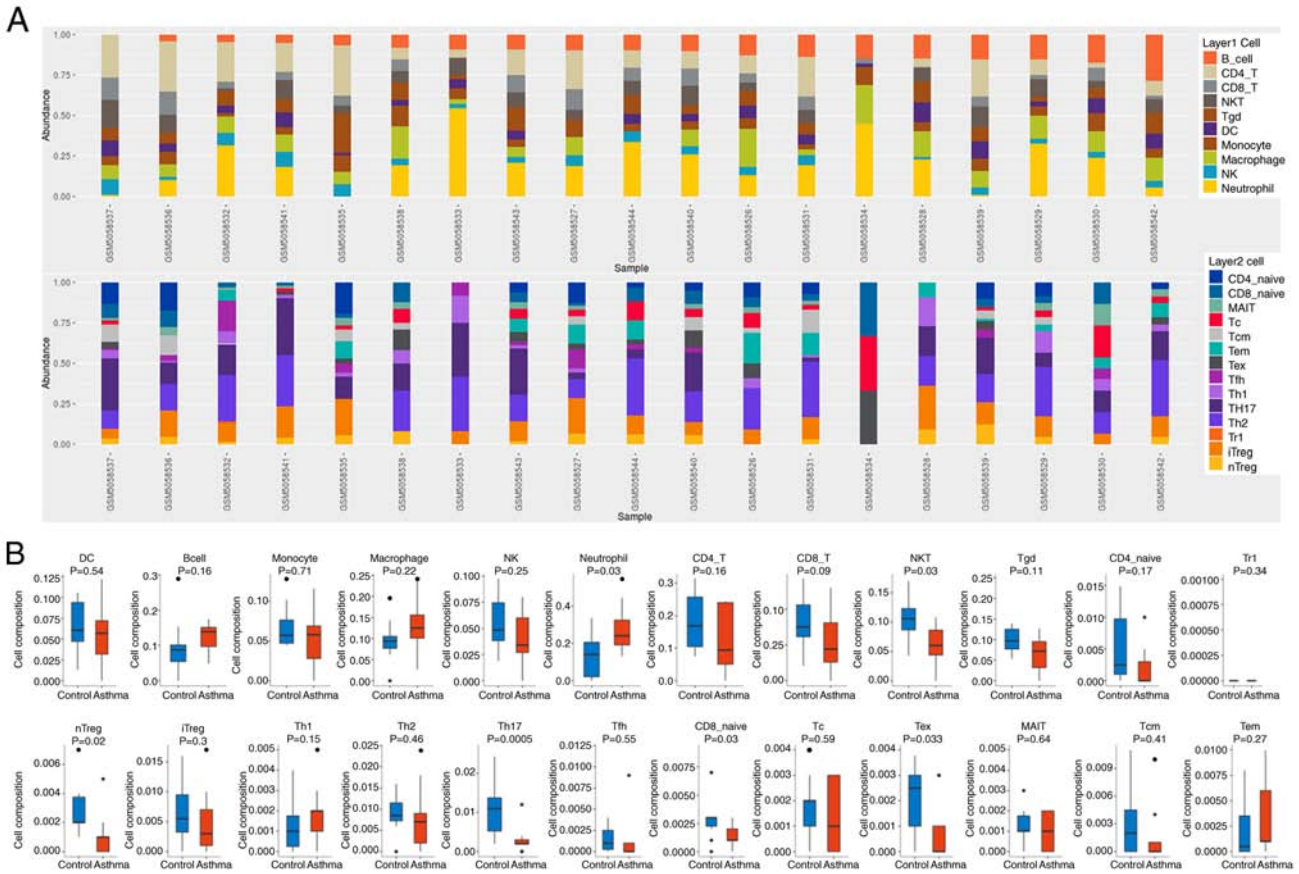


Figure 2. ImmuCellAI analysis of 19 samples from GSE165934. (A) The proportions of 24 immune cells in control samples (GSM5058526-GSM5058534) and asthma samples (GSM5058535-GSM5058544), with colored squares representing different types of immune cells. (B) Immune cell abundance was analyzed and examined between asthma (red) and normal (blue) tissues by ImmuCellAI. The black dots represent outliers. DC, dendritic cells; NK, natural killer cells; NKT, natural killer T cells; Tr1, Type 1 regulatory T cells; nTreg, natural regulatory T cells; iTreg, induced regulatory T cells; Th1, T helper 1 cells; Tfh, T follicular helper cell; Tc, cytotoxic T cells; Tex, exhausted T cells; MAIT, mucosal-associated invariant T cells; Tcm, central memory T cells; Tem, effector memory T cells.

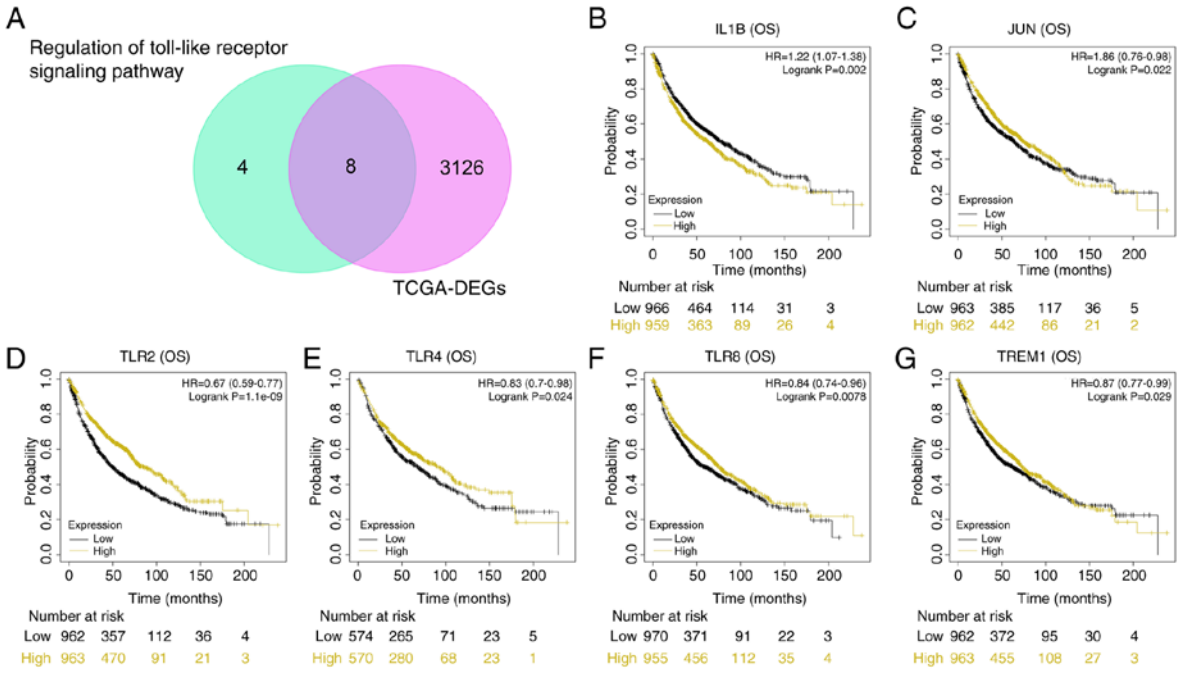


Figure 3. Screening and survival analysis of the overlapping genes. (A) Venn diagram of genes regulated by regulation of Toll-like receptor signaling pathway and TCGA-DEGs, with 8 intersection genes in the middle. (B-G) OS analysis on (B) IL1B, (C) JUN, (D) TLR2, (E) TLR4, (F) TLR8 and (G) TREM1 in lung cancer. The black line indicates low expression and the gold line indicates high expression. TCGA, The Cancer Genome Atlas; DEGs, differentially expressed genes; OS, overall survival; TREM1, triggering receptor expressed on myeloid cells 1.

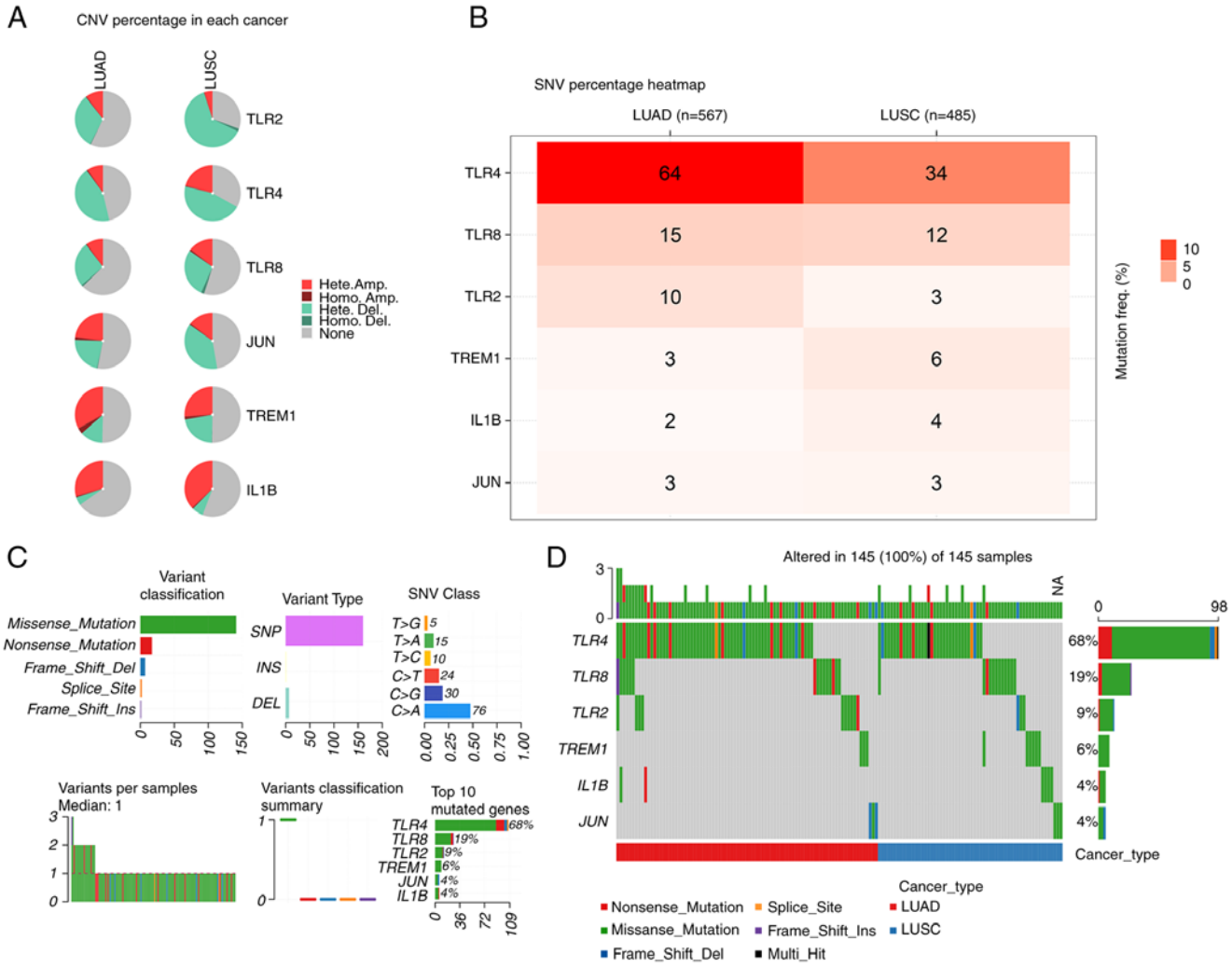


Figure 4. CNV and SNV analyses on 6 mutated genes. (A) Percentage of CNVs for the 6 mutated genes in LUSC and LUAD. (B) Percentage of SNVs for the 6 mutant genes in LUSC and LUAD. (C) The categories of mutation types based on various classes and tumor mutational burden in the sample. (D) The 6 mutated genes are depicted in a waterfall plot arranged by cancer type, with their mutational patterns arranged by mutation frequency. Different colors denote distinct mutation types. CNV, copy number variation; SNV, single nucleotide variation; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TREM1, triggering receptor expressed on myeloid cells 1.

was apparent that TREM1, pT-stage and pTNM-stage were independent prognostic variables to construct the predictive nomogram, and that TREM1 was considered as the hub gene (Fig. 5A and B). A composite nomogram was designed with TREM1, pT-stage and pTNM-stage to predict 1-, 3- and 5-year OS rates in patients with NSCLC (Fig. 5C). The presentation of the calibration plot for patient survival prediction demonstrated the predicted results of the prognostic nomogram matched with the observed results, suggesting that the model had good prognostic prediction for patients (Fig. 5D).

Association between TREM1 expression and clinical factors in LUSC and LUAD. Comparing different patient samples, including both LUAD and LUSC tissue samples, TREM1 expression levels were found to be lower in primary tumor compared with those in normal tissues (Fig. 6A and E). However, among different clinical factors of LUAD and LUSC, TREM1 expression had no significant association with individual cancer stages, patient's smoking habits or nodal metastasis status (Fig. 6B-D and F-H).

Immunoassay on TREM1 in lung cancer. Fig. 7A shows the association between TREM1 and 6 immune cell types in LUSC and LUAD. A positive correlation was denoted by Cor>0, and a negative correlation by Cor<0. In LUAD, TREM1 expression was negatively correlated with tumor purity, B cells, $CD8^+$ T cells and $CD4^+$ T cells. The expression of TREM1 was positively correlated with the expression of macrophages, neutrophils and dendritic cells. In LUSC, TREM1 expression was inversely correlated with tumor purity. The expression of TREM1 was positively correlated with the expression of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and dendritic cells. Fig. 7B shows the fraction of immune cells that infiltrated tumors in samples with high and low TREM1 expression. Myeloid dendritic cells had higher TIMER scores in TREM1-expressing samples. In addition, the TIMER score was higher in the high TREM1 expression group. The differences in expression of the 8 immune checkpoint molecules between the two groups were not obvious. However, it was observed that the level of immune checkpoint molecules was higher in samples with high TREM1 expression (Fig. 7C).

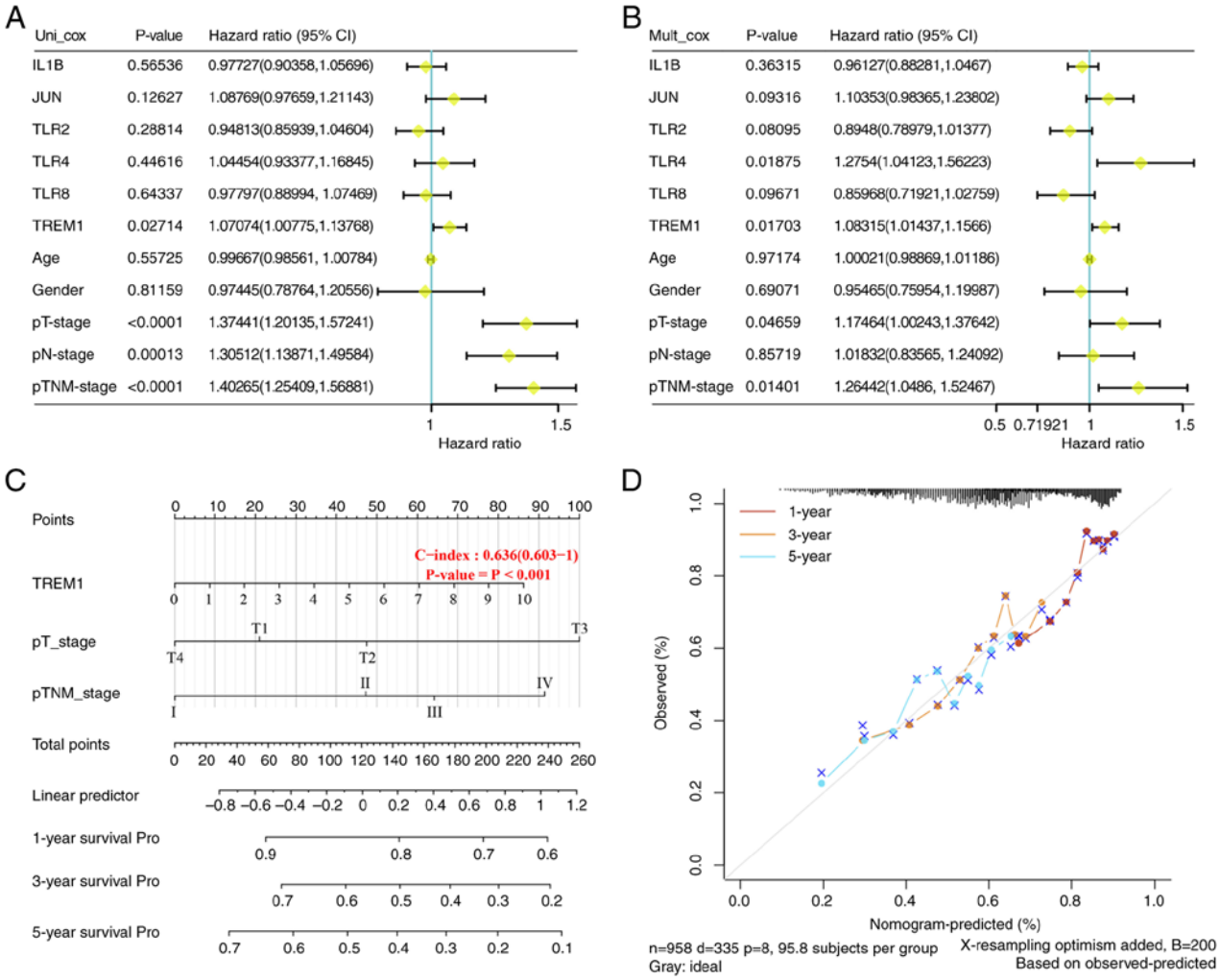


Figure 5. TREM1 could be a prognostic biomarker in lung cancer. (A) Univariate Cox regression analysis showed that TREM1, pT-stage, pN-stage and pTNM-stage were significant prognostic variables. (B) Multivariate Cox regression analysis showed that the significant prognostic variables were TLR4, TREM1, pT-stage, and pTNM-stage. (C) Nomogram with independent indicators, with a scale marked on the corresponding line segment of each variable, representing the range of possible values of the variable. The longer the line segment, the greater its contribution to the prognosis. (D) Calibration curve of the nomogram model, and the diagonal gray line is the ideal nomogram. TREM1, triggering receptor expressed on myeloid cells 1. Pro, prognosis.

Over the past few years, TMB has been a significant prognostic biomarker; however, its prognostic value in NSCLC has remained unclear. Through TCGA, the correlation between TMB and the levels of TREM1 in NSCLC was comprehensively analyzed to determine the impact of TREM1 in the development of NSCLC. The present results suggested that TREM1 had a negative association with the TMB score in NSCLC (Fig. 7D).

TREM1 inhibits cell proliferation, invasion and migration via the Toll-like receptor pathway in lung cancer. Results of RT-qPCR and western blotting experiments showed downregulation of TREM1 in lung cancer cells (H292, A549 and H1299) compared with human bronchial epithelial-like cells (16HBE), especially in A549 and H1299 cells (Fig. 8A and B). Subsequently, TREM1 was overexpressed in A549 and H1299 cells, and the overexpression efficiency was examined by RT-qPCR and western blotting. The findings indicated a marked increase in TREM1 expression within A549 and H1299 cells (Fig. 8C and D). CCK-8 experiments were performed in H1299 and A549 cell lines, and it was observed that overexpressed TREM1 significantly inhibited the proliferation of A549 and H1299 cells (Fig. 8E and F). Since TREM1 was most significantly expressed in H1299 cells and A549 cells, a Transwell assay using these two cell lines was performed, which showed that overexpression of TREM1 significantly inhibited the invasion and migration of H1299 as well as A549 cells (Fig. 8G-J). Furthermore, the effect of overexpressed TREM1 on the related proteins of the Toll-like receptor pathway, including TLR2, TLR4 and MyD88, was explored. RT-qPCR results suggested that the mRNA levels of MyD88, TLR2 and TLR4 were reduced in lung cancer cells after overexpression of TREM1 (Fig. 8K and L). Western blotting results suggest the protein levels of MyD88, TLR2 and TLR4 were also reduced ($P<0.05$) in lung cancer cells after overexpression of TREM1, indicating that TREM1 negatively regulated MyD88, TLR2 and TLR4 (Fig. 8M).

Discussion

Asthma can cause a series of reactions such as wheezing and chest tightness, which usually occur at night or in the

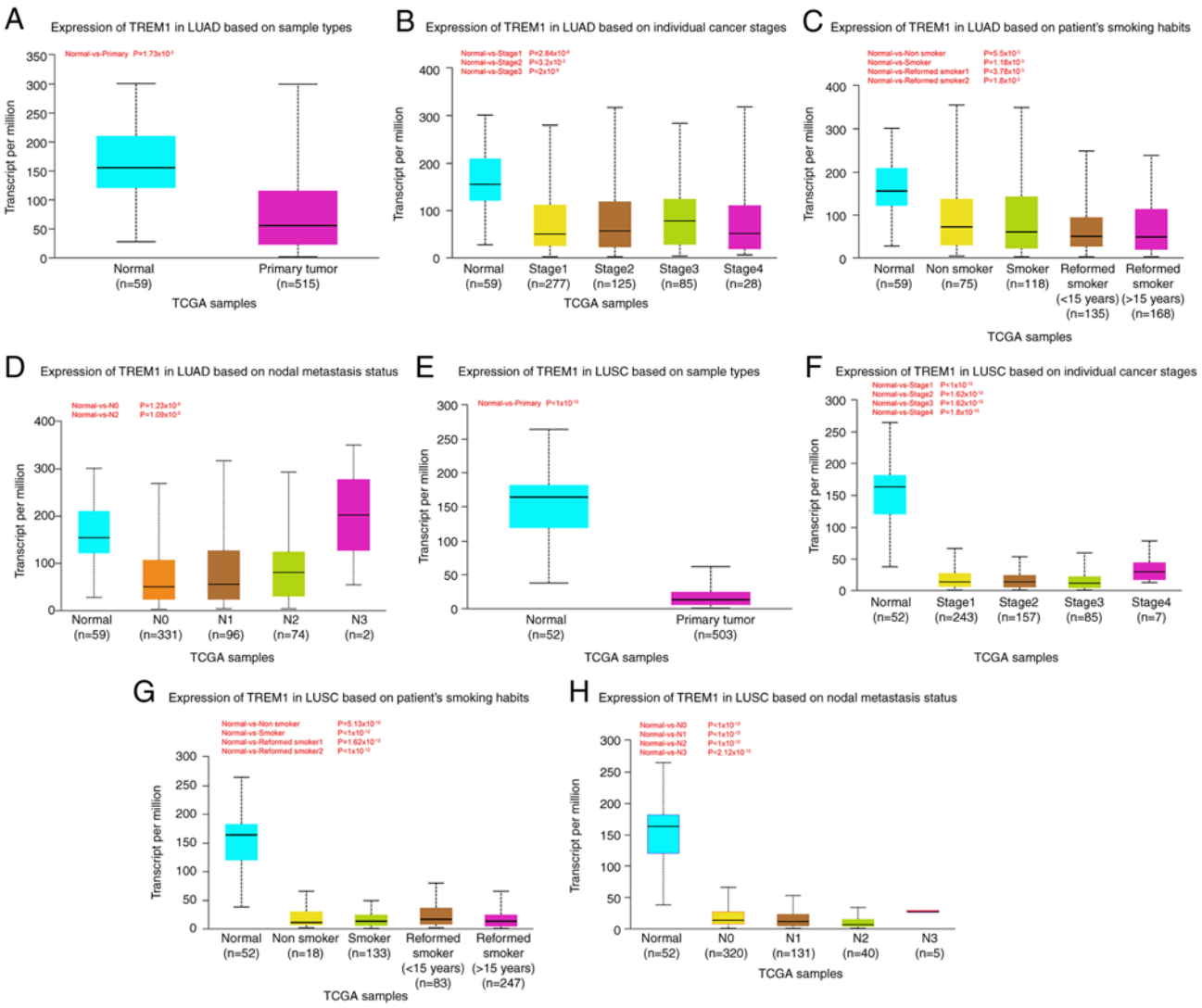


Figure 6. Relative expression of TREM1 in subgroups of patients with LUAD and LUSC. Expression of TREM1 based on sample types, cancer stages, smoking habits and nodal metastasis status in (A-D) LUAD and (E-H) LUSC. Data retrieved from the University of Alabama at Birmingham Cancer Data Analysis Portal. LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; TREM1, triggering receptor expressed on myeloid cells 1.

early morning and can seriously affect the quality of life of patients (24). According to the number of exacerbations during oral systemic corticosteroid therapy, asthma can be classified into four categories: Intermittent, mild, moderate and severe persistent asthma (25). The chronic inflammatory state in the lungs of asthmatics is considered to cause oxidative damage that may contribute to the development of lung cancer (26). Jiang *et al* (7) suggested that proper control of asthma symptoms not only reduces asthma attacks but also helps reduce the incidence of lung cancer. Therefore, identifying the key genes related to both lung cancer and asthma could reveal the molecular mechanism behind their connection.

In the present study, 1,275 DEGs were extracted from the GSE165934 database, and the PPI network of DEGs yielded 460 nodes. The enriched pathways of node genes included the 'glucocorticoid receptor pathway' and the 'Toll-like receptor signaling pathway'. Glucocorticoids are a common therapy for controlling airway inflammation in asthma and work by their attachment to intracellular glucocorticoid receptors, thereby promoting enhanced production of anti-inflammatory genes and blocking the activation of pro-inflammatory genes in asthmatic airways (27). Inhaled corticosteroids have been successful in treating the majority of asthmatic patients, improving lung function and reducing exacerbations (28,29). Toll-like receptors are essential for identifying invading pathogens and activating the immune system (30,31). Wu *et al* (32) showed that a combination of Toll-like receptor-related genes could be a promising indicator for asthma prognosis. In addition, a study by Pandey *et al* (33) found genetic variants in the Toll-like receptor signaling pathway related to childhood asthma. Further research on the molecular mechanisms of these pathways could be crucial for developing asthma therapies.

Combining genes regulated by the regulation of Toll-like receptor signaling pathway, TCGA-DEGs and OS analysis, 6 genes (IL1B, JUN, TLR2, TLR4, TLR8 and TREM1) were identified for further analysis. Mutation profiling showed that patients with LUAD and LUSC exhibited different types of mutations. TLR4 had the highest mutation frequency in patients with LUAD and LUSC (68%), followed by TLR8 (19%), TLR2 (9%), TREM1 (6%), JUN (4%), and IL1B (4%).

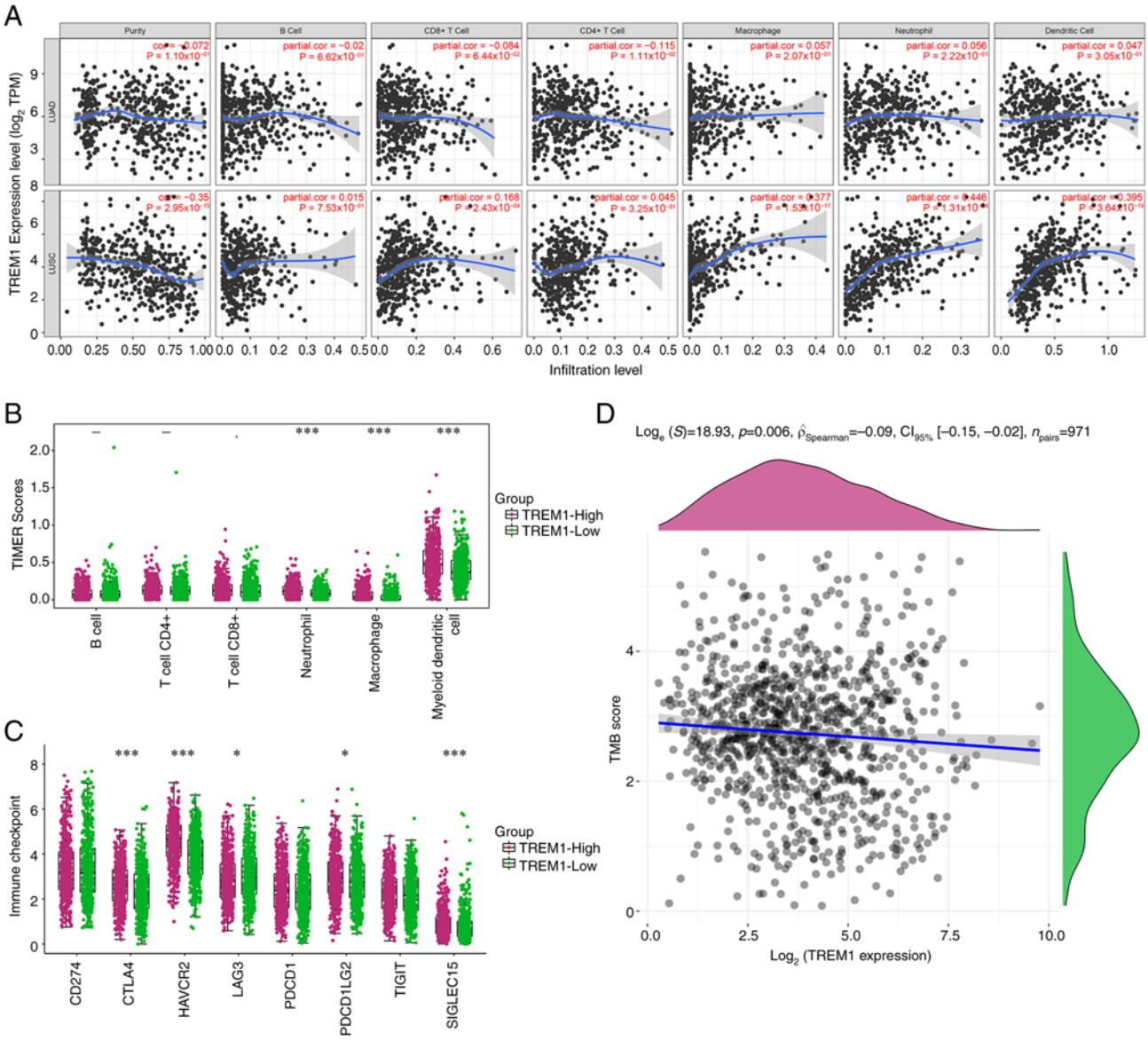


Figure 7. Immunoassay of TREM1 expression. (A) Correlation of TREM1 expression with immune cell infiltration. Correlation coefficients and P-values are indicated in the upper right corner. (B) TIMER scores of immune cells. Samples with high and low TREM1 expression are represented in purple and green, respectively. (C) Expression distribution of immune checkpoint genes. (D) Correlation of TREM1 expression level with TMB score. *P<0.05 vs TREM1-Low; ***P<0.001 vs TREM1-Low. TMB, tumor mutational burden; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TREM1, triggering receptor expressed on myeloid cells 1; TIMER, Tumor Immune Estimation Resource.

Poltorak *et al* (34) reported that disruptive mutations in TLR4 are associated with the emergence of gram-negative sepsis while maintaining the majority of immune system components. Additionally, it was discovered that the majority of the missense mutations of these altered genes were found in patients with LUAD and LUSC, and missense mutations with a high frequency might alter the structure and function of proteins (35), which suggested a possible role in the pathogenesis of LUAD and LUSC. In the present study, in patients with LUAD and LUSC, SNPs were the primary mutation variant type, and C>T was the most common DNA nucleotide substitution compared with other SNV classes.

Through a series of bioinformatics analyses, TREM1 was discovered as the hub gene related to both asthma and lung cancer, suggesting it could serve as a prognostic indicator of lung cancer. Five members of the immunoglobulin superfamily make up the TREM family, including TREM1, TREM2, TREM3, and TREM-like transcripts-1 (TLT1) and -2 (TLT2) (36). TREM1 and TREM2 are immunoglobulin superfamily receptors that typically regulate innate immunity through inflammatory responses (37). Liu *et al* (38) showed that peripheral TREM1 induction amplified pro-inflammatory responses to the brain- and gut-derived immunogenic components after a stroke. Bernal-Martínez *et al* (39) suggested that TREM1 performed a significant role in the pathophysiology of acute inflammatory disorders with various etiologies, including acute myocardial infarction, atherosclerosis and viral illnesses. Chen *et al* (40) showed that TREM1/Dap12-based chimeric antigen receptor-T cells exhibited powerful anticancer activity both *in vitro* and *in vivo* by designing a chimeric immune receptor. Furthermore, TREM1 was differentially related to the clinical features of patients with LUSC and LUAD. In the

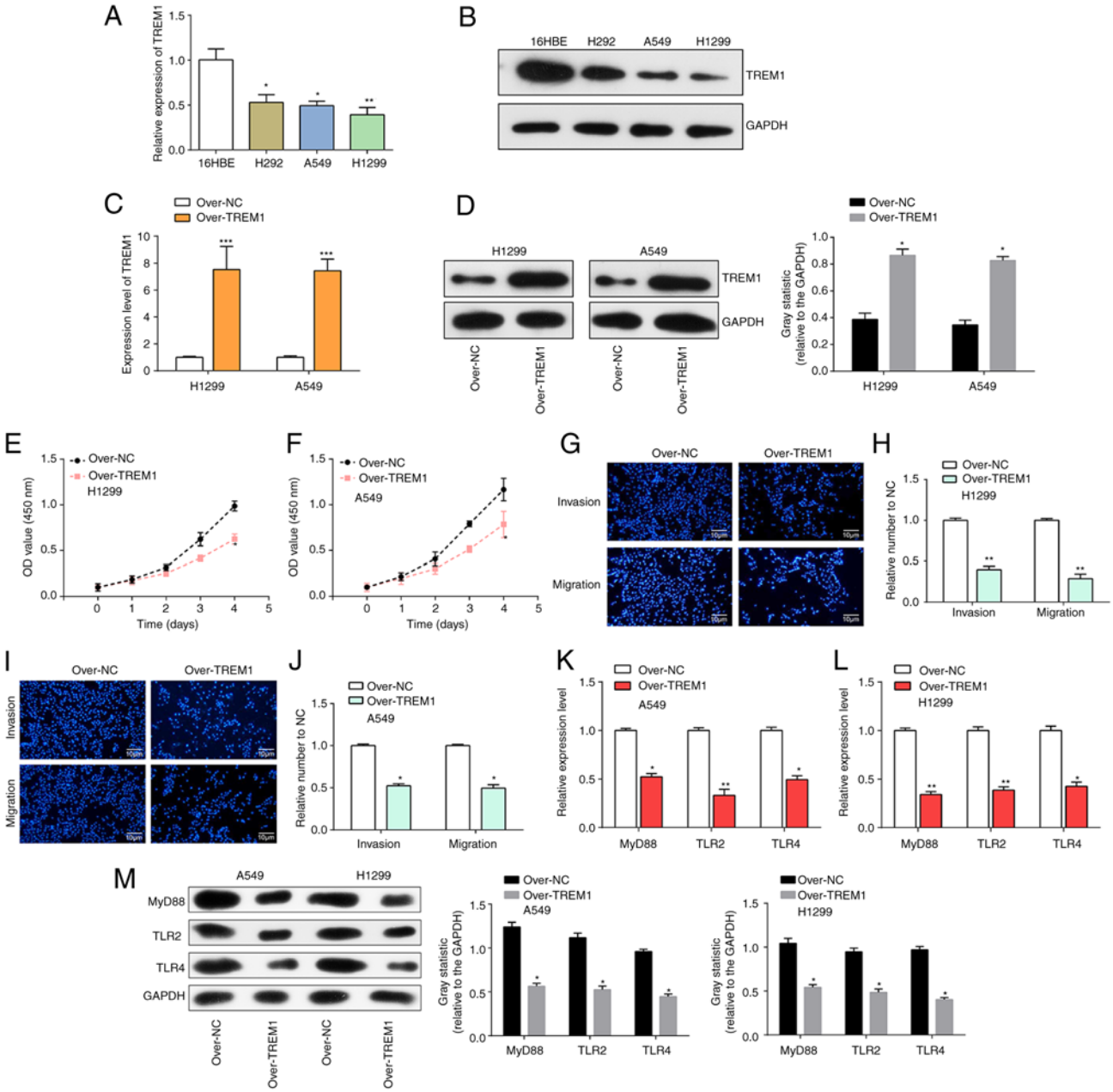


Figure 8. TREM1 could inhibit lung cancer progression via the Toll-like receptor pathway in lung cancer cells. (A) TREM1 expression in lung cancer cell lines. (B) The protein expression of TREM1 in lung cancer cell lines was detected by western blot. (C) The overexpression efficiency of TREM1 in lung cancer cell lines. (D) Overexpression efficiency of the TREM1 gene in A549 and H1299 cells was detected by western blotting. (E and F) The effect of overexpressed TREM1 on cell proliferation. The effect of overexpressed TREM1 on cell invasion and migration in (G and H) H1299 and (I and J) A549 cell lines. The expression levels of MyD88, TLR2 and TLR4 were downregulated by overexpression of TREM1 in (K) A549 and (L) H1299 cells. (M) The protein bands show the regulation of MyD88, TLR2 and TLR4 by overexpressed TREM1. Scale, 10 μm. *P<0.05; **P<0.01; ***P<0.001. TREM1, triggering receptor expressed on myeloid cells 1; NC, negative control; OD, optical density.

present study, it was found that overexpression of TREM1 could block cell migration, invasion and proliferation in lung cancer, and reduce the expression of proteins related to the Toll-like receptor signaling pathway, suggesting that TREM1 is a lung cancer suppressor gene involved in the Toll-like receptor signaling pathway.

As immune cells are crucial for the development, metastasis, prognosis and treatment of tumors (41), immune infiltration assays should be performed to investigate how immune cells and tumors interact. Immune checkpoint molecules expressed on immune cells can inhibit immune cell activity and prevent the body from mounting successful antitumor immune responses, leading to the development of tumor immune escape (42). The present study found that the expression levels of immune checkpoint genes were higher in the high TREM1 expression group. TMB, which includes the total amount of base substitution, insertion and deletion mutations in somatic proteins, is also a critical prognostic biomarker for immune checkpoint inhibitors in a number of cancer types, such as lung cancer, melanoma and colorectal cancer (43). Increased somatic mutation can lead to neoantigen expression and tumorigenesis, which activates $CD8^{+}$ cytotoxic

T cells and triggers the antitumor effect of the T cell-dependent immune response (44). TMB has been recognized as a novel biomarker of immunotherapy response and a candidate for the prediction of response to immune checkpoint inhibitors (45). Cheng *et al* (46) reported that the degree of TREM1 expression was significantly inversely linked with TMB in NSCLC . A high TMB score is considered to increase the number of neoantigens that are present on the surface of tumor cells, enhancing immunogenicity and improving the response of malignancies to immune checkpoint inhibitor therapy. Therefore, the suppressor gene, TREM1, may be used in a treatment for lung cancer.

The present bioinformatics-based approach identified a hub gene, TREM1, involved in the molecular mechanism underlying asthma and lung cancer. Further analysis showed that TREM1 was downregulated in lung cancer cells as a tumor suppressor gene, however, its overexpression could significantly reduce the proliferation of lung cancer cells by regulating the Toll-like receptor pathway. The present study reveals the pathogenesis between asthma and lung cancer, and provides a new potential biomarker for the treatment and prognosis of lung cancer. However, the present study has certain limitations, as the results of the current study have not been validated in samples from patients with asthma and lung cancer. Additionally, the mechanism of action of TREM1 downstream genes targeting the Toll-like receptor pathway in lung cancer remains unclear and requires further analysis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZY conceived the idea for and designed the present study. WZ acquired the data, and KS was responsible for data analysis and interpretation. Statistical analysis was performed by WZ and ZY. KS drafted the manuscript, and WZ, ZY and KS were responsible for the revision of the manuscript for intellectual content. WZ and KS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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