MMP12 serves as an immune cell–related marker of disease status and prognosis in lung squamous cell carcinoma

Wei Zhang[1], Guo-Sheng Li[2], Xiang-Yu Gan[1], Zhi-Guang Huang[1], Rong-Quan He[3], Hong Huang[4], Dong-Ming Li[1], Yu-Lu Tang[1], Deng Tang[1], Wen Zou[1], Jun Liu[2], Yi-Wu Dang[1], Gang Chen[1], Hua-Fu Zhou[2], Jin-Liang Kong[4] and Hui-ping Lu[1]

[1] Department of Pathology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China
[2] Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China
[3] Department of Medical Oncology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China
[4] Department of Respiratory and Critical Care, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China

ABSTRACT

Background. Worldwide, lung squamous cell carcinoma (LUSC) has wreaked havoc on humanity. Matrix metallopeptidase 12 (*MMP12*) plays an essential role in a variety of cancers. This study aimed to reveal the expression, clinical significance, and potential molecular mechanisms of *MMP12* in LUSC.

Methods. There were 2,738 messenger RNA (mRNA) samples from several multicenter databases used to detect *MMP12* expression in LUSC, and 125 tissue samples were validated by immunohistochemistry (IHC) experiments. Receiver operator characteristic (ROC) curves, Kaplan–Meier curves, and univariate and multivariate Cox regression analyses were used to assess the clinical value of *MMP12* in LUSC. The potential molecular mechanisms of *MMP12* were explored by gene enrichment analysis and immune correlation analysis. Furthermore, single-cell sequencing was used to determine the distribution of *MMP12* in multiple tumor microenvironment cells.

Results. *MMP12* was significantly overexpressed at the mRNA level ($p < 0.05$, SMD = 3.13, 95% CI [2.51–3.75]), which was verified at the protein level ($p < 0.001$) by internal IHC experiments. *MMP12* expression could be used to differentiate LUSC samples from normal samples, and overexpression of *MMP12* itself implied a worse clinical prognosis and higher levels of immune cell infiltration in LUSC patients. *MMP12* was involved in cancer development and progression through two immune-related signaling pathways. The high expression of *MMP12* in LUSC might act as an antigen-presenting cell–associated tumor neoantigen and activate the body's immune response.

Conclusions. *MMP12* expression is upregulated in LUSC and high expression of *MMP12* serves as a risk factor for LUSC patients. *MMP12* may be involved in cancer development by participating in immune-related signaling pathways and elevating the level of immune cell infiltration.

Subjects Bioinformatics, Molecular Biology, Oncology, Pathology, Respiratory Medicine
Keywords Lung squamous cell carcinoma, MMP12, Gene expression, Prognosis, Clinical value, Immunity

INTRODUCTION

Since cancer cells have multiple means of evading elimination by the immune system, there is still a shortage of effective cures for cancer. Lung cancer is one of the deadliest malignancies in the world and is the primary cause of death among cancer patients (*Siegel, Miller & Jemal, 2020*; *Mridha et al., 2022*; *Adams et al., 2023*). Lung squamous cell carcinoma (LUSC) belongs to non-small-cell lung cancer (NSCLC), and clinical statistics show that the number of patients with LUSC accounts for about 30% of patients with NSCLC (*Li et al., 2022a*; *Ding et al., 2022*; *Chen et al., 2022a*; *Pan et al., 2022*). In recent years, conventional platinum-based two-drug therapy and combination chemotherapy have remained the first-line treatments for advanced LUSC. However, due to aggressiveness, late diagnosis, and poor treatment response, LUSC poses a colossal treatment challenge, with an overall five-year survival rate of only about 18% for patients (*Yang et al., 2022*; *Wang et al., 2022*; *Liu et al., 2022b*; *Huang et al., 2022*; *Ji et al., 2022*; *Xiao et al., 2022*). Therefore, there is an urgent need to further explore the potential biomarkers of LUSC and provide a reliable basis for studying cancer immunotherapy targets.

Matrix metallopeptidase 12 (*MMP12*), also known as macrophage metalloelastase, is produced primarily by macrophages and is a member of the matrix metalloproteinase family. MMP12 is involved in various normal physiological functions, including the catabolism of the extracellular matrix and elastin degradation. It is also involved in the progress of various diseases, such as inflammatory lung diseases and arthritis (*Nénan et al., 2005*). Previously, a high expression of *MMP12* was found to be closely associated with the occurrence and progression of cancers at different sites, such as lung adenocarcinoma (LUAD) (*Lv et al., 2015*), colorectal cancer (*Yu et al., 2021*), hepatocellular carcinoma (*Guo & Jiang, 2022*), cervical cancer (*Lin et al., 2021*), and breast cancer (*Cheng et al., 2021*). In particular, *MMP12* expression is elevated in squamous carcinomas at other anatomical sites, such as esophageal squamous cell carcinoma (ESCC) (*Mao et al., 2022*) and oral squamous cell carcinoma (OSCC) (*Chen et al., 2022b*). *MMP12* promotes macrophage proliferation *via* the ERK/P38 MAPK pathway, and the knockdown of *MMP12* inhibits the development of some tumor cells (*Lv et al., 2015*). A growing body of evidence suggests a clear association between *MMP12* upregulation and the development and progression of multiple malignancies. Previous studies showed that *MMP12* expression was upregulated in 57 cases of LUSC tissues, and that high expression of *MMP12*, risk score, age, tumor stage, and TMN stage were significantly associated with prognosis of LUSC patients. However, the aforementioned studies have certain limitations (*Ma et al., 2020*; *Zhang et al., 2020*). For example, the studies were conducted based on small samples (only 720 samples from public databases), lacked internal sample validation, and did not separately analyze the immune mechanisms involved in *MMP12*. Therefore, further comprehensive investigation of the clinical value and mechanism of *MMP12* in LUSC is warranted.

In this study, we first determined the specific expression levels of *MMP12* in LUSC by analyzing large samples from multicenter and immunohistochemistry (IHC) results. Secondly, we focused on the potential molecular mechanisms and critical clinical implications of *MMP12* in LUSC. Finally, we extensively validated the significant correlation

of *MMP12* expression with immune signaling pathways, immune cell levels, and immune cell infiltration. In conclusion, this study revealed, for the first time, the clinical significance and potential mechanisms of *MMP12* in LUSC, providing new insights into the possible association between *MMP12* and LUSC immune cells.

MATERIALS AND METHODS

Data acquisition and data processing

The high-throughput data related to LUSC for this study were obtained from multiple publicly available databases: the Gene Expression Omnibus (GEO), ArrayExpress, The Cancer Genome Atlas (TCGA), and Genotype-Tissue Expression (GTEx) databases. Among them, microarray and RNA-Seq data could be obtained directly from the GEO database (http://www.ncbi.nlm.nih.gov/geo). Data for patients diagnosed with LUSC were downloaded from TCGA (http://xena.ucsc.edu/) and included RNA-Seq and clinical information (including clinical characteristics and survival data). A total of 33 criteria-compliant data sets with 2,738 samples, including 1,348 LUSC samples and 1,390 non-LUSC samples, were included in this study (Supplementary Material 1). In addition, we obtained four datasets (GSE37745, GSE29013, GSE30219, and GSE73403) involving a total of 221 LUSC cases to analyze whether *MMP12* expression was associated with overall survival (OS) in LUSC patients.

The "limma" package (*Ritchie et al., 2015*) was used to process the data sets from public databases and to normalize them using $\log_2 (x + 1)$. Since the experimental batches differed for each data set, this study combined 33 LUSC cohorts into 10 new fellows based on the same platform. For example, GSE31552 and GSE44077 were merged into the reorganization cohort GPL6244 since both data sets were from the same platform: GPL6244. Finally, the "SVA" package (*Leek & Storey, 2007*) was used to eliminate batch effects.

Evaluation of MMP12 protein expression in LUSC tissues based on internal IHC staining

To clarify the differences in MMP12 protein expression between LUSC and non-LUSC patients, we collected 125 tissue samples (106 LUSC samples; 19 control samples) from the First Affiliated Hospital of Guangxi Medical University. A total of 125 internal samples were divided into three tissue microarray sections (Nos. LUC481, LUC1021, and LUC1502, constructed by Guilin Fanpu Biotech, Guangxi Zhuang Autonomous Region, China) for further IHC experiments. The relevant operations were performed according to the manufacturer's standards. First, tissue sections were fixed by formalin and embedded in paraffin, then sections were dewaxed, and antigen repair was performed with ethylenediaminetetraacetic acid buffer (pH = 9.0) at boiling state. After inactivation of endogenous enzyme activity, rabbit antihuman MMP12 monoclonal antibody (EPR11944[B], ab170414, dilution ratio 1:100) was added and stored overnight at 4 °C ambient. Next, a second antibody-labeled horseradish peroxidase (D-3004-15, Changdao Biotechnology Co., Ltd., Shanghai, China) was added to the tissue sections and incubated at 25 °C for 30 min. Subsequently, the slides were stained with diaminobenzidine, taking care to rinse the slides with PBS between steps. After hematoxylin restaining, the slides

were dehydrated with a gradient concentration of ethanol solution and finally sealed with neutral gum. The sections were scored by microscopy, with brown representing positive and blue representing negative. The staining scores were judged as follows: 0 (no staining), 1 (mild staining), 2 (moderate staining), and 3 (severe staining). Different numbers of positive cells represented different scores: 0 (number <5%), 1 (5%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (number > 75%). The total IHC score was generated by the product of the sample staining score and the percentage of positive cells, and the above assessments were performed independently by two senior pathologists. The raw data of IHC staining were presented in Supplementary Material 2. This study was approved and supported by the Medical Ethics Review Committee of the First Affiliated Hospital of Guangxi Medical University (2021 [KY-E-246]).

MMP12 expression and its clinical relevance in LUSC

The ability of *MMP12* expression to discriminate between tumor and control tissues could be determined using receiver operator characteristic (ROC) curves and summary ROC (sROC) curves based on sensitivity, specificity, and area under the curve (AUC) values. It was generally considered that larger sensitivity, specificity, and AUC values indicate a more remarkable ability of *MMP12* to differentiate between cancer and control tissues. Based on Yoden Index, true positive rate, false positive rate, true negative rate, false negative rate and cut-off values were calculated for each data set , and Stata (v15.0) was used to plot ROC curves.

In addition, this study further analyzed the association between *MMP12* expression and the survival outcomes of LUSC patients by establishing Kaplan–Meier curves and univariate and multivariate Cox regression analyses. The clinical indicators (gender, age, tumor stage, node stage, metastasis stage, clinical stage, and *MMP12*) and prognostic information utilized in the above analyses were available from the Xena database.

MMP12 potential mechanism in LUSC

Cistrome Data Browser (*Zheng et al., 2019*) contains a vast number of human transcription factors (TF), histone modifications, and chromatin accessibility samples. It is currently one of the most comprehensive chromatin immunoprecipitation sequence (ChIP-Seq)-related databases. To explore the potential molecular mechanism of *MMP12* in LUSC, this study predicted the TFs of target genes based on the Cistrome Data Browser and the standardized mean difference (SMD). In addition, ChIP-Seq data in the Cistrome Data Browser were used to validate our results and IGV (2.16.0) was used to visualize them. ChIP-Seq data must pass all quality tests and target gene score > 1. JASPAR (*Fornes et al., 2020*) is a free and publicly available TF database that focuses on collecting relevant TFs with DNA-binding site motifs. Therefore, the relevant TF motif data and the potential promoter region of *MMP12* in this study could be directly obtained from JASPAR and NCBI, respectively. Subsequently, the FIMO tool in the MEME suite was selected to mine the upstream transcription start site (TSS) of *MMP12* and the potential binding sequences of these motifs.

Gene Set Enrichment Analysis (GSEA) was performed using the "clusterProfiler" package (*Wu et al., 2021*), hoping to further explore the potential signaling pathways that

MMP12 may involve in LUSC. The signaling pathways shown in this analysis were obtained from the Kyoto Encyclopedia of Genes and Genomes database. To predict the relationship between the core genes of the enriched pathways, an interaction database platform, STRING (v11.0), was used to construct a protein–protein interaction (PPI) network (*Crosara et al., 2018*). This search was performed with "Homo sapiens" as the set species, the confidence score was set to 0.4, and other settings were set as default.

Correlation of *MMP12* expression with the tumor microenvironment (TME)

Based on the TCGA-GTEx and GPL570 data sets, a single sample Gene Set Enrichment Analysis (ssGSEA) was used to analyze the differences in multiple immune cell scores between the high and low *MMP12* expression groups. Subsequently, we analyzed the correlation between *MMP12* and individual immune cell scores one by one and initially assessed the potential of *MMP12* for immune prediction.

The Tumor Immune Estimation Resource (TIMER) (*Li et al., 2020*; *Chen et al., 2022c*; *Liu et al., 2022a*; *Li et al., 2022b*) is an online database that allows comprehensive detection of immune cell infiltration levels. The data for six immune-infiltrating cells were downloaded from the database. The six immune cells included B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells. In addition, to explore *MMP12* expression levels in various cells (*e.g.*, monocytes/macrophage), the Cancer Single-cell Expression Map (*Zeng et al., 2022*) was used to systematically investigate the heterogeneity of *MMP12* in different cells.

Statistical analysis

The Wilcoxon rank-sum test and the SMD were used for comparisons between various groups. Among them, the Wilcoxon rank-sum test should be used when the combined data set was skewed and the variance of the totals to which the two samples belong was different. When the results show I^2 values < 50% or chi-square test p - values > 0.05, there was less heterogeneity between the data sets, and we need to use a fixed-effects model for SMD calculations. The results of the SMD were statistically significant when the corresponding 95% confidence interval (CI) did not contain 0. The following criteria were used to determine differentially expressed genes: high expression: SMD > 0 and 95% CI excluding 0; low expression: SMD < 0 and 95% CI excluding 0. *Begg*'s test and *Egger*'s test evaluated the publication bias of SMD results, and $p < 0.1$ indicated a significant publication bias. Sensitivity analysis was used to evaluate the reliability of the analysis results. For hazard ratio (HR), the presence of a 95% CI of 1 or $p > 0.05$ indicated no statistical significance. In addition to the above statistical methods, the Spearman correlation coefficient was used to detect the correlation between *MMP12* and immune cells.

In this study, $p < 0.05$ represented a statistically significant difference. Stata (v15.0) was used for *Egger*'s tests, and sensitivity analysis, and R (v4.1.0) was used to complete all the remaining computational and visualization steps. The following was the research flow of this study (Fig. 1A).
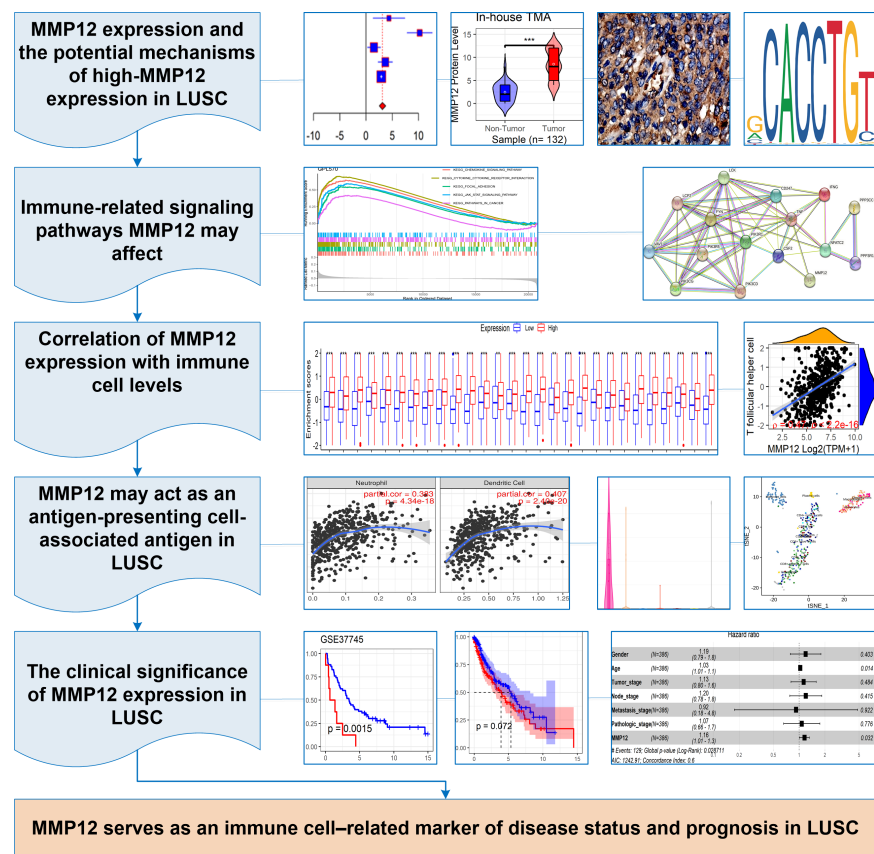
Figure 1 The research overflow of this study.

RESULTS

MMP12 expression level differences between LUSC and its control tissues

In the 10 data sets included in this study, statistically significant differences in *MMP12* expression between the LUSC group and control tissues were detected in nine cohorts (except for "GSE6044"). The results showed that *MMP12* mRNA expression levels were increased in the LUSC group in nine of the combined data sets mentioned above ($p < 0.05$; Fig. 2A). Then, a random-effects model showed that *MMP12* mRNA expression was elevated in the LUSC group but not in the control group (SMD = 3.13, 95% CI [2.51–3.75]; Fig. 2B), and no significant publication bias was detected ($p > 0.1$; Fig. 2C and Supplementary Material 3). Sensitivity analysis showed a small effect of low sample size on the overall *MMP12* expression in LUSC and a large heterogeneity of the data (Supplementary Material 4). We believed that the greater heterogeneity was related to the data coming from different experimental platforms, and a random-effects model was used in this study.

Furthermore, the expression level of MMP12 in LUSC was further verified using internal immunohistochemical experiments. Compared to the non-LUSC group, MMP12 protein levels were significantly upregulated in the LUSC group samples ($p < 0.001$; Fig. 2D),
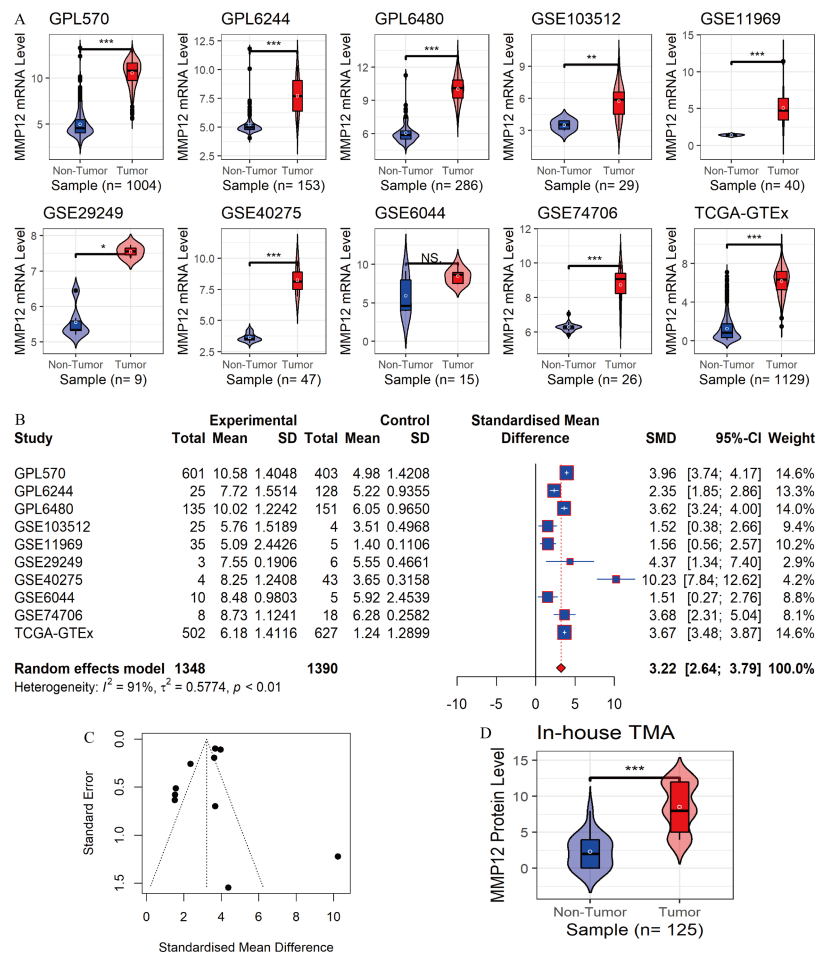
Figure 2 **Differential expression levels of MMP12 between LUSC and its control tissues.** (A) Violin plots of *MMP12* mRNA expression between LUSC and its control tissues in each data set. (B) Forest plot of *MMP12* mRNA expression between LUSC and its control tissues. (C) Funnel plot for detecting SMD publication bias. (D) Violin plot of MMP12 protein expression between LUSC and its control tissues. ns/NS$p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Full-size ◄ DOI: 10.7717/peerj.15598/fig-2

consistent with the mRNA expression levels. Further, under the microscope, as seen in Figs. 3A–3D, MMP12 protein could be easily observed in LUSC tissues (Figs. 3B and 3D) but not in control tissues (Figs. 3A and 3C).

A potential molecular mechanism for the high expression of MMP12 in LUSC

As shown in the flow of Fig. 3E, based on the Cistrome Data Browser for calculation, we selected 11 TFs with a selection score greater than or equal to 0.5. Subsequently, the results of the SMD calculation and co-expression analysis determined that *SNAI2* was a potential TF regulating *MMP12* expression and positively correlated with *MMP12* expression. The peak signal of *SNAI2* ChIP-Seq was located within 2 kb upstream of *MMP12*, further validating the above conclusion (Supplementary Material 5).
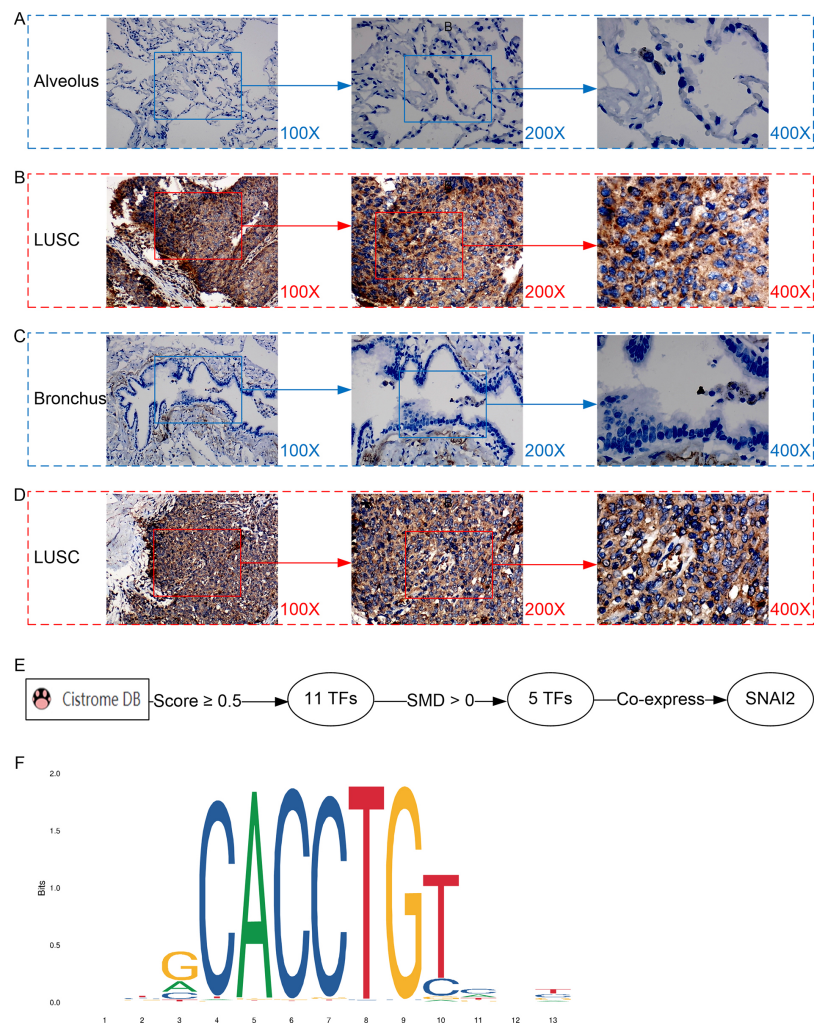
Figure 3 Protein expression and potential molecular mechanisms of MMP12 in LUSC. (A–D) Microscopic images showing anti-MMP12 antibodies exhibiting different levels of staining in alveolar, bronchial, and LUSC tissue. (E) After screening, *SNAI2* was identified as a possible transcription factor that regulated *MMP12* expression. (F) Motif map of *SNAI2*.

Full-size ⊞ DOI: 10.7717/peerj.15598/fig-3

The motif of *SNAI2* was shown in Fig. 3F. Combined with JASPAR and FIMO tools, we obtained the binding sequence of *SNAI2* upstream of *MMP12* TSS:GCACCTGTC. This study also identified two potential binding sites between the *SNAI2* motif and the TSS of *MMP12* (Supplementary Material 6).

MMP12 might affect immune-related signaling pathways

In this study, GSEA was used to explore the potential mechanisms of *MMP12* in multiple signaling pathways. The peaks of the curves appeared in the high-expression group, implying that these pathways were positively correlated with *MMP12* expression and that these signaling pathways became more active when *MMP12* expression was upregulated. Based on the LUSC samples from the data sets TCGA-GTEx (Fig. 4A) and GPL570
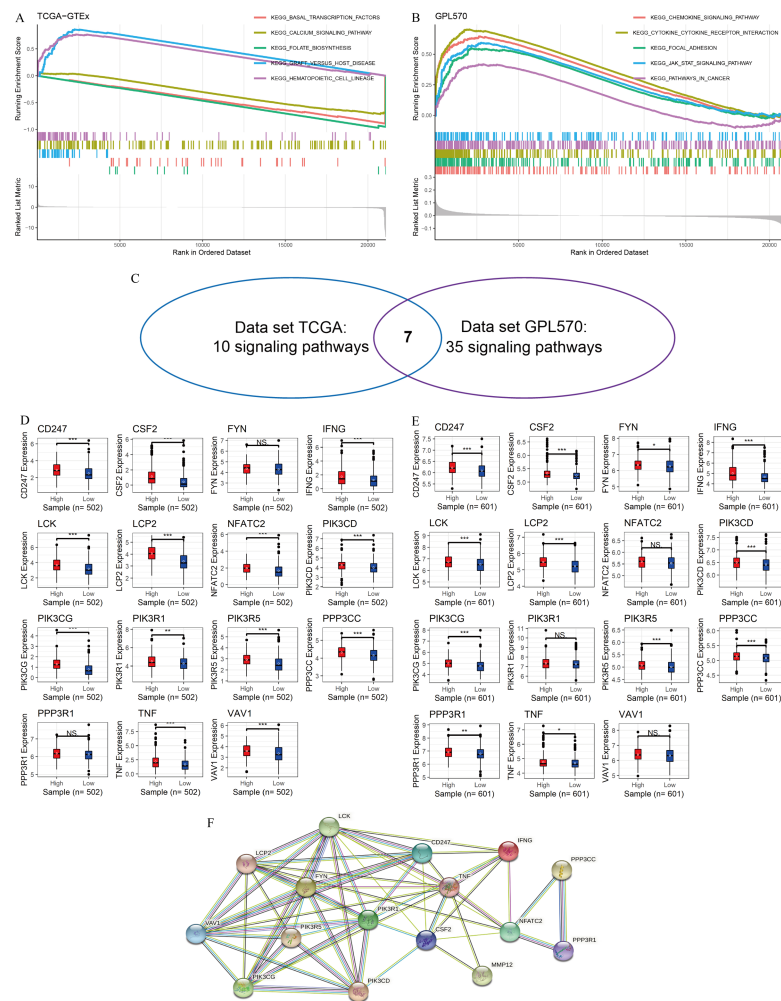
Figure 4 *MMP12* may affect immune-related signaling pathways. (A–C) LUSC samples based on data sets TGCA-GTEx (A) and GPL570 (B) *MMP12* might affect immune-related signaling pathways and their intersection (C). (D–E) LUSC samples based on data sets TGCA-GTEx (D) and GPL570 (E). LUSC samples: the expression differences of the core genes of the two pathways in the high and low *MMP12* expression groups. (F) Protein–protein interaction analysis of the core genes of the pathway. $^{ns/NS}p > 0.05$, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Full-size ⌷ DOI: 10.7717/peerj.15598/fig-4

(Fig. 4B), GSEA suggested that *MMP12* was associated with multiple signaling pathways, and the results of the two data sets overlapped to obtain seven common pathways (Fig. 4C). Notably, two pathways (the T-cell receptor signaling pathway and the natural killer cell–mediated cytotoxicity) showed a direct association of *MMP12* with immune cells, suggesting that *MMP12* was likely to be involved in essential mechanisms of cancer through immune-related signaling pathways (Supplementary Material 7).

Subsequently, for the core genes enriched to both pathways, differential expression of most genes was observed between the high and low *MMP12* expression groups, and all of these genes were upregulated in the high *MMP12* expression group ($p < 0.05$; Figs. 4D and 4E). Finally, this study performed a PPI analysis of the core genes enriched in both

Zhang et al. (2023), *PeerJ*, DOI 10.7717/peerj.15598

9/24

pathways and found that these genes closely interacted with each other (Fig. 4F). The black and green connecting lines between *MMP12* and tumor necrosis factor (TNF) and *CSF2* suggested their co-expression and previous studies between the genes, respectively. We further explored previous studies finding that *MMP12* affects the activity of the *TNF* signaling pathway and monocyte-derived *MMP12* is induced by *CSF2* (*Björnfot Holmström et al., 2017*; *Mao et al., 2022*). Furthermore, we found that LCK proto-oncogene (LCK) had the highest number of PPI nodes with other genes, implying that *LCK* might be the central gene in both signaling pathways and had multiple different interactions with other genes, followed by *PIK3R1* and *TNF*.

MMP12 positively correlated with immune cell levels

LUSC patients in the TCGA-GTEx cohort were divided into high and low *MMP12* expression groups using median *MMP12* expression. The correlation between *MMP12* expression and 28 immune cells was investigated using ssGSEA. The results showed significant differences in immune cell scores between the high and low *MMP12* expression groups, and higher levels of immune cells could be observed in the high *MMP12* expression group ($p < 0.01$; Fig. 5A). Considering that the association between them could still not be intuitively determined by difference analysis alone, we further explored the correlation between *MMP12* and these immune cell scores. As can be found in Fig. 5B, the expression level of *MMP12* tended to be positively correlated with the immune cell score ($\rho > 0.15$, $p < 0.01$). Most of these results were validated by the GPL570 data set (Figs. 6A and 6B).

MMP12 might serve as an antigen-presenting cell–associated LUSC antigen

Considering that the immune response plays a crucial role in antitumor, this study further verified the potential association between *MMP12* expression and immune cells in TME using different methods. Based on the TIMER algorithm (Fig. 7A), we found that *MMP12* expression was significantly negatively correlated with tumor purity ($\rho < 0$, $p < 0.001$) and that upregulation of *MMP12* expression was associated with increased infiltration levels of five immune cell types (except CD4+ T cells) ($\rho > 0$, $p < 0.05$). From the previous results, it was clear that *MMP12* in LUSC was positively correlated not only with the level of specific immune cells (*e.g.*, CD 8 + T cells) but also with the level of non-specific immune cell infiltration (*e.g.*, neutrophils), suggesting that it might have the potential to activate the immune system.

Further analysis showed that multicenter single-cell sequencing data results also suggested that *MMP12* expression was evident in immune cells in several classes of TME-related cells, such as dendritic cells (DC) and monocytes/macrophages (Figs. 7B–7D and Supplementary Material 8). This suggested that the high expression of *MMP12* in LUSC might act as an antigen-presenting cell–associated tumor neoantigen and activate the body's immune response.

MMP12 discriminated between LUSC tissues and normal lung tissues

The important clinical value of *MMP12* in TME has already been observed, so this study expected that *MMP12* could further demonstrate a valuable clinical role in LUSC by ROC
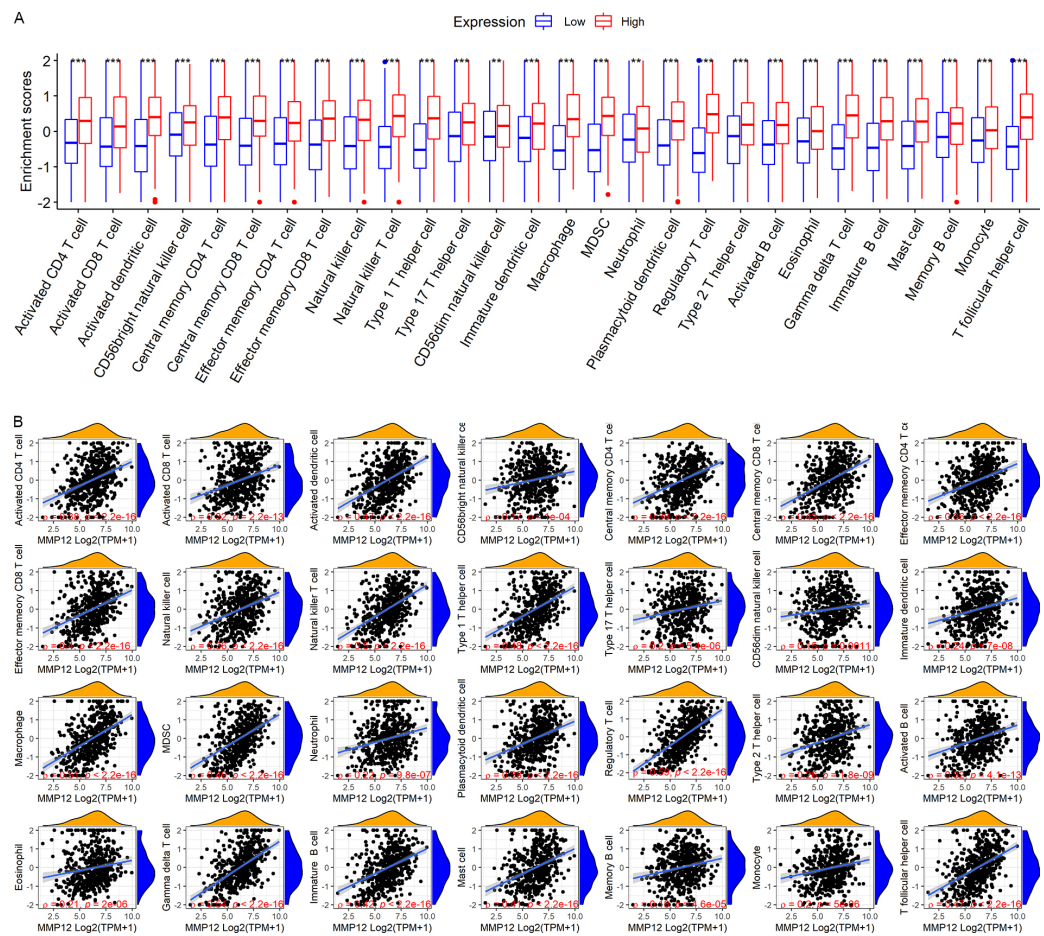
Figure 5 Based on LUSC samples from the TCGA-GTEx data set, the correlation between *MMP12* and immune cell levels. (A) Different immune cell scores between the high and low *MMP12* expression groups. (B) Correlation between *MMP12* and immune cell scores. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Full-size 🖼 DOI: 10.7717/peerj.15598/fig-5

and sROC curves. In the included 10 combined data sets, *MMP12* mRNA expression possessed a very high precision to distinguish LUSC samples from control samples according to the ROC curves of nine of the cohorts (AUC > 0.9) (Fig. 8A). The sROC analysis showed that *MMP12* expression made it feasible to distinguish LUSC samples from control tissue samples (sensitivity = 0.96, specificity = 0.94, AUC = 0.98) (Fig. 8B). These results suggested that *MMP12* had significant potential in the identification of LUSC.

Correlation of *MMP12* with the prognosis of LUSC patients

As shown above, *MMP12* might be a potential marker for the identification of LUSC. Therefore, based on GEO and TCGA data, this study explored the relationship between *MMP12* expression and the prognosis of LUSC patients. As shown in Figs. 9A and 9C, high *MMP12* mRNA expression was associated with poor OS, and the OS of LUSC patients with *MMP12* overexpression was poorer. A trend showed that overexpressed *MMP12* could be a prognostic risk factor for LUSC patients, and only results based on the GSE37745
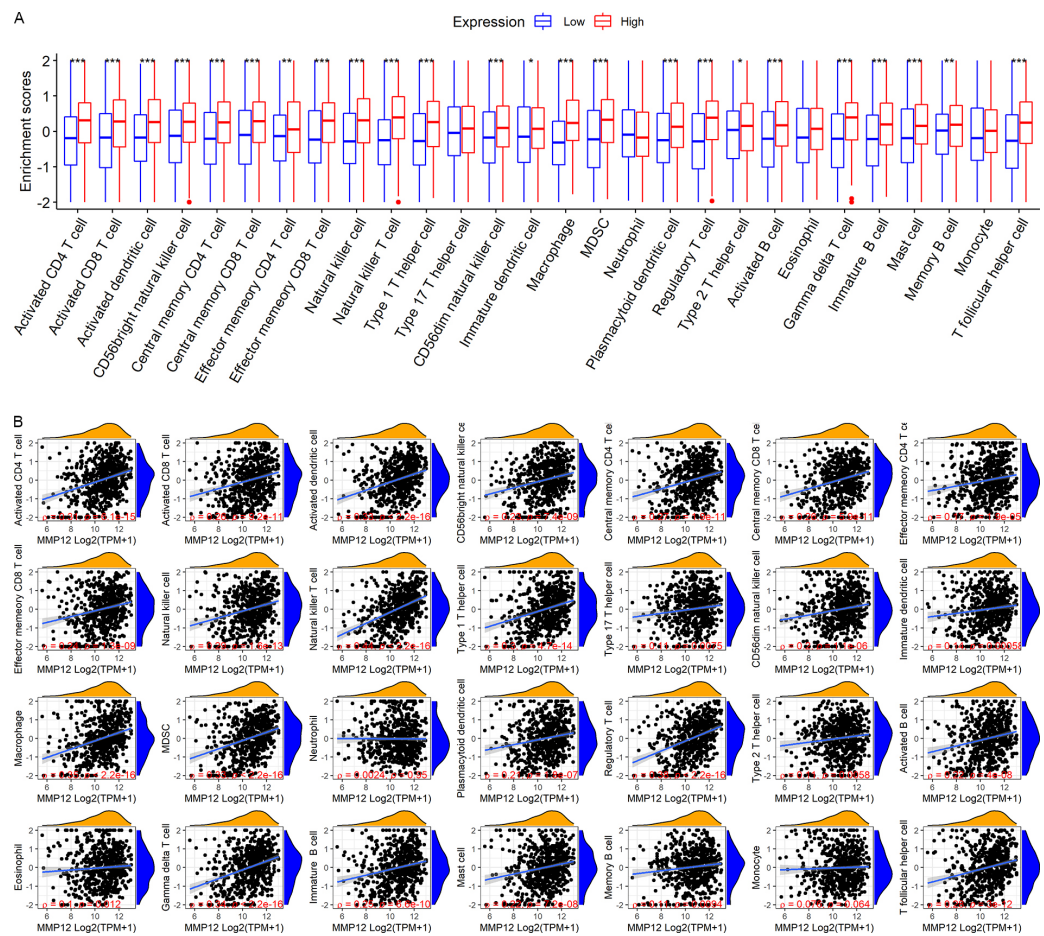
Figure 6 Based on LUSC samples from the **GPL570** data set, the correlation between *MMP12* and immune cell levels. (A) Different immune cell scores between the high and low *MMP12* expression groups. (B) Correlation between *MMP12* and immune cell scores. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Full-size 🖼 DOI: 10.7717/peerj.15598/fig-6

cohort were statistically significant ($p = 0.026$). Subsequently, the four GEO data sets were further combined and it was also demonstrated by prognostic analysis that overexpression of *MMP12* leads to poorer prognosis in LUSC patients (Fig. 9B). The four GEO data sets covered 116 samples; compared to patients with reduced *MMP12* expression, patients with elevated *MMP12* expression tended to have a poorer OS (HR = 1.66, 95% CI [1.01–2.75]).

Further, the effect of different clinical indicators (gender, age, tumor stage, node stage, metastasis stage, pathologic stage, and *MMP12* expression level/value on the OS of LUSC patients was investigated by establishing Cox regression analysis. The results showed that both increased age and upregulated MMP12 protein expression resulted in more adverse OS in LUSC patients ($p < 0.05$; Fig. 9D), and the multivariate Cox regression analysis showed similar results ($p < 0.05$; Fig. 9E).
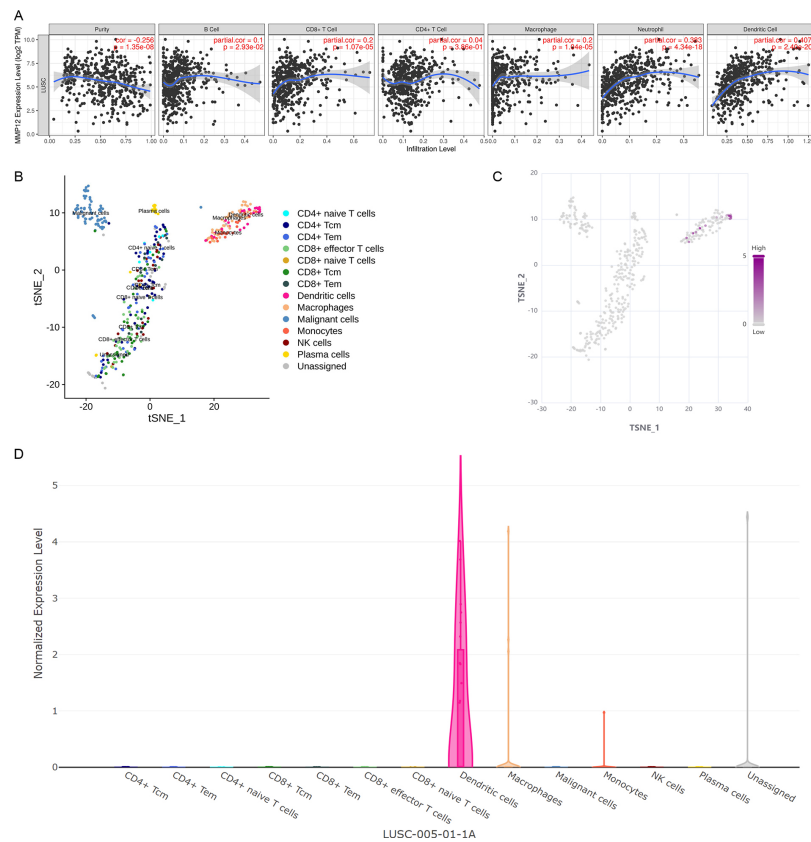
Figure 7 **MMP12 might play a role as an antigen-presenting cell–associated LUSC antigen.** (A) *MMP12* was closely associated with antigen-presenting cells. (B) *MMP12* was expressed at high levels in immune cells. (C) *MMP12* was highly expressed in antigen-presenting cells. (D) The expression of *MMP12* was significantly increased in DC and monocytes/macrophages.

Full-size 🖾 DOI: 10.7717/peerj.15598/fig-7

DISCUSSION

To our knowledge, a comprehensive study on *MMP12* in LUSC has not previously appeared; this paper fills this part of the gap in several aspects, including differential expression, potential molecular mechanisms, immune relevance, and clinical significance. In this study, 1,348 LUSC samples and 1,390 control samples were collected for a comprehensive analysis, which showed elevated levels of *MMP12* mRNA in LUSC. The IHC staining results suggested that MMP12 expression was elevated in LUSC tumor cells at the protein level, and this result was supported by *Ella et al. (2018)*. *MMP12* overexpression in LUSC exhibited significant prognostic and predictive values. Furthermore, *MMP12* might participate in immune-related signaling pathways and was closely associated with immune-infiltrating cells in TME, implying that this gene might play a critical role in immune response.

The differential expression of *MMP12* in cancer has received much attention, and its high expression has been reported several times. *Elshimi et al. (2019)* observed that MMP12 was involved in tumor aggressiveness and metastasis and that overexpressed blood mRNA *MMP12* could be used for the early diagnosis of hepatocellular carcinoma. *Lin et al. (2021)*
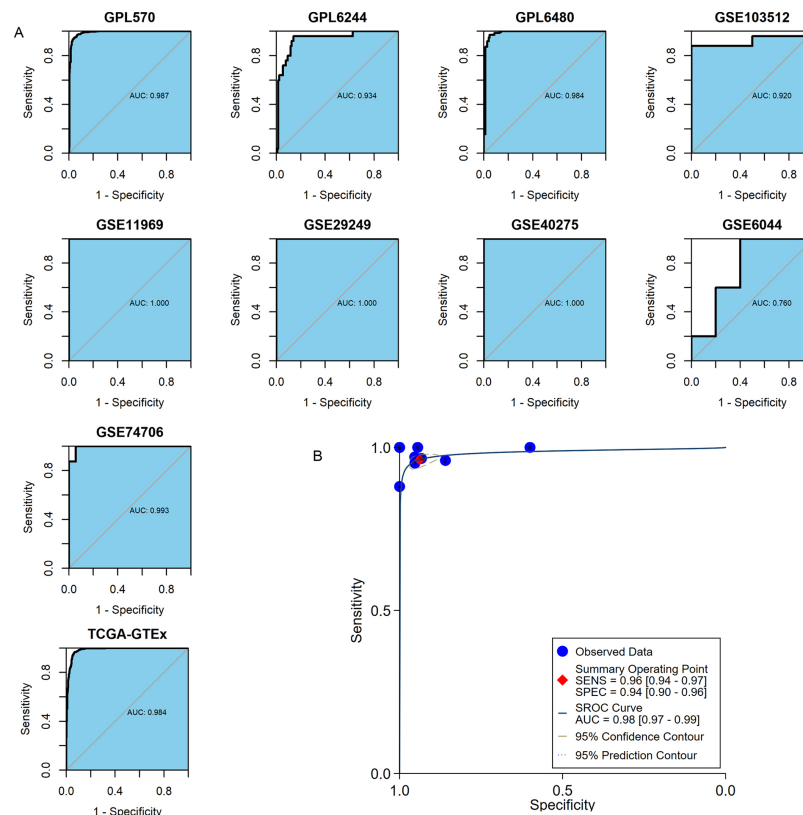
Figure 8 The ability of *MMP12* to distinguish cancerous tissues from control tissues. (A) Receiver operating characteristic curves. (B) Summary receiver operating characteristic curve.

Full-size 🖼 DOI: 10.7717/peerj.15598/fig-8

also found that *MMP12* was highly expressed in cervical cancer and that inhibition of *MMP12* expression could significantly reduce the metastasis of cervical cancer cells. *Ella et al. (2018)* found that *MMP12* produced by tumor cells promoted proliferation of lung tumors. Meanwhile, previous studies have shown that high expression of *MMP12* is closely associated with poor prognosis in ESCC and OSCC. Therefore, we focused our attention on squamous tumors in the lung. In LUSC, *Zhang et al. (2020)* only mentioned *MMP12* differentially expressed in this disease; however, the research was based on limited samples (*n* of samples = 620), and *MMP12* was not explored deeply enough in LUSC. In our study, upregulation of *MMP12* expression in multiple cancer data sets (*n* of samples = 2738) was detected using differential analysis and SMD. Furthermore, we validated this result at the protein level using in-house IHC experiments (*n* of samples = 125), with tissue samples originating from the First Affiliated Hospital of Guangxi Medical University. These new attempts exemplified the strengths of this study.

Although dysregulation of *MMP12* in cancer is common, its underlying molecular mechanisms remain unclear. This study provided a basis for revealing the underlying molecular mechanisms of *MMP12*. Through the computation and screening of the Cistrome Data Browser, *SNAI2* was determined as a potential TF regulating the upregulation of *MMP12* expression in LUSC. Previous studies have shown that *SNAI2* can induce the
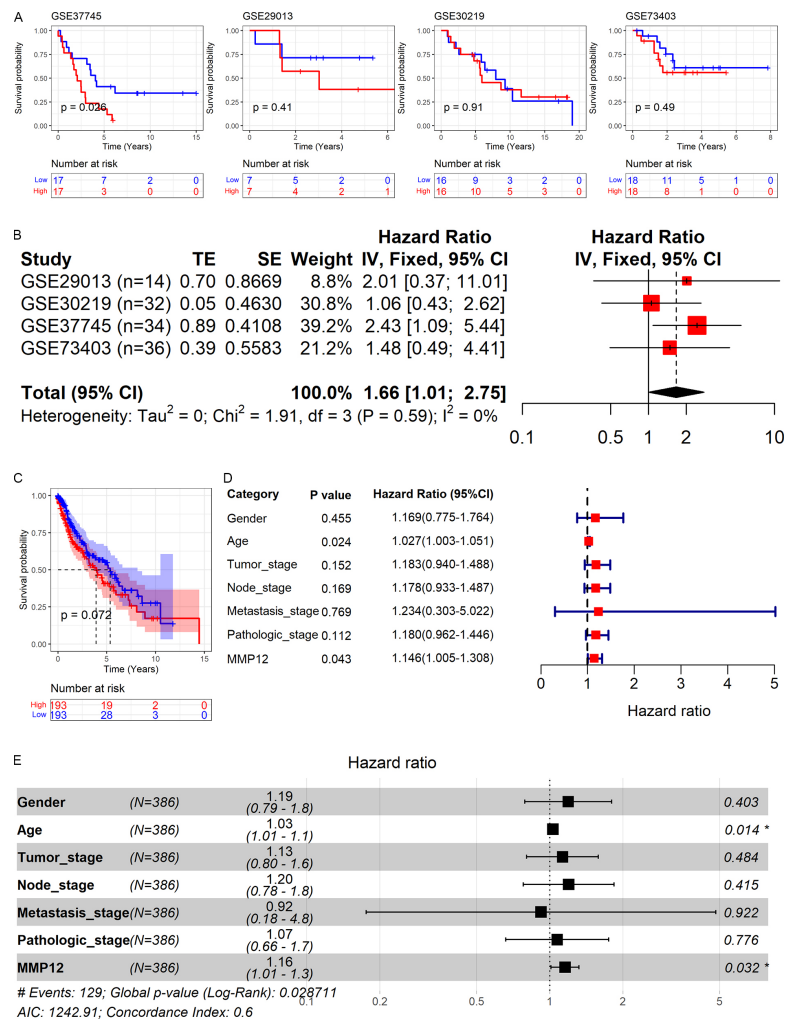
Figure 9 **Correlation of *MMP12* with the prognosis of LUSC patients.** (A) Kaplan–Meier curve for GEO data. (B) Univariate Cox regression analysis for the GEO data. (C) Kaplan–Meier curve for TCGA data. (D) Univariate Cox regression analysis for TCGA data. (E) Multivariate Cox regression analysis of TCGA data.

Full-size ▣ DOI: 10.7717/peerj.15598/fig-9

invasiveness of cancer cells (*Fan et al., 2020*); predictably, *MMP12* might be involved in LUSC metastasis as a downstream gene of *SNAI2*, and this proposition was reinforced by ChIP-Seq data in the Cistrome Data Browser. To the best of our knowledge, this finding has never been reported before, reflecting this study's novelty. Subsequently, based on the LUSC samples from the TCGA-GTEx and GPL570 data sets, we found two signaling pathways showing a direct association of *MMP12* with immune cells (the T-cell receptor signaling pathway and natural killer cell–mediated cytotoxicity). This result focused our attention on the relevance of *MMP12* to immune cells in TME. Furthermore, the leading genes in the two signaling pathways were upregulated in the high-*MMP12* expression group. This result suggested that *MMP12* might be involved in the underlying mechanisms of cancer through immune-related signaling pathways. Interestingly, based on the results

of PPI analysis, *LCK* was identified as a key gene in this network, implying that *LCK* played a crucial role in the immune-related pathways involved in *MMP12*. A study showed that the LCK protein is a constitutive component of the T-cell receptor (TCR) (*Jury et al., 2003*). LCK can regulate the TCR's function to recognize pathogens' antigens and generate immune responses (*Cai et al., 2020*). Based on interconnected cues, we inferred that *MMP12* was further involved in the underlying mechanisms of LUSC by participating in T lymphocyte-dominated adaptive reactions.

Since the previous results show that *MMP12* is involved in the potential mechanisms of cancer through immune-related signaling pathways, we focused on the cells associated with TME. Immune cells account for a large proportion of TME (*Wu et al., 2019*). *MMP12* was significantly and positively correlated with immune cell levels, and immune cell scores were higher in the *MMP12* high-expression group, suggesting that *MMP12* was very closely associated with immune cells. We further validated this using the TIMER algorithm (*Chen et al., 2022c*; *Li et al., 2020*; *Li et al., 2022b*; *Liu et al., 2022a*) and multicenter single-cell sequencing, and the results were mostly consistent with our expectations; upregulation of *MMP12* expression was not only associated with increased infiltration levels of five immune cell types but was also widely present in DCs as well as monocytes/macrophages. DCs are involved in antitumor immunity by activating T cells, and macrophages can participate in immune regulation through phagocytosis, antigen presentation, and the secretion of cytokines (*Wynn & Vannella, 2016*). MMP12 in DCs and macrophages plays an essential role in the processes of many diseases. For treating allergic rhinitis, *Zhou et al. (2022)* speculated that MMP12 might be a biomarker reflecting DC activity. *Weng et al. (2018)* found that MMP12 secreted by M2-type macrophages was involved in the regression of liver fibrosis. Furthermore, the close association of *MMP12* with immune cells suggests its possible involvement in tumorigenesis and development. For example, inflammatory cytokines activate mesenchymal stromal cells in breast cancer and induce elevated levels of *MMP12* expression by neutrophils in TME (*Yu et al., 2017*). *MMP12* can affect ESCC-related pathway activity (*e.g.*, Toll-like receptor signaling pathway, TNF signaling pathway, and IL-17 signaling pathway), and is closely associated with the dysregulation of macrophage M0, B cells, eosinophils, and mast cells (activated or quiescent) (*Mao et al., 2022*). Overall, this study found that *MMP12* was highly expressed not only in tumor cells but also in some APCs of LUSC. *MMP12* possessed the potential to activate the body's immune response as an antigen-presenting cell–associated tumor neoantigen, suggesting that *MMP12* might be a potential new immunotherapeutic target in LUSC.

Considering the tantalizing clinical significance of elevated *MMP12* expression in LUSC, this study attempted to provide valuable findings. *MMP12* expression could distinguish LUSC with high accuracy and thus demonstrated an excellent potential as a diagnostic biomarker. Multiple assays have shown that elevated *MMP12* expression in LUSC was associated with shorter survival time, and Cox regression analysis further revealed that age was also a significant factor in the shortened OS of LUSC patients. As mentioned above, the upregulation of *MMP12* expression can promote the growth and migration of various tumors (including LUSC) (*Elshimi et al., 2019*; *Xu et al., 2018*);

however, *MMP12's* protective effect on tumors has also been reported (*Liu et al., 2021*). This difference may result from several factors, including differences in tumor types and stages of development, or even different cells that express *MMP12*. For example, *MMP12* from vulvar squamous cell carcinoma possessed a more aggressive profile; *MMP12* levels were higher in well-differentiated grade I tumors (produced by macrophages) compared to grade III tumors (*Decock et al., 2011*).

There were still some limitations in this study: (1) We could not secure a sufficient number of samples for analyzing the differential expression of MMP12 at the mRNA and protein levels and its correlation with prognosis. (2) Although we explored the potential mechanism of *MMP12* action in LUSC in the signaling pathway and upstream TF, more in-depth studies were not conducted. (3) More *in vivo* and *in vitro* experiments should be added to further investigate and demonstrate *MMP12* as a potential biomarker of immune cells in LUSC.

CONCLUSION

In conclusion, this study provides a comprehensive assessment of *MMP12* and determines that *MMP12* expression is upregulated in LUSC. High expression of *MMP12* serves as a risk factor for LUSC patients and may be an immune-related predictive and prognostic marker for LUSC. *MMP12* may be involved in cancer development by participating in immune-related signaling pathways and elevating the level of immune cell infiltration. In addition, *MMP12* is significantly expressed in DCs and monocytes/macrophages, which may provide new directions for the development of future immunotherapy for LUSC.

Abbreviations

LUSC	lung squamous cell carcinoma
NSCLC	non-small-cell lung cancer
MMP12	Matrix metallopeptidase 12
LUAD	lung adenocarcinoma
mRNA	messenger RNA
GEO	the Gene Expression Omnibus
TCGA	The Cancer Genome Atlas
GTEx	Genotype-Tissue Expression
IHC	immunohistochemistry
ROC	receiver operator characteristic
AUC	area under the curve
TF	transcription factor
ChIP-Seq	chromatin immunoprecipitation sequence
SMD	standardized mean difference
TSS	transcription start site
GSEA	gene set enrichment analysis
PPI	protein-protein interaction
TME	tumor microenvironment
TIMER	The Tumor Immune Estimation Resource
CI	confidence interval

HR	Hazard Ratio
LCK	LCK proto-oncogene
DC	dendritic cells
OS	overall survival
TCR	T cell receptor

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

Gang Chen is an Academic Editor for PeerJ.

Author Contributions

- Wei Zhang conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Guo-Sheng Li conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Xiang-Yu Gan performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

- Zhi-Guang Huang conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Rong-Quan He conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Hong Huang performed the experiments, prepared figures and/or tables, and approved the final draft.
- Dong-Ming Li conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Yu-Lu Tang conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Deng Tang performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Wen Zou performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Jun Liu performed the experiments, prepared figures and/or tables, and approved the final draft.
- Yi-Wu Dang performed the experiments, prepared figures and/or tables, and approved the final draft.
- Gang Chen analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Hua-Fu Zhou analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Jin-Liang Kong analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Hui-ping Lu conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Studies concerning human subjects have been approved and supported by the Medical Ethics Review Committee of the First Affiliated Hospital of Guangxi Medical University(2021[KY-E-246]). All patients who provided internal samples signed an informed consent form.

Data Availability

The following information was supplied regarding data availability:

The data is available at TCGA, LUSC (https://portal.gdc.cancer.gov/projects/TCGA-LUSC) and NCBI GEO: GSE29249, GSE103512, GSE74706, GSE40275, GSE6044, GSE11969, GSE19188, GSE19804, GSE30219, GSE18842, GSE101929, GSE157010, GSE106937, GSE18385, GSE33532, GSE50081, GSE43580, GSE37745, GSE28571, GSE29013, GSE10245, GSE2109, GSE27556, GSE31552, GSE44077, GSE51852, GSE33479, GSE101420, GSE73403, GSE40074, GSE40588.

Zhang et al. (2023), *PeerJ*, DOI 10.7717/peerj.15598

19/24

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15598#supplemental-information.

REFERENCES

Adams SJ, Stone E, Baldwin DR, Vliegenthart R, Lee P, Fintelmann FJ. 2023. Lung cancer screening. *Lancet* **401**:390–408 DOI 10.1016/s0140-6736(22)01694-4.

Björnfot Holmström S, Clark R, Zwicker S, Bureik D, Kvedaraite E, Bernasconi E, Nguyen Hoang AT, Johannsen G, Marsland BJ, Boström EA, Svensson M. 2017. Gingival tissue inflammation promotes increased matrix metalloproteinase-12 production by CD200R(low) monocyte-derived cells in periodontitis. *Journal of Immunology* **199**:4023–4035 DOI 10.4049/jimmunol.1700672.

Cai Y, Liu R, Lu X, Zhang Q, Wang X, Lian H, Wang H. 2020. Correlation in gene expression between the aggravation of chronic obstructive pulmonary disease and the occurrence of complications. *Bioengineered* **11**:1245–1257 DOI 10.1080/21655979.2020.1839216.

Chen C, Tang D, Gu C, Wang B, Yao Y, Wang R, Zhang H, Gao W. 2022a. Characterization of the immune microenvironmental landscape of lung squamous cell carcinoma with immune cell infiltration. *Disease Markers* **2022**:2361507 DOI 10.1155/2022/2361507.

Chen X, Lei H, Cheng Y, Fang S, Sun W, Zhang X, Jin Z. 2022b. CXCL8, MMP12, and MMP13 are common biomarkers of periodontitis and oral squamous cell carcinoma. *Oral Diseases* Online ahead of print DOI 10.1111/odi.14419.

Chen Y, Sun J, Luo Y, Liu J, Wang X, Feng R, Huang J, Du H, Li Q, Tan J, Ren G, Wang X, Li H. 2022c. Pharmaceutical targeting Th2-mediated immunity enhances immunotherapy response in breast cancer. *Journal of Translational Medicine* **20**:615 DOI 10.1186/s12967-022-03807-8.

Cheng T, Chen P, Chen J, Deng Y, Huang C. 2021. Landscape analysis of matrix metalloproteinases unveils key prognostic markers for patients with breast cancer. *Frontiers in Genetics* **12**:809600 DOI 10.3389/fgene.2021.809600.

Crosara KTB, Moffa EB, Xiao Y, Siqueira WL. 2018. Merging in-silico and in vitro salivary protein complex partners using the STRING database: a tutorial. *Journal of Proteomics* **171**:87–94 DOI 10.1016/j.jprot.2017.08.002.

Decock J, Thirkettle S, Wagstaff L, Edwards DR. 2011. Matrix metalloproteinases: protective roles in cancer. *Journal of Cellular and Molecular Medicine* **15**:1254–1265 DOI 10.1111/j.1582-4934.2011.01302.x.

Ding Y, Bian TT, Li QY, He JR, Guo Q, Wu CY, Chen SS. 2022. A new risk model for CSTA, FAM83A, and MYCT1 predicts poor prognosis and is related to immune infiltration in lung squamous cell carcinoma. *American Journal of Translational Research* **14**:7705–7725.

Ella E, Harel Y, Abraham M, Wald H, Benny O, Karsch-Bluman A, Vincent D, Laurent D, Amir G, Izhar U, Shapira OM, Yoon D, Lee HS, Sugarbaker DJ, Burt B, Peled

A, Wald O. 2018. Matrix metalloproteinase 12 promotes tumor propagation in the lung. *Journal of Thoracic and Cardiovascular Surgery* **155**:2164–2175 DOI 10.1016/j.jtcvs.2017.11.110.

Elshimi E, Sakr MAM, Morad WS, Mohammad L. 2019. Optimizing the diagnostic role of alpha-fetoprotein and abdominal ultrasound by adding overexpressed blood mRNA matrix metalloproteinase-12 for diagnosis of HCV-related hepatocellular carcinoma. *Gastrointest Tumors* **5**:100–108 DOI 10.1159/000495838.

Fan H, Wang X, Li W, Shen M, Wei Y, Zheng H, Kang Y. 2020. ASB13 inhibits breast cancer metastasis through promoting SNAI2 degradation and relieving its transcriptional repression of YAP. *Genes and Development* **34**:1359–1372 DOI 10.1101/gad.339796.120.

Fornes O, Castro-Mondragon JA, Khan A, Van der Lee R, Zhang X, Richmond PA, Modi BP, Correard S, Gheorghe M, Baranašić D, Santana-Garcia W, Tan G, Chèneby J, Ballester B, Parcy F, Sandelin A, Lenhard B, Wasserman WW, Mathelier A. 2020. JASPAR 2020: update of the open-access database of transcription factor binding profiles. *Nucleic Acids Research* **48**:D87–D92 DOI 10.1093/nar/gkz1001.

Guo ZY, Jiang LP. 2022. Matrix metalloproteinase 12 (MMP12) as an adverse prognostic biomarker of vascular invasion in hepatic cell carcinoma. *European Review for Medical and Pharmacological Sciences* **26**:2238–2249 DOI 10.26355/eurrev_202204_28454.

Huang L, Xie T, Zhao F, Feng Y, Zhu H, Tang L, Han X, Shi Y. 2022. DLX2 is a potential immune-related prognostic indicator associated with remodeling of tumor microenvironment in lung squamous cell carcinoma: an integrated bioinformatical analysis. *Disease Markers* **2022**:6512300 DOI 10.1155/2022/6512300.

Ji L, Moghal N, Zou X, Fang Y, Hu S, Wang Y, Tsao MS. 2022. The NRF2 antagonist ML385 inhibits PI3K-mTOR signaling and growth of lung squamous cell carcinoma cells. *Cancer Medicine* **12**:5688–5702 DOI 10.1002/cam4.5311.

Jury EC, Kabouridis PS, Abba A, Mageed RA, Isenberg DA. 2003. Increased ubiquitination and reduced expression of LCK in T lymphocytes from patients with systemic lupus erythematosus. *Arthtitis and Rheumatism* **48**:1343–1354 DOI 10.1002/art.10978.

Leek JT, Storey JD. 2007. Capturing heterogeneity in gene expression studies by surrogate variable analysis. *PLOS Genetics* **3**:1724–1735 DOI 10.1371/journal.pgen.0030161.

Li G, Wu L, Yu J, Zhai S, Deng H, Wang Q. 2022a. Identification and validation of three-gene signature in lung squamous cell carcinoma by integrated transcriptome and methylation analysis. *Journal of Clinical Oncology* **2022**:9688040 DOI 10.1155/2022/9688040.

Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. 2020. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Research* **48**:W509–W514 DOI 10.1093/nar/gkaa407.

Li Y, Fu W, Geng Z, Song Y, Yang X, He T, Wu J, Wang B. 2022b. A pan-cancer analysis of the oncogenic role of ribonucleotide reductase subunit M2 in human tumors. *PeerJ* **10**:e14432 DOI 10.7717/peerj.14432.

Lin CL, Ying TH, Yang SF, Chiou HL, Chen YS, Kao SH, Hsieh YH. 2021. MTA2 silencing attenuates the metastatic potential of cervical cancer cells by inhibiting AP1-mediated MMP12 expression via the ASK1/MEK3/p38/YB1 axis. *Cell Death & Disease* **12**:451 DOI 10.1038/s41419-021-03729-1.

Liu BX, Yang J, Zeng C, Chen Y. 2022a. MACC1 correlates with tumor progression and immune cell infiltration of colon adenocarcinoma and is regulated by the lncRNA ZFAS1/miR-642a-5p axis. *Journal of Clinical Oncology* **2022**:8179208 DOI 10.1155/2022/8179208.

Liu Y, Cheng L, Li C, Zhang C, Wang L, Zhang J. 2021. Identification of tumor microenvironment-related prognostic genes in colorectal cancer based on bioinformatic methods. *Scientific Reports* **11**:15040 DOI 10.1038/s41598-021-94541-6.

Liu Y, Sun M, Xiong Y, Gu X, Zhang K, Liu L. 2022b. Construction and validation of prognosis nomogram for metastatic lung squamous cell carcinoma: a population-based study. *Technology in Cancer Research & Treatment* **21**:15330338221132035 DOI 10.1177/15330338221132035.

Lv FZ, Wang JL, Wu Y, Chen HF, Shen XY. 2015. Knockdown of MMP12 inhibits the growth and invasion of lung adenocarcinoma cells. *International Journal of Immunopathology and Pharmacology* **28**:77–84 DOI 10.1177/0394632015572557.

Ma X, Ren H, Peng R, Li Y, Ming L. 2020. Identification of key genes associated with progression and prognosis for lung squamous cell carcinoma. *PeerJ* **8**:e9086 DOI 10.7717/peerj.9086.

Mao JT, Lu Q, Jing PY, Li ZL, Yang XQ, Zhang JP, Li Z. 2022. Comprehensive analysis of prognostic value and immune infiltration of MMP12 in esophageal squamous cell carcinoma. *Journal of Clinical Oncology* **2022**:4097428 DOI 10.1155/2022/4097428.

Mridha MF, Prodeep AR, Hoque A, Islam MR, Lima AA, Kabir MM, Hamid MA, Watanobe Y. 2022. A comprehensive survey on the progress, process, and challenges of lung cancer detection and classification. *Journal of Healthcare Engineering* **2022**:5905230 DOI 10.1155/2022/5905230.

Nénan S, Boichot E, Lagente V, Bertrand CP. 2005. Macrophage elastase (MMP-12): a pro-inflammatory mediator? *Memorias Do Instituto Oswaldo Cruz* **100**(**Suppl 1**):167–172 DOI 10.1590/s0074-02762005000900028.

Pan J, Huang Z, Lin H, Cheng W, Lai J, Li J. 2022. M7G-related lncRNAs predict prognosis and regulate the immune microenvironment in lung squamous cell carcinoma. *BMC Cancer* **22**:1132 DOI 10.1186/s12885-022-10232-z.

Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* **43**:e47 DOI 10.1093/nar/gkv007.

Siegel RL, Miller KD, Jemal A. 2020. Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians* **70**:7–30 DOI 10.3322/caac.21590.

Wang X, Huang Z, Li L, Wang G, Dong L, Li Q, Yuan J, Li Y. 2022. DNA damage repair gene signature model for predicting prognosis and chemotherapy outcomes in lung squamous cell carcinoma. *BMC Cancer* **22**:866 DOI 10.1186/s12885-022-09954-x.

Weng SY, Wang X, Vijayan S, Tang Y, Kim YO, Padberg K, Regen T, Molokanova O, Chen T, Bopp T, Schild H, Brombacher F, Crosby JR, McCaleb ML, Waisman A, Bockamp E, Schuppan D. 2018. IL-4 receptor alpha signaling through macrophages differentially regulates liver fibrosis progression and reversal. *EBioMedicine* **29**:92–103 DOI 10.1016/j.ebiom.2018.01.028.

Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo X, Yu G. 2021. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation* **2**:100141 DOI 10.1016/j.xinn.2021.100141.

Wu X, Hu W, Lu L, Zhao Y, Zhou Y, Xiao Z, Zhang L, Zhang H, Li X, Li W, Wang S, Cho CH, Shen J, Li M. 2019. Repurposing vitamin D for treatment of human malignancies via targeting tumor microenvironment. *Acta Pharmaceutica Sinica B* **9**:203–219 DOI 10.1016/j.apsb.2018.09.002.

Wynn TA, Vannella KM. 2016. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* **44**:450–462 DOI 10.1016/j.immuni.2016.02.015.

Xiao Z, Shi G, Xi S, Singh AK, Willette-Brown J, Li X, Zhu F, Su L, Wu X, Schrump DS, Hu Y. 2022. A TNFR1-UBCH10 axis drives lung squamous cell carcinoma dedifferentiation and metastasis through a cell-autonomous signaling loop. *Cell Death & Disease* **13**:885 DOI 10.1038/s41419-022-05308-4.

Xu L, Lu C, Huang Y, Zhou J, Wang X, Liu C, Chen J, Le H. 2018. SPINK1 promotes cell growth and metastasis of lung adenocarcinoma and acts as a novel prognostic biomarker. *BMB Reports* **51**:648–653 DOI 10.5483/BMBRep.2018.51.12.205.

Yang L, Wei S, Zhang J, Hu Q, Hu W, Cao M, Zhang L, Wang Y, Wang P, Wang K. 2022. Construction of a predictive model for immunotherapy efficacy in lung squamous cell carcinoma based on the degree of tumor-infiltrating immune cells and molecular typing. *Journal of Translational Medicine* **20**:364 DOI 10.1186/s12967-022-03565-7.

Yu J, He Z, He X, Luo Z, Lian L, Wu B, Lan P, Chen H. 2021. Comprehensive analysis of the expression and prognosis for MMPs in human colorectal cancer. *Frontiers in Oncology* **11**:771099 DOI 10.3389/fonc.2021.771099.

Yu PF, Huang Y, Han YY, Lin LY, Sun WH, Rabson AB, Wang Y, Shi YF. 2017. TNF α-activated mesenchymal stromal cells promote breast cancer metastasis by recruiting CXCR2(+) neutrophils. *Oncogene* **36**:482–490 DOI 10.1038/onc.2016.217.

Zeng J, Zhang Y, Shang Y, Mai J, Shi S, Lu M, Bu C, Zhang Z, Zhang Z, Li Y, Du Z, Xiao J. 2022. CancerSCEM: a database of single-cell expression map across various human cancers. *Nucleic Acids Research* **50**:D1147–D1155 DOI 10.1093/nar/gkab905.

Zhang J, Zhang J, Yuan C, Luo Y, Li Y, Dai P, Sun W, Zhang N, Ren J, Zhang J, Gong Y, Xie C. 2020. Establishment of the prognostic index of lung squamous cell carcinoma based on immunogenomic landscape analysis. *Cancer Cell International* **20**:330 DOI 10.1186/s12935-020-01429-y.

Zheng R, Wan C, Mei S, Qin Q, Wu Q, Sun H, Chen CH, Brown M, Zhang X, Meyer CA, Liu XS. 2019. Cistrome data browser: expanded datasets and new tools for gene regulatory analysis. *Nucleic Acids Research* **47**:D729–D735 DOI 10.1093/nar/gky1094.

Zhou Y, Xu M, Gong W, Kang X, Guo R, Wen J, Zhou D, Wang M, Shi D, Jing Q.
2022. Circulating MMP-12 as potential biomarker in evaluating disease severity and
efficacy of sublingual immunotherapy in allergic rhinitis. *Mediators of Inflammation*
2022:3378035 DOI 10.1155/2022/3378035.

Zhang et al. (2023), *PeerJ*, DOI 10.7717/peerj.15598

24/24