Potential applications of prognostic and immunological marker transmembrane serine proteinase 2 in prediction, prevention and personalized treatment of lung cancer

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Transmembrane serine proteinase 2 (TMPRSS2), which is an essential serine protease for priming spike protein of SARS-CoV-2, was found in low expression in many cancer tissue including lung cancer. However, the mechanism of severely downregulated in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) was not reported yet; the correlation between TMPRSS2 and prognosis in LUAD and LUSC is also not clear. In our present research, we found that TMPRSS2 was severely downregulated in LUAD and LUSC, and the expression of TMPRSS2 in LUAD is much lower than that of LUSC. Low TMPRSS2 expression was an independent prognostic factor for poor OS in LUAD, but not in LUSC patients. Promoter hypermethylation is one of the results of TMPRSS2 downregulated in LUAD and LUSC, whereas copy-number alteration is another reason for TMPRSS2 downregulated in LUAD but not LUSC. Then, low TMPRSS2 expression has higher prognostic value in LUAD and may be due to different immune environments and different enriched immune cells subgroups. *European Journal of Cancer Prevention* 32: 65–68 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Nonsmall cell lung cancer is one of the leading causes of cancer death in the world (Herbst *et al*., 2018). Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the two most common subtypes (Munkhbaatar *et al*., 2020). Different subtypes have distinct sites of origin, histology, genetic, and epigenetic changes (Justilien *et al*., 2014). These differences are closely related to their unique responses to therapy (Justilien *et al*., 2014). Therefore, it is meaningful to investigate the difference in their molecular mechanisms.

Transmembrane serine proteinase 2 (TMPRSS2) as is a kind of type II transmembrane serine protease plays a key role in tumor growth, invasion, and metastasis (Ko *et al*., 2020), which is an essential serine protease for priming spike protein of SARS-CoV-2 (Ziegler *et al*., 2020). TMPRSS2 was found in low expression in many cancer tissue including lung cancer (Kong *et al*., 2020). However, the mechanism of severely downregulated in LUAD and LUSC was not reported yet; the correlation between TMPRSS2 and prognosis in LUAD and LUSC is also not clear.

In this study, we investigated the expression of TMPRSS2 and its relationship with prognosis in LUAD and LUSC. We also explored the possible molecular mechanisms underlying TMRPSS2 low expression in LUAD and LUSC. Our objective was to investigate whether TMPRSS2 can be used as a molecular marker and prognostic indicator of LUAD and LUSC.

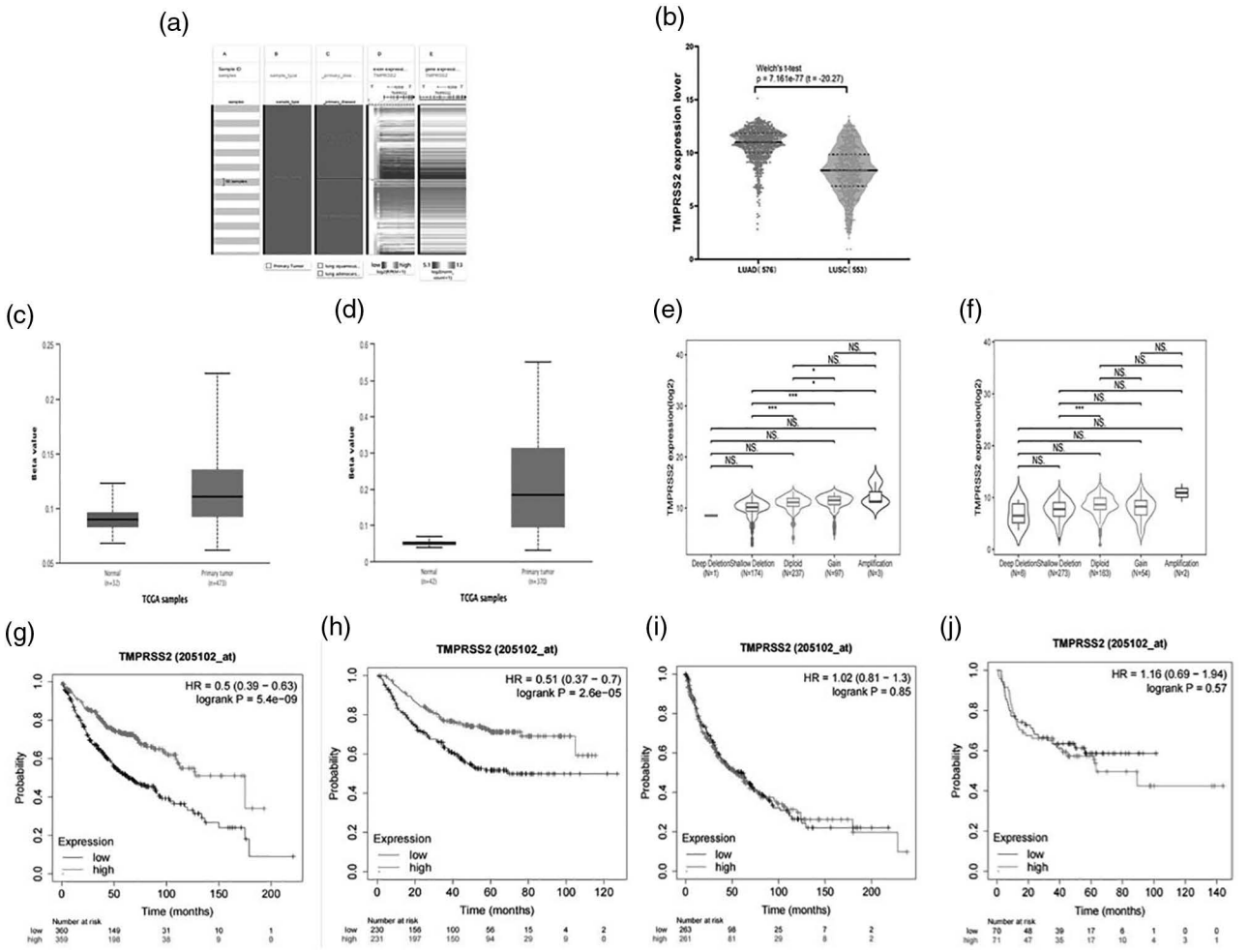
Materials and methods

TMPRSS2 expression, DNA methylation, copy-number alterations (CNA), and the relationship between TMRPSS2 expression and immune infiltration and type markers of immune cells and survival data related to TMRPSS2 in LUAD and LUSC cancer patients were performed using the bioinformatic analysis including the University of California Santa Cruz (UCSC) Xena. cBioPortal for Cancer Genomics (Cerami *et al*., 2012), Kaplan–Meier plotter database (Győrffy *et al*., 2010), UALCAN database (Chandrashekar *et al*., 2017), and TIMER database (Li *et al*., 2017).

Statistical analysis

The statistical results of the survival analysis were obtained from a log-rank test, and the correlations of TMPRSS2 with immune infiltration and type markers of immune cells were evaluated using Spearman's

Fig. 1.



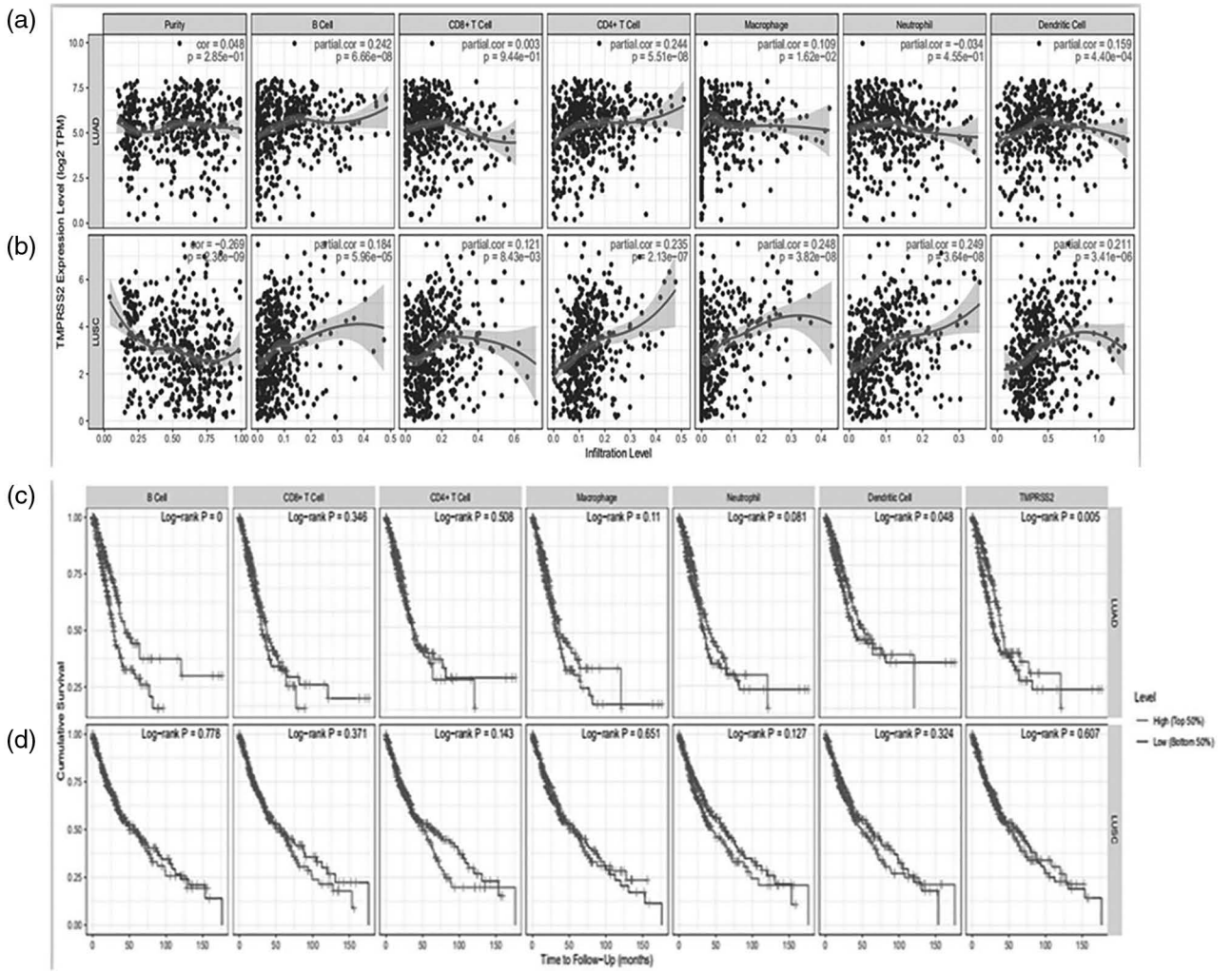
The expression levels of TMPRSS2 in LUAD and LUSC. (a) Heatmap of TMPRSS2 mRNA and exon expression in patients with primary LUSC or LUAD. Data were obtained from TCGA-LUSC and TCGA-LUAD. (b) Box plots of TMPRSS2 expression in LUSC and in LUAD tissues. The analysis was performed using UCSC Xena Browser.(*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). (c and d) Promoter methylation levels were high in LUAD and LUSC (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).(e and f) Comparison of TMPRSS2 mRNA expression in different CNA groups in LUAD and LUSC. (g–j) Low TMPRSS2 expression in the Kaplan–Meier plotter database had favorable OS and DFI in LUAD (g and h) and LUSC (i and j). CNA, copy number alteration; DFI, disease-free interval; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TCGA, The Cancer Genome Atlas; TMPRSS2, transmembrane serine proteinase 2; UCSC, the University of California Santa Cruz.

correlation. Student's *t*-test was used to compare two independent samples. *P*-values <0.05 were considered statistically significant.

Results and discussion

In this article, we compare TMPRSS2 expression in LUAD and LUSC; TMPRSS2 mRNA RNAseq and exon RNAseq data in The Cancer Genome Atlas (TCGA)-LUAD and TCGA-LUSC were extracted for analysis. Heatmap and following comparison showed that TMPRSS2 expression was significantly higher in LUSC than that in LUAD tissues in UCSC Xena database (Fig. 1a and b). Since the significant decreases of TMPRSS2 expressions in LUAD and LUSC were observed, we would like to know what causes the difference in TMPRSS2 expression. DNA methylation and CNA are important event of the genome and are closely related to the process of the disease (Wulfridge *et al.*, 2019). In particular, hypermethylation and missing copy number can lead to decrease in related genes' expression. Therefore, we used UALCAN database to verify the methylation level of TMPRSS2 promoter in LUAD and LUSC, and cBioPortal database to analyze the change in TMPRSS2 copy number in LUAD and LUSC. Interestingly, the methylation levels of TMPRSS2 promoter in LUAD and LUSC were significantly higher than that in normal tissue (Fig. 1c and d). The change in copy number is even more significant in LUAD. Based on the expression differences of TMPRSS2 in LUAD and

Fig. 2.



TMPRSS2 expression is correlated with the level of immune infiltration in LUAD and LUSC. (a and b) TMPRSS2 expressions were positively correlated with B cell, CD4 + T cell, neutrophil and dendritic cell immune infiltration levels of LUAD (a), the level of immune infiltration of macrophage in LUSC (b). (c and d) Kaplan–Meier plots of immune infiltration and TMPRSS2 expression levels in LUAD (c) and LUSC (d). LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TMPRSS2, transmembrane serine proteinase 2.

LUSC, we wonder whether TMPRSS2 can be used as a biomarker for prognostic judgment between LUAD and LUSC. In the Kaplan–Meier plotter database, we also found that TMPRSS2 expression was associated with a favorable prognosis in LUAD cancer patients [Fig. 1g and h; overall survival hazard ratio (OS HR) (95% confidence interval{CI}) = 0.5 (0.39–0.63), P = 5.4e–09; recurrence-free interval (RFI) HR (95% CI) = 0.51 (0.37–0.7), P = 2.6e–05], but not be LUSC cancer patients [Fig. 4i and j; OS HR (95% CI) = 1.02 (0.81–1.3), P = 0.86; RFI HR (95% CI) = 1.16 (0.69–1.94), P = 0.57].

As is known to all, patients with lung cancer are more susceptible to infection than normal individuals. However, TMPRSS2, the expression of tissue in lung cancer patients, is lower than that in normal individuals. We speculate that the difference in viral susceptibility may lie in the immune microenvironment of tumor patients. Therefore, we tested whether the transcription levels of TMPRSS2 in LUAD and LUSC were correlated with immune infiltration. The Tumor Immune Estimation Resource (TIMER) database was used to analyze the correlations between TMPRSS2 level and LUAD and LUSC. As shown in Fig. 2a, the expression of TMPRSS2 was positively correlated with the level of immune infiltration of B cell (r = 0.242, P = 6.66e–08), CD4 + T cell (r = 0.244, P = 5.51e–08), macrophage (r = 0.109, P = 1.62e–02), and Dendritic cell (r = 0.159, P = 4.4e–04), and had no correlation with CD8 + T cell (r = 0003, P = 0.944) and neutrophil in LUAD (r = –0.034, P = 0.455). However, in the LUSC (Fig. 2b), the expression of TMPRSS2 was not only correlated with the above

immune cells including B cell (r = 0.184, P = 5.96e−05), CD4 + T cell (r = 0.235, P = 2.13e08), macrophage (r = 0.248, P = 3.82e−08), and Dendritic cell (r = 0.211, P = 3.41e−06), but also with CD8 + T cells (r = 0.121, P = 8.43e−03) and neutrophils (r = 0.249, P = 3.64e−08). This suggests that TMPRSS2 plays a strong role in regulating immune cell infiltration in LUAD, with a particularly strong effect on B cell and Dendritic infiltration. We further generated Kaplan–Meier plots using the TIMER database in order to explore the relationship between immune cell infiltration and TMPRSS2 expression in LUAD and LUSC. We found B cell infiltration (P = 0), Dendritic cell infiltration (P = 0.048), and TMPRSS2 expression (P = 0.005) to significantly correlate with LUAD prognosis (Fig. 2c), whereas no significant correlation between prognosis and immune cell infiltration and TMPRSS2 expression was observed in LUSC (Fig. 2d). This suggests that TMPRSS2 plays a strong role in regulating immune cell infiltration in LUAD, with a particularly strong effect on B cell and Dendritic infiltration.

Conclusion

To sum up, TMPRSS2 expression decreased significantly in LUAD and LUSC, and changes in methylation levels of TMPRSS2 promoters and CNA are the likely causes. Elevated TMPRSS2 was positively significantly in LUAD rather than LUSC. Elevated TMPRSS2 was positively correlated with immune infiltration and prognoses of rather than LUAD and LUSC. However, this research also has some limitations, due to the limitation of the database; we did not continue to analyze the deep relationship between TMPRSS2 and immune infiltration. In addition, experiments are urgently needed to verify the analysis results in our research.

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Conflicts of interest

There are no conflicts of interest.

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