Original Article

TMEM213 as a novel prognostic and predictive biomarker for patients with lung adenocarcinoma after curative resection: a study based on bioinformatics analysis

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Background: Lung cancer is the leading cause of cancer-related death worldwide. Few effective biomarkers for lung adenocarcinoma have been adapted for clinical practice to assist in prognosis evaluation and treatment plan implementation. Our study's goal was to find a new biological marker associated with the prognosis of lung adenocarcinoma after curative resection and the benefit of adjuvant chemotherapy (ACT).

Methods: Using the clinical information and RNA-Seq expression from The Cancer Genome Atlas (TCGA) database, prognostic genes were screened out and analyzed by Subpopulation Treatment Effect Pattern Plot (STEPP) in GSE42127 to filter out the drug-related gene. The relationship between the gene expression and clinicopathological parameters was assessed in the TCGA database. The prognostic significance was evaluated by Cox proportional hazards (PHs) regression analysis with 1,000 bootstrap replications. Gene set enrichment analysis (GSEA) was performed using high-throughput RNA sequencing data in TCGA and functional gene sets derived from the Molecular Signatures Database (MSigDB).

Results: A total of 297 prognostic genes were analyzed by STEPP in GSE42127. The results indicated a beneficial effect of adjuvant paclitaxel-carboplatin in patients with high *TMEM213* expression. Its expression correlated with gender (P=0.013), and Kaplan-Meier analysis showed that patients with high *TMEM213* expression had significantly longer overall survival (OS) (P=0.014, 0.027, and 0.000). Multivariate analysis showed *TMEM213* to be an independent predictor for improved OS of patients (P=0.020), and the result was verified with the bootstrapping methodology and online "Kaplan-Meier Plotter" database analysis. Moreover, enriched pathway analysis indicated that *TMEM213* expression was associated with the two gene sets of KEGG_DRUG_METABOLISM_CYTOCHROME_P450 and KEGG_ABC_TRANSPORTERS.

Conclusions: Based on bioinformatics analysis, we found that *TMEM213* expression independently predicted better OS for lung adenocarcinoma. Patients in the high *TMEM213* group appear to benefit more from adjuvant paclitaxel-carboplatin, but this needs further validation.

Keywords: *TMEM213*; lung adenocarcinoma; prognosis; adjuvant chemotherapy (ACT)

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Introduction

Lung cancer is the major cause of cancer death worldwide (1). The vast majority of lung cancer is non-small cell lung cancer (NSCLC), of which adenocarcinoma accounts for 50%, being the most common histological type (2). Currently, the American Joint Committee on Cancer (AJCC) staging system is the primary reference for guiding clinical decisions and is by far the very best predicting factor of prognosis in patients with NSCLC. Over 20% of early-stage patients eventually progress to disease recurrence and metastasis, which suggests that current survival predictions are deficient and in need of improvement (3). This also suggests that occult metastases are present as early as at the time of surgical intervention. Therefore, adjuvant chemotherapy (ACT) is recommended to improve the prognosis of patients who undergo a complete resection. Although the use of adjuvant paclitaxel-carboplatin has not been demonstrated in prospective studies, research has shown that it can be considered as the ACT for resected NSCLC (4-6). Nevertheless, some findings regarding the use of adjuvant paclitaxel-carboplatin have raised controversy. One phase III trial denies the benefit of adjuvant paclitaxel and carboplatin in resected early-stage lung cancer (7). Another study reported that stage II–IIIA patients usually receive platinum-based ACT after surgical resection, but only 4–15% survival benefit has been observed (8). This suggests that NSCLC is very heterogeneous, and this potential heterogeneity is not well-reflected in the current staging system, which significantly confuses treatment of patients. Therefore, it is essential to find a novel and effective prognostic factor to differentiate patients who might benefit from adjuvant paclitaxel-carboplatin and those who are not sensitive to it and need other, more active treatments.

The involvement of transmembrane proteins (TMEMs) in malignancy has recently attracted the interest of researchers. TMEMs are a group of 310 different proteins predicted to be components of cellular membranes, such as lysosomes, mitochondrial membranes, and the Golgi apparatus. The role of most TMEM proteins remains unclear, mainly due to the difficulty in purification and extraction of TMEM proteins (9). Moreover, many TMEM proteins have been functionally designated as transmembranous anion channels (10). Members of TMEMs are frequently abnormally expressed in various cancers, such as hepatocellular carcinoma (*TMEM7*) (11), gastric carcinoma (*TMEM16A*) (12), renal cell carcinoma (*TMEM22*) (13), lymphomas (*TMEM176*) (14), glioma (*TMEM97*) (15), and ovarian cancer (*TMEM45A* and *TMEM158*) (16,17). *TMEM213* belongs to a member of the TMEM family. Kamiński found that *TMEM213* SNPs may be involved in the pathogenesis of coronary artery disease (18). Sang Yun analyzed the differences in gene expression between peripheral T-cell lymphoma and normal reactive lymph nodes and found that *TMEM213* was significantly up-regulated (19). It has also been reported that significant deregulation of expression of *TMEM213* in clear cell renal cell carcinoma (ccRCC) may be involved in tumorigenesis and associated with the invasion and metastasis of ccRCC (9). However, there has been no investigation yet conducted concerning the relationship between *TMEM213* expression and clinicopathological features and the prognosis of lung adenocarcinoma.

It has been demonstrated that gene-expression profiles obtained by cDNA microarray analysis can provide detailed features of individualized cancers and such information might improve the clinical tactics for neoplastic diseases by developing novel drugs and providing the foundation of personalized treatment (20). In this study, a screening approach was performed on RNA-Seq expression data from The Cancer Genome Atlas (TCGA) database and microarray datasets from the Gene Expression Omnibus (GEO) database to find a possible target in the TMEM family whose expression could serve as an independent prognostic predictor of lung adenocarcinoma after curative resection and benefit from adjuvant paclitaxel-carboplatin.

Methods

Patient screening process

Clinical information of patients with lung adenocarcinoma was obtained from the TCGA database. There were 522 patients screened for eligibility, excluding 174 patients with non-RO excision; 2 patients with neoadjuvant chemotherapy; 26 patients with IIIB, IV or not staged; 28 patients with a history of malignant tumors or multiple primary tumors; and 19 patients without expression profiles. The final 273 patients with both clinical information and expression profiles were included in the analysis (*Figure 1*).

Screening prognostic genes

Patients with lung adenocarcinoma in the TCGA database were selected, and survival analysis was performed for all

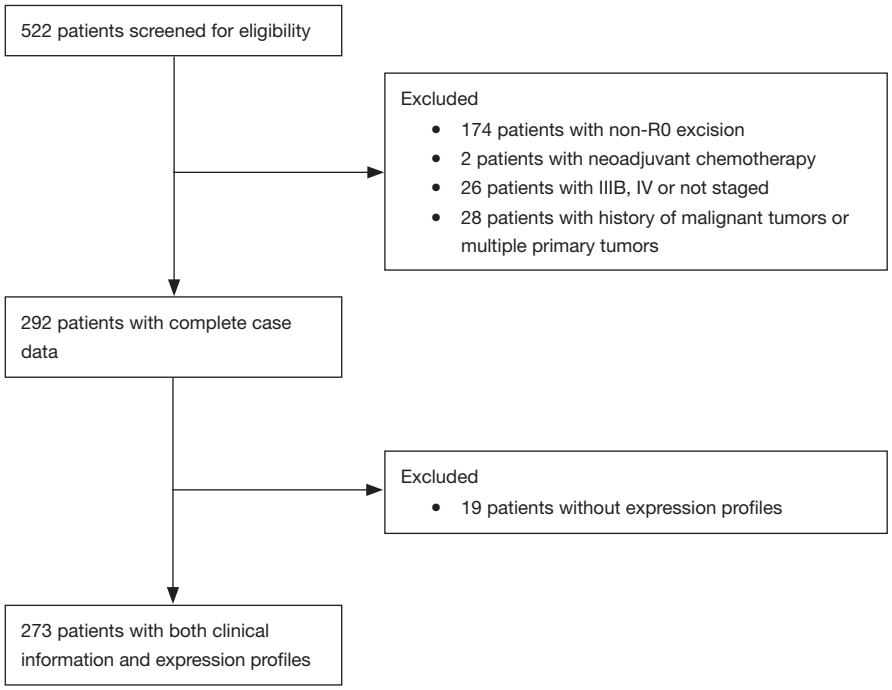

Figure 1 Eligibility and analysis.

genes using the R software (R 3.0.2). Overall survival (OS) was defined by the time from the beginning of surgery to death or the last follow-up date. Using the MAX STAT function package, the log-rank statistic was calculated based on the Conditional-Monte Carlo method. The optimal cut-off value for the predicted prognosis between the 10th and 90th is the binary value for each gene ($P<0.05$). Based on Univariate COX analysis, genes with P values of <0.05 were selected for the follow-up analysis.

Screening drug-related genes by the Subpopulation Treatment Effect Pattern Plot (STEPP)

In the NCBI GEO datasets, GSE42127 is the dataset related to ACT and prognosis, which was attached as the supplementary information in relevant research (PMID: 23357979) (21). Interactions between ACT and each screened gene were analyzed with STEPP methodology in GSE42127. STEPP is a graphical tool that helps researchers explore the heterogeneity of treatment effects based on the value of a consecutive baseline covariate in overlapping subpopulations (22). Briefly, STEPP uses a sliding-windows method to define several overlapping subpopulations of the patient based on a continuous covariate, such as gene expression, and plots the resulting treatment effects estimated within each subpopulation (23). STEPP analyses were performed using the R (http://cran.r-project.org/) software with Package "STEPP." Finally, the drug-related gene was screened according to the STEPP pattern and P value.

Statistical analysis

R statistical software (version 3.0.2) was used for statistical analyses. All statistical tests were two-tailed and P values less than 0.05 were considered statistically significant. *TMEM213* expression was a binary variable (low/high) for analysis. Other categorical variables included gender, T-stage, N-stage, and TNM stage. Age was a numeric variable. The relationships between *TMEM213* expression levels with categorical variables were examined using the Chi-squared test or Fisher's exact test. Welch's two independent sample *t*-test or nonparametric Mann-Whitney U test was used to compute P values in comparing continuous variables. The Kaplan-Meier (KM) survival curve was reported, and the time-to-event data were compared using the log-rank test. Univariate analysis analyzing OS and independent variables, including

Table 1 Characteristics of TCGA cohort

Characteristic	Number of patients (%)
Age (years)	
Median [range]	66 [33–84]
Gender	
Male	128 (46.9)
Female	145 (53.1)
Race	
White	198 (72.5)
Others	75 (27.5)
T stage	
T1–T2	249 (91.2)
T3–T4	24 (8.8)
N stage	
N0/Nx	188 (68.9)
N1/N2	85 (31.1)
TNM stage	
I	166 (60.8)
II/IIIA	107 (39.2)

TCGA, The Cancer Genome Atlas.

TMEM213 and other clinic-pathological parameters (CPPs) were conducted using the Cox proportional hazard (PH) regression model. Corresponding hazards ratios (HRs) and 95% confidence interval (CI) were reported. Multivariable Cox regression models were further conducted by adjusting for other CPPs (age, gender, T-stage, and N-stage). *TMEM213* was modeled as dichotomous according to the relative fit of multivariate models adjusted for the standard prognostic factors. The stepwise selection was conducted, and the final model was selected based on the Akaike information criterion (AIC). Internal validation was conducted using the bootstrapping method (1,000 replications). Corresponding estimate and 95% CI were also reported.

The external validation was conducted using the online Kaplan-Meier plotter database analysis (24). OS was estimated by median *TMEM213* expression for lung adenocarcinoma patients. All other parameters were set at default settings, except for the "treatment group." It was set for "only surgical margins negative" to replicate our current study cohort conditions best.

Enriched pathway analysis

Gene set enrichment analysis (GSEA) assay was performed to investigate the biological characteristics shared by different *TMEM213* expression levels. GSEA was performed using the software GSEA v2.2.2 (www.broadinstitute.org/software/gsea). The *TMEM213* expression level was annotated as high or low phenotype, and C2: curated gene setsCP: KEGG: KEGG gene sets from the Molecular Signatures Database (MSigDB) were utilized. All other parameters were set to default.

Results

Patient characteristics

Clinical information of 273 lung adenocarcinoma patients was obtained from publicly available TCGA database (*Table 1*). The median age was 66 (range, 33–84) years. Males accounted for 46.9% (n=128), females accounted for 53.1% (n=145), and 72.5% were white (n=198). T1/T2 and T3/T4 accounted for 91.2% (n=249) and 8.8% (n=24), respectively. N0/Nx and N1/N2 accounted for 68.9% (n=188) and 31.1% (n=85), respectively. Stage I and II/IIIA accounted for 60.8% (n=166) and 39.2% (n=107), respectively. The median survival duration was 26.4 months (range, 1.47–241.6 months).

TMEM213 is a prognostic gene that is associated with drug efficacy

Based on the TCGA database of 273 patients, 1,743 genes were screened out of 17,165 genes using Max stat package according to the following procedure. In the area between the 10th and 90th binary value, the selected cut point can obtain the optimal log-rank test (P<0.05). Then, based on the above 1,743 genes, univariate Cox analysis and KM curve (log-rank test) were used to screen out 297 genes with prognostic value.

For these 297 genes, STEPP analysis was performed in GSE42127 (*Table S1*), and its main clinical information is listed in *Table S2*. The results showed that in the TMEM family, as *TMEM213* expression increased, the OS rate at 3 years significantly increased in the ACT group compared with the non-ACT group (P=0.044). The difference in OS rate at 3 years was also statistically significant (P=0.044). Although the hazard ratio for OS did not achieve statistical significance, we did observe a better survival rate (P=0.137) (*Figure 2 A,B,C*).

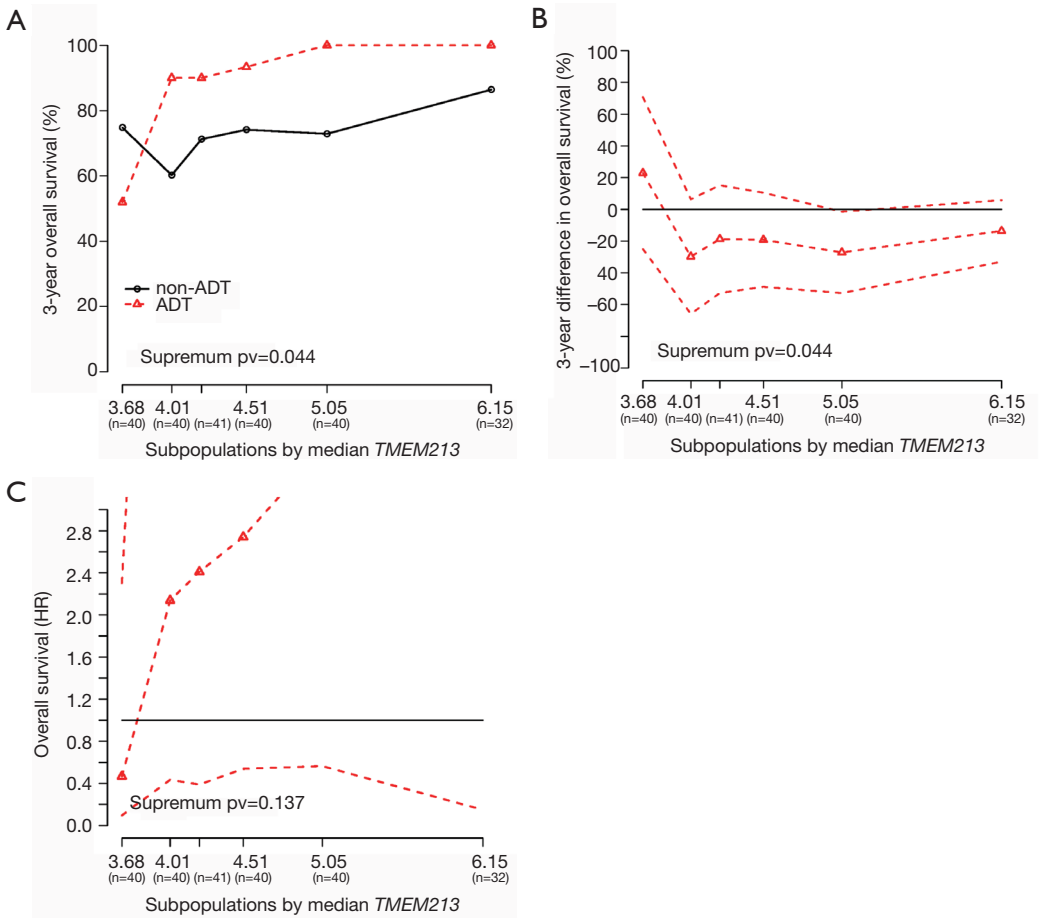

Figure 2 The effect of adjuvant paclitaxel-carboplatin on OS in GSE42127. (A) STEPP of 3-year OS (%) with ACT and non-ACT according to patients' subpopulations clustered by *TMEM213*; (B) STEPP of 3-year difference in OS (%) between patients with non-ACT and ACT according to patients' subpopulations clustered by *TMEM213*; (C) STEPP of OS hazard ratio between patients with non-ACT and ACT according to patients' subpopulations clustered by *TMEM213*. OS, overall survival; STEPP, Subpopulation Treatment Effect Pattern Plot; ACT, adjuvant chemotherapy.

Association of TMEM213 expression with clinicopathological variables

The correlation between clinical pathological parameters and *TMEM213* expression in the TCGA database is shown in *Table 2*. There was no significant difference among patients with different *TMEM213* expression levels while controlling for patients' age, size, lymph node involvement, and TNM stage. Nevertheless, *TMEM213* expression was statistically significantly associated with gender (P=0.013). High *TMEM213* expression was more commonly seen among females.

Influence of TMEM213 expression on survival and external validation

In order to assess the relationship between *TMEM213* expression and prognosis with lung adenocarcinoma in TCGA database, Kaplan-Meier analysis showed patients with increased *TMEM213* expression had longer OS (log-rank P=0.014, 0.027, and 0.000 for dichotomous, trichotomous and maximally selected log-rank modeling of *TMEM213* expression, respectively) (*Figures 3*,*S1*). Univariate analysis using Cox PH models showed that lower T stage, lower N stage, and higher *TMEM213*

Table 2 The correlation of *TMEM213* expression with the clinical pathological characteristics of TCGA-LUAD cohort

Characteristics	Low expression of *TMEM213*, n (%)	High expression of *TMEM213*, n (%)	P value
Age (years)			0.263
Median [range]	65 [38–83]	67 [33–84]	
Gender			0.013
Female	62 (45.6)	83 (60.6)	
Male	74 (54.4)	54 (39.4)	
Race			0.474
White	96 (70.6)	102 (74.5)	
Others	40 (29.4)	35 (25.5)	
T stage			0.193
T1–T2	121 (89.0)	128 (93.4)	
T3–T4	15 (11.0)	9 (6.6)	
N stage			0.725
N0/Nx	95 (69.9)	93 (67.9)	
N1/N2	41 (30.1)	44 (32.1)	
TNM stage			0.746
I	84 (61.8)	82 (59.9)	
II/IIIA	52 (38.2)	55 (40.1)	

TCGA-LUAD, The Cancer Genome Atlas Lung Adenocarcinoma.

expression significantly predicted prolonged OS (P=0.001, 0.001, and 0.015, respectively). In the multivariable Cox PH analysis, *TMEM213* expression [hazard ratio (HR) =0.623, P=0.020], T stage (HR =2.936, P=0.001), and N stage (HR =2.332, P=0.001) were associated with prolonged OS, and this prognostic model for lung adenocarcinoma was further validated using 1,000 bootstrapping replications (*Table 3*).

For external validation, patients with high expression of *TMEM213* showed significantly longer OS than those with low expression level (log-rank P=0.045) in the online Kaplan-Meier plotter database (*Figure 4*).

GSEA analysis for TMEM213 expression in lung adenocarcinoma

To investigate the possible function and mechanism of *TMEM213* in lung adenocarcinoma, the enriched pathway was analyzed (*Figure 5*). High-throughput RNA sequencing data from the lung adenocarcinoma group in TCGA and GSE42127 were used for GSEA. The results showed that the expression of *TMEM213* was related to the metabolic pathway of P450, ABC transporter, butyric acid, arachidonic acid, tryptophan, fatty acid, histidine, and bile acid synthesis and other metabolic pathways (*Tables 4*,*S3*).

Discussion

Several studies have shown that prognostic gene signatures can also be predictive of benefit from ACT, but few of them tested cohorts using adjuvant paclitaxel-carboplatin treatments (21,25,26). The NATCH trial failed to demonstrate patients who underwent resection could benefit from adjuvant paclitaxel-carboplatin. This may well be due to the fact that most patients in this study are stage I disease who are not known to benefit from chemotherapy (7). The Cancer and Leukemia Group B 9633 trial, comparing adjuvant paclitaxel-carboplatin with observation in patients with stage IB disease, was not powered to address improvement in OS with chemotherapy. Nonetheless, it could be considered as a treatment option in selected stage IB patients who had tumors ≥4.0 cm in diameter (27). In this study, we tried to identify a possible

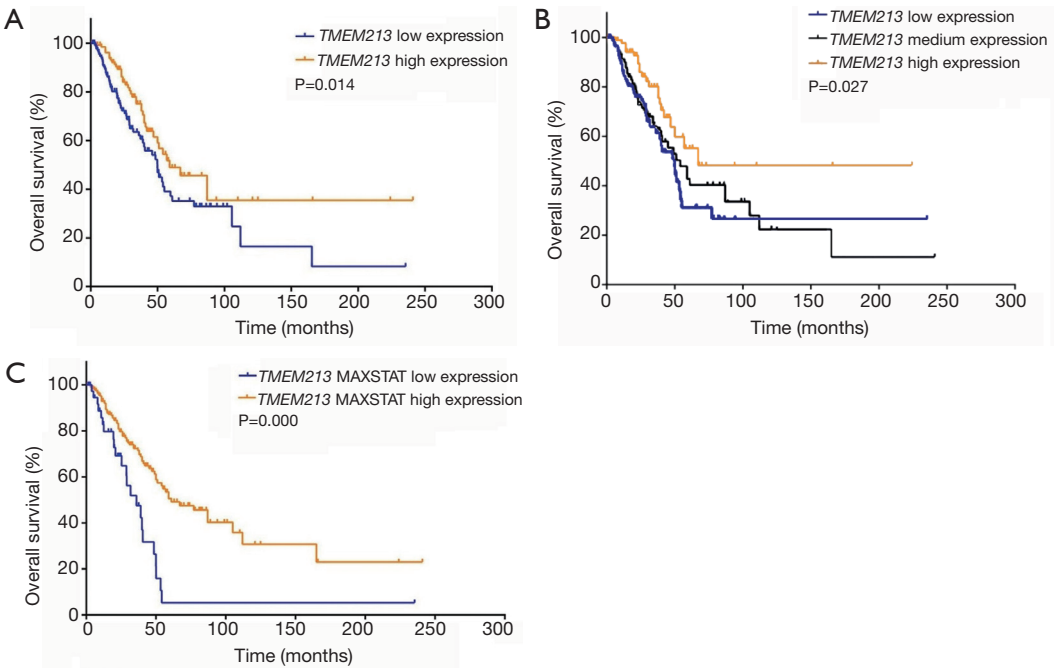

Figure 3 Kaplan-Meier survival curves between the expression of *TMEM213* and the overall survival time of patients with lung adenocarcinoma after surgery. The X-axis represents the survival time (months) and the Y-axis represents cumulative survival rate [Log-Rank P=0.014, 0.027 and 0.000 for dichotomous (A), trichotomous (B) and maximally selected Log-Rank modeling (C) of *TMEM213* expression, respectively].

Table 3 Univariate and multivariate analysis of overall survival in TCGA by Cox regression model and 1,000 bootstrapping

Characteristics	No.		Univariant analysis			Multivariant analysis			
	Patients	Events	HR	95% CI	P value	HR	95% CI	P value	Bootstrapping 95% CI
Age (years)	273	101	1.007	0.987–1.027	0.507	–	–	–	–
Gender			1.098	0.742–1.624	0.639				
Female	145	51							
Male	128	50							
T stage			3.134	1.740–5.618	0.001	2.936	1.631–5.287	0.001	1.659–6.801
T1–T2	249	87							
T3–T4	24	14							
N stage			2.419	1.634–3.580	0.001	2.332	1.574–3.456	0.001	1.489–4.730
N0/Nx	188	52							
N1/N2	85	49							
TMEM213 expression			0.610	0.410–0.908	0.015	0.623	0.418–0.928	0.020	0.278–0.845
Low	136	60							
High	137	41							

TCGA, The Cancer Genome Atlas; HR, hazard ratio.

target in the TMEM family and elucidate its potential as a prognostic biomarker for the benefit from carboplatin plus taxane-based ACT in resected lung adenocarcinoma patients.

Bioinformatics analysis is a well-established method which utilizes online expression profile datasets and has been widely used to help researchers identify potential biomarkers. We analyzed the expression profile of lung adenocarcinoma in TCGA and screened a microarray dataset of GSE42127 by STEPP. We found that *TMEM213* expression was significantly correlated with gender, and survival analysis showed that patients with high *TMEM213* expression had significantly better OS than those with low *TMEM213* expression. Univariate and multivariate Cox analysis indicated that *TMEM213* was an independent predictor for OS improvement. The STEPP approach was developed to allow researchers to investigate the heterogeneity of treatment effects on survival outcomes across values of a (continuously measured) covariate, such as *TMEM213* expression (22). Our study showed that with the increase of *TMEM213* expression, the treatment group could benefit from adjuvant paclitaxel-carboplatin, and, as the value of gene expression increased, so did the survival benefit.

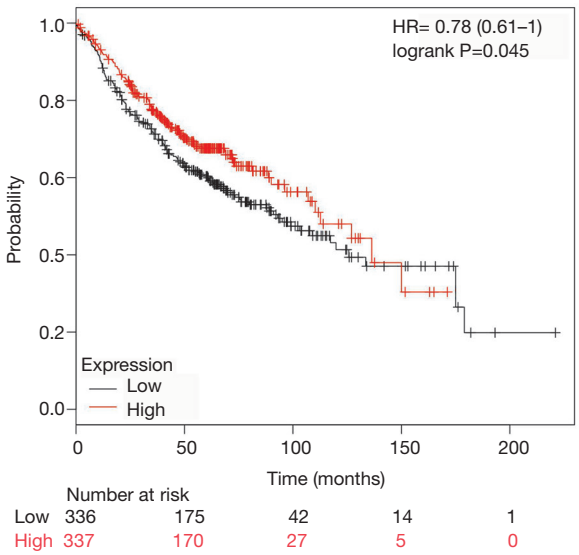

Figure 4 Kaplan-Meier Plotter database of *TMEM213* expression. Patients with high expression of *TMEM1213* showed significant longer overall survival than those with low expression.

Our finding was reliable and generalizable for the following reasons. First, the cutoff value of *TMEM213* expression, whether it was selected by the median method, the trisection method, or the most selective log-rank statistical analysis method, all showed that the patients with high expression had a good prognosis. Second, our Cox regression model was robust, and our findings were further

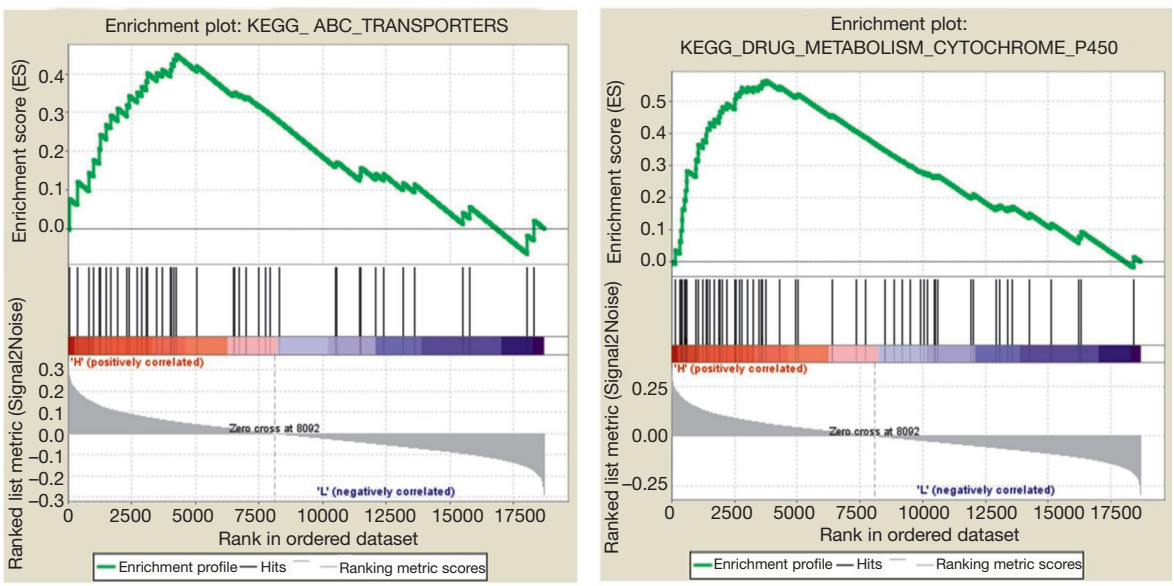

Figure 5 Enriched pathway analyzes *TMEM213* expression. The GSEA results showing the correlation of *TMEM213* levels and lung adenocarcinoma related gene sets in MSigDB. GSEA, Gene Set Enrichment Analysis; MSigDB, Molecular Signatures Database.

Table 4 *TMEM213* enrich pathways

Name	ES	NES	NOM P value	FDR P value
KEGG_BUTANOATE_METABOLISM	0.624	2.002	0.004	0.046
KEGG_ARACHIDONIC_ACID_METABOLISM	0.520	1.920	0.002	0.063
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	0.564	1.834	0.002	0.068
KEGG_HISTIDINE_METABOLISM	0.578	1.836	0.008	0.082
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	0.558	1.794	0.010	0.083
KEGG_FATTY_ACID_METABOLISM	0.579	1.863	0.004	0.084
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	0.552	1.691	0.028	0.149
KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS	0.586	1.696	0.008	0.163
KEGG_TRYPTOPHAN_METABOLISM	0.477	1.625	0.014	0.209
KEGG_ABC_TRANSPORTERS	0.449	1.589	0.023	0.237

KEGG, Kyoto Encyclopedia of Genes and Genomes; ES, enrichment score; NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate.

confirmed by 1,000 internal bootstrap replications and external validation using an online high-throughput dataset.

Our findings have value for clinical application. We found that the prognostic value of *TMEM213* signature was independent of T and N stage in the stepwise Cox regression. Presently, the TNM stage has been viewed as an important factor for predicting survival, but clinically, we can observe that patients with the same TNM stage might have a different prognosis. This highlights the importance of identifying more personalized biomarkers for a more precise survival prediction of patients with lung adenocarcinoma. Additionally, most current molecular signatures for lung cancer are prognostic only and do not provide any estimation as to whether a patient would benefit from ACT. STEPP results suggest that the expression of *TMEM213* can guide our future selection and predictive efficacy of postoperative ACT in patients with radical lung adenocarcinoma. However, this trend still needs further validation in perspective, well-balanced studies with large sample size.

The characteristics of *TMEM213* involving lung adenocarcinoma have not been reported in any functional studies. We speculate that it might be related to the growth of lung cancer. Alveolar type II cells can secrete surfactant, play an important role in ion transport, and contribute to the regulation of alveolar fluid and epithelial repair after lung injury (28). Lin found that lung adenocarcinoma can initiate in alveolar type II cells (29). One study found that *TMEM213* expression was significantly upregulated in alveolar type II cells under a hypoxic environment induced by submerged condition (30). Yu found that long-term exposure to hypoxia repressed tumor progression of the lung cancer from A549 cells (31), and thus presumed that the high expression of *TMEM213* gene may have the effect of inhibiting lung cancer. In addition, it is generally believed that *TMEM213* is located in the endoplasmic reticulum (the reliability is 44.4%), and it has an endoplasmic relocation signal (9,32). Endoplasmic reticulum stress has a significant effect on the proliferation and growth of almost all types of cancer, including lung cancer (32). Hung found that dehydrocostuslactone (DHE) caused the apoptosis of NSCLC cells via the stress response of the endoplasmic reticulum (33). Li indicated that chaetocin induced the stress response signal of the endoplasmic reticulum and led to death receptor-5 dependent apoptosis in NSCLC (34). Thus, it is important to investigate the functional roles of *TMEM213*.

Enriched pathway analysis revealed the high *TMEM213*-expression-enriched gene signature of "DRUG_METABOLISM_CYTOCHROME_P450" and "ABC_TRANSPORTERS" from the KEGG pathway database. Both gene sets were associated with clinical drug resistance of paclitaxel. Paclitaxel is a potent anti-cancer agent that binds to β-tubulin and prevents mitosis

through microtubule overstabilization. It is an effective chemotherapeutic agent to treat ovarian, breast, gastric, and lung cancers, and the response rate of paclitaxel-based chemotherapy for lung cancer is 30–40% (35). The P450 enzymes in tumor cells may inactivate the anticancer drug, and the overexpression of the ABC transporters may encode the efflux pump, which leads the drug to be discharged, thereby hindering the retention of the drug in the cell, which can have a negative effect on chemotherapeutic-mediated tumor cell death. Recent studies *in vitro* have shown that paclitaxel treatment increases the level of P450 in human hepatocytes as well as ABC transporters in colon tumor cells, and thus may influence their own metabolism and elimination (36). It has also been reported that a weekly paclitaxel chemotherapy regimen can induce P450 enzyme and ABC protein expression. This can maintain the plasma drug concentration of paclitaxel targeting the proliferating endothelial cells within the tumor as opposed to neoplastic cells themselves so that the role of paclitaxel chemotherapy is more effective (37). We found that high expression of *TMEM213* was positively correlated with P450 pathway and ABC transporter pathway; these might lead to pharmacokinetically relevant changes in drug metabolism and elimination such that a modification in the concentration of paclitaxel might be beneficial.

Several limitations to this study needed to be noted. First, this study analyzed the online data of lung adenocarcinoma on TCGA and GEO but did not use clinical samples to validate. Second, our study did not investigate the mechanism behind the predictive and prognostic value of *TMEM213* in lung adenocarcinoma. Experimental studies on cancer cell lines and xenograft models will provide more information to understand its functional role better. Third, our conclusions need to be validated by prospective controlled trials with large sample sizes guided by a priori conducted power analysis.

Conclusions

In summary, this study provided the first evidence that *TMEM213* presence in lung adenocarcinoma is related to gender. High expression of *TMEM213* suggests a good prognosis. Survival analysis with online data supports the results above. Furthermore, we found that high *TMEM213* expression is more likely to benefit from adjuvant paclitaxel-carboplatin. Taken together, *TMEM213* may serve as a potential candidate biomarker and therapeutic target for lung adenocarcinoma. Future studies will focus on the verification of our findings in clinical practices and the functional roles of this innovative signature.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

Table S1 STEPP analysis P values for 247 genes

Genes	P-val	HRp-val
CHRNA1	0.001	0.01
FAM177B	0.004	0.014
PTPRR	0.008	0.009
LGR5	0.012	0.139
CDT1	0.014	0.203
RHOJ	0.016	0.176
CLN6	0.018	0.046
FOXF2	0.021	0.27
IP6K1	0.023	0.039
UBE2G1	0.026	0.462
IL27RA	0.041	0.075
CCT6A	0.043	0.131
TMEM213	0.044	0.137
CENPK	0.056	0.034
NKRAS1	0.071	0.105
C17orf53	0.076	0.349
ZDHHC21	0.079	0.284
FCRLA	0.08	0.344
ZNF14	0.089	0.169
SNCAIP	0.09	0.297
KIAA0226L	0.092	0.225
CYP3P1	0.101	0.183
TMS5F1	0.101	0.411
INSIG1	0.104	0.096
RPN2	0.104	0.16
CKAP2L	0.11	0.102
PEG10	0.116	0.043
CLCN7	0.118	0.325
FAM54A	0.128	0.534
FOXJ3	0.143	0.27
YPEL1	0.144	0.282
TFDP2	0.154	0.419
FYTTD1	0.158	0.201
AK4	0.16	0.198
TDO2	0.16	0.59
VNN1	0.16	0.386
GUCY1A3	0.166	0.14
CSGALNACT1	0.169	0.364
ZNF486	0.199	0.129
CDCA3	0.222	0.506
RFX5	0.23	0.327
GBE1	0.231	0.276
ITGA4	0.233	0.109
EIF4G1	0.237	0.632
TMC5	0.242	0.484
LRRC46	0.246	0.3
NLRC3	0.247	0.565
ZWINT	0.252	0.122
ABCF3	0.264	0.067
SH3BP5	0.269	0.375
SENP6	0.271	0.647
CX8	0.274	0.486
UBE2V2	0.285	0.864
ZNF429	0.291	0.457
RXRB	0.297	0.287
TPST2	0.301	0.324
ST3GAL2	0.306	0.165
SLC4A5	0.307	0.049
BIRC5	0.31	0.616
MTHFD1	0.313	0.151
FANCD2	0.318	0.31
IL24	0.318	0.298
FBLN5	0.319	0.245
CTNND2	0.328	0.637
FBXL16	0.329	0.124
NGEF	0.329	0.814
PARP15	0.329	0.365
ZNF441	0.336	0.168
STAP1	0.338	0.322
MGARP	0.347	0.698
DENND4C	0.353	0.928
NCAPH	0.357	0.293
ADK	0.358	0.467
UBQLN1	0.364	0.439
SLCO4A1	0.366	0.553
LPL	0.377	0.485
ZNF28	0.378	0.355
CCR7	0.381	0.896
KHDRBS2	0.381	0.789
CD3E	0.393	0.338
AKT1S1	0.407	0.8
FAM111B	0.411	0.373
FAM83D	0.412	0.331
METTL21A	0.412	0.838
ADGRF4	0.417	0.306
SYNDIG1L	0.424	0.705
UNC5CL	0.428	NA
ARL14	0.431	0.419
TPSNP1	0.432	0.531
SPAG1	0.433	0.775
REV3L	0.436	0.359
RWDD3	0.436	0.217
ZNF294P	0.441	0.11
ZNF253	0.442	0.58
KIF23	0.443	0.745
DLAT	0.444	0.332
SMARCD3	0.444	0.176
CMTM7	0.445	0.794
ASAH1	0.447	0.416
CENPM	0.45	0.233
PSTPIP2	0.453	0.65
AP3B1	0.464	0.68
RCCD1	0.464	0.429
CCM2	0.468	0.522
SPTB	0.47	0.58
FOSL1	0.472	0.677
RFXAP	0.472	0.467
IRX6	0.476	0.872
APTG1	0.485	0.562
MMD	0.487	0.114
SMC2	0.489	0.286
JAM2	0.492	0.159
KLRG2	0.506	0.571
B3GALT2	0.507	0.649
CISD2	0.514	0.869
CEP55	0.519	0.837
ORC6	0.525	0.924
SPRR2D	0.529	0.675
B7H2A1	0.533	0.741
MAGEH1	0.533	0.328
SUSD3	0.533	0.641
C3orf21	0.534	0.229
LDLRAD3	0.537	0.207
LOA5	0.538	0.825
TMEM231	0.542	0.183
CYSLTR1	0.543	0.793
DOK1	0.546	0.477
ATXN1L	0.56	0.421
CCDC184	0.56	0.115
TSNARE1	0.575	0.149
TTC33	0.582	0.204
C1orf132	0.584	0.471
CIT	0.585	0.809
CYP2A6	0.585	0.109
KIFC1	0.585	0.296
SPRR2E	0.588	0.234
GYG1	0.595	0.625
KIF6A	0.597	0.921
MRPL46	0.608	0.325
DDIAS	0.612	0.606
FRZB	0.612	0.439
ESRP2	0.62	0.187
FBXO45	0.62	0.872
CDH26	0.621	0.573
CIDEC	0.622	0.542
CREG2	0.623	0.661
INSL4	0.627	0.634
CERKL	0.629	0.537
ANXA10	0.633	0.544
ST6GALNAC6	0.638	0.643
BRSB9	0.652	0.613
ESPL1	0.653	0.589
HEY2	0.658	0.591
L6/A4	0.658	0.796
CDCA2	0.659	0.826
MNDA	0.659	0.747
CAMK1D	0.663	0.283
FBLN1	0.666	0.205
CYP20A1	0.671	0.923
IRX5	0.671	0.414
MRPL51	0.672	0.287
AXIN2	0.673	0.194
FAM150A	0.673	0.756
PDIGF1	0.676	0.519
AVEN	0.681	0.167
HJURP	0.681	0.961
LCK	0.681	0.788
MDAP1	0.681	0.514
IL16	0.687	0.368
MCM5	0.687	0.32
KIF2C	0.695	0.381
PKP2	0.699	0.914
FAM204A	0.7	0.164
PEX26	0.704	0.969
GRIA4	0.706	0.245
E2F7	0.711	0.15
MAL	0.715	0.494
CLIP4	0.724	0.538
ECE2	0.729	0.881
GJB2	0.729	0.845
TRMT10B	0.733	0.453
PTCHD4	0.737	0.547
TMEM252	0.739	0.729
CAPZA1	0.756	0.43
TROAP	0.76	0.881
CD36	0.764	0.342
NLRP1	0.766	0.714
MEI1	0.767	0.404
KCNK17	0.773	0.889
NAA50	0.781	0.557
ARMC9	0.783	0.27
MED23	0.785	0.91
TNFRSF13B	0.795	0.511
B7H3A3	0.799	0.832
CDCA8	0.799	0.597
FAM117A	0.81	0.231
C16orf59	0.812	0.508
MELK	0.812	0.517
NAPSA	0.815	0.394
1-Sep	0.819	0.867
HMMR	0.821	0.685
PAOX	0.821	0.843
PRM1K	0.821	0.75
KIAA1524	0.828	0.462
GTPBP8	0.833	0.906
CENPF	0.848	0.246
FAM184A	0.848	0.767
CRISP3	0.852	0.123
REM1	0.861	0.769
CRO7	0.863	0.759
BLZF1	0.864	0.989
KIF20A	0.869	0.838
CD28	0.874	0.559
FYN	0.874	0.256
CYP4Z2P	0.877	0.905
ERO1A	0.878	0.89
ICAM3	0.881	0.296
TK1	0.882	0.443
STEAP2	0.893	0.852
PLPPR1	0.899	0.657
PLEK2	0.907	0.577
ATP8B3	0.909	0.425
LDLR	0.917	0.961
AHNAK2	0.925	0.182
GGTLC1	0.932	0.759
LPK	0.932	0.687
CROCC	0.941	0.299
BACE1	0.942	0.928
ZNF682	0.945	0.911
ZKSCAN4	0.949	0.656
KIF14	0.95	0.802
RHPN2	0.951	0.92
ZNF43	0.951	0.95
C12orf76	0.954	0.933
GMAP8	0.955	0.951
ZNF610	0.958	0.732
FAM21C	0.961	0.913
DHFR	0.962	0.793
MTF1	0.962	0.656
KIAA0922	0.966	0.608
SLC4A8	0.97	0.912
MYO16	0.972	0.983
TMSF19	0.978	0.895
SMAD9	0.987	0.77
HELLS	0.995	0.477
ADGRG6	0.996	0.691
UCK2	0.997	0.974

STEPP: Subpopulation Treatment Effect Pattern Plot; NA, not available.

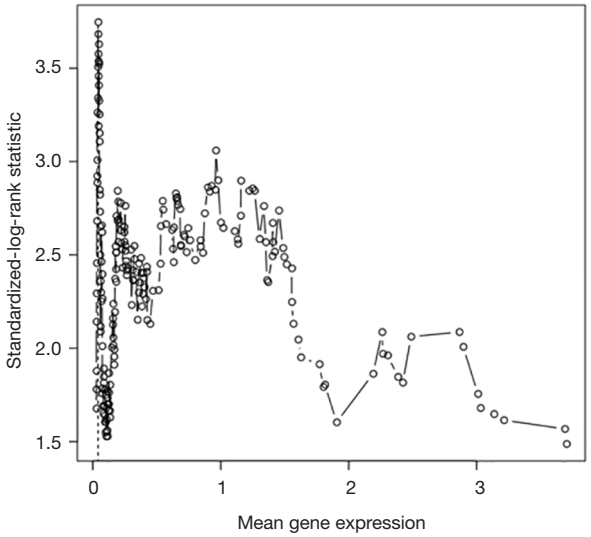

Figure S1 The MAXSTAT method selects the cut point of *TMEM213* expression.

Table S2 Characteristics of GSE42127 cohort

Characteristics	AdjuChemo, No. (%)	Non-AdjuChemo, No. (%)
Age (years)		
Median (range)	64.2 (42.3–82.1)	66.4 (45.8–86.2)
Gender		
Female	16 (41.0)	49 (52.1)
Male	23 (59.0)	45 (47.9)
TNM stage		
I	22 (56.4)	67 (71.3)
II	6 (15.4)	16 (17.0)
≥ IIIA	11 (28.2)	11 (11.7)

GEO, Gene Expression Omnibus; GSE, GEO series; AdjuChemo, adjuvant chemotherapy.

Table S3 *TMEM213* enrich pathways

Name	ES	NES	NOM P value	FDR P value
KEGG_BUTANOATE_METABOLISM	0.473	1.495	0.048	0.416
KEGG_ARACHIDONIC_ACID_METABOLISM	0.498	1.415	0.041	0.555
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	0.443	1.197	0.234	0.536
KEGG_HISTIDINE_METABOLISM	0.571	1.605	0.012	0.409
KEGG_FATTY_ACID_METABOLISM	0.571	1.762	0.016	0.366
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	0.565	1.733	0.018	0.246
KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS	0.582	1.440	0.068	0.530
KEGG_ABC_TRANSPORTERS	0.337	1.047	0.365	0.681
KEGG_ALPHA_LINOLENIC_ACID_METABOLISM	0.689	1.585	0.008	0.390
KEGG_PROPANOATE_METABOLISM	0.541	1.724	0.010	0.180
KEGG_TIGHT_JUNCTION	0.366	1.564	0.006	0.386

KEGG, Kyoto Encyclopedia of Genes and Genomes; ES, enrichment score; NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate.