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Varied clinical significance of ATP-binding cassette C sub-family members for lung adenocarcinoma

Linbo Zhang, Ping Huang, Chunxia Huang, Lingmei Jiang, Zhijie Lu, Peng Wang*

Abstract

Lung adenocarcinoma (LUAD) is a lethal malignancy worldwide and a major public health concern. We explored the potential clinical significance for LUAD of ATP-binding cassette (ABC), sub-family C, consisting of ABCC1–6, 8–12, and cystic fibrosis transmembrane conductance regulator (CFTR).

Five hundred LUAD patients from The Cancer Genome Atlas database were used for analysis, including differential expression and diagnostic and prognostic significance. Oncomine and MERAV databases were used to validate differential expression and diagnostic significance. A risk score model was constructed using prognosis-related ABCC members. Prognosis-related genes were further explored to correlate their expression with tumor stage progression. Interaction networks, including biological processes and metabolic pathways, were constructed using Cytoscape software and STRING website.

ABCC1–3 consistently showed high expression in tumor tissues (all $P \leq 0.05$). Most datasets indicated that ABCC5, 10, and 11 were highly expressed in tumor tissues whereas ABCC6, 9, and CFTR were highly expressed in nontumor tissues (all $P \leq 0.05$). Diagnostic significance of ABCC3 and ABCC5 was consistently assessed and validated in three datasets (all area under the curve $>$ 0.700) whereas ABCC6, 8, 10, 11, and CFTR were assessed in The Cancer Genome Atlas dataset and validated in one dataset (all area under the curve $>$ 0.700). Prognostic analysis indicated that ABCC2, 6, and 8 mRNA expression was associated with survival of LUAD (all adjusted $P \leq .037$). The risk score model constructed using ABCC2, 6, and 8 suggested prognostic significance for survival predictions. ABCC2 expression was associated with tumor stage, whereas ABCC6 and 8 were not. Interaction networks indicated that they were involved in establishment of localization, ion transport, plasma membrane, apical plasma membrane, adenylyl nucleotide binding, ABC transporters, ABC transporter disorders, ABC-family-protein-mediated transport, and bile secretion.

Differentially expressed ABCC2 and ABCC5 might be diagnostic whereas ABCC2, 6, and 8 may be prognostic biomarkers for LUAD, possibly through ABC-family-mediated transporter disorders.

Abbreviations: ABCC = ATP-binding cassette C, AUC = area under the curve, CFTR = cystic fibrosis transmembrane conductance regulator, LUAD = lung adenocarcinoma, NSCLC = nonsmall cell lung cancer, ROC = receiver operating characteristic, TCGA = The Cancer Genome Atlas.

Keywords: ATP-binding cassette C sub-family, biomarker, diagnosis, lung adenocarcinoma, prognosis

1. Introduction

It is estimated by the World Health Organization that there are 1.69 million deaths from lung cancer each year worldwide, which is significantly more than for other types of cancer.[1] Nonsmall cell lung cancer (NSCLC), including 2 major subtypes: lung adenocarcinoma (LUAD), the most common type of lung cancer, and lung squamous cell carcinoma, comprise >85% of all lung cancers.[1–3] LUAD and lung squamous cell carcinoma originate from distinct cells and show distinct molecular traits, indicating different biological and pathological characteristics, which induces distinct therapeutic strategies.[1] Lung cancer is a malignant disease with heterogeneous characteristics at both molecular and histological levels and metastasis is always shown in the diagnosis process, which lead to the unsatisfactory survival rate.[4] Even though there are new treatments, such as targeted chemotherapy or immunological therapy, for LUAD, its prognosis is still poor.[5] Thus, further explorations to determine potential diagnostic and prognostic molecular biomarkers for lung cancer, including LUAD, are important.

The ATP-binding cassette (ABC) transporter superfamily is one of the largest transporter gene families and is made up of membrane proteins that translocate a variety of substrates across both extra- and intra-cellular membranes.[6] Genetic variation in

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this family of genes can cause or contribute to a number of human disorders with Mendelian and complex inheritance.[6] This superfamily contains 8 subfamilies: ABCA–H.[7] The ABCC subfamily consists of 12 members: ABCC1–6, cystic fibrosis transmembrane conductance regulator (CFTR), and ABCC8–12,[6] and these members are reported to be involved in transporting drugs, ions, toxins, and endogenous compounds.[8] ABCC1 is the first cloned drug-transporting ABCC protein, and is highly expressed in doxorubicin-selected multidrug resistant LUAD cell line H69AR.[9,10] Functional experiments concerning ABCC3 using an ABCC3$^{-/-}$ mouse model have suggested that ABCC3 participates in the hepatic sinusoidal excretion of glucuronidated compounds.[11,12] ABCC4 participates in the removal of many cell endogenous and xenobiotic organic anionic compounds with potential function in the extracellular signaling pathway.[13] ABCC5, like ABCC4, is a cyclic nucleotide organic anion transporter regulating the efflux of many substances, such as certain monophosphate nucleotide metabolites and purine analogs.[14] ABCC6-dependent molecular mechanisms can influence the circulation or formation of NTPs, which is supported by pyrophosphate, a factor preventing pseduxanthoma elasticum.[15]

CFTR uses this enzymatic activity to control ATP occupancy rather than actively transport substrates.[16] ABCC10 expression has been detected in NSCLC cells after exposure to paclitaxel.[17] ABCC12, may arise from a gene duplication event and probably play a role in the male meiotic prophase, spermatid development or sperm function.[18,19] To date, there has been little exploration of the potential association between mRNA expression of ABCC subfamily members and tumor characteristics. Therefore, we conducted the present study to explore the potential relationships between ABCC family members and LUAD.

2. Materials and methods

2.1. The mRNA expression analysis

The mRNA expression of ABCC family members in normal lung tissue and primary lung tissue was downloaded from MERAV (http://merav.wi.mit.edu/).[20] The LUAD dataset in The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/) was downloaded and used for the present analysis. In addition, datasets of Landi Lung[21] in the Oncomine database were used to validated mRNA expression of ABCC family members in lung.

2.2. Analysis of diagnostic and prognostic significance

Diagnostic significance was evaluated using mRNA expression in tumor and nontumor tissues. Area under the curve (AUC) $\geq$0.7 and $P \leq .05$ were considered to have diagnostic significance. The TCGA dataset was used to evaluate the diagnostic significance of ABCC family members. Moreover, seven datasets in Oncomine database were used to validate the diagnostic significance of ABCC family members as well.

Prognostic significance of ABCC family members was evaluated in the TCGA database. ABCC expression was divided into low and high expression at median expression cut off. Univariate and multivariate Cox proportional hazard regression models were used to evaluate the P values and hazard ratio of ABCC family members in LUAD. Prognosis-related clinical factors were adjusted in the multivariate Cox proportional regression model, inducing adjusted P values. Prognosis-related genes, adjusted $P \leq .05$, were further combined for joint-effect analysis.

2.3. Risk score model construction and disease progression analysis

Prognosis-related genes were used to construct a risk score model using the following formula: risk score = $\beta_1 \times \text{expression}_1 + \beta_2 \times \text{expression}_2 + \ldots + \beta_n \times \text{expression}_n$.[22–24] β originated from the multivariate Cox regression model. Risk scores, survival status, gene expression, Kaplan-Meier plot and time-dependent receiver operating characteristic (ROC) curves were recruited in the risk score model. In addition, prognosis-related genes were used to explore the relationships between gene expression and disease progression.

2.4. Interaction networks analysis

A coexpression network was constructed and used to evaluate correlations among all the ABCC members. A gene–gene interaction network was constructed using geneMANIA plugin in Cytoscape software.[25,26] A protein–protein interaction network was constructed using the STRING website (https://string-db.org/).[27] Moreover, candidate mechanism-related networks including biological processes, cellular components, molecular functions and metabolic pathways were constructed using BinGO and CluoGO plugins in Cytoscape software.[28,29]

2.5. Statistical analysis

Diagnostic ROC curves, Kaplan-Meier plots, and scatter plots were drawn using Graphpad software version 7.0. Survival analysis was performed using the Kaplan-Meier method and Cox proportional regression model using SPSS version 24.0. Hazard ratio and 95% confidence interval were included in the survival plots. $P \leq .05$ was considered statistically significant.

3. Results

3.1. The mRNA expression and demographic characteristic analysis

The mRNA expression of ABCC family members indicated that ABCC1–4, CFTR, and ABCC9 showed high expression in primary lung tumors (Fig. 1A–D, G, I) whereas other members showed high expression in normal lung tissues (Fig. 1E, F, H, J–L). In addition, demographic data showed that 500 LUAD patients were recruited in the dataset, which contained 270 males and 230 females (21 patients data missing); 268 persons with stage I, 119 with stage II, 80 with stage III and 25 with stage IV LUAD (8 patients data missing, Table 1). Tumor stage was associated with overall survival ($P < .0001$, Table 1) whereas age and sex were not (both $P > .05$, Table 1). The mRNA expression suggested that ABCC1–3, 5, and 10–12 showed high expression in tumor tissues (Fig. 2A–C, E, J–L) whereas ABCC4, 6, 8, and 9 and CFTR showed high expression in normal tissues (Fig. 2D, F–I).

3.2. Diagnostic significance assessment and Oncomine database validation

Diagnostic significance was evaluated using the TCGA database via ROC curves. ROC curves suggested that ABCC3, 5, 6, and

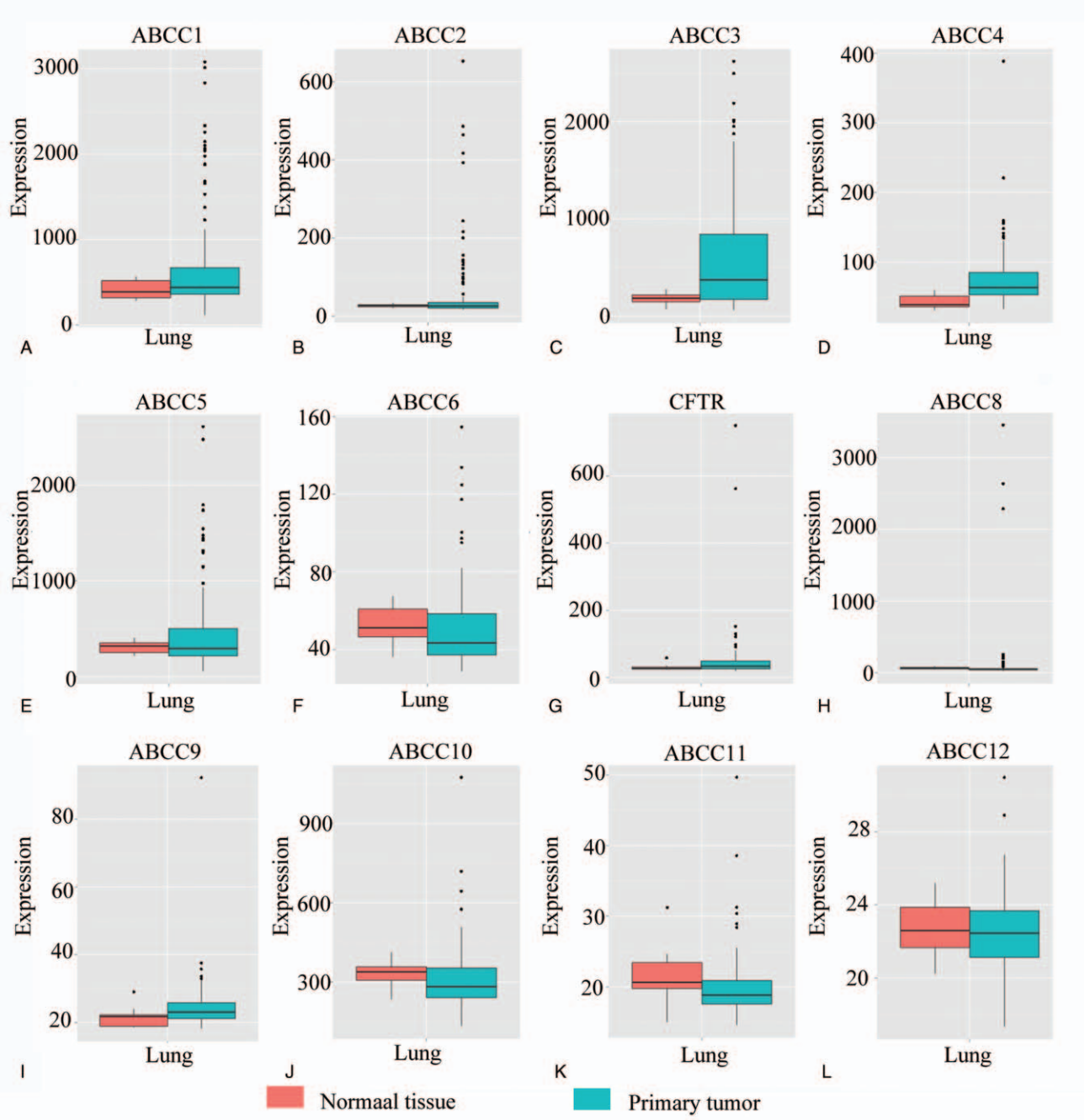

Figure 1. Expression of ABCC subfamily in lung tumor and normal lung tissues. A–L: expression of ABCC1–6, cystic fibrosis transmembrane conductance regulator, and ABCC8–12 in lung tumor and normal lung tissues, respectively. ABCC = ATP-binding cassette C.

8–11 and CFTR had potential diagnostic significance for LUAD (AUC = 0.902, 0.714, 0.780, 0.756, 0.883, 0.846 and 0.852, respectively, all $<.001$, Fig. 3C, E–K) whereas other members did not (Fig. 3A, B, L). The Oncomine database validated mRNA expression and diagnostic significance. Each member was validated using 2 other datasets (Figs. 4 and 13). Differential expression indicated that ABCC3, 5, 6, 8, 10, and 11 showed high expression in LUAD (Fig. 4A–C, E, G, H), whereas CFTR and ABCC9 showed low expression in LUAD (Fig. 4D, F). Moreover, other members showed diagnostic significance for LUAD (all AUC > 0.700, $P \leq .009$, Fig. 4I–M, O, P) but ABCC9 did not (AUC = 0.669, $P = .029$, Fig. 4N). Another dataset indicated that ABCC3, 5, and 9–11 showed high expression in LUAD (Fig. 13 A, B, F–H) whereas ABCC6, 8, and CFTR showed low expression (Fig. 13C–E). Only ABCC3 and ABCC5 showed diagnostic significance (AUC = 0.955 and 0.732, respectively, Fig. 13I–J) whereas others did not (all AUC < 0.700, Fig. 13K–P).

3.3. Survival analysis

Univariate Cox and Kaplan-Meier analysis found that ABCC2, 6, 8, 11, and 12 showed prognostic significance (all $P < .05$, Table 2,

Table 1

Demographic and clinicopathologic characteristics of lung adenocarcinoma patients.

Variables	Patients (N = 500)	Overall survival			
		No. of event	MST (month)	HR (95%CI)	Log-rank *P* value
Gender					
Male	270	96	1454	Ref.	.754
Female	230	86	1528	1.048 (0.783–1.403)	
Age (yr)*					
<65	215	74	1499	Ref.	.386
≥65	264	99	1454	1.143 (0.845–1.546)	
Tumor stage†					
Stage I	268	65	2620	Ref.	**<.0001**
Stage II	119	54	1209	**2.473 (1.719–3.559)**	**<.0001**
Stage III	80	45	879	**3.495 (2.383–5.126)**	**<.0001**
Stage IV	25	16	826	**3.819 (2.201–6.629)**	**<.0001**

95%CI = 95% confidence interval, HR = hazard ratio, MST = median survival time, Ref = reference.

* Twenty-one data were missing.

† Eight data were missing.

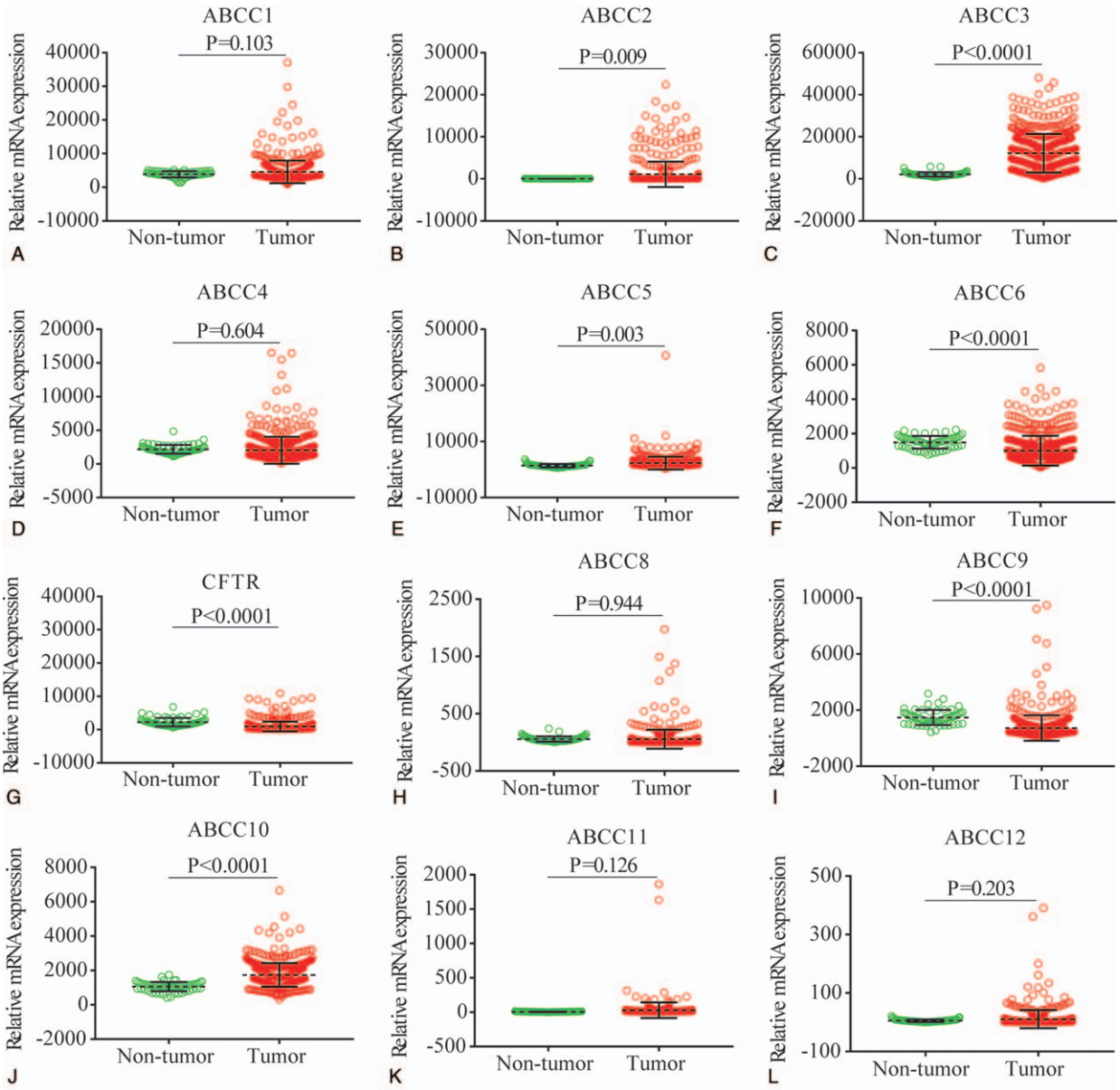

Figure 2. The mRNA expression of ABCC subfamily in lung adenocarcinoma and nontumor tissues in The Cancer Genome Atlas dataset. A–L: expression of ABCC1–6, cystic fibrosis transmembrane conductance regulator, and ABCC8–12 in lung adenocarcinoma and non-tumor tissues, respectively. ABCC = ATP-binding cassette C.

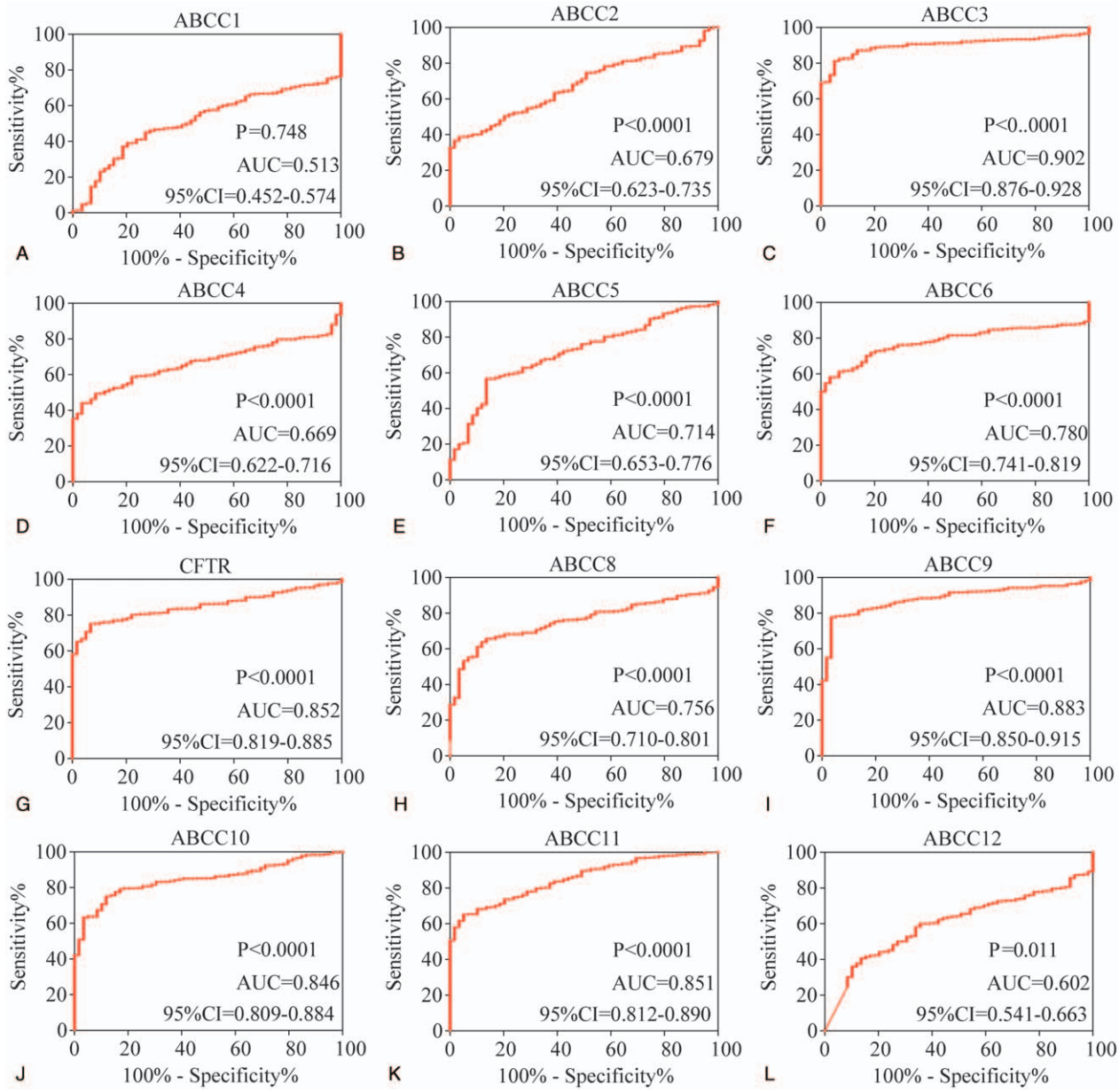

Figure 3. Diagnostic ROC curves of ABCC subfamily in lung adenocarcinoma and nontumor tissues in The Cancer Genome Atlas dataset. A–L: diagnostic ROC curves of ABCC1–6, cystic fibrosis transmembrane conductance regulator, and ABCC8–12 in lung adenocarcinoma and nontumor tissues, respectively. ABCC = ATP-binding cassette C, ROC = receiver operating characteristic.

Fig. 5B, F, H, K, L) whereas other members did not (all $P > .05$, Table 2, Fig. 5A, C–E, G, I, J). Multivariate Cox analysis indicated that ABCC2, 6, and 8 showed prognostic significance ($P = .026$, .037, and .019, respectively, Table 2). In addition, prognosis-related genes, ABCC2, 6, and ABCC8, were combined for join-effect analysis (Table 3). Joint-effect analysis found that they had prognostic significance for patient survival as well (Fig. 6A–D, Table 3).

3.4. Risk score model construction and disease progression analysis

Risk score model was constructed using ABCC2, 6, and ABCC8 (Fig. 7A–C). β coefficients used in the model were shown in Table 4. ABCC2, 6, and 8 mRNA expression, patient survival, and risk score ranking were shown in Figure 7A. Kaplan–Meier plot indicated that this model showed prognostic significance for survival prediction of LUAD (log-rank $P < .0001$, Fig. 7B). Survival ROCs indicated that it predicted significance for survival at 1 to 4 years (all AUC > 0.06, Fig. 7C).

After that, prognosis-related genes ABCC2, 6, and 8 were further explored for correlation between their expression and tumor stage (Fig. 8A–C). ABCC2 expression was associated with all the stages (all $P < .05$, Fig. 8A). Nonetheless, ABCC6 expression was partly associated with tumor stage (Fig. 8B) and ABCC8 expression was not associated with any stages (all $P > .05$, Fig. 8C).

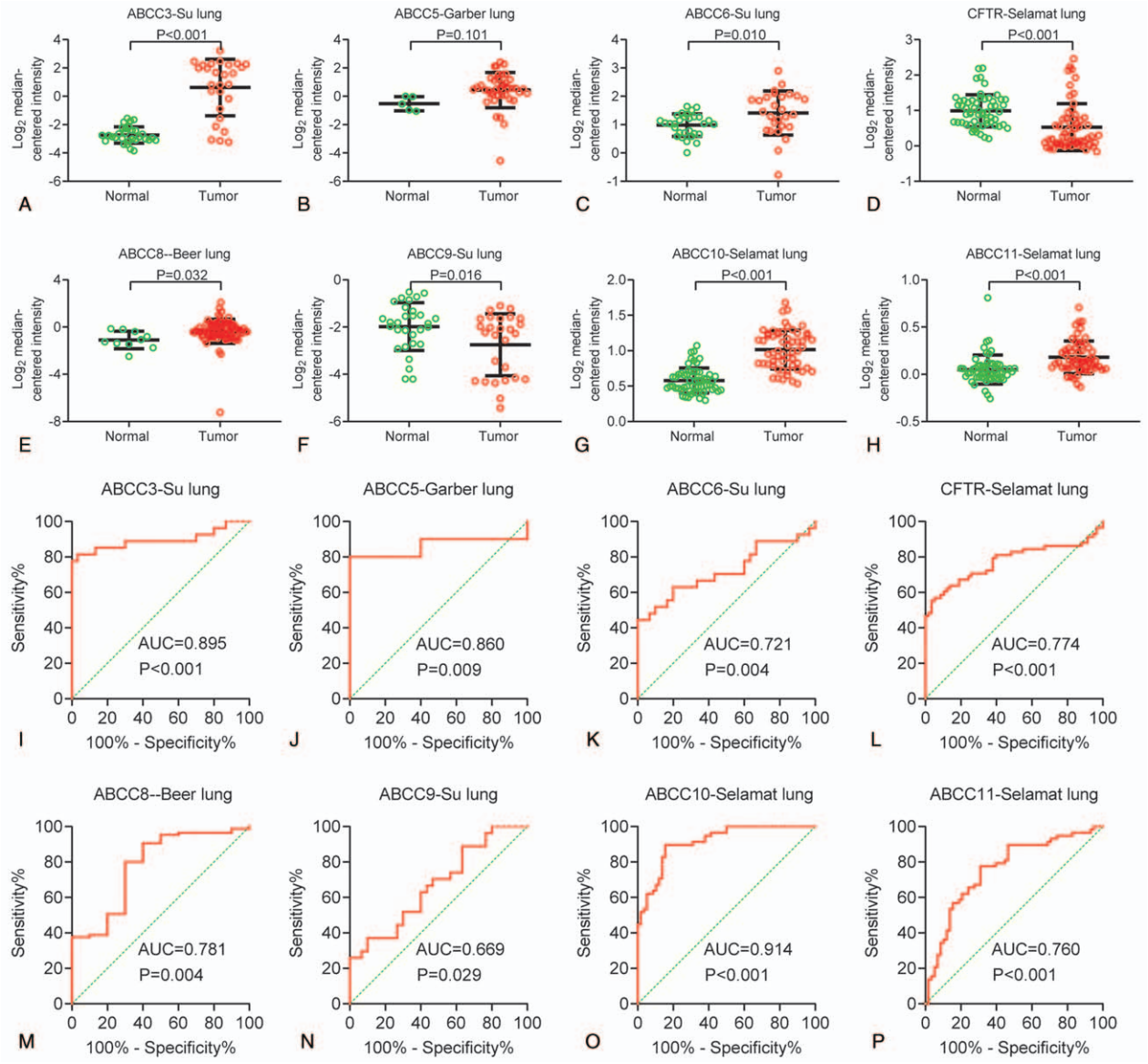

Figure 4. The mRNA expression and diagnostic ROC curves of ABCC subfamily in lung adenocarcinoma and nontumor tissues in Su, Garber, Selamat, and Beer datasets. A–H: mRNA expression of ABCC3, 5, 6, CFTR, and ABCC8–11, respectively; I–P: diagnostic ROC curves of ABCC3, 5, 6, CFTR, and ABCC8–11, respectively. ABCC = ATP-binding cassette C, CFTR = cystic fibrosis transmembrane conductance regulator, ROC = receiver operating characteristic.

3.5. Interaction networks construction

Coexpression analysis using mRNA expression indicated that ABCC1 was positively correlated with ABCC2; ABCC3 positively correlated with ABCC6; and ABCC11 positively correlated with ABCC12 ($r=0.49$, 0.39 and 0.43, respectively, Fig. 9A). The gene–gene interaction network suggested that ABCC8 was coexpressed with ABCC9 and ABCC4 showed physical interaction with CFTR; these members had more interactions in shared protein domains (Fig. 9B). The protein–protein interaction network suggested that ABCC1, 3, 5, 6, 10, and 12 showed the most coexpression relationships with other members (Fig. 9C).

The enriched biological processes network indicated that these ABCC members were involved in localization, establishment of localization, ion transport, and transmembrane transport (Fig. 10). The enriched cellular components network indicated that these ABCC members were involved in membrane integrity, intrinsic to membrane, plasma membrane, cell fraction, and apical plasma membrane (Fig. 11). The enriched molecular functions network indicated that these ABCC members were involved in adenylyl nucleotide binding, purine nucleoside binding, ATP binding, ATPase activity, and NTP activity (Fig. 12). The enriched metabolic pathways indicated that ABCC members were involved in ABC transport, ABC transporter disorders, ABC-family-protein-mediated transport, and bile secretion (Fig. 8D).

4. Discussion

Our present study analyzed potential significance of 12 ABCC family members, ABCC1–6, 8–12, and CFTR, in LUAD using the TCGA dataset and other datasets in Oncomine. ABCC1–3 had

Table 2

Prognostic analysis of ABCC family genes in lung adenocarcinoma.

Variables	Patients (n = 500)	Overall survival					
		No. of event	MST (month)	HR (95%CI)	Crude *P* value	HR (95%CI)	Adjusted *P* value*
ABCC1							
Low expression	250	87	1516	Ref.	.228	Ref.	.580
High expression	250	95	1235	1.196 (0.894–1.600)		1.087 (0.809–1.460)	
ABCC2							
Low expression	250	85	1531	Ref.	**.027**	Ref.	**.026**
High expression	250	97	1235	**1.389 (1.038–1.860)**		**1.398 (1.040–1.877)**	
ABCC3							
Low expression	250	89	1499	Ref.	.259	Ref.	.391
High expression	250	93	1421	1.183 (0.884–1.583)		1.137 (0.848–1.525)	
ABCC4							
Low expression	250	97	1229	Ref.	.102	Ref.	.820
High expression	250	85	1622	0.784 (0.585–1.050)		0.966 (0.716–1.303)	
ABCC5							
Low expression	250	95	1600	Ref.	.844	Ref.	.225
High expression	250	87	1293	1.030 (0.769–1.379)		1.201 (0.893–1.616)	
ABCC6							
Low expression	250	105	1268	Ref.	**.008**	Ref.	**.037**
High expression	250	77	1778	**0.671 (0.500–0.902)**		**0.728 (0.541–0.981)**	
CFTR							
Low expression	250	89	1492	Ref.	.243	Ref.	.734
High expression	250	93	1499	1.190 (0.889–1.593)		1.052 (0.784–1.412)	
ABCC8							
Low expression	250	103	1229	Ref.	**0.003**	Ref.	**0.019**
High expression	250	79	1653	**0.635 (0.472–0.852)**		**0.695 (0.513–0.943)**	
ABCC9							
Low expression	250	96	1379	Ref.	0.624	Ref.	0.576
High expression	250	86	1622	0.930 (0.695–1.244)		0.919 (0.684–1.235)	
ABCC10							
Low expression	250	103	1421	Ref.	0.210	Ref.	0.062
High expression	250	79	1622	0.829 (0.618–1.112)		0.754 (0.560–1.015)	
ABCC11							
Low expression	250	81	1531	Ref.	**0.034**	Ref.	0.393
High expression	250	101	1268	**1.373 (1.025–1.840)**		1.142 (0.843–1.547)	
ABCC12							
Low expression	250	105	1209	Ref.	**0.013**	Ref.	0.182
High expression	250	77	1778	**0.689 (0.513–0.925)**		0.814 (0.602–1.101)	

95%CI = 95% confidence interval, ABCC = ATP-binding cassette C, CFTR = cystic fibrosis transmembrane conductance regulator, HR = hazard ratio, MST = median survival time, NA = not available.
* *P* values were adjusted for tumor stage.

consistently high expression in tumor tissues. However, most datasets indicated that ABCC5, 10, and 11 were highly expressed in tumor tissues whereas ABCC6, 9, and CFTR were highly expressed in nontumor tissues. ABCC4 and ABCC8 expression was not in accordance in these datasets. The diagnostic significance of ABCC3 and ABCC5 was assessed in the TCGA dataset and consistently validated in the other two datasets. Prognostic analysis of the multivariate Cox hazard regression model indicated that ABCC2, 6, and 8 mRNA expression was associated with survival of LUAD . Risk score model constructed using ABCC2, 6, and 8 suggested prognostic significance for survival prediction. ABCC2 expression was associated with all the tumor stages, whereas ABCC6 was partly associated and ABCC8 was not. Interaction networks including biological processes, cellular components, molecular functions and metabolic pathways indicated they were involved in establishment of localization, ion transport; membrane integrity, plasma membrane; ATP binding, ATPase activity, and NTP activity; and ABC-family- protein–mediated transport, and bile secretion.

Lung cancer is a leading cause of cancer mortality worldwide, including in China.[30,31] NSCLC is the major subtype, accounting for approximately 85% of cases of lung cancer, and LUAD is the major cause of NSCLC.[32] Although advances in cancer research and treatment have improved in recent years, the prognosis of NSCLC is still unsatisfactory, with the only 14% 5-year survival rate.[33] The ABC transporters are recognized as an ABC superfamily on the basis of similarity of the sequence and organization of ABC domains, even though the integral membrane proteins probably have evolved independently several times and therefore lead to varied subfamilies.[34] ABCC is one of the 8 subfamilies of the ABC superfamily and participates in several biological processes, such as ion transport, toxin excretion, and signal transduction.[35]

ABCC1 and ABCG2 have cancer stem cell properties and their expression is decreased in breast cancer stem cells in the presence of curcumin.[36] Targeted as a candidate gene by miRNA-7, ABCC1 expression is negatively associated with miRNA-7 expression in miRNA-7 mimic and inhibitor experiments.[37] It has been concluded that miRNA-7 mediates SCLC chemoresistance by

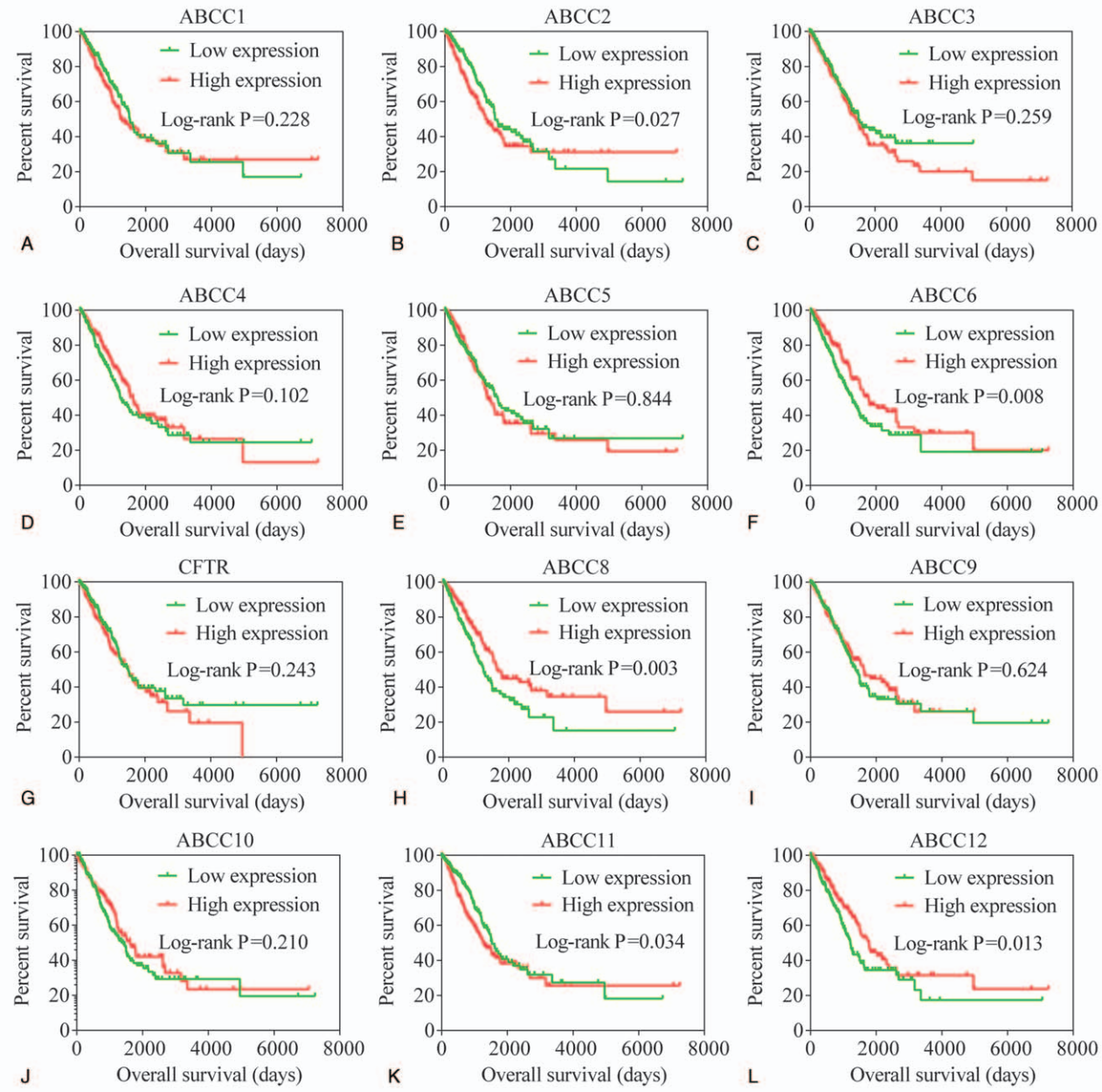

Figure 5. Kaplan-Meier plots of ABCC subfamily in TCGA dataset. A–L: Kaplan-Meier plots of ABCC1–6, cystic fibrosis transmembrane conductance regulator, and ABCC8–12 in TCGA dataset, respectively. ABCC = ATP-binding cassette C, TCGA = The Cancer Genome Atlas.

repressing ABCC1 expression, and might be a novel prognostic biomarker as well as therapeutic target.[37] However, ABCC1 expression has not been reported in NSCLC and LUAD. Our study did not find any diagnostic and prognostic significance of ABCC1 in LUAD.

A study focusing on the genetic variants of ABCB1, ABCC2, and ABCG2 in colorectal cancer suggested that ABCB1 and ABCG2 haplotypes are associated with prognosis but ABCC2 is not.[38] However, Andersen et al found that ABCC2 expression is increased significantly in adenoma with mild to moderate dysplasia; in carcinoma tissues compared with unaffected tissues from the same person; and in carcinoma and distant unaffected tissues from colorectal cancer patients compared with healthy individuals.[39] They also found that ABCG2 mRNA expression is decreased in adenoma and carcinoma tissues compared with healthy individuals, and indicated that these ABC transporters participate in the early processes of colorectal carcinogenesis.[39] The present study similarly found potential significance of ABCC2 expression in LUAD. Although the significance of ABCC2 is suggested in different cancers, we speculate that ABCC2 expression is involved in tumor initiation or progression. Further investigations about ABCC2 may be helpful for the elucidation of the understanding of LUAD.

Chen et al conducted a meta-analysis of polymorphisms of ABCB1 rs1128503, ABCC2 rs717620, and ABCC3 rs4148416 in osteosarcoma. They suggested that ABCC3 rs4148416 polymorphism is related to poor response in osteosarcoma and ABCB1 rs1128503 polymorphism to good response in

Table 3

Joint-effect analysis of prognosis-related genes for overall survival.

Group	*ABCC2*	*ABCC6*	*ABCC8*	Overall survival			
				Events /total	MST (Months)	Adjusted HR (95%CI)	Adjusted *P* value*
I	High	Low		60/132	1046	Ref.	**.013**
II	High	Low		82/236	1492	0.721 (0.513–1.013)	.059
	Low	High					
III	Low	High		40/132	2027	**0.553 (0.370–0.827)**	**.004**
I	High		Low	57/120	999	Ref.	**.004**
ii	High		Low	86/260	1653	**0.644 (0.457–0.907)**	**.012**
	Low		High				
iii	Low		High	39/120	1622	**0.512 (0.338–0.775)**	**.002**
A		Low	Low	72/151	1081	Ref.	**.010**
B		Low	High	64/198	1499	**0.670 (0.477–0.941)**	**.021**
		High	Low				
C		High	High	46/151	1790	**0.585 (0.400–0.857)**	**.006**
a	High	Low	Low	42/80	879	Ref.	**.003**
b	Low	Low	Low	63/163	1235	0.767 (0.515–1.142)	.191
	High	Low	High				
	High	High	Low				
c	Low	High	Low	53/184	1778	**0.504 (0.334–0.761)**	**.001**
	Low	Low	High				
	High	High	High				
d	Low	High	High	24/73	1790	**0.500 (0.300–0.832)**	**.008**

95%CI = 95% confidence interval, ABCC2/6/8 = ATP-binding cassette C 2/6/8, HR = hazard ratio, MST = median survival time, NA = not available.

* *P* values were adjusted for tumor stage.

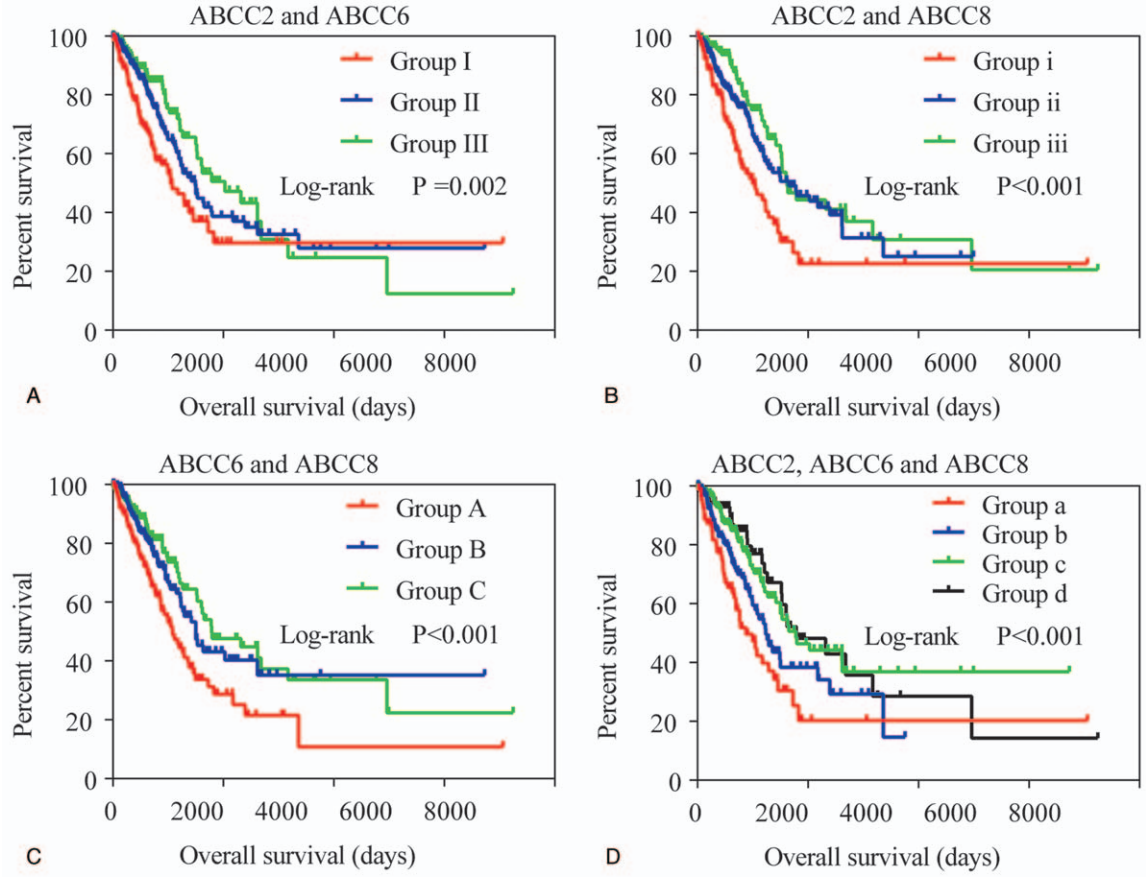

Figure 6. Joint-effect analysis of ABCC2, 6, and 8 in TCGA dataset. A–D: joint-effect analysis of ABCC2 and 6; ABCC2 and 8; ABCC6 and 8; ABCC2, 6, and 8 in TCGA dataset, respectively. ABCC = ATP-binding cassette C, TCGA = The Cancer Genome Atlas.

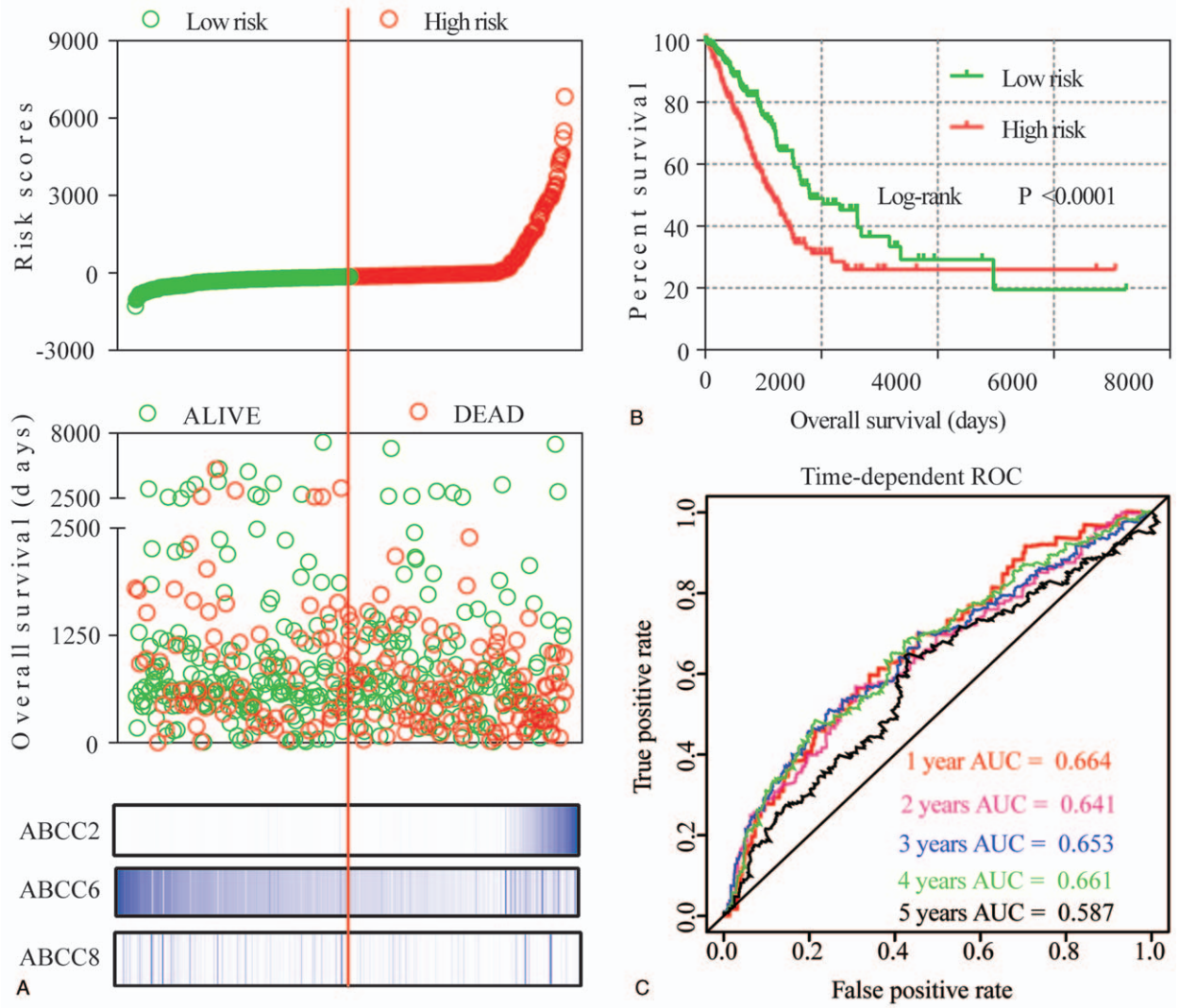

Figure 7. Risk score model constructed using ABCC2, 6, and 8 in The Cancer Genome Atlas dataset. A: risk score model, including risk score ranking, patients survival, and ABCC2, 6, 8 expressions; B: Kaplan-Meier plot of risk score model; C: time-dependent receiver operating characteristic curves of risk score model in 1 to 5 years. ABCC = ATP-binding cassette C.

Caucasians rather than Asians.[40] Stewart et al demonstrated that the calcium permeable ion channel transient receptor potential cation channel, subfamily C, member 1 regulates expression of ABCC3 at both basal and epidermal-growth-factor-induced levels.[41] Parallel with the above study, we found that ABCC3 mRNA expression has potential diagnostic value for LUAD. This finding contributes to the further elucidation of LUAD mechanisms.

Research regarding ABCC4 expression indicated that mRNA and protein expression of ABCC4 is elevated in human nasal-type natural killer (NK)/T-cell lymphoma YTS and SNK-6 cells compared to normal NK cells.[42] This study provides novel

Table 4

Risk score model construction for overall survival.

Variables	β	SE	Wald	HR (95%CI)	*P* value
Tumor stage I			48.589		<.0001
Stage II	0.826	0.187	19.432	2.284 (1.004–3.298)	<.0001
Stage III	1.196	0.197	36.736	3.308 (2.247–4.871)	<.0001
Stage IV	1.349	0.284	22.495	3.852 (2.206–6.726)	<.0001
ABCC2	0.308	0.152	4.127	1.361 (1.011–1.831)	.042
ABCC6	-0.222	0.157	2.000	0.801 (0.589–1.089)	.157
ABCC8	-0.308	0.159	3.747	0.735 (0.538–1.004)	.053

ABCC2/6/8 = ATP-binding cassette C 2/6/8, 95%CI = 95% confidence interval, β = coefficient, HR = hazard ratio, SE = standard error.

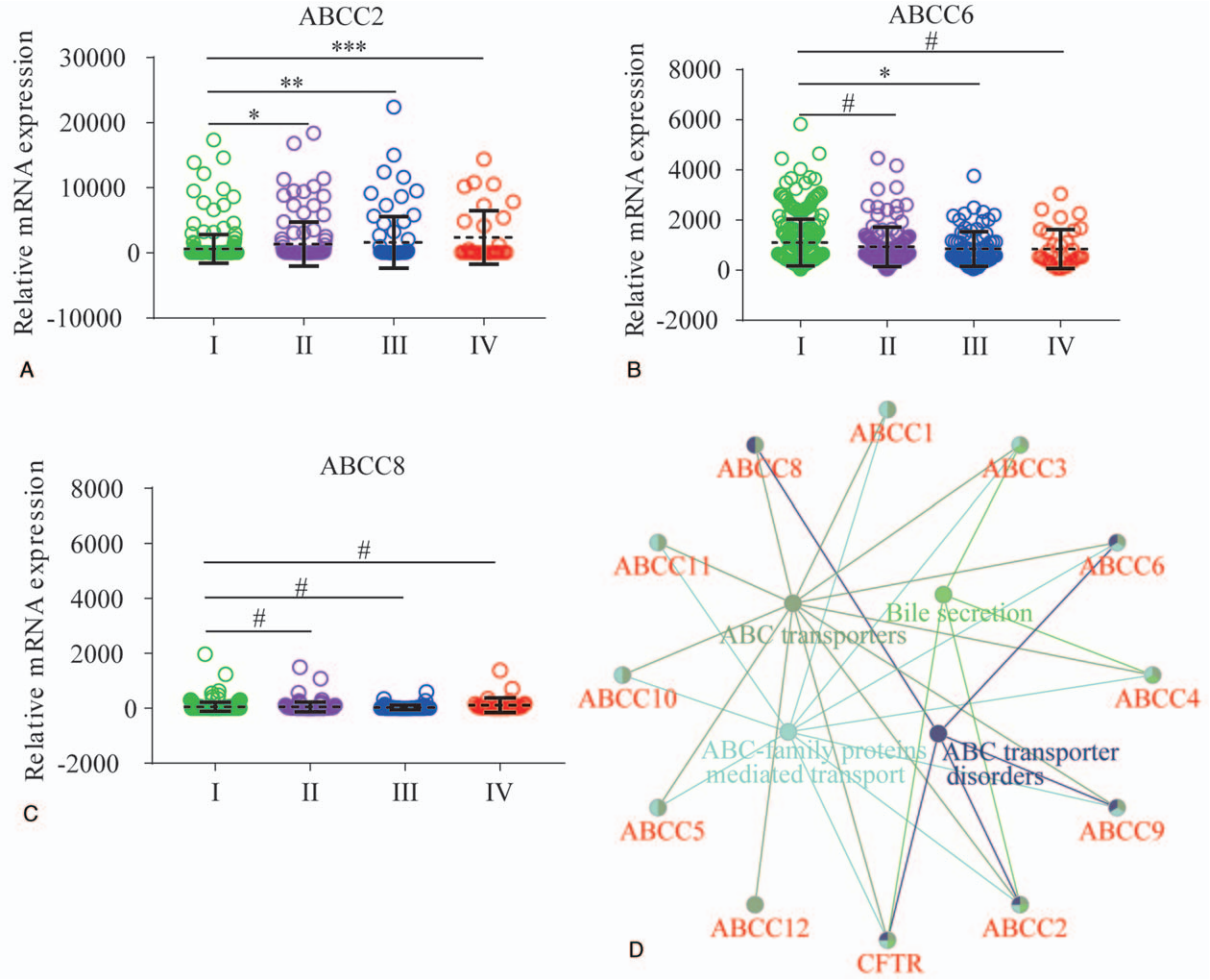

Figure 8. Scatter plots of ABCC expressions with tumor stage and ABCC with metabolic pathways. A–C: scatter plots of ABCC2, 6, and 8 expression with tumor stage; D: interaction network between ABCC members and metabolic pathways. ABCC = ATP-binding cassette C.

approaches for the treatment of NK/T-cell lymphoma. ABCC9 mutations or gene polymorphisms have been documented in Cantú syndrome,[43] hippocampal sclerosis of aging pathology,[44] and acute myocardial infarction.[45] However, the present study did not find diagnostic and prognostic significance of ABCC4 and ABCC9 in LUAD. More studies concerning their potential value in tumors are needed.

Targeted as target gene by miRNA-129-5p using bioinformatics and reporter gene assays, ABCB1, ABCC5, and ABCG1 were associated with chemoresistance of gastric cancer due to the potential significance of miRNA-129-5p as a therapeutic target.[46] Mourskaia et al found that ABCC5 may serve as a mediator of breast cancer skeletal metastasis and may be a candidate therapeutic target for bone metastasis of breast cancer.[47] Nonetheless, our study found potential diagnostic significance of ABCC5 rather than prognostic significance for LUAD. Previous studies have reported the diagnostic value of ABCC5 in other tumors. Therefore, the diagnostic significance of it in tumors need further exploration.

ABCC6 and ABCC9 were significantly downregulated whereas ABCC1, 4, 5, 10–12 were upregulated in post-treatment tumors compared to nontumor tissues.[48] In addition, ABCC1 and ABCC8 expression at the intratumoral level was significantly associated with tumor grade and hormone receptor expression in both male and female patients.[48] Besides, ABCC6, 8, 10, and 11 mRNA expression had diagnostic significance and ABCC2, 6, and 8 mRNA expression had prognostic significance for LUAD. These findings are similar to those of Hlaváč et al who showed that ABCC8 plays a role in cancer and chemoresistance in breast cancer, and has diagnostic and prognostic significance in LUAD. Li et al demonstrated that CFTR is downregulated in NSCLC tissues compared to normal lung tissues,[49] which is in accordance with our result that CFTR was highly expressed in non-tumor tissues in LUAD patients. Unfortunately, our study did not indicate any prognostic significance for LUAD. Nevertheless, we found that CFTR showed diagnostic significance in LUAD, which has not been reported previously.

Although our study is believed to be the first to report the diagnostic and prognostic significance of the ABCC subfamily in LUAD, there were still some limitations to the study. First, our results need to be validated in other medical centers and populations. Second, both in vivo and in vitro experiments are needed to explore the specific mechanisms of action of ABCC members in LUAD. Finally, more clinical factors related to patient survival and disease progression should be included in future studies. Clinical trials and target therapy targeting this family should be further warranted as well.

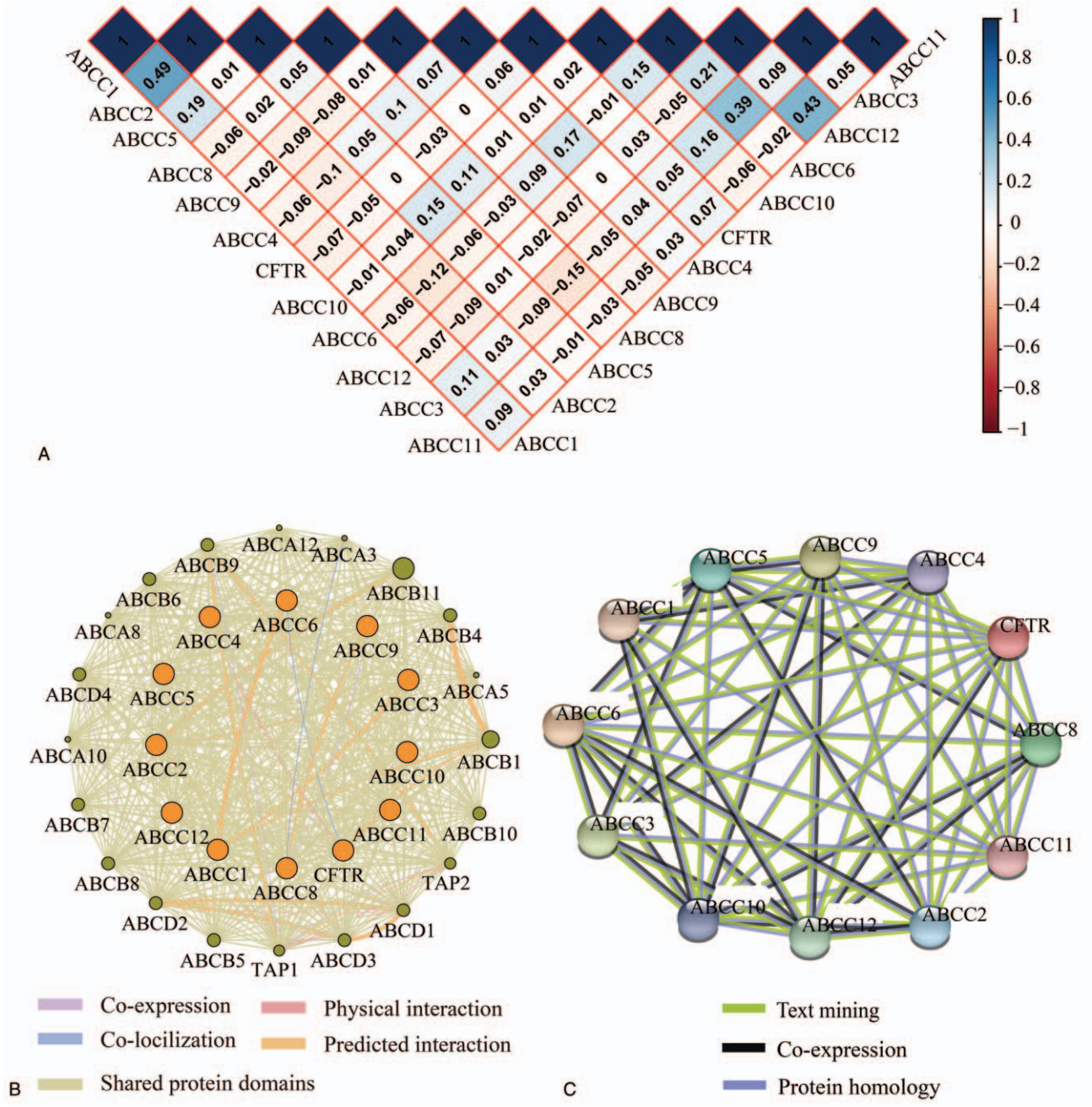

Figure 9. Coexpression matrix and interaction networks among ABCC members. A: coexpression matrix among ABCC members; B: gene–gene interaction network among ABCC members; D: protein–protein interaction network among ABCC members. ABCC = ATP-binding cassette C.

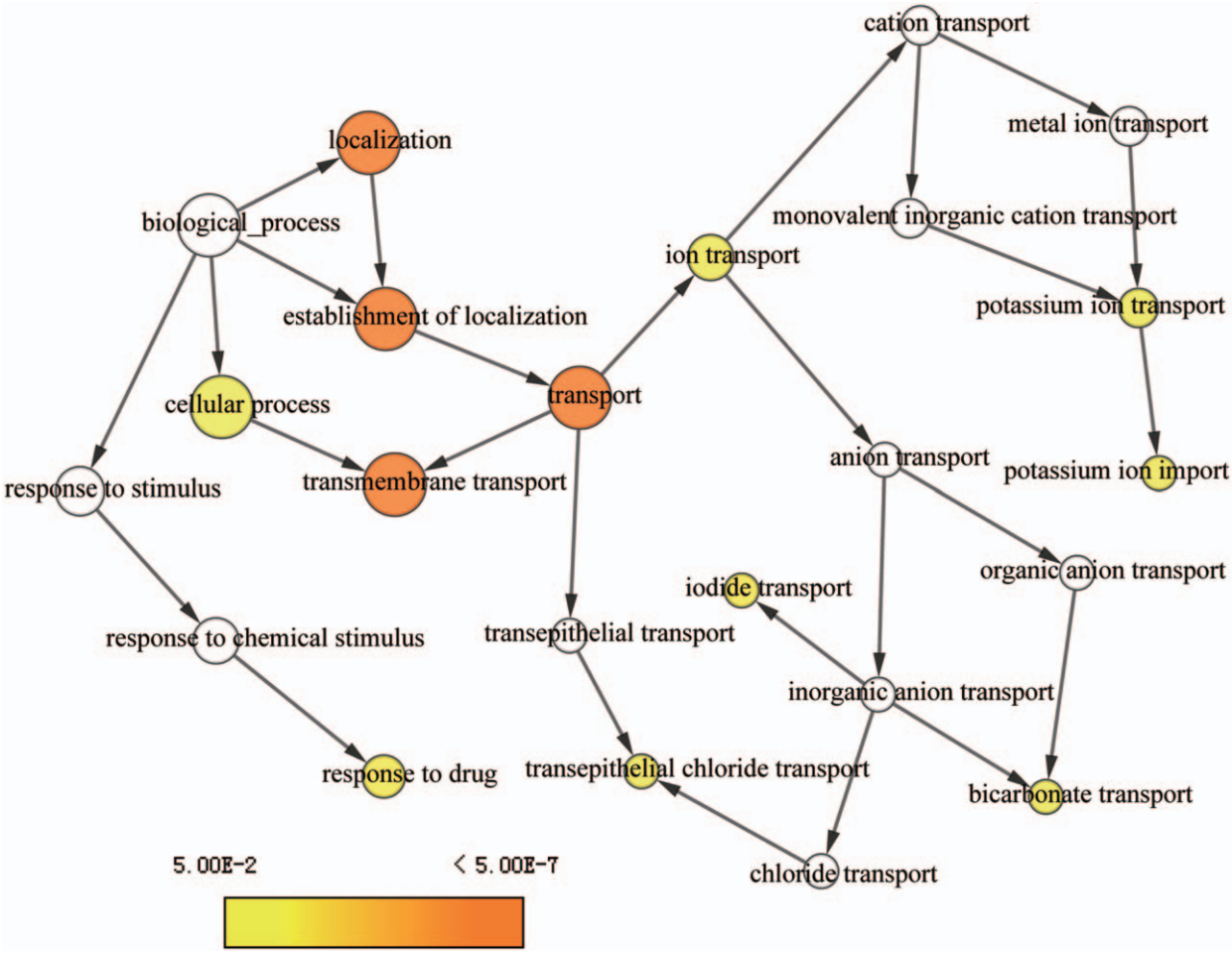

Figure 10. Biological processes network ATP-binding cassette C members are involved in.

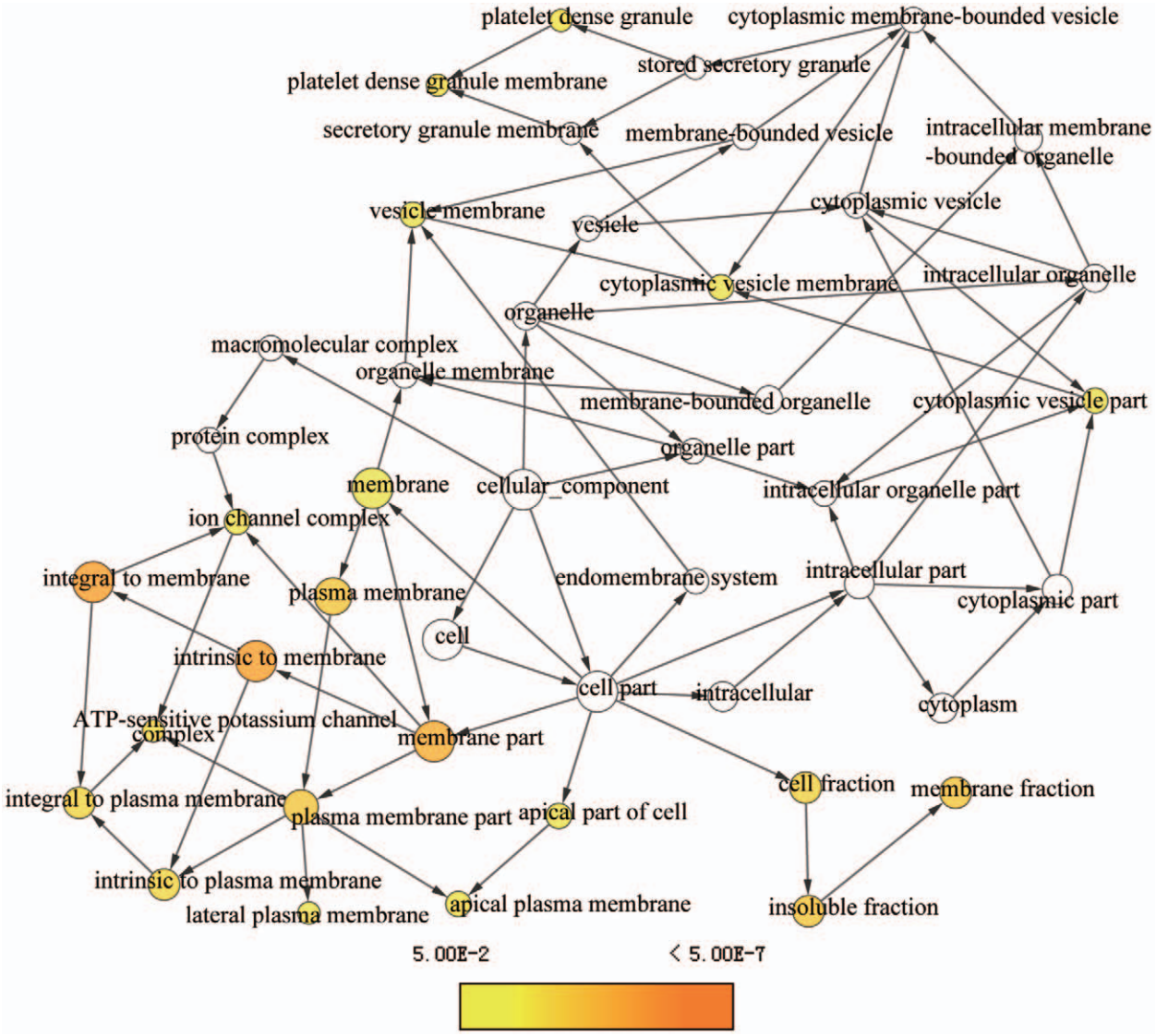

Figure 11. Cellular components network ATP-binding cassette C members are involved in.

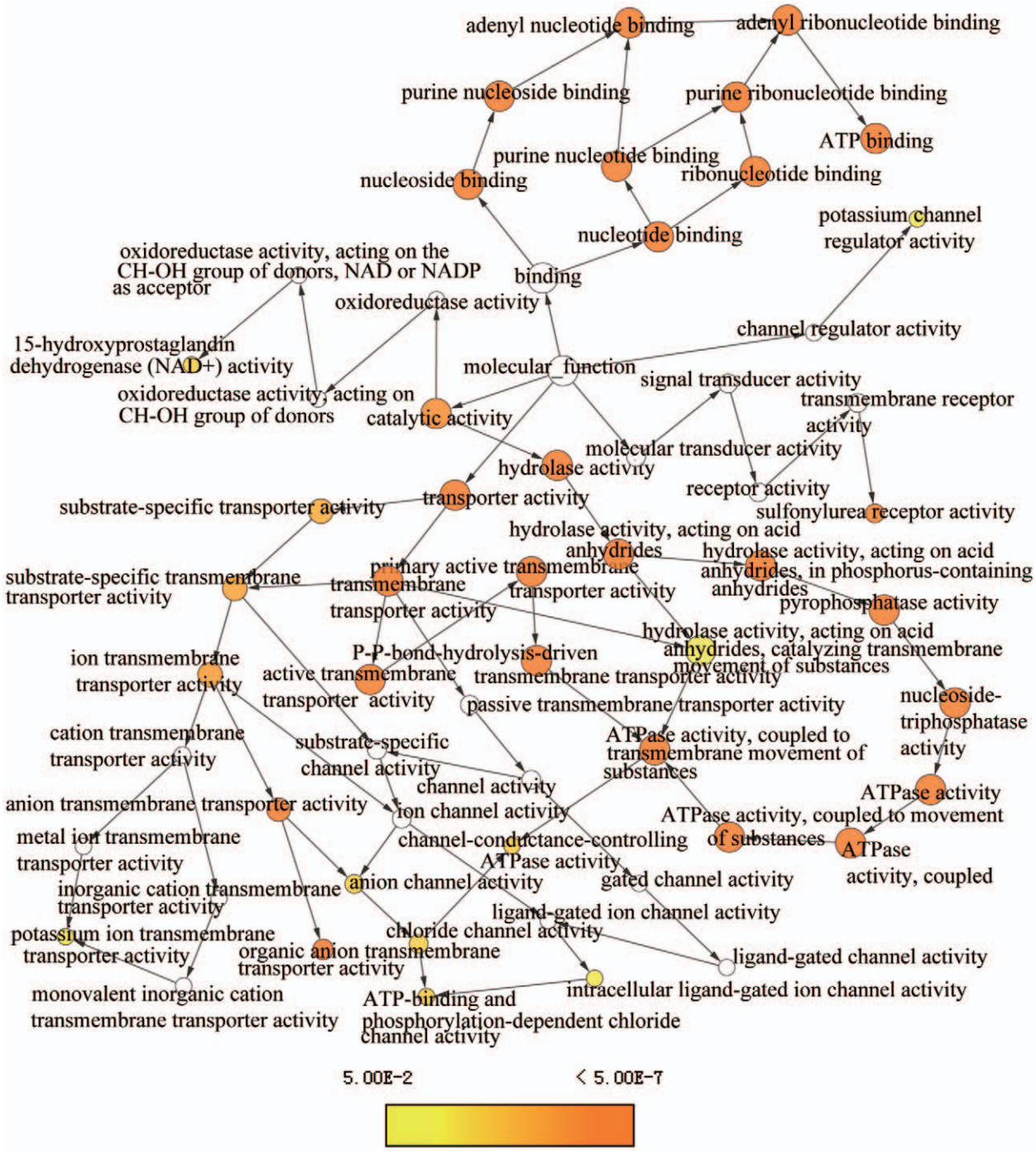

Figure 12. Molecular functions network ATP-binding cassette C members are involved in.

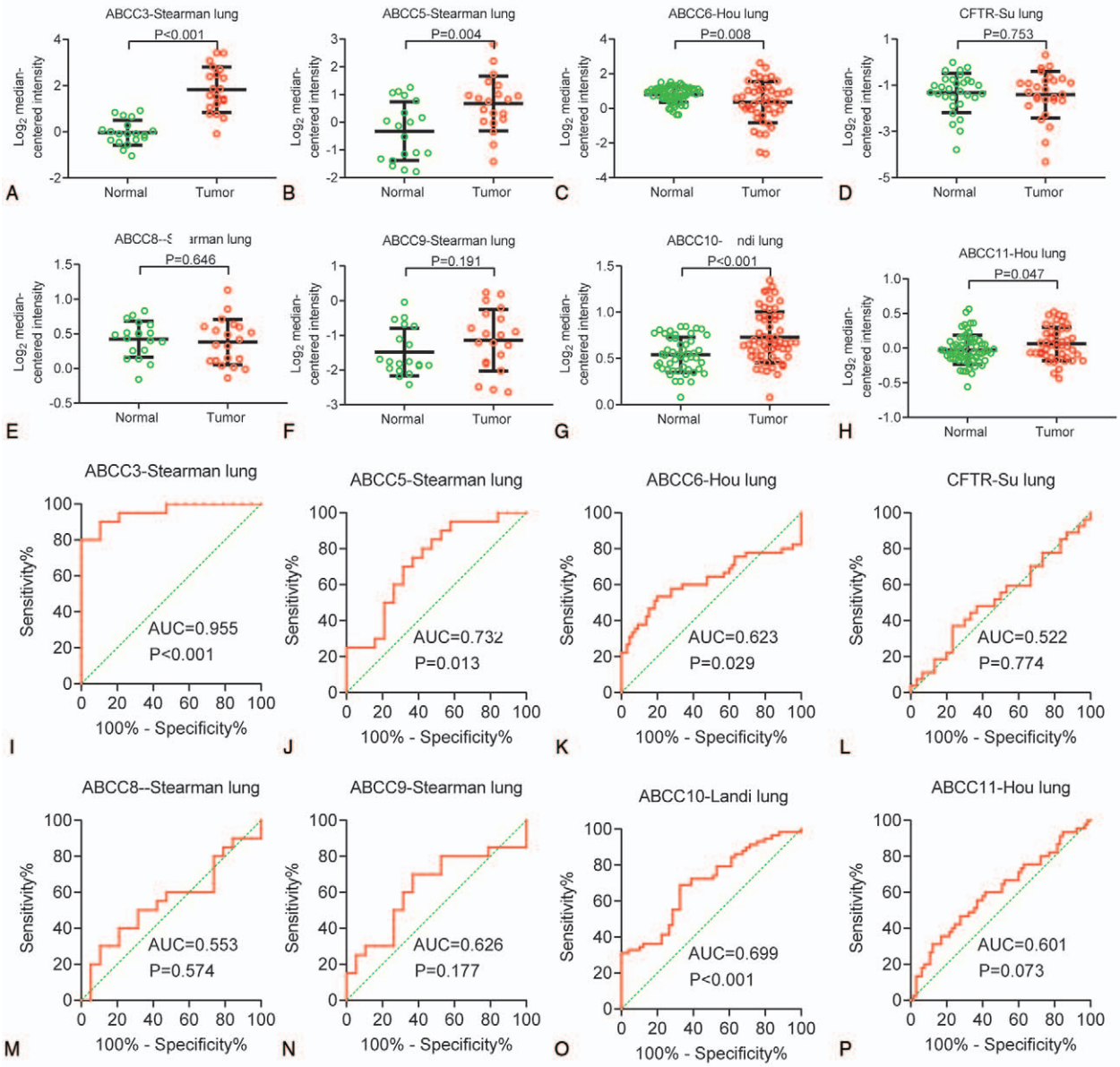

Figure 13. The mRNA expression and diagnostic ROC curves of ABCC subfamily in lung adenocarcinoma and nontumor tissues in Stearman, Hou, Su, and Landi datasets. A–H: mRNA expression of ABCC3, 5, 6, CFTR and ABCC8–11, respectively; I–P: diagnostic ROC curves of ABCC3, 5, 6, CFTR and ABCC8–11, respectively. ABCC = ATP-binding cassette C, CFTR = cystic fibrosis transmembrane conductance regulator, ROC = receiver operating characteristic.

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Validation: Chunxia Huang, Lingmei Jiang.
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Writing – original draft: Peng Wang, Linbo Zhang.
Writing – review & editing: Peng Wang, Linbo Zhang.

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