Original Article

High integrin α3 expression is associated with poor prognosis in patients with non-small cell lung cancer

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Background: We previously showed that α3β1 integrin is a novel cancer biomarker and drug target in non-small cell lung cancer (NSCLC). This study characterized the integrin α3 (ITGA3) expression on patient specimens.

Methods: Tissue microarrays (TMAs) were prepared from archival tissue blocks containing 161 patients, which included 91 adenocarcinoma (LUAD), 46 squamous carcinomas (LUSC), and 24 other histology types. TMA sections were stained and scored for ITGA3 expression by immunohistochemistry (IHC). Kaplan-Meier curves and log-rank tests were used to compare overall survival (OS) between IHC score groups. Propensity-score-weighted Kaplan-Meier curves and weighted Cox models were used to adjust for covariate imbalance between IHC score groups. Logistic regression was used to determine ITGA3 transcriptome expression in NSCLC in The Cancer Genome Atlas (TCGA).

Results: ITGA3 IHC expression (1+ to 3+) was detected in 107/161 (66.5%) of the NSCLC samples, and was associated with poor prognosis at the edge of significance (HR =1.30, 95% CI: 0.99–1.71, P=0.056), but significant (P<0.05) in subgroups of female patients, smokers and tumors with grade I and II differentiation using propensity-score-weighted survival analysis after adjusting for confounders. Multivariate survival analysis based on multiple imputation for missing variables showed ITGA3 expression, old age and metastasis were associated with poor prognosis (P<0.05). ITGA3 IHC expression was associated with poor prognosis in LUSC (HR =2.27, P<0.05) but not in LUAD (HR =1.49, P=0.16). Median ITGA3 expression was significantly higher in LUAD than LUSC (P<0.0001) in the TCGA transcriptome datasets. Using a higher cutoff than LUSC (70.6 *vs.* 19.5 FPKM), high ITGA3 RNA expression was also associated with poor prognosis in LUAD (P=0.023). ITGA3 interacted with key genes regulating epithelial to mesenchymal transition, angiogenesis, invasion and metastasis in both LUAD and LUSC.

Conclusions: High ITGA3 IHC expression was associated with poor prognosis in NSCLC patients. Further study is warranted for targeting α3β1 integrin in NSCLC.

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Introduction

Non-small cell lung cancer (NSCLC) accounts for 80–85% of lung cancer, which is the leading cause of cancer-related deaths worldwide (1,2). Despite advances in early detection and treatment, the prognosis for patients with NSCLC remains poor (3). Novel therapeutic targets and treatment strategies are needed to improve the clinical outcomes for NSCLC patients, especially those diagnosed with unresectable, locally advanced or metastatic NSCLC. α3β1 integrin is a promising cancer biomarker and drug target in NSCLC (4). Like other integrins, α3β1 integrin is a heterodimeric transmembrane glycoprotein receptor without a cytoplasmic kinase domain, and binds to various ligands such as fibronectin, laminin, collagen, epiligrin, thrombospondin and chondroitin sulfate proteoglycan 4 (CSPG4) (5). α3β1 integrin mediates the adhesion, cytoskeleton function, and metastasis of NSCLC via cross-talk with EGFR and other receptor tyrosine kinase (RTK) (4,6-8). The integrin α3 (ITGA3) subunit binds to the integrin β1 (ITGB1) subunit as its exclusive binding partner. α3β1 integrin is weakly detected in the basement membranes of normal alveolar walls but is highly expressed in primary lung cancer cells (9,10) and tumor-derived exosomes (4,11). Overexpression of α3β1 integrin has been detected in multiple tumor types and is associated with tumorigenesis, invasion, metastasis, as well as resistance to cancer treatment in several cancer types, including NSCLC (4,12), breast cancer (13), cervical cancer (14), glioma (15), and other cancer type metastasis to the lung (16,17). Almost all these studies were done on preclinical models. Due to the poor sensitivity and specificity of early commercially available antibodies, limited study has been reported on the clinical significance of ITGA3 expression using archival formalin fixed, paraffin embedded (FFPE) patient samples (14). Furthermore, there are conflicting data on the prognosis of ITGA3 expression in NSCLC (12,18). We have recently generated and characterized LXY30, a high affinity peptide ligand for targeting α3β1 integrin-expressing, live tumor cells and tumor-derived exosomes in NSCLC, regardless of histology and tumor genotypes (4,19). Of note, LXY30 has been optimized to bind to live tumor cells but not to live normal cells (4). However, LXY30 does not bind to tumor cells on the archival, FFPE specimens. In the current study, we determined the expression pattern and prognosis of ITGA3 expression by immunohistochemistry (IHC) using commercially available antibodies on archived NSCLC tumors.

Methods

Tissue microarrays (TMAs)

TMAs were prepared from 200 FFPE tissue blocks using an IRB approved protocol (IRB 293828, UC Davis Cancer Center Biorepository) (*Figure 1*). Three 0.6 mm (diameter) by 0.4 mm (length) cylindrical cores of tumor and adjacent non-malignant (control) lung tissues were collected from each case and placed in the same block (quick-ray manual tissue microarrayer). Three cores of normal liver and kidney tissues were also included on the same block as normal controls. One TMA section was stained with hematoxylin and eosin (H&E) to visualize the presence of tumor and normal lung tissue. The deidentified database for the TMAs has been annotated with patient demographics (age at diagnosis, gender, race/ethnicity), stage, histopathology, and survival outcomes.

IHC stain

TMA slides were stained by IHC for ITGA3 integrin expression. 4-μm FFPE tissue sections were cut for each TMA. Slides were deparaffinized and pretreated with Heat-Induced-Epitope-Retrieval solution (pH 9) (DAKO). Slides are preincubated in Dual Endogenous Enzyme Block (DEEB) blocking solution in a DAKO Autostainer Link48. Primary antibody for ITGA3 (ab131055, dilution 1:200; Abcam) were used for IHC. The slides were incubated for 30 minutes and then washed, which was followed by detection by EDL solution (DAKO EnVision and Dual Link System-HRP). Hematoxylin was used as the counterstain. IHC slides were scored by two readers (QL and WM)

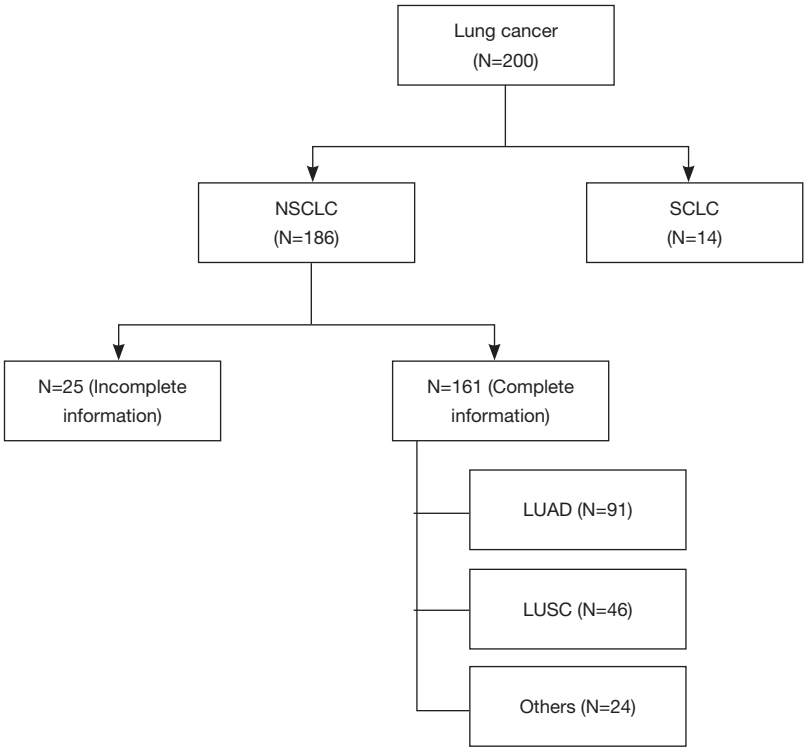


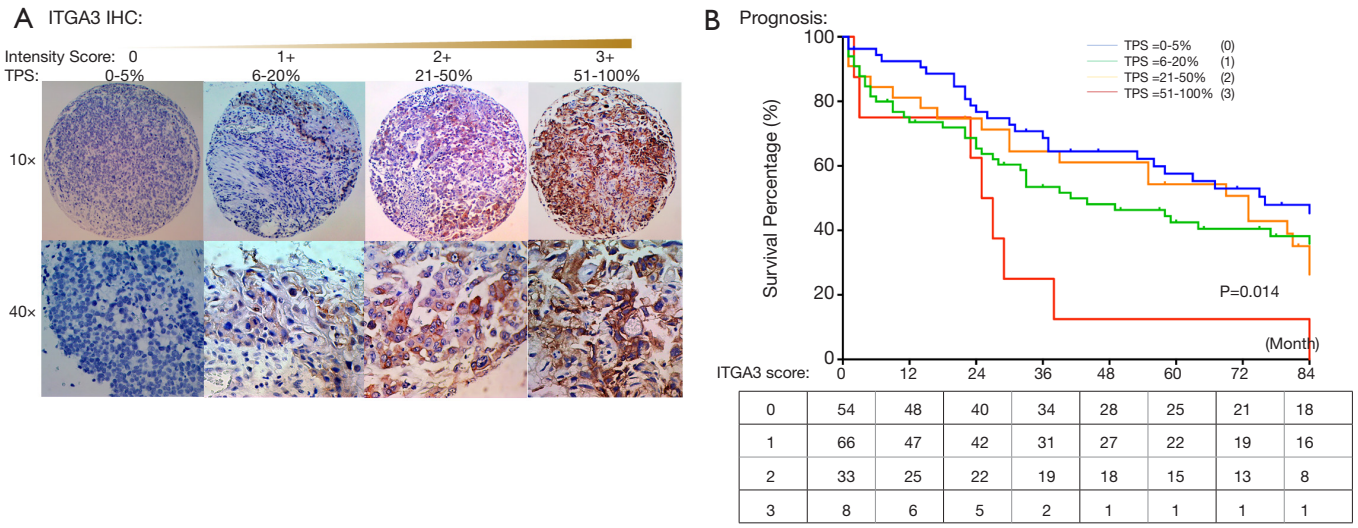
Figure 1 Study flow chart. The distribution of lung cancer types of TMA is illustrated. TMA, tissue microarray.

and verified by two pathologists (TW and CZ) blinded to clinicopathologic information. Staining for ITGA3 was semi-quantified and assessed using both intensity and percentage of positive cells. Staining intensity was graded as 0= negative (no cells stained); 1= weak; 2= moderate; 3= strong. The percentage of positive cells was defined as 0 (0–5%), 1+ (6–20%), 2+ (21–50%), and 3+ (51–100%) (*Figure 2A*). After the initial analysis, study pathologists recommended to measure the ITGA3 expression by tumor proportion score (TPS), i.e., the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The specimen was considered to have ITGA3 expression if TPS >5% and no ITGA3 expression if TPS ≤5%.

Statistical analysis

All baseline demographics and patient characteristics were compared between different ITGA3 expression level groups by Pearson chi-square tests except for age, which was considered a continuous variable and therefore analyzed with Kruskal-Wallis test. Multivariate logistic regression with backward variable section was applied to patient and tumor characteristics to examine factors associated with expression (TPS >5%) versus no (TPS ≤5%) ITGA3 expression levels.

The Kaplan-Meier method and log-rank test were first used to compare survival outcomes between different ITGA3 expression levels. Univariate survival analysis and multivariate analysis were performed with Cox proportional hazards models using overall survival (OS) as outcomes. Factors found to be significant in univariate analysis were included and selected by backward method in multivariate analysis (except ITGA3 IHC expression which was always included). To ensure the proportional hazards assumption holds, the assumption was examined using Schoenfeld residuals. To account for potential confounding and covariate imbalances between different ITGA3 expression groups, we used propensity score-weighted Kaplan-Meier estimator, incorporating the inverse of the propensity score (20). The propensity score was used to balance the covariate distribution between groups for studies with either causal or non-causal purposes (21). For survival outcomes, this method has been shown to produce less biased treatment effect estimates than stratification or covariate adjustment



0	54	48	40	34	28	25	21	18
1	66	47	42	31	27	22	19	16
2	33	25	22	19	18	15	13	8
3	8	6	5	2	1	1	1	1

Figure 2 Atlas of ITGA3 expression by IHC. (A) Atlas of representative pictures of 0, 1, 2, and 3 IHC scores for ITGA3 expression in NSCLC TMAs. (B) Kaplan-Meier curves of overall survival were stratified by ITGA3 expression score (0, 1, 2, and 3). ITGA3, integrin α3; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; TMA, tissue microarray.

based on the propensity score (22). Significant covariates in univariate and multivariate survival analyses were used in the propensity score model using logistic regression. We also performed subgroup analyses for different patient characteristics groups. As there was high proportion of unknown metastasis status (63% of all patients) in the database, and the above analyses created an additional level for patients with unknown metastasis status, we performed additional sensitivity analyses using the multiple imputation method for unknown metastasis based on missing at random (MAR) assumption (23). All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria). All P values were two-sided, and a P≤0.05 was considered statistically significant.

TCGA data access and analysis

The transcriptome expression data from NSCLC patients were accessed on September 9th, 2019 in The Cancer Genome Atlas (TCGA) database (http://www.cancergenome.nih.gov) including 500 lung adenocarcinoma (LUAD) and 494 lung squamous cell carcinoma (LUSC) samples. Fragments per kilobase of exon per million reads mapped (FPKM) values were chosen as the representative measure of gene expression (24). Survival data was obtained for the individual patient barcodes matched to these FPKM values and was used to separate NSCLC subtypes into high and low expression groups, on which log-rank tests for significant difference (P<0.05) were conducted.

Results

Patient characteristics

Figure 1 illustrates the distribution of the 200 cases of lung cancer on the TMA. Excluding those cases with small cell lung cancer (SCLC) and NSCLC without complete clinical information, the expression of ITGA3 was analyzed in 161 cases. All the statistics for baseline demographics and patient characteristics are summarized in *Table 1*.

High ITGA3 expression was associated with poor prognosis

ITGA3 IHC expression was detected in 107/161 (66.5%) of the NSCLC samples. Weak (1+), moderate (2+) and strong (3+) ITGA3 expression was detected in 66 (41.0%), 33 (20.5%), and 8 (5.0%) cases, respectively (*Figure 2A*). Kaplan-Meier curves indicated that weak (1+) and strong (3+) ITGA3 expression was significantly associated with poor OS in NSCLC patients (P<0.05) (*Figure 2B*). Overall, NSCLC patients whose tumors expressed ITGA3 by IHC (1+ to 3+) was associated with poorer prognosis compared

Table 1 Patient characteristics

Characteristics	N=161	Percentage (%)
Age (mean ± SD; years old)	65.9±10	
Range (years old)	32–86	
Gender		
Female	94	58.3%
Male	67	41.6%
Race/ethnicity		
NHW	121	75.2%
Black	7	4.3%
Asian	4	2.5%
Other	29	18%
Smoking history		
Smoker	100	62.1%
Never smoker	61	37.9%
Histology type		
LUAD	91	56.5%
LUSC	46	28.6%
Others	24	14.9%
Grade		
I	33	20.5%
II	43	26.7%
III	51	31.7%
Not known	34	21.11%

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

to those patients whose tumors did not express ITGA3 (*Figure 3A*). We also performed propensity-score-weighted survival analyses between different ITGA3 expression levels for different patient characteristics groups. We found that association between ITGA3 IHC expression (weak to strong) and poor prognosis is significant in subgroups of female patients (HR =1.49, 95% CI: 1.02–2.18, P=0.04) (*Figure 3B*), smokers (HR =1.77, 95% CI: 1.21–2.57, P=0.003) (*Figure 3C*) and differentiation grade I & II (HR =2.07, 95% CI: 1.39–3.08, P<0.001) (*Figure 3D*), but not in male patients (P=0.50), non-smokers (P=0.22), differentiation grade III (P=0.66) (*Table 2*). Sensitivity analyses using multiple imputation demonstrated similar conclusions for these subgroups.

Multivariate survival analysis demonstrated that poor prognosis is associated with older age and metastasis (*Table 2*). Propensity-score-weighted survival analysis demonstrated that the significance of association between ITGA3 IHC expression (1+ to 3+) and prognosis is near the edge (HR =1.30, 95% CI: 0.99–1.71, P=0.056) for all NSCLC patients after adjusting for confounders (*Table 3*).

Due to a high proportion of unknown metastasis, sensitivity analyses using multiple imputation method for unknown metastasis were performed and demonstrated that ITGA3 IHC expression (1+ to 3+) was significantly associated with poor prognosis (HR =1.67, 95% CI: 1.05–2.64, P=0.029) for all NSCLC patients after adjusting for confounders and imputing for unknown metastasis (*Table 4*).

ITGA3 expression in histology subgroups of NSCLC

We found the median score of ITGA3 IHC was not significantly higher in LUAD than LUSC (P=0.44) (*Figure 4A*), and high ITGA3 IHC score was associated with a statistically significant difference in LUSC (P<0.05) (*Figure 4B*) but not LUAD (P=0.16) (*Figure 4C*). Representative ITGA3 IHC stains on LUAC and LUSC are showed (*Figure 4D*). After adjusting for confounders, propensity-score-weighted survival analysis demonstrated that the association of ITGA3 IHC expression (weak to strong) and poor prognosis was statistically significant in LUSC patients (HR =1.76, 95% CI: 1.10–2.81, P=0.018) but not in LUAD patients (P=0.13).

Poor prognosis was associated with tumor metastasis

We found that ITGA3 expression was significantly higher in the NSCLC tumors from patients with metastatic tumors (N=19) compared to those patients without metastatic tumors (N=41). Due to a high proportion of unknown metastasis status (63% of all patients) in the database, the wide confidence interval of HR for metastasis was observed, although the association is still significant. We performed additional sensitivity analysis using multiple imputation method for unknown metastasis, and the significance of metastasis on poor prognosis was confirmed by sensitive analysis (HR =2.89, 95% CI: 1.48–5.65, P=0.003) (*Table 3*).

ITGA3 expression was associated with no smoking history

Multivariate logistic regression with backward variable section was applied to patient and tumor characteristics

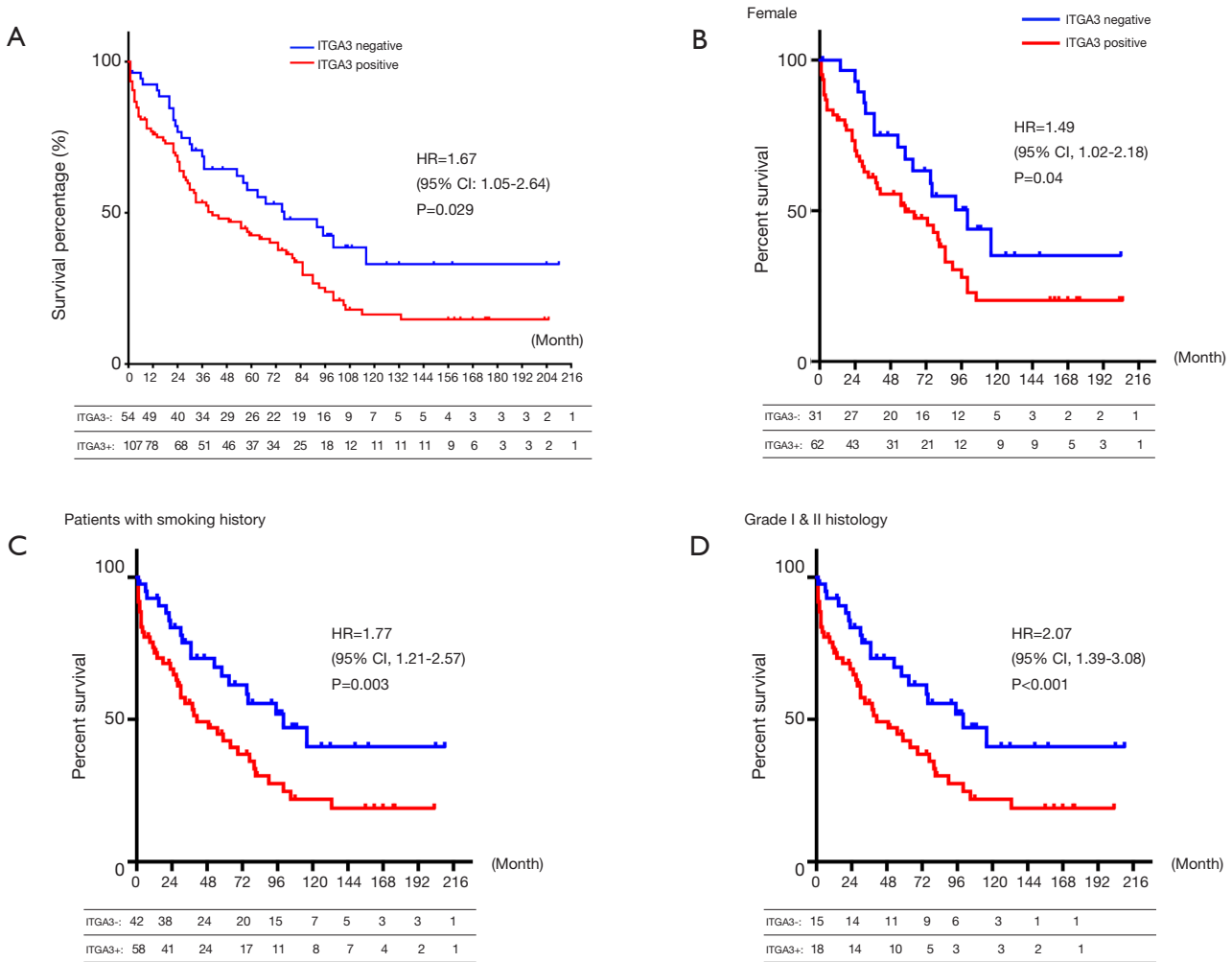


Figure 3 High level integrin α3 expression was associated with poor prognosis in NSCLC patients. Kaplan-Meier curves of overall survival were stratified by ITGA3 expression (negative *vs.* positive) (A), female (B), patients with smoking history (C), and grade I histology (D). ITGA3, integrin α3; NSCLC, non-small cell lung cancer.

to examine factors associated with expression (TPS >5%) versus no (TPS ≤5%) ITGA3 expression levels (*Table 4*). We found ITGA3 expression was associated with no smoking history (OR =2.96, 95% CI: 1.40–6.23, P=0.004), but not significantly associated with other factors.

High α3 integrin expression by transcriptome sequencing was associated with poor prognosis in patients with NSCLC

We also validated our findings in an independent cohort of NSCLC tumors. We analyzed the RNA expression of ITGA3 gene in 494 LUSC and 500 LUAD samples using the transcriptome expression data in TCGA database. We found that the median RNA expression level of ITGA3 was significantly higher in LUAD than LUSC (43.80 *vs.* 17.40, P<0.0001) (*Figure 5A*). Similar to our finding in IHC, high ITGA3 RNA expression was associated with a statistically significant poor prognosis in LUSC (P<0.05) (*Figure 5B*). Using a higher cutoff than LUSC (70.6 *vs.* 19.5 FPKM), high ITGA3 RNA expression was also associated with poor prognosis in LUAD (P=0.023) (*Figure 5C*). ITGA3 shared many interactive genes mediating cell adhesion and motility in both LUSC and LUAD (*Figure 6A,B*, *Table 5*), respectively. Furthermore, ITGA3 interacted with many key genes regulating epithelial to mesenchymal transition, angiogenesis, invasion and metastasis in both LUAD and

Table 2 Univariate, multivariate, and propensity score-weighted survival analysis for overall survival using Cox proportional hazards models

Factors	Univariate			Multivariate		
	HR	95% CI	P value	Adjusted HR	95% CI	P value
ITGA3 IHC score						
TPS ≤5%	1.00			1.00		
TPS >5%	1.68	1.09–2.57	0.018	1.39	0.90–2.14	0.137
Age (in year)	1.03	1.01–1.05	0.003	1.03	1.01–1.05	0.009
Sex						
Male	1.00					
Female	0.65	0.44–0.95	0.027			
Race						
Non-white	1.00					
White	1.96	1.27–3.02	0.003			
Smoke						
No	1.00					
Yes	0.67	0.46–0.99	0.042			
COPD						
No	1.00					
Yes	0.71	0.46–1.09	0.116			
Histology						
LUAD	1.00					
LUSC	1.16	0.76–1.76	0.502			
Other/unknown	0.70	0.38–1.29	0.256			
Differentiation grade						
Well (I)	1.00					
Moderate (II)	1.66	0.91–3.02	0.098			
Poor (III)	1.67	0.90–3.09	0.105			
Unknown	1.25	0.64–2.41	0.514			
Metastasis						
No	1.00			1.00		
Yes	60.95	8.01–463.74	<0.001	58.8	7.74–447.01	<0.001
Unknown	78.40	10.85–566.76	<0.001	73.3	10.15–529.46	<0.001
ITGA3 IHC score*						
All						
TPS ≤5%				1.00		
TPS >5%				1.30	0.99 –1.71	0.056

Table 2 (*continued*)

Table 2 (*continued*)

Factors	Univariate			Multivariate		
	HR	95% CI	P value	Adjusted HR	95% CI	P value
Subset analysis of smokers						
TPS ≤5%				1.00		
TPS >5%				1.77	1.21–2.57	0.003
Subset analysis of non-smokers						
TPS ≤5%				1.00		
TPS >5%				0.78	0.52–1.17	0.223
Subset analysis of LUSC						
TPS ≤5%				1.00		
TPS >5%				1.76	1.10–2.81	0.018
Subset analysis of LUAD						
TPS ≤5%				1.00		
TPS >5%				1.34	0.91–1.95	0.134
Subset analysis of Female						
TPS ≤5%				1.00		
TPS >5%				1.49	1.02–2.18	0.040
Subset analysis of LUSC						
TPS ≤5%				1.00		
TPS >5%				1.14	0.78–1.68	0.501
Subset analysis of differentiation grade I&II						
TPS ≤5%				1.00		
TPS >5%				2.07	1.39–3.08	<0.001
Subset analysis of differentiation grade III						
TPS ≤5%				1.00		
TPS >5%				1.12	0.69–1.81	0.656

Variables significant in univariate analyses were included in multivariate analysis and further selected by backward method. *, data are collected by propensity score-weighted survival analysis. ITGA3, integrin α3; IHC, immunohistochemistry; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

LUSC as illustrated in *Figure* 7.

Discussion

α3β1 integrin is a promising biomarker for lung cancer detection and a potential drug target, being one of the most commonly expressed integrin subtypes found on tumor cells mediating metastasis and treatment resistance (6). Using a novel peptide ligand LXY30 that has higher affinity and longer half-life than natural integrin (4,19), we recently showed that ITGA3 was expressed in about 90% of live tumor cells and exosomes isolated from patients with metastatic NSCLC (4). However, LXY30 does not work in FFPE specimens. We have screened several

Table 3 Sensitivity analyses with multivariate survival analysis for overall survival using Cox proportional hazards models

Multivariate analysis	HR	95% CI	P value
IHC score of ITGA3			
TPS ≤5% (negative)	1.00		
TPS >5% (positive)	1.67	1.05–2.64	0.029
Age (in year)	1.03	1.00–1.06	0.014
Metastasis (N=60)			
No (N=41)	1.00		
Yes (N=19)	2.89	1.48–5.65	0.003

Multiple imputation method was used for unknown metastasis based on missing at random (MAR) assumption. ITGA3, integrin α3; IHC, immunohistochemistry.

Table 4 Association between covariates and positive (TPS >5%) *vs.* negative (TPS ≤5%) ITGA3 expression levels in NSCLC patients

Smoking history	Odds ratio (95% CI)	P value
Yes	1.00	
No	2.96 (1.40–6.23)	0.004

Multivariate logistic regression was applied to model the event of high ITGA3 expression, and variables were selected by backward method. ITGA3, integrin α3; NSCLC, non-small cell lung cancer.

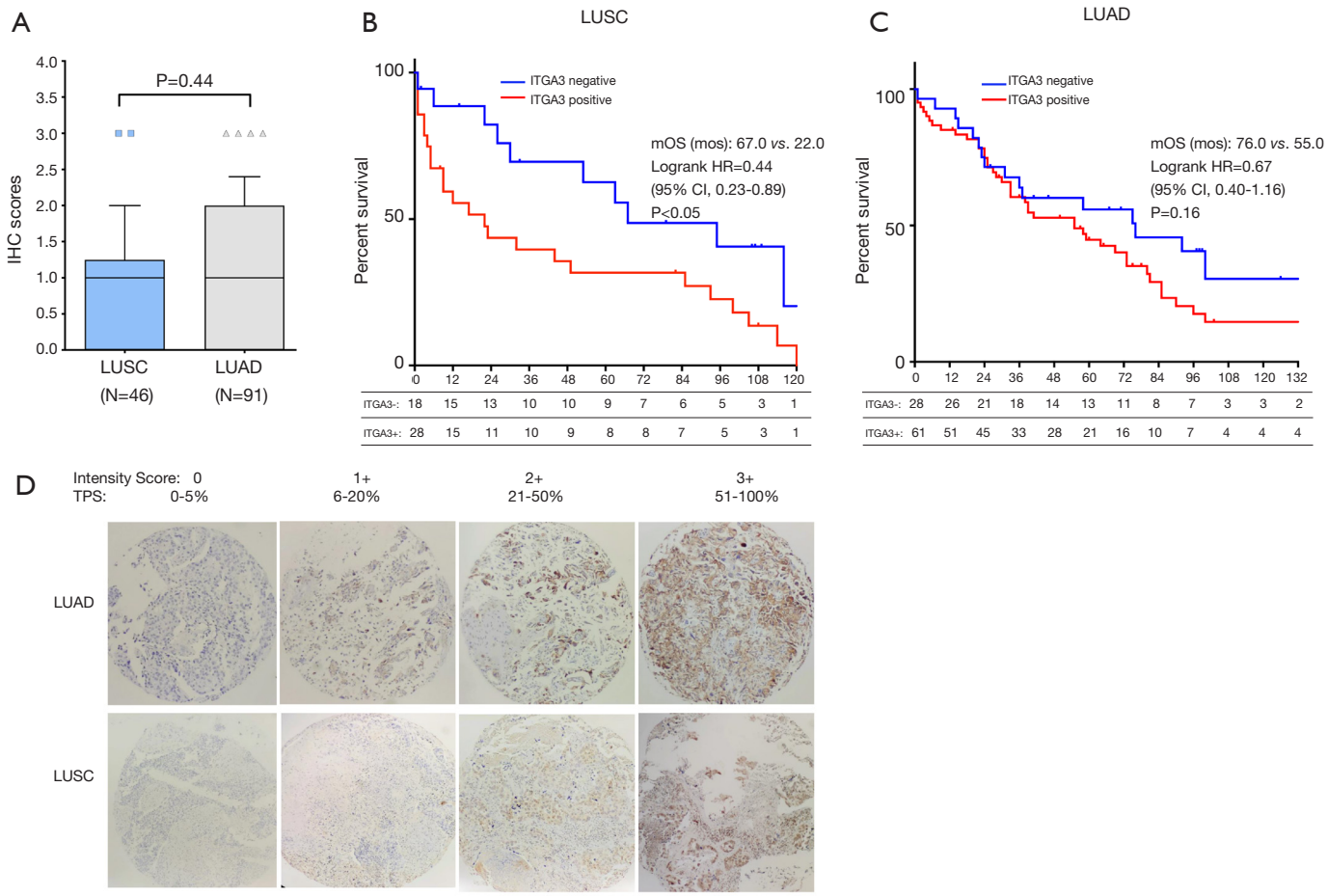


Figure 4 ITGA3 expression by histology subtypes. (A) The median ITGA3 expression was higher in LUAD than LUSC. Kaplan-Meier curves of overall survival were stratified by ITGA3 expression (negative *vs.* positive) in LUSC (B) and LUAD (C), respectively. (D) Representative ITGA3 IHC stains on LUAC and LUSC (×10). ITGA3, integrin α3; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

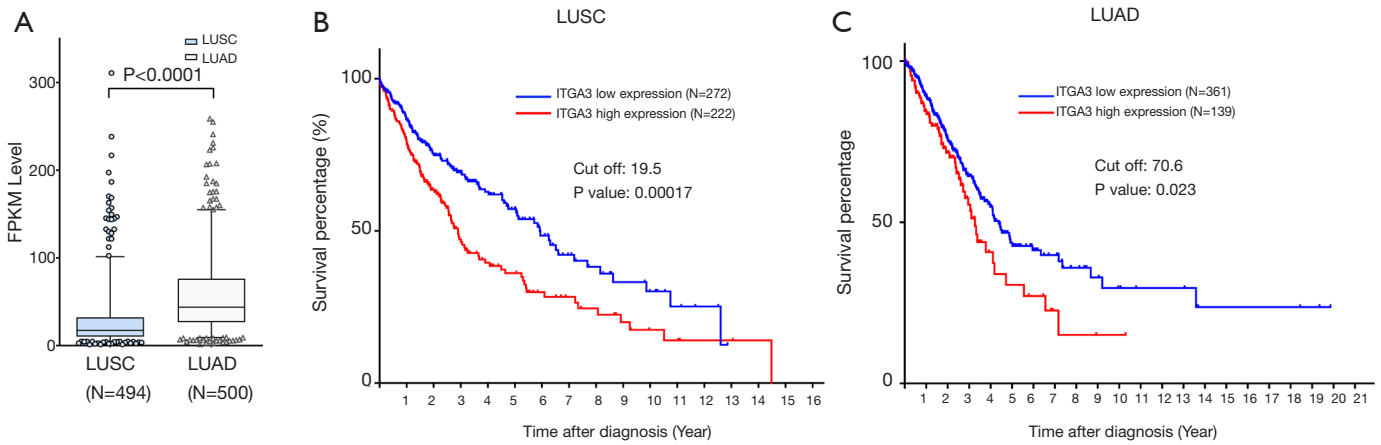


Figure 5 ITGA3 expression by RNA in LUSC and LUAD in TCGA RNA seq database. The transcriptome expression data from lung cancer patients were analyzed in TCGA database (http://www.cancergenome.nih.gov) including 499 LUSC and 500 LUAD samples. (A) The median RNA expression of ITGA3 was significantly higher in LUAD than LUSC. Kaplan-Meier curves of overall survival were stratified by ITGA3 RNA expression (low *vs*. high at different cutoffs) in LUSC (B) and LUAD (C), respectively. ITGA3, integrin α3; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

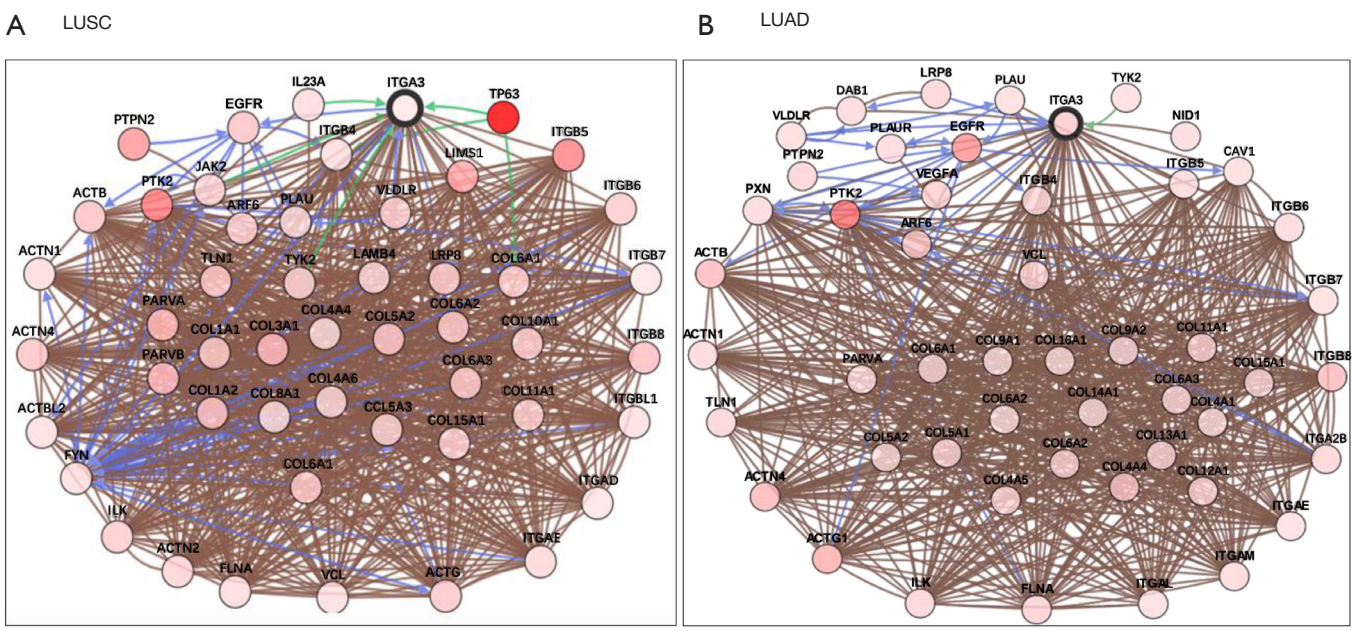


Figure 6 Interactive networks of ITGA3 expression in TCGA RNA sequencing databases. Interactive networks of ITGA3 expression in LUSC (A) and LUAD (B). The gene names and functions are summarized in *Table 5*. ITGA3, integrin α3; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

commercially available anti-ITGA3 antibodies and selected the Abcam ab131055 antibody in this study. We found that ITGA3 was expressed in the majority (66.5%) of NSCLC specimens by IHC (*Figure 2*). We further categorized the ITGA3 IHC expression in our study as weak (1+), moderate (2+) and strong (3+), which were detected in 66 (41.0%), 33 (20.5%), and 8 (5.0%) cases, respectively (*Figure 2*). The frequencies and patterns of ITGA3 expression in our

Table 5 Summary of key genes interacted with ITGA3 in the TCGA transcriptome databases

Gene ID	Interactive networks	Function	LUSC	LUAD
ACTB	Actin beta	Cell motility, structure and integrity	Yes	Yes
ACTBL2	Actin beta like 2	Cytoskeletal protein; cell motility, structure and integrity	Yes	
ACTG1	Actin gamma 1	Actin protein family; cytoskeleton	Yes	Yes
ACTN2	Actinin alpha 2	Cytoskeletal protein	Yes	
ACTN4	Actinin alpha 4	Cytoskeletal protein; cell motility, structure and integrity	Yes	Yes
ARF6	ADP ribosylation factor 6	Vesicular trafficking; activators of phospholipase D	Yes	Yes
CAV1	Caveolin-1	Integrin signaling		Yes
COL10A1	Collagen, type 10, alpha 1	Collagens family; Strengthen and support tissues	Yes	
COL11A1	Collagen, type 11, alpha 1	Collagens family; Strengthen and support tissues	Yes	Yes
COL12A1	Collagen, type 12, alpha 1	Collagens family; Strengthen and support tissues		Yes
COL13A1	Collagen, type 13, alpha 1	Collagens family; Strengthen and support tissues		Yes
COL14A1	Collagen, type 14, alpha 1	Collagens family; Strengthen and support tissues		Yes
COL15A1	Collagen, type 15, alpha 1	Collagens family; Strengthen and support tissues	Yes	Yes
COL1A1	Collagen, type 1, alpha 1	Collagens family; Strengthen and support tissues	Yes	
COL1A2	Collagen, type 1, alpha 2	Collagens family; Strengthen and support tissues	Yes	
COL3A1	Collagen, type 3, alpha 1	Collagens family; Strengthen and support tissues	Yes	
COL4A4	Collagen, type 4, alpha 4	Collagens family; Strengthen and support tissues	Yes	Yes
COL4A5	Collagen, type 4, alpha 5	Collagens family; Strengthen and support tissues		Yes
COL4A6	Collagen, type 4, alpha 6	Collagens family; Strengthen and support tissues	Yes	
COL5A1	Collagen, type 5, alpha 1	Collagens family; Strengthen and support tissues		Yes
COL5A2	Collagen, type 5, alpha 2	Collagens family; Strengthen and support tissues	Yes	Yes
COL5A3	Collagen, type 5, alpha 3	Collagens family; Strengthen and support tissues	Yes	
COL6A1	Collagen, type 6, alpha 1	Collagens family; Strengthen and support tissues		Yes
COL6A2	Collagen, type 6, alpha 2	Collagens family; Strengthen and support tissues		Yes
COL8A1	Collagen, type 8, alpha 1	Collagens family; Strengthen and support tissues		Yes
COL8A2	Collagen, type 8, alpha 2	Collagens family; Strengthen and support tissues		Yes
COL9A2	Collagen, type 9, alpha 2	Collagens family; Strengthen and support tissues		Yes
DAB1	DAB adaptor protein 1	Regulator of reelin signaling; brain development		Yes
EGFR	Epidermal growth factor receptor	Cell migration, adhesion and proliferation	Yes	Yes
FLNA	Filamin A, alpha	Cytoskeletal protein; cell motility, structure and integrity	Yes	Yes
FYN	FYN proto-oncogene	Integrins signaling pathway; activates Ras	Yes	
IGTAL	Integrin, alpha L	Integrin family; Mediate cell-matrix or cell-cell adhesion		Yes
ILK	Integrin-linked kinase	Integrin-mediated signal transduction	Yes	Yes
ITGA2B	Integrin alpha-lib	Integrin family; Mediate cell-matrix or cell-cell adhesion		Yes
ITGAD	Integrin, alpha D	Integrin family; Mediate cell-matrix or cell-cell adhesion	Yes	

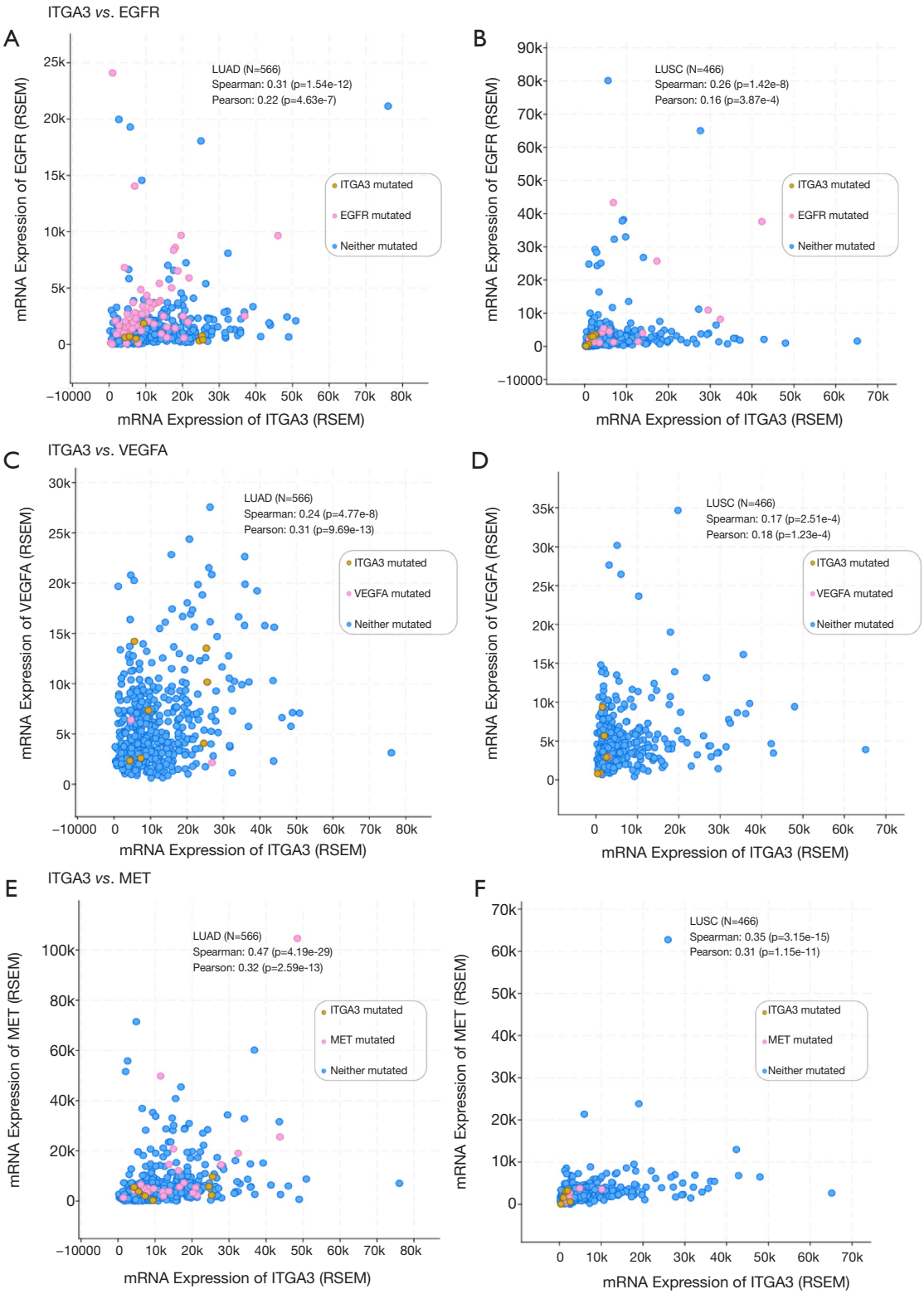
Table 5 (*continued*)

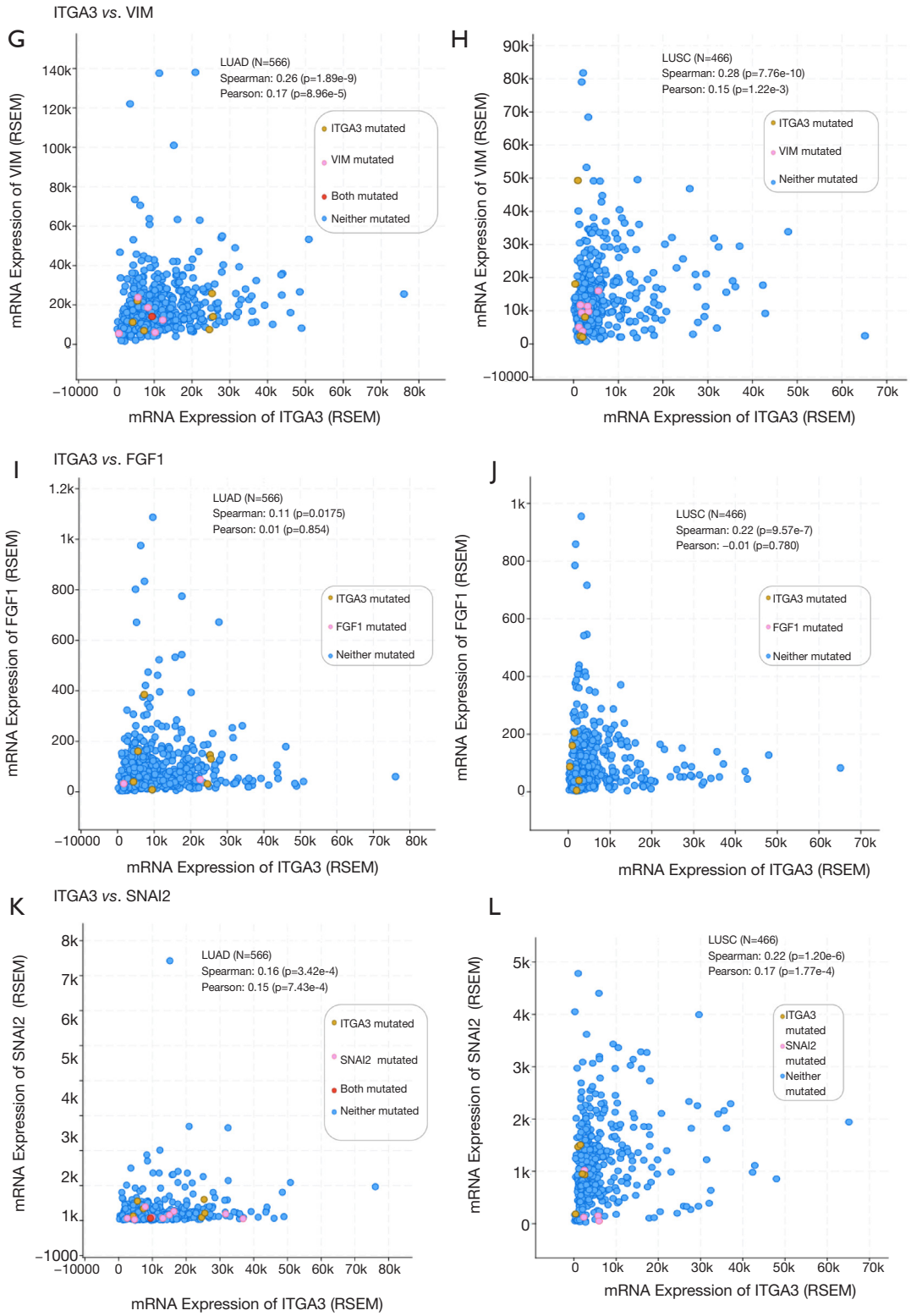
Table 5 (*continued*)

Gene ID	Interactive networks	Function	LUSC	LUAD
ITGAE	Integrin, alpha E	Integrin family; Mediate cell-matrix or cell-cell adhesion	Yes	Yes
ITGB4	Integrin subunit beta 4	Integrin family; Mediate cell-matrix or cell-cell adhesion	Yes	Yes
ITGB5	Integrin subunit beta 5	Integrin family; Mediate cell-matrix or cell-cell adhesion		Yes
ITGB6	Integrin subunit beta 6	Integrin family; Mediate cell-matrix or cell-cell adhesion	Yes	Yes
ITGB7	Integrin subunit beta 7	Integrin family; Mediate cell-matrix or cell-cell adhesion	Yes	Yes
ITGB8	Integrin subunit beta 8	Integrin family; Mediate cell-matrix or cell-cell adhesion	Yes	Yes
ITGBL1	Integrin subunit beta like 1	Integrin family; Mediate cell-matrix or cell-cell adhesion	Yes	
JAK2	Janus kinase 2	Cell growth and division; hematopoietic cells development	Yes	
LAMB4	Laminin subunit beta 4	mediate cell attachment and migration	Yes	
LIMS1	LIM zinc finger domain containing 1	Integrin signaling	Yes	
LRP8	Low-density lipoprotein receptor-related protein 8	Low-density lipoprotein receptor family	Yes	Yes
NID1	Nidogen-1	Basement membrane glycoprotein		Yes
PARVA	Alpha-parvin	Actin-binding proteins		Yes
PLAU	Plasminogen activator	Converts plasminogen to plasmin	Yes	
PLAU	Plasminogen activator, urokinase	Converts plasminogen to plasmin		Yes
PLAUR	Plasminogen activator, urokinase receptor	Receptor for urokinase plasminogen activator		Yes
PTK2	Protein tyrosine kinase 2	Cellular adhesion and cell growth	Yes	Yes
PTPN2	Protein tyrosine phosphatase non-receptor type 2	Cell growth, differentiation; catalytic activity	Yes	Yes
PXN	Paxillin	Adhere cells to the extracellular matrix		Yes
TLN1	Talin-1	Cytoskeletal protein	Yes	Yes
TP63	Tumor protein P63	Transcription factor; cell growth and early development	Yes	
TYK2	Tyrosine kinase 2	Non-receptor tyrosine kinase; cytokine signaling	Yes	Yes
VCL	Vinculin	Cytoskeletal protein; adhesion formation and maturation	Yes	Yes
VEGFA	Vascular endothelial growth factor A	Proliferation and migration of vascular endothelial cells; angiogenesis		Yes
VLDLR	Very low-density lipoprotein (VLDL) receptor	Lipoprotein receptor	Yes	Yes

ITGA3, integrin α3; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

study are within the range of two different antibodies in the Human Protein Atlas (https://www.proteinatlas.org) (*Table 6*). These data further support our effort in targeting ITGA3 using LXY30 in patients with live NSCLC. Propensity-score-weighted survival analysis demonstrated that the significance of association between ITGA3 IHC expression (1+ to 3+) and prognosis is near the edge (HR =1.30, 95% CI: 0.99–1.71, P=0.056) after adjusting for confounders. However, propensity-score-weighted survival analysis demonstrated that the association of ITGA3 IHC expression and poor prognosis was not statistically significant in either LUSC patients (P=0.11) or LUAD





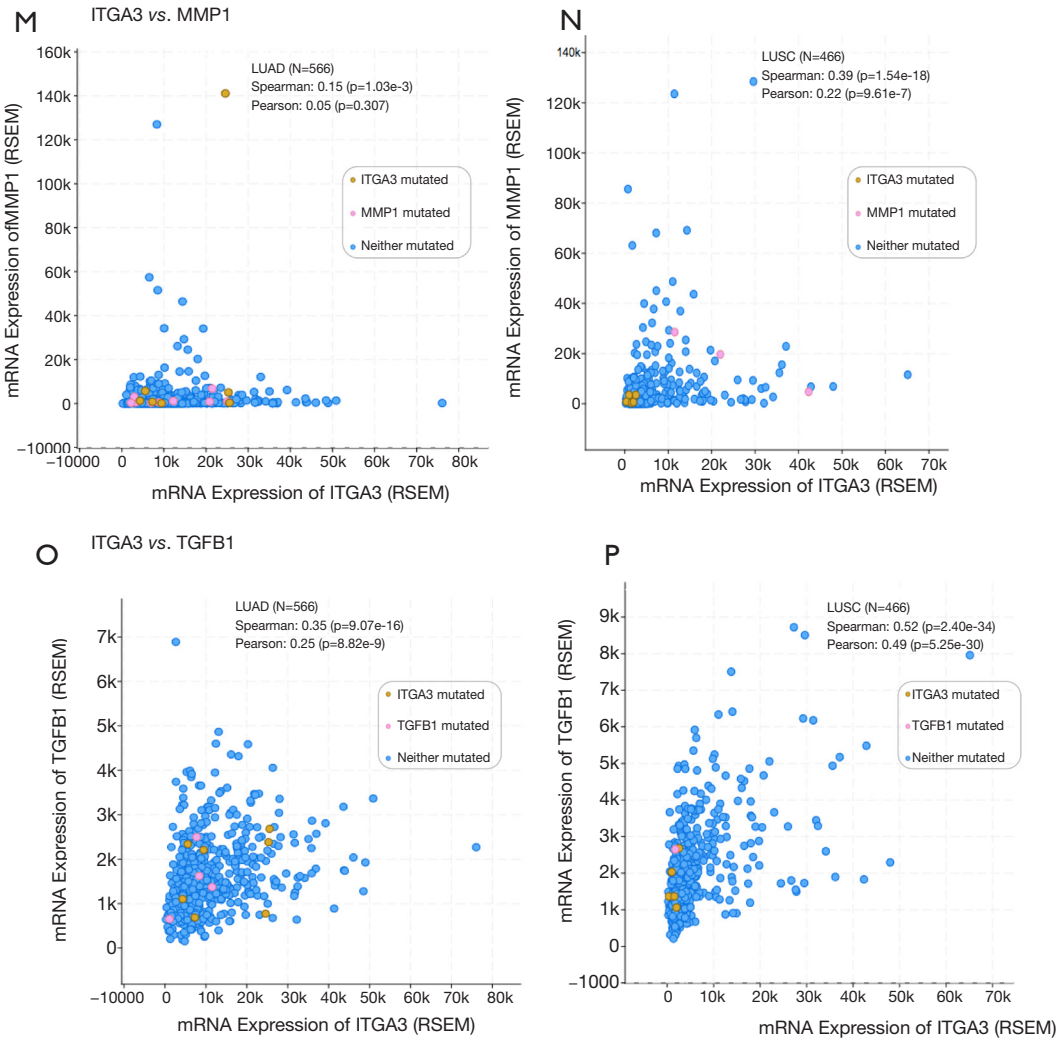


Figure 7 ITGA3 regulates malignant behaviors in LUSC and LUAD. Summary of selected key genes mediating EMT, angiogenesis, invasion and metastasis that had significant interaction with ITGA3 in TCGA RNA sequencing database. https://www.cbioportal.org/; last data access on 5/7/2020. ITGA3, integrin α3; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

patients (P=0.07). Thus, histology alone should not be used to select NSCLC for ITGA3 expression.

The expression of specific integrin subtypes has been linked to organotropic metastasis of circulating tumor cells and exosomes in epithelial tumors (11,12,17). ITGA3 and β1 subunits were detected in 13 (54%) and 24 (100%) of exosomes isolated from 24 human metastatic cell lines, while the normal tissues or PBMCs did not express any integrin (4). Although there are a significant number of patients with an unknown metastasis status, we found that ITGA3 IHC expression was only significantly associated with non-smoking history in both univariate and multivariate analyses.

The TCGA datasets are publicly accessible resources containing sequencing data of human tumors and normal tissues using NGS and other clinically relevant modern genomic technologies. Since its conception, TCGA datasets have been proven invaluable tools to cancer research community to study the role of specific genes and genomic changes in the biology and prognosis of specific cancer types (https://cancergenome.nih.gov). Using the TCGA RNASeq dataset, we found a similar pattern of high ITGA3

Table 6 Comparison of integrin α 3 IHC expression in NSCLC with published datasets

Antibody name	Marker	Host species	Positive stain rate	Intensity	No. cases	Percentage
HPA008572 (the human protein atlas)	Sigma Aldrich	Rabbit	92%	Strong	4	33%
				Moderate	6	50%
				Weak	1	8%
				Negative	1	8%
CAB018594 (the human protein atlas)	Santa Cruz biotech	Mouse	41%	Strong	1	8%
				Moderate	3	25%
				Weak	1	8%
				Negative	5	42%
AB131055 (current study)	abCAM	Rabbit	67%	Strong	8	5%
				Moderate	32	20%
				Weak	66	41%
				Negative	54	34%

ITGA3, integrin α3; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer.

expression in LUAD compared to LUSC. The interactive networking analysis showed that LUAD and LUSC shared many cell adhesion and motility genes while distinct genes interaction was also noted for LUAD and LUSC, respectively (*Figure 6*). Similar to our recent report, ITGA3 interacts with EGFR in LUAD (4). Furthermore, TP63 and PTK2 (FAK) modulates the ITGA3 expression in LUSC, which warrant further validation.

Our study has several translational potentials. First, we have recently shown that a novel, potent peptide LXY30 has high affinity for binding to α3β1 integrin, which can increase the sensitivity of cancer detection, molecular diagnosis and *in vivo* targeted delivery of imaging dye and cancer drugs in α3β1 integrin-expression NSCLC (4). The ITGA3 IHC can be used to identify candidate NSCLC patients using archived FFPE tissue specimens for targeted therapy although the caution should be excised to select the primary antibody for ITGA3. Second, we have confirmed that ITGA3 expression is associated with metastasis of NSCLC tumors. Analysis of TCGA databases showing that ITGA3 significantly interacted with many key genes regulating cell adhesion, motility, epithelial to mesenchymal transition, angiogenesis, invasion and metastasis in both LUAD and LUSC (*Table 5* and *Figure 7*). Further study is warranted to delineate the mechanism by which ITGA3 may mediate these malignant processes, and to develop therapeutic strategies for NSCLC patients.

Our study has several limitations. First, we could not explain the impact of sex, histology and smoking status on ITGA3 expression. Second, there were no molecular and immune biomarker data (such as PD-L1 IHC, tumor mutation burden) of tumors available for this dataset, which included NSCLC specimens from before the era of precision oncology. We plan to study the association of ITGA3 expression with molecular and immune biomarkers in future studies.

In conclusion, we have shown that the expression of ITGA3 subunit in patients with NSCLC is associated with poor prognosis and tumor metastasis. Further research is warranted for targeting α3β1 integrin in NSCLC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr-19-633). 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. The study was approved by the institutional review board (IRB) at the University of California, Davis (Protocol No. 226210) and conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. DeSantis CE, Miller KD, Goding Sauer A, et al. Cancer statistics for African Americans, 2019. CA Cancer J Clin 2019;69:211-33.
2. Li T, Kung HJ, Mack PC, et al. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. J Clin Oncol 2013;31:1039-49.
3. Postmus PE, Kerr KM, Oudkerk M, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017;28:iv1-21.
4. Xiao W, Ma W, Wei S, et al. High-affinity peptide ligand LXY30 for targeting alpha3beta1 integrin in non-small cell lung cancer. J Hematol Oncol 2019;12:56.
5. Millard M, Odde S, Neamati N. Integrin targeted therapeutics. Theranostics 2011;1:154-88.
6. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 2010;10:9-22.
7. Legate KR, Wickstrom SA, Fassler R. Genetic and cell biological analysis of integrin outside-in signaling. Genes Dev 2009;23:397-418.
8. Morse EM, Brahme NN, Calderwood DA. Integrin cytoplasmic tail interactions. Biochemistry 2014;53:810-20.
9. Bartolazzi A, Cerboni C, Flamini G, et al. Expression of alpha 3 beta 1 integrin receptor and its ligands in human lung tumors. Int J Cancer 1995;64:248-52.
10. Guo L, Zhang F, Cai Y, et al. Expression profiling of integrins in lung cancer cells. Pathol Res Pract 2009;205:847-53.
11. Hoshino A, Costa-Silva B, Shen TL, et al. Tumour exosome integrins determine organotropic metastasis. Nature 2015;527:329-35.
12. Yoshimasu T, Sakurai T, Oura S, et al. Increased expression of integrin alpha3beta1 in highly brain metastatic subclone of a human non-small cell lung cancer cell line. Cancer Sci 2004;95:142-8.
13. Shirakihara T, Kawasaki T, Fukagawa A, et al. Identification of integrin alpha3 as a molecular marker of cells undergoing epithelial-mesenchymal transition and of cancer cells with aggressive phenotypes. Cancer Sci 2013;104:1189-97.
14. Du Q, Wang W, Liu T, et al. High Expression of Integrin alpha3 Predicts Poor Prognosis and Promotes Tumor Metastasis and Angiogenesis by Activating the c-Src/Extracellular Signal-Regulated Protein Kinase/Focal Adhesion Kinase Signaling Pathway in Cervical Cancer. Front Oncol 2020;10:36.
15. Nakada M, Nambu E, Furuyama N, et al. Integrin alpha3 is overexpressed in glioma stem-like cells and promotes invasion. Br J Cancer 2013;108:2516-24.
16. Morini M, Mottolese M, Ferrari N, et al. The alpha 3 beta 1 integrin is associated with mammary carcinoma cell metastasis, invasion, and gelatinase B (MMP-9) activity. Int J Cancer 2000;87:336-42.
17. Zhou B, Gibson-Corley KN, Herndon ME, et al. Integrin alpha3beta1 can function to promote spontaneous metastasis and lung colonization of invasive breast carcinoma. Mol Cancer Res 2014;12:143-54.

18. Adachi M, Taki T, Huang C, et al. Reduced integrin alpha3 expression as a factor of poor prognosis of patients with adenocarcinoma of the lung. J Clin Oncol 1998;16:1060-7.
19. Xiao W, Li T, Bononi FC, et al. Discovery and characterization of a high-affinity and high-specificity peptide ligand LXY30 for in vivo targeting of alpha3 integrin-expressing human tumors. EJNMMI Res 2016;6:18.
20. Imbens GW. The role of the propensity score in estimating dose-response functions. Biometrika 2000;87:706-10.
21. Li F, Zaslavsky AM, Landrum MB. Propensity score weighting with multilevel data. Stat Med 2013;32:3373-87.
22. Austin PC. The performance of different propensity score methods for estimating marginal hazard ratios. Stat Med 2013;32:2837-49.
23. Rubin DB. Multiple Imputation for Nonresponse in Surveys. 1987.
24. Wagner GP, Kin K, Lynch VJ. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. Theory Biosci 2012;131:281-5.

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