

The Clinical Significance of Integrin Subunit Alpha V in Cancers: From Small Cell Lung Carcinoma to Pan-cancer

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Abstract

Background: Little is known about the relationship between integrin subunit alpha V (*ITGAV*) and cancers.

Materials & Methods: Using large sample size from multiple sources, the clinical roles of *ITGAV* expression in pan-cancer were explored.

Results: Upregulated *ITGAV* mRNA expression was revealed in multiple cancers ($p < .05$). Decreased mRNA (SMD = -1.05) and increased protein levels of *ITGAV* were detected in small cell lung cancer (SCLC) ($n = 865$). *ITGAV* expression made it feasible to distinguish cancers from non-cancers (e.g., for SCLC and non-SCLC, AUC = 0.88, sensitivity = 0.78, specificity = 0.84), and represented a risk role in cancers' prognosis ($p < .05$).

Conclusion: *ITGAV* served as a potential marker for prognosis and identification of cancers including SCLC.

1. Introduction

Lung cancer is one of the most common cancers. Past research predicted that there would be more than 2,200,000 newly diagnosed lung cancer patients in 2020 worldwide, accounting for more than 10% of cancer cases[1, 2]. With an estimated 1,800,000 deaths in 2020, lung cancer is the leading cause of cancer death[1, 2]. Although several clinical management approaches have been applied, including surgery, chemotherapy, and molecular targeted therapy, lung cancer's five-year survival rate is still just 10–20%[2]. Nearly 13–15% of lung cancer cases are small cell lung cancer (SCLC)[3], whose two-year survival has remained nearly unchanged (from 14% in 2009 to 15% in 2014) in America[1]. There is an urgent need to identify possible markers for distinguishing and treating SCLC.

SCLC is characterized by rapid growth and early metastasis[4, 5], and exploring markers from this perspective is likely to be a feasible strategy. Integrins regulate the localization and activity of proteolytic enzymes that promote cancer cell invasion and migration during tumor progression and metastasis. Integrin subunit alpha V (*ITGAV*) belongs to the integrin alpha chain family, and its encoded product is one component of integrins, which can bind with five integrin β subunits ($\beta 1$, $\beta 3$, $\beta 5$, $\beta 6$, and $\beta 8$)[6, 7]. Data from several studies suggest that *ITGAV* is highly expressed in several cancers, and demonstrates risk roles in proliferation and migration of cancer cells[8, 9]. However, no report on *ITGAV* and SCLC has been found in the literature, which makes it worth researching.

Based on many public and in-house samples, and for the first time, this study attempts to explore the expression, clinical significance, and underlying mechanisms of *ITGAV* in SCLC. With regard to conspicuous clinical values of *ITGAV* in SCLC, extensive pan-cancer analysis was also performed, contributing to the understanding of *ITGAV*'s pathology mechanisms and its potential application value in cancers.

2. Materials And Methods

The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, China.

2.1. Collection of samples and performance of immunohistochemistry

Datasets (microarrays and RNA sequencing) for SCLC-related analyses were screened in several public databases, including ArrayExpress, Oncomine, Gene Expression Omnibus, and the GDC Data Portal. Strategies for searching datasets were: “(lung or bronch*) and (small cell) and (mRNA or gene).” The included criteria for datasets were: (1) *homo sapiens*-related cohort; (2) lung/bronchus tissues or cells; and (3) mRNA expression profile including *ITGAV* expression. The exclusion criteria were: (1) duplicate and/or incomplete expression profile, and (2) less than three samples in a combined dataset.

Clinical samples were collected and sliced for immunohistochemical detection of the ITGAV protein at the First Affiliated Hospital of Guangxi Medical University. Then, in-house samples were used to perform immunohistochemistry. The antibody—rabbit monoclonal to ITGAV (EPR16800)—applied in the study was purchased from Abcam plc. Immunohistochemical experiments were carried out according to the manufacturer’s instructions. Details about experimental methods and protein level scoring criteria can be found in our previous study[10].

For pan-cancer analyses, a dataset and its associated clinical information from the Xena database (developed by the University of California, Santa Cruz) was utilized in the study. Six types of samples (normal tissue, normal solid tissue, primary tumor, primary solid tumor, and primary blood derived cancer), bone marrow, and primary blood derived cancer (peripheral blood) were included for further research. Abbreviations of cancers involved in the pan-cancer dataset are listed in **Supplementary material 1**.

2.2. Data standardization and elimination of batch effects between various datasets

All mRNA expression-associated data were transformed by $\log_2(x + 1)$. Datasets with the same platform (e.g., GSE32036 and GSE4127 from GPL6884) were merged for a combined dataset after eliminating batch effects[11–14], which was similar to the published study[10].

2.3 The expression of *ITGAV* in SCLC

Wilcoxon tests and a pooled standardized mean difference (SMD) were applied to assess the differential *ITGAV* expression between the SCLC group and the non-SCLC control group. A random effect model was used when there was significant heterogeneity between the datasets, which was judged by an I^2 value of $> 50\%$ and/or a chi-square test p -value of < 0.1 . Otherwise, a fixed effect model was used for the SMD calculation. The SMD value had statistical significance only when its 95% confidence interval excluded

zero. A Begg's test was used to detect whether there was obvious publication bias (evaluated by $p > 0.1$) for the SMD analysis.

2.4 The clinical significance of *ITGAV* expression in SCLC

Wilcoxon tests were applied to identify whether *ITGAV* expression was relevant to the clinical features of SCLC patients. Kaplan-Meier curves and univariate Cox analysis were used to detect prognosis values of *ITGAV* expression in SCLC. In Kaplan-Meier curves, the optimal cut-point (using survminer software package) was used for classification of high-*ITGAV* and low-*ITGAV* expression. Receiver operating characteristic curves (ROCs) and a summary ROC were used to detect the ability of *ITGAV* expression in distinguishing SCLC from non-SCLC, which was judged by the area under the curve (AUC) values (ranging from 0 to 1).

2.5 The underlying mechanisms resulting in low-*ITGAV* expression in SCLC

A gene with a \log_2 (fold change) ≥ 1 through the limma package [15–17] and SMD < 0 , was identified as having low-expression genes (LEGs) in SCLC. A gene that was positively related to *ITGAV* expression (Pearson coefficient ≥ 3 , $p < 0.05$) in $> 30\%$ (4/12) datasets was considered to have *ITGAV*-positively-related genes (*ITGAV*-PRGs). Based on ChIP-Seq data, the Cistrome data browser was feasible to predict TFs possibly regulating *ITGAV* expression. A TF encoded by a gene with triple identity—LEG, *ITGAV*-PRG, and predict TF—was considered a potential TF regulating *ITGAV* expression.

2.6 The potential mechanisms of *ITGAV* in SCLC

Based on *ITGAV*-related LEGs, underlying mechanisms of *ITGAV* expression in SCLC were explored using ontology terms and signaling pathways. Ontology terms in the study included disease ontology and gene ontology, while pathways were from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome. Gene set enrichment analysis (GSEA) for GO and KEGG was also carried out to verify findings from term and pathway analyses.

To detect associations between *ITGAV* expression and tumor immune microenvironment (TME), the relationship between *ITGAV* expression and immune infiltration levels (using Pearson coefficient) was explored based on the ESTIMATE algorithm[17–20] and CIBERSORT algorithm[12, 21, 22]. Immune checkpoints were promising in the treatment of cancers[23], and thus, the study also evaluated their associations with *ITGAV* expression to preliminarily assess the immunotherapy potential of *ITGAV* in SCLC.

2.7 Statistical pan-cancer analyses

The SMD was applied in SCLC-related rather than pan-cancer-associated calculations, as there was just one dataset for each cancer in the pan-cancer dataset and it was not suitable for performing SMD evaluation. Except for SMD, TF prediction, and enrichment analyses, statistical methods used in pan-

cancer analyses were the same as in research on SCLC. All data for pan-cancer analyses are shown in **Supplementary material 2**.

2.8 Statistical analysis

In this study, unless there was an additional explanation, $p < 0.05$ indicates statistical significance. Violin plots, forest plots, Kaplan-Meier curves, ROCs, box plots, GO plots, and heatmaps were generated by a series of Bioconductor packages[24, 25] in R software (v4.1.0).

3. Results

3.1 Overview of samples included in the study

For SCLC, 28 datasets from public databases were finally selected and divided into 12 combined datasets based on the same platforms. The 12 datasets contained 363 SCLC samples and 532 non-SCLC control samples. Also, 26 SCLC and 29 non-SCLC in-house samples were included for exploring *ITGAV* protein levels in SCLC. Thus, 1,022 samples (n of SCLC = 389, n of non-SCLC = 561) were eventually selected for research of *ITGAV* on SCLC. Selection processes and details of SCLC-related samples can be seen in **Supplementary materials 3–4**.

In analyses of pan-cancer, *ITGAV* expression in 26 cancers (with not less than three samples for each cancer) were explored, involving 21,989 samples (n of cancer = 11,537; n of non-cancer = 10,452). Then, 12,106 samples (in 39 cancers) with OS information and 5,666 samples (in 32 cancers) with DFI data were utilized for survival analyses (**Supplementary material 2**).

3.2 *ITGAV* expression in SCLC and non-SCLC

Compared to the non-SCLC group, *ITGAV* mRNA expression was downregulated in the SCLC group, which was reported by 8/12 datasets included in the study (*Wilcoxon* test, $p < .05$, Fig. 1A). The consistent conclusion was also sustained by the random effects model (SMD = - 1.05, 95% CI did not contain 0, Fig. 1B), and no significant publication bias was detected for SMD results (*Begg's* test, $p = .891$, Fig. 1C). At the protein level, positive *ITGAV* expression was detected in SCLC tissues rather than non-SCLC tissues (Figs. 2A–L), and as a result, higher *ITGAV* protein levels were found in SCLC tissues (*Wilcoxon* test, $p < .001$, Fig. 1D).

3.3 The clinical significance of *ITGAV* expression in SCLC

3.3.1 The correlation between *ITGAV* expression and clinical parameters

No statistical correlation between *ITGAV* expression and TNM stage, clinical stage, or age was found in SCLC (data not shown), indicating that the expression of *ITGAV* in SCLC was likely not affected by these factors and that *ITGAV* was a factor independent of these indicators.

3.3.2 The prognosis values of *ITGAV* expression in SCLC

As seen in Figs. 2M–N, high-*ITGAV* expression was associated with unfavorable overall survival and disease-free survival, and the latter was statistically significant. Therefore, *ITGAV* expression represented a risk role in SCLC prognosis.

3.3.3 The identification effects of *ITGAV* expression in SCLC

With mRNA expression of 12 datasets (containing 895 samples), *ITGAV* demonstrated conspicuous effects in distinguishing SCLC from non-SCLC (AUC = 0.88, sensitivity = 0.78, specificity = 0.84; Fig. 2O), suggesting its potential in identifying SCLC.

3.4 The underlying mechanisms resulting in low-*ITGAV* expression in SCLC

After calculation, 3,629 LEGs were identified, with the absolute value of \log_2 (fold change) ≥ 1 and SMD < 1 (data not shown). Also, 525 genes were considered *ITGAV*-PRGs in $> 30\%$ (4/12) datasets (data not shown). In the Cistrome data browser, 74 predicted TFs likely regulated *ITGAV* expression, and 423 *ITGAV*-positively-related low-expression genes (*ITGAV*-PRLEGs) were merged from LEGs and *ITGAV*-PRGs. Through the intersection of LEGs, *ITGAV*-PRGs, and predicted TFs, three TFs—ZEB2, IK2F1, and EGR2—were screened (Fig. 3A). All three TFs were considered potential TFs regulating *ITGAV* expression, as they had ChIP-Seq peaks upstream of the transcription start site of *ITGAV* (Fig. 3B).

3.5 Potential mechanisms of *ITGAV* in SCLC

3.5.1 Enrichment analyses of *ITGAV*-PRLEGs in SCLC

Through disease ontology, *ITGAV*-PRLEGs were associated with lung diseases, small cell lung carcinoma, and non-small cell lung carcinoma (Fig. 4A). They tend to participate in the composition of “collagen-containing extracellular matrix,” “focal adhesion,” and “endocytic vesicle” (cell components); are involved in biological processes such as “response to interferon-gamma,” “neutrophil mediated immunity,” and “extracellular matrix organization”; and affect multiple molecular functions (e.g., “integrin binding,” “collagen binding,” and “growth factor binding”).

ITGAV-PRLEGs clustered in immune and virus-related KEGG pathways, some examples of which were “phagosome,” “Th1 and Th2 cell differentiation,” and “Coronavirus disease—COVID-19” (Fig. 4B). *ITGAV*-PRLEGs also aggregated in immune and ECM-associated Reactome pathways (Fig. 4B). Thus, a close relationship between *ITGAV*-PRLEGs and immunity can be seen. This finding was also supported by GSEA results (Figs. 5A–B).

3.5.2 Analysis of the relationship between *ITGAV* expression and TME

Based on the ESTIMATE algorithm, *ITGAV* expression in SCLC was positively related to stromal scores and immune scores (Fig. 5C), although there was no statistical significance for ESTIMATE score and tumor purity (data not shown).

To further explore the relevance of *ITGAV* expression with immune cell infiltration levels, the CIBERSORT algorithm was applied. As a result, *ITGAV* expression was negatively related to activated immune infiltration levels of several immune cells (e.g., CD8 T cells, M2 macrophages, and active mast cells) and positively associated with resting immune cells (e.g., memory CD4 T cells, and dendritic cells; Fig. 5D). However, such findings were seen in the non-SCLC group rather than the SCLC group, as no statistical significance was detected for the latter (Fig. 5D). *ITGAV* showed close correlation with immune stroma and immune cells, to some extent suggesting its potential mechanisms in SCLC.

3.6. *ITGAV* in pan-cancer

With regard to differently expressed *ITGAV* and its clinical significance in SCLC, we attempted to perform a similar exploration in pan-cancer analysis, which could contribute to the understanding and application of *ITGAV* in cancers.

3.6.1 The expression of *ITGAV* between SCLC and non-SCLC

Various expressions can be seen between cancer and non-cancer groups. Elevated *ITGAV* expression was found in 19 cancers, such as glioblastoma multiforme, glioma, and brain lower grade glioma (LGG; Fig. 6A). Such a phenomenon also existed in LUAD and LUSC (both belonged to NSCLC; Fig. 6A), contrary to SCLC. Eight cancers (e.g., uterine corpus endometrial carcinoma) were found with low-*ITGAV* expression in cancer groups rather than non-cancer groups (Fig. 6A).

3.6.2 The correlation between *ITGAV* expression and clinical parameters

For most of the 39 cancers, there was no statistical correlation between *ITGAV* expression and TNM stage or clinical stage, except for adrenocortical carcinoma, LUAD, sarcoma, thyroid carcinoma, and LGG (Fig. 6B). This revealed that *ITGAV* expression was an independent factor in SCLC.

3.6.3 The clinical significance of *ITGAV* expression in pan-cancer

In the 39 cancers explored in the study, *ITGAV* expression was relevant to the overall survival of 9/39 cancers: LGG, glioma, liver hepatocellular carcinoma (LIHC), mesothelioma, stomach adenocarcinoma, pancreatic adenocarcinoma (PAAD), stomach, and esophageal carcinoma, LUAD, and kidney renal papillary cell carcinoma ($p < 0.05$; Fig. 6C). Interestingly, *ITGAV* expression consistently played risk roles in all nine cancers, indicating the risk factor of *ITGAV* for cancer patients' overall survival. *ITGAV* expression was also identified as a risk factor for PAAD's disease-free survival ($p < 0.05$; Fig. 6D), although this was not seen in other cancers.

Similar to in SCLC, *ITGAV* expression showed significantly distinctive effects between cancers and non-cancers, particularly for cholangiocarcinoma, esophageal carcinoma, glioblastoma multiforme, glioma, acute myeloid leukemia, LGG, PAAD, and high-risk Wilms tumors (all AUCs > 0.9; Fig. 7). AUC values for other cancers can be seen in **Supplementary material 5**. In a summary ROC for all 34 cancers, AUC was up to 0.86, suggesting that *ITGAV* expression has the potential to screen cancers (Fig. 7).

3.6.4 Immune-related analyses of *ITGAV* in pan-cancer

Through analyses of immune infiltration levels of pan-cancer, the *ITGAV* expression in 11 cancers was relevant to stromal score, immune score, and ESTIMATE score (Pearson correlation coefficient >0.2, $p < .05$; Table 1), and the first three of them were COAD, COADREAD, and KIPAN (Fig. 8A). Interestingly, no negative associations, but consistent positive relationships, were observed in the series of findings.

Table 1
Pearson correlation of *ITGAV* expression and immune infiltration levels in cancers.

Cancers	Stromal score		Immune score		ESTIMATE score	
	Correlation coefficient	<i>p</i>	Correlation coefficient	<i>p</i>	Correlation coefficient	<i>p</i>
GBM	0.239	0.003	0.275	0.001	0.269	0.001
GBMLGG	0.244	< 0.001	0.231	< 0.001	0.243	< 0.001
LGG	0.248	< 0.001	0.21	< 0.001	0.231	< 0.001
LUAD	0.413	< 0.001	0.207	< 0.001	0.325	< 0.001
COAD	0.762	< 0.001	0.593	< 0.001	0.722	< 0.001
COADREAD	0.747	< 0.001	0.574	< 0.001	0.705	< 0.001
KIPAN	0.51	< 0.001	0.268	< 0.001	0.403	< 0.001
READ	0.71	< 0.001	0.535	< 0.001	0.668	< 0.001
PAAD	0.573	< 0.001	0.339	< 0.001	0.479	< 0.001
OV	0.343	< 0.001	0.272	< 0.001	0.332	< 0.001
BLCA	0.404	< 0.001	0.264	< 0.001	0.356	< 0.001

Notes: GBM, glioblastoma multiforme; GBMLGG, glioma; LGG, brain lower grade glioma; LUAD, lung adenocarcinoma; COAD, colon adenocarcinoma; COADREAD, colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma; KIPAN, pan-kidney cohort; rectum adenocarcinoma; PAAD, pancreatic adenocarcinoma; OV, ovarian serous cystadenocarcinoma; BLCA, bladder urothelial carcinoma.

Based on the CIBERSORT algorithm, the relevance between *ITGAV* expression and multiple immune cells is evident. Positive associations (Pearson correlation coefficient >0.2 , $p < .05$) of *ITGAV* expression with resting CD4 memory T cells, M1 macrophages, and M2 macrophages were observed at least 10 cancers (Fig. 8B). At ≥ 10 cancers, negative relationships can be observed (Pearson correlation coefficient ≤ -0.2 , $p < .05$) of *ITGAV* expression with memory B cells, CD8 T cells, follicular helper T cells, regulatory T cells,

and activated NK cells (Fig. 8B). In addition, *ITGAV* expression was related to many immune checkpoints' expression in a series of cancers (Fig. 8C), and revealed the immunotherapy potential of *ITGAV*.

4. Discussion

Using large samples from multiple sources, this study comprehensively identified different *ITGAV* expression in SCLC and revealed the conspicuous prognosis and identification values of *ITGAV* expression in this disease. Several TFs that may regulate *ITGAV* expression in SCLC were also identified, which has not been reported before. Focusing on TME, the study explored the molecular mechanisms of *ITGAV* in SCLC. Further, the extensive pan-cancer analysis, with tens of thousands of samples, verified differential expression of *ITGAV* and its clinical significance in multiple cancers.

Differential expression of *ITGAV* in cancer is common, and its high expression is predominant. Upregulated *ITGAV* expression has been identified in multiple cancers, such as gastric cancer cells[7], breast cancer[8], and hepatocellular carcinoma[9]. Downregulated *ITGAV* expression has also been emphasized in epithelial ovarian cancer[26]. Thus, it is necessary to comprehensively explore *ITGAV* expression in many cancers. In this study, among 35 cancers (including SCLC), 80% (28/35) demonstrated different expression levels between cancer groups and non-cancer groups, and high expression was observed in 68% (16/28) of the latter. One point that should be noted was that low-*ITGAV* mRNA and high-*ITGAV* protein levels were detected. These seemingly inconsistent results were in fact reasonable, since (1) polypeptides can be produced by an mRNA molecule, but their translation rates may vary in space and time; (2) mRNA molecules from the same gene may feasibly produce various amounts of protein due to translational heterogeneity[27]; (3) the protein product of a gene tended to be more stable than its mRNA, as the latter degrades more easily[28]. Therefore, inconsistent *ITGAV* mRNA and protein levels are still rational in some conditions.

Low-*ITGAV* mRNA expression in SCLC likely resulted from downregulated expression of three TFs—ZEB2, IK2F1, and EGR2. The three TFs may be regulators of *ITGAV* expression, as they not only demonstrated decreased expression and close positive relationships with *ITGAV* expression in SCLC, but also had ChIP-Seq peaks upstream of the transcription start site of *ITGAV*. Previous reports have demonstrated that some of the three TFs are important factors in SCLC. For instance, Wang et al.[29] found that ZEB2 participated in promoting the occurrence of extracellular matrix in SCLC, thus contributing to the progression of the disease. However, to the best of our knowledge, no relevant research on the regulation of ZEB2, IK2F1, and EGR2 for *ITGAV* exists, which to some extent indicates the novelty of this study.

ITGAV expression demonstrated conspicuous clinical significance in quite a few cancers. The gene has been recognized as having an important risk role in cancer progression. For example, Cheuk et al.[8] showed high-*ITGAV* expression causing breast cancer metastasis; Kemper et al.[30] identified that *ITGAV* expression contributed to PAAD; Loeser et al.[6] revealed the relationship between *ITGAV* expression and unfavorable prognosis for patients with esophageal adenocarcinoma. However, no similar research has

been reported for SCLC. Although reduced *ITGAV* mRNA expression was detected in SCLC, we believe that the roles of *ITGAV* in SCLC resulted from its high protein levels for several reasons.

First, a gene's function is usually due to its coding protein rather than its mRNA[27]. Second, its association with SCLC's poor prognosis (found in the current study) supported *ITGAV* as a risk factor in SCLC. Further pan-cancer analyses demonstrated that *ITGAV* expression was related to poor OS of patients with LGG, glioma, LIHC, mesothelioma, stomach adenocarcinoma, PAAD, stomach, and esophageal carcinoma, LUAD, and kidney renal papillary cell carcinoma; moreover, it was also associated with shorter disease-free survival of PAAD patients. Among these cancers, *ITGAV* has been identified as a risk factor in LIHC[9], mesothelioma[31], and PAAD[30], while no reports about the remaining six cancers indicated novelty of our study. The finding that *ITGAV* expression makes it feasible to distinguish multiple cancer tissues from their controls suggests the potential of *ITGAV* expression in screening cancers. *ITGAV* may serve as an essential marker of prognosis and identification of multiple cancers.

Through disease ontology, *ITGAV*-PRLEGs are involved in some diseases, including small cell lung carcinoma and non-small cell carcinoma, suggesting associations between *ITGAV* and lung cancers. For *ITGAV*-PRLEGs, the keywords for gene ontology were "adhesion," "extracellular matrix," and "immunity." Cell-cell interactions and cell adhesion are key mediators of lung cancer progression, including immune evasion and metastatic events[32]. Integrins take part in cell surface adhesion and signaling and have essential functions in cancer progression. The protein product encoded by *ITGAV* is a subunit of integrins (the other is the β subunit), and thus participates in cancer development[6, 7]. For example, upregulated *ITGAV* stimulated the synergistic effect of integrin and selectin, which promoted adhesion between PAAD and peritoneal mesothelial cells, finally leading to the growth of pancreatic cancer[30]. It can be seen that the classic role of *ITGAV* in cell adhesion may be one of its potential mechanisms in SCLC. Little is known about its immune-related role in cancers, although this finding was clear in this study.

ITGAV's roles in cancers may be linked to TME. In our study, signaling pathways of KEGG, Reactome, and GSEA consistently demonstrated that *ITGAV*-PRLEGs were clustered in immune-related pathways. Moreover, *ITGAV* expression was positively associated with scores for all immune stromal cells, immune cells, and estimated scores (tumor purity), suggesting its close associations with TME. Of the multiple components of TME, immune cells were recognized as predominant factors in regulating cancer progression[33]. Positive associations between *ITGAV* expression and resting CD4 memory T cells, M1 macrophages, and M2 macrophages were observed in more than 10 cancers. Macrophages demonstrated a dual role in tumor progression. On one hand, based on proinflammatory cytokines and cytotoxic activities, they tend to inhibit tumor growth; on the other hand, they are more likely to stimulate tumor proliferation, angiogenesis, and metastasis, and as a result trigger tumor progression[33]. Thus, a positive correlation between *ITGAV* expression and macrophage infiltration levels may support its relationship with poor prognosis in cancer patients.

More importantly, negative relationships between *ITGAV* expression and infiltration levels of memory B cells, CD8 T cells, and activated NK cells were observed. Interestingly, both CD8 T cells and activated NK

cells were prominent factors (particularly CD8 T cells) in controlling tumor progression[34, 35], and their negative relationship with *ITGAV* supported the risk roles *ITGAV* played in most of the cancers. However, the immune-related mechanism of *ITGAV* is complex, which is indicated in the negative correlation of *ITGAV* with memory B cells, follicular helper T cells, and regulatory T cells (the three were considered to play a dual role in immune response)[35]. Taken together, the correlation between *ITGAV* and immune response (at least with immune cell infiltration levels) is clear, but more efforts are needed to investigate the mechanism.

ITGAV has potential for immunotherapy, and immune checkpoint inhibitor therapy is one of the most promising cancer treatment methods available[36]. Studies have shown that immune checkpoints are thought to be involved in immunosuppression, thereby preventing immune cells from eliminating cancer cells. Dysregulated expression of checkpoints in the TME is common[37]. *ITGAV* is related to a variety of immune checkpoints and is mainly positively correlated, which suggests its potential for use in immunotherapy.

Some limitations of this study should be noted: (1) there was a lack of in-house samples for exploring *ITGAV* protein levels in pan-cancer; (2) no pure body fluid samples were used to verify the ability of *ITGAV* to screen cancer; and (3) the complicated molecular mechanisms of *ITGAV* on cancers (such as immune infiltration levels) still need experimental verification in future research.

5. Conclusion

In summary, diverse *ITGAV* expression (mainly upregulated) in multiple cancers, its clinical significance, and its potential molecular mechanisms in dozens of cancers were identified in the study. *ITGAV* expression served as a risk factor for patient prognosis and distinct effects for patients with some cancers. *ITGAV* may play a role in cancers by participating in immunity-related signaling pathways and influencing the infiltration levels of several immune cells. This study provided useful insights for a better understanding of the pathogenesis mechanism of *ITGAV* in cancers, and *ITGAV* as a potential marker for cancer prognosis and identification.

Future Perspective: Globally, cancer is one of the most common factors of human health and survival. Tumor patients benefit from the current medical methods. Unfortunately, treatments for quite a few cancers (e.g., small cell lung carcinoma) are limited, resulting in poor survival prognosis of patients. Exploring potential markers for screening and treatment of cancers is necessary in cancers research and promising in cancers clinical management. This study comprehensively identified different *ITGAV* expression in small cell lung carcinoma and other cancers. It also revealed the conspicuous prognosis and identification values of *ITGAV* expression in multiple cancers, suggesting *ITGAV*'s potential in cancer prognosis and identification.

Declarations

Ethics approval and consent to participate: Ethical approval was obtained from the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, China. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication: Not applicable.

Availability of data and materials: Datasets from public databases can be obtained from SangerBox (<http://vip.sangerbox.com/>), ArrayExpress (<https://www.ebi.ac.uk/arrayexpress/>), GEO (<https://www.ncbi.nlm.nih.gov/gds/?term=>), GTE-x (<https://www.gtexportal.org/home/index.html>), and the TCGA Research Network (www.cancer.gov/tcga) with dataset IDs (e.g., GSE32036). Data contained in in-house tissues can be obtained from the corresponding author.

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Figures

Figure 1

The expression of ITGAV in small cell lung carcinoma (SCLC). Panel A: Violin plots of ITGAV expression in SCLC. Panel B: A forest plot of evaluating standard mean difference (SMD) of ITGAV expression between SCLC and non-SCLC groups. Panel C: A funnel plot with Begg's test for publication bias test. Panel D: A violin plot of ITGAV protein levels between SCLC and non-SCLC groups. TMA, tissue microarray.

Figure 2

ITGAV protein levels, and the clinical significance of ITGAV expression in small cell lung carcinoma (SCLC). Panels A–L: The protein levels of ITGAV in non-SCLC (A–F) and SCLC (G–L) tissues under the microscope by in-house tissue microarrays. The left figure of each two combined figures is 200x, and the right figure is 400x. Panel M: A Kaplan-Meier curve of overall survival between high- and low- ITGAV expression groups. Panel N: A Kaplan-Meier curve of disease-free survival between high- and low-ITGAV expression groups. Panel O: Summary receiver operating characteristic curve for identifying small cell lung carcinoma based on ITGAV expression. SENS, sensitivity; SPEC, specificity; AUC, area under the curve.

Figure 3

Identification of potential transcription factors regulating ITGAV expression. A: Veen plots for screening predicted transcription factors for ITGAV. B: For the three transcription factors, there existed binding sites with the potential promoter region of ITGAV.

Figure 4

Disease Ontology terms, Gene Ontology terms, Kyoto Encyclopedia of Genes and Genomes and Reactome pathways of ITAGV-positively-related downregulated expression genes. DO: Disease Ontology, CC: cellular component; BP: biological process; MF: molecular function.

Figure 5

Gene set enrichment analysis (GSEA), and immune infiltration levels analyzes. Panels A–B: GSEA for Kyoto Encyclopedia of Genes and Genomes (panel A), and Reactome pathways (panel B) of ITGAV-positively-related downregulated expression genes. Panels C–D: Analyzes of immune infiltration levels based on ESTIMATE (panel C) and CIBERSORT (panel D) algorithms. SCLC, small cell lung carcinoma.

Figure 6

The expression of ITGAV, and its associations with clinical parameters and prognosis in pan-cancer. Panel A: The expression of ITGAV mRNA expression in pan-cancers. * $p < 0.05$. Panel B: Associations of ITGAV in clinical parameters in multiple cancers. Panel C: The correlation of ITGAV expression with the overall survival of cancers patients. Panel D: The correlation of ITGAV expression with the disease-free survival of cancers patients.

Figure 7

Receiver operating characteristic curves and a summary receiver operating characteristic curve for identifying cancers from non-cancers based on ITGAV expression. SENS, sensitivity; SPEC, specificity; AUC, area under the curve.

Figure 8

The association of ITGAV expression with immune infiltration level, in pan-cancer and its correlation with the overall survival of cancers patients.

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