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ALOX5可作为与免疫细胞浸润相关的非小细胞肺癌预后生物标志物

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[摘 要] **目的：**肺癌免疫治疗疗效与免疫细胞浸润密切相关。花生四烯酸5-脂氧合酶(arachidonic acid 5-lipoxygenase，ALOX5)可激活炎症反应并触发各种细胞死亡模式；然而，ALOX5与肺癌免疫细胞浸润的相关性尚不清楚。本研究利用在线数据库分析ALOX5在非小细胞肺癌(non-small cell lung cancer，NSCLC)中的表达，探讨其与NSCLC免疫细胞浸润的相关性及其与预后的关系。**方法：**分析TIMER、GEPIA和UALCAN等在线数据库中NSCLC和正常组织*ALOX5* mRNA和蛋白表达的差异；应用Kaplan-Meier数据库探讨ALOX5的预后价值，GeneMANIA和String网站探索与*ALOX5*基因表达相关联的基因及蛋白质；对TCGA数据库挖掘出的差异基因进行基因本体(Gene Ontology，GO)和京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes，KEGG)富集分析；应用基因集富集分析(gene set enrichment analysis，GSEA)软件预测ALOX5可能参与的信号通路，TIMER数据库分析ALOX5对免疫细胞浸润水平的影响。**结果：**与正常肺组织相比，*ALOX5*在NSCLC组织中呈低表达(P<0.05)，且影响NSCLC患者预后。基因互作网络分析发现：与*ALOX5*基因相互作用的基因主要有毛状样蛋白(coactosin like protein 1，*COTL1*)、白三烯C4合酶(leukotriene C4 synthase，*LTC4S*)和环加氧酶2(prostaglandin endoperoxide synthase 2，*PTGS2*)等脂质氧化和促炎介质生成的相关基因，蛋白质-蛋白质相互作用(protein-protein interaction，PPI)网络分析结果与之一致。GO、KEGG富集分析发现：*ALOX5*基因参与多种免疫细胞功能活化的生物过程，并参与免疫反应功能通路。GSEA结果显示*ALOX5*可能通过影响细胞因子与细胞因子受体的相互作用、自然杀伤细胞介导的细胞毒性和T细胞受体等信号通路，从而激活免疫反应，介导与免疫相关的预后。肺腺癌及肺鳞状细胞癌中*ALOX5* mRNA表达与肿瘤浸润性免疫细胞(B细胞、$CD8^+$T细胞、$CD4^+$T细胞等)浸润水平均呈正相关(均P<0.05)，并且与经典的T细胞免疫检查点抑制剂基因标志物呈正相关(P<0.001)。**结论：***ALOX5*基因在NSCLC中表达显著下调，可影响NSCLC预后和肿瘤免疫细胞浸润水平。*ALOX5*基因可能是潜在的与免疫细胞浸润相关的NSCLC预后生物标志物。

[关键词] 花生四烯酸5-脂氧合酶；非小细胞肺癌；预后生物标志物；免疫细胞浸润水平

Role of ALOX5 in non-small cell lung cancer: A potential therapeutic target associated with immune cell infiltration

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ABSTRACT

Objective: The efficacy of immunotherapy for lung cancer is closely related to immune cell infiltration. Arachidonic acid 5-lipoxygenase (ALOX5) can activate inflammatory responses and trigger various cell death patterns; however, the relevance of ALOX5 to immune cell infiltration in lung cancer is unclear. The expression of ALOX5 in non-small cell lung cancer (NSCLC) is analyzed using an online database to explore the correlation between ALOX5 and immune cell infiltration in NSCLC and its relationship with prognosis.

Methods: Differences in *ALOX5* expression in NSCLC and normal lung tissues were analyzed by online databases such as TIMER, GEPIA and HPA; the UALCAN database was used to reveal the relationship between ALOX5 and clinical features; Kaplan-Meier database was applied to explore the prognostic value of ALOX5; GeneMANIA and String Website was used to explore genes and proteins associated with *ALOX5* expression, respectively; the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were used to analyze *ALOX5* differential genes which were picked up through the TCGA database; GSEA software was applied to predict the signal pathways that *ALOX5* may be involved in; and the TIMER database was used to analyze the effect of ALOX5 expression on the level of immune cell infiltration.

Results: Compared with the normal lung tissues, the *ALOX5* expression was low in NSCLC tissues (P<0.05), and which affected the prognosis of lung cancer patients. The expression level of *ALOX5* was related to clinical features such as sex, age, metastasis, and pathological staging in NSCLC patients (all P<0.05). The gene interaction network analysis found that the genes interacting with *ALOX5* mainly included the genes related to lipid oxidation and pro-inflammatory mediators such as coactosin like protein 1 (*COTL1*), leukotriene C4 synthase (*LTC4S*), and prostaglandin endoperoxide synthase 2 (*PTGS2*), and the protein-protein interaction analysis results were consistent. GO and KEGG analysis found that *ALOX5* was involved in the biological process of activation of immune cell function and was involved in immune response function pathways. The GSEA analysis showed that *ALOX5* may activate immune responses and mediate immune-related prognosis by affecting the cytokine-cytokine receptor interactions, natural killer-mediated cytotoxicity, and T cell receptor signaling pathways. The *ALOX5* mRNA expressions in lung adenocarcinoma and lung squamous cell carcinoma were positively correlated with the tumor infiltration immune cells (B cells, $CD8^+$ T cells, $CD4^+$ T cells, etc.) (all P<0.05), and the *ALOX5* mRNA expression was positively correlated with the expression of classic T cell immune checkpoint inhibitor genes (P<0.001).

Conclusion: The *ALOX5* gene expression in NSCLC is significantly downregulated, and which can affect NSCLC prognosis and immune cell infiltration levels. *ALOX5* gene may be a potential biomarker of NSCLC prognosis associated with immune cell infiltration.

KEY WORDS arachidonic acid 5-lipoxygenase; non-small cell lung cancer; prognostic biomarkers; immune cell infiltration level

肺癌是全球癌症相关死亡的主要原因，全球癌症统计数据显示2020年肺癌新发病例达220.7万，仅次于乳腺癌；死亡例数为179.6万，位居各种癌症之首[1-2]。肺癌分为非小细胞肺癌(non-small cell lung cancer，NSCLC)和小细胞肺癌(small cell lung cancer，SCLC)，其中以NSCLC为主，约占85%。NSCLC主要病理类型有肺腺癌(lung adenocarcinoma，LUAD)和肺鳞状细胞癌(lung squamous carcinoma，LUSC)[3]。近年来，靶向癌细胞的药物研发取得了一定的进展，但这些治疗方法仍然不能使晚期NSCLC患者获得理想的生存期[4]。肿瘤微环境(tumor microenvironment，TME)是指肿瘤中出现的非癌变细胞及其组分，包括它们产生和释放的分子[5]。肿瘤细胞与TME之间不断的相互作用在肿瘤的发生、发展、转移和对治疗的反应中起决定性的作用。研究[6]表明TME在患者特定治疗干预的反应方面也起关键作用，TME的免疫学分析可能可以预测预后和免疫治疗反应。

花生四烯酸5-脂氧合酶(arachidonic acid 5-lipoxygenase，ALOX5)是一种含铁的非血红素双加氧酶，可催化花生四烯酸(arachidonic acid，AA)等多不饱和脂肪酸的过氧化作用[7]，在炎症反应、细胞死亡及肿瘤发生、发展过程中具有重要作用。既往研究[8-12]发现：ALOX5可影响肿瘤的侵袭和迁移，在胃癌、结直肠癌、乳腺癌、慢性髓细胞白血病等癌症的发生、发展过程及临床预后中发挥重要的作用。另外，*ALOX5*已被证明是免疫相关基因，其基因多态性与肺癌患病风险增加相关[13]。然而，*ALOX5*基因在NSCLC发生、发展中的作用及其潜在的分子机制尚不清楚。本研究拟整合多种生物信息学方法，分析*ALOX5*基因在NSCLC中的表达及其对NSCLC预后的影响，并探讨其可能存在的分子机制，为NSCLC的诊治及预后判断提供新的依据。

1 资料与方法

1.1 使用的网站及数据库

GEPIA网站(http://gepia. cancer-pku. cn/index.html)：在GEPIA数据库中研究LUAD/LUSC与正常相邻肺组织样本*ALOX5*基因的表达情况。采用Spearman相关系数法进行相关分析，确定*ALOX5*基因与程序性细胞死亡蛋白-1(programmed death-1，PD-1)及其配体(PD-L1)、细胞毒T淋巴细胞相关抗原4(cytotoxic T lymphocyte-associated antigen-4，CTLA-4)的关系。

Tumor Immune Estimation Resource (TIMER)网站(https://cistrome. shinyapps. io/timer/)：利用TIMER数据库评估*ALOX5*基因在多种类型癌症中的表达。分析*ALOX5*基因与LUAD、LUSC中免疫细胞浸润的相关性。

基因本体(Gene Ontology，GO)和京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes，KEGG)富集分析、基因集富集分析(gene set enrichment analysis，GSEA)：通过GO和KEGG分析探讨*ALOX5*基因在肺癌中的生物学功能。利用GSEA研究*ALOX5*基因的潜在作用机制。GO、KEGG由R包ClusterProfiler执行。GSEA富集分析应用GSEA软件进行。

CIBERSORT(https://cibersort. stanford. edu/)网站：采用1个已验证的包含547个基因和22个人类免疫细胞亚群的白细胞基因签名矩阵来描述免疫细胞组成。通过CIBERSORT测量肿瘤浸润免疫细胞(tumor infiltrating immune cells，TIICs)的比例，并检查*ALOX5*基因表达和免疫细胞亚群之间的相关性。

Kaplan-Meier Plotter (http://kmplot.com)网站：包含3 452例临床肺癌患者基因表达数据和生存信息的在线数据库，可分析*ALOX5*基因在肺癌中的预后价值。

PrognoScan (http://www. abren. net/PrognoScan/)网站：通过PrognoScan数据库分析*ALOX5*基因表达与NSCLC生存率之间的相关性。

采用GeneMANIA(http://www.genemania.org)数据库构建*ALOX5*的基因-基因相互作用网络。利用STRING在线数据库(https://string-db. org/)构建ALOX5的蛋白质-蛋白质相互作用(protein-protein interaction，PPI)网络。

1.2 统计学处理

Kaplan-Meier、PrognoScan和GEPIA网站的结果显示为*HR*和*P*或Cox *P*值(来自log-rank检验)。基因表达差异采用独立样本*t*检验。基因表达的相关性采用Spearman相关分析。*ALOX5*基因与铁死亡相关基因(ferroptosis-related genes，*FRGs*)的相关热图由R软件包与Spearman相关的pheatmap生成。$P<0.05$为差异有统计学意义。

2 结　果

2.1 NSCLC患者*ALOX5*表达降低

通过TIMER数据库分析*ALOX5*基因在泛癌中的表达情况(图1A)。在GEPIA和UALCAN数据库中，*ALOX5* mRNA在LUAD和LUSC组织中的表达均低于正常肺组织(图1B、1C)。直接用TCGA数据库获得的数据显示：相较于邻近正常组织，*ALOX5*基因在LUAD和LUSC组织中表达均明显降低(均*P*<

0.001，图1D)。此外，在58对LUAD和50对LUSC肿瘤标本中，与相邻的正常组织相比，*ALOX5*基因在和LUSC中的表达均明显降低(均P<0.001，图1E)。

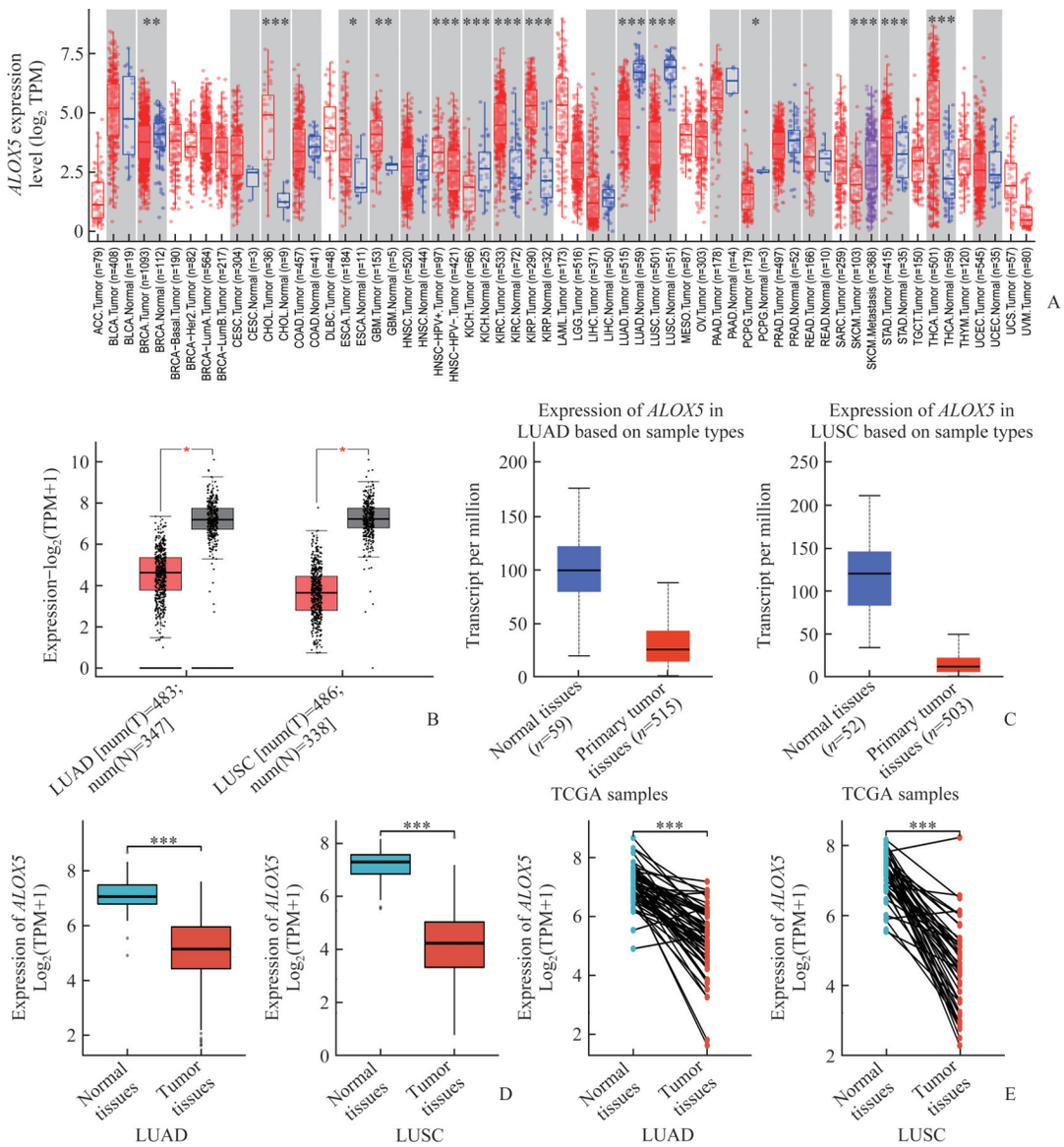

图1 肺癌中*ALOX5*的表达

Figure 1 Expression of *ALOX5* in lung cancer

A: Expression of *ALOX5* in different types of cancers is investigated in the TIMER database; B: Compared to the normal tissues, *ALOX5* expression is reduced in the lung cancer tissues in the GEPIA database; C: Expression of *ALOX5* in lung cancer is investigated in the UALCAN database; D: Expression analysis of *ALOX5* is analyzed in the lung cancer tissues and the normal tissues adjacent to the cancer in TCGA database; E: TCGA database and statistical analysis of *ALOX5* expression in 58 pairs of LUAD tissues and paraneoplastic normal tissues and 50 pairs of LUSC tissues and paraneoplastic normal tissues. *P<0.05, **P<0.01, ***P<0.001. *ALOX5*: Arachidonic acid 5-lipoxygenase gene; TPM: Transcripts per million; LUAD: Lung adenocarcinoma; LUSC: Lung squamous carcinoma; TCGA: The Cancer Genome Atlas; num(T): Number of tumor tissues; num(N): Number of normal tissues.

2.2 肺癌患者*ALOX5*表达降低与预后相关

KaplanMeier plotter数据库显示：*ALOX5*基因表达降低与LUAD患者较好的总生存期(overall survival，OS)和无进展生存期(progression-free survival，PFS)相关，而在LUSC患者中不相关(图2A)。另外，在LUAD和LUSC中均显示*ALOX5*基因表达高低与进展后生存期(post progression survival，PPS)无显著相关性(图2A)。此外，PrognoScan数据库显示：在GSE31210队列中，LUAD中*ALOX5*基因表达升高与较差的OS、无复发生存时间(recurrence

free survival，RFS)、疾病特异性生存期(disease-specific survival，DSS)显著相关，而在GSE4573队列中也显示NSCLC中*ALOX5*基因表达升高与较差的DSS显著相关(图2B)。为了更好地了解*ALOX5*基因表达在肺癌中的预后价值和潜在机制，利用Kaplan-Meier数据库探讨*ALOX5* mRNA表达与临床特征之间的关系。结果显示：在LUAD中，*ALOX5*高表达与较差的OS相关，而不论性别和吸烟与否，*ALOX5*高表达是OS和FPS的危险因素。

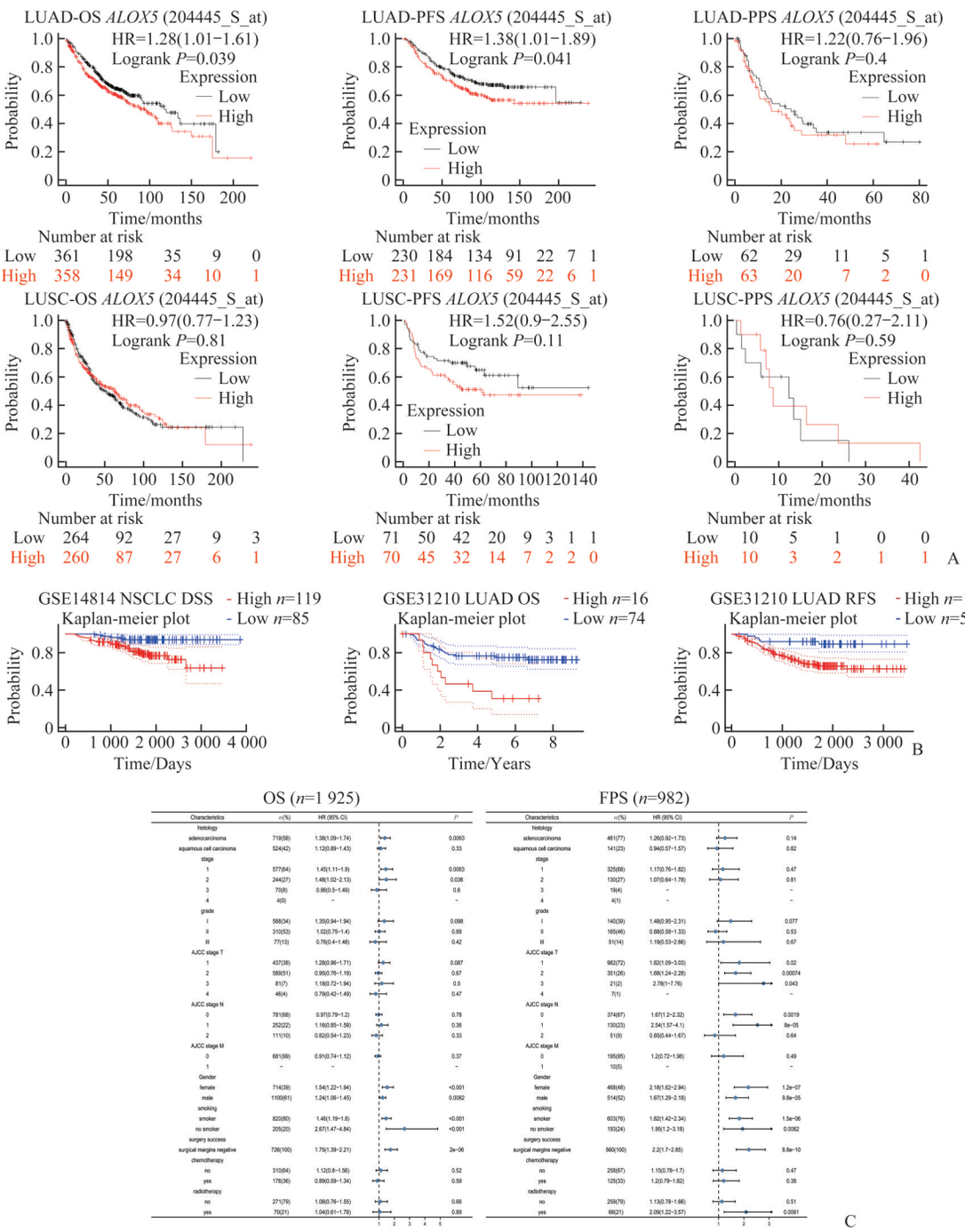

图2　评价*ALOX5*预后价值的生存曲线

Figure 2　Survival curves to evaluate the prognostic value of *ALOX5*

A: Survival curves of OS, PFS, and PPS for LUAD and LUSC by Kaplan-Meier plotter; B: Survival curves for DSS, OS, and RFS in the PrognoScan database; C: Correlation between *ALOX5* expression and the clinicopathological parameters in the LUAD and LUSC patients by forest plots. *ALOX5*: Arachidonic acid 5-lipoxygenase gene; LUSC: Lung squamous carcinoma; LUAD: Lung adenocarcinoma; OS: Overall survival; PFS: Progression-free survival; PPS: Post progression survival; RFS: Recurrence free survival; DSS: Disease-specific survival.

2.3 *ALOX5*相互作用基因和蛋白质的鉴定及遗传改变

利用GeneMANIA构建*ALOX5*与改变的邻近基因的基因-基因相互作用网络。结果显示：与*ALOX5*密切相关的前20个基因包括*COTL1*、*ALOX5AP*、*ALOX15B*和*LTC4S*等(图3A)。利用STRING数据库生成ALOX5的PPI网络(图3B)共有21个节点，包括COTL1、LTC4S和ALOX5AP等(图3B)。基于TCGA数据库，探讨*ALOX5*与FRGs的关系。*ALOX5*在LUAD中与*SLC40A1*、*TFRC*、*FTL*、*FTH1*、*CP*、*ACO1*呈显著正相关(图3C)。此外，在LUSC中*ALOX5*与*TFRC*、*TFR2*、*IREB2*呈显著负相关，而与*SLC40A1*、*FTL*、*CP*和*ACO1*呈正相关(图3D)。

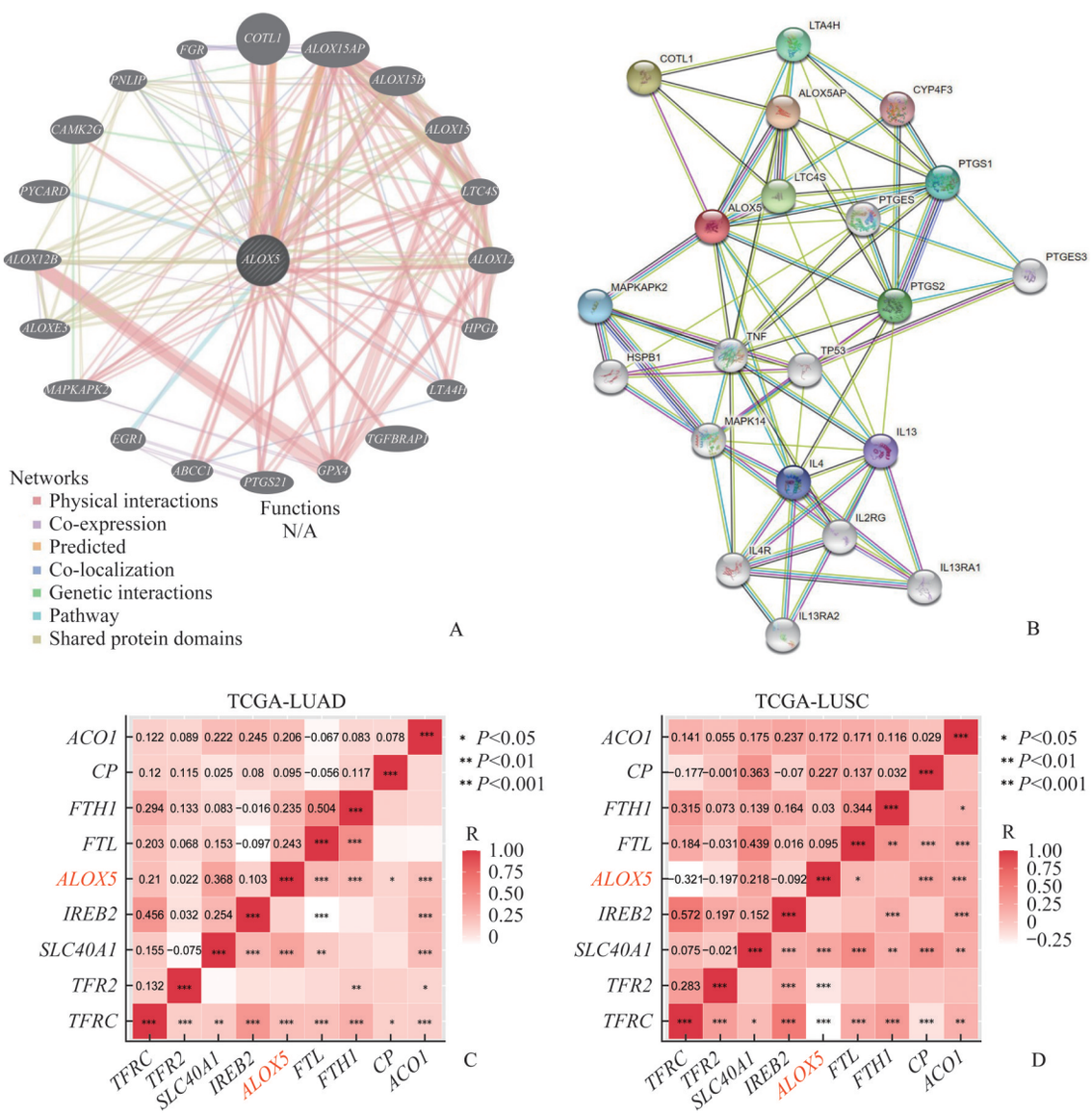

图3 *ALOX5*基因相互作用的基因和蛋白质

Figure 3 Genes and proteins interacted with *ALOX5* gene

A: Construction of the gene-gene interaction network of *ALOX5* by GeneMANIA; B: PPI network of *ALOX5* generated by STRING; C: Heatmap showing the correlation of *ALOX5* with FRGs in LUAD; D: Heatmap showing the correlation of *ALOX5* with FRGs in LUSC. **P*<0.05, ***P*<0.01; ****P*<0.001. *ALOX5*: Arachidonic acid 5-lipoxygenase gene; LUSC: Lung squamous carcinoma; LUAD: Lung adenocarcinoma; TCGA: The Cancer Genome Atlas; PPI: Protein-protein interaction; FRGs: Ferroptosis-related genes.

2.4 GO、KEGG富集分析

LUAD和LUSC中与*ALOX5*正相关的前30个基因见图4A、4B。然后，选择前300个与*ALOX5*基因显著正相关的基因，通过clusterProfiler包进行GO和KEGG分析。GO术语可分为3类：分子功能(molecular function，MF)、生物过程(biological process，BP)和细胞成分(cell component，CC)。构建了表征GO分析中最富集的前15个BP、MF和CC项的柱状图(图4B)。结果显示：在LUAD中与*ALOX5*共表达的基因介导的BP主要包括中性粒细胞活化、免疫反应中的中性粒细胞活化和中性粒细胞脱颗粒等；在LUSC中主要为T细胞活化、淋巴细胞活化的调节、白细胞活化的阳性调节等(图4C、4D)。KEGG通路分析还发现：在LUAD和LUSC中，*ALOX5*参与细胞因子及其受体相互作用、细胞因子信号通路、Th1和Th2细胞分化等免疫相关通路(图4E、4F)。

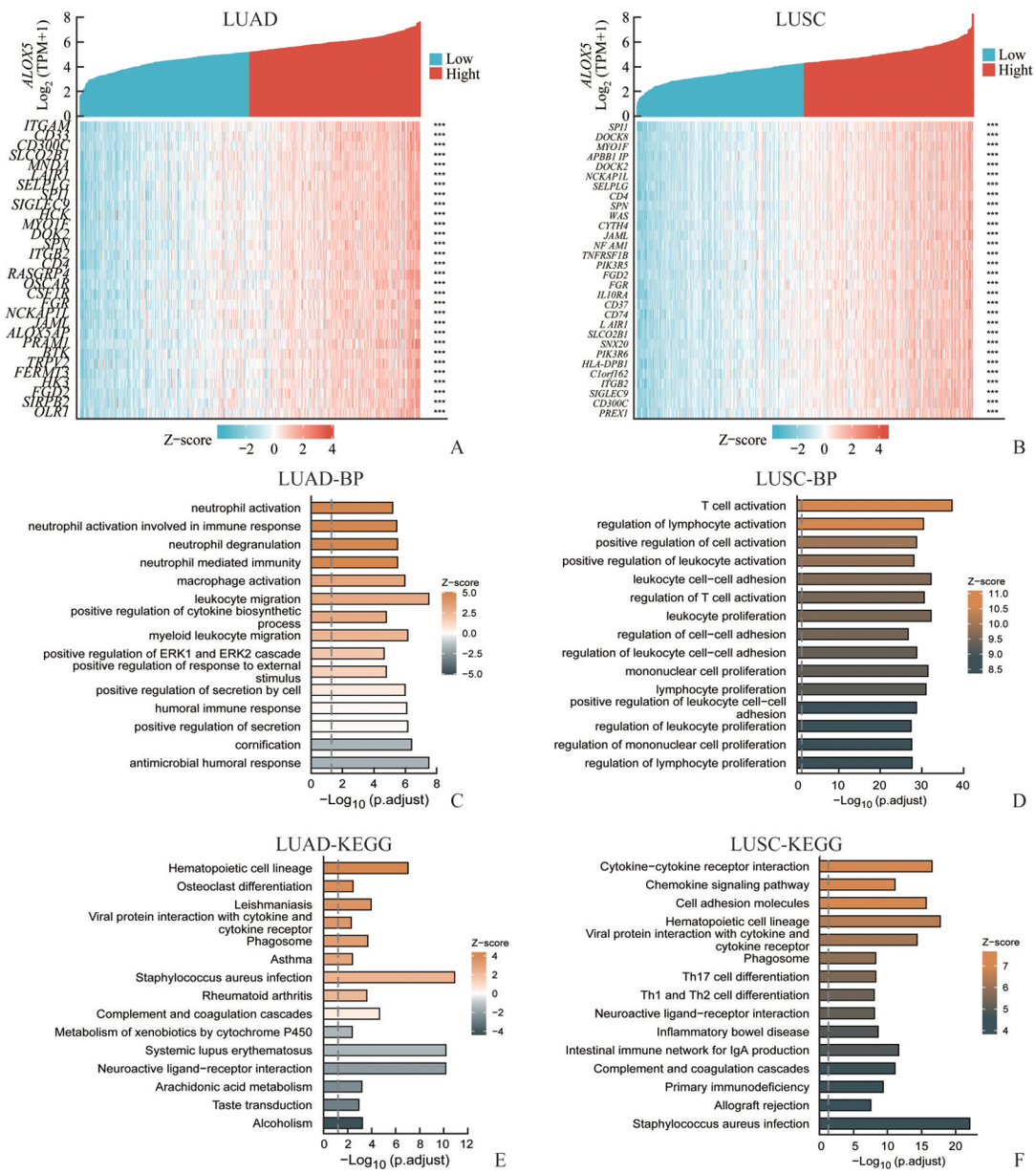

图4　*ALOX5*的GO和KEGG富集分析

Figure 4　GO and KEGG enrichment analysis of *ALOX5*

A: Heat map of the top 30 genes positively associated with *ALOX5* in LUAD; B: Heat map of the top 30 genes positively associated with *ALOX5* in LUSC; C: Top 15 BP class enrichment in LUAD; D: Top 15 BP class enrichment in LUSC; E: Top 15 KEGG-enriched pathways in LUAD; F: Top 15 KEGG enrichment pathways in LUSC. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; *ALOX5*: Arachidonic acid 5-lipoxygenase gene; LUSC: Lung squamous carcinoma; LUAD: Lung adenocarcinoma; BP: Biological process.

2.5 GSEA鉴定*ALOX5*相关信号通路

在KEGG中，GSEA揭示了多个在肺癌中富集的免疫功能基因集。在LUAD中，包括细胞因子和细胞因子受体的相互作用、自然杀伤细胞介导的细胞毒性、B细胞受体信号通路、T细胞受体信号通路等相关基因集(图5A~5F)。在LUSC中，*ALOX5*主要富集于细胞因子和细胞因子受体的相互作用、自然杀伤细胞介导的细胞毒性、T细胞受体信号通路(图5G~5L)。

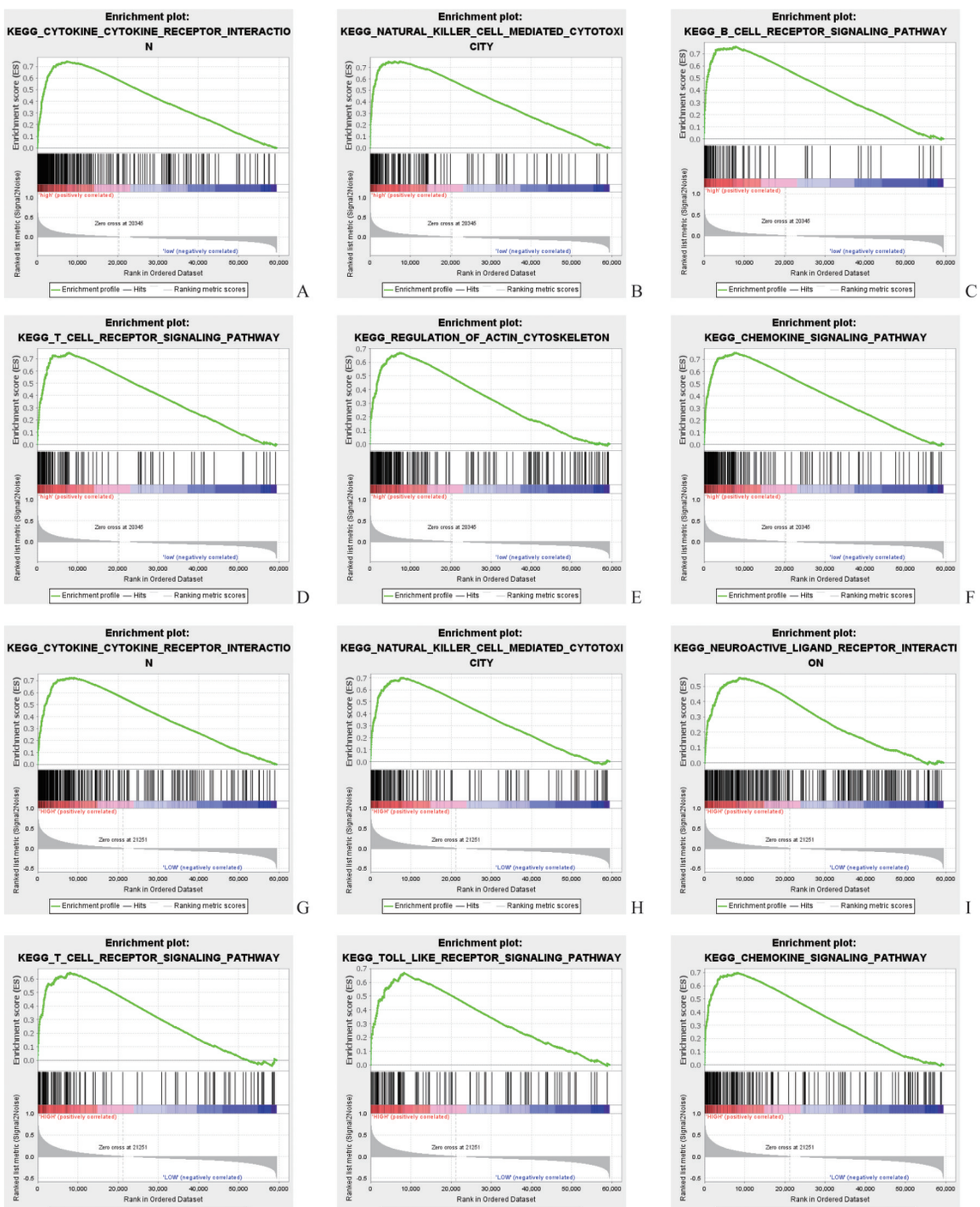

图5 GSEA分析显示的肺腺癌及肺鳞状细胞癌中*ALOX5*的基因富集通路

Figure 5 GSEA analysis showing gene enrichment pathway of *ALOX5* in lung adenocarcinoma and lung squamous carcinoma

A−F: Enriched plot in LUAD; G−L: Enriched plot in LUSC. *ALOX5*: Arachidonic acid 5-lipoxygenase gene; LUSC: Lung squamous carcinoma; LUAD: Lung adenocarcinoma; GSEA: Gene set enrichment analysis.

2.6 *ALOX5*表达与多种免疫标志物的相关性研究

为了加深对*ALOX5*与免疫应答之间相互作用的理解，使用TCGA数据库和TIMER数据库验证*ALOX5*表达与LUAD和LUSC中不同的免疫特征之间的相关性。用于表征各种免疫细胞的基因见表1。调整肿瘤纯度后，*ALOX5*表达与LUSC和LUAD中不同类型的免疫细胞中的大多数免疫标志物显著相关。

分析*ALOX5*表达与6种TIICs(包括B细胞、$CD8^+$ T细胞、$CD4^+$ T细胞、巨噬细胞、中性粒细胞和树突状细胞)之间的相关性，结果显示*ALOX5*表达水平与LUAD和LUSC中TIICs均呈显著正相关(图6A)。ssGSEA分析结果显示：*ALOX5*表达与肺癌免疫细胞浸润相关性的棒棒糖图见图6，*ALOX5*与LUAD和LUSC中巨噬细胞、活化树突状细胞、中性粒细胞等的浸润水平呈正相关(图6B)。进一步研究发现*ALOX5*表达与T细胞检查点PD-1、PD-L1和CTLA-4的表达均有显著相关性(均P<0.001，图6C、6D)。

表1　*ALOX5*与免疫细胞基因标志物的相关性分析

Table 1　Correlation analysis of *ALOX5* with immune cell gene markers

Description	Gene markers	LUAD				LUSC			
		None		Purity		None		Purity	
		cor	*P*	cor	*P*	cor	*P*	cor	*P*
B cell	*CD19*	0.23	***	0.09	0.054	0.48	***	0.34	***
	CD79A	0.20	***	0.06	0.172	0.49	***	0.34	***
T cell (general)	*CD3D*	0.40	***	0.26	***	0.59	***	0.49	***
	CD3E	0.45	***	0.33	***	0.67	***	0.58	***
	CD2	0.48	***	0.37	***	0.65	***	0.56	***
$CD8^+$ T cell	*CD8A*	0.31	***	0.19	***	0.56	***	0.48	***
	CD8B	0.24	***	0.14	**	0.45	***	0.42	***
Monocyte	*CD86*	0.67	***	0.62	***	0.73	***	0.66	***
	CSF1R	0.75	***	0.71	***	0.79	***	0.73	***
TAM	*CCL2*	0.40	***	0.32	***	0.51	***	0.44	***
	CD68	0.69	***	0.65	***	0.62	***	0.54	***
	IL10	0.46	***	0.36	***	0.54	***	0.47	***
M1 macrophage	*IRF5*	0.60	***	0.55	***	0.27	***	0.27	***
	PTGS2	−0.10	*	−0.10	*	0.12	**	0.05	0.278
M2 macrophage	*CD163*	0.62	***	0.56	***	0.71	***	0.65	***
	VSIG4	0.70	***	0.67	***	0.68	***	0.61	***
Neutrophils	*CEACAM8*	0.39	***	0.39	***	0.16	***	0.14	**
	ITGAM	0.80	***	0.77	***	0.75	***	0.69	***
	CCR7	0.47	***	0.36	***	0.64	***	0.56	***
Natural killer cell	*KIR2DL1*	0.08	0.054	0.03	0.556	0.26	***	0.21	***
	KIR2DL3	0.14	**	0.06	0.176	0.31	***	0.27	***
	KIR3DL1	0.10	**	0.03	0.572	0.40	***	0.36	***
	KIR3DL2	0.16	***	0.08	0.063	0.34	***	0.28	***
	KIR2DS4	0.13	**	0.06	0.159	0.27	***	0.23	***
Dendritic cell	*HLA-DPB1*	0.68	***	0.63	***	0.80	***	0.74	***
	HLA-DRA	0.66	***	0.61	***	0.75	***	0.69	***
	HLA-DPA1	0.66	***	0.61	***	0.79	***	0.73	***
	CD1C	0.56	***	0.51	***	0.58	***	0.45	***
	ITGAX	0.70	***	0.66	***	0.77	***	0.70	***

*P<0.05, **P<0.01, ***P<0.001. cor: Correlation coefficient; LUSC: Lung squamous carcinoma; LUAD: Lung adenocarcinoma; TAM: Tumor-associated macrophage; None: Unadjusted tumor purity; Purity: Adjustment of tumor purity.

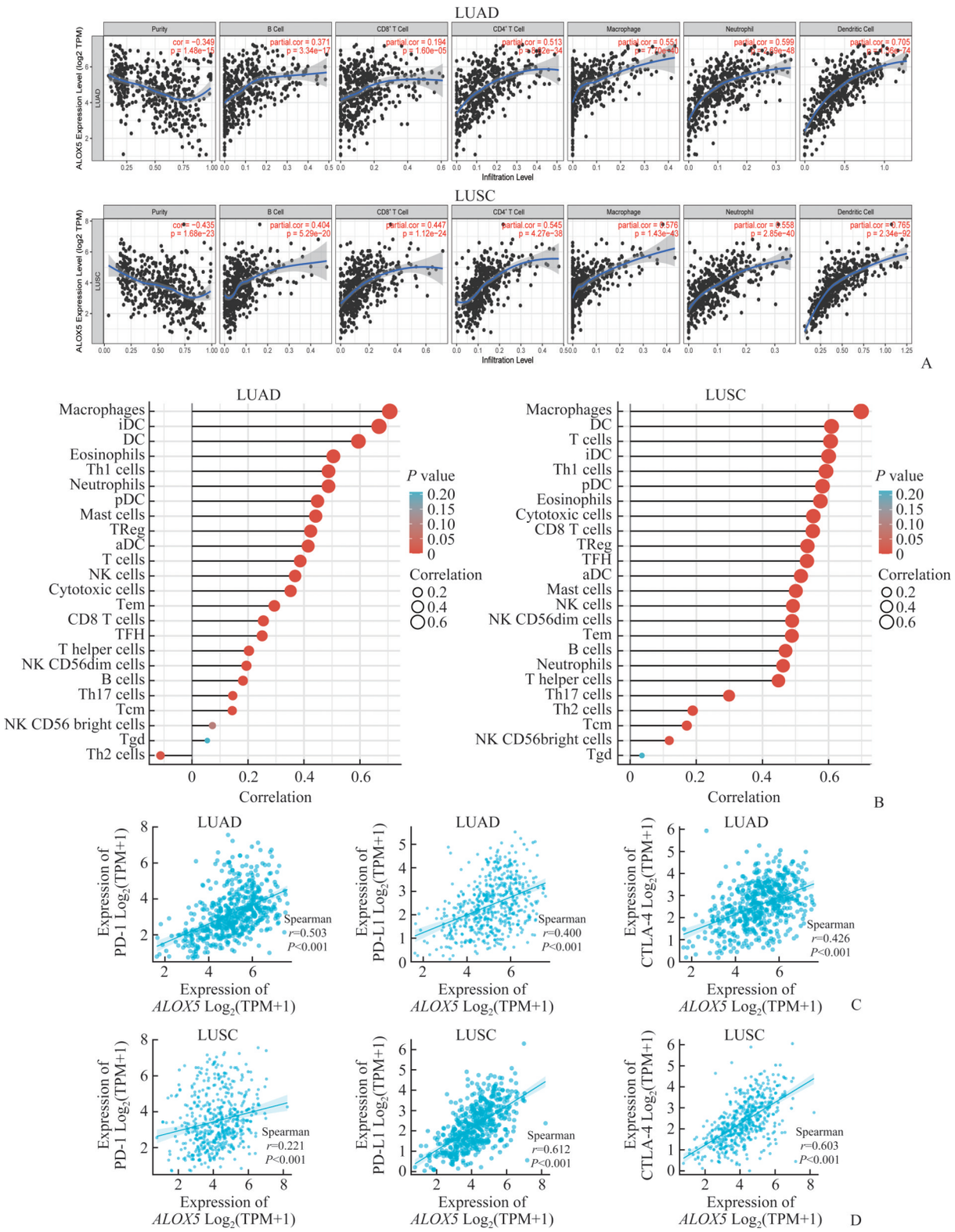

图6 *ALOX5*表达与免疫细胞浸润水平的相关性

Figure 6 Correlation of *ALOX5* expression with the levels of immune cell infiltration

A: *ALOX5* is significantly correlated with tumor purity, and positively correlated with different immune cell infiltration in TIMER database; B: Lollipop plot shows the correlation between *ALOX5* expression and immune cell infiltration in lung cancer using ssGSEA analysis; C and D: Scatter plot shows *ALOX5* expression correlating with PD-1, PD-L1 and CTLA-4 in LUAD (C) and LUSC (D) in the TCGA database. *ALOX5*: Arachidonic acid 5-lipoxygenase gene; LUSC: Lung squamous carcinoma; LUAD: Lung adenocarcinoma; GSEA: Gene set enrichment analysis; TCGA: The Cancer Genome Atlas; TPM: Transcripts per million; PD-1: Programmed death-1; PD-L1: Programmed cell death-ligand 1; CTLA-4: Cytotoxic T lymphocyte antigen 4.

3 讨　论

ALOX5是触发脂质过氧化的关键酶，可激发炎症反应，并引发各种细胞死亡模式，包括凋亡、焦亡和铁死亡[7]。研究[14]发现：ALOX5是介导细胞死亡的重要的酶，依赖于ERK-1磷酸化，介导核膜脂质过氧化，导致细胞溶解分子的核易位，诱导DNA损伤和细胞死亡。既往研究[8]发现：在多种肿瘤类型中，*ALOX5*基因表达上调并介导转移或化学治疗(以下简称“化疗”)耐药，导致不良预后。*ALOX5*基因在胃癌患者中表达上调，并且通过其代谢物5-羟基二十碳四烯酸(5-HETE)激活胃癌细胞，降低化疗疗效。*ALOX5*基因可能通过抑制STAT/K-RAS信号通路，提高急性髓系白血病的化疗敏感性，在急性髓系白血病中表现出抗肿瘤作用[15]。*ALOX5*基因通过调节PI3K/AKT通路，促进膀胱癌细胞的增殖和转移[16]。本研究发现：*ALOX5*基因在NSCLC中表达明显下调，*ALOX5*基因表达下调只在LUAD中与较好的OS和PFS显著相关，而在LUSC中预后差异不显著，提示*ALOX5*基因可以作为LUAD预后生物标志物。

M2型巨噬细胞不仅介导肿瘤细胞转移，还可以释放多种抑制T淋巴细胞的细胞因子影响其功能，促进肿瘤细胞免疫逃逸，导致不良预后[17]。既往研究[18]发现：肺泡巨噬细胞比间质巨噬细胞表达更高水平的*ALOX5*和产生更多白三烯(leukotriene，LT)B4。*ALOX5*抑制剂zileuton的使用可使转移灶的数量减少。本研究采用ssGSEA及TIMER分析的结果显示*ALOX5*表达与巨噬细胞浸润水平相关性最高，主要为与M2巨噬细胞呈显著正相关。Wculek等[11]研究发现：中性粒细胞是小鼠乳腺癌模型(前)转移肺微环境中建立的主要成分和驱动因素。从机制方面看，LTs通过选择性地扩大具有高致瘤潜能的癌细胞亚池，有助于远处组织的定植。抑制介导LTs生成的限速酶ALOX5可消除中性粒细胞前转移活性，从而减少转移。本研究GO分析发现：在LUAD中，与ALOX5相关的基因富集的功能最主要的包括中性粒细胞活化、免疫反应中的中性粒细胞活化、中性粒细胞脱颗粒。另外，本研究发现与*ALOX5*相互作用及共表达的基因主要有*COTL1*、*ALOX5AP*、*LTC4S*等，相应表达的蛋白质也是COTL1、ALOX5AP、LTC4S等。研究[19-20]发现*COTL1*基因在乳腺癌细胞中表达上调，其介导的微丝排列导致乳腺癌细胞迁移和肿瘤转移。*ALOX5AP*基因可通过增强M2巨噬细胞极化和浆液性卵巢癌微环境中的免疫抑制来预测预后不良[21]。肺癌组织中的癌细胞和外泌体通过*LTC4S*基因介导LTC4生成LTD4，从而刺激癌细胞迁移和生存[22]。以上研究为进一步探索*ALOX5*基因在NSCLC发生、发展中的分子机制提供了依据，在多个方面解释了NSCLC转移的可能机制，但NSCLC发生、发展及转移的具体机制仍需要分子生物学实验来验证。

近年来，TME的免疫逃逸机制及免疫疗法在肿瘤治疗中发挥越来越重要的作用[23]。有研究[24]报道免疫细胞浸润对NSCLC的临床和预后具有重要作用，将免疫细胞浸润作为特征纳入预后模型，有助于临床医生对患者的预后进行更准确的预测。本研究发现：LUAD和LUSC中*ALOX5* mRNA表达水平与B细胞、$CD8^+$ T细胞、$CD4^+$ T细胞、树突状细胞的浸润水平相关。这些结果提示*ALOX5*有可能参与NSCLC的免疫细胞浸润过程。肿瘤浸润性淋巴细胞(tumor infiltrating lymphocyte，TILs)反映了癌症中的适应性抗肿瘤免疫反应，通常与良好的预后相关[25]。在NSCLC中，$CD8^+$ T细胞是富集于肿瘤细胞巢周围的基质组织区域最丰富的亚群，较高的间质$CD8^+$ T细胞密度，尤其在PD-L1阳性的肿瘤中，与较长的生存期显著相关[26-27]。本研究发现*ALOX5*与不同类型的T细胞基因标志物呈显著相关。GSEA富集分析也发现：在LUAD和LUSC中均显示出*ALOX5*的功能富集于免疫功能基因集，包括细胞因子及其受体的相互作用、自然杀伤细胞介导的细胞毒性、T细胞受体信号通路等。此外，本研究还发现*ALOX5*与经典的T细胞免疫检查点抑制剂基因标志物*CD274*、*PDCD1*和*CTLA4*呈显著正相关。上述结果提示，*ALOX5*可作为潜在的与免疫细胞浸润相关的可预测免疫治疗预后的生物标志物。

总之，*ALOX5*可能作为一种潜在的新的肺癌预后生物标志物。此外，*ALOX5*与肺癌患者TME中免疫细胞浸润及免疫功能存在密切相关关系，这些发现具有潜在的价值，不仅可以促进对*ALOX5*作用的理解，而且可以促进其在肺癌预后和免疫治疗中的转译使用。这些结果提示*ALOX5*可能成为肺癌免疫相关治疗的新靶点。为进一步阐明*ALOX5*在NSCLC中的功能和作用，还需分子生物学实验进行验证和分析。

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