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Pan-Cancer Analysis Reveals the Prognostic Value and the Immune Response of Galanin

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Abstract

Lung cancer is one of the most common cancers with high cancer-related mortality worldwide. Increasing evidence revealed that Galanin (GAL) and galanin receptors become biomarker in several endocrine tumors. In this study, we first performed pan-cancer analysis for GAL's expression and prognosis using The Cancer Genome Atlas (TCGA) and found that GAL might be a potential oncogene in lung cancer and endometrial cancer.

We performed bioinformatics analysis on lung cancer from TCGA, and jointly analyzed with online databases such as LinkedOmics, TIMER and TISIDB. We found that lower expression of GAL was accompanied with worse outcomes of patients with lung cancer. Moreover, GAL expression was significantly correlated with a variety of the Tumor-Infiltrating Immune Cells (TIICs). GAL level was significantly positively associated with tumor immune cell infiltration, biomarkers of immune cells and immune checkpoint expression in lung cancer.

Collectively, our findings elucidated that GAL correlated with poor prognosis and tumor immune infiltration in lung cancer.

Keywords: TCGA; Galanin; NSCLC; Immune infiltration; Prognosis

Introduction

Lung cancer is one of the most common cancers with high cancer-related mortality worldwide [1,2]. Non-Small Cell Lung Cancer (NSCLC) accounts for 85% of all lung cancers, while approximately 15% of that was Small Cell Lung Cancer (SCLC). Lung Squamous Cell Carcinoma (LUSC) comprises approximately 30% of NSCLC, 5-year Overall Survival (OS) of which was only 18% because of the poor prognosis [3]. Treatment with targeted therapy, such as Tyrosine Kinase Inhibitors (TKIs), can prolong the survival of patients with driver mutation positive Lung Adenocarcinoma (LUAD), while few people with LUSC benefit from targeted therapy [4]. Clinically, the patients with advanced/metastatic LUSC without still mainly receive the first-line therapy for standard platinum-based doublet chemotherapy [5,6].

Data from KEYNOTE-010 indicated that 23% to 28% of patients with advanced NSCLC have a high level of Programmed Death Ligand 1 (PD-L1) expression determined by PD-L1 Tumor Proportion Score (TPS) ≥ 50% [7,8]. Immune checkpoint blockade therapy targeting PD-1/PD-L1 has become the hotspot of the therapeutic landscape of metastasis lung cancer [9]. However, a number of patients still occurred drug resistance [10]. Therefore, novel immune targets are urgently required to improve patient outcomes when combined with existing immunotherapies in lung cancers.

G Protein-Coupled Receptors (GPCRs) have been demonstrated to act as tumor suppressor genes and correlated with carcinogenesis in cell biology and therapeutic outcome in the clinical setting. GPCRs controlled not only immune cells but also non-immune cells microenvironment of specific tissues and organs [11]. Additionally, GPCRs can regulate inflammatory gene expression and transcription factors involved in inflammatory signaling cascades, such as CREB, ERKs, NFAT, c-Jun, STAT3, and NFκB [12,13].

Galanin (GAL) is a 29 to 30 amino acid peptide, which distributed extensively in the central and peripheral nervous system [14,15]. Galanin has three at least three specific G protein-coupled

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receptor subtypes, GalR1-3, each of which belongs to the G-Protein-Coupled Receptor (GPCR) superfamily [16]. Galanin modulates a variety of human diseases and pathological processes, including seizure, Alzheimer's disease, mood disorders, anxiety, alcohol intake in addiction, metabolic diseases, pain, cognition, nociception, memory, feeding, neurotransmitter, hormone secretion and cell proliferation and solid tumors [16-18].

Galanin and galanin receptors have increasingly become biomarker in several endocrine tumors, for example pancreatic, hypothalamic and pituitary tumors [19-21]. According to clinical data, besides somatostatin receptor agonists' octreotide and serotonin, galanin also can be utilized in the treatment of pancreatic tumor. Small cell lung carcinoma has also been shown to express galanin and GalR1 and in some cases GalR2 and the modulation of GalR2 on tumor growth has been shown [22]. Previous studies have shown that Colorectal Cancer (CRC) tissue has higher GAL expression than normal colonic mucosa [23]. Another study investigated GAL expression in stage II-III CRCs and made a conclusion that GAL is an indicator of tumor recurrence in CRC patients [24]. However, the prognostic value of GAL expression in tumor is not completely understood.

In this present study, we comprehensively measured the correlation between GAL expression and prognosis of cancer patients in databases. Further, we investigated the association of GAL with tumor-infiltrating immune cells in the different tumor microenvironments *via* Tumor Immune Estimation Resource (TIMER). The findings in this report shed light on the important role of GAL in lung cancers as well as provide a potential relationship and an underlying mechanism between GAL and tumor-immune interactions.

Materials and Methods

Data acquisition and processing

We downloaded the RNA expression profiles and clinical data of LUAD&LUSC patients from the TCGA database (https://portal.gdc.cancer.gov/), TCGA provided 1,145 lung cancer (LUAD&LUSC) samples containing prognostic information.

Linked omics database analysis

The LinkedOmics database (http://www.linkedomics.org/login.php) is to analyze 32 TCGA cancer-associated multidimensional data sets. The GAL-related genes were screened from the TCGA LUAD&LUSC cohort through the LinkFinder module in the database by the Pearson correlation coefficient. Functional enrichment analysis was performed to examine Gene Ontology biological process (GO_BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways based on Gene Set Enrichment Analysis (GSEA).

TIMER database analysis

Tumor Immune Estimation Resource (TIMER) database (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for systematic analysis of immune infiltrates including 32 cancer types. TIMER utilized a deconvolution method to analyze the abundance of Tumor-Infiltrating Immune Cells (TIICs) of the LUAD&LUSC samples from the TCGA and GEO dataset.

TISIDB database analysis

The TISIDB database (http://cis.hku.hk/TISIDB) is a web portal of multiple heterogeneous data types, which is to estimate the interaction between tumor and immune system. TISIDB analyze associations for GAL with immunomodulator in lung cancer. P value <0.05 was considered statistically significant.

Kaplan-Meier plotter database analysis

Kaplan-Meier plotter is an online database can assess the effect of genes on the survival of 10,461 cancer samples. The correlation between GAL expression and survival in HNSC, LIHC, BRCA, BLCA, CHOL, UCEC, KICH, KIRC, PAAD and Lung cancer (LUAD+LUAD) was analyzed by Kaplan-Meier plotter (http://kmplot.com/analysis/). The Hazard Ratio (HR) with 95% confidence intervals and log-rank P-value were measured. Log rank p value <0.05 was considered as statistically significant.

Statistical analysis

Survival curves were generated by the Kaplan-Meier plots displayed with HR and P or Cox P-values from a log-rank test. The correlation of gene expression was evaluated by Spearman's correlation and statistical significance, and P-values <0.05 were considered statistically significant.

Result

Pan-cancer analysis of GAL expression

To explore the potential roles of GAL in carcinogenesis, we first analyzed its expression in 33 types of human cancer. Compared with normal samples, GAL was significantly upregulated in 9 cancer types, including BLCA, BRCA, CHOL, HNSC, LIHC, LUAD, LUSC, PRAD, UCEC, and was markedly downregulated in 2 cancer types, involving KIRC, KICH. However, no significant difference in CESC, COAD, ESCA, GBM, CNIRP, PAAD, PCPG, READ, STAD and THCA was observed. As presented, GAL expression in BLCA, CHOL, LUSC, LUAD, LIHC, BRCA, or UCEC was statistically increased when compared with corresponding normal controls. While GAL expression in KIRP, HNSC or PRAD, was obviously decreased.

Galanin as a prognostic biomarker correlated with immune infiltrates

Furthermore, survival analysis for GAL in HNSC, LIHC, BRCA, BLCA, CHOL, UCEC, KICH, KIRC, PAAD, LUSC and LUAD was conducted. Both Overall Survival (OS) and Disease-Free Survival (DFS) were included. For OS, high expression of GAL in LUAD and UCEC had unfavorable prognosis. For DFS, upregulated expression of GAL in LUAD, BRCA and UCEC suggested poor prognosis. No statistical significance of GAL for the prognosis of other cancer types was observed.

Across the 24 immune cell subtypes, we found the fraction of Th2 cells to be most positively correlated with GAL in BLCA and Lung Cancer, while the fraction of Th1 cells to be most positively correlated with GAL in BRCA and KICH. Besides, Th17 cells are most negatively associated with GAL in BLCA, CHOL, KICH and KIRC.

Biological function of GAL in NSCLC

We constructed the network for GAL based on string data. The results showed that GALR1, GALR2, GALR3, CPRM1, NMU, POMC, NPY, SST, PDYN and NPY1R, were closely associated with GAL expression. Among these genes, GALR3, NMU, NPY1R, NPY and POMC were associated with GAL in Lung Cancer. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) was to analyze the functions of GAL and the genes significantly associated with GAL on the Database for Annotation, Visualization and Integrated Discovery (DAVID). For obtaining the biological function of GAL in Lung Cancer, the co-expression pattern of GAL in TCGA-

LUAD and TCGA-LUSC were deployed based on the LinkedOmics web portal.

The heat maps show the top 50 genes positively and negatively associated with GAL in LUAD and LUSC respectively. LUAD co-expressed genes of GAL join mainly in Chromosome segregation, ncRNA processing, ribonucleoprotein complex biogenesis, spindle organization, rRNA metabolic process, translational elongation, protein localization to chromosome, DNA replication, translational initiation, mitotic cell cycle phase transition, tRNA metabolic process, telomere organization, DNA recombination, mitochondrial respiratory chain complex assembly, protein folding, interleukin-5 production, adrenergic receptor signaling pathway, protein localization to cell surface, etc. While in LUSC co-expressed genes of GAL join mainly in mitochondrial gene expression, translational initiation, protein localization to endoplasmic reticulum, rRNA metabolic process, ribonucleoprotein complex biogenesis, ncRNA processing, translational elongation, RNA localization, ribonucleoprotein complex subunit organization, cytoplasmic translation, tRNA metabolic process, RNA catabolic process, nucleobase-containing compound transport, mitochondrial RNA metabolic process, interstrand cross-link repair, mitochondrial respiratory chain complex assembly, protein localization to chromosome, NADH dehydrogenase complex assembly, protein folding, establishment of protein localization to membrane, ribonucleoprotein complex localization, protein-containing complex disassembly, etc. KEGG pathway analysis indicated enrichment in Ribosome, Ribosome biogenesis in eukaryotes, RNA transport, Proteasome, Maturity onset diabetes of the young, Purine metabolism, Retinol metabolism, Drug metabolism, *Staphylococcus aureus* infection, Asthma, B cell receptor signaling pathway, T cell receptor signaling pathway, Inflammatory bowel disease, etc. These results show that GAL expression network widely absorb on the prognosis and immune activation of NSCLC.

Association of GAL with immune infiltration level in NSCLC

Then we explored the affection of GAL expression of various immune cell infiltration in NSCLC from the TIMER database. The significant positive associations between GAL expression with Th2 cells, DC, Mast cells, Eosinophils, NK CD65bright cells, Th17 cells, DC, Th1 cells, NK CD56 cells, NK cells, pDC, T cells, T helper cells, Tcm, B cells, CD4 T cells, CD8 T cells, dendritic cells, macrophages and neutrophils were all proved by Pearson correlation analysis. Additionally, low-expression GAL tumors harbored significantly higher portion of follicular T cells, aDC, B cells, DC, Eosinophils, iDC, Macrophages, Mast cells, Neutrophils, NK CD65 bright cells, NK CD56 cells, NK cells, pDC, T helper cells, Tem, TFH, Tgd, Th1 cells, Th17 cells, Th2 cells than GAL-High tumors.

Relation between GAL with immune molecules

Correlations between immune molecules including BTLA_exp, TGFB1_exp, CSF1R_exp, CD40_exp, CD70_exp, CD276_exp, CD_28 and PDCD1_exp with GAL in LUAD. There were correlations between GAL expression and immune infiltration, including CD160_exp, CD244_exp, VTCN1_exp and TGFBR1_exp in LUSC. Therefore, it was confirmed that GAL participating widely in modulating various immune molecules in NSCLC to affect immune infiltration in the tumor microenvironment.

To further explore the role of GAL in tumor immune, we determined the expression correlation of GAL with biomarkers of immune cells in NSCLC using TIMER database. GAL was significantly positively correlated with M1 macrophage's biomarkers (NOS2, COX2), dendritic cell's biomarkers (HLA-DPB1), Th1 cell's biomarkers (STAT6), Tfh cell's biomarker (BCL6) and Th17 cell's biomarker (STAT3) in LUAD. While in LUSC, GAL was significantly positively correlated with T cell's biomarker (CD2), Monocyte (CSF1R), M1 Macrophage (COX2, IRF5), Neutrophils' biomarkers (CD66b, CD11b, CCR7), Natural killer cell' s biomarkers (KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1), Dendritic cell's biomarkers (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, BDCA-1, BDCD-4), Th1 cell's biomarkers (STAT4, STAT1, IFN-γ, TNF-α), Th2 cell's biomarkers (STAT6, STAT5A), Tfh (BCL6), T17 cell's biomarkers (STAT3), Treg's biomarkers (STAT5B, TGFβ), T cell exhaustion's biomarkers (PD-1,GZMB). In addition, multivariate Cox survival analysis revealed T cell CD4+, Treg, B cell, NK cell, macrophages, and neutrophils to be independent prognostic biomarkers for LUAD, and none of them to be the independent prognostic biomarkers for LUSC. These findings partially support that GAL is positively linked to immune cell infiltration.

GAL is an independent prognostic indicator of NSCLC

We examined the association between GAL expression and clinicopathological characteristics of patients with NSCLC. We found that patients with high GAL expression had more advanced TNM stage, poor differentiation, poor overall survival and progression-free survival than patients with low GAL expression. Further, smoke was correlated with high GAL expression.

We used univariate and multivariate Cox analyses to assess whether GAL was an independent prognostic factor for patients with NSCLC. Univariate Cox analysis indicated that age at initial diagnosis, pathologic stage; stage T2-4, stage N1-3 and stage M were remarkably associated with OS ($p<0.05$). Subsequent multivariate Cox analysis further showed that age, stage T3 & 4, stage N2 & 3 and stage M1 were independent predictors of OS ($p<0.05$). These results indicated that GAL, as an independent prognostic indicator, might be useful for clinical prognosis evaluation.

Discussion

GAL is a 29 to 30 amino acid peptide and it is widely connected to the central and peripheral nervous systems. Three GAL receptors (GAL1-3) have been identified as G-protein-coupled member. Although Gal has been found to modulate the progression of small cell lung carcinoma and colorectal cancer, the role of GAL in cancer is still unclear.

In this study, we analyzed expression levels of GAL in 33 types of human cancer from TCGA. GAL was significantly upregulated in BLCA, BRCA, CHOL, HNSC, LIHC, LUAD, LUSC, PRAD, UCEC, and was downregulated in KIRC and KICH. Additionally, the TCGA data showed that GAL expression in BLCA, CHOL, LUSC, LUAD, LIHC, BRCA, or UCEC was statistically increased when compared with normal adjacent tissues. While GAL was obviously decreased in cancer tissues of KIRP, HNSC and PRAD compared with normal tissues.

Furthermore, analysis of the TCGA database revealed that Overall Survival (OS) and Progress-Free Survival (RFS) for GAL in HNSC, LIHC, BRCA, BLCA, CHOL, UCEC, KICH, KIRC, PAAD, LUAD and LUSC. High expression of GAL can be used as an independent risk factor for OS in LUAD and UCEC. For DFS, upregulated expression of GAL in LUAD, BRCA and UCEC suggested poor prognosis. All these statistics strongly suggest that GAL is a prognostic biomarker in

lung cancer and endometrial cancer.

It is known that galanin treatment significantly upregulated the response of polymorphonuclear neutrophils of human and murine to interleukin 8 [25]. Andrea Ramspacher et al. elucidate the effect of galanin on the expression of cytokines and chemokines and GAL increased the expression of IL-1β, TNF-α, IL-10, IL-18, CCL3 and CXCL8 in nonactivated monocytes [26]. Further, Galanin can be classified as an immunomodulatory peptide has specific immune-related functions and human immune cells displayed galanin and its receptor [27-29]. Here we found the fraction of Th2 cells was most positively correlated with GAL in BLCA and Lung Cancer. What's more, the fraction of Th1 cells was most positively correlated with GAL in BRCA and KICH. Besides, Th17 cells are most negatively associated with GAL in BLCA, CHOL, KICH and KIRC. Thus, our results indicated that GAL has the potential function in tumor immunology.

It is reported that Galanin stimulates Ca2+ Mobilization, inositol phosphate accumulation, and clonal growth and involved in the activation of MAPK pathway in Small Cell Lung Cancer (SCLC) [30,31]. Then we tried to explore the role of GAL in NSCLC and conducted a network of GAL-related genes based on the string database in Lung cancer. The most relevant biological and signaling pathway was "G protein-coupled signaling". The mitogenic neuropeptide galanin was revealed that has an interaction with GALR2 to activate G proteins and a G12/Rho pathway in SCLC [23]. Regulators of G Protein Signaling (RGS) Proteins were reported to play a role in both innate and adaptive immune processes [32]. Therefore, we explored whether GAL is correlated with immune infiltration in NSCLC.

Through analyzing the database from TISIDB, we found immune molecules including BTLA_exp, TGFB1_exp, CSF1R_exp, CD40_exp, CD70_exp, CD276_exp, CD_28 and PDCD1_exp, correlated with GAL in LUAD. Further, our results demonstrated that there is moderate to strong positively relation between GAL with M1 macrophage's biomarkers (NOS2, COX2), dendritic cell's biomarkers (HLA-DPB1), Th1 cell's biomarkers (STAT6), Tfh cell's biomarker (BCL6) and Th17 cell's biomarker (STAT3) in LUAD. While in LUSC , GAL was significantly positively correlated with T cell's biomarker (CD2), Monocyte (CSF1R), M1 Macrophage (COX2, IRF5), Neutrophils' biomarkers (CD66b, CD11b, CCR7), Natural killer cell's biomarkers (KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1), Dendritic cell's biomarkers (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, BDCA-1, BDCD-4), Th1 cell's biomarkers (STAT4, STAT1, IFN-γ, TNF-α), Th2 cell's biomarkers (STAT6, STAT5A), Tfh (BCL6), T17 cell's biomarkers (STAT3), Treg's biomarkers (STAT5B, TGFβ), T cell exhaustion's biomarkers (PD-1,GZMB). Together these findings suggest that the GAL plays an important role in the regulation of immune infiltrating cells in NSCLC.

We examined the association between GAL expression and clinicopathological characteristics of patients with lung cancer. We found that patients with high GAL expression were correlated with TNM stage, differentiation, overall survival, smoke and progression-free survival. Univariate Cox analysis indicated that age, pathologic stage, stage T, stage N and stage M were remarkably associated with OS ($p<0.05$). Multivariate Cox analysis further showed that age, stage T3 and 4, stage N2 and 3, and stage M1 were independent predictors of OS ($p<0.05$). These results indicated that GAL, as an independent prognostic indicator, might be useful for clinical prognosis evaluation.

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