Analysis of the Expression and Clinical Significance of VIPR1 in Lung Adenocarcinoma Based on TCGA Database

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Abstract

Background: The expression of VIPR1 is associated with the prognosis of many malignant tumors. To analyze the expression and clinical significance of vasoactive intestinal peptide Types I (VIPR1) in lung adenocarcinoma based on The Cancer Genome Atlas (TCGA) database.

Methods and results—The RNASeq data, clinical data and prognosis data of lung adenocarcinoma and normal lung tissue were downloaded from TCGA database. The expression difference of VIPR1 mRNA in lung adenocarcinoma and normal lung tissue $p < 0.05$, and the correlations of the expression levels of VIPR1 in lung adenocarcinoma with clinical pathological characteristics and prognosis were analyzed. The possible regulatory signaling pathways of VIPR1 were predicted by the gene set enrichment analysis (GSEA). CIBERSORT was used to analyze the expression of immune cells in tumor tissues and normal tissues. TIMER was used to analyze the relationship between VIPR1 and immune cell expression. The expression levels of VIPR1 mRNA in lung adenocarcinoma tissues were significantly lower than that in normal lung tissues. The expression levels of VIPR1 mRNA were significantly correlated with gender, T staging—N staging and pathological grade $p < 0.05$ but not with age, M staging and survival status. Survival analysis showed that the survival time of patients with low expression of VIPR1 was significantly lower than that of patients with high expression. In addition, the expression level of VIPR1 in lung adenocarcinoma was negatively correlated with the infiltration level of myeloid inhibitory cells ($r = -0.448, p < 0.01$). Through GSEA functional enrichment analysis, it was found that VIPR1 was mainly related to cell cycle pathway, p53 signal pathway, DNA replication pathway, RNA degradation pathway and so on.

Conclusions—VIPR1 gene may be a new target for the clinical prognosis and targeted therapy of lung adenocarcinoma.

Background:

vasoactive intestinal peptide (VIP), as a vasoactive intestinal peptide, participates in smooth muscle relaxation of lung and intestinal epithelium [1], which can promote the survival of peripheral neurons and inhibit neuronal death after injury [2]. It can inhibit the proliferation and metastasis of human renal cell carcinoma A498 cells by up-regulating p53 and down-regulating the expression of NF- κ B, VEGF and CD-34 [3]. At the same time, it also has immunomodulatory function and is a specific chemokine and activator of macrophages, B cells and T cells [4]. It can also inhibit the production of many pro-inflammatory factors and chemokines, including TNF- α , IL-6, IL-12, MIP-1 and MIP-2, and promote the production of some antagonists such as anti-inflammatory cytokines such as IL-10 and IL-1R [3]. Among them, TNF- α , IL-12, MIP-1, MIP-2 and IL-10 are mainly regulated by VIPR1 [5]. VIPR1 is the most important receptor for VIP, which is mainly found in some regions of the human brain. It is also expressed in rat and human pulmonary macrophages, pulmonary venous vascular smooth muscle and airway epithelium from bronchioles to respiratory bronchioles [6]. The expression of VIPR1 was different in different tumor tissues, and the expression level in breast cancer was 5 times higher than that in normal breast tissue [7],

but lower in hepatocellular carcinoma [8], thyroid cancer [9], gastric cancer [10] and non-small cell lung cancer [11, 12]. In breast cancer, VIP can induce cell proliferation by acting on VIPR1 [13], while in hepatocellular carcinoma, patients with high expression of VIPR1 have a relatively good prognosis [8]. More and more studies have proved that VIPR1 plays an important role in the differentiation and treatment of human cancer, such as antagonizing the binding of VIP and VIPR1 in prostate cancer, inhibiting tumor cell growth [8], functional imaging of tumor tissue and targeted chemotherapy in breast cancer[14]. Up-regulation of VIPR1 expression in lung adenocarcinoma can inhibit the growth of H1299 cells[15, 16]. In recent years, immunotherapy has increasingly become a hot spot in the treatment of lung cancer, and its effect is also related to immune cell infiltration in the tumor microenvironment. At present, there are no studies on the role of VIPR1 in immune infiltration of lung adenocarcinoma, so this study intends to use a variety of bioinformatics methods to study the role of VIPR1 in lung adenocarcinoma, and explore its effect on the pattern of immune cell infiltration, so as to provide clinical prognostic markers of new immune-related genes.

Materials And Methods:

Data collection, collation and analysis:

The transcriptional group data and related clinical data of lung adenocarcinoma patients were downloaded from TCGA database (<https://portal.gdc.cancer.gov/>). The data download format was standardized FPKM format, of which 497 cases were tumor samples and 54 cases were normal control samples. To analyze the difference of gene expression between tumor tissue and normal tissue, and to explore the relationship between VIPR1 expression level and total survival time of patients with lung adenocarcinoma by univariate Cox regression analysis. T staging, M staging, N staging, age, gender and pathological grade were selected as the clinicopathological features of lung adenocarcinoma. The box chart was used to show the difference of gene expression between normal group and tumor group, and the difference was statistically significant ($p < 0.05$).

The Human Protein Atlas:

Analysis of the distribution of VIPR1 in normal lung tissue and lung adenocarcinoma tissue (scale: 50 μ m) based on THE HUMAN PROTEIN ATLAS database (HPA, <https://www.proteinatlas.org/>).

Gene enrichment analysis

GSEA4.0.1 software is used to analyze and explore the important signal pathways related to VIPR1. According to the expression level of VIPR1, they were divided into high expression group and low expression group. Get the c2.cp.kegg.v7.4.symbols.gmt dataset through the GSEA website. The default weighted enrichment statistics method was used for enrichment analysis and the number of random combinations was set to 1000.

Immune infiltration assessment:

CIBERSORT (<http://cibersort.stanford.edu/>) was used to estimate the composition of 22 kinds of immune cells in lung adenocarcinoma and normal lung tissues. Through deconvolution algorithm, CIBERSORT software can calculate the composition of immune cells in complex cells based on standardized gene expression data.

Analysis of the relationship between VIPR1 and immune infiltrating cells:

The relationship between VIPR1 expression and immune cell infiltration such as CD4 + T cells, B cells, neutrophils and myeloid inhibitory cells was analyzed by Immune plate of TIMER2.0 database (<http://timer.cistrome.org/>).

Statistical analysis

The R software (version 4.1.0) was used for data analysis. The expression of VIPR1 in lung adenocarcinoma and normal lung tissues was compared by wilcoxon rank sum test, the expression of VIPR1 in different TNM stages of lung adenocarcinoma was compared by one-way ANOVA, and the correlation between the two groups was tested by spearman correlation test. The relationship between VIPR1 and total survival time (OS) was analyzed by Kmuri M curve and tested by log-rank method. The test level is $\alpha = 0.05$.

Results:

Relationship between VIPR1 gene expression level and clinical characteristics of patients with lung adenocarcinoma

The clinical and transcriptional data of patients with lung adenocarcinoma were downloaded from TCGA public database. After analysis, we found that there was differential expression of VIPR1 in lung adenocarcinoma and normal lung tissues, and the expression in normal lung tissue (figure 1.K) was higher than that in lung adenocarcinoma tissue (figure 1.M). We continued to analyze the relationship between the high and low expression of VIPR1 and the prognosis of patients. The results showed that the patients with high expression of VIPR1 had a better prognosis than those with low expression (figure. 1. F). Further analysis showed that the expression of VIPR1 was correlated with gender, T staging, N staging and clinical grade (figure 1. D, G, H, J), but not with age, survival status and M staging.

Functional enrichment Analysis of VIPR1

The results of GSEA enrichment analysis showed that the samples with low expression of VIPR1 were significantly enriched in cell cycle pathway, p53 signal pathway, DNA replication pathway and RNA degradation pathway (figure 2, table 1).

Table 1

Functional enrichment analysis of VIPR1 gene

NAME	SIZE	ES	NES	NOM p-val
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	42	0.5655199	1.99602	0
KEGG_CELL_CYCLE	124	-0.757659	-2.37	0
KEGG_ALZHEIMERS_DISEASE	165	-0.645564	-2.2276	0
KEGG_PROTEASOME	46	-0.890569	-2.2286	0
KEGG_PYRIMIDINE_METABOLISM	97	-0.626209	-2.1845	0.001949318
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	56	-0.654081	-2.167	0
KEGG_OOCYTE_MEIOSIS	112	-0.583873	-2.1869	0
KEGG_RNA_DEGRADATION	59	-0.641009	-2.1709	0
KEGG_PROTEIN_EXPORT	24	-0.797611	-2.1206	0
KEGG_HUNTINGTONS_DISEASE	180	-0.624188	-2.0782	0.002105263
KEGG_HOMOLOGOUS_RECOMBINATION	28	-0.780059	-2.0784	0
KEGG_BASAL_TRANSCRIPTION_FACTORS	35	-0.656776	-2.0484	0.003913894
KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	134	-0.519087	-2.0372	0
KEGG_PURINE_METABOLISM	156	-0.497453	-2.0228	0.001912046
KEGG_PARKINSONS_DISEASE	127	-0.715276	-2.0111	0.004106776
KEGG_DNA_REPLICATION	36	-0.857378	-2	0
KEGG_PENTOSE_PHOSPHATE_PATHWAY	27	-0.680321	-1.9653	0.00212766
KEGG_MISMATCH_REPAIR	23	-0.779243	-1.9494	0.003891051
KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	85	-0.511501	-1.9324	0
KEGG_CITRATE_CYCLE_TCA_CYCLE	31	-0.732301	-1.9498	0.001992032
KEGG_SPLICEOSOME	127	-0.645736	-1.9536	0.001926782
KEGG_P53_SIGNALING_PATHWAY	68	-0.503025	-1.9107	0.001865672
KEGG_RIBOFLAVIN_METABOLISM	15	-0.63668	-1.9005	0.004048583
KEGG_RNA_POLYMERASE	28	-0.658956	-1.9025	0.003846154
KEGG_CYSTEINE_AND_METHIONINE_METABOLISM	34	-0.58691	-1.8713	0.002
KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS	53	-0.48055	-1.7782	0.00408998
KEGG_N_GLYCAN_BIOSYNTHESIS	46	-0.523136	-1.7458	0.025896415
KEGG_BASE_EXCISION_REPAIR	35	-0.628301	-1.7196	0.01372549

There is a difference in the expression of immune cells between lung adenocarcinoma and normal tissues

As shown in figure 3, The proportion of immature B cells, plasma cells, activated memory CD4+T cells, follicular helper T cells, regulatory T cells, M1 macrophages and resting dendritic cells in lung

adenocarcinoma tissues was higher than that in normal lung tissues. Normal lung tissue is rich in inactivated memory CD4+T cells, inactivated NK cells, monocytes, M0 macrophages, M2 macrophages, activated dendritic cells, resting mast cells, neutrophils, but no immature CD4+T cells were found.

There is a correlation between the expression level of VIPR1 gene and the expression of immune infiltrating cells

After TIMER2.0 analysis, we found that the epigenetic expression of VIPR1 motifs was negatively correlated with tumor-related fibrillar cells ($Rho=-0.103$, $p < 0.01$) and myeloid inhibitory cells ($Rho=-0.488$, $p < 0.01$) (figure. 4).

Discussion:

The prevention, diagnosis and treatment of lung cancer is still a difficult problem today. Through the analysis of the TCGA database, we found that the expression of VIPR1 was low in lung adenocarcinoma, while the patients with high expression had a better prognosis and the research of others is also consistent[15]. VIPR1 is the main receptor of VIP. VIP can inhibit the production of TNF- α , IL-6 and VEGF[17]. Some studies have shown that PGE2, TNF- α , IL-6, IL-1 and VEGF combined with GM-CSF can induce CD33+ monocytes isolated from healthy subjects to differentiate into MDSC[18]. Myeloid inhibitory cells (MDSC) are developed from myeloid progenitor cells (CMP). They are mainly divided into two categories: mononuclear myeloid suppressor cells (M-MDSC) and polymorphonuclear myeloid suppressor cells (PMN-MDSC) [19, 20]. Inhibitory immune infiltration microenvironment is one of the main reasons for tumor cell immune escape and poor efficacy of immunotherapy. MDSC is one of the main cells that constitute inhibitory immune infiltration microenvironment, which can inhibit the role of T cells, B cells, macrophages and dendritic cells, and directly act on epithelial cells to promote their growth and mutation[17]. PMN-MDSC can produce ROS, which can directly destroy the binding of T cell TCR to MHC, while M-MDSC can consume T cells' important nutrients, such as L-arginine, thus inhibiting the function of T cells. It has been reported that inhibition of MDSC cell infiltration can inhibit the progression of lung cancer [21]. Tumor-associated fibroblasts (CAF) are the most abundant stromal cells in the tumor microenvironment, which can secrete a variety of chemokines to promote tumor cell growth, invasion and proliferation [22]. High infiltration of MDSC and CAF in tumor tissue often indicates a poor prognosis.

In this study, by analyzing the download data of TCGA database, we found that VIPR1 was low-expressed in lung adenocarcinoma, which suggested that VIPR1 may be an important tumor suppressor gene in lung adenocarcinoma. Further analysis of the relationship between VIPR1 and clinical characteristics of lung adenocarcinoma showed that the expression of VIPR1 was significantly correlated with gender, T staging, N staging and clinical grade. At the same time, the survival curve showed that the patients with high expression of VIPR1 had a better prognosis. These results suggest that the low expression of VIPR1 may be an unfavorable factor for the prognosis of lung adenocarcinoma. In order to further explore the mechanism of VIPR1 in lung adenocarcinoma, we found that the high expression of VIPR1 can inhibit the

invasion of MDSC and CAF in tumor tissue by TIMER analysis. GSEA enrichment analysis showed that the samples with low expression of VIPR1 were mainly enriched in cell cycle, P53 signal pathway, DNA replication pathway and RNA degradation pathway. Therefore, we speculate that low expression of VIPR1 may promote the development of lung adenocarcinoma mainly by regulating cell cycle, P53 signal, DNA and RNA replication and degradation to promote MDSC and CAF infiltration. Therefore, we speculate that the high expression of VIPR1 may improve the prognosis of patients by inhibiting the production of MDSC.

Conclusion

VIPR1 gene may become a clinical prognostic index and a new target for targeted therapy of lung adenocarcinoma.

Declarations

Acknowledgements

Not applicable

Author contributions

GJZ and HQW conceived and designed the experiments. XBL and YFW analyzed the data. XBL and XWW wrote the manuscript.

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Availability of data

All data generated or analyzed during the present study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Research involving human and/or animal participants

This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

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Figures

Figure 1

The relationship between VIPR1 expression level and clinical characteristics of lung adenocarcinoma patients

Figure 2

VIPR1 functional enrichment analysis

Figure 3

Differences in immune cell composition between normal lung tissue and lung adenocarcinoma tissue

Figure 4

Correlation between VIPR1 expression level and immune infiltration level