B Cell Receptor Signaling Pathway Mutation as Prognosis Predictor of Immune Checkpoint Inhibitor for Lung Adenocarcinoma

Anqi Lin
Department of Oncology, Zhujiang Hospital, Southern Medical University, 253 Industrial Avenue, Guangzhou, 510282, Guangdong

Jianbo Fang
Department of Oncology, Zhujiang Hospital, Southern Medical University, 253 Industrial Avenue, Guangzhou, 510282, Guangdong

Quan Cheng
Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha 410008, Hunan

Zaoqu Liu
Department of Interventional Radiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan

Peng Luo
Department of Oncology, Zhujiang Hospital, Southern Medical University, 253 Industrial Avenue, Guangzhou, 510282, Guangdong

Jian Zhang (zhangjian@i.smu.edu.cn)
Department of Oncology, Zhujiang Hospital, Southern Medical University, 253 Industrial Avenue, Guangzhou, 510282, Guangdong

Research Article

Keywords: lung adenocarcinoma, B cell receptor, Immune checkpoint inhibitor, biomarker, microenvironment

Posted Date: May 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1628375/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Purpose: The advent of immune checkpoint inhibitors (ICIs) has generated a revolutionary breakthrough in the therapeutic treatment of many solid tumors, including lung adenocarcinoma (LUAD). However, the response rate of ICI therapy in patients with LUAD is low, so a clinical challenge has arisen in effectively using biomarkers to screen patients who can benefit from ICIs therapy.

Methods: In this study, we divided patients according to BCR signaling pathway gene non-synonymous mutant or not, and established univariate and multivariate Cox regression models based on a LUAD cohort treated with ICI (Miao-LUAD), and examined the relationship between the mutation status of the BCR signaling pathway and the prognosis of immunotherapy. Then, combining the data from The Cancer Genome Atlas (TCGA) LUAD cohort, the Rizvi-LUAD, the Samstein-LUAD and the Zhujiang Hospital of Southern Medical University LUAD (Local-LUAD) cohort, the mutation panorama, immunogenicity, tumor microenvironment (TME) and pathway enrichment analysis between the BCR signaling pathway mutant group (BCR signaling MUT) and the BCR signaling pathway wild group (BCR signaling WT) were comprehensively compared.

Results: It was found that, compared with the BCR signaling WT, the BCR signaling MUT had a significantly improved progression-free survival (PFS) and overall survival (OS), higher immunogenicity and immunoreactivity, and a pathway activation environment that was not conducive to the growth of tumor cells.

Conclusion: These results revealed that the mutation state of the BCR signaling pathway has potential as a biomarker to predict the efficacy of ICIs.

Introduction

Among all cancer-related deaths, those caused by lung cancer rank first both globally and in China, posing a significant threat to human health.(Gao et al. 2020; Siegel et al. 2022) Generally, lung cancer can be divided into two types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Lung adenocarcinoma (LUAD) is the main histological type in NSCLC, accounting for 40% of total lung cancer instances. Although surgery is the first choice of treatment for patients with early-stage LUAD, more than 70% of patients have reached advanced stages by the time they see a doctor. Chemotherapy and radiotherapy are the traditional treatment methods for patients with advanced LUAD. According to the statistics, the 5-year survival rate of patients with advanced LUAD under traditional treatment is less than 5%. (Arbour and Riely 2019) Therefore, there is a need to urgently prioritize the early diagnosis of lung cancer and the application of more effective treatment methods. The advent of immune checkpoint inhibitions (ICIs) therapy has brought about a revolutionary treatment for many solid tumors including lung cancer. Tumor cells can up-regulate the expression of programmed cell death ligand 1 (PD-L1), which is an immune checkpoint molecule specifically combined with the programmed cell death 1 (PD-1), so as to escape immune surveillance.(Chen et al. 2012) The ICIs targets the PD-1 or the PD-L1 axis, thus
to restore the anti-tumor activity of T cells. (Azoury et al. 2015) In the treatment of NSCLC, the use of ICI alone or together with chemotherapy has demonstrated a remarkable curative effect, and it has become the standard treatment scheme for patients with advanced NSCLC. According to the results of a phase III clinical trial, (Borghaei et al. 2021) the 5-year survival rate of NSCLC patients who have previously received platinum therapy has increased by more than 5 times (13.4% vs 2.6%) after PD-1 inhibitor treatment, compared with the docetaxel treatment group. Combined chemotherapy and anti-PD-1 antibodies were used in patients with NSCLC with ERBB-2 mutation; the median progression-free survival (PFS) reached 9 months and the median overall survival (OS) reached 24 months. (F et al. 2022) Compared with chemotherapy, the median OS of the PD-L1 inhibitor treatment group in phase II POPLAR clinical trials and phase III OAK clinical trials was prolonged to 12.6 months and 13.3 months, respectively. (Mazieres et al. 2021) However, due to factors including the exhaustion of T cells, the defect of antigen presentation, the influence of other immune checkpoint molecules and damage to the IFN-γ signaling pathway, the effective rate of ICIs therapy rarely exceeds 40%. (Kalbasi and Ribas 2020; Y et al. 2021; Juarez-Garcia et al. 2022) Therefore, it is very important to screen potential immune therapy beneficiaries through clinical usefully biomarkers.

At present, many studies have revealed potential biomarkers for predicting the efficacy of ICIs therapy, (M et al. 2021) including biomarkers related to tumors, tumor microenvironments, and peripheral blood cells, but they each have specific limitations. For example, the higher the expression level of PD-L1, the better the efficacy of PD-1/PD-L1 blocking. (Garon et al. 2019; Middleton et al. 2020) However, some studies have found that NSCLC patients benefit from nivolumab immune checkpoint therapy regardless of the expression level of PD-L1. Moreover, the expression of PD-L1 is a dynamic process, it can be induced by IFN-γ secreted by tumor infiltrating lymphocytes (TILs), and it could be affected by the process of treatment. (Wojas-Krawczyk and Kubiatowski 2020) Results consistently show that the expression of PD-L1 as a biomarker is not comprehensive. As for the non-synonymous tumor mutation burden (TMB), which is documented positively correlated with the OS of ICIs therapy, (M et al. 2021) however at present, the detection quality of TMB is uneven and there is no unified cutoff standard. (Büttner et al. 2019) Some studies have even shown that high TMB does not suggest a positive ICIs curative effect, (McGrail et al. 2021) and further study is required on TMB as a prognostic predictor. Therefore, we need new biomarkers to predict the potential efficacy of ICIs for LUAD patients.

B cell receptor (BCR) is a membrane immunoglobin that transmits signals downstream by binding extracellular antigens and ligands, thus regulating the proliferation, activation, differentiation, cell selection and apoptosis of B cells. (Dal Porto et al. 2004) B cells infiltrated in a tumor microenvironment are thought to have a dual function in tumor promotion and anti-tumor immunity. (Ss et al. 2021) While B cells activated by the BCR signaling pathway can act as antigen-presenting cell, presenting antigens to the cell surface, and then activate tumor-specific CD8 + T cells, thus enhancing anti-tumor immunity. (Ghosh et al. 2021) It is also reported in the literature that plasma cells differentiated from B cell can infiltrate tumors and play an anti-tumor role by producing the tumor-specific antibody IgG1. (Ye and Lee 2022) However, an overactivated BCR signaling pathway is also considered an important reason for the occurrence and development of B-cell-derived malignant tumors such as chronic lymphocytic leukemia.
and diffuse large B-cell lymphoma. (Protós-Pelejá et al. 2022; Taylor et al. 2022) Clinically, there are many inhibitors of BCR and related pathways, such as Bruton’s tyrosine kinas inhibition. (Jebaraj et al. 2022) However, at present, the relationship between the BCR signaling pathway and LUAD is not clear, and there is a lack of research on the relationship between the mutation of this pathway and the efficacy of ICIs. Therefore, we hope to explore the relationship between the mutation state of the BCR pathway and the efficacy of ICIs through analysis of clinical, genome and transcriptome information.

In this study, we analyzed a TCGA-LUAD data set, Samstein-LUAD, Rizvi-LUAD and Miao-LUAD ICI-treated data sets, as well as Local-LUAD data sets; compared the prognosis of ICI treatment after grouping according to the mutation status of the BCR signaling pathway; comprehensively analyzed the relationship between the BCR signaling pathway mutation and ICI treatment efficacy from the gene level to the tumor microenvironment level using the bioinformatics method; and hypothesized about the related mechanism.

Materials And Methods

LUAD sample collection

We collected two data sets of LUAD patients treated with ICI, namely Rizvi-LUAD (Rizvi et al. 2018) and Miao-LUAD. (Samstein et al. 2019) Both ICI-treated cohorts included mutation data and prognosis data. In addition, we downloaded the LUAD dataset from the TCGA database using the TCGAbiolinks R package, (Colaprico et al. 2016) which included mutation data, expression data and clinical data.

We collected 70 LUAD samples from Zhujiang Hospital of Southern Medical University, and used targeted sequencing (HapOncoTM680 Panel) to obtain the mutation data (see Supplementary Table 1 for Panel on targeted sequencing). The written informed consent of all participants was obtained, and this study was approved by the Zhujiang Hospital Research Ethics Committee of Southern Medical University.

Status Analysis of BCR receptor signaling pathway

The B cell receptor signaling pathway gene set (KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY) comes from the MsigDB database (Supplementary Table 2). (Liberzon et al. 2011) We preprocessed the somatic mutation data of patients (deleting synonymous mutation data and retaining non-synonymous mutation data). (Mayakonda et al. 2018) Then, we counted the number of gene mutations in the B cell receptor signaling pathway in every LUAD patient. If the number of gene mutations in the B cell receptor signaling pathway in LUAD patients is zero, then the patient is a B cell receptor signaling pathway wild-type (WT). Otherwise, the patient is a B cell receptor signaling pathway mutant-type (MUT). The baseline clinical information of Miao-LUAD, TCGA-LUAD were shown in the Supplementary Fig. 1–2.

Immunogenicity, immune infiltration and pathway enrichment analysis
Drawing on previously published research results, Immunogenicity data includes tumor TMB and neoantigen loads (NAL). (Thorsson et al. 2019) In addition, we collected the gene sets of DNA damage repair-related pathways, and counted the number of mutations in each patient's DNA damage repair (DDR)-related pathways. Immune infiltration analysis is mainly immune cell analysis and immune-related pathway score analysis. In the analysis of immune cells, we applied a CIBERSORT algorithm (Chen et al. 2018) to estimate the relative abundance of 22 kinds of immune cells in each patient's TME. Immune-related scores come from a previously published study. Pathway enrichment analysis includes gene set enrichment analysis (GSEA) and single sample gene set enrichment analysis (ssGSEA). (Yu et al. 2012; Hänzelmann et al. 2013)

**Statistical test**

The Mann-Whitney U test evaluates the statistical difference between the two groups of categorical variables, and Fisher's exact test evaluates the statistical differences between the two groups of categorical variables. The univariate and multivariate Cox risk proportional regression model and Kaplan-Meier analysis were used to explore the prognosis of the B cell receptor signaling pathway for patients with LUAD who received ICI treatment. All data analysis and data visualization in this study are based on R software (Version 3.6). The p value is bilateral and p < 0.05 has statistical difference.

**Results**

LUAD patients with BCR signaling pathway mutation have better PFS and OS after ICI treatment

Figure 1A is a brief flowchart of this study. According to whether the BCR signaling pathway gene is non-synonymous mutated or not, the 47 patients in the Miao-TCGA cohort were divided into a BCR signaling MUT group (31) and a BCR signaling WT group (16). The results of the univariate Cox regression model showed that the BCR signaling pathway was a protective factor in the mutant group, which could predict better PFS and OS. After adjusting for related clinical confounding factors such as gender and smoking, the results of the multivariate Cox regression model confirmed that the mutation of the BCR signaling pathway can be used as an independent protective factor for LUAD patients receiving ICI treatment (Fig. 2A). Next, we carried out a Kaplan-Meier survival analysis of the Miao-LUAD cohort between BCR signaling MUT and WT groups to establish whether the mutant state of this pathway can effectively predict the prognosis of ICI treatment. The results showed that the BCR signaling MUT group had significantly longer PFS (Logrank test, HR = 0.25, 95% CI [0.03–2.07], p = 0.018) (Fig. 2B) and longer OS (Logrank test, HR = 0.48, 95% CI [0.20–1.14], p = 0.032) (Fig. 2C).

**Overview of gene mutation between BCR signaling MUT and WT groups**

In the Miao-LUAD cohort, the somatic mutant genes were compared with the top 20 mutation frequencies across BCR signaling MUT patients and BCR signaling WT patients (Fig. 3A). We found that in the BCR signaling MUT group, KRAS (52% vs 0%; p < 0.05), CSMD3 (42% vs 0.06%; p < 0.05), and COL11A1 (39% vs 0%; p < 0.05) genes had significantly higher mutation rates than in the WT group.
vs 0; p < 0.05), the mutation frequency of the three genes increased significantly, among which only KRAS gene is a tumorigenic gene. Then, mutually exclusive co-occurrence analysis was conducted on these 20 genes (Fig. 3B). From this it was clear that KRAS and EGFR are mutually exclusive (p < 0.05), a result consistent with the conclusions of previous studies. It was also worth noting that there was no statistically significant difference in gender and the proportion of smokers between the MUT and WT groups, suggesting that these two clinical factors may not be related to the mutation of the BCR signaling pathway.

The BCR signaling MUT group indicates higher immunogenicity compared with the BCR signaling WT group

Sufficient immunogenicity is the basis of the immune response. In order to explore the relationship between the mutation status of the BCR signaling pathway and immunogenicity, we compared the TMB, NAL and mutation numbers of DNA damage repair (DDR) related pathways. The results of TMB analysis showed that among the Local-LUAD, Rizvi-LUAD, Samstein-LUAD and TCGA-LUAD, the TMB of the BCR signaling MUT group was significantly higher than that of the BCR signaling WT group (Fig. 4A-D; all p < 0.05). Furthermore, in the TCGA-LUAD, the analysis results on the number of DDR related pathways mutations also support the hypothesis that the BCR signaling MUT group had higher immunogenicity; that is, the BCR signaling MUT group had higher number of BER, DDR, DSB, FA, HR, SSB, NHEJ, NER and MMR pathway gene mutations than the BCR signaling WT group (Fig. 4E; all p < 0.05). In addition, the TCGA-LUAD cohort BCR signaling MUT group had higher NAL than the BCR signaling WT group, and the number of NAL measures the number of neoantigen in tumor cells, so as to evaluate the tumor ability to induce a tumor-specific immune response.

Differences in the tumor microenvironments between BCR signaling MUT and BCR signaling WT

Based on the mutation data and transcriptome data of the TCGA-LUAD cohort, a comparative evaluation was conducted between the mutation status of the BCR signaling pathway and the 22 kinds of immune cells infiltrated in tumors, using a CIBERSORT algorithm (Fig. 5A). The result showed that the numbers of CD8+ T cells, activated CD4 memory T cells and activated mast cells in BCR signaling MUT group were higher (all p < 0.05). However, the numbers of regulatory T cells (Tregs), monocytes, resting CD4 T memory cells, resting mast cells and resting dendritic cells increased significantly in BCR signaling WT group (all p < 0.05). Then the immune-related scores (Fig. 5B) was calculated for the TCGA-LUAD cohort, and the TH2 cell score and IFN-γ response score were significantly higher in the BCR signaling MUT group (all p < 0.05). Interestingly, although there is no statistically significant difference in the infiltration degree of M1 macrophages between the BCR signaling MUT and BCR signaling WT groups, we can still see the increased infiltration trend of M1 macrophages in MUT group, which may be related to the small sample
size. Consistent with this, the macrophage regulation score of BCR signaling MUT group is significantly higher than that of BCR signaling WT group (p < 0.05).

**Pathway enrichment analysis between BCR signaling MUT and BCR signaling WT**

Based on transcriptome data of TCGA-LUAD cohort, ssGSEA and GSEA were used to enrich and analyze the functional gene sets. The results of ssGSEA (Fig. 5C) showed that the ssGSEA scores of several cytokines and chemokines that inhibit anti-tumor immunity, such as IL-1β, IL-10, CXCL2, CXC2 and TGF-β, are significantly lower in BCR signaling MUT group (all p < 0.05). The ssGSEA score of arachidonic acid and fatty acid metabolism pathways in BCR signaling MUT group was also significantly lower than that in WT group (all p < 0.05). In addition, GSEA results confirmed the significant activation of pathways related to the inhibition of tumor occurrence and development in BCR signaling MUT group (p < 0.05, ES > 0) (Fig. 6A), such as the SMAD signaling pathway and the FoxO signaling pathway. Whereas, the signaling pathways related to the promotion of tumor occurrence and development were significantly down-regulated in BCR signaling MUT group (p < 0.05, ES < 0) (Fig. 6A), such as signaling by WNT in cancer, and constitutive signaling by aberrant PI3K in cancer. Similarly, within BCR signaling MUT group the signal pathways relating to angiogenesis were significantly down-regulated (p < 0.05, ES < 0) (Fig. 6B), such as vascular endothelial cell proliferation, vascular endothelial growth factor signaling pathway, Hedgehog signaling pathway and fibroblast growth factor receptor signaling pathway. The results of pathway enrichment analysis indicated that BCR signaling MUT group had a pathway enrichment environment which was not conducive to tumor growth and metastasis.

**Discussion**

Based on the analysis of mutation data and clinical data of the ICI-treated Miao-LUAD cohort, this study found that mutation of the BCR signaling pathway was a potential predictor of ICIs efficacy. Compared with WT group, BCR signaling MUT group had better PFS and OS. By analyzing the transcriptome data of the TCGA-LUAD cohort, we found that BCR signaling MUT group had higher immunogenicity and more CD4 + and CD8 + T cell infiltration degree, as well as higher immune-related signature scores. The results of the pathway enrichment analysis also showed that the signaling pathways of BCR signaling MUT group to promote the proliferation and metastasis of tumor cells was significantly down-regulated, and conversely the signaling pathways for inhibiting the proliferation and metastasis of tumor cells was significantly up-regulated. These conclusions support our initial hypothesis, that is, the mutation state of the BCR signaling pathway can be used as a potential biomarker to predict the efficacy of ICIs therapy for LUAD patients.

TME is a complex system mainly composed of tumor cells, surrounding immune and inflammatory cells, tumor-related fibroblasts, nearby interstitial tissues, capillaries, and various cytokines and chemokines. (Dougan and Dougan 2017) Before ICIs baseline treatment, TILs infiltrated in the TME; in particular, CD8 + T cells were reported to be positively correlated with ICIs treatment efficacy,(Jacquelot et al. 2017;
Zhang et al. 2021; Huang et al. 2021) and CD8 + T cells were considered to be specific anti-tumor T cells. They can produce IFN-γ, TNF and granzyme B through the specific combination of T cell receptors with tumor cells, thus killing tumor cells.(Reiser and Banerjee 2016) The main factors that limit the function of CD8 + T cells are whether T cells can differentiate into sufficient CD8 + T cells, and whether CD8 + T cells can infiltrate into a TME smoothly.(Nolz 2015) CD4 + T cells play an important role in helping CD8 + T cells to enhance anti-tumor immunity.(Tran et al. 2014; Cui et al. 2021) Once activated by tumor-associated antigen, CD4 + T cells can promote the maturation of dendritic cells, present more antigens, and promote the differentiation of naive CD8 + cells into effector CD8 + T cells, thus increasing the number of CD8 + T cells. CD4 + T cells can also simultaneously act as cytotoxic cell by secreting IFN-γ and TNF or express receptors of TNF family factors such as FASL and TRAIL, mediating a direct cytotoxic immune response. MHC class II restricted antigen recognition of CD4 + T cells, as distinct from MHC class I restricted antigen recognition of CD8 + T cells, is an important supplementary part of antigen recognition in anti-tumor immunity. Interestingly, in the absence of CD4 + T cells, the ability of CD8 + T cells to kill tumor cells is also limited. According to a recent study, the number of memory CD4 + T cell subsets in peripheral blood before treatment is a strong predictor of the efficacy of immunotherapy for NSCLC. To sum up, the synergy between CD4 + T cells and CD8 + T cells is the key to anti-tumor immunity. In this study, we analyzed the infiltration degree of 22 kinds of immune cells in the TCGA-LUAD cohort via a CIBERSORT method. There are more CD8 + T cells and activated memory CD4 + T cells infiltrating in BCR signaling MUT group. On the contrary, the degree of Tregs, which can directly inhibit CD8 + T cells infiltration in LUAD,(Ap et al. 2013) infiltration in BCR signaling MUT group is significantly lower than that in WT group. The role of mast cells in tumor immunity has been controversial. According to epidemiological surveys, the existence of mast cells in TME is negatively correlated with the progress of lung cancer.(Sinnamon et al. 2008; Hodges et al. 2012) Mast cell-derived TNF can kill tumor cells;(Liu et al. 2011) however, the humanized-mouse melanoma model proves that tumor-infiltrated mast cells are related to the tolerance of anti-PD-1 antibody treatment, which is a negative factor for immunotherapy efficacy.(R et al. 2021) In our results, there are significant differences in the degree of mast cell infiltration between the BCR signaling MUT group and WT group, but both only for a small number. Further research is required to determine the role it plays. In general, the anti-tumor immune microenvironment clearly activated in BCR signaling MUT group can explain why this group has significantly prolonged PFS and OS.

Immunogenicity of tumors is considered to be a prerequisite for exerting anti-tumor immunity.(Wang et al. 2019) Based on the analysis of RNA expression data and mutation data, and after comparing TMB, NAL and DDR-related pathways mutations of BCR signaling MUT group and WT group, which are representative markers for evaluating immunogenicity, it was found that BCR signaling MUT group had higher immunogenicity. In addition, correspondingly, the results of immune-related characteristic scores show that the BCR signaling MUT group has a higher IFN-γ score. IFN-γ secreted by TILs activates cellular immunity and induces cell cycle arrest and death of cancer cells.(Schoenborn and Wilson 2007) Furthermore, the role of macrophages in anti-tumor immunity varies according to their phenotype, while IFN-γ can induce the differentiation of type I macrophages, and make it secrete IL-12 to induce an anti-
tumor immune response. (Ostrand-Rosenberg et al. 2012) Studies have shown that IFN-γ secreted by T cells, stimulated by tumor antigens, can spread widely, and play an important role in clearing metastatic tumor cells. (Hoekstra et al. 2020) A recent study has proven that IFN-γ activates the immune response by activating the tumor intrinsic STING pathway in NSCLC, which in turn is related to the increase of DNA damage, (Della Corte et al. 2020; Xiong et al. 2022, p.) consistent with our previous results that BCR signaling MUT group had higher DDR-related pathways mutations.

Chemokines and cytokines secreted by immune cells in TME, such as IL-12, IFN-γ, CXCL-9, and CXCL-10, are important components of LUAD anti-tumor immunity. (Lin et al. 2021) However, in our ssGSEA results, cytokines and chemokine produce pathways related to immunosuppression, such as IL-10, IL-1β, CCL2 and CXCL2, were significantly down-regulated in BCR signaling MUT group, functions of these factors which are secreted by cancer cells and cancer-related immune cells are completely opposite with those mentioned at the beginning. (Paval et al. 2022) These factors recruit tumor-associated macrophages (TAMs), Tregs and Myeloid-derived suppressor cells (MDSCs) into the TME, helping tumor cells escape detection by the immune system and promoting the development and metastasis of tumors. (Shimizu et al. 2010; Srivastava et al. 2012) There is evidence that IL-10 secreted by cancer cells activated type II macrophages, the latter promoting tumor growth by inhibiting T cells. Tumor cells can also recruit a large number of Tregs and MDSCs for themselves by secreting IL-10 and TGF-β and up-regulating the expression of CXCR2 ligand. (Shimizu et al. 2010; Zaynagetdinov et al. 2012) Both Tregs and MDSCs are notorious immunosuppressive cells, which jointly inhibit the infiltration and function of CD8 + T cells and mediate the adaptive immune tolerance of tumors. Anti-tumor immunity can be further weakened through the up-regulation of IL-10, which can promote the differentiation of native CD4 T cells into Tregs. (Zdanov et al. 2016) IL-1β is a pro-inflammatory factor, and in the NSCLC mouse model, IL-1β derived from mast cells and tumor-associated neutrophils has been proven to be beneficial to tumor growth. (McLeod et al. 2016; Lilis et al. 2019) The effects of IL-1β are significantly related to a poor prognosis for NSCLC. (Kim et al. 2013) At the same time, a drug clinical trial using IL-1β inhibitor showed that, after the secretion of IL-1β is inhibited, the mortality rate related to NSCLC decreased significantly. (Ridker et al. 2017a, b) In addition, in the study of the mouse model of pleural cancer with lung cancer metastasis, researchers found that the vicious circle of IL-1β and CCL2 working together explained the occurrence and development of malignant pleural effusion in NSCLC. (Giannou et al. 2015; Aglioti et al. 2017; Marazioti et al. 2018)

In addition, in our GSEA results, some classic signaling pathways that promote tumor occurrence and development were significantly down-regulated in BCR signaling MUT group, (Akiri et al. 2009; Ivy et al. 2009; Vanhaesebroeck et al. 2010; Xi and Chen 2014) such as signaling by WNT in cancer and constitutive signaling by aberrant PI3K in cancer. It is worth noting that the pathways related to tumor angiogenesis, which is the basis for the survival and metastasis of tumor cells, were also significantly down-regulated in BCR signaling MUT group. (Chinchilla et al. 2010; Desai and Adjei 2016; Hu et al. 2022) Tumor angiogenesis in solid tumors can also prevent tumor-specific T cells from infiltrating TME. (Bellone and Calcinootto 2013) Finally, the analysis revealed that the signal pathways related to tumor inhibition were significantly up-regulated in MUT group, such as positive regulation of pathway restricted SMAD
protein phosphorylation; (Markowitz and Roberts 1996) activated SMAD protein inhibited the activation of TGF-β signal pathway, which was reported to be related to the enhancement of tumor invasiveness and metastasis in advanced NSCLC. Correspondingly, in ssGSEA results, BCR signaling MUT group had lower scores of TGF-β signaling pathway. FoxO signaling pathway not only inhibits the abnormal expression of Fox family proteins, but also, and consequently, inhibits the role of these proteins in promoting tumor growth. (Katoh et al. 2013) In addition, we also found that the MUT group had lower scores in ssGSEA, a signaling pathway related to fatty acid metabolism, which was related to immune failure and tumor metastasis. (Zhao et al. 2019) To sum up, the enrichment results show that the MUT group has a pathway expression environment that is not conducive to tumor growth and metastasis. These results may indicate the weakness in the viability of tumor cells in LUAD patients in BCR signaling MUT group, compared with WT group.

Generally speaking, our study comprehensively analyzed the relationship between the mutation state of BCR signaling pathway and the prognosis of LUAD patients treated with ICI, from the perspectives of survival analysis, gene mutation, immune infiltration and pathway enrichment, and deduced the related mechanism; however this study still has several limitations. First, because the survival data of the LUAD cohort treated with ICI is very limited, the relationship between the BCR signaling pathway and ICI treatment survival was only able to be explored in the Miao-LUAD cohort. Secondly, we did not carry out related cell experiments and animal experiments to further verify our results; this is an area of research that we hope to supplement in the future. Thirdly, because of the quantity and complexity of mutation sites in the BCR signaling pathway, we did not explore the specific molecular mechanism between mutation and ICI efficacy, but we compared the TME differences between the wild group and the mutant group, and formed a hypothesis for the mechanism, using immune infiltration analysis and pathway enrichment analysis combined with previous literature reports. Finally, we hope that our findings can provide assistance for future cancer mechanism investigation.

**Conclusion**

In this study, we found that there is a significant relationship between the mutation state of BCR signaling pathway and the therapeutic effect of ICI. As a result, the BCR signaling MUT group had better PFS and OS. With further analysis of expression data, mutation data and clinical data, it was found that the MUT group had higher immunogenicity and a more anti-tumor activated microenvironment. The results of pathway enrichment analysis reveal that the MUT group has a pathway enrichment environment which is definitely unfavorable for tumor growth and metastasis, and these results may explain why the BCR signaling MUT group has longer PFS and OS. To sum up, these results support the mutation status of the BCR signaling pathway as a potential biomarker to predict the efficacy of ICI therapy for LUAD.

**Abbreviations**

ICIs (Immune checkpoint inhibitors), LUAD (lung adenocarcinoma), TCGA (The Cancer Genome Atlas), MUT (mutant-type), WT (wild-type), TME (tumor microenvironment), TILs (tumor infiltrating
lymphocytes), PD-L1 (programmed cell death ligand 1), PD-1 (programmed cell death 1), NSCLC (non-small cell lung cancer), SCLC (small cell lung cancer), TMB (tumor mutation burden), BCR (B cell receptor), NAL (neoantigen loads), GSEA (gene set enrichment analysis), ssGSEA (single sample gene set enrichment analysis), PFS (Progression-free survival), OS (overall survival), TAMs (tumor-associated macrophages), Tregs (regulatory T cells), MDSCs (Myeloid-derived suppressor cells).

Declarations

Funding

This work was supported by the Natural Science Foundation of Guangdong Province (Grant No. 2018A030313846 and 2021A1515012593), the Science and Technology Planning Project of Guangdong Province (Grant No. 2019A030317020) and the National Natural Science Foundation of China (Grant No. 81802257, 81871859, 81772457, 82172750 and 82172811).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

AL, JF, QC and ZL wrote the article; PL and JZ designed the research; AL and JF performed the research; AL, JF, QC, ZL, PL, and JZ Writing-review and editing.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The patients/participants provided their written informed consent to participate in this study and the research presented here has been performed in accordance with the Declaration of Helsinki and has been approved by the ethics committee of the Zhujiang Hospital of Southern Medical University.

Consent to participate

Informed consent was obtained from all individual participants included in the study.
Consent to publish

Not applicable.

References


**Figures**

**Figure 1**

(A) Work flowchart of clinical cohort establishment and subsequent analysis in this study
Figure 2

The predictive value of clinical characteristics and the mutation status of the BCR signaling pathway for ICI efficacy. (A) Forest plot of the results of the univariate and multivariate Cox regression analyses in the Miao-LUAD cohort (ICI-treated cohort). The main portion of the forest plot presents the hazard ratios (HR) and 95% confidence intervals (95%CI). The p value represents the statistical significance of the variable. The HR indicates whether the factors are predictors of favorable (HR < 1) or poor (HR > 1) outcomes. KM
survival curves for (B) PFS and (C) OS in 47 LUAD patients from the Miao-LUAD cohort. LUAD, lung adenocarcinoma; PFS, progression-free survival; OS, overall survival

Figure 3

Genomic profiles of 47 LUAD patients in the Miao-LUAD cohort. (A) The 20 genes with the highest mutation frequencies and corresponding clinical information. (B) Mutual exclusion co-occurrence analysis of the top 20 mutated genes
Figure 4

Comparison of TMB between the MUT and WT groups in the (A) Local-LUAD cohort, (B) Rizvi-LUAD cohort, (C) Samstein-LUAD cohort, and (D) TCGA-LUAD cohort. (E) Comparison of DDR related signaling pathways alterations between the MUT and WT groups in the TCGA-LUAD cohort. (F) Comparison of NAL between the MUT and WT groups in the TCGA-LUAD cohort. MUT, B cell receptor signaling pathway
mutant type; WT, B cell receptor signaling pathway wild type; TMB, tumor mutation burden; DDR, DNA damage repair; NAL, neoantigen load

Figure 5

(A) Comparison of the proportions of immune cells estimated by the CIBERSORT method between MUT and WT groups in the TCGA-LUAD cohort. (B) Comparison of immune related scores between MUT and WT groups in the TCGA-LUAD cohort.
WT groups in the TCGA-LUAD cohort. The immune related scores are Th2 Cell, Macrophage Regulation and IFN-gamma response. (C) Results of ssGSEA analysis between MUT and WT groups in the TCGA-LUAD cohort (*p<0.05; **p<0.01; ***p<0.001; and ****p<0.0001; Wilcoxon rank-sum test)

Figure 6
(A-B) Comparison of GSEA analysis between MUT and WT groups in the TCGA-LUAD cohort. (C) Potential mechanism underlying the prognostic value of the BCR signaling pathway mutation

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFig1.pdf
- SupplementaryFig2.pdf
- SupplementaryTable1.xlsx
- SupplementaryTable2.xlsx