Association Between FSIP2 Mutation and an Improved Prognosis in Patients With Skin Cutaneous Melanoma Treated With Immune Checkpoint Inhibitors

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Primary research

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Abstract

Background

Immune checkpoint inhibitors (ICIs) have been remarkably successful in skin cutaneous melanoma (SKCM), however the response to treatment varies greatly among different patients. Considering that the efficacy of ICI treatment is affected by many factors, we selected the Fibrosheath interacting protein 2 (FSIP2) gene and systematically analyzed its potential as a predictor of ICI treatment prognosis.

Methods

Patient data were collected from an ICI treatment cohort (n = 120) and The Cancer Genome Atlas (TCGA)-SKCM cohort (n = 467). The data was divided into an FSIP2-mutant (MT) group and FSIP2-wild-type (WT) group according to the FSIP2 mutation status. In this study we analyzed the patients' overall survival rate, tumor mutational burden (TMB), neoantigen load (NAL), copy number variation (CNV), cell infiltration data and immune-related genes. We used gene set enrichment analysis (GSEA) to delineate biological pathways and processes associated with the prognosis of immunotherapy.

Results

The prognosis of SKCM patients with FSIP2-MT receiving ICIs was significantly better than that of those with FSIP2-WT. The patients in the FSIP2-MT group had higher tumor immunogenicity and lower regulatory T cell (Treg) infiltration. Results of GSEA showed that pathways related to tumor progression (MAPK and FGFR), immunomodulation, and IL-2 synthesis inhibition were significantly downregulated in the FSIP2-MT group.

Conclusion

Our research suggests that the FSIP2 gene has the potential to predict the prognosis of ICI treatment. The higher tumor immunogenicity and lower Treg levels may be closely related to the fact that patients with FSIP2-MT can benefit more from ICI treatment.

Introduction

Skin cutaneous melanoma (SKCM) is a common skin tumor caused by uncontrolled proliferation of epidermal melanocytes, notorious for its rapid progression and poor prognosis. According to the 2018 global cancer statistics (Bray et al. 2018), skin cancer (melanoma) accounts for approximately 21.6% of new cases of skin cancer and 46% of all skin cancer deaths. Although traditional treatments including surgery, radiotherapy and chemotherapy have made great progress in recent years, the efficacy of these traditional treatments is not satisfactory due to the resistance of SKCM to chemotherapy and

radiotherapy and the side effects caused by the treatments. The five-year survival rate is 20% for patients with metastatic melanoma, and the 10-year survival rate is only 10%(Long et al. 2016; O et al. 2019); thus, a more effective treatment is urgently needed.

In recent years, the discovery of CTLA-4, PD-1/PD-L1 and other immune checkpoint molecules has given us a deeper understanding of immunosuppression limiting antitumor immunity and provided new ideas for tumor immunotherapy. Monoclonal antibodies called immune checkpoint inhibitors (ICIs) have been prepared against immune checkpoint molecules. ICIs have been used to treat a variety of malignant tumors, including SKCM, and were recognized with the Nobel Prize in 2018(TB et al. 2020). Clinical studies show that the five-year survival rate of patients with metastatic SKCM treated with an anti-PD-1 monoclonal antibody (nivolumab) is 34%; when treated with an anti-PD-1 antibody and anti-CTLA-4 antibody (nivolumab and ipilimumab), the five-year survival rate rises to 44%(Larkin et al. 2019). Obviously, patients benefit more from ICIs than from traditional treatment. Although ICIs have shown good clinical efficacy, only a small number of patients benefit from long-term treatment(Rotte et al. 2015), and the factors affecting the efficacy of ICIs are still uncertain.

Fibrosheath interaction protein 2 (FSIP2) is an important part of the fiber sheath, which makes up the cytoskeletal structure of the main part of the sperm flagellum. The sheath is a scaffold of glycolytic enzymes and signaling proteins and plays an important role in vitality regulation(K et al. 2016; Martinez et al. 2018). Although the expression of FSIP2 is testis specific(Brown et al. 2003), we have found that FSIP2 has a higher mutation frequency not only in male reproductive system tumors, such as testicular germ cell tumors, but also in Paget disease, liver cancer and other cancers(G et al. 2019; K et al. 2015; Zhang et al. 2014). There are few studies on the relationship between FSIP2 and cancer, but some studies have shown that FSIP2 is not only an important part of AKAP4 but also influences the function of PKA by docking on AKAP4. AKAP4 has been found to be highly expressed in a variety of cancers, and the regulatory subunit PKAI of PKA has also been shown to play important roles in promoting the proliferation and transformation of tumors and the generation of immunosuppressive microenvironments in the tumor microenvironment (TME)(Brown et al. 2003; M et al. 2015; Martinez et al. 2018).

The TME is the cellular environment in which tumors exist and includes peripheral blood vessels, extracellular matrix components, and other nontumor cell nuclear signaling molecules. The growth and metastasis of tumors are inseparably linked to the TME in which the tumors are located(Hui and Chen 2015). A number of studies have pointed out that the efficacy of ICIs is related to the infiltration of lymphocytes (CD8 + T cells; CD4 + T cells, etc.) and expression of cytokines (IFN- γ , IL-2, IL-17, etc.) in the TME(Abiko et al. 2015; Garriss et al. 2018). We speculate that FSIP2 may regulate the expression of PKA by affecting the expression of AKAP4, which in turn influences immune infiltration in the TME, and this process also provides suitable immune targets for immunotherapy, suggesting that the prognosis of ICI treatments may be related to FSIP2 mutations.

At present, there is no systematic analysis addressing the relationship between FSIP2 and the efficacy of ICIs in the treatment of SKCM, so we hope to collect and analyze existing retrospective ICI treatment

cohort data to clarify the association between them. Therefore, we divided our patients into two groups according to FSIP2 gene mutation, systematically compared tumor immunogenicity, the TME, the expression of immune-related genes and signaling pathways between tumors with mutant FSIP2 (FSIP2-MT) or wild-type FSIP2 (FSIP2-WT), providing a theoretical basis for formulating new treatment options.

Methods

2.1. Clinical cohorts and gene expression data

To evaluate the relationship between FSIP2 gene mutation and the prognosis of SKCM patients who received ICIs, an immunotherapy cohort with clinical and whole-exon sequencing (WES) data was collected (Miao et al. 2018). We reserved SKCM patients who received ICI treatments (anti-CTLA-4 therapies; anti-PD-1/PD-L1 therapies; or combined therapies) (n = 120) for further analysis. The R package TCGAAbiolinks (Colaprico et al. 2016) was used to download The Cancer Genome Atlas (TCGA)-SKCM cohort (n = 467) with somatic mutation and survival data (overall survival, OS) from the Genomic Data Commons (<https://portal.gdc.cancer.gov/>). To analyze the immunogenicity of tumors, we collected neoantigen load (NAL) data from the TCGA-SKCM cohort (Thorsson et al. 2018) and downloaded SKCM cell line WES data, from Genomics of Drug Sensitivity in Cancer (GDSC) (Yang et al. 2012). We used gene expression data (Illumina HiSeq, RNASeq) downloaded with TCGAAbiolinks for immune cell infiltration analysis. Copy number variation (CNV) data were obtained through Broad GDAC Firehose (<http://gdac.broadinstitute.org/>).

2.2. kaplan-meier (km) Analysis

We screened out patients with SKCM and divided them into mutant FSIP2 (FSIP2-MT) and wild-type FSIP2 (FSIP2-WT) groups according to FSIP2 gene mutation data. KM analysis was used to determine whether prognosis differed between the two groups after ICI therapy. To determine whether the prognosis of SKCM patients given traditional treatment is affected by FSIP2 mutation, we also conducted a KM analysis of TCGA-SKCM based on the FSIP2 gene mutation status.

2.3. tumor Immunogenicity Analysis

The occurrence and development of cancer are always accompanied by changes in DNA. The number of nonsynonymous mutations (Mb) in each trillion bases is the tumor mutational burden (TMB), which has been suggested to be related to the clinical efficacy of ICI treatment (D et al. 2020). Neoantigens are new peptides produced by somatic mutation. These newly generated peptides can drive immune responses against cancer cells by being recognized as foreign substances in the body (Barroso-Sousa et al. 2020). Therefore, the neoantigens load (NAL) is also considered to be the basic determinant of the immunotherapy response. Data on the in TCGA-SKCM have been reported in the literature (Thorsson et al. 2018). Consistent with other studies (Chalmers et al. 2017), we used nonsynonymous mutations in an ICI-

treated cohort (Miao et al), TCGA-SKCM and GDSC-SKCM as raw mutation counts and divided by 38 Mb to quantify the TMB. Based on the R package ComplexHeatmap(Gu et al. 2016), we visualized the mutation panorama and clinical characteristics of the immunotherapy cohort and TCGA-SKCM cohort (the top 20 mutated genes).

2.4. copy Number Alteration (cnv) Analysis

Genomic Identification of Significant Targets in Cancer (GISTIC) is an algorithm used to identify mutation sites that may be associated with cancer pathogenesis. It can visualize regions in the genome to show amplification and missing bases in thousands of samples(Zheng et al. 2020). We used GenePattern(Reich et al. 2006) to analyze the Affymetrix SNP 6.0 microarray data of TCGA-SKCM that we downloaded and performed CNV visualization on the GISTIC2.0 analysis results with the R package Maftools(Mayakonda et al. 2018). When performing GISTIC2.0 analysis, except for the confidence level set at 0.99 and not excluding the X chromosome before analysis, the GISTIC2.0 analysis used the default (default settings) parameter settings.

2.5. tumor Immune Status And Drug Sensitivity Analyses

The effectiveness of immunotherapy is affected by multiple factors, such as tumor immunogenicity and antigen presentation efficiency, so we used CIBERSORT(Newman et al. 2015) (<http://cibersort.stanford.edu/>) to analyze downloaded TCGA-SKCM cell infiltration data. We mainly analyzed the infiltration statuses of 22 kinds of immune cells, including B cells, NK cells, T cell subsets (CD8 + T cells, CD4 + T cells, T helper cells, regulatory T cells (Tregs), and gamma delta T cells), monocytes and macrophages (M0, M1, and M2). In addition, we also compared the mRNA expression levels of immune-related genes in the FSIP2-MT and FSIP-WT groups created from the TCGA-SKCM cohort. Immune cell-related genes(Hao et al. 2018), immune-related genes and their functional classification(Thorsson et al. 2018) have been reported in the literature. The expression levels of these genes were quantified as log2 (FPKM + 1), and the fold-change (FC) cutoff was selected to be greater than 1.49 or less than 0.67. To analyze the effect of FSIP2 gene mutation on conventional treatment, we downloaded the data for SKCM cell lines with drug sensitivity data from GDSC and compared the differences in the sensitivities to different drugs between FSIP2-MT and FSIP-WT groups.

2.6. gene Set Enrichment Analysis (gsea)

Through the R package edgeR(Robinson et al. 2010), we performed GSEA on gene expression data (raw count) in the TCGA-SKCM queue downloaded from TCGAbiolinks. We used the clusterProfiler R package(Yu et al. 2012) to annotate the gene dataset, and $p < 0.05$ was used as the threshold for gene ontology (GO) terms, Kyoto Encyclopedia of Genes and Genomes (KEGG) results and Reactome results to be considered significantly different.

2.7.statistical Analysis

In this study, R software (version 3.6.1) was used for statistical analysis of data, and all statistical tests were set as two-sided tests. $P < 0.05$ was considered significant. We used the Mann-Whitney U test to compare the differences in the TMB, immune cell abundance, immune-related gene expression, and age between FSIP2-WT and FSIP2-MT groups. Fisher's exact test was used to compare differences between the top 20 mutation rates of the FSIP2-WT and FSIP2-MT groups in the immunotherapy cohort and the TCGA-SKCM cohort. In the immunotherapy cohort, we used Fisher's exact test to compare sex and treatment response between the FSIP2-WT and FSIP2-MT groups. In the TCGA-SKCM cohort, we also used Fisher's exact test to compare sex, race, ethnicity and clinical stage between the FSIP2-WT and FSIP2-MT groups. The KM method and log-rank test were used for survival analysis. The visualization box plot in this paper was generated with the R package ggpubr(Kassambara 2018), and the CNV visualization false discovery rate (FDR) was 0.05.

Results

3.1.FSIP2-MT is associated with a good ICI prognosis

We divided the somatic mutation and survival data (OS) from the clinical ICI treatment cohort(Miao et al. 2018) ($n = 120$) and TCGA-SKCM cohort ($n = 467$) into two groups based on the FSIP2 mutation status. In this way, the ICI treatment cohort was divided into FSIP2-MT ($n = 15$) and FSIP2-WT ($n = 105$) groups, and the TCGA-SKCM cohort was also divided into FSIP2-MT ($n = 58$) and FSIP2-WT ($n = 409$) groups. Survival analysis of these data showed that among the patients receiving ICIs, those in the FSIP2-MT group were more sensitive to ICI treatment ($p = 0.038$; hazard ratio (95% confidence interval (CI)): 0.43 (0.23–0.82); Fig. 1a). In contrast, traditional SKCM treatment options are generally surgical resection or chemotherapy(Coit et al. 2019). The survival analysis results for the TCGA-SKCM cohort showed that when receiving conventional treatment, OS was not significantly different between the FSIP2-MT and FSIP2-WT groups ($p = 0.978$; hazard ratio (95% CI): 1.01 (0.68–1.49); Fig. 1b).

3.2.Relationship among clinical characteristics, gene mutations and FSIP2 gene mutations in patients

Based on the mutation status of FSIP2, we compared differences in clinical characteristics between the FSIP2-MT and FSIP2-WT groups. Figure 2a shows that in the immunotherapy cohort from the literature, there were no significant differences in sex, treatment response or OS; however, patients with FSIP2 gene mutations tended to have an older age ($p = 0.047$). As shown in Fig. 2b, in the TCGA-SKCM cohort, excluding the influencing factor of receiving ICI treatment, there were no significant differences in age, stage, race or ethnicity between the two groups, but there were more men in the FSIP2-MT group ($p = 0.043$).

In addition, in Fig. 2, we also show the gene mutation panoramas of the ICI treatment cohort and the TCGA-SKCM cohort. As shown in Fig. 2a, among the top 20 mutated genes in the ICI treatment cohort,

except for the higher mutation frequencies of the MUC16, USH2A, DNAH7 and PKHD1L1 genes in the FSIP2-MT group, there were no significant differences in other gene mutations between the two groups. The main FSIP2 mutation types in the ICI-treated cohort were missense (84.2%) and nonsense (15.8%). Figure 2b shows that among the top 20 mutated genes in the TCGA-SKCM cohort, except for the BRAF gene, the other genes had a higher mutation frequency in the FSIP2-MT group, and the main mutation type was missense (89.6%); other mutation types, including splice site (1.3%), frameshift (3.9%), inframe ins/del (1.3%) and nonsense (3.9%) mutations, accounted for small percentages of the total mutation rate.

3.3.patients With Fsip2-mt Have An Elevated Tmb And Nal

As shown in Fig. 3a, we analyzed the TMB in the ICI-treated cohort and TCGA-SKCM cohort according to the FSIP2 gene mutation status. The results showed that the FSIP2-MT group had a higher TMB than the FSIP2-WT group, and there was a significant difference. The SKCM cell line data including WES data downloaded from GDSC (n = 52) were also divided into two groups according to the FSIP2 gene mutation status: FSIP2-MT (n = 3) and FSIP2-WT (n = 49). The TMB levels of the two groups were analyzed, and the results also suggested that the FSIP2-MT group had a higher TMB. The accumulation of mutations in the cancer genome may lead to tumor-specific production of “neoantigens” that are not affected by central T cell tolerance. Therefore, we analyzed the NAL of the TCGA-SKCM cohort, and the results showed that the FSIP2-MT group had a higher NAL. The higher TMB and NAL in patients with FSIP2-MT may be related to their better response to ICIs.

3.4.fsip2-mt Results In A Relatively Low Cnv

We analyzed the downloaded TCGA-SKCM queue data by GISTIC2.0 after grouping according to the mutation status of FSIP2. As shown in Fig. 3b, compared with normal samples, the TCGA-SKCM cohort samples showed significant amplifications on chromosomes 1, 3 to 8, 11 to 12, 15 to 17 and 22, while deletions were found on chromosomes 1 to 6, 8 to 12, 14 to 16 and 19. For the FSIP2-MT group, the amplified regions were mainly located on chromosomes 1, 6, 12 and 15, and the deleted regions were located on chromosomes 1, 3, 9 and 15. However, the amplification regions in the FSIP2-WT group were mainly located on chromosomes 1, 3 to 9, 11 to 13, 15 to 17, and 22, and the deleted regions were located on chromosomes 1, 3 to 9, 11 to 13, 15 to 17, 19, 22 and X. Compared with those of the FSIP2-MT group, the distribution and peak value of the amplified/deleted regions in the FSIP2-WT group were significantly higher, and the results were similar to those of the TCGA-SKCM cohort.

3.5.relationship Between Fsip2 And The Tumor Immune Status

The effect of ICI therapy depends not only on the immunogenicity of the tumor itself but also on the immune status of the tumor. The infiltration of immune cells, such as CD8 + T cells, Tregs, NK cells and macrophages (M0, M1, and M2), also affects the efficacy of ICI treatment. As shown in Fig. 4a and Fig. 4b, we analyzed the statuses of infiltrating immune cells and immune genes between the FSIP2-WT group and the FSIP2-MT group in the TCGA-SKCM cohort and marked the cells and genes with significant differences. In addition, we also analyzed the infiltration statuses of several specific immune cell populations. As shown in Fig. 4c, CIBERSORT analysis results for the FSIP2-MT and FSIP2-WT groups in the TCGA-SKCM cohort showed that except for memory B cells, CD8 + T cells, and Tregs, which were significantly upregulated in the FSIP2-WT group, and M2 macrophages, which were significantly upregulated in the FSIP2-MT group, there were no significant differences in immune cell infiltration between the two groups.

3.6.relationship Between Fsp2 And The Expression Of Immune-related Genes

The immune status of tumors is regulated by immune-related genes, so the expression of immune-related genes also affects the prognosis of ICI therapy. According to the immune-related gene sets reported in the literature, we evaluated the expression of immune-related genes between the FSIP2-MT group and FSIP2-WT group in the TCGA-SKCM cohort. As shown in Fig. 4d, the expression levels of the CD8A and CD8B genes, which are related to immune cell activity (cytolytic activity), in the FSIP2-WT group were significantly increased, and the expression levels of the PDCD1 and TIGIT genes, which are related to immune checkpoints, were also significantly increased. In addition, there was no significant difference in the expression of chemokine genes (CCL5, CXCL10, and CXCL9), other immune cell activity-related genes (GZMA, PRF1, and GZMB) or immune checkpoint-related genes (CD274, CTLA-4, HAVCR2, IDO1, LAG3, and PDCD1LG2) between the FSIP2-MT and FSIP2-WT groups.

3.7.effect Of Fsp2 On Chemotherapy Sensitivity

As shown in Fig. 5, we analyzed SKCM cell lines drug sensitivity data obtained from GDSC. After grouping the data according to the mutation status of FSIP2, we compared the difference in the 50% inhibitory concentration (IC50) between the FSIP2-MT group and FSIP2-WT group for 18 commonly used antineoplastic drugs. The results showed that except for that of bleomycin, the IC50s of the FSIP2-MT group were significantly higher than those of the FSIP2-WT group for the other 17 antineoplastic drugs.

3.8.pathway Analysis Of Fsp2-mt And Fsp2-wt

After GSEA of the TCGA-SKCM cohort, we screened out significantly upregulated or downregulated pathways that might be related to the prognosis of ICI treatment. The results are shown in Fig. 6. The pathways related to tumor progression, such as positive regulation of the MAPK cascade and FGFR

(FGFR1, FGFR2, FGFR2c, FGFR3, and FGFR3c) ligand binding and activation, were significantly downregulated in the FSIP2-MT group ($ES < 0$, $p < 0.05$), suggesting a better prognosis. In addition, we observed that the negative immune-regulation pathways, such as negative regulation of IL-2 production, negative regulation of the immune response, and negative regulation of lymphocyte-mediated immunity, were also significantly downregulated in the FSIP2-MT group.

Discussion

In recent years, we have made a series of breakthroughs in the treatment of tumors. With the discovery of immune checkpoints, increasing numbers of ICIs have been put into clinical use (FF et al. 2020). Traditional treatments, such as surgery plus radiotherapy and chemotherapy, have been combined with immunotherapy to significantly improve the OS and quality of life of tumor patients. As the treatment regimen for SKCM has also undergone great changes due to the emergence of new drugs, such as ICIs (including PD-1 checkpoint inhibitors, CTLA-4 checkpoint inhibitors, etc.), some patients have achieved long-term remission (Larkin et al. 2015). However, there is still a large number of patients who cannot achieve sustained remission, and the median OS time of those patients with metastatic melanoma is only 6–10 months (FF et al. 2020). Therefore, we analyzed the factors that may affect the efficacy of ICIs, including tumor immunogenicity (the TMB and NAL), CNV, the TME and immune-related gene expression, in FSIP2-MT and FSIP2-WT groups. The results showed that the FSIP2-MT group had higher immunogenicity (a significantly higher TMB and NAL) and fewer immunosuppressive cells (Tregs). In addition, GSEA of the TCGA-SKCM cohort showed that pathways related to immunosuppression and tumor progression were significantly downregulated in the FSIP2-MT group.

Immunogenicity refers to the ability to promote the body's immune response (Blankenstein et al. 2012). Considering that tumor immunogenicity is affected by a variety of factors, we analyzed the TMB, NAL and CNV data we collected from the ICI-treated cohort and TCGA-SKCM cohort to systematically evaluate the immunogenicity of SKCM. Previous studies have shown that patients with a high TMB benefit more from ICI treatment (Samstein et al. 2019; Snyder et al. 2017), but there is no clear conclusion as to whether the TMB can be used as an indicator for screening patients who are sensitive to ICIs (Hellmann et al. 2019). In addition, closely related to the TMB, the NAL generated by cells with somatic mutations can also predict the efficacy of ICI treatment (K et al. 2020). On the other hand, CNV has been shown to have potential predictive value for immunotherapy in recent years (Liu et al. 2019). Existing research results indicate that the correlation between CNV and the TMB is weak in predicting the therapeutic effect of ICIs. However, both the TMB and CNV have the ability to predict the prognosis of ICI treatment, suggesting that the TMB and CNV are independent prognostic predictors of ICI treatment (Hieronimus et al. 2018; Liu et al. 2019). In our study, there were also significant differences in the distribution and peak value of amplified/deleted regions between the FSIP2-MT group and the FSIP2-WT group, suggesting that the patients in the FSIP2-MT group and FSIP2-WT group responded differently to ICIs.

The occurrence of an immune reaction is not only closely related to tumor immunogenicity but also requires the participation of the antigen presentation process, which is inextricably linked to the

TME(Blankenstein et al. 2012; Wang et al. 2019). CIBERSORT analysis results showed that compared with the FSIP2-MT group, the FSIP2-WT group had a higher level of Tregs. Tregs are a type of cell that specifically function in immunosuppression, inhibiting the activation and expansion of lymphocytes that are abnormal or hyperreactive(Sakaguchi et al. 2008). On the one hand, Tregs play very important roles in normal physiological and pathophysiological processes, including antitumor and antimicrobial immunity, transplantation, and allergy(Takahashi and Sakaguchi 2003). On the other hand, they can also suppress the body's immune response to tumors and contribute to the development of an immunosuppressive TME(Elkord et al. 2010). Many studies have shown that in many tumors, such as ovarian cancer, pancreatic ductal adenocarcinoma, lung cancer, and SKCM, high expression of Tregs is associated with a poor prognosis. Tregs may exert their immunosuppressive activity through inhibitory cytokines (TGF- β , IL-10, and IL-35), immune checkpoint and inhibitory receptors (CTLA-4, PD-1, TIGIT, etc.) and direct cytotoxicity. We generally believe that ICIs achieve antitumor effects by suppressing immune checkpoints and activating cytotoxic T lymphocytes (CTLs) or effector T cells (Teff), but they may also affect Tregs, which are part of the immune system. Recent studies on Tregs have also pointed out that commonly used ICIs, such as anti-CTLA-4 antibodies, can activate Tregs while activating CTLs, and studies of PD-1 checkpoint inhibitors suggest that nivolumab can abrogate the suppressive function of Tregs, which suggests that Tregs also play a vital role in the process of immunotherapy(Chaudhary and Elkord 2016).

After analyzing immune-related genes in the TCGA-SKCM cohort, we found that the expression of the immune checkpoint genes PDCD1 and TIGIT, which are related to immunosuppression and T cell depletion in tumors, was increased significantly in the FSIP2-WT group(Johnston et al. 2014; Yeong et al. 2019). We speculate that the upregulated immune checkpoint molecule expression is caused by the elevated level of Tregs in the FSIP2-WT group, which creates an immunosuppressive environment. It is generally believed that patients with CD8 + CTLs that express high levels of immune checkpoint molecules, such as CTLA-4 and PD-1, tend to benefit more from ICI therapy than patients with CD8 + CTLs with low checkpoint molecule expression(Daud et al. 2016). That is, highly expressed immune checkpoint-related genes, such as PDCD1 and TIGIT, can theoretically provide targets for ICI treatment, suggesting a better prognosis. This is contrary to our results.

According to existing studies, cAMP-dependent RI/PKA activation induced by adenosine or PEG2 is an important mechanism by which Tregs play a role in tumor immunosuppression(M et al. 2015). After collecting data from the ICI-treated cohort and the TCGA-SKCM cohort for analysis, we found that the FSIP2 mutations found in both cohorts were mainly missense mutations. This may cause FSIP2 to lose its original function and further affect Treg-induced PKA-mediated immunosuppression of antitumor immunity by reducing the expression of AKAP4 and the attachment of PKA to AKAP4(Brown et al. 2003; Martinez et al. 2018).

Our GSEA of TCGA-SKCM data also indicated that in the FSIP2-MT group, pathways related to tumor progression (MAPK and FGFR), immunomodulation, and IL-2 synthesis inhibition were significantly downregulated. According to existing research results, inhibition of the MAPK pathway achieves good results in tumor immunotherapy, which may be related to the downregulation of immunosuppressive

factor expression(Deken et al. 2016; Loi et al. 2016). Another study also noted that FGFR blockers not only showed good antitumor effects but also improved the effect of immunotherapy when combined with anti-PD-1 therapy(Palakurthi et al. 2019). In clinical studies, the combination of IL-2 and anti-CTLA-4 therapy also enhanced the antitumor effect(Kohlhapp et al. 2015; West et al. 2013). Therefore, the results of the GSEA also suggest that the FSIP2-MT group can achieve better ICI treatment efficacy.

Conclusions

In this study, we found that compared with that in the FSIP2-WT group, the prognosis of immunotherapy in the FSIP2-MT group was better. The FSIP2-MT group had higher tumor immunogenicity and lower Treg levels, and GSEA also suggested that the FSIP2-MT group responded better to ICI treatment. We tried to elucidate the possible mechanism by which FSIP2 mutation and the tumor immune microenvironment affect the prognosis of SKCM patients receiving ICIs. On the other hand, this work also provides theoretical guidance for further improving the efficacy of ICIs in SKCM patients with or without FSIP2 mutations. However, there are few studies or data on FSIP2 mutations and tumor immunotherapy. The association between FSIP2 mutation and SKCM still needs to be verified in further experiments.

Declarations

Ethics approval:

Not applicable

Consent for publication:

Not applicable

Availability of data and material:

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

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Authors' contributions:

Jian Zhang and Peng Luo conceived and designed the study. Haoxuan Ying and Anqi Lin performed the experiments. Anqi Lin contributed significantly to the data analyses and Haoxuan Ying wrote the paper. Haoxuan Ying, Anqi Lin and Peng Luo reviewed and edited the manuscript. All authors read and approved the manuscript.

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Conflicts of interest / Competing interests:

The authors declare that there is no conflict of interests regarding the publication of this article.

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Figures

Figure 1

Kaplan-Meier analysis of SKCM patients in the ICI-treated cohort and TCGA-SKCM cohort. The acquired cohorts were grouped according to the mutation status of FSIP2, with yellow indicating the FSIP2-WT group and blue indicating the FSIP2-MT group. (a) Kaplan-Meier curve of the overall survival (OS) of SKCM patients receiving ICIs. The overall survival of the FSIP2-MT group ($n = 15$) was significantly longer than that of the FSIP2-WT group ($n = 105$) ($p = 0.038$; hazard ratio (95% CI): 0.43 (0.23-0.82)). (b) Kaplan-

Meier curve of the overall survival of SKCM patients in the collected TCGA-SKCM cohort. The FSIP2-MT group (n = 58) and FSIP2-WT group (n = 399) had no significant difference in overall survival (p = 0.978; hazard ratio (95% CI): 1.01 (0.68-1.49)).

Figure 2

Clinical characteristics and gene mutation panoramas of the SKCM patients in the ICI-treated cohort and TCGA-SKCM cohort. The acquired ICI-treated cohort and TCGA-SKCM cohort were divided into an FSIP2-MT group and FSIP2-WT group according to the FSIP2 mutation status. (a). We performed Fisher's exact test on the FSIP2-WT and FSIP2-MT groups of the ICI-treated cohort based on age, sex, treatment response, overall survival and TMB. Except for a higher average age, longer OS time and higher TMB in the FSIP2-MT group, there were no significant differences between the two groups. A comparative analysis of the top 20 mutated genes showed that there were significant differences between the two groups in the mutation frequencies of the MUC16, FATA4, USH2A, CSMD1, DNAM7, and PKHD1L1 genes. The mutation types of the FSIP2 gene were mainly missense mutations (84.2%) and nonsense mutations (15.8%). (b). We performed Fisher's exact test on the FSIP2-WT and FSIP2-MT groups of the TCGA-SKCM cohort based on age, sex, disease stage, ethnicity, race, overall survival, TMB and neoantigen load. Except for patient sex, TMB and NAL, no parameters showed significant differences between the two groups. A comparative analysis of the top 20 mutated genes showed that, except for the BRAF gene, none of the remaining 19 genes showed significant differences in mutation status. The main mutation type of the FSIP2 gene was missense mutation (89.6%), and the other types were splice site (1.3%), frame shift (3.9%), inframe ins/del (1.3%) and nonsense (3.9%) mutations (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).

Figure 3

Tumor immunogenicity and CNV analyses of the TCGA-SKCM cohort. (a) We used the Mann-Whitney U test to compare the TMB levels (along the y-axis) of the FSIP2-MT group (gray) and the FSIP2-WT group (yellow) in the ICI-treated cohort, TCGA-SKCM cohort and GDSC-SKCM cohort and the tumor neoantigen load (along the y-axis distribution) in the TCGA-SKCM cohort. The numbers in parentheses indicate the total number of patients included in the analysis of each dataset, with * indicating significant differences. The results showed that the FSIP2-MT group had significantly higher TMB levels than the FSIP2-WT group, and the NAL in the FSIP2-MT group was significantly higher. (b) The CNV of the TCGA-SKCM cohort was analyzed by using GISTIC2.0. We set the x-axis as the chromosome number and the y-axis as the G-score. The amplified part is displayed above the x-axis, and the markedly amplified part is marked with red; the deleted part is displayed below the x-axis, and the markedly deleted part is marked with blue (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).

Figure 4

FSIP2 mutation affects tumor immune resistance. (a) Heat map of infiltrating immune cells in the FSIP2-MT and FSIP2-WT groups in the SKCM-TCGA cohort. We marked different infiltrating immune cells with different colors. The FC cutoff was set to be greater than 1.49 or less than 0.67, and row annotation represents the immune cells to which genes belong. (b) Immune gene heat maps for the FSIP2-MT group and the FSIP2-WT group in the SKCM-TCGA cohort. Similarly, the FC cutoff was selected to be greater than 1.49 or less than 0.67, and the row annotation represents the function of the gene. Genes shown in black font have FC > 1.49 or < 0.67 and p < 0.05; genes shown in white font were not significantly different between the two groups. (c) The TCGA-SKCM cohort grouped according to the FSIP2 mutation status, with yellow for the FSIP2-WT group and gray for the FSIP2-MT group. The Mann-Whitney U test was used to analyze differences in the infiltration levels of 22 kinds of immune cells between the two groups, and the results with significant differences are marked with *. (d) Immune-related genes analyzed according to functional classification (chemokine, cytolytic activity, and immune checkpoint) using the Mann-Whitney U test. The results with significant differences are marked with * (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).

Figure 5

Drug sensitivity analysis of GDSC-SKCM cell line data. SKCM cell lines with drug sensitivity data obtained from GDSC were grouped according to the FSIP2 mutation status, with yellow for the FSIP2-WT group and gray for the FSIP2-MT group. We used the Mann-Whitney U test to analyze the differences in the IC50 values of conventional chemotherapeutic drugs between the FSIP2-MT and FSIP2-WT groups, and the results with significant differences are marked with * (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Figure 6

Gene set enrichment analysis delineates biological pathways and processes associated with the prognosis of immunotherapy in the TCGA-SKCM cohort. GSEA validated the decreased activity of (a) the IL-2 synthesis negative regulation pathway, (b) the MAPK activation pathway, (c) the negative immune regulation pathway, and (d) the FGFR activation pathway in the FSIP2-MT group.