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FAT3 Mutation Is Associated With Tumor Mutation Burden and Poor Prognosis in Esophageal Cancer

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Objective: To explore the mutated genes in esophageal cancer (ESCA), evaluate their relationship with tumor mutation burden (TMB) and prognosis of ESCA, and analyze the advantages of FAT3 as a potential prognostic marker in ESCA.

Methods: The somatic mutation landscape was analyzed according to ESCA samples from the TCGA and ICGC database. The differences of TMB between the mutant type and the wild type of frequently mutated genes were compared by Mann-Whitney U test. The association of gene mutations with prognosis was analyzed by the Kaplan-Meier method. The relative abundance of 22 tumor-infiltrating lymphocyte subsets in ESCA was calculated by the CIBERSORT algorithm.

Results: FAT3 was a high frequency mutation in both TCGA and ICGC samples from the somatic mutation landscape. Then, the mutation type of FAT3 had significantly higher TMB in patients with ESCA compared with the wild type ($P<0.05$). Meanwhile, the prognosis of the FAT3 mutation type was significantly worse in patients with ESCA ($P<0.05$), and the FAT3 mutation status might be an independent factor for prognosis of patients with ESCA (HR: 1.262–5.922, P=0.011). The GSEA analysis revealed the potential mechanism of FAT3 mutation on the occurrence and development of ESCA. Finally, naive B cells were significantly enriched in FAT3 mutation samples of the ESCA microenvironment ($P<0.05$).

Conclusions: FAT3 mutation is related to TMB and poor prognosis in ESCA. FAT3 mutation may be a prognostic marker of ESCA and reveals the potential mechanism of FAT3 mutation on ESCA.

Keywords: FAT3, esophageal cancer, tumor mutation burden, prognostic marker, bioinformatics

INTRODUCTION

Esophageal cancer (ESCA) is a common malignant tumor of the digestive tract in the world. It has the characteristics of high malignancy and poor prognosis. The main pathological types of ESCA include esophageal squamous cell carcinoma (ESCC), which is more common in Asian countries, and esophageal adenocarcinoma (EADC), which is more common in Western countries (1–3). ESCA is mainly characterized by progressive dysphagia, and most patients diagnosed with esophageal cancer have entered the advanced stage. Despite some progress in ESCA surgical techniques, chemotherapy and radiotherapy protocols, and perioperative management, the 5-year survival rate of patients with ESCA is about 19%, which is a serious threat to human life and health (4, 5).

Tumor mutation burden (TMB) is the total number of somatic gene variants detected per million bases of genomic DNA, including base substitutions, insertions, or deletions. Tumor cells can produce many specific mutations at the genetic level, and every 150 non-synonymous mutations may produce one to two neoantigens, which can be recognized by the autoimmune system, thus activating T cells and causing immune response (6–8). The more non-synonymous mutations, the more neoantigens will be recognized by the autoimmune system, and the stronger the autoimmune effect can be caused. Therefore, the higher the TMB, the easier it will benefit from immunosuppressive therapy (9, 10). Several clinical studies have demonstrated that TMB can predict the efficacy of immunotherapy for solid tumors, such as melanoma (11), bladder cancer (12), and small cell lung cancer (13).

In this study, the gene mutations of ESCA were analyzed by using the single-nucleotide variants (SNV)data from The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC), and then the common high-frequency mutation genes were screened out. Then, we explored the association of high-frequency mutant genes with TMB and overall survival, and conducted gene enrichment analysis on mutant genes closely related to prognosis. Finally, the association of key mutant genes with immune infiltration was explored. This study may identify a marker for ESCA, which had the potential as a target for immunotherapy.

MATERIALS AND METHODS

Data Resources

The SNV data of ESCA were downloaded from the TCGA database (https://portal.gdc.cancer.gov/), containing 184 samples. The simple somatic mutation data of ESCA were downloaded from the ICGC database (https://dcc.icgc.org/releases/current/Projects/ESCA-CN), containing 298 samples from China. The clinical information data of ESCA were downloaded from the TCGA.

The clinical information data were collated to obtain 144 samples, including survival time, survival status, age, gender, TNM, and stage.

Identification of Frequently Mutated Genes

MAF files were conducted by VarScan for somatic variants for ESCA samples from the TCGA database, and waterfall plots were used to visualize somatic variants for ESCA samples by the maftools package (14). TSV files were annotated by hg19 reference genome and visualized using the GenVisR package for somatic variants for ESCA samples from the ICGA database. Then, the top 30 high-frequency mutated genes were selected by ranking the frequency of gene mutations from high to low in the TCAG database and the ICGC database. The common frequently mutated genes were intersected that were both of the top 30 high-frequency mutated genes in the TCAG database and ICGC database.

Relationship Between Gene Mutation and TMB and Prognosis

The whole-exome sequencing (WES) data were used to calculate the TMB score in esophageal cancer from the TCGA database. The total number of non-synonymous somatic variants was divided by exome size to calculate the TMB score from the TCGA database (15). The differences of TMB between the mutant type and the wild type of frequently mutated genes were compared to explore the relationship between gene mutation and TMB. The association of gene mutations with survival prognosis was analyzed by the Kaplan-Meier method. Univariate and multivariate Cox regression was analyzed to prove that FAT3 was an independent factor. The FAT3 mutation status and some clinical indicators such as age, gender, stage, TMB, and other genes mutation status were included in the analysis.

Gene Set Enrichment Analysis

The transcriptome data from the TCGA database were used in gene set enrichment analysis (GSEA). According to the FAT3 mutation status, the samples were divided into mutation and wild groups. The GSEA was conducted by GSEA software (version 4.0.3) from Broad Institute (16). The gene set "c2.all.v7.1.symols.gmt" in the MsigDB database was used as the reference gene set. Permutations for each analysis were set as 1000 times. Pathways with normal P-value < 0.01 were considered significantly enriched.

Association of Gene Mutations With Tumor-Infiltrating Immune

The relative abundance of 22 tumor-infiltrating lymphocyte subsets in ESCA patients with a different FAT3 mutation status was calculated using the CIBERSORT algorithm (17). The relative abundance of tumor-infiltrating immune was calculated after correction of gene expression, and samples with P-value<0.05 were included in the study. The bar and violin plots were used to show the association of gene mutations with tumor-infiltrating immune.

Statistical Analysis

The relationship between gene mutation and TMB was analyzed by using the GraphPad Prism software (version 8.0). The

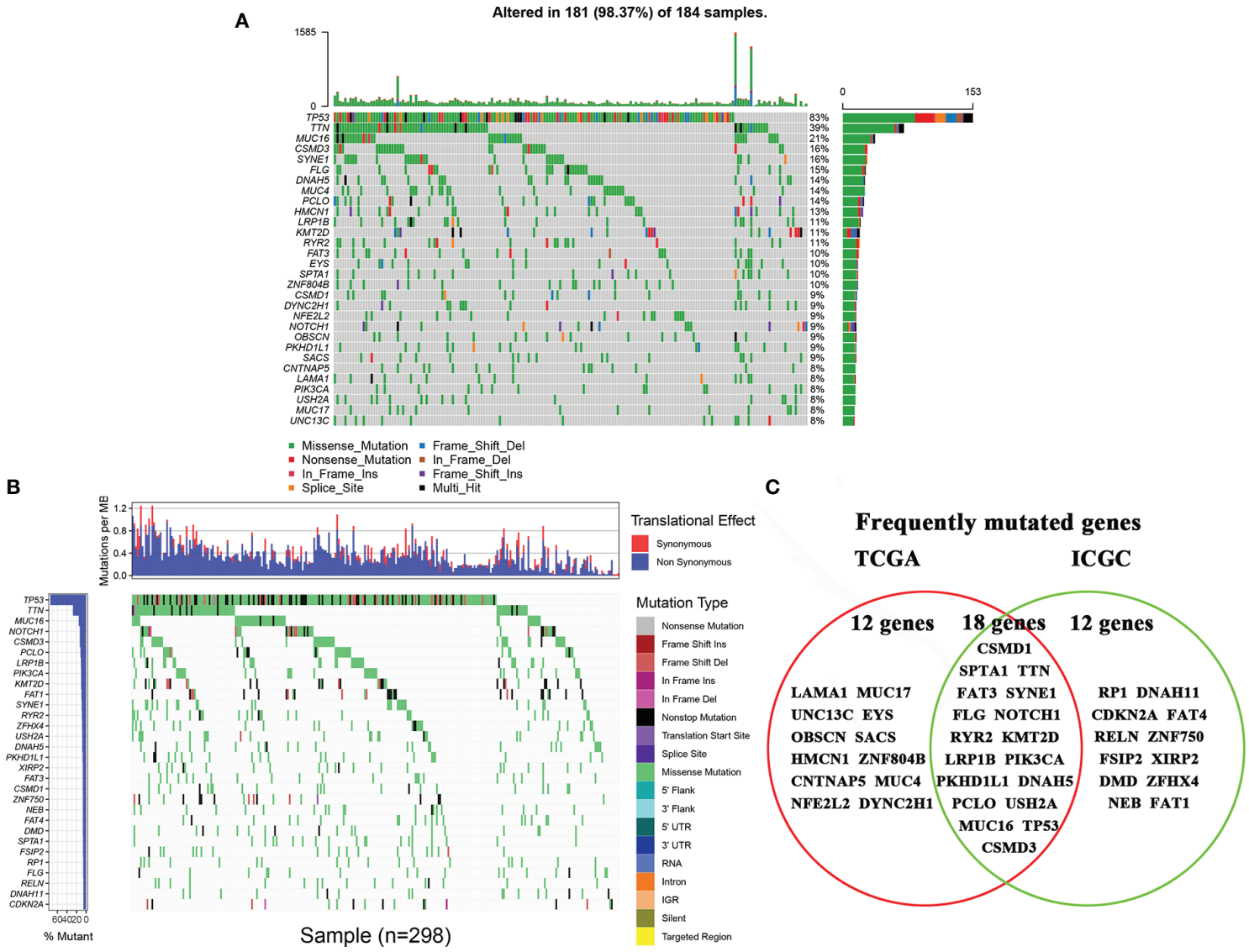

FIGURE 1 | Landscapes of frequently mutated genes in esophageal cancer. **(A)** Oncoplot displaying the landscapes of frequently mutated genes in ESCA from the TCGA database. Genes are ordered according to their mutation frequency (left panel), and different mutation types were presented as indicated by the annotation bar (bottom). **(B)** Waterfall plot displaying the landscapes of frequently mutated genes in ESCA from the ICGC database. Genes are ordered according to their mutation frequency (left panel), and different mutation types were presented as indicated by the annotation bar (right panel). **(C)** Venn diagram displaying the common frequently mutated genes that were both of the top 30 high-frequency mutated genes in the TCAG database and ICGC database.

relationship between gene mutation and TMB was analyzed by Mann-Whitney U test. Univariate and multivariate Cox analyses were performed with IBM SPSS Statistics (version 20), and the method "forward:LR" was used in multivariate Cox analysis. Other analyses, such as mutation information analysis, survival analysis, and tumor-infiltrating immune analysis, were performed using R software (version 3.6.1). The survival curves were drawn by the Kaplan-Meier method, and log-rank test was used to evaluate the survival analysis. *P*-values<0.05 were considered significant.

RESULTS

Identification of Frequently Mutated Genes in ESCA

The top 30 high-frequency mutant genes from the TCGA database were showed in **Figure 1A**. TP53, TTN, MUC16, CSMD3, and SYNE1 were the five genes with the highest mutation frequency in the TCGA database. Meanwhile, the top 30 high-frequency mutant genes from the ICGC database were showed in **Figure 1B**. TP53, TTN, MUC16, NOTCH1, and CSMD3 were the five genes with the highest mutation frequency in the ICGC database. Finally, we obtained 18 frequently mutated genes in ESCA that were represented in both the TCGA and ICGA databases, containing CSMD1, SPTA1, TTN, FAT3, SYNE1, FLG, NOTCH1, RYR2, KMT2D, LRP1B, PIK3CA, PKHD1L1, DNAH5, PCLO, USH2A, MUC16, TP53, and CSMD3 (**Figure 1C**).

Relationship Between Gene Mutation and TMB and Prognosis

The TMB score was calculated based on SNV data of ESCA from the TCGA database. Compared with the wild type of genes, the mutation type of CSMD1, SPTA1, TTH, FAT3, SYNE1, LRP1B, RYR2, PCLO, MUC16, CSMD3, and USH2A had significantly higher TMB in patients with ESCA (*P*<0.05, **Figure 2**). Based on the median of the TMB score, the samples were divided into a high-TMB group and a low-TMB group. Based on the Kaplan-Meier analysis, the high-TMB group was related to a negative prognosis in ESCA (*P*<0.05, **Figure 3A**). For mutant genes with significantly higher TMB, we conducted survival analysis and found that the prognosis of CSMD1, FAT3, and LRP1B mutation type were significantly worse in patients with ESCA (P<0.05, **Figures 3B–D**). The results of survival analysis by other gene mutation status were not statistically significant (P>0.05, **Supplementary Figures 1A–H**). The CSMD1, FAT3, and LRP1B mutation status and some clinical indicators such as age, gender, stage, and TMB were included in the Cox regression analysis. The FAT3 mutation status was statistically significant in Cox regression analysis (**Table 1**), which might be an independent prognosis factor for patients with ESCA (HR: 1.262-5.922, P=0.011).

GSEA of FAT3 Mutation

Gene enrichment analysis was performed with TCGA to explore the function role of FTA3 mutation. The results of GSEA analysis showed samples with FAT3 mutation enriched in "ERBB2 Breast Preneoplastic UP", "Kras Oncogenic Signature", "Malignant Skin Tumor DN", "MCV6 LCP With H3K27ME3", and "MET Signaling" (**Figure 4**). These pathways revealed the potential mechanism of FAT3 mutation on the occurrence and development of ESCA and provided support for further exploring the role of FAT3 mutation in tumors.

Association of FAT3 Mutation With Tumor-Infiltrating Immune

Research has shown that TMB may be a potential marker for predicting the efficacy of immunotherapy (18); therefore, we explored the relationship between FAT3 mutation and tumor-infiltrating immune in ESCA. The immune infiltration of the ESCA microenvironment was calculated using the CIBERSORT algorithm, and the Stacked bar showed the proportion of 22 immune cells in each sample of ESCA (**Figure 5A**). Compared with the FAT3 wild type, naive B cells were significantly enriched in the FAT3 mutation type of the ESCA microenvironment (*P*<0.05,

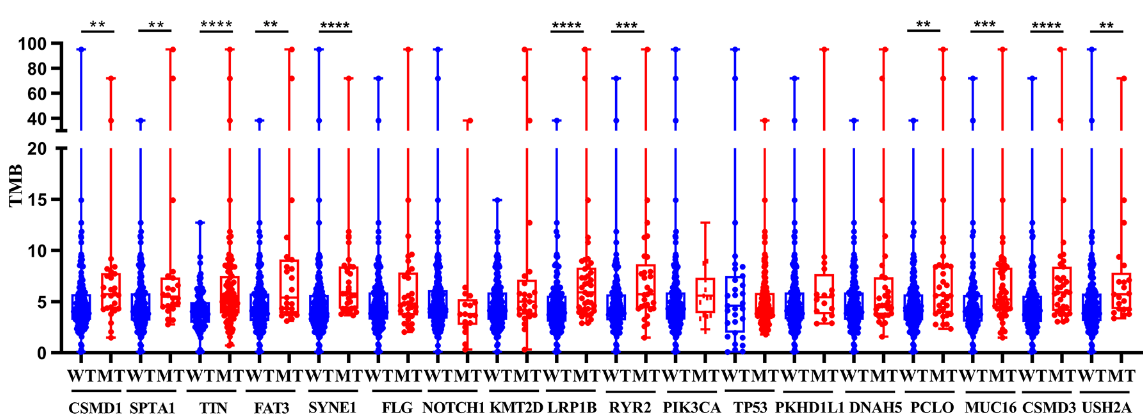

FIGURE 2 | Gene mutations are associated with TMB. Compared with the wild type of genes, the mutation type of CSMD1, SPTA1, TTH, FAT3, SYNE1, LRP1B, RYR2, PCLO, MUC16, CSMD3, and USH2A had significantly higher TMB in patients with ESCA. **p < 0.01; ***p < 0.001; ****p<0.0001. WT, wild type; MT, mutant type.

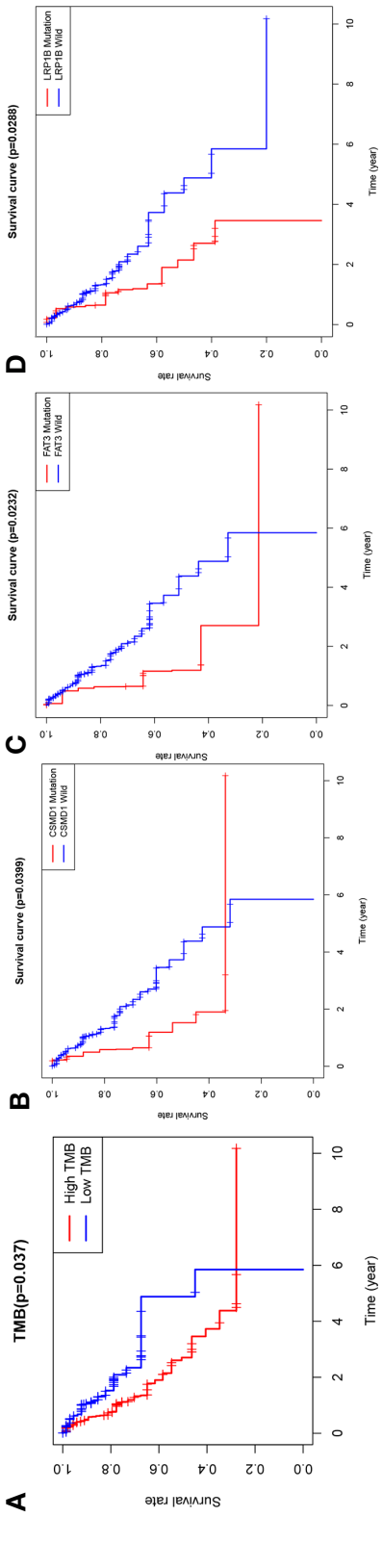

FIGURE 3 | Gene mutations are associated with clinical prognosis. **(A)** The high-TMB group was related to a negative prognosis in ESCA. **(B–D)** The association of gene mutations with survival prognosis was analyzed by Kaplan-Meier method. A total of 144 samples containing complete clinical information were included. *P*-values<0.05 were considered significant. WT, wild type; MT, mutant type.

Figure 5B). Moreover, activated memory $CD4^+$ T cells had the strongest positive correlation with $CD8^+$ T cells, and activated Mast cells had the strongest negative correlation with resting Mast cells from the correlation matrix (**Figure 5C**). For naive B cells, the strongest positive correlation were the memory B cells and the strongest negative correlation were activated memory $CD4^+$ T cells (**Figure 5C**).

DISCUSSION

In this study, the somatic mutation landscape was analyzed according to ESCA samples from the TCGA database containing 184 samples and the ICGC database containing Chinese samples. FAT3 was a high frequency mutation in both TCGA and ICGC samples from the somatic mutation landscape. Then, the mutation type of FAT3 had significantly higher TMB in patients with ESCA compared with the wild type. Meanwhile, the prognosis of the FAT3 mutation type was significantly worse in patients with ESCA, and the FAT3 mutation status was statistically significant in Cox regression analysis, which might be an independent prognosis factor for patients with ESCA. The GSEA analysis revealed the potential mechanism of FAT3 mutation on the occurrence and development of ESCA, and provided support for further exploring the role of FAT3 mutation in tumors. Finally, naive B cells were significantly enriched in FAT3 mutation samples of the ESCA microenvironment.

FAT3 was a cadherin gene located on chromosome 11q14.3-q21. Experiments on homologs of rodent FAT3 showed that its mRNA was highly expressed in embryonic rat brain, but its expression was relatively low in adult brain tissue (19). In mice, the expression of FAT3 was limited to the developing central nervous system, with the highest expression in olfactory bulb and retina (20). FAT3 might control the polarized development of tissues through cytoskeleton, thereby affecting the emergence of asymmetric cell morphology during retinal development (21). The point mutation of FAT3 can cause pancreatic tumor in human cancer (22). Studies have shown that FAT3 was a key mutation gene in multiple tumors, including ovarian cancer, breast cancer (23), lung adenocarcinoma (24), and pancreatic acinar cell carcinoma (25). Studies have reported that the whole genome expression of lung tumor cell populations of transgenic mice was analyzed by laser capture microdissection, and it was found that FAT3 mRNA was significantly down-regulated in lung adenocarcinoma. It was speculated that such molecular switches may promote the transformation of epithelial dysplasia into lung glands cancer (26). FAT3 was relatively less studied and was thought to participate in the development of human cancer through a pathway similar to that of the Ena/VASP proteins (22). These studies might indicate that FAT3 mutation played an important role in the occurrence and development of tumors.

ESMO collected the TMB levels of 104,814 patients with 30 kinds of solid tumors and identified the highest level of TMB, namely cutaneous malignant melanoma, followed by non-small cell lung cancer and other squamous cell carcinoma (27). The higher the level of TMB, the better the effect of immunotherapy

TABLE 1 | Univariate and multivariate Cox analysis of esophageal cancer patients by the IBM SPSS Statistics (version 20).

Factors	Univariate		Multivariate	
	HR (95%CI)	P-value	HR (95%CI)	P-value
Age (year)(<60,≥60)	1.326(0.712–2.471)	0.374		
Gender (male, female)	0.831(0.325–2.125)	0.7		
Stage (stage I and II, stage III and IV)	3.743(1.929–7.261)	<0.001	3.992(2.043–7.800)	<0.001
TMB (low, high)	1.971(1.031–3.767)	0.04	–	0.257
LRP1B (wide, mutant)	2.073(1.063–4.044)	0.032	–	0.409
FAT3 (wide, mutant)	2.353(1.102–5.026)	0.027	2.734(1.262–5.922)	0.011
CSMD1 (wide, mutant)	2.169(1.019–4.614)	0.044	–	0.551

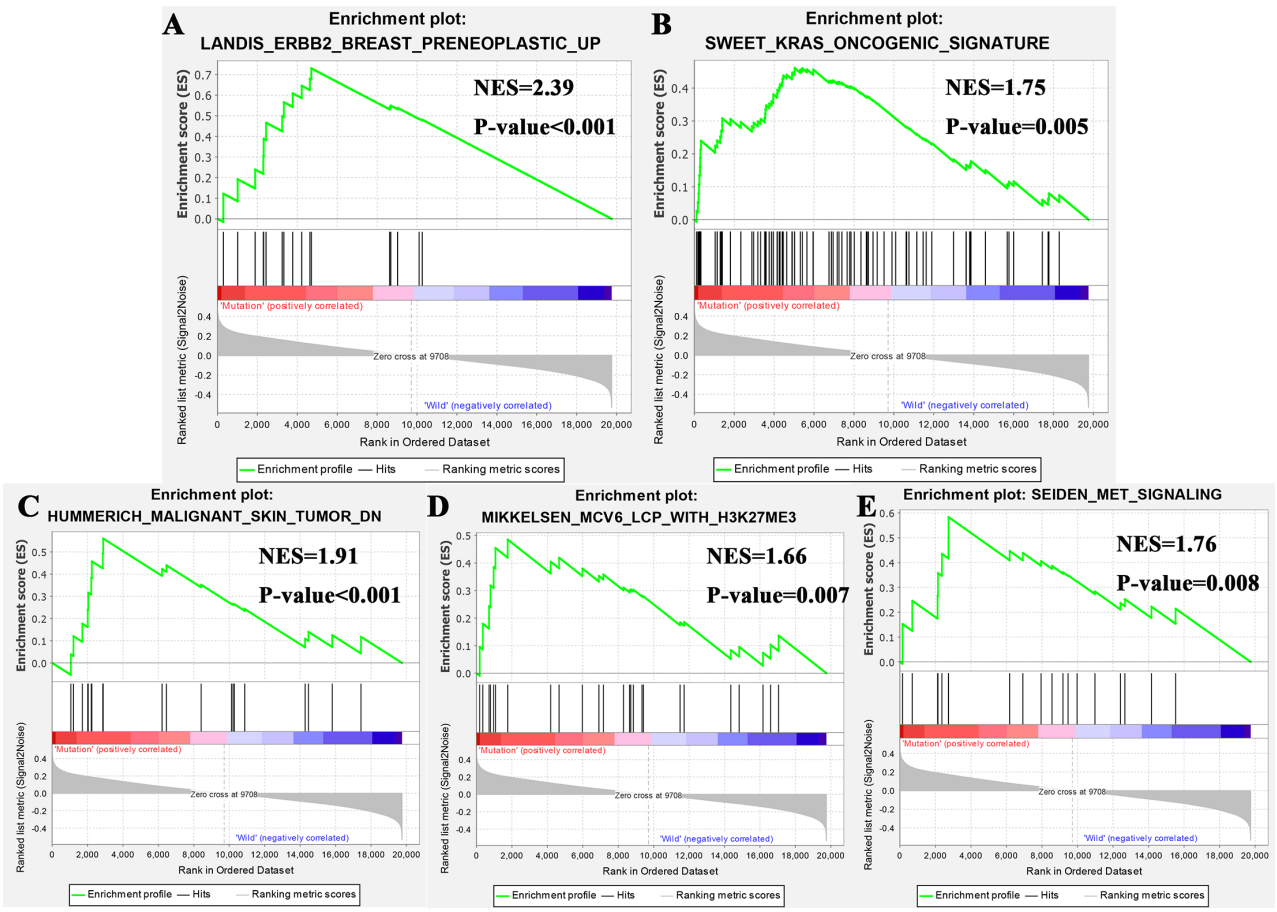

FIGURE 4 | Significantly enriched pathways associated with FAT3 mutation. GSEA was performed with TCGA to explore the function role of FTA3 mutation. The GSEA analysis showed that samples with FAT3 mutation enriched in **(A)** ERBB2 Breast Preneoplastic UP, **(B)** Kras Oncogenic Signature, **(C)** "Malignant Skin Tumor DN, **(D)** MCV6 LCP With H3K27ME3 pathway, and **(E)** MET Signaling pathway. NES, normalized enrichment score.

(10). And this was confirmed in patients with high levels of TMB in melanoma and lung squamous carcinoma (11). The mutation type of FAT3 had significantly higher TMB in patients with ESCA compared with the wild type in this study. The high-TMB group was related to a negative prognosis in ESCA from the Kaplan-Meier analysis. These results may suggest that immunotherapy for ESCA patients with high TMB will obtain higher benefits compared with patients with low TMB.

GSEA analysis showed samples with FAT3 mutation enriched in "ERBB2 Breast Preneoplastic UP", "Kras Oncogenic Signature", "Malignant Skin Tumor DN", "MCV6 LCP With H3K27ME3", and "MET Signaling". The "ERBB2 Breast Preneoplastic UP" showed that the TGF-beta pathway was intrinsically suppressed in ErbB2/Neu tumors *via* a mechanism involving loss of TGF-beta-Receptor-I/ALK5 (28). "Kras Oncogenic Signature" revealed that FAT3 mutation may affect

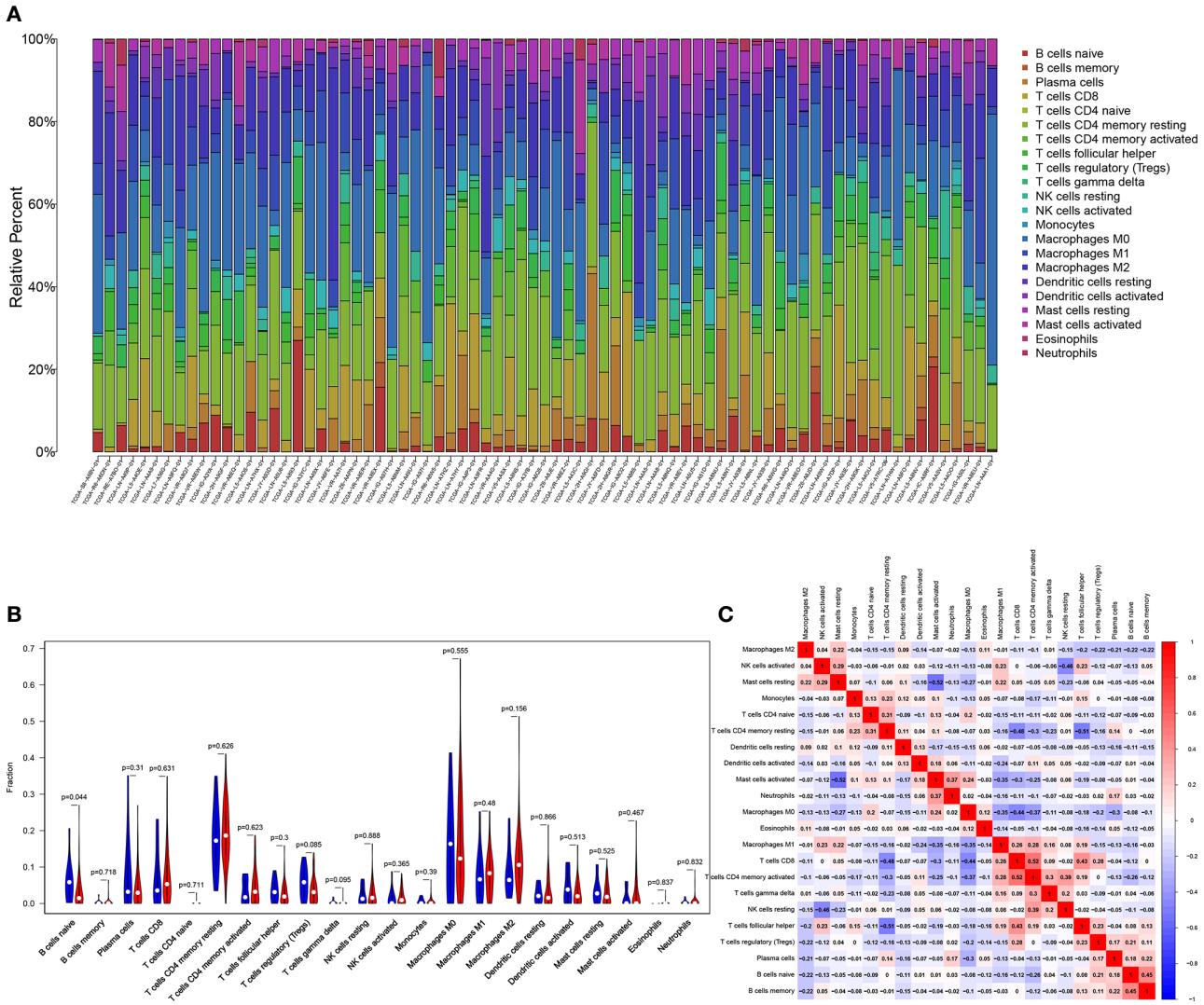

FIGURE 5 | Association of FAT3 mutation with tumor-infiltrating Immune. **(A)** Stacked bar chart shows the proportion of 22 immune cells in each sample of ESCA. **(B)** Violin plot displays the differentially infiltrated immune cells between FAT3-mutant groups and FAT3-wild group. Blue color: FAT3-wild group; Red color: FAT3-mutant group. **(C)** Correlation matrix of immune cell proportions. Red color: positive correlation; Blue color: negative correlation.

ESCA through the KRAS pathway (29). "Malignant Skin Tumor DN" showed that the mechanism of FAT3 mutation in the occurrence and development of ESCA was related to that of malignant skin (30). "MCV6 LCP With H3K27ME3" was related to cell differentiation (31). The MET signaling pathway played an important role in cell migration, apoptosis, proliferation, and differentiation. It can promote tumor cells to form a more aggressive cell phenotype to avoid immunity, and enhance the survival and invasion ability of tumor cells (32, 33).

At present, several studies have confirmed that there are differences in the expression of immune cells in tumor tissues. The proportion of M1 macrophages was higher in nasopharyngeal carcinoma, while memory B cells and resting memory $CD4^+$ T cells were relatively lower (34). The proportion of M0 macrophages and activated memory $CD4^+$ T cells was higher, while neutrophils and monocytes were lower in prostate cancer tissues (35). The expression of activated memory $CD4^+$ T cells, M0 macrophages, and M1 macrophages in colorectal cancer was higher than that in normal tissues (36). In this study, naive B cells were significantly enriched in the FAT3 mutation type of the ESCA microenvironment compared with the FAT3 wild type. For naive B cells, the strongest positive correlation was the memory B cells and the strongest negative correlation was activated memory CD4 T cells. B cells may play an important role in tumor immunity of ESCA. More than 90% of gastroesophageal adenocarcinoma tumor tissues had more tumor infiltrating B cells (37). Studies had found that there were a variety of memory B cells in pancreatic cancer tissues, such as IgG $^+$, IgG2a/b $^+$, IgA $^+$ memory B cells, and cells, that were not conducive to tumor progression (38). Shi et al. (39) found that margin-infiltrating B lymphocytes at the edge of cancer

presented an atypical memory phenotype ($IgD^{-}IgG^{+}CD27^{-}CD38^{-}$). Margin-infiltrating B lymphocytes could secrete IFN-γ and IL-12p40, promote Th1 immune response, and exert anti-tumor activity. Nielsen et al. (40) showed that $CD20^{+}$ tumor-infiltrating lymphocytes similarly had an atypical memory phenotype ($CD27^{-}$) in ovarian cancer and that the cells highly expressed antigen-presenting molecules (MHC-I, MHC-II, CD40, CD80, CD86) and co localized with $CD8^{+}$ T cells, suggesting that it presented tumor antigens and activated tumor killer T cells, resulting in tumor suppression. $CD4^{+}$ T lymphocytes not only can help $CD8^{+}$ cytotoxic T lymphocytes but also can help NK cells to kill tumors. For example, it had been found that $CD4^{+}$ T lymphocytes were better than $CD8^{+}$ T lymphocytes in rejecting solid tumors in *in vitro* experiments (41, 42). $CD4^{+}$ T lymphocytes can both inhibit tumor growth and promote tumor growth (43, 44). The above research results indicated that $CD4^{+}$ T lymphocytes were complex and changeable in the anti-tumor cell immunity. These results may indicate the potential mechanism of immune microenvironment affecting ESCA.

This study also has some limitations. Firstly, there are few FAT3 mutation-type samples compared with wild-type samples. Secondly, due to technical limitations, no clinical samples were collected to verify the results.

CONCLUSIONS

In conclusion, through the analysis of somatic mutation data in the TCGA and ICGC databases, FAT3 mutation was a high frequency mutation gene in ESCA. Then, the association of FAT3 mutation with TMB and prognosis was obtained. FAT3 mutation was an independent risk factor in ESCA. Furthermore, the GSEA of FAT3 mutation and the relationship between FAT3 mutation and tumor-infiltrating immune were explored. These results indicated that FAT3 mutation was a prognostic marker of ESCA, and revealed the potential mechanism of FAT3 mutation on ESCA.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

WH and SL conceived and designed the study. ZG, XY, and CS performed the analysis procedures. XY, ZG, QW, and YW analyzed the results. JH, X-PL, and SL contributed analysis tools. ZG, and XY contributed to the writing of the manuscript. All authors have reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.603660/full#supplementary-material

Supplementary Figure 1 | Gene mutations aren't associated with clinical prognosis. **(A–H)** The association of gene mutations with survival prognosis was analyzed by Kaplan-Meier method. A total of 144 samples containing complete clinical information were included. *P*-values<0.05 were considered significant. WT, wild type; MT, mutant type.

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