Rnf43 Mutation As A Biomarker For Immune Checkpoint Inhibitor Efficacy In Colorectal Cancer

Nan Zhang
The Affiliated Luoyang Central Hospital of Zhengzhou University
XinPeng Shi
The Affiliated Luoyang Central Hospital of Zhengzhou University
WenCui Ju
The Affiliated Luoyang Central Hospital of Zhengzhou University
YunFeng Lou (louyunfeng258@163.com)
The Affiliated Luoyang Central Hospital of Zhengzhou University, The Affiliated Luoyang Central Hospital of Zhengzhou University
XiaoYong Luo
The Affiliated Luoyang Central Hospital of Zhengzhou University, The Affiliated Luoyang Central Hospital of Zhengzhou University

Research Article

Keywords: immune checkpoint inhibitors, biomarker, colorectal cancer, Rnf43,mutation

Posted Date: June 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-536739/v1

Abstract

Background: Immune checkpoint inhibitors can prolong the survival of patients with advanced colorectal cancer and have been approved for the treatment of metastatic colorectal cancer patients with mismatch repair defects and high microsatellite instability (dMMR-MSI-H). However, there are still many deficiencies in their clinical application, such as their benefit in a limited population, low efficiency, and lack of accurate markers. Therefore, finding accurate biomarkers has become an urgent problem in the immunotherapy of colorectal cancer. Our research aimed to find biomarkers that can accurately predict the population with potential benefit.

Methods: We analysed data from a colon cancer immunotherapy cohort (n = 110, ICI cohort), including mutation data and clinical data, to identify the mutated gene closely related to Immune Checkpoint Inhibitors (ICIs). Next, we further verified the relationship between gene mutation and clinical features, such as Tumor Mutational Burden (TMB) and Microsatellite Instability (MSI) status in the TCGA colorectal cancer data set (non-ICI cohort). Then, CIBERSORT was used to analyse the relationships between gene mutation and both immune cell infiltration and immune genes, and GSEA was used to analyse the effect of gene mutation on pathway activation levels. In addition, we analysed the Whole-exome Sequencing (WES) and drug sensitivity data of colorectal cancer cell lines in the GDSC database.

Conclusions: Our results show that Rnf43 mut can be used as a biomarker to predict the efficacy of ICI for colorectal cancer, and it can be used for clinical screening of patients who benefit from ICI for colorectal cancer.

Introduction

Following its initial success for melanoma treatment, immunotherapy rapidly became the main treatment for a variety of solid cancers, including colorectal cancer (CRC) [1]. Two kinds of anti-programmed cell death 1 (PD1) blocking antibodies, pembrolizumab and nivolumab, have shown curative effects in patients with metastatic colorectal cancer with mismatch repair defects and high microsatellite instability (dMMR-MSI-H) status [2–3], and they have achieved good results and been approved by the FDA. Some patients with advanced colorectal cancer have obtained long-term benefit from immunotherapy. How to identify these patients by accurately predicting the biomarkers for immunotherapeutic efficacy is an urgent problem needing to be solved [4–5].

Currently,there are some limitations in assessing mismatch repair defects and microsatellite instability, such as different detection methods and different interpretation standards[6]. Real-world data indicate that only approximately 4% of mCRC patients have dMMR-MSI-H status and that the efficacy rate in patients with dMMR-MSI-H tumours is 50% [2]. Therefore, more accurate predictors are needed to expand the population of pateints with colorectal cancer that may benefit from immunotherapy, and there is no specific predictor of colorectal cancer.

It has been confirmed that gene mutations have a relationship with immunotherapy, for example,TP53 mutation,increased PD-L1 expression specificity and increased mutation burden in lung adenocarcinoma [7]. KRAS mutation and TET1 mutation have been proven to be new predictors of disease and ICI responses in different cancer types[8]. In NSCLC, patients with EGFR mutations have poor responses to immunotherapy [9], and patients with NRTK3 and ZFHX3 mutations have prolonged OS after ICI treatment [10–11].

The purpose of this study was to identify mutated genes closely related to the efficacy of immunotherapy for colorectal cancer. By analysing an ICI cohort and a non-ICI cohort of patients with colorectal cancer, we found that the Overall Surviva(OS) of patients with Rnf43 mutation was prolonged after immunotherapy, while the survival of patients with Rnf43 mutation was not improved in the non-ICI cohort. In addition, Rnf43 mutation was associated with enhanced tumour immunogenicity, activated antitumour immunity and increased T cell infiltration. In addition, it increased MSI, TMB and the expression of immune-related genes.

Method

Sample collection and analysis of clinical characteristics

To assess the predictive effect of single gene mutations on ICI efficacy, we assembled a discovery cohort from Robert M Samstein et al (https://www.cbioportal.org) based on the ICI treatment of 110 patients with colorectal cancer [12], and the research samples were sequenced using the MSK-IMPACT panel authorized by the US Food and Drug Administration (FDA) [13]. The OS data of the ICI cohort were analysed by univariate Cox regression excluding genes with fewer than 5 mutations. To further verify the predictive effect of single gene mutations on the efficacy of ICIs, we collected data from the TCGA data portal (https://www.cbioportal.org) and downloaded the WES data and clinical data of 594 patients with colorectal cancer for analysis.

Analysis of gene mutations and tumour immunogenicity

TMB and MSI data of the ICI cohort and non-ICI cohort were used to analyse the correlations between gene mutations and predictive markers of immune efficacy; the CIBERSORTx deconvolution method was used to analyse the abundance of tumour infiltrating immune cells [14]. The abundances of 22 kinds of infiltrating immune cells in Rnf43-mut and Rnf43-wt tumours were compared, and the R package ggpubr was used to visualize the results. In addition, the differences between mutated and wild-type immune-related genes and some important biomarkers were analysed.

Pathway enrichment analysis

The R package clusterProfiler was used to analyse and visualize the changes in pathway activation levels caused by gene mutations [15–16]. The R package GSVA was used to analyse TCGA cancer

transcriptome data by ssGSEA, and the R package pheatmap was used to visualize pathway differences in Rnf43-mut tumours.

Clinical results

Our clinical results are mainly Progression Free Survival(PFS) and OS. PFS and OS were evaluated from the start date of immunotherapy to the date of progression or death from any cause. OS in the ICI cohort was calculated from the start date of ICI treatment, while OS in the TCGA cohort was calculated from the date of first diagnosis.

Statistical analysis

The Mann-Whitney U test was used to analyse changes in TMB, MSI, immune cell infiltration abundances and important markers caused by Rnf43 mutation; the Kaplan-Meier method, log-rank test and Cox proportional hazards regression analysis were used to analyse the PFS and OS of patients with Rnf43-mut and Rnf43-wt tumours; the R package limma [17] and DESeq2 were used to analyse gene expression data from TCGA and GDSC cancer cell lines and the edgeR package was used to analyse the effects of Rnf43 mutation on immune-related genes. The level of significance was set at 0.05, and all statistical tests were two-tailed. R v.3.6.1 was used for statistical analysis.

Results

3.1. Rnf43 mutation enrichment in patients with good response to ICIs

To explore the relationship between gene mutations and ICI efficacy, we assembled a discovery cohort from Robert M Samstein et al (https://www.cbioportal.org) to analyse the mutation data and clinical data of 110 patients with colorectal cancer treated with ICIs. By univariate regression analysis, we found that Rnf43 mutation was closely related to the curative effect of ICIs (excluding cases with a mutation frequency less than 5%), and a P value less than 0.05 indicated significance. In addition, we estimated the ability of Rnf43 mutation to predict PFS and OS benefits in the ICI cohort and TCGA-CRC cohort. Survival analysis showed that there was a significant difference in OS between the Rnf43-mut and Rnf43-wt groups in the ICI cohort after adjusting for age, sex, medication type and tumour type (P = 0.00043, Fig. 1A), but there was no significant difference in PFS and OS between these groups in the TCGA cohort after adjusting for age, sex and tumour stage (Fig. 1B). These results suggest that the improvement in OS by Rnf43 mutation in the ICI cohort may be due to immunotherapy.

TMB and MSI scores can be used as biomarkers for good prognosis of colorectal cancer after ICI treatment. In our study, we analysed the effect of Rnf43 mutation on the prognosis of colorectal cancer. In the ICI cohort, the Rnf43-mut group had a higher TMB than the Rnf43-wt group (P = 1.2e-11, Fig. 1Ca). Similarly, in the TCGA cohort, the Rnf43-mut group had a higher TMB and MSI score than the Rnf43-wt group (P = 3.773e-05, Fig. 1Cb; P = 6.605e-10, P = 1.223e-10; Fig. 1D), indicating that patients with Rnf43

mutation may benefit from ICI treatment. Therefore, Rnf43 mutation can be used as a predictive biomarker in patients with colorectal cancer to identify the population that would benefit from ICI treatment.

In addition, by analyzing GDSC esophageal cancer cell line data, we found that FGFR tyrosinase kinase inhibitor AZD4547 is more sensitive to patients with Rnf43 mutation, which suggests that patients with Rnf43 mutation may be effective to AZD4547(P = 0.008, Fig. 1E).

3.2 Relationships between Rnf43 mutation and clinical features

The results of hierarchical analysis of the ICI cohort and non-ICI cohort are shown in Fig. 2A. The results show that in the ICI cohort, there were significant differences in sex and that in the non-ICI cohort, there were significant differences in age and tumour stage. We previously determined by Cox regression analysis that these differences had no effect on the efficacy of ICI treatment in the Rnf43-mut group. These results indicate that in the TCGA cohort, Rnf43 mutation was related to early stage. We evaluated the relationship between Rnf43 mutation and clinical features (Fig. 2B, Fig. 2C). The figure shows the mutational landscape of the ICI and non-ICI cohorts and the relationship between the top 20 mutated genes and clinical features. The results showed that sex, age, stage, drug type and pathological type had no significant effect on the mutation status of Rnf43. Our previous analysis showed that patients with Rnf43 mutation had higher MSI and TMB, which indicated that the effect of Rnf43 on the immune response was independent of other factors. In the ICI cohort and TCGA cohort, the mutation frequency in the Rnf43-mut group was higher than that in the Rnf43-wt group. The majority of Rnf43 mutations in the ICI cohort were missense mutations (44.87%), while the majority of Rnf43 mutations in the TCGA cohort were frameshift deletion mutations (51.56%).

3.3 Rnf43 mutation enhances antitumour immunity and immunogenicity

To evaluate the effect of Rnf43 mutation on the immune microenvironment, we used CIBERSORTx to analyse the abundances of infiltrating immune cells in the TCGA-CRC cohort. We found that in the Rnf43-mut group, the abundances of infiltrating M1 macrophages, NK cells, CD8 + T cells and total T cells were significantly higher than those in the Rnf43-wt group (Fig. 3A). We further explored the antitumour immunity and immunogenicity patterns of patients with Rnf43 mutation in the TCGA cohort. The results showed that some immune-related genes, such as ADRM1, CXCR6, VNN2, and CLIC2 were enriched in the Rnf43-mut group (Fig. 3C). In addition, some stimulatory immunomodulators, such as the chemokines CXCL9 and CXCL10, cytolytic activity-related genes PRF1 and GZMA, and immune checkpoint biomarkers CD274 and PDCD1, were significantly upregulated in the Rnf43-mut group (Fig. 3B) [18].

3.4 Rnf43 mutation affects immune-related pathways

To explore whether Rnf43 mutation affects important pathways, we used the clusterProfiler package to analyse the GSEA results for the TCGA-CRC cohort. The results showed that Rnf43 mutation activated

immune-related pathways, such as antigen presentation, NK cells, and immune regulation. In addition, it activated immune checkpoint-related pathways, such as the CTLA4, PD-1 and MSI pathways (Fig. 4A), and metabolic pathways such as the fatty acid and P450 pathways were also found to be downregulated, thus alleviating immunosuppression (Fig. 4B).

Next, to further study the effect of Rnf43 mutation on pathway activation, we conducted ssGSEA in the TCGA-CRC cohort and found that there were significant differences in the activation levels of some pathways between the Rnf43-mut and Rnf43-wt groups. The enrichment degree of antigen presentation, NK cells, CTLA4, PD-1 and colon cancer MSI in the Rnf43-mut group was significantly higher than that in the Rnf43-wt group, and these pathways had significant positive regulatory effects on antitumour immunity (Fig. 4C). These results indicate that Rnf43 mutation has a positive regulatory effect on ICI treatment by affecting the activation level of some pathways.

Discussion

We collected colorectal cancer sequencing data from the MSKCC and TCGA databases and screened out the mutated genes closely related to the efficacy of ICIs from the mutation data and clinical data of the MSKCC data set based on ICI treatment response. After verification in the TCGA-CRC cohort, we found that among the evaluated genes, mutation of the Rnf43 gene was most closely related to ICI treatment of colorectal cancer. Rnf43 mutation was more common in patients with good ICI responses, and the effect of Rnf43 mutation on the immune microenvironment was analysed. We found that patients with Rnf43 mutation showed higher MSI and TMB and longer OS times associated with immunotherapy. In addition, we evaluated the changes in immune cell infiltration and immune-related genes in the context of Rnf43 mutation. Rnf43 mutation was related to high infiltration levels of immune cells, upregulated expression of immune-related genes, and effects on immune,MSI and PD-1 related pathways. Through analysis of the sequencing data from these databases, the close relationship between Rnf43 mutation and the efficacy of ICIs for colorectal cancer was reinforced, and the combination strategy of epigenetic targeting and immunotherapy was firmly supported.

Although an increasing number of studies have confirmed that gene mutations are closely related to immunotherapy efficacy [8–11], most of the research has been performed in lung cancer and pan-cancer cohorts, and there is a lack of data analysis in colorectal cancer, which has specific considerations for immunotherapy, such as MSI status [19–20], instead of PD-L1 expression and TMB. Currently, the most widely studied approach is detection of PD-L1 expression in tumours [21]. Interestingly, in some tumour types, such as NSCLC, gastric cancer and gastroesophageal junction tumours, the expression of PD-L1 may be a useful predictive marker, but in colorectal cancer, the expression of PD-L1 is not related to response or survival rate [2]. Moreover, previously identified predictive biomarkers, such as MSI, PD-L1, and TMB, are not perfect, and their limitations make them insufficiently accurate. The PD-L1 detection methods, cut-off values, and interpretation standards required by different immunotherapeutic drugs are different [21]. For example, immunotherapy may not be effective in patients with MSI-H tumours but may not be completely ineffective in patients with MSI-L tumours [20]. Although a number of studies support

the predictive value of TMB [22], the TMB detection methods are quite different, and clear cut-off values and detection methods are lacking. Therefore, detection of a gene mutation has higher specificity than these other biomarkers in guiding immunotherapy, and we can easily identify genomic alterations in patients through sequencing results.

This is the first report of Rnf43 gene mutation, which is closely related to immunotherapy efficacy in colorectal cancer. Our results suggest that Rnf43 mutation can be used as a predictor of immunotherapy efficacy in colorectal cancer. It has been reported that Rnf43, also called E3 ubiquitin ligase Rnf43 or circular E3 ubiquitin transferase Rnf43, belongs to the Goliath and Godzilla family, a small group of proteins with transmembrane domains and characteristic ubiquitin ligase domains that participate in a negative feedback mechanism in the Wnt/β-catenin signalling pathway to inhibit tumours. Rnf43 inhibits Wnt/β-catenin signalling mainly through ubiquitination and degradation of receptors involved in lysosomal pathways. Inactivation of Rnf43 results in a lack of or decrease in frizzled degradation, which enhances upregulation of the Wnt/β-catenin signalling pathway. Mutations in Rnf43 have been reported in different cancers. We describe the structure, function and most common mutations of Rnf43 in different cancers. Human tissue RNA sequencing showed that the Rnf43 gene was highly expressed in normal non-tumour tissue from some organs, such as the duodenum, small intestine, colon and prostate [23]. It was also found that Rnf43 mutation was negatively correlated with BRAF and KRAS mutations, which was consistent with a previous report that patients with KRAS and BRAF mutations had lower clinical activity of ICIs.

In recent years, studies have shown that the Th1 chemokines CXCL9 and CXCL10 can increase tumour invasion of effector T cells and block the programmed death ligand 1 checkpoint [24]; Nauphan et al found that the NF-κB signalling pathway activated immune-related genes, thereby activating cellular immunity [25]; Hassan Wael et al found that Notch1 significantly reduced cell proliferation through apoptosis and controlled cell proliferation and apoptosis [26]; In addition, Fokhrul et al and Hossain et al found that metabolic pathways play an important role in the differentiation and function of immune cells through the following mechanism: bone marrow-derived suppressor cells (MDSCs) inhibit T cell immunity by increasing activated fatty acid oxidation (FAO) and fatty acid uptake, promote tumour cell migration and proliferation, and promote cell growth. Inhibition of FAO alone can significantly delay T cell-dependent tumour growth and enhance the effect of adoptive T cell antitumour therapy [27]. In this study, Rnf43 mutation induced upregulation of the chemokines CXCL9 and CXCL10, cytokines, and immune checkpoint markers; activation of immune pathways; and inhibition of metabolic pathways. These results were consistent with those of previous studies.

Although the predictive value of Rnf43 mutation for ICI efficacy in colorectal cancer is significant, there are some limitations of this study. We analysed the predictive value of Rnf43 mutation for immunotherapy efficacy only in colorectal cancer, and this value is unknown for other tumours. It is necessary to expand this analysis to other tumours in the future to further analyse the predictive value of Rnf43 mutation for immunotherapy efficacy.

Conclusions

Our results suggest that Rnf43 mutation can significantly prolong the survival of patients receiving immunotherapy. Therefore, Rnf43 mutation can be used as a predictive biomarker for immunotherapy efficacy in colorectal cancer to predict which patients may benefit from immunotherapy. In the future, we still need to further explore the underlying molecular mechanism and carry out clinical research to expand the predicted range of tumours.

Declarations

Ethical Approval and Consent to Participate - The data of this study are all from public databases, which do not involve ethics.

Consent for Publication – All participants agreed to publish,and with no copyright issues.

Availability of data and materials – I have uploaded the original data in the supplement files.

Competing interests – There is no interest competition in this research.

Funding – There is no Funding in this research.

Authors'contributions-conceived,designed:NZ,YFL;analysis and visualization:NZ,XPS;supervision:WCJ; writing and editing:NZ,XYL.All authors contributed to the article and approved the submitted version,All authors reviewed the manuscript.Thank each participant for their contribution to this study.

References

[1]Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. (2017) 541:321–30.

[2]Karuna Ganesh, Zsofia K Stadler, Andrea Cercek,et al.Immunotherapy in colorectal cancer: rationale, challenges and potential.Nat Rev Gastroenterol Hepatol. 2019 Jun;16(6):361-375.

[3]Aaron J Franke, William Paul Skelton,et al.Immunotherapy for Colorectal Cancer: A Review of Current and Novel Therapeutic Approaches.J Natl Cancer Inst.2019 Nov 1;111(11):1131-1141.

[4]Pawel Wrobel, Shahid Ahmed.Current status of immunotherapy in metastatic colorectal cancer.Int J Colorectal Dis.2019 Jan;34(1):13-25.

[5] Sharma P, Hulieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. (2017) 168:707– 23.

[6]Dung T Le, Jennifer N Durham, Kellie N Smith, et al.Mismatch Repair Deficiency Predicts Response of Solid Tumors to PD-1 Blockade.Science. 2017 Jul 28;357(6349):409-413.

[7]Zhong-Yi Dong, Wen-Zhao Zhong, Xu-Chao Zhang,et al.Potential Predictive Value of TP53 and KRAS Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma.Clin Cancer Res.2017 Jun 15;23(12):3012-3024.

[8]Hao-Xiang Wu, Yan-Xing Chen, Zi-Xian Wang,et al.Alteration in TET1 as Potential Biomarker for Immune Checkpoint Blockade in Multiple Cancers. J Immunother Cancer,2019 Oct 17;7(1):264.

[9]Mei Ji, Yan Liu, Qing Li, Xiaodong Li,et al.PD-1/PD-L1 expression in non-small-cell lung cancer and its correlation with EGFR/KRAS mutations.Cancer Biol Ther.2016 Apr 2;17(4):407-13.

[10]Yuchun Niu, Anqi Lin, Peng Luo,et al.Prognosis of Lung Adenocarcinoma Patients With NTRK3 Mutations to Immune Checkpoint Inhibitors.Front Pharmacol. 2020 Aug 12;11:1213.

[11]Jiexia Zhang, Ningning Zhou,Anqi Lin,et al.ZFHX3 mutation as a protective biomarker for immune checkpoint blockade in non-small cell lung cancer.Cancer Immunol Immunother.2020 Jul 11.

[12]Robert M Samstein, Chung-Han Lee, Alexander N Shoushtari,et al.Tumor mutational load predicts survival after immunotherapy across multiple cancer types.Nat Genet,2019 Feb;51(2):202-206.

[13]Donavan T Cheng, Talia N Mitchell, Ahmet Zehir, Ronak H Shah, et al.Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology.J Mol Diagn.2015 May;17(3):251-64.

[14]Binbin Chen, Michael S Khodadoust, Chih Long Liu, Aaron M Newman, et al.Profiling Tumor Infiltrating Immune Cells With CIBERSORT.Methods Mol Biol. 2018;1711:243-259.

[15]Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16(5):284-7.

[16]A S, T P, M VK,et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles[J].Proceedings of the National Academy of Sciences of the United States of America 2005 102(43):15545-15550.

[17]Matthew E Ritchie, Belinda Phipson, Di Wu, Yifang Hu,et al.Limma Powers Differential Expression Analyses for RNA-sequencing and Microarray Studies.Nucleic Acids Res. 2015 Apr 20;43(7):e47.

[18]Tsuyoshi Hamada, Thing Rinda Soong, Yohei Masugi,et al.TIME (Tumor Immunity in the MicroEnvironment) classification based on tumor CD274 (PD-L1) expression status and tumor-infiltrating lymphocytes in colorectal carcinomas.Oncoimmunology.2018 Mar 19;7(7):e1442999.

[19]Mehmet Sitki Copur.Immunotherapy in Colorectal Cancer.Oncology (Williston Park) .2019 Oct 28;33(10):686506.

[20]Malek Kreidieh, Deborah Mukherji,et al.Expanding the Scope of Immunotherapy in Colorectal Cancer: Current Clinical Approaches and Future Directions.Biomed Res Int.2020 Jan 25;2020:9037217.

[21]Sandip Pravin Patel,Razelle Kurzrock.PD-L1 Expression as a Predictive Biomarkers in Cancer Immunotherapy.Mol Cancer Ther.2015 Apr;14(4):847-56.

[22]Samstein RM, Lee CH, Shoushtari AN. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nature genetics. 2019;51(2):202–206.

[23]Stefano Serra, Runjan Chetty.Rnf43.J Clin Pathol.2018 Jan;71(1):1-6.

[24]Peng D, Kryczek I, Nagarsheth N,et al.Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature. 2015 Nov 12;527(7577):249-53.

[25]N Auphan, J A DiDonato, C Rosette,et al.Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis.Science. 1995 Oct 13;270(5234):286-90.

[26]Hassan Wael, Ryoji Yoshida, Shinji Kudoh,et al.Notch1 Signaling Controls Cell Proliferation, Apoptosis and Differentiation in Lung Carcinoma Lung Cancer,2014 Aug;85(2):131-40.

[27]Fokhrul Hossain, Amir A Al-Khami, Dorota Wyczechowska,et al.Inhibition of Fatty Acid Oxidation Modulates Immunosuppressive Functions of Myeloid-Derived Suppressor Cells and Enhances Cancer Therapies.Cancer Immunol Res. 2015 Nov;3(11):1236-47.

Figures

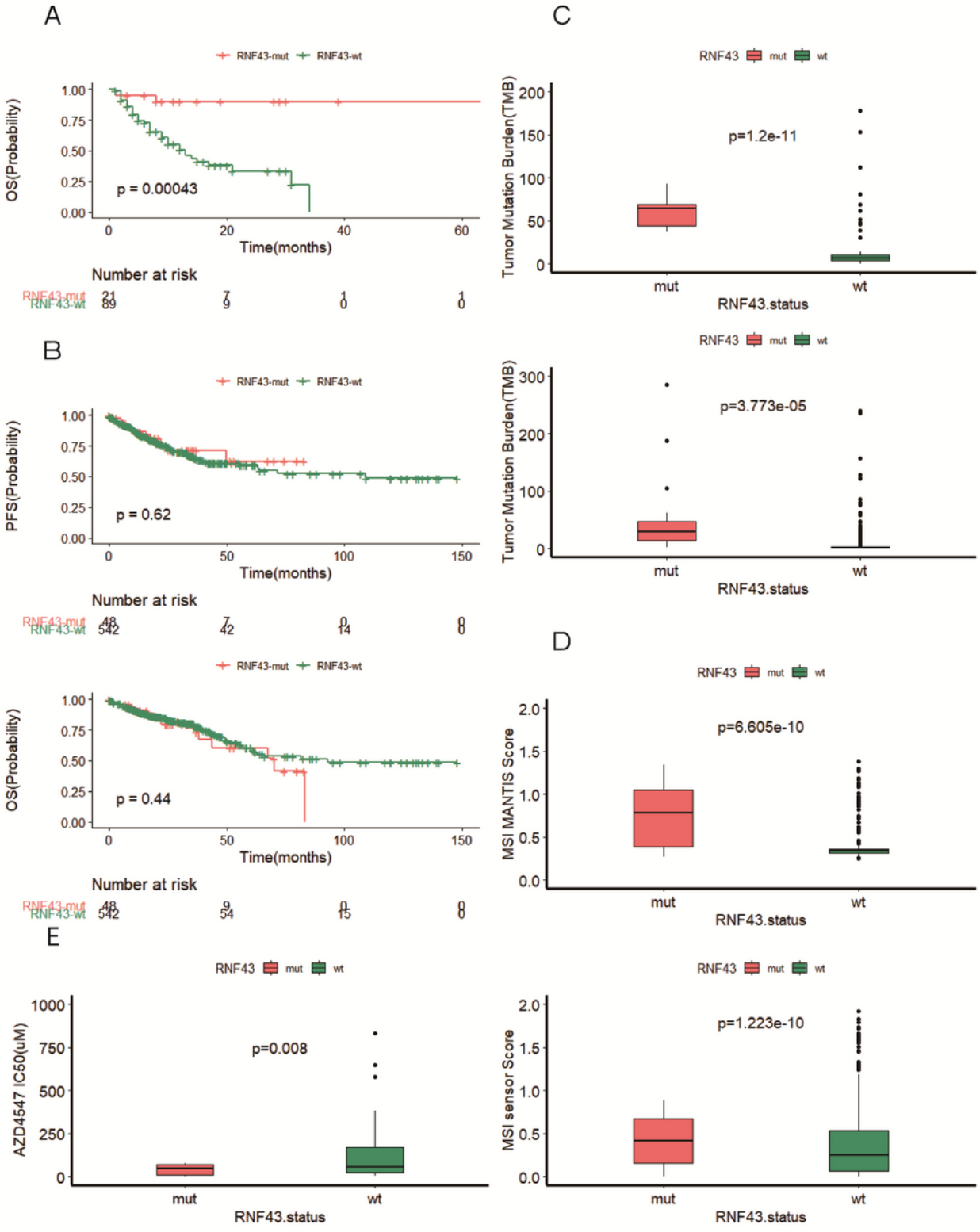

Figure 1

Predictive value of Rnf43 mutation for the efficacy of ICI therapy in colorectal cancer.(A.Effect of Rnf43 mutation on OS in the ICI cohort of patients with colorectal cancer.B.Effect of Rnf43 mutation on PFS and OS in the TCGA cohort (non-ICI cohort) of patients with colorectal cancer.C.Effect of Rnf43 mutation on TMB in colorectal cancer.D.Effect of Rnf43 mutation on MSI in the non-ICI cohort of patients with colorectal cancer (two detection methods)).

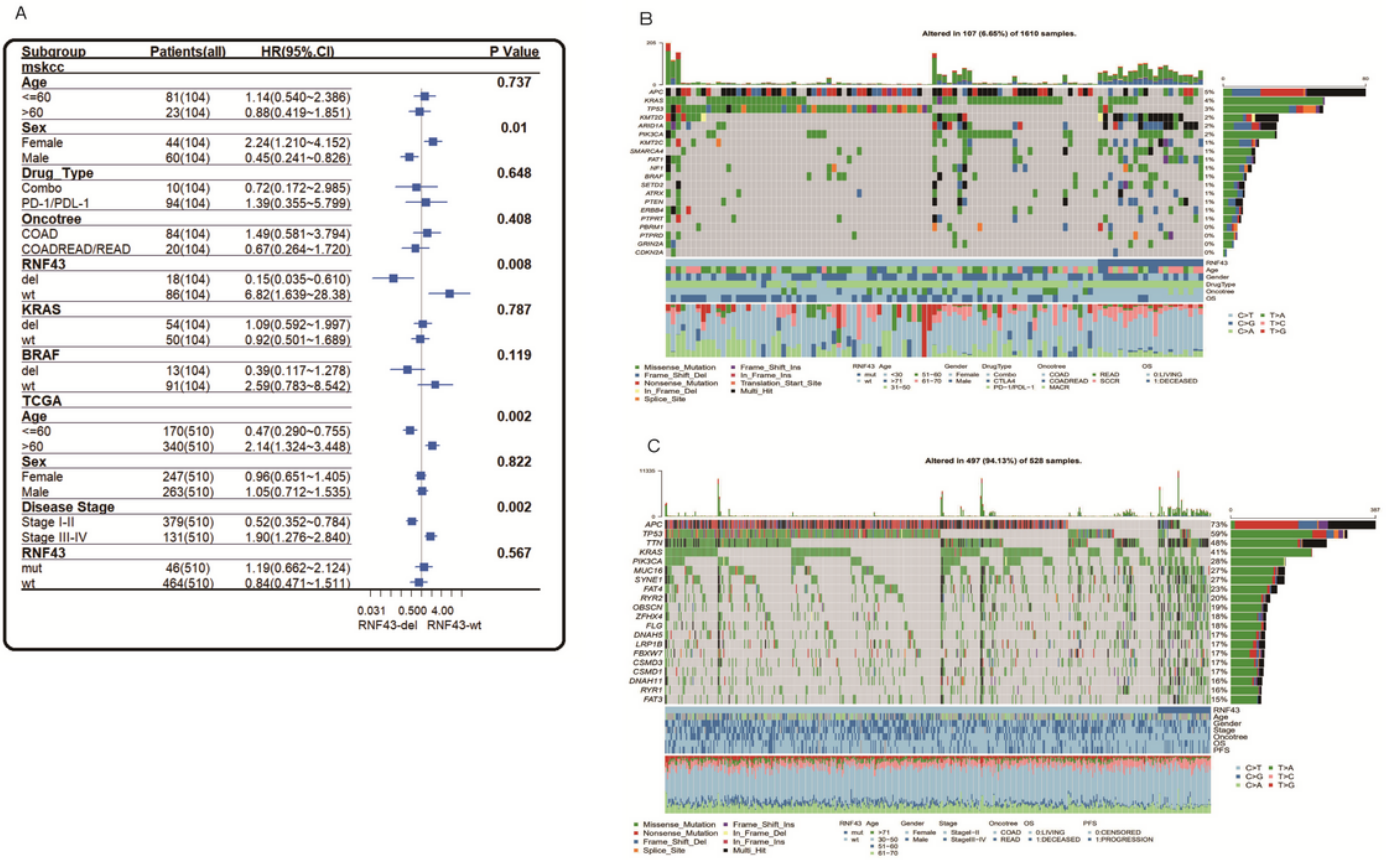

Figure 2

Mutational landscape: somatic mutation and its relationship with clinical features(A.Hierarchical analysis of the ICI and non-ICI cohorts.B.The mutational landscape of the ICI cohort showed the top 20 significant mutations and the clinical features between Rnf43-mut and Rnf43-wt.C.The TCGA cohort mutational landscape showed the top 20 significantly mutated genes and the clinical characteristics in the Rnf43-mut and Rnf43-wt groups.)

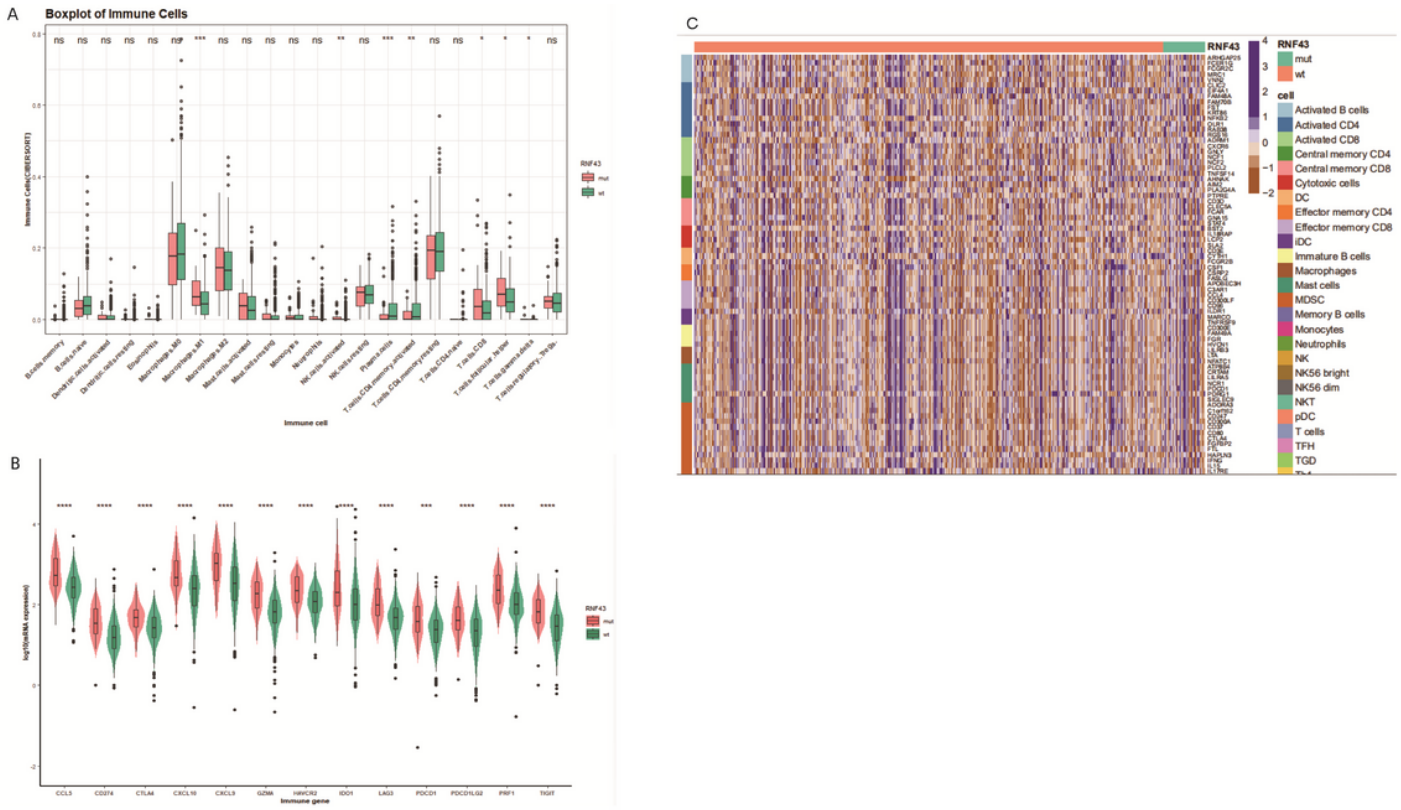

Figure 3

Relationship between RNF43 mutation and both immune cell infiltration and immune-related gene expression(A.Abundances of 22 kinds of infiltrating immune cells in the Rnf43-mut and Rnf43-wt groups in the TCGA cohort. B. The differences in the expression of some important immune-related genes, such as chemokines, cytolytic activity-related genes and immune checkpoint markers, between the Rnf43-mut and Rnf43-wt groups in the TCGA cohort. C.Significant differences in immune-related genes according to the Rnf43 mutation status were found.)

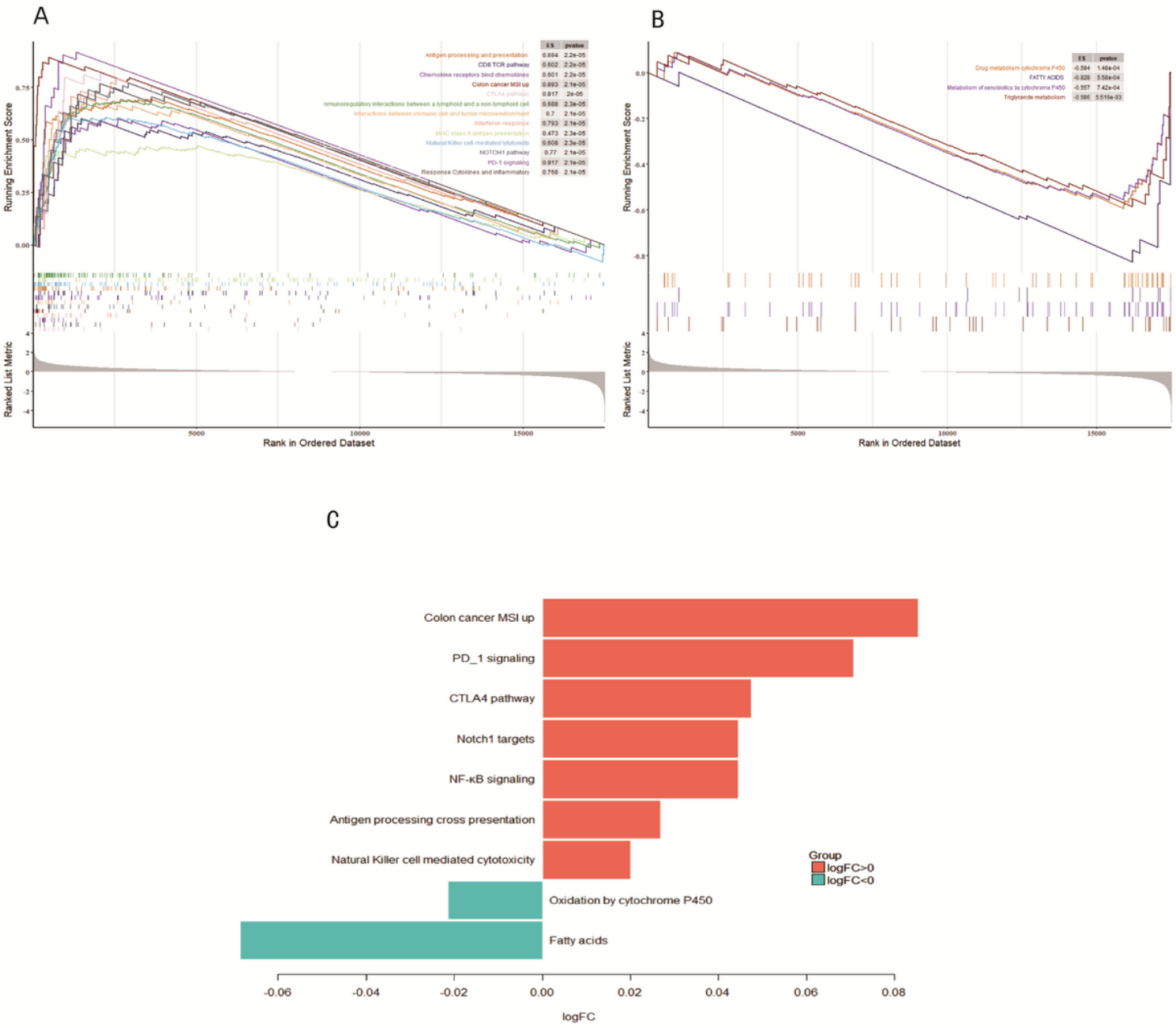

Figure 4

Relationship between RNF43 mutation and both immune cell infiltration and immune-related gene expression(A.Abundances of 22 kinds of infiltrating immune cells in the Rnf43-mut and Rnf43-wt groups in the TCGA cohort. C. The differences in the expression of some important immune-related genes, such as chemokines, cytolytic activity-related genes and immune checkpoint markers, between the Rnf43-mut and Rnf43-wt groups in the TCGA cohort.C.Significant differences in immune-related genes according to the Rnf43 mutation status were found.)

Supplementary Files

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

- TCGAclinicaldata.pdf
- mskcclClclinicaldata.pdf
- mskcclClmutations.pdf
- supplementarylegend.docx