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Original Article

Association of ATRX mutations with immunologically active characteristics in patients with MSI-prone tumors

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Abstract: Objectives: The role of DNA damage repair deficiency in improving immune checkpoint inhibitors (ICIs) efficacy has been widely recognized. Studies have confirmed the association of gene mutations in homologous recombination (HR) with an immune-activated microenvironment. Given the crucial role of the tumor microenvironment in ICIs response, our study aimed to identify specific HR gene mutations that influence the tumor microenvironment and thus serve as potential biomarkers for ICIs in tumors that are prone to occur with microsatellite instability (MSI) events (MSI-prone tumors). Methods: The multi-omics and clinical data of MSI-prone tumors were extracted from ICIs-treated and non-ICIs-treated cohorts. We depicted the mutation landscape of HR genes in MSI-prone tumors and identified the prognosis related HR gene mutations. We integrated multiple immunotherapy-related indicators by bioinformatics methods to characterize the anti-tumor immunity and tumor microenvironment. Results: ATRX, ARID1A, BRCA2 and ATM were the common top four frequently mutated HR genes in MSI-prone tumors, among which ATRX mutations were identified to have prognostic value for ICIs treatment. The bioinformatics analyses suggested that patients with ATRX mutilations (ATRX-mt) have enhanced anti-tumor immunity and inflamed tumor microenvironment in MSI-prone tumors. MSI-stratified analyses revealed the immunologically active features in both microsatellite instability-high (MSI-H) and non-MSI-H populations. There may exist a synergistic effect between ATRX mutations and MSI-H status in immune activation. Conclusions: Our work found the association of ATRX mutations with immunologically active characteristics in MSI-prone tumors. The combined use of ATRX mutations and MSI-H status might have potential clinical utility for ICIs selection in MSI-prone tumors.

Keywords: Immune checkpoint inhibitors, ATRX mutations, immune activation, microsatellite instability

Introduction

At present, the study of immune checkpoint inhibitors (ICIs) have achieved striking developments in the treatment of advanced solid tumors, especially tumors prone to occur with microsatellite instability (MSI) events (MSI-prone), such as colorectal, endometrial and gastric carcinomas. MSI is a hypermutable phenotype of short repetitive sequences in the genome caused by DNA mismatch repair deficient (MMRd) [1]. According to the microsatellite instability status, tumors can be divided into three subtypes: microsatellite instability-high (MSI-H), microsatellite instability-low (MSI-L) and microsatellite stable (MSS) tumors. Results from preclinical and clinical trials have proved that MSI-H tumors have an inflammatory tumor microenvironment and improved ICIs response [2]. As a result, MSI-H was approved by the US Food and Drug Administration as a pan-cancer biomarker for clinical use of the anti-PD1 monoclonal antibody pembrolizumab regardless of tumor site or histology [3]. Nevertheless, the therapeutic responses to ICIs vary considerably among patients with MSI-prone tumors. In MSI-H populations, only about 50% patients are responsive to ICIs [4]. Therefore, it is of great clinical value to identify other biomarkers used for aiding MSI-H in predicting ICIs response in MSI-prone tumors.

In addition to MSI-H, other DNA damage repair (DDR) deficiency factors could improve the sensitivity to ICIs [5]. The impairment in DDR genes has been traditionally recognized to promote carcinogenesis and tumor growth [6]. However, these defects may also shape the tumor immu-

nogenicity by (1) enhancing antigenicity through increased genomic alterations, (2) activating T cells and triggering T cell killing functions through the production of adjuvant and co-stimulatory molecules, especially type I interferon and pro-inflammatory cytokines, (3) as well as influencing expression of immune checkpoints and death receptors [6-8]. A growing body of evidence has reported the correlation of homologous recombination (HR) deficiency with increased immunogenicity in several cancer types [7]. Therefore, specific HR gene mutations might help to distinguish the patients who would receive benefit from ICIs in MSI-prone tumors. In this study, we aimed to delineate the mutation characteristics of HR genes in MSI-prone tumors and to identify specific HR gene mutations that could serve as biomarkers for ICIs efficacy in MSI-prone tumors.

Materials and methods

Data sources

MSI-H has been widely detected in colon adenocarcinoma (COAD), stomach adenocarcinoma (STAD) and uterine corpus endometrial carcinoma (UCEC). These three tumor types have been recognized as being MSI-prone in multiple studies [1, 9]. Therefore, we integrated these three tumor types as MSI-prone tumors in the present study. We have depicted the HR genes mutation features of MSI-prone tumors and explored their association with clinical outcomes in both ICIs-treated and non-ICIs-treated cohorts. The genomic and clinical data of MSI-prone tumors in ICIs-treated cohorts were gathered from the Samstein et al published study [10]. The non-ICIs-treated cohorts contained two datasets including MSI-prone tumors from The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering (MSK) Cancer Center datasets [11]. In addition, we collected the RNA-seq data of MSI-prone tumors from the TCGA cohort for the analyses of anti-tumor immunity and tumor microenvironment. The MSI-H status of MSI-prone tumors from TCGA cohort was retrieved from The Cancer Immunome Atlas (TCIA, https://www.tcia.at/home) [12]. In the TCGA cohort, the frequency of MSI-H status in all three tumor types was greater than 10% (UCEC, 31.5%; STAD, 19.1% and COAD, 18.4%). The flow chart of this study design was shown in **Figure 1**.

Homologous recombination (HR) related genes

We defined 30 HR-related genes in our study, each of which was associated with the HR pathway activity or was included in HR biomarker in clinical trials [13-16]. The HR related genes were as follow: ARID1A, ATM, ATRX, BAP1, BARD1, BLM, BRCA1, BRCA2, BRIP1, CHEK1, CHEK2, MRE11A, MRE11, NBN, PALB2, RAD50, RAD51, RAD51B, WRN, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, FANCA, RAD51D, XRCC2, RAD52 and RAD54L. The nonsynonymous somatic mutations, including nonsense mutations, missense mutations, frame shift insertion and deletion, in-frame insertion and deletion, nonstop mutations and splice site mutations, were taken into consideration.

Immunotherapy-related anti-tumor immunity

We utilized the score of cytolytic activity (CYT) [17], inflammation signature [18], immunologic constant of rejection (ICR) [19] and IFN-γ signaling [20] to characterize the anti-tumor immunity. All these indicators were proven to correlate with immunotherapy efficacy. CYT score was estimated by the geometric mean of transcripts per kilobase million (TPM) expression of two key cytolytic effectors (GZMA and PRF1). The method of single sample gene set enrichment analysis (ssGSEA) was used to quantify the score of inflammation signature and IFN-γ signaling [21]. We selected four gene panels from ICIs-treated clinical trials to measure IFN-γ signaling, including KEYNOTE-012 [22], KEYNOTE-059 [23], CheckMate-275 [24] and POPLAR [25]. As previously described [26], ICR score was calculated by the mean of the log2 transformed TPM values of ICR signature genes. The activities of TGF-β pathway were assessed by the method of PROGENy [27].

Tumor microenvironment (TME)

According to a recent study, we characterized tumor microenvironment (TME) properties with 29 knowledge-based functional gene expression signatures (Fegs) of conserved immunotherapeutic predictive function [28]. The 29 Fegs represented four TME properties: anti-tumor microenvironment, pro-tumor microenvironment, angiogenesis fibrosis and malignant cell properties. CIBERSORT was employed to evaluate the abundance of tumor-infiltrating lymphocytes (TILs) [29].

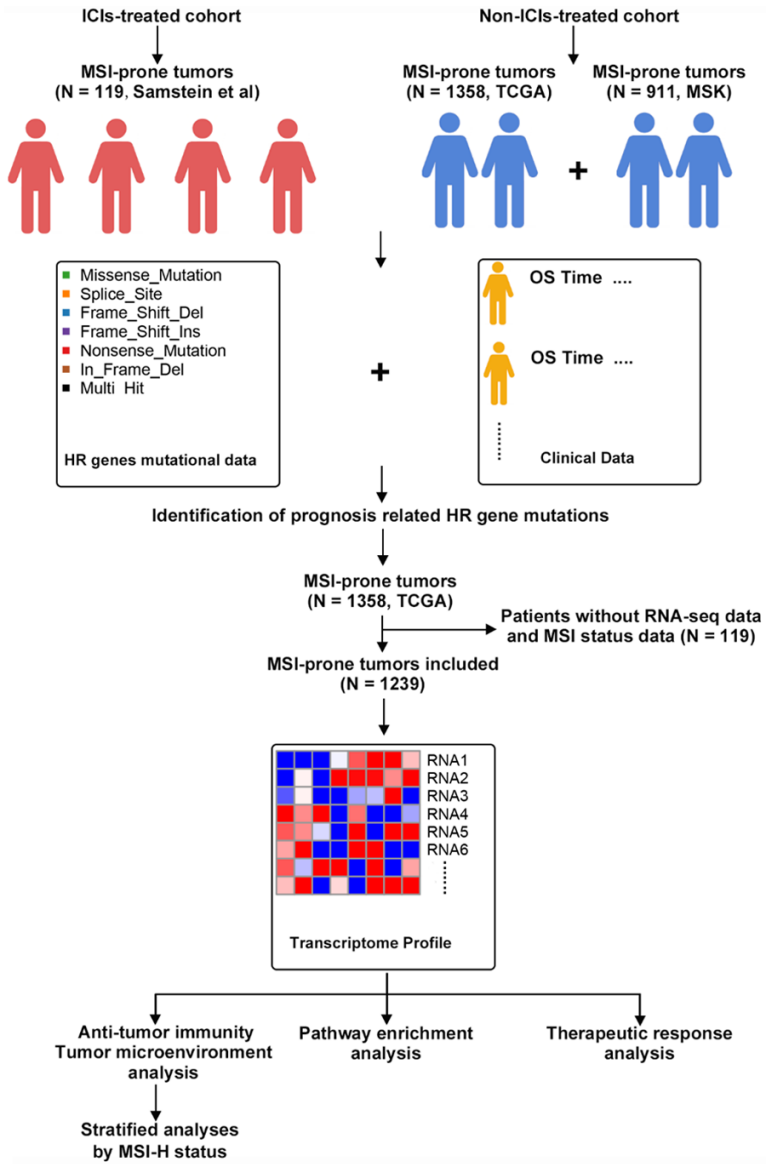


Figure 1. Flow chart of the study design. We identified the prognosis related homologous recombination (HR) gene mutations in immune checkpoint inhibitors (ICIs)-treated (Samstein et al) and non-ICIs-treated cohort (TCGA and MSKCC). Based on RNA-seq data from TCGA cohort, we investigated the influence of prognosis related HR genes mutations on anti-tumor immunity, tumor microenvironment, pathway enrichment and therapeutic response.

Gene set enrichment analysis

We performed the gene set enrichment analysis (GSEA) by the software of GSEA (version 4.0.3) with the gene set of "c2.cp.kegg.v7.4.symbol". The gene set variation analysis (GSVA) is a robust method to estimates variation of molecular pathways and gene expression signatures in an unsupervised manner for microarray and RNA-seq data of a sample population [21]. The gene sets of "c2.cp.kegg.v7.4.symbols" and "h.all.v7.4.symbols" were downloaded from MSigDB database for running GSVA. The R package *limma* was further used to identify pathways or hallmarks that differed between the groups with the cutoff value of FDR < 0.05 [30].

Therapeutic response analyses

The R package *pRRophetic* was implemented to predict the chemotherapy drug sensitivity of each patient. Based on drug sensitivity data from GDSC (https://www.cancerrxgene.org/), the *pRRophetic* package can predict clinical chemotherapeutic response by establishing statistical models from the expression profiles from human cancer cell lines. The half-maximal inhibitory concentration (IC50) value of each sample was calculated by ridge regression, and 10-fold cross-validation based on GDSC training set was used to evaluate the prediction accuracy [31]. The response to the anti-PD1 and anti-CTLA4 was predicted via subclass mapping (Submap) in GenePattern (https://cloud.genepattern.org) [32, 33]. In this manner, we collected an open clinical dataset of 47 melanoma patients who responded to ICIs [6].

Statistical analysis

The differences between subgroups were examined by Mann-Whitney U test or Kruskal-Wallis test. Due to the delayed clinical effect of immunotherapy, we adopted the non-proportional-hazard survival model to assess the prognosis of patients receiving ICIs. According to recent literature, the delayed treatment effect of immunotherapy usually occurs after approximately four months [34]. As a result, the approach of long-term survival inference was used to compare the survival rate of ICIs-

treated patients after four months by R packages *ComparisonSurv*. The differences of survival rate between subgroups in non-ICIs-treated cohorts were compared by the Log-rank test of Kaplan-Meier analysis. A two-tailed $P < 0.05$ was considered as significant. All statistical analyses were carried out with R software (version 4.0.4).

Results

Identification of ATRX mutations related to favorable prognosis from ICIs treatment in MSI tumors

We first explored the mutational frequency of HR genes in ICIs-treated and non-ICIs-treated MSI-prone tumor cohorts. As shown in Figure S1A, the ATRX, ARID1A, ATM and BRCA2 were the top four most frequently mutated HR genes. We further studied the impact of these four HR genes mutations on prognosis after ICIs treatment in Samstein et al ICIs-treated cohort which consisted of 84 COAD and 35 STAD patients. We found that ATRX mutant patients showed better long-term survival than ATRX wildtype patients (**Figure 2A-D**). Besides, we have also evaluated the predictive value of ATRX mutations for survival outcome in other ICIs-treated cohorts with various tumor types. Two ICIs-treated cohorts (MSK whole ICIs-treated cohort from Samstein et al and ICIs-treated NSCLC cohort from Rizvi et al) were collected [10, 35]. The MSK whole ICIs-treated cohort contained 1,610 patients with various cancer types, while Rizvi cohort was comprised of 240 Non-Small cell lung cancer (NSCLC) patients. Consistently, ATRX mutations were related to prolonged long-term survival in these two cohorts (MSK whole ICIs-treated cohort, $P = 0.0563$; Rizvi cohort, $P = 0.0014$) (**Figure 2E**, **2F**). Moreover, NSCLC patients with ATRX mutations had higher proportion of durable clinical benefit (DCB, MT vs WT: 57.2% vs 27.0%, $P = 0.025$) in Rizvi cohort (**Figure 2G**). Higher tumor mutation burden (TMB) was observed in all three ICIs-treated cohorts (**Figure 2H**). Finally, we investigated the relationship between ATRX mutations and overall survival in two non-ICIs-treated MSI-prone tumors cohorts. The MSK non-ICIs-treated cohort included 911 patients (COAD: 710; STAD: 135; UCEC: 94) and TCGA cohort contained 1,358 MSI-prone tumors (COAD: 397; STAD: 433; UCEC: 528). Similar to the above result, we determined the prognosis value of ATRX mutations in these two non-ICIs-treated cohorts (Figure S1B). Taken together, we found that ATRX mutations frequently occur in MSI-prone tumors and imply better survival benefits from ICIs treatment.

ATRX mutations were associated with enhanced anti-tumor immunity and immune-active tumor microenvironment in TCGA cohort

A total of 1,239 patients with complete whole exome sequencing (WES), RNA-seq and MSI status data were enrolled to assess the impact of ATRX mutations on anti-tumor immunity and tumor microenvironment (TME). The patients with ATRX nonsynonymous mutations were defined as ATRX-mt, while those lacking ATRX nonsynonymous mutations as ATRX-wt. As displayed in **Figure 3A**, ATRX-mt patients had elevated TMB, higher immune checkpoint genes expression and increased score of several anti-tumor immunity indicators. However, the transforming growth factor beta (TGF-β) pathway activity, reported to decrease tumor response to PD-L1 blockade by restricting T cell infiltration [36], was significantly down-regulated in ATRX-mt patients.

We continued to compare the TME traits between ATRX-mt and ATRX-wt patients. Bagaev et al. have used 29 functional gene expression signatures (Fegs) to divide the TME into four subtypes: anti-tumor immune infiltration, pro-tumor immune infiltration, angiogenesis fibroblasts and malignant cell properties [28]. The patients harboring immune-favorable TME subtypes may achieve the most benefit from immunotherapy. In general, ATRX-mt patients showed higher levels of anti-tumor immune infiltrate and lower level of pro-tumor immune infiltration (**Figure 3B**). Additionally, we noticed that the Fegs related to cancer-associated fibroblast (CAF) activation and angiogenesis were down-regulated in ATRX-mt patients (**Figure 3B**).

Finally, we employed CIBERSORT to estimate the infiltration of immune cell fractions between ATRX-mt and ATRX-wt patients. As expected, the anti-tumor lymphocyte cells such as CD8 T cells, activated memory CD4 T cells, T follicular helper, gamma delta T cells and M1 macrophages were more abundant in ATRX-mt patients, while the proportions of tumor promoting cells including naive B cells, resting memory CD4 T cells, regulatory T cells (Tregs)

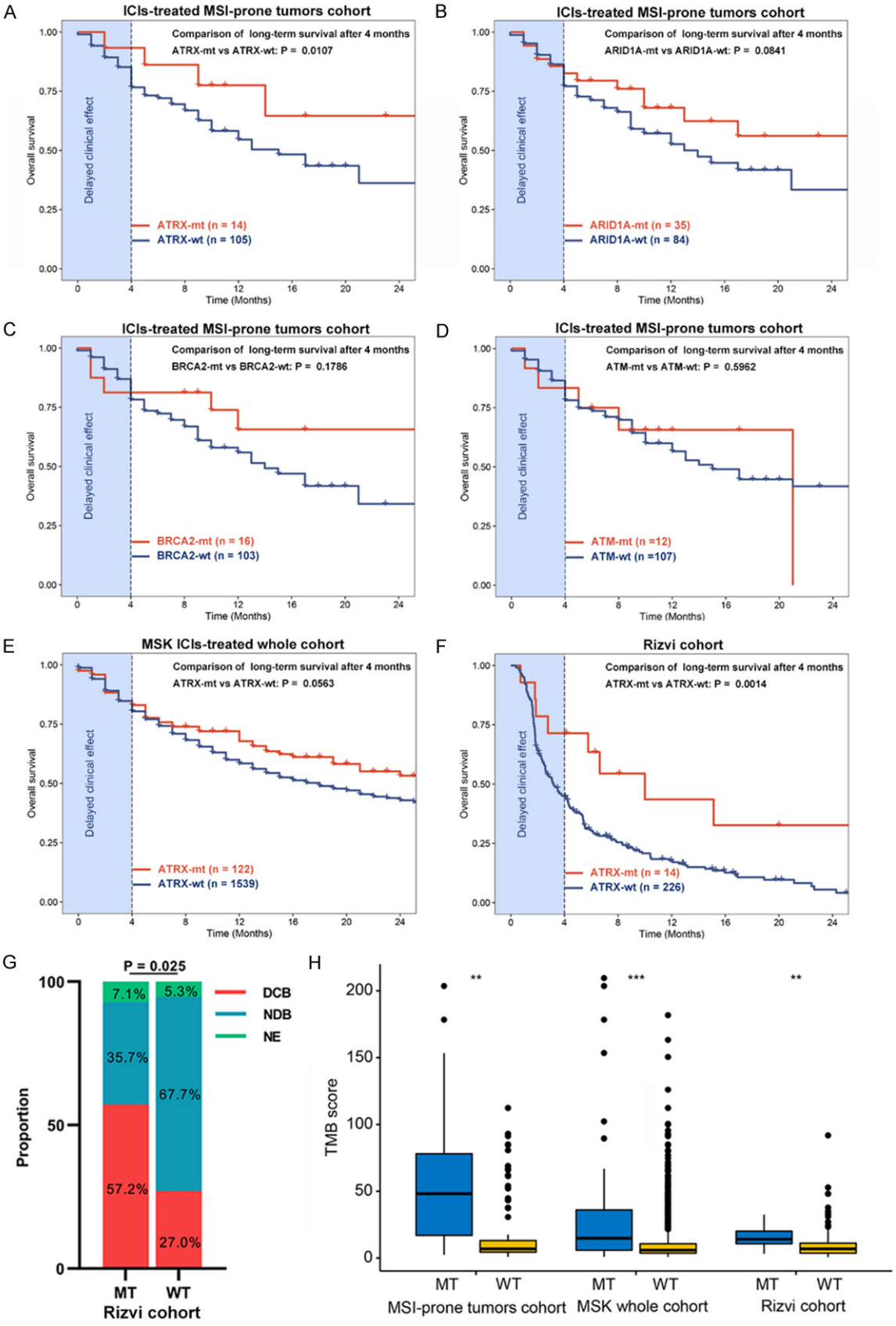

A
ICIs-treated MSI-prone tumors cohort
Comparison of long-term survival after 4 months
ATRX-mt vs ATRX-wt: P = 0.0107
Delayed clinical effect
Overall survival
ATRX-mt (n = 14)
ATRX-wt (n = 105)
Time (Months)
B
ICIs-treated MSI-prone tumors cohort
Comparison of long-term survival after 4 months
ARID1A-mt vs ARID1A-wt: P = 0.0841
ARID1A-mt (n = 35)
ARID1A-wt (n = 84)
C
ICIs-treated MSI-prone tumors cohort
Comparison of long-term survival after 4 months
BRCA2-mt vs BRCA2-wt: P = 0.1786
BRCA2-mt (n = 16)
BRCA2-wt (n = 103)
D
ICIs-treated MSI-prone tumors cohort
Comparison of long-term survival after 4 months
ATM-mt vs ATM-wt: P = 0.5962
ATM-mt (n =12)
ATM-wt (n =107)
E
MSK ICIs-treated whole cohort
Comparison of long-term survival after 4 months
ATRX-mt vs ATRX-wt: P = 0.0563
ATRX-mt (n = 122)
ATRX-wt (n = 1539)
F
Rizvi cohort
Comparison of long-term survival after 4 months
ATRX-mt vs ATRX-wt: P = 0.0014
ATRX-mt (n = 14)
ATRX-wt (n = 226)
G
P = 0.025
DCB
NDB
NE
Proportion
7.1%
35.7%
57.2%
5.3%
67.7%
27.0%
MT
WT
Rizvi cohort
H
TMB score
MT
WT
MSI-prone tumors cohort
MSK whole cohort
Rizvi cohort

Figure 2. Long-term survival analysis of HR related gene mutations in ICIs-treated cohorts. A-D. The impact of four frequently mutated HR genes (ATRX, ARID1A, BRCA2 and ATM) in microsatellite instability (MSI)-prone tumors on the long-term survival rates after four months of ICIs treatment. ATRX mutations were identified as the specific HR gene mutations related to better survival outcome from ICIs-treatment. E, F. ATRX mutations were associated with favorable clinical outcomes from ICIs treatment in two ICIs-treated cohorts with various tumor types (MSK whole ICIs-treated cohort and Rizvi ICIs-treated NSCLC cohort). G. Proportions of patients achieving durable clinical benefit (DCB) in ATRX-mt and ATRX-wt patients from the Rizvi cohort. H. Boxplot showing tumor mutation burden (TMB) between ATRX-mt and ATRX-wt patients in the three ICIs-treated cohorts.

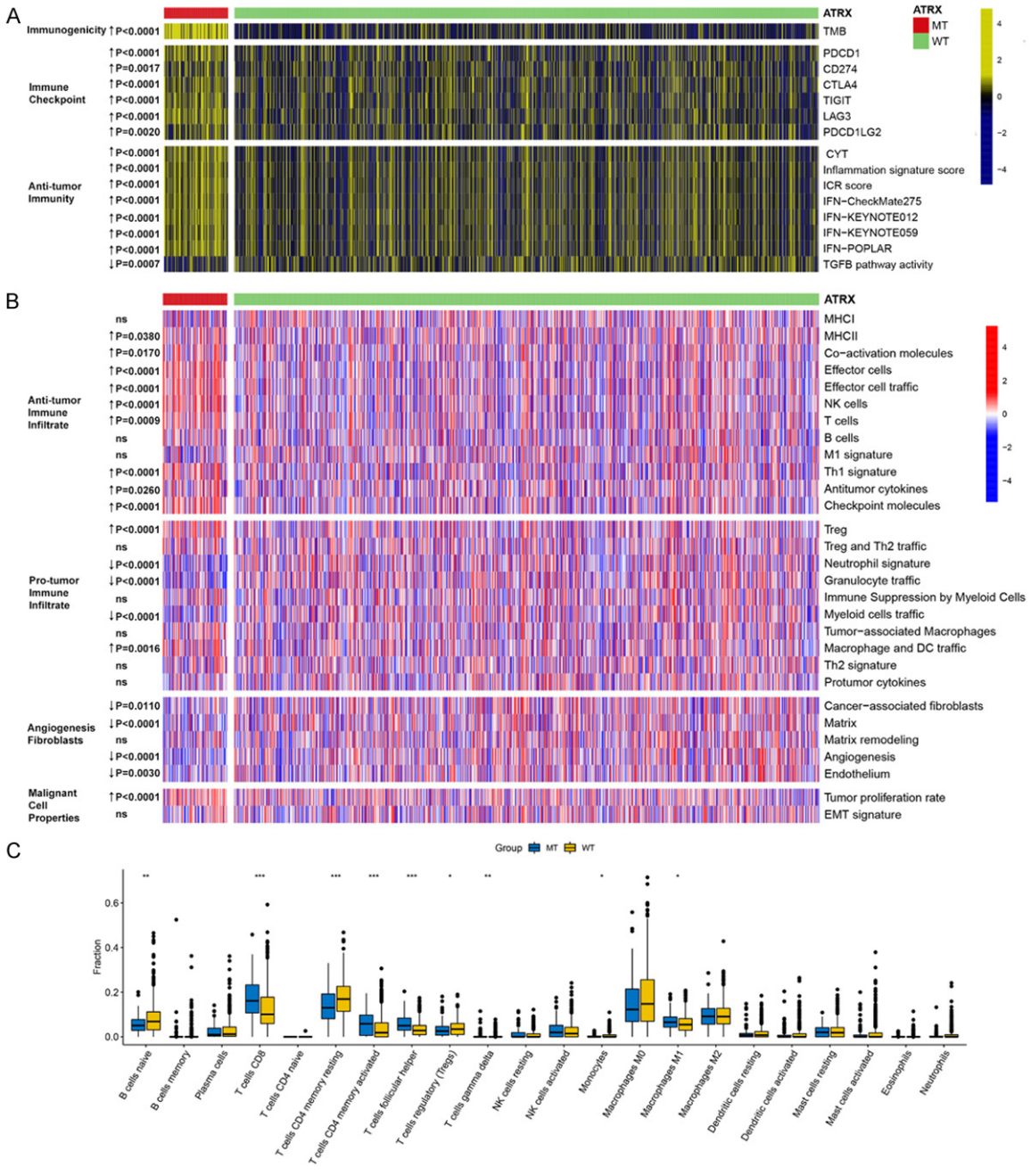


Figure 3. Association of ATRX mutations with anti-tumor immunity and tumor microenvironment (TME). A. Heatmap depicting several anti-tumor immunity indicators including TMB, immune checkpoint genes expression, cytolytic activity (CYT), inflammation signature score, immunologic constant of rejection (ICR) score, IFN-γ signaling score and TGF-β pathway activity. The score of these anti-tumor immunity indicators has been log2 transformed. B. Heatmap showing the enrichment score of 29 functional gene expression signatures (Fegs). The 29 Fegs were used to clas-

sify the TME into four subtypes: anti-tumor microenvironment, pro-tumor microenvironment, angiogenesis fibrosis and malignant cell properties. The up and down arrows on the left side of heatmap represented up-regulation and down-regulation in ATRX-mt patients, respectively. The number on the left side of indicated the *P* value (ATRX-mt vs ATRX-wt). C. Boxplot comparing the infiltration of 22 immune cells between ATRX-mt and ATRX-wt patients. CIBERSORT was used to assess the abundance of these immune cells. ***P < 0.001, **P < 0.01, *P < 0.05.

and monocytes were significantly decreased (**Figure 3C**).

ATRX-mt patients showed immune activation features in both MSI-H and non-MSI-H populations

In order to examine whether the immune activation features in ATRX-mt patients depended on MSI-H status, we divided the 1,239 MSI-prone tumors in TCGA cohort into four subgroups including MSI-H patients with ATRX mutations (ATRX-mt/MSI-H, G1, N = 61), MSI-H patients lacking ATRX mutations (ATRX-wt/MSI-H, G2, N = 234), Non-MSI-H patients with ATRX mutations (ATRX-mt/Non-MSI-H, G3, N = 62) and Non-MSI-H patients lacking ATRX mutations (ATRX-wt/Non-MSI-H, G4, N = 882) (**Figure 4A**).

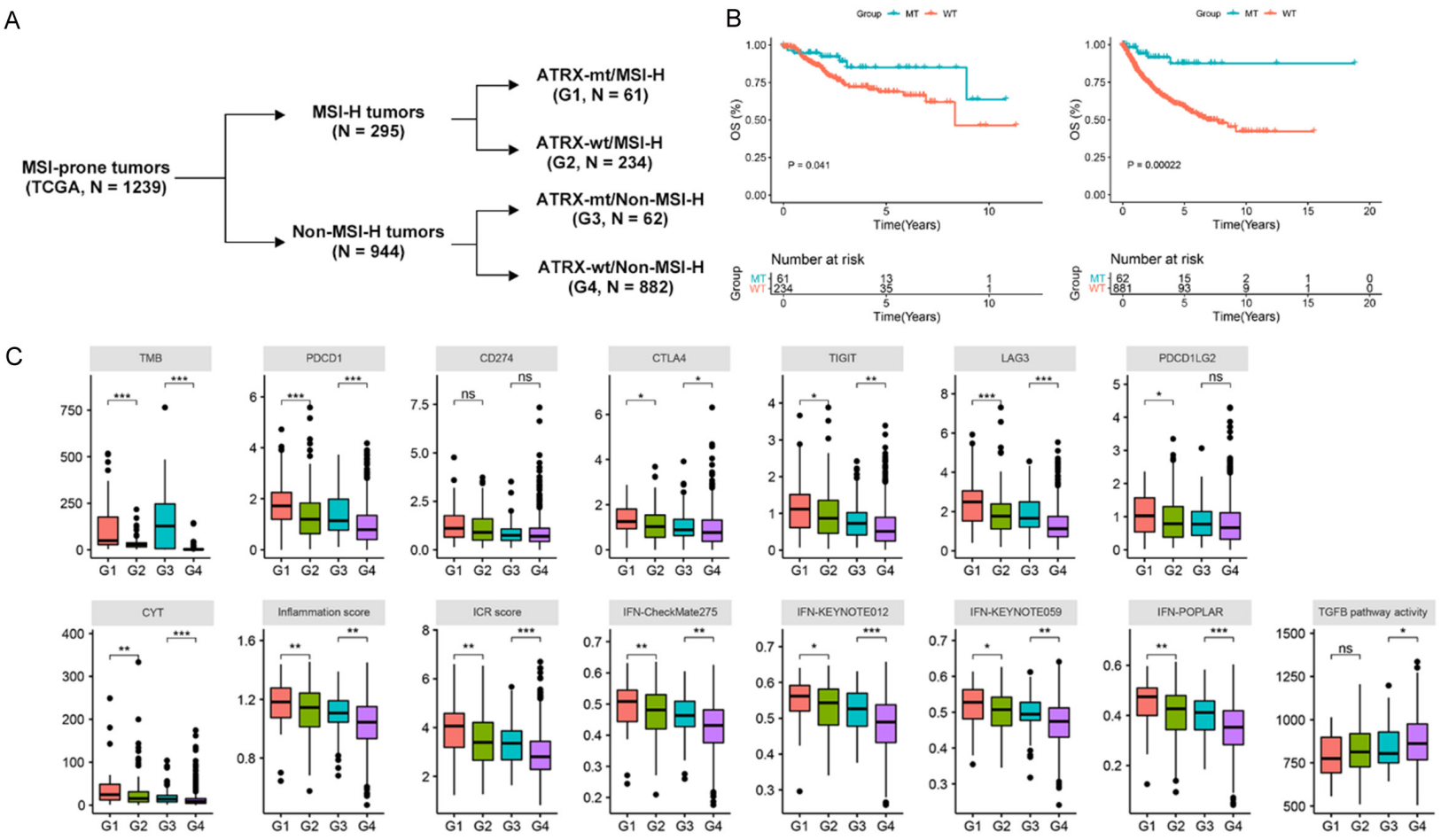
The MSI-stratified Kaplan-Meier analysis suggested that ATRX mutations were correlated with better OS in both MSI-H and non-MSI-H patients (**Figure 4B**). When comparing anti-tumor immunity and TME traits among the four groups, we discovered that both G1 (ATRX-mt/MSI-H) and G3 (ATRX-mt/Non-MSI-H) patients showed immunologically active features (**Figure 4C** and **4D**). Analyses of immune cell abundance further revealed the enrichment of anti-tumor lymphocytes and decreased fraction of pro-tumor cells in ATRX-mt patients (G1 and G3 patients) (**Figure 4E**). In particular, ATRX-mt/MSI-H patients, (G1) patients showed the highest level of immune-activated characteristics among four groups. There might be a synergistic effect between ATRX mutations and MSI-H status on immune activation.

Gene set enrichment analysis

The GSEA analyses suggested that immune-active pathways, including antigen processing and presentation, natural killer cell mediated cytotoxicity, RIG I like receptor signaling pathway and T cell receptor signaling pathway, were up-regulated in ATRX-mt patients (**Figure 5A**). Gene set variation analysis (GSVA) based on KEGG and tumor hallmark dataset also showed the up-regulation of immune-active pathways and down-regulation of hallmarks associated with immunosuppression (angiogenesis, hypoxia and TGF BETA signaling) (**Figure 5B**).

Drug sensitivity analysis

We performed subclass mapping analysis to investigate the difference in the likelihood of response to ICIs between ATRX-mt and ATRX-wt patients by using ICIs-treated 47 melanoma patients [6]. ATRX-mt patients showed highly similar immune profiles to the melanoma patients who respond to anti-PD1 blockade (Bonferroni-corrected P = 0.008) (**Figure 6A**). Given the important role of ATRX in HR, we then examined the effect of ATRX mutations on the expression of ATRX in the TCGA cohort. We found that ATRX-mt patients had lower gene expression levels than ATRX-wt patients (**Figure 6B**). Han et al. have reported that loss of ATRX increased the sensitivity to temozolomide, the major chemotherapeutic agent used for glioblastoma treatment [37]. We thus compared the estimated IC_{50} levels of twelve common chemotherapy drugs available from GDSC by ridge regression and 10-fold cross-validation. As displayed in **Figure 6C**, ATRX-mt patients had lower IC_{50} levels, namely being more sensitive to these twelve chemotherapy drugs. With the better response to both chemotherapy drugs and ICIs in ATRX-mt patients, we further evaluated the clinical benefits from ATRX mutations in the Janjigian cohort which contained 40 patients who received chemotherapy followed by ICIs [38]. ATRX-mt patients had higher TMB than ATRX-wt patients (Figure S2A). Among the three patients with ATRX mutations, two achieved complete response (CR) to ICIs, and the proportion of ORR in ATRX-mt patients was higher than ATRX-wt patients (ATRX-mt, 66.7% vs ATRX-wt, 21.1%). Furthermore, we also found longer overall survival in ATRX-mt patients after combination treatment (Figure S2B). Taken together, ATRX-mt patients might be more sensitive to both chemotherapy and anti-PD1 immunotherapy. The combination use of chemotherapy and ICIs might be a worthwhile option for patients carrying ATRX mutations.


A
MSI-prone tumors
(TCGA, N = 1239)
MSI-H tumors
(N = 295)
Non-MSI-H tumors
(N = 944)
ATRX-mt/MSI-H
(G1, N = 61)
ATRX-wt/MSI-H
(G2, N = 234)
ATRX-mt/Non-MSI-H
(G3, N = 62)
ATRX-wt/Non-MSI-H
(G4, N = 882)
B
Group MT WT
OS (%)
P = 0.041
P = 0.00022
Time(Years)
Number at risk
MT 61 13 1
WT 234 35 1
MT 62 15 2 1 0
WT 881 93 9 1 0
C
TMB
PDCD1
CD274
CTLA4
TIGIT
LAG3
PDCD1LG2
CYT
Inflammation score
ICR score
IFN-CheckMate275
IFN-KEYNOTE012
IFN-KEYNOTE059
IFN-POPLAR
TGFB pathway activity
G1 G2 G3 G4

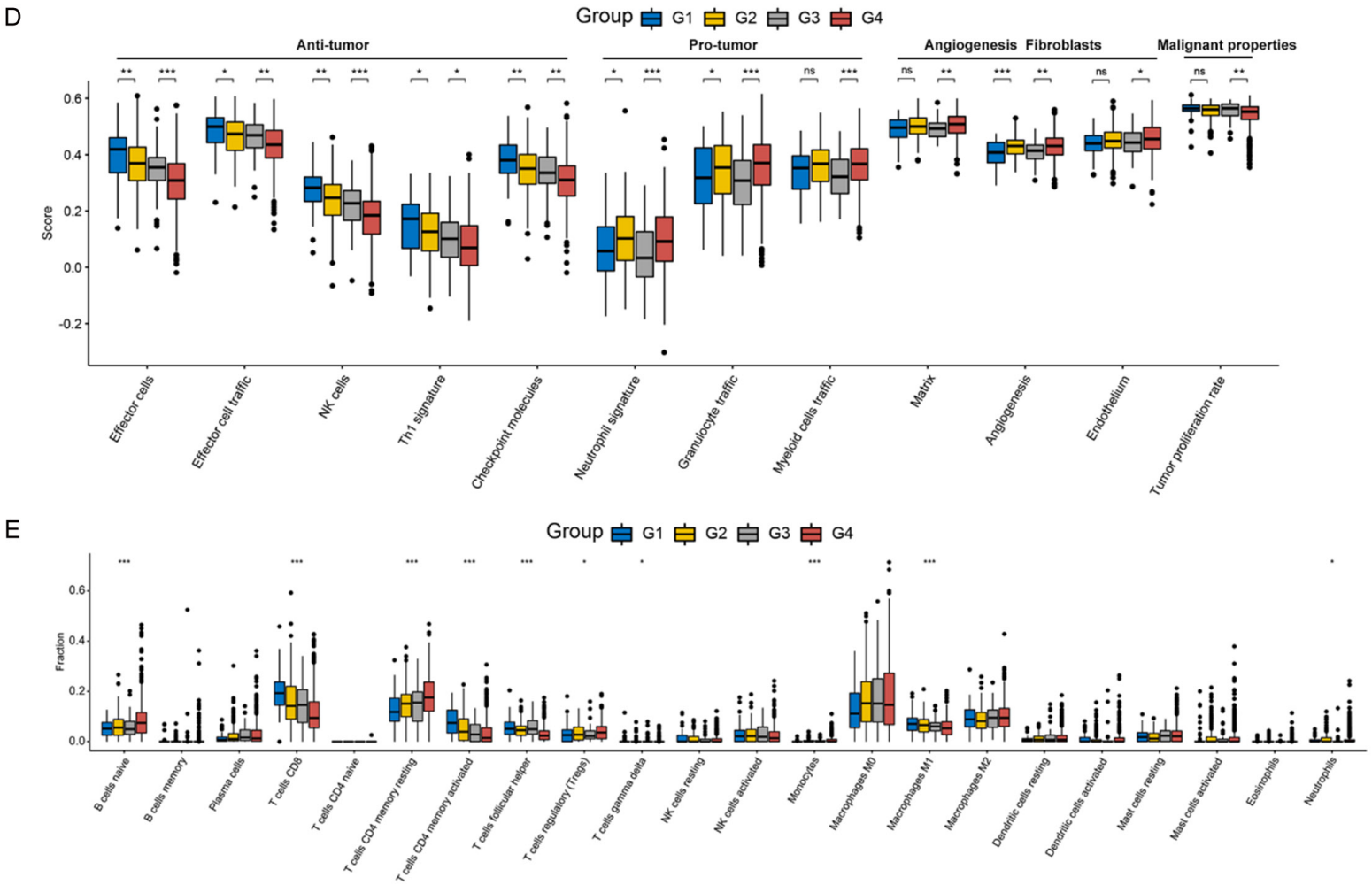


Figure 4. The MSI status stratified analyses of anti-tumor immunity and tumor microenvironment features between ATRX-mt and ATRX-wt patients. (A) The framework of the MSI status stratified analyses. The patients were divided into four groups including ATRX-mt/MSI-H patients (G1, N = 61), ATRX-wt/MSI-H patients (G2, N = 234), ATRX-mt/Non-MSI-H (G3, N = 62) and ATRX-wt/Non-MSI-H patients (G4, N = 882). (B) Kaplan-Meier analysis comparing the overall survival between G1 and G2 groups and G3 and G4 groups. (C, D) Boxplots showing differences in anti-tumor immunity (C) and tumor microenvironment (D) indicators score across four groups. (E) The proportions differences of 22 immune cells between four groups. ***P < 0.001, **P < 0.01, *P < 0.05.

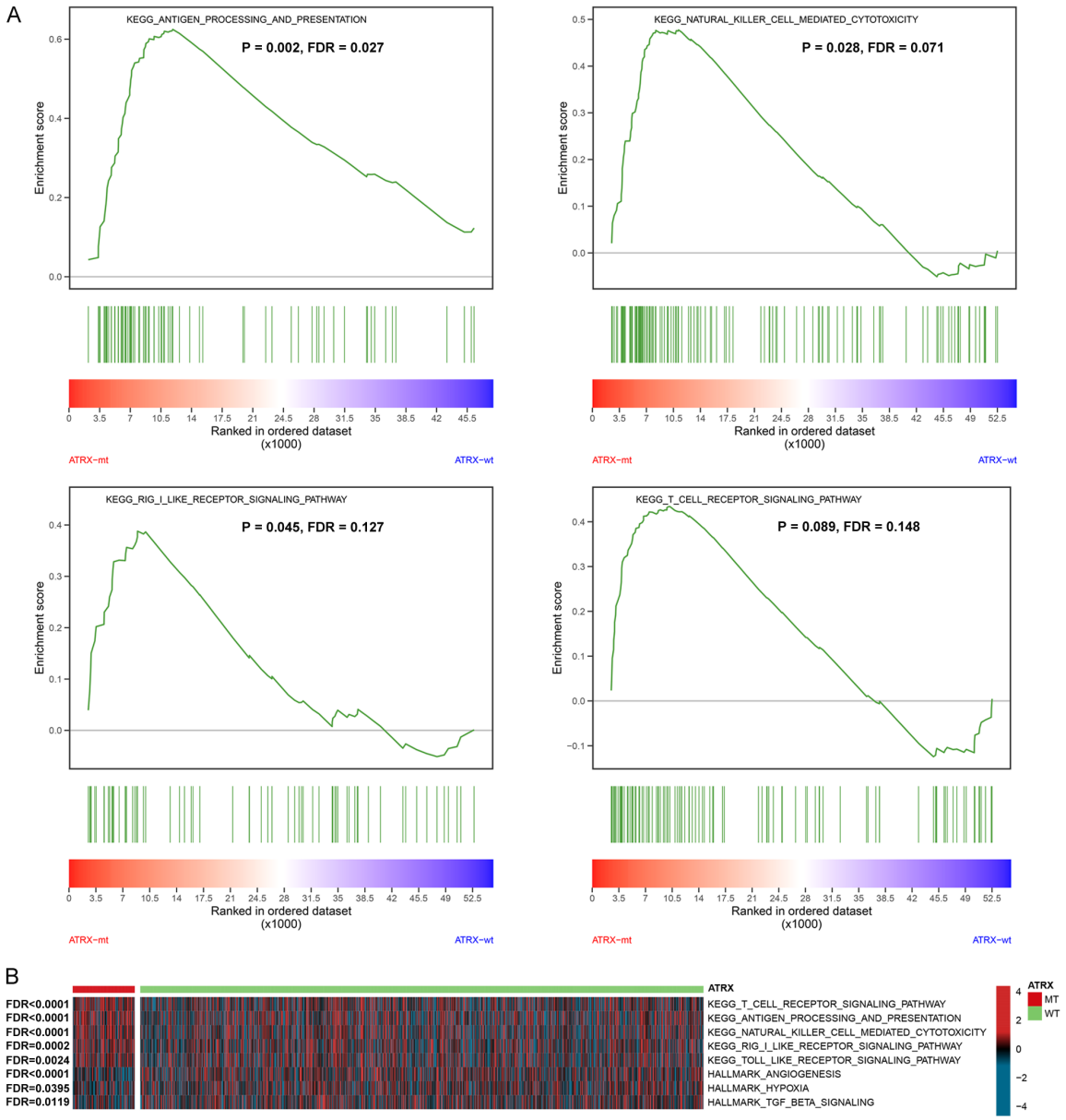


Figure 5. Gene set enrichment analysis (GSEA). A. GSEA plot of KEGG pathways enriched in ATRX-mt patients including antigen processing and presentation, natural killer cell mediated cytotoxicity, RIG I like receptor signaling pathway and T cell receptor signaling pathway. Nominal *P*-values and the false-discovery rate (FDR) are indicated. B. Heatmap of the immune-related KEGG pathways and tumor hallmarks quantified by gene set variation analysis (GSVA). The method of limma was used to make the differential analysis. The cutoff value of FDR < 0.05 was considered to be significant.

Discussion

In the present work, we analyzed the mutational spectrum of HR related genes in MSI-prone tumors and found the association of ATRX mutations with immunologically active characteristics that were independent of MSI-H.

ATRX is a tumor suppressor gene which encodes the SWI/SNF-like chromatin remodeling protein. It has been recently reported that SWI/SNF gene mutations contributed to ICIs efficacy in several types of cancer. PBRM1 mutations were related with a higher ORR and longer survival in a clinical trial of renal cell carcinoma patients [39]. Patients with ARID1A, ARID1B, and ARID2 mutations were more likely to benefit from ICIs therapy in non-small cell lung cancer [40]. The mechanism of SWI/SNF gene mutations in improving ICIs efficacy is

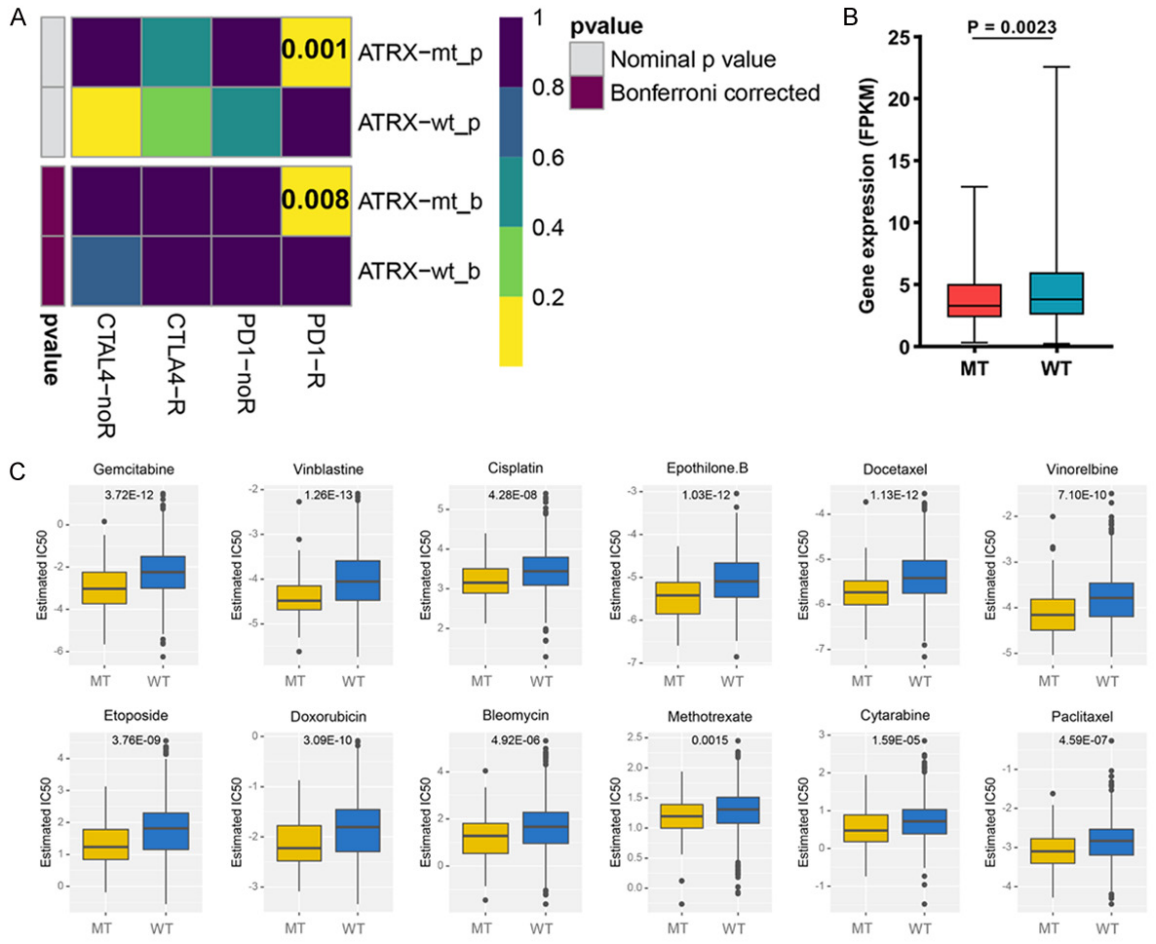


Figure 6. Analysis of drug sensitivity prediction. A. Subclass analysis revealed that ATRX-mt patients could be more sensitive to the anti-PD-L1 and anti-PD1 agents (Bonferroni-corrected P = 0.008). B. Comparison of ATRX expression between ATRX-mt and ATRX-wt patients in TCGA MSI-prone tumors cohort. C. Boxplots showing the estimated IC_{50} level of twelve common chemotherapy drugs between ATRX-mt and ATRX-wt patients. The IC_{50} level represent the drug sensitivity, and the lower value implies that the more sensitive the patient would be to treatment.

complex. For example, loss of ARID2 or BRD7 increased tumor cell sensitivity to IFN-γ and thus enhanced chemokines production that engaged effector T cells [41]. ARID1A interacted with MSH2 to promote MMR during DNA replication. ARID1A inactivation compromised MMR, resulting in increased TMB, elevated cytotoxic T cell infiltration and PD-L1 expression [42]. As a SWI/SNF family gene, ATRX plays critical roles in maintaining DDR including homologous recombination and non-homologous end joining activity [43, 44]. Besides, ATRX can maintain genetic stability and facilitate appropriate DNA replication [45, 46]. A recent study has addressed that ATRX deficiency increased the sensitiveness of non-small cell lung cancer to ICIs [47]. The lung cancer mouse model with ATRX deficiency exhibited significantly shrunken tumor volume, longer survival and enhanced infiltration of T cells compared with the control group after anti-PD1 and anti-CLTA4 intervention. However, few studies have explored the association of ATRX mutations with ICIs efficacy in MSI-prone tumors. In this work, we found the significant correlation of ATRX mutations with enhanced antitumor immunity and inflamed TME. Remarkably, we noticed the synergistic effect of ATRX mutations and MSI-H on immune activation. The augment of immune activation in patients carrying both ATRX mutations and MSI-H status might result from the synergistic effect of co-mutations in two DDR pathways (HR and MMR). Similar synergistic effects of gene deficiencies in two DDR pathways (HR and MMR or HR and BER) was also reported in previous studies,

better predicting ICIs efficacy than do a single mutated DDR pathway [48]. Therefore, the combined use of ATRX mutations and MSI-H status might help to better identify the good responders to ICIs in MSI-prone tumors.

The increased sensitivity to common chemotherapy drugs was another hallmark of ATRX-mt patients. The accumulating preclinical literature has demonstrated that cytotoxic chemotherapy could aid immunotherapy by robust immune stimulation, enhancement of tumor-specific antigens presentation and inducing expression of PD-L1 [49, 50]. Plenty of clinical evidence has also confirmed the efficacy of combination use of ICIs with standard-of-care chemotherapies [51]. We thus tested our hypothesis in metastatic esophagogastric cancer patients receiving combination use of ICIs and chemotherapy. This implicated a possibility that ATRX-mt patients may benefit from the combination of chemotherapy and ICIs. Nevertheless, it requires further validation by more experimental and clinical studies.

There were several limitations in this retrospective study. First of all, due to the datasets availability, we only investigated the predictive value of ATRX mutations in one ICIs-treated MSI-prone tumors cohort. Although we also found the association between ATRX mutations and favorable clinical outcomes in the ICIs-treated cohorts across various cancer types, these results might over exaggerate the conclusion drawn for patients with MSI-prone tumors. Larger clinical cohorts of ICIs treatment especially containing more endometrial cancer patients are warranted to validate our findings herein. Secondly, the analyses of influence of ATRX mutations on immunologically active features were based on the RNA-seq data from TCGA. The ICIs-treated clinical trials with gene expression data are required to expand our findings. Finally, we only observed the association between ATRX mutations with the factors that improved ICIs efficacy. The underlying molecular mechanism by which ATRX mutations can induce immunologically active characteristics and the synergistic effects of ATRX mutations and MSI-H still needs further experimental exploration.

Conclusion

Our study has identified the specific HR-related gene mutations, ATRX mutations, that are correlated with survival benefits from ICIs in MSI-prone tumors. The ATRX mutant patients showed an immunologically active phenotype including enhanced anti-tumor immunity, inflamed TME characteristics and immune cell infiltration. The synergistic effect between ATRX mutations and MSI-H status on immune activation implied a possible combination use of these two biomarkers for guiding ICIs selection in MSI-prone tumors.

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Disclosure of conflict of interest

None.

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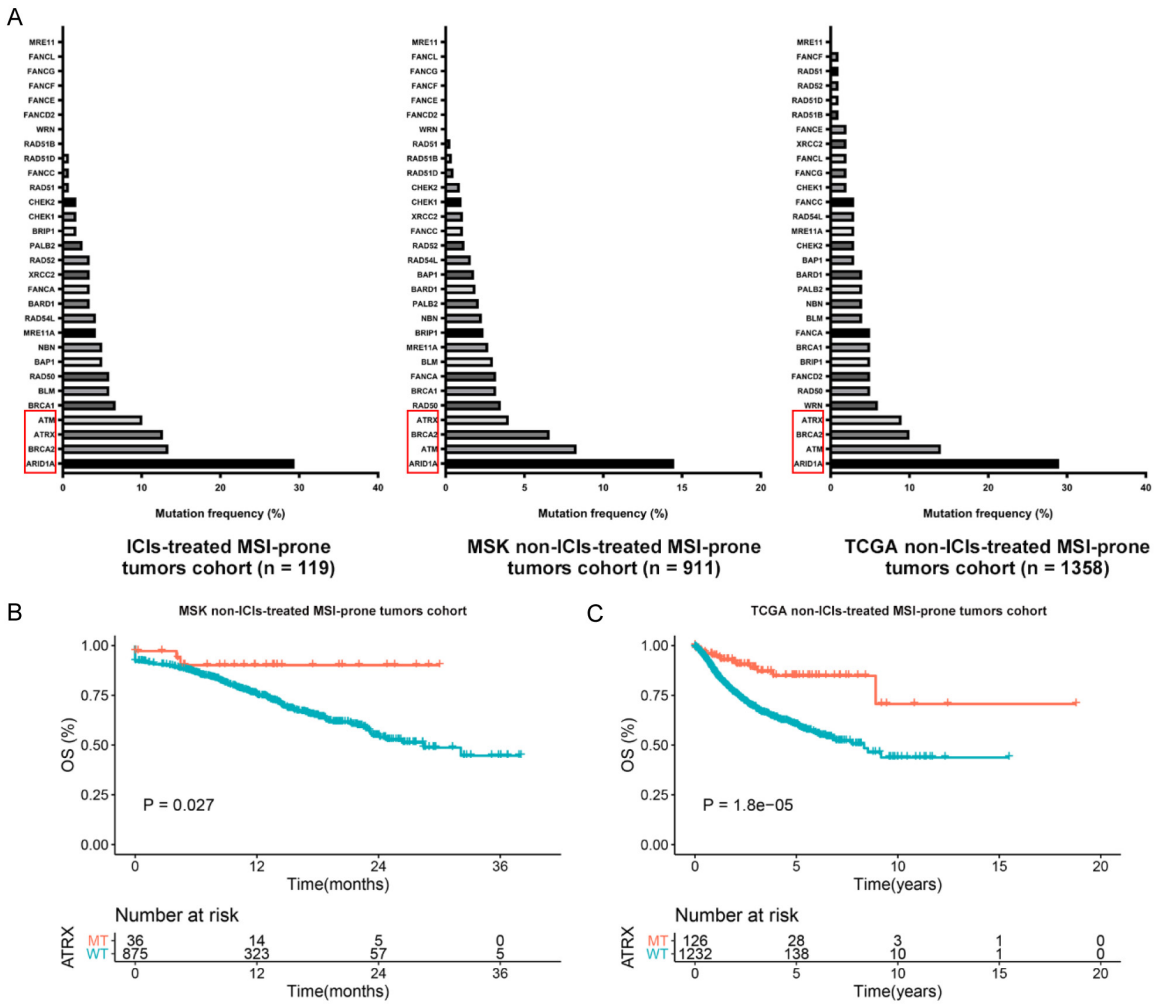

Figure S1. (A) The mutation frequency of 30 HR related genes in three MSI-prone tumors cohorts. (B, C) Kaplan-Meier analysis comparing overall survival between ATRX-mt and ATRX-wt patients in two non-ICIs-treated MSI-prone tumors cohorts (MSK cohort, B; TCGA cohort, C).

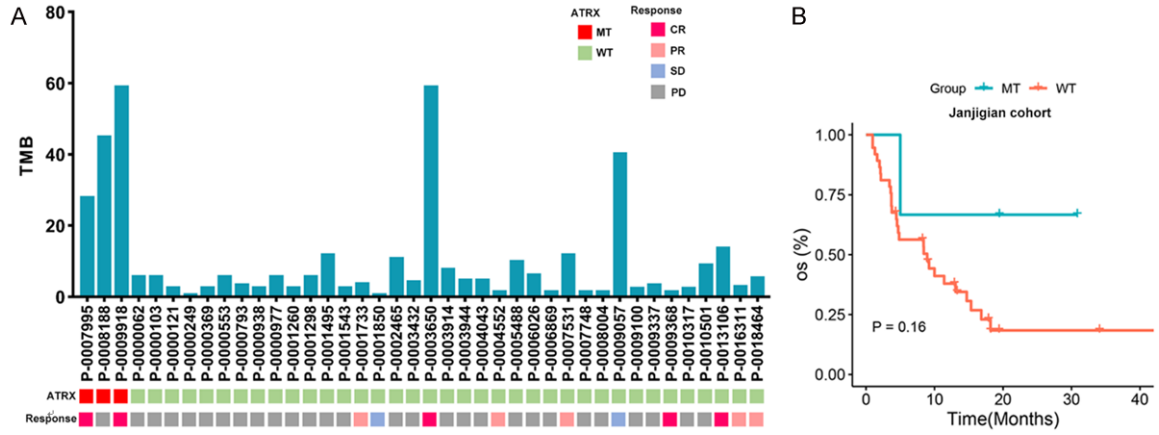

Figure S2. The association between ATRX mutations and clinical benefits in Janjigian cohort. A. Histogram of TMB in 40 patients with metastatic esophagogastric cancer who have received chemotherapy followed by ICIs. The annotation tracks below x-axis indicated ATRX mutations and ICIs response. B. Kaplan-Meier curves of overall survival for ATRX-mt and ATRX-wt patients in Janjigian cohort.