



# *DNAH7* mutations benefit colorectal cancer patients receiving immune checkpoint inhibitors

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**Background:** Colorectal cancer (CRC) is a malignant tumor associated with a high mortality rate. While the advent of immune checkpoint inhibitors (ICIs) has been a gamechanger, only a small percentage of CRC patients benefit from ICIs. The pathological mechanism of CRC is not well understood, but somatic mutations, especially missense mutations, are believed to play an important role. This study examined the relationship between ICIs in colorectal cancer and missense mutations in the axonemal dynein heavy chain gene 7 (*DNAH7*).

**Methods:** A clinical cohort (n=690) and the CRC data from the publicly available Cancer Genome Atlas (TCGA) were examined. Gene Set Enrichment Analysis, ESTIMATE analysis, and clinical correlation analysis were performed to explore the effects and mechanisms of *DNAH7* mutation on immunotherapy in colorectal cancer.

**Results:** The results showed that CRC patients with *DNAH7* mutations can benefit more from ICIs ( $P<0.05$ ). Patients with *DNAH7* mutation had higher ESTIMATE scores, immune scores, and matrix scores, compared to patients without the *DNAH7* mutation ( $P<0.001$ ). The transport of small molecules, keratinization, asthma, autoimmune thyroid disease, allograft rejection, and other pathways were significantly enriched in *DNAH7* mutated tissues ( $P<0.05$ ). The top key genes associated with the *DNAH7* mutation included AQP8, MS4A12, GUCA2B, and ZG16 ( $P<0.01$ ).

**Conclusions:** The current study not only demonstrated the significance of *DNAH7* as a risk factor and prognostic feature in CRC, but also revealed that *DNAH7* mutations might affect the clinical efficacy of ICIs by impacting the tumor immune microenvironment.

**Keywords:** Colorectal cancer (CRC); *DNAH7*; mutation; immune checkpoint inhibitors (ICIs); tumor

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## Introduction

Colorectal cancer (CRC) is a prevalent malignancy with nearly 1.4 million new cases each year globally (1). Despite medical advances, the efficacy of diagnosis and treatments

remains unsatisfactory (2). In recent years, immunotherapy has been widely used with some success. While immune checkpoint inhibitors (ICIs) are effective in defective mismatch repair (MMR) or highly microsatellite unstable

(MSI-H) metastatic CRC, it has not produced good results in patients with good MMR or microsatellite stable (MSS) tumors (3). The progressive accumulation of somatic mutations within cells can lead to the occurrence of CRC (4). Hence, it is important to determine the key somatic mutations of CRC, which may be important markers for the prognosis and prediction of CRC, or may be novel therapeutic targets. Specific mechanisms of DNA repair include polymerase proofreading, base excision repair, and MMR (5).

Axonemal dynein heavy chain 7 (*DNAH7*) is an inner arm component of human cilia, which is induced during ciliated cell differentiation. There are two kinds of cilia, one is called motile cilia and the other is called primary cilia. Motile cilia are responsible for cleaning, and their absence causes inflammation. As sensing organelles, primary cilia sense mechanical stress, optical, chemical and other signals from the extracellular environment. The abnormal expression of *DNAH7* can cause the loss of cilia, and then become a pathogenic factor of tumors. The down-regulation of primary cilia expression is related to the occurrence of colorectal cancer, ovarian cancer, and kidney cancer. *BRVF V600E* is a gene closely related to the occurrence and development of CRC, which gene sequence has significant sequence homology with the region of a variety of axonal dynein heavy chain proteins, such as *DNAH7*. Therefore, *DNAH7* may be closely related

to the occurrence, development and prognosis of CRC. But the role of *DNAH7* in CRC has not been thoroughly investigated. Therefore, we tried to research the role of *DNAH7* in CRC.

This investigation explored the relationship between *DNAH7* gene mutations and prognosis in CRC patients. Analysis of data from The Cancer Genome Atlas (TCGA) revealed that missense mutations were a major component of the overall mutations in CRC. Indeed, missense mutations were the main form of mutation observed in *DNAH7*. Patients were divided into two groups, one group with *DNAH7* mutations and the other group with no *DNAH7* mutations. The results showed that *DNAH7* mutation is a risk factor for CRCs, and the mutation load and immunogenicity were higher in such patients. Moreover, functional enrichment analysis revealed the distinct pathway between CRC patients with *DNAH7* mutations and those with unmutated *DNAH7*. Furthermore, CRC patients with the *DNAH7* mutation benefited more from ICI therapy compared with patients who did not have the *DNAH7* mutation. We present the following article in accordance with the TRIPOD reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-6166/rc>).

## Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

## Data download

The rectal adenocarcinoma (READ) and colonic adenocarcinoma (COAD) datasets, including 690 colorectal cancer (CRC) samples, and all corresponding clinical data, were obtained from The Cancer Genome Atlas (TCGA) database (<http://gdc-portal.nci.nih.gov/>). There was no record of survival time for 63 samples, 3 samples had no record of survival status, and 3 samples had no record of gender, and these samples were excluded. Finally, 582 CRC samples and 44 normal control samples were included for analysis. The data of “Masked Mutation” was used as the data of Somatic Mutation of CRCs. VarScan software (6) was used to preprocess the data. The somatic mutation data was visualized by R maftools package (7). The gene expression data (fragments per kilobase per million (FPKM) value) of the patient’s RNA sequencing were downloaded and converted into transcript per million (TPM) values.

## Highlight box

### Key findings

- This study demonstrated the significance of *DNAH7* as a risk factor and a prognostic feature in CRC, and that *DNAH7* affects the clinical efficacy of ICIs by affecting the tumor immune microenvironment.

### What is known and what is new?

- CRC is a malignant tumor with a high mortality rate. The advent of ICIs has brought satisfactory results in CRC treatment. However, only a small percentage of CRC patients benefit from ICIs.
- This study demonstrated the connection between ICIs and *DNAH7* mutations in CRC.

### What is the implication, and what should change now?

- This study revealed that *DNAH7* may affect the clinical efficacy of ICIs by affecting the tumor immune microenvironment, and *DNAH7* mutation may be used as a prognostic indicator and potential target for CRC treatment in clinical practice.

### ***Copy number alteration (CNV) analysis***

The Masked Copy Number Segment data of patients were downloaded and the R TCGAAbiolinks package was used to analyze the copy number alteration in the *DNAH7* gene in CRC samples (8). GenePattern5, GISTIC2.0 analysis was performed on the downloaded CNV segments (9). The default settings were used for the GISTIC2.0 analysis excepted for a few parameters (e.g., confidence level of 0.99; X chromosomes not excluded before analysis). The R package maftools were used to visualize the results of the GISTIC2.0 analysis.

### ***Calculation and correlation analysis of somatic mutation and tumor mutation burden (TMB) score***

For each tumor sample, we detected the total number of somatic mutations in the tumor and translated it into the TMB of the sample. The TMB was defined as the number of coded, somatic, base replacement, and insert-deletion mutations per megabyte in the genome used non-synonymous and code transfer insertion deletion less than the 5% detection, obtained from the TCGA dataset. The TMB value was calculated for each sample and the Wilcoxon rank-sum test was used to compare the TMB between the *DNAH7* mutated patients and *DNAH7* unmutated patients.

### ***Differentially expressed genes (DEGs) and clinical correlation analysis***

To analyze the influence of *DNAH7* mutation on the occurrence and development of CRC, samples were divided into a mutated group and an unmutated group according to the status of *DNAH7* mutation. The overall differences between the two groups of samples were analyzed using principal component analysis (PCA) (10). The R DESeq2 package (11) was used to analyze the DEGs between the two groups, with log fold change (logFC) >1.0 and P<0.05 as the threshold. The results of the differential analysis are displayed in heatmaps and volcano plots.

### ***Functional enrichment analysis and gene set enrichment analysis (GSEA)***

Gene Ontology (GO) analysis, including molecular function, biological processes, and cellular components, was conducted for large-scale functional enrichment studies.

Kyoto Encyclopedia of Genes and Genomes (KEGG) is a widely used database that stores information about biological pathways, genomes, diseases, and drugs. The ClusterProfiler R software package (12) was used to perform GO annotation analysis and KEGG pathway enrichment analysis of signature genes. A false discovery rate (FDR) <0.05 was considered statistically significant.

GSEA (13) was performed to distinguish the biological processes among different groups of CRC samples, and to explore whether a specific gene set was statistically different between two biological states (14). The MSIGDB database (<https://www.gsea-msigdb.org/gsea/msigdb/>) was accessed to download the “C2.cp.kegg.v6.2.-Symbols” gene set for GSEA, and an adjusted P value <0.05 was considered statistically significant.

### ***Comparison of immune cell infiltration levels and immune correlation score between two groups***

Based on gene expression profiles, ESTIMATE analysis was conducted to quantify immunocompetence (level of immune invasion) of the tumor sample. The immunoactivity and stroma score of each tumor was assessed using the R ESTIMATE package (15). The Mann-Whitney U test was used to compare the level of immune cell infiltration between the two groups of samples.

### ***Screening of hub genes and construction of the protein-protein interaction (PPI) network***

The PPI network of selected genes was constructed using the STRING online tool (16) and Cytoscape (V3.7.2) was used to visualize the network model (17). The genes with credibility score greater than 0.4 were selected. The Maximal Clique Centrality (MCC) algorithm was used to determine the nodes in the set in the common expression network, which is the most effective method (18). The Cytoscape cytoHubba plug-in was used to calculate the MCC of each node (18). The genes with the first 8 MCC values were selected as hub genes.

### ***DNA damage related signaling pathway analysis***

We evaluated and compared differences in enrichment fractions in DNA damage repair related pathways between *DNAH7* mutated and unmutated groups in the CRC cohort. Among them, the gene set related to DNA damage repair pathway was obtained from the Broad Institute's

Molecular Signatures Database (MSigDB) (14).

### ***Clinical prediction model validation of the DNAH7 gene mutation***

Univariate and multivariate Cox analysis was used to examine the independent predictive ability of risk scores for overall survival (OS) in patients with the *DNAH7* gene mutation combined with clinicopathological features. Subsequently, we constructed survival predictions in patients in the TCGA dataset using nomograms. The Harrell's Consistency index (C-index) was measured to quantify differentiated performance. By comparing the predicted and observed actual survival rates of the nomogram, a calibration curve was obtained.

### ***Statistical analysis***

R software (version 4.0.2) was used for all statistical data analysis. The Mann-Whitney U test was used to compare continuous variables between two groups. The Wilcoxon rank-sum test was used to analysis differences in the non-normally distributed variables, and the independent Student *t*-test was used to analysis normally distributed variables. The Chi-square test or Fisher's exact test was applied to compare and estimate the statistical significance between the two groups of categorical variables. Pearson correlation analysis was used to calculate the correlation coefficients of different genes. Survival analysis was performed using the R survival package, and survival differences were analyzed using the Kaplan-Meier survival curve. Differences in survival time between the two groups were evaluated using the log-rank test. Univariate and multivariate Cox analyses were used to determine independent prognostic factors. The receiver operating characteristic (ROC) curve was plotted using the R pROC package (19) and the area under the ROC curve (AUC) was calculated to assess the accuracy of the risk score in estimating prognosis. A P-value <0.05 was defined as statistically significant and all statistical P values were bilateral.

## **Results**

### ***Analysis of the overall degree of mutation in CRC patients***

The CRC mutation data were downloaded from TCGA database to explore the frequency of the *DNAH7* gene

mutation. CRC mutations were predominantly missense mutations involving single nucleotide variants (SNVs), with C>T being the most common (*Figure 1A,1B*). Patients in the dataset were subsequently divided into a *DNAH7* mutated group (*Figure 1C*) and a *DNAH7* unmutated group (*Figure 1D*). The waterfall plots show the mutation level of related genes. Analysis of amino acid changes in the *DNAH7* protein showed that the main mutated forms were missense mutation forms (*Figure 1E*).

### ***DNAH7 gene copy number alteration (CNV)***

*Figure 2A* shows the CNV heat map of the *DNAH7* mutant gene, in which the red color indicates an increase in CNV.

*Figure 2B* showed the circos diagram of the *DNAH7* gene CNV of CRC. Visual analysis of the CNV results of GISTIC2.0 based on CRC queue (*Figure 2C,2D*) showed that CNV increased the most digits at 13P1.2 in the CRC *DNAH7* mutation group, while CNV decreased the most multiples at 16p13.3.

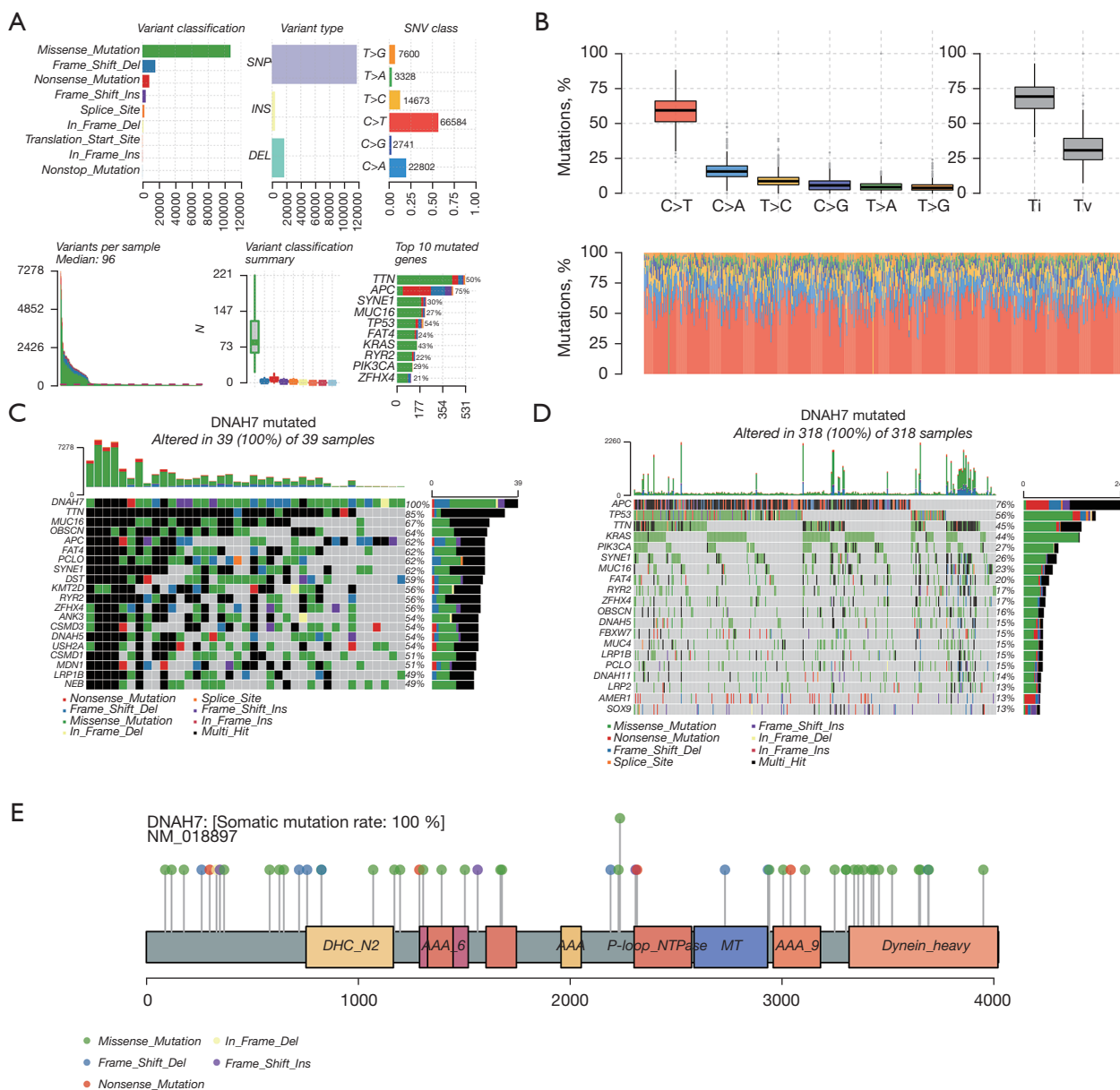
### ***Correlation parsing of biological features of DNAH7 gene mutation level***

The expression of the *DNAH7* gene was significantly different between the tumor group and the non-tumor group (P=0.003, *Figure 3A*). There was a significant increase in the TMB value (P<0.001, *Figure 3B*), and a non-significant difference in the MSI value (P=0.114, *Figure 3C*).

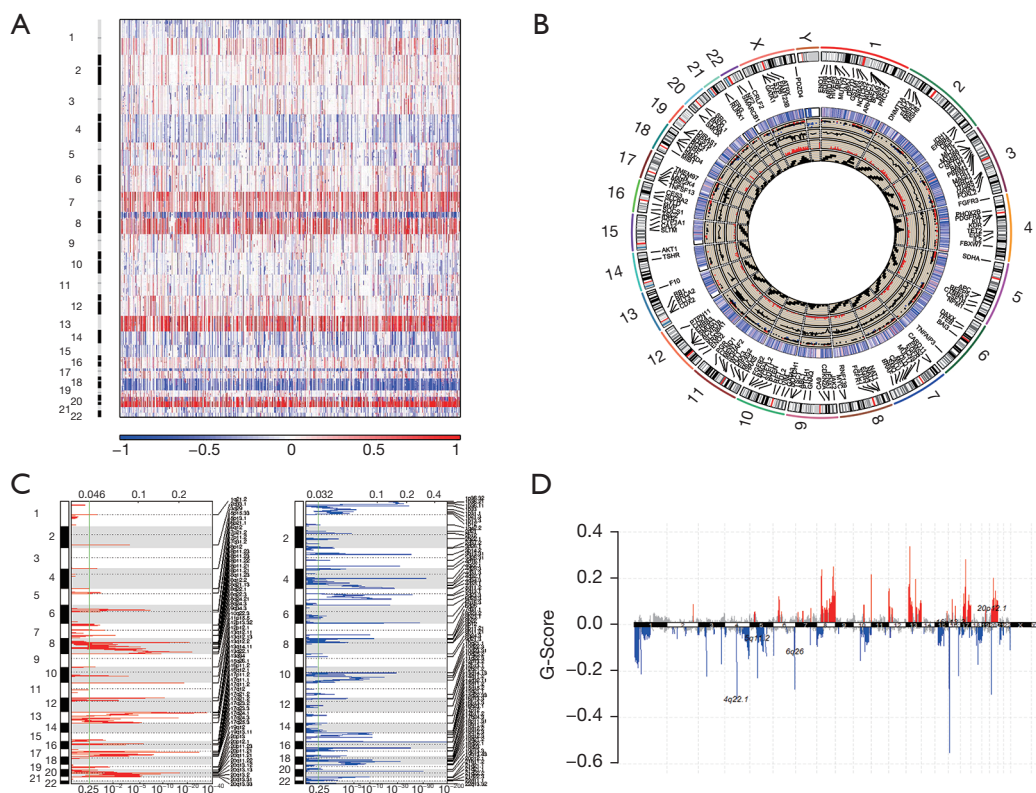
Subsequently, we explored the influence of *DNAH7* mutation on different biological features. The results demonstrated that the *DNAH7* mutated group was significantly different from the non-mutated group in signatures 1, 6, and 15 (*Figure 3D,3E*).

### ***Drug susceptibility analysis and pathway analysis of the DNAH7 gene mutation***

The influence of gene mutations on drug susceptibility was examined. Various drugs were relevant to *DNAH7* gene mutation levels, in particular, the Druggable Genome (*Figure 4A*). Pathway analysis revealed that *DNAH7* mutations were strongly associated with the RTK-RAS pathway and the NOTCH pathway in CRCs (*Figure 4B*), and related genes in the RTK-RAS pathway (*Figure 4C*) and NOTCH pathway (*Figure 4D*) had a high mutation rate in CRC samples.



**Figure 1** Mutation level and clinical correlation analysis of the *DNAH7* gene in CRC. (A) Statistical summarizing of mutation information in CRC. Mutation types are classified according to different categories, with missense mutation being the predominant mutation. SNPs appeared more frequently than INS or DEL. Specific samples showed the burden of tumor mutations and the top ten mutated genes. (B) The relative proportion of various SNV mutations in CRC, among which C>T was the most common. (C) Gene mutation distribution map of the *DNAH7* mutated group. (D) Gene mutation distribution map of the *DNAH7* unmutated group. (E) Variation distribution map of *DNAH7* protein amino acid in the CRC dataset. Ti, transitions; Tv, transversions; CRC, colorectal cancer; SNPs, single nucleotide polymorphisms; INS, insertions; DEL, deletions; SNV, single nucleotide variant.



**Figure 2** *DNAH7* gene copy number alteration in CRC. (A) A CNV heat map of the *DNAH7* mutant gene, where red represents increased CNV and blue represents decreased CNV. (B) Circos plots of CRC *DNAH7* mutated patients. GISTIC analysis of CRC *DNAH7* mutation patients, where red represents increased CNV and blue represents decreased CNV. (C) A visual analysis of the CNV results based on the CRC queue via GISTIC2.0 analysis of CRC patients with *DNAH7* mutations. (D) In CRC *DNAH7* mutation patients, CNV variation occurred in several genes, with red representing increased CNV and blue representing decreased CNV. CRC, colorectal cancer; CNV, copy number variation.

### Differential gene expression in patients with *DNAH7* mutations

To explore the influence of the *DNAH7* gene mutation on the occurrence and development of CRC, patients in the TCGA dataset were divided into a mutated group and an unmutated group based on *DNAH7* gene mutation. Differential expression gene analysis demonstrated that there were 1,764 significantly upregulation genes and 583 downregulation genes in the CRC datasets (Figure 5A, 5B).

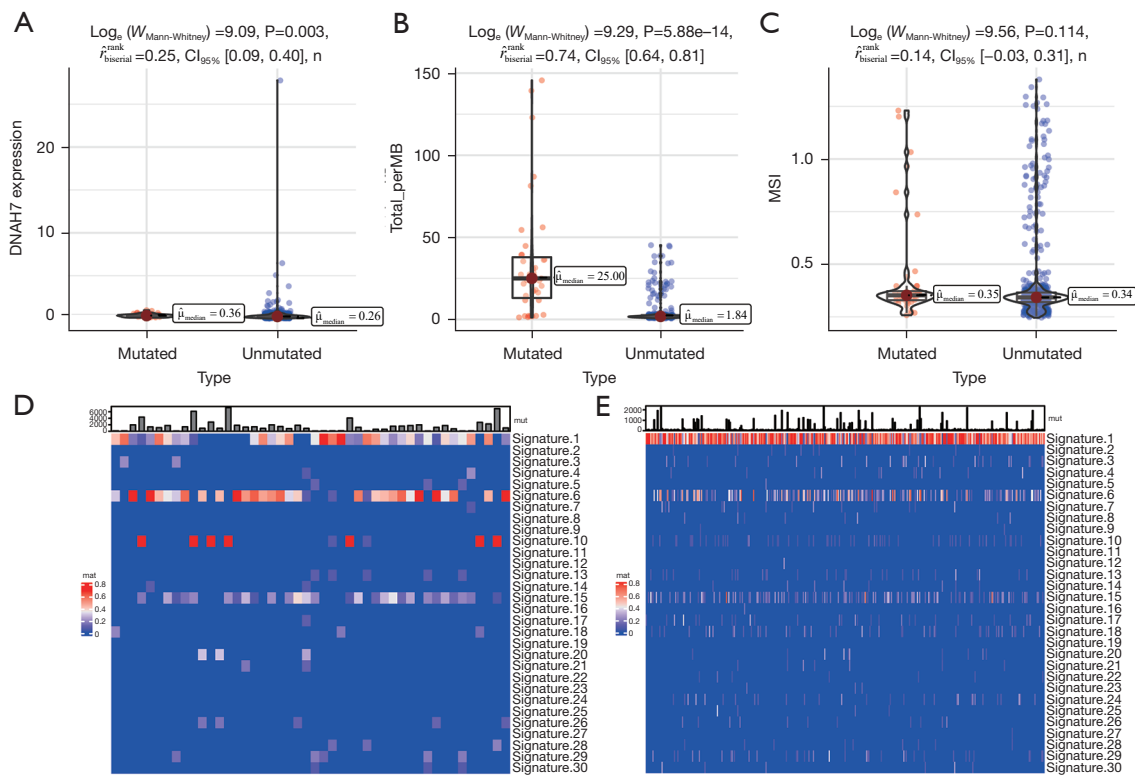
Functional enrichment analysis was performed and the GO results (Table 1) showed that the differentially expressed genes were strongly related to biological processes such as receptor ligand activity, apical part of the cell, and organic anion transport (Figure 5C, 5D). KEGG analysis (Table 2) suggested that differentially expressed genes mainly affected pathways such as bile secretion and neuroactive ligand-

receptor interaction (Figure 5E, 5F).

GSEA (Figure 6A-6F) revealed that pathways such as transport of small molecules, keratinization, asthma, autoimmune thyroid disease, allograft rejection, and others were significantly enriched in *DNAH7* mutant tissues (Table 3).

### Construction of the related regulatory network and the PPI network map

A PPI network of the DEGs was constructed using the STRING database and the interaction relationship between genes was imported into the Cytoscape software to obtain Figure 7A. The top ten genes from the PPI network were selected as hub genes via the Cytoscape's cytoHubba plug-in and the MCC algorithm (Figure 7B). Figure 7C shows the co-expression heat map of the top10 key genes and *DNAH7*. Figure 7D shows the molecular correlation



**Figure 3** Analysis of the biological features of *DNAH7* gene mutations in colorectal cancer. (A) *DNAH7* expression was substantially higher in *DNAH7* mutated patients compared to *DNAH7* unmutated patients ( $P=0.003$ ). (B) The level of TMB was significantly increased in *DNAH7* mutated patients compared to unmutated patients ( $P<0.001$ ). (C) There was no distinct difference in the MSI value between *DNAH7* mutated and unmutated patients ( $P=0.114$ ). (D) COSMIC signature heat map analysis of *DNAH7* mutated patients in the CRC dataset. (E) COSMIC signature heat map analysis of *DNAH7* unmutated patients in the TCGA dataset. CI, confidence interval; MSI, microsatellite instability; TMB, tumor mutation burden; CRC, colorectal cancer; TCGA, The Cancer Genome Atlas.

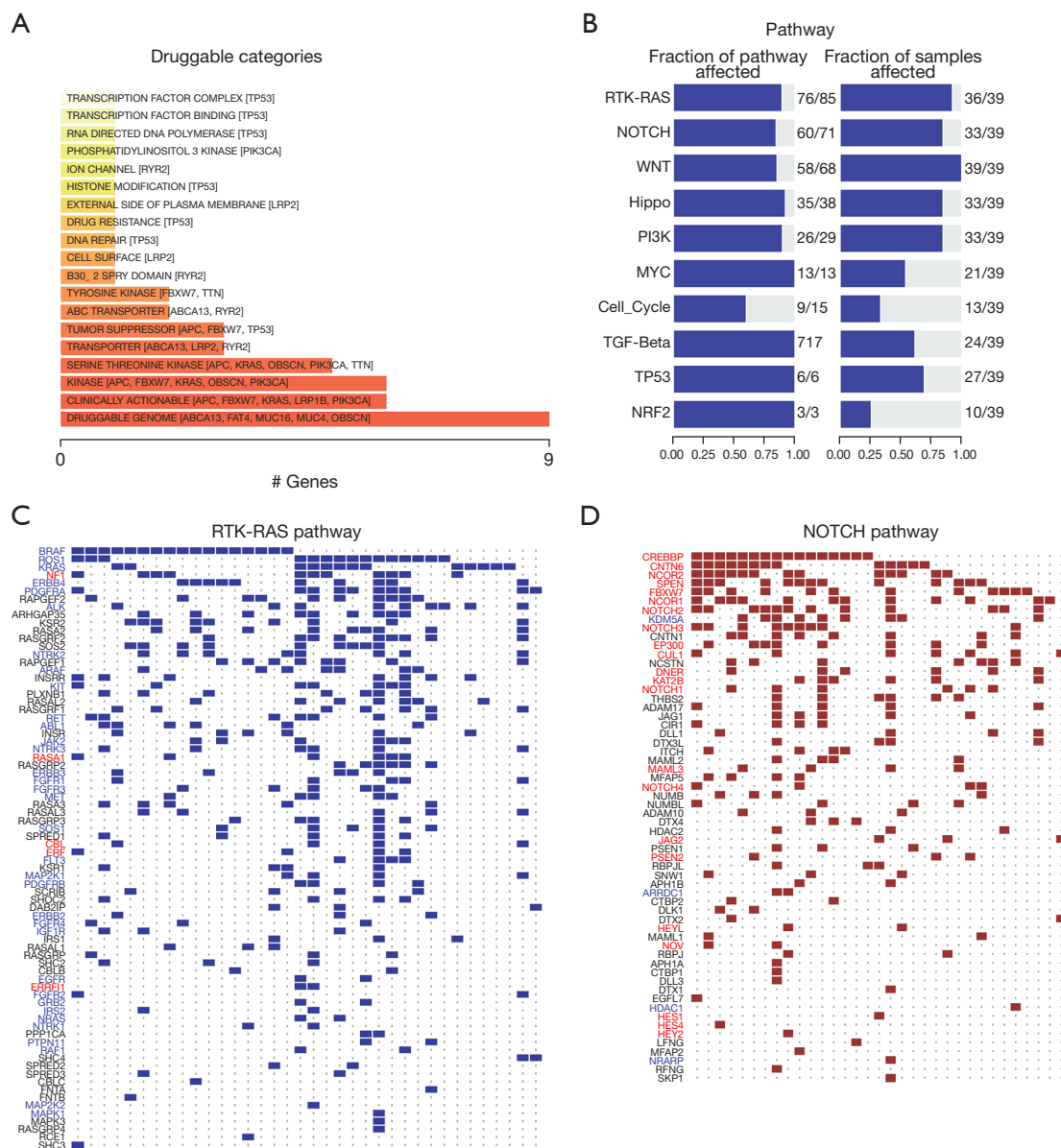
between the top10 key genes and *DNAH7*, among which, AQP8, MS4A12, GUCA2B, and ZG16 have the strongest correlation with *DNAH7*.

#### ***The effect of the DNAH7 gene mutation on immunological characteristics of colorectal cancer patients***

We further investigated the influence of the *DNAH7* gene mutation on the immunological characteristics of CRC. In the *DNAH7* mutated group, the ESTIMATE scores, immune scores, and stromal scores were significantly increased ( $P<0.001$ ; *Figure 8A-8C*), but tumor purity was significantly decreased ( $P<0.001$ ; *Figure 8D*).

#### ***Correlation analysis of the DNAH7 gene mutation and clinical features***

The clinical correlation of *DNAH7* gene mutations was analyzed. According to the clinicopathological features, both univariate and multivariate Cox regression analyses demonstrated that the level of *DNAH7* mutation was a hazard factor for CRC ( $P=0.014$  and  $0.007$ , and *Figure 9A* and *Figure 9B*, respectively) (*Table 4*). In the CRC data sets, *DNAH7* mutation had a significant impact on patient prognosis ( $P=0.014$ , *Figure 9C*). To further clarify the influence of *DNAH7* gene mutation on CRC, we incorporated the *DNAH7* gene mutation and



**Figure 4** Drug susceptibility analysis of the *DNAH7* gene mutation in CRC. (A) An analysis of the relationship between the level of gene mutation in *DNAH7* in CRC and various drugs bases on the drug-gene interaction database. (B) An analysis of the changes in the level of gene mutation in diverse oncogenic signaling pathways in the CRC data sets. (C) Mutation distribution of major genes in the RTK-RAS pathway in patients with CRC. (D) Mutation distribution of major genes in the NOTCH pathway in patients with CRC. CRC, colorectal cancer.

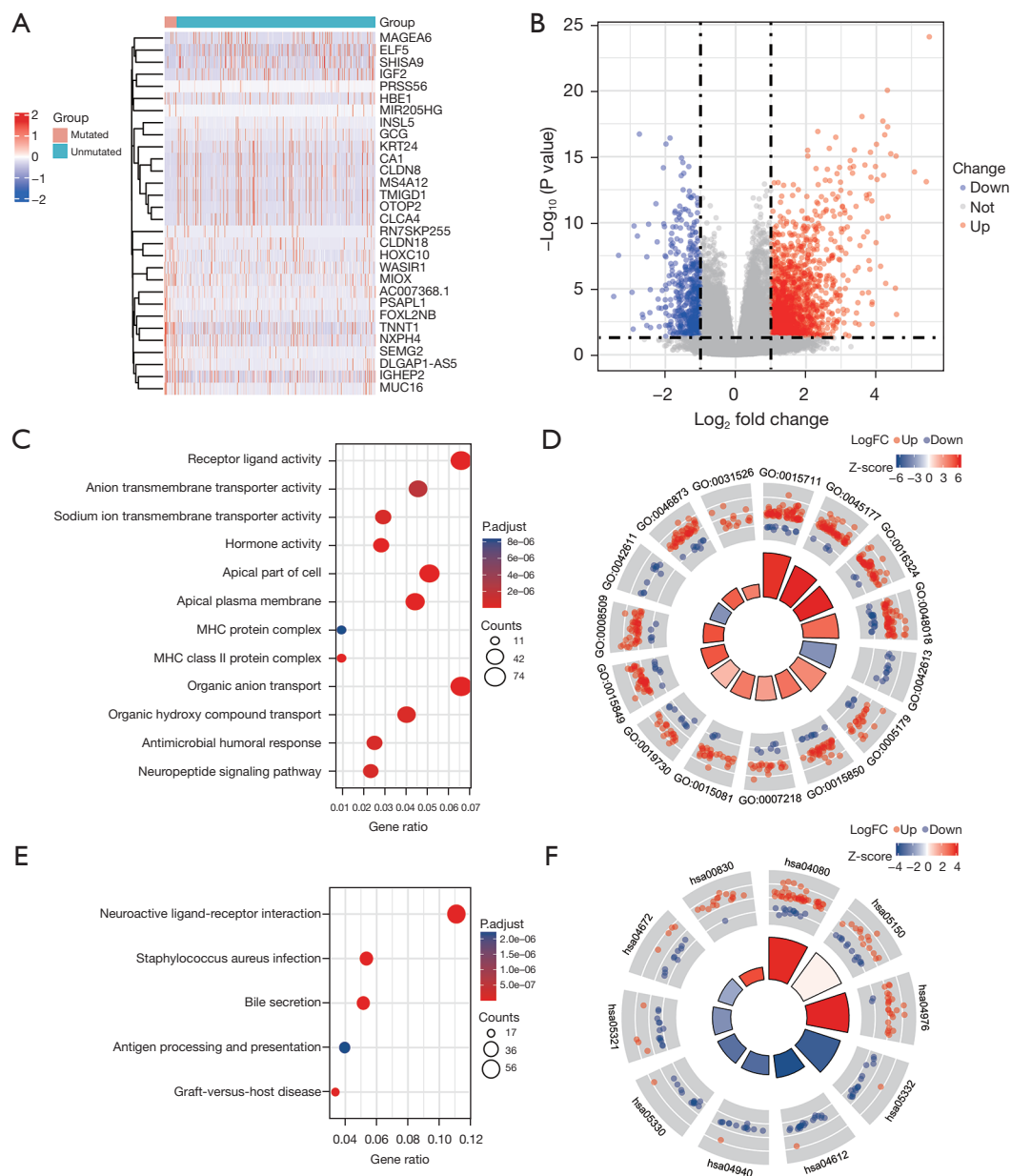
clinicopathological features into the model and constructed a nomogram (Figure 9D) to predict OS of CRC patients. The decision curve analysis (DCA) showed that the rosette map of *DNAH7* gene mutation had a good prediction effect (Figure 9E). The calibration curve showed a good consistency between the estimated OS values and the actual

observed values at 1-, 3- and 5-year through nomogram predicted survival probability (Figure 9F).

### Discussion

CRC is the most common digestive system malignant





**Figure 5** Identification of the DEGs in *DNAH7* mutated patients and functional enrichment analysis of these genes. (A,B) A volcano map and heatmap showing the expression of DEGs in the *DNAH7* mutated and unmutated groups. (C,D) Gene Ontology analysis showed that the DEGs were closely related to receptor ligand activity, apical part of the cell, organic anion transport, and other biological processes. (E,F) Kyoto Encyclopedia of Genes and Genomes analysis suggested that the DEGs mainly affected neuroactive ligand-receptor interaction, *Staphylococcus aureus* infection, bile secretion, and other pathways. DEGs, differentially expressed genes.

tumor, with a high mortality rate. Although there are comprehensive treatment methods such as surgical operation, iatrochemistry, targeting, and so on, the efficacy is not ideal, and distant metastasis occurs frequently. The 5-year survival in patients with metastatic CRC is less than

20% (20).

Recently, studies have suggested that immunotherapy is beneficial to CRC because of the high immunogenicity conferred by the high mutational load of CRC. Immunotherapy has been shown to be effective in

**Table 1** GO analysis of differential DEGs

Ontology	ID	Description	P value	p.adjust	q value
BP	GO:0015711	Organic anion transport	8.02e-14	4.01e-10	3.68e-10
BP	GO:0015850	Organic hydroxy compound transport	1.63e-10	4.08e-07	3.74e-07
BP	GO:0007218	Neuropeptide signaling pathway	3.39e-10	5.65e-07	5.18e-07
BP	GO:0019730	Antimicrobial humoral response	6.06e-10	5.77e-07	5.29e-07
CC	GO:0045177	Apical part of cell	7.39e-12	3.60e-09	2.89e-09
CC	GO:0016324	Apical plasma membrane	3.38e-11	8.24e-09	6.62e-09
CC	GO:0042613	MHC class II protein complex	1.12e-10	1.81e-08	1.46e-08
CC	GO:0042611	MHC protein complex	6.89e-08	8.39e-06	6.75e-06
MF	GO:0048018	Receptor ligand activity	1.09e-11	9.09e-09	7.44e-09
MF	GO:0005179	Hormone activity	5.92e-11	2.47e-08	2.02e-08
MF	GO:0015081	Sodium ion transmembrane transporter activity	2.07e-09	5.74e-07	4.70e-07
MF	GO:0008509	Anion transmembrane transporter activity	9.74e-09	2.03e-06	1.66e-06

GO, gene ontology; DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function.

**Table 2** KEGG analysis of differential DEGs

ID	Description	P value	p.adjust	q value
hsa04080	Neuroactive ligand-receptor interaction	1.22e-11	1.75e-09	1.39e-09
hsa05150	Staphylococcus aureus infection	1.46e-11	1.75e-09	1.39e-09
hsa04976	Bile secretion	1.79e-11	1.75e-09	1.39e-09
hsa05332	Graft-versus-host disease	1.55e-10	1.14e-08	9.05e-09
hsa04612	Antigen processing and presentation	3.78e-08	2.22e-06	1.77e-06

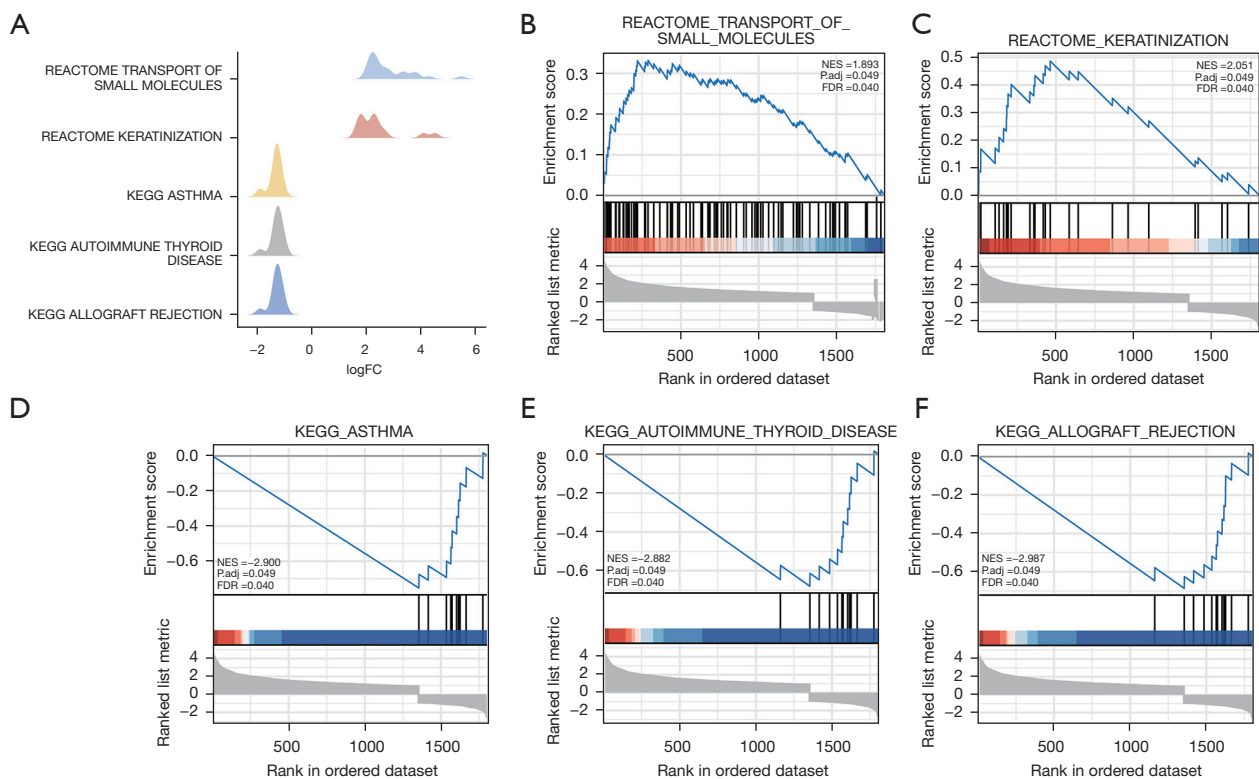
KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

metastatic CRC with deficient MMR or high microsatellite instability (MSI-H) (3,21). Unfortunately, there is currently no effective predictive biomarker for the effectiveness of immunotherapy in CRC patients. Therefore, it is important to identify the key somatic mutations and improve the efficacy of immunotherapy. The clinical data and mutation data was obtained from a TCGA CRC cohort and examined. Survival analysis indicated that *DNAH7* mutated patients had better immunotherapy prognosis compared to *DNAH7* unmutated patients, suggesting that ICIs are effective in *DNAH7* mutated CRC. We further demonstrated that *DNAH7* likely affects the efficacy of ICIs by acting on the tumor immune microenvironment.

The study revealed that *DNAH7* mutations were the major type of somatic mutations in CRC, with missense mutations being the most common. The *DNAH7* mutated

group had a greater increase in copy number alteration compared to the *DNAH7* unmutated group, and the TMB level was significantly increased in *DNAH7* mutated patients. Furthermore, significant changes were detected in the mutated *DNAH7* gene at signature 1, 6, and 15 (*Figure 3D, 3E*).

In addition, we demonstrated that various drugs were associated with the level of *DNAH7* gene mutation, such as clinically actionable, kinase, serine threonine kinase, and especially the druggable genome (*Figure 4A*). Oncogenic signaling pathway analysis revealed that CRC samples with *DNAH7* mutations had a high number of related genes that were mutated in the RTK-RAS pathway (*Figure 4C*) and NOTCH pathway (*Figure 4D*). RAS is a key protein in the regulation of cell proliferation and is commonly found in cancers. A previous study reported that the RTK-RAS pathway is related to the pathogenesis of CRC (22)



**Figure 6** GSEA of the differentially expressed genes in *DNAH7* mutated colorectal cancer. (A) GSEA of the differentially expressed genes. *DNAH7* mutant tumor tissues were closely related to (B) transport of small molecules; (C) keratinization; (D) asthma; (E) autoimmune thyroid disease; (F) allograft rejection; and other pathways. KEGG, Kyoto encyclopedia of genes and genomes; GSEA, gene set enrichment analysis.

and mutations in the RTK-RAS pathway led to decreased relapse free survival (RFS) in CRC patients (23). The Cancer Genome Atlas Pan-Cancer Analysis Project examined 123 patients with non-MSI, highly metastatic CRC, and found significant changes in the RTK-RAS, Notch, Wnt, p53, PI3K, TGF- $\beta$ , and Myc pathways (24). These changes in the RTK-RAS and Notch pathway have been shown to be strongly related to poor OS in patients whose colorectal liver metastases were resected (25).

The effect of the *DNAH7* gene mutation on the occurrence and development of tumors was examined using patients from the TCGA-CRC cohort. A total of 1,764 upregulation genes and 583 downregulation genes were identified in the CRC data sets (Figure 5A,5B). Additionally, GO analysis revealed that the differentially expressed genes were enriched in biological processes including receptor ligand activity, apical part of the cell, and organic anion transport (Figure 5C,5D). KEGG analysis showed that the differentially expressed genes prominently affected

neuroactive ligand-receptor interaction, *Staphylococcus aureus* infection, bile secretion, and other pathways (Figure 5E,5F).

By establishing the PPI network and the interaction relationship, we identified the top 10 key genes, among which, AQP8, MS4A12, GUCA2B, and ZG16 have the strongest correlation with *DNAH7*. The expression of these latter genes is associated with the OS of CRC patients (26,27). The *DNAH7* gene mutation, which was first mentioned in male infertility, causes asthenospermia syndrome and is a susceptibility gene for testicular cancer (28,29). Therefore, the *DNAH7* gene may be a novel biological marker for CRC. Analysis of the effects of *DNAH7* gene mutation on the immunological characteristics of CRC revealed that the ESTIMATE score, immune score, and stromal score were all elevated, while tumor purity decreased significantly in *DNAH7* mutated CRC. Finally, both univariate and multivariate Cox regression analysis demonstrated that *DNAH7* gene

**Table 3** Results of GSEA analysis

ID	enrichmentScore	P value	p.adjust	q value
REACTOME_TRANSPORT_OF_SMALL_MOLECULES	0.33217744	0.00104493	0.04933586	0.03994807
REACTOME KERATINIZATION	0.48623374	0.00120482	0.04933586	0.03994807
WP_ALLOGRAFT_REJECTION	-0.4744149	0.00537634	0.04933586	0.03994807
REACTOME_INTERFERON_SIGNALING	-0.75628843	0.00520833	0.04933586	0.03994807
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	-0.40111294	0.0047619	0.04933586	0.03994807
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	-0.71254582	0.0047619	0.04933586	0.03994807
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	-0.59114226	0.00454545	0.04933586	0.03994807
KEGG_GRAFT_VERSUS_HOST_DISEASE	-0.70690146	0.00454545	0.04933586	0.03994807
KEGG_LEISHMANIA_INFECTION	-0.75571668	0.00434783	0.04933586	0.03994807
KEGG_VIRAL_MYOCARDITIS	-0.60687597	0.00429185	0.04933586	0.03994807
REACTOME_INTERFERON_GAMMA_SIGNALING	-0.75529543	0.00429185	0.04933586	0.03994807
KEGG_TYPE_I_DIABETES_MELLITUS	-0.691749	0.00420168	0.04933586	0.03994807
REACTOME_MHC_CLASS_II_ANTIGEN_PRESENTATION	-0.75487465	0.00420168	0.04933586	0.03994807
WP_EBOLA_VIRUS_PATHWAY_ON_HOST	-0.6430013	0.00395257	0.04933586	0.03994807
KEGG_ALLOGRAFT_REJECTION	-0.68643059	0.00395257	0.04933586	0.03994807
KEGG_AUTOIMMUNE_THYROID_DISEASE	-0.68017463	0.003663	0.04933586	0.03994807
KEGG_ASTHMA	-0.75319622	0.003367	0.04933586	0.03994807

GSEA, gene set enrichment analysis.

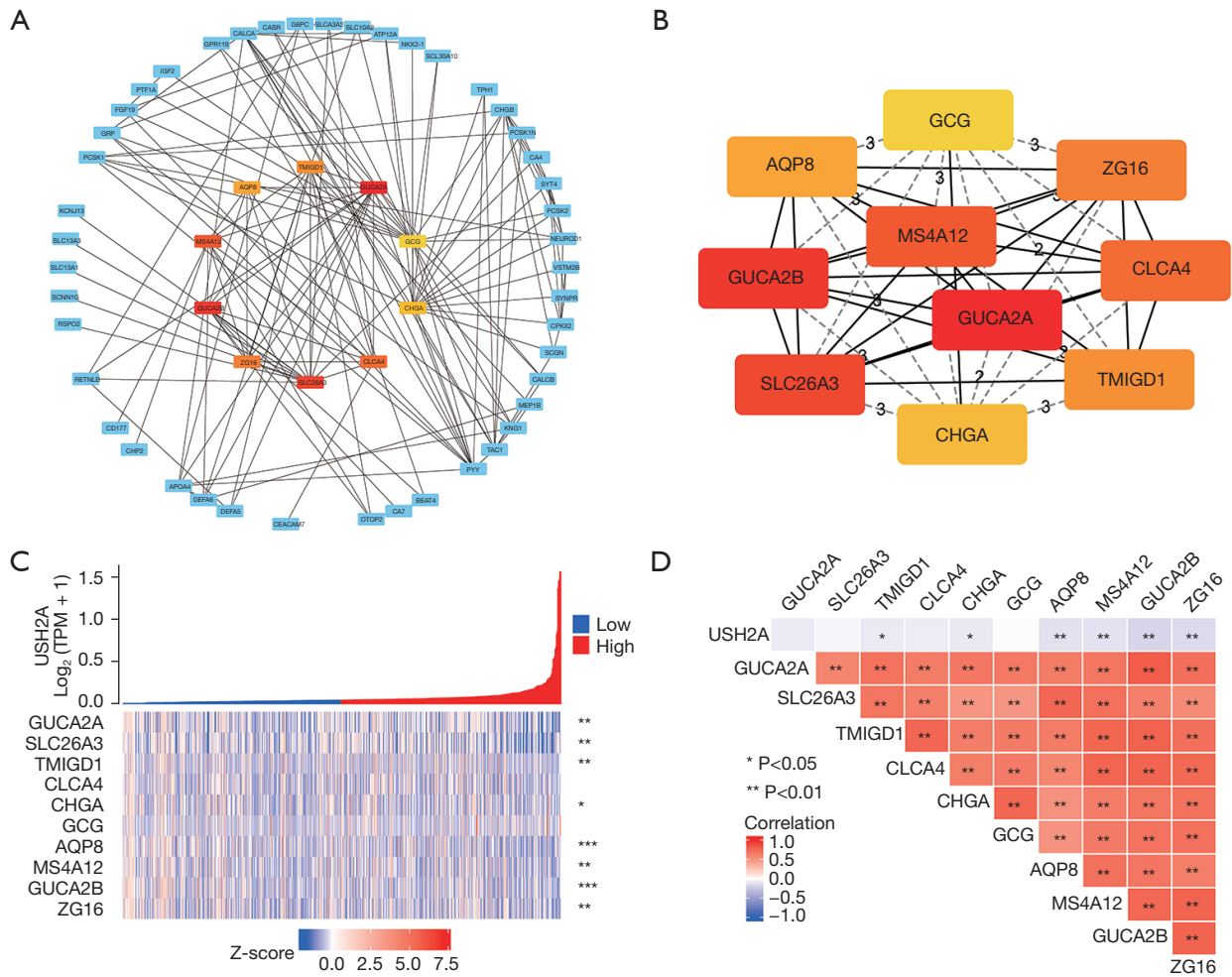
mutation level was a risk factor for CRC. A longer OS was observed in *DNAH7* mutant patients than unmutant *DNAH7* patients. The OS benefit was approximately 131 months (about 3,932 days) in *DNAH7* mutant patients. Therefore, *DNAH7* mutation has good predictive value for the OS of CRC patients.

There were some limitations to this study. First, although we included two independent cohort studies with clinical and mutational data, there was a lack of relevant clinical studies. Second, while the functional pathways of the *DNAH7* mutation were analyzed, further validation *in vitro* and *in vivo* is required. Third, there may have been batch

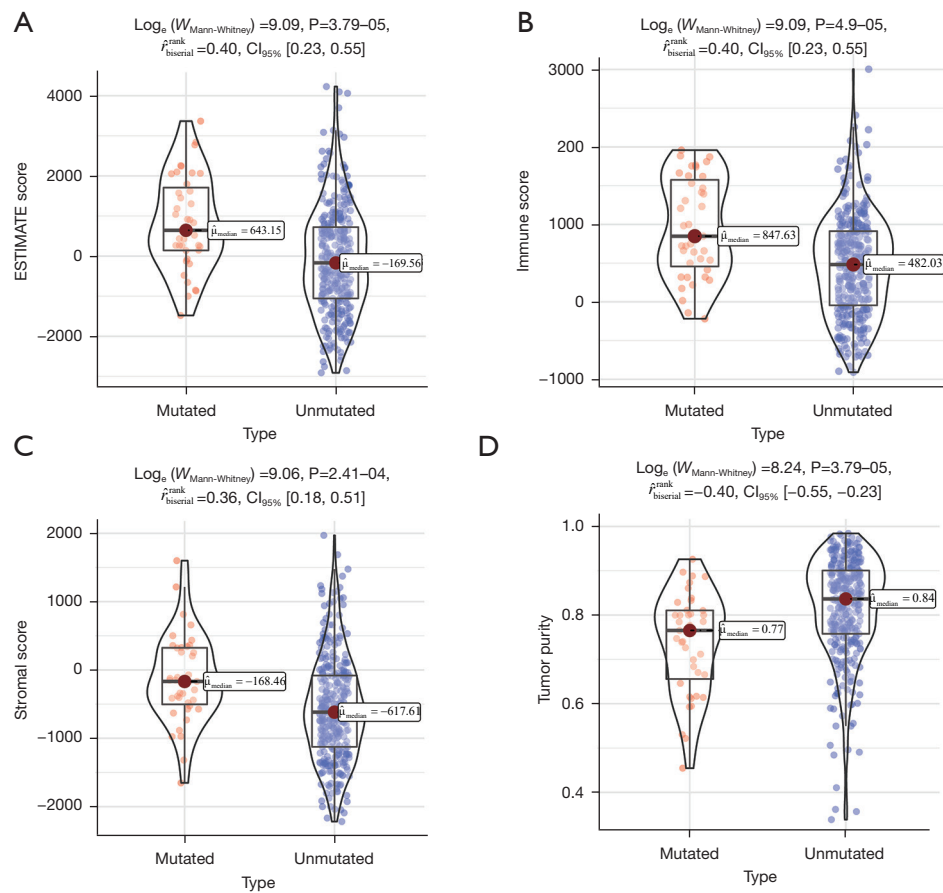
differences that could not be avoided nor removed during analysis due to the large number of data sets.

## Conclusions

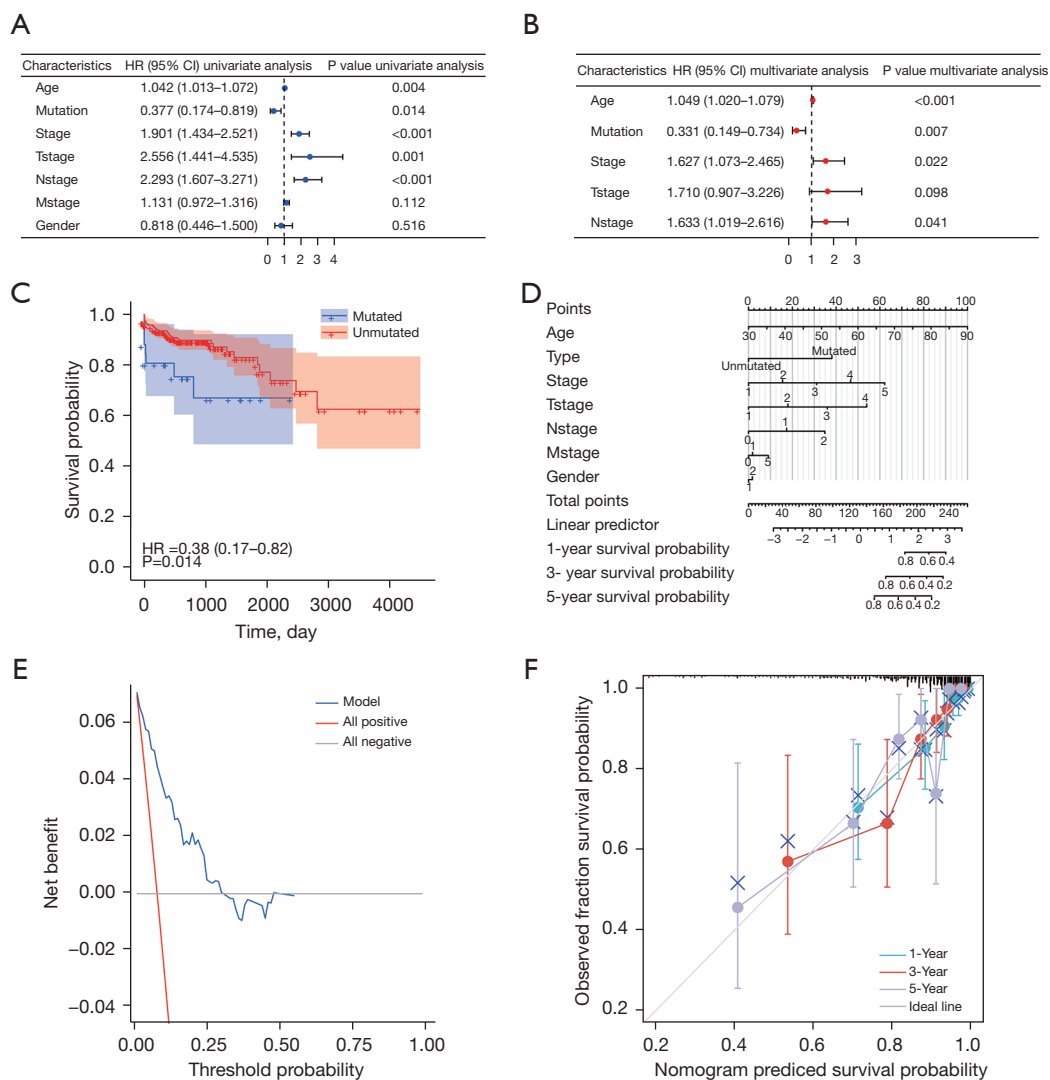
The results herein demonstrated that CRC with *DNAH7* mutations benefited more from ICI therapy than CRC without the *DNAH7* mutation. Furthermore, the *DNAH7* mutated group had a higher mutation load and immunogenicity compared to the unmutated group. Indeed, *DNAH7* mutation may be a potential prognostic indicator and/or therapeutic target for the clinical treatment of CRC.



**Figure 7** Gene-gene interaction network and the PPI network. (A) The STRING database was used to analyze the PPI network of differentially expressed genes, where each node represents a different gene. (B) The top 10 genes were selected as key genes based on the MCC algorithm. (C) Co-expression heat maps of the top 10 key genes and *DNAH7*. (D) Molecular correlation diagram of the top 10 key genes and *DNAH7*. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001. PPI, protein-protein interaction; MCC, maximal clique centrality.



**Figure 8** The effect of the *DNAH7* gene mutation on the immunological characteristics of colorectal cancer patients. (A) The ESTIMATE scores in the *DNAH7* mutated group were significantly higher than that in the unmutated group ( $P < 0.001$ ). (B) The immune scores in the *DNAH7* mutated group were significantly higher than that in the unmutated group ( $P < 0.001$ ). (C) The stromal scores in the *DNAH7* mutated group were significantly elevated compared to that in the unmutated group ( $P < 0.001$ ). (D) The tumor purity in the *DNAH7* mutated group was significantly decreased compared to the unmutated group ( $P < 0.001$ ). CI, confidence interval.



**Figure 9** The influence of *DNAH7* gene mutation on clinicopathological features in colorectal cancer data sets. (A) The effects of *DNAH7* gene mutations and different clinicopathological characteristics on OS were examined by univariate Cox analysis. (B) The effects of *DNAH7* gene mutations and clinicopathological characteristics on OS were examined by multivariate Cox analysis. (C) Survival analysis showed that *DNAH7* gene mutations had significant influence on prognosis in the CRC data set (P=0.014). (D) The nomogram was constructed using *DNAH7* mutation gene and clinicopathological characteristics. (E) The DCA of the *DNAH7* gene mutation nomogram. (F) The calibration curve of the *DNAH7* gene mutation nomogram. The abscissa represents the predicted survival probability and the ordinate represents the fraction survival probability. This experiment was repeated 1,000 times each time. The curve shows that the nomogram has good value of prediction of the 1-, 3-, and 5-year survival of patients with CRC. HR, hazard ratio; CI, confidence interval; OS, overall survival; CRC, colorectal cancer; DCA, decision curve analysis.

**Table 4** *DNAH7* gene mutation for predicting patients' OS by univariate and multivariate Cox analysis in The TCGA database

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	314	1.042 (1.013–1.072)	0.004*	1.049 (1.020–1.079)	<0.001*
Type	314	–	–	–	–
Mutated	33	Reference	–	–	–
Unmutated	281	0.377 (0.174–0.819)	0.014*	0.331 (0.149–0.734)	0.007*
Stage	314	1.901 (1.434–2.521)	<0.001*	1.627 (1.073–2.465)	0.022*
T stage	314	2.556 (1.441–4.535)	0.001*	1.710 (0.907–3.226)	0.098
N stage	314	2.293 (1.607–3.271)	<0.001*	1.633 (1.019–2.616)	0.041*
M stage	314	1.131 (0.972–1.316)	0.112	–	–
Gender	314	0.818 (0.446–1.500)	0.516	–	–

\*, P<0.05. OS, overall survival; TCGA, The Cancer Genome Atlas; CI, confidence interval; T, tumor; N, node; M, metastasis.

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## Footnote

**Reporting Checklist:** The authors have completed the TRIPOD reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-6166/rc>

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-6166/coif>). The authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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