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Original Article

Differential isocitrate dehydrogenase 1 and isocitrate dehydrogenase 2 mutation-related landscape in intrahepatic cholangiocarcinoma

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Abstract

Background: Patients with intrahepatic cholangiocarcinoma (ICC) are prone to recurrence and poor survival. Targeted therapy related to isocitrate dehydrogenase (IDH) is an extremely important treatment. IDH1 and IDH2 mutations are generally thought to have similar effects on the tumor landscape. However, it is doubtful whether these 2 mutations have exactly the same effects on tumor cells and the tumor microenvironment.

Methods: All collected tumor samples were subjected to simultaneous whole-exon sequencing and proteome sequencing.

Results: IDH1 mutations accounted for 12.2%, and IDH2 mutations accounted for 5.5%, all missense mutations. Tumors with IDH mutations had lower proportions of KRAS and TP53 mutations. Mutated genes were obviously enriched in the kinase pathway in the tumors with IDH2 mutations. The signaling pathways were mainly enriched in the activation of cellular metabolic activities and an increase of inhibitory immune cells in the tumors with IDH mutations. Moreover, tumors had unique enrichment in DNA repair in IDH1 mutants and secretion of biological molecules in IDH2 mutants. Inhibitory immune cells might be more prominent in IDH2 mutants, and the expression of immune checkpoints PVR and HLA-DQB1 was more prominent in IDH1 mutants. IDH mutants were more related to metabolism-related and inflammation-immune response clusters, and some belonged to the DNA replication and repair cluster.

Conclusions: These results revealed the differential IDH1 and IDH2 mutation-related landscapes, and we have provided an important reference database to guide ICC treatment.

Key words: intrahepatic cholangiocarcinoma; isocitrate dehydrogenase; whole-exon sequencing; proteome.

Implications for practice

Targeted therapy related to isocitrate dehydrogenase (IDH) mutation is an extremely important treatment for intrahepatic cholangiocarcinoma. IDH1 and IDH2 are isomerases with highly similar structures, and many studies have combined them for analysis. However, our study found that tumors with IDH1 and IDH2 mutations might exhibit differential functional and immune microenvironment characteristics. Our study laid a promising foundation for precise treatment and combination therapies involving IDH1/2 mutation-associated inhibitors. In the treatment of intrahepatic cholangiocarcinoma, different treatment schemes should be adopted for different IDH mutation states.

Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common malignant tumor in the liver, with an incidence increasing year-by-year globally.[1] Although chemotherapy remains the main first-line therapy scheme, there is only an approximately 9% 5-year overall survival rate.[2] Therefore, there is an urgent need to identify appropriate treatment targets based on the characteristics of ICC. Many clinical trials are currently being conducted on targeted therapy and combination drugs for ICC.

Abnormalities in metabolic function exist in tumor cells, which may be caused by structural or functional abnormalities of some metabolic enzymes. Several metabolic pathways

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that play an important role in tumor development have been identified, among which the isocitrate dehydrogenase (IDH) mutation-related pathway is an important pathway for tumor cells to utilize "metabolic reprogramming" to create favorable growth conditions for themselves. The IDH family can be divided into 2 major groups according to their catalytic properties: NAD^+-dependent IDH3 and $NADP^+$-dependent IDH1 and IDH2. IDH1 and IDH2 mutations are common in tumors and they are usually regarded as having similar functions in tumorigenesis and development.[3] For many malignancies, the presence of IDH mutations is an important prognostic and therapeutic choice factor, such as acute myeloid leukemia,[4] chondrosarcomas,[5] and gliomas.[6] IDH1 mutations are present in approximately 20% of ICCs,[7] and targeted therapy related to IDH1 mutations is an extremely important treatment for ICC. An orally administered IDH1 mutation inhibitor, ivosidenib, has been approved for ICC therapy by the Food and Drug Administration. However, while it increased progression-free survival, it did not improve overall survival in one phase III clinical trial.[8] In some patients, drug resistance may develop within a few months,[2] and there are few studies on phenotypic changes and malignant tumor behavior related to IDH2 mutations. Thus, uncovering the landscape of IDH1/2 mutation-associated tumor characteristics is essential for improving the treatment of ICC, and IDH2-related drugs and combination therapies deserve further exploration.

Many studies have focused on distinguishing the differences between tumors with and without IDH mutations based on genomic and transcriptomic analyses,[9] but the changes in the genomic and transcriptomic levels may not fully correspond to the changes at the proteomic level, and there are limited studies of comprehensive and holistic protein alterations. In addition, IDH1 and IDH2 are isomerases with similar structures, and abundant studies have combined them for analysis, but it is still doubtful whether mutations of the 2 have exactly the same effects on tumor cells and the tumor microenvironment. Therefore, from the perspective of the genome and proteome, we elaborated on the differential genetic and protein changes in tumors with IDH1 or IDH2 mutations, revealed the relevant cellular pathway landscape and tumor immune microenvironment, and provided potential targets and protocols for combination drugs.

Materials and methods

Patient selection

This study included 255 patients diagnosed with ICC (Supplementary Table S1). Fifty-seven patients without distant metastasis (the Zhe cohort) who had not received preoperative chemotherapy or radiotherapy underwent radical surgical resection. The pathological diagnosis was confirmed to be ICC by pathological experts. All patients received regular postoperative treatment with gemcitabine + platinum (GP regimen) or capecitabine. All samples were simultaneously subjected to whole exon sequencing (WES) and proteomic sequencing. All patients in this study signed written informed consent and were approved by the Medical Ethics Committee of the Affiliated Hospital of Southwest Medical University (No. KY2019053) and NanFang Hospital of Southern Medical University (No. NFEC-2021-067 and No. NFEC-2018-028). Human data have been performed in accordance with the Declaration of Helsinki. Other patients without distant metastasis who had both WES and proteomic sequencing data were collected from a public database (the Fu cohort) maintained by the Zhongshan Hospital of Fudan University.[10]

WES and proteome sequencing processing

WES was performed in adherence to both previous literature reports and the manufacturer's experimental protocols.[11] Genomic DNA was isolated from tumor samples and matched pairs using the DNeasy Blood and Tissue Kit. The quality of dsDNA was assessed using the NanoDrop 2000 after quantification with the dsDNA HS assay kit. Libraries were prepared using the KAPA Hyper Prep kit. The captured libraries were PCR amplified using the KAPA HiFi HotStart ReadyMix. The KAPA Library Quantification Kit was used to quantify the purified libraries. Genomic DNA sequencing was performed on the Illumina NovaSeq 6000 platform, generating 150-bp paired-end reads. The sequencing data were multiplexed using bcl2fastq. Low-quality or N bases were removed using Trimmomatic.[12] The data were then aligned to the hg19 reference human genome using Burrows-Wheeler Aligner[13] and further processed into vcf file by the Genome Analysis Toolkit.[14] ANNOVAR software[15] was used to annotate the vcf file, and then the R package maftools[16] was used to visualize and analyze the WES results after converting the files into maf files.

Tandem mass tag-based proteome sequencing and processing were also conducted according to the manufacturer's experimental protocols and the previous literature.[17] Proteins were extracted via tissue cleavage utilizing lysate. Peptides were subsequently obtained after denaturation with 6 M urea and 2 M thiourea, centrifugation, and labeled with TMTpro 16 plex. The peptide samples were then analyzed using a Q Exactive HF mass spectrometer and annotated by UniProt database. The proteins with undetected protein expression levels in more than half of the samples were deleted, and then the random forest model was used to supplement other null values by the R package missForest.[18]

Differential expression analysis and enrichment analysis

Differential proteins in the proteome were analyzed using the R package limma.[19] Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted using the R package clusterProfiler.[20] The R package clusterProfiler was also used to perform gene set enrichment analysis (GSEA) relying on the MSigdb and REACTOME databases. Single-sample GSEA (ssGSEA) from the R package GSVA[21] was used to evaluate the enrichment score of each sample.

Immune cell types enrichment analysis and patient clinical clusters

The proportion and type of immune cells in the tissue were estimated using the R package xCell.[22] The immune cell enrichment score and immune score (ImmuneScore) for each sample were also evaluated using xCell. The patients were clustered based on their main clinical biological types using the R package ConsensusClusterPlus,[23] differential analyses between groups were performed using limma, and GO, KEGG, and ssGSEA enrichment analyses were performed.

Statistical analysis

Fisher's exact tests and chi-square tests were used to assess correlations or differences between variables, Wilcoxon signed-rank tests and Student's *t* tests were used to compare

differences between 2 groups, and Kruskal-Wallis tests and analysis of variance were used to compare differences between multiple groups. Kaplan-Meier survival analysis using the log-rank test to compare survival differences between groups. A bilateral $P < .05$ was considered statistically significant.

Results

Characteristics of the ICC cohort

WES was performed on samples collected from the cohort with ICC to identify IDH mutations. Among them, the frequency of IDH1 mutations was 9% (the Zhe cohort) and 13% (the Fu cohort), and the frequency of IDH2 mutations was 7% (the Zhe cohort) and 5% (the Fu cohort), and IDH3 mutations were not found (Figure 1A). Notably, the patient populations with IDH1 and IDH2 mutations did not overlap. The IDH1 and IDH2 mutations were all missense mutations, with IDH1 mutations occurring at R132 and IDH2 mutations occurring at R172. There was no significant correlation between the presence of an IDH mutation and sex, age, or TNM stage (all $P > .05$). There was no significant difference in survival analysis between patients with mutated IDH1, patients with mutated IDH2, and patients with wild-type IDH (the Zhe cohort and the Fu cohort, all $P > .05$).

Genomic characteristics in IDH mutation-associated ICC

The common mutations associated with ICC mainly included KRAS and TP53.[2] The Zhe cohort with IDH mutations had lower proportions of TP53 (11%) and KRAS (0%) mutations. In contrast, wild-type IDH samples had higher proportions of TP53 (23%) and KRAS (12%) mutations (Figure 1A). Further differential analysis was conducted between the 2 groups (Figure 1B), and there was a relatively higher mutation frequency of TSPYL5 and HMCN2 in the tumors with IDH mutations (all $P < .05$). Conversely, IDH wild-type tumors had relatively more OR51M1 and KLHL38 mutations (all $P < .05$). Compared with the IDH wild-type and IDH2 mutated-type (Supplementary Figure S1A), the IDH1 mutated-type had a higher shared mutation frequency of HCAR3 and MAGEC1 (all $P < .05$). Compared with the IDH wild-type and IDH1 mutated-type (Supplementary Figure S1A), IDH2 mutated-type had a higher shared mutation frequency of CFHR1 and OR4N2 (all $P < .05$). Gene interaction analysis in samples revealed that PTGFRN and STK33 mutated gene pairs in IDH1 mutated samples were mutually exclusive (all $P < .05$), while these mutated gene pairs in IDH2 mutated samples were co-occurrence (all $P < .05$). Pathway analysis of mutation-related genes (Supplementary Figure S1B-E) showed that the major aggregation of signaling pathways was similar, but the proportion of WNT and Hippo signaling pathways was different between IDH1/2 mutants. Furthermore, we considered the possibility of combined therapy or potential targets of IDH based on mutated genes. We analyzed potential drug targets (Supplementary Figure S1F-I), and the results showed that relatively more mutated genes clustered in the transporter pathways in the IDH1 mutants, while relatively more mutated genes clustered in the kinase pathways in the IDH2 mutants.

Proteomic differences in IDH mutation-associated ICC

We further explored the protein-level changes in ICC with IDH mutations. Differential analysis of the proteomic data in the Zhe cohort (Figure 2A) revealed relatively higher CRYAA and POLQ expression and lower PRUNE1 and ANKIB1 expression in association with IDH mutations (all $P < .05$). GO (Figure 2B) and KEGG (Figure 2C) analyses showed significant enrichment in immune molecule production and cell-cell adhesion (all $P < .05$), as well as metabolic pathways such as lipid metabolism, and biosynthesis of amino acids (all $P < .05$). GSEA (Figure 2D, 2E) and ssGSEA also revealed that IDH mutations were associated with disorders of cellular metabolic activities, such as bile acid metabolism and hormone metabolism, and immune-related pathways, such as co-stimulation by the CD28 family and interferon response (all $P < .05$).

The genomic characteristics of ICC samples with IDH mutations were significantly different between the IDH1 and IDH2 mutations. Therefore, we analyzed tumors with IDH1 and IDH2 mutations separately. The results of the Zhe cohort showed that compared to tumors with IDH2 mutations and wild-type IDH, tumors with IDH1 mutations exhibited relatively higher expression of MAGEA4 and CAPN6 (all $P < .05$) and relatively lower expression of CYP3A5 and ORM1 (all $P < .05$). GO (Figure 3A) and KEGG (Figure 3B) analyses revealed disordered biological processes, including the nitrogen metabolism and motor proteins signaling pathways (all $P < .05$). Moreover, ssGSEA (Figure 3C) showed that DNA repair and interleukin-2 signaling pathways were differentially enriched (all $P < .05$). We used the Fu cohort to further validate the distinct characteristics of IDH1 mutations. The results of the Fu cohort by differential expression and enrichment analysis (Supplementary Figure S2A-C) found that IDH1 mutants were differentially enriched in glyoxylate and dicarboxylate metabolism, amino acid metabolism, DNA repair, and interleukin-33 signaling pathways (all $P < .05$). In conclusion, the proteomic profiles associated with IDH1 mutations were differentially enriched in interleukin immune pathways, amino acid metabolism, and DNA repair processes.

On the other hand, compared with IDH1 mutations and wild types, tumors with IDH2 mutations showed relatively higher expression of SAA1 and CST4 (all $P < .05$) and relatively lower expression of MAGEA4 and IGF2BP1 (all $P < .05$). GO (Figure 3D) and KEGG (Figure 3E) analyses identified enriched pathways, including complement cascades, nicotinate and nicotinamide metabolism, ferroptosis, and lipid metabolism (all $P < .05$). Additionally, ssGSEA (Figure 3F) showed that the oxidative phosphorylation signaling pathway and adipogenesis were differentially enriched (all $P < .05$). We used the Fu cohort to further validate the distinct characteristics of the IDH2 mutations. The results of the Fu cohort by differential expression and enrichment analysis (Supplementary Figure S2D-F) found that IDH2 mutants were enriched in the lumen-related pathways, proteoglycans, cellular senescence, focal adhesion, lipolysis, and xenobiotic metabolism (all $P < .05$). In conclusion, the proteomic profile associated with IDH2 mutations was differentially enriched in the secretion of biological molecules and lipid metabolism responses.

Immunological characteristics in IDH mutation-associated ICC

IDH mutations in many tumors are also related to immune processes, so we further explored whether IDH mutation-associated ICC has unique immune-related aspects. By analyzing the immune components using the xCell algorithm applied to proteomics, the results of the Zhe cohort (Figure 4A)

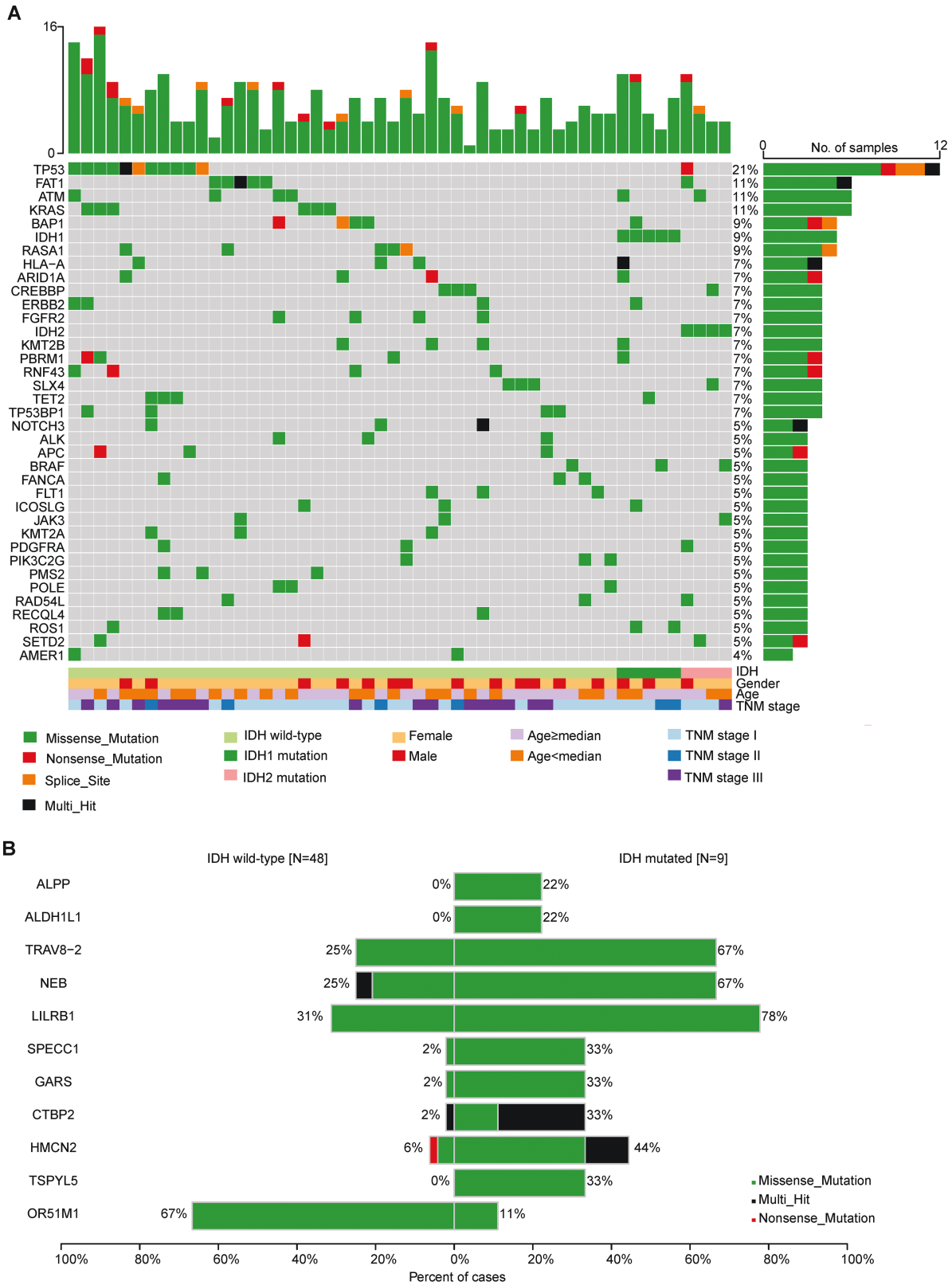

Figure 1. The genomic landscape of patients in Zhe cohort ($n = 57$). (A) Significantly mutated genes in ICC patients. (B) Differentially mutated genes between IDH mutated and IDH wild-type patients.

showed that patients with IDH mutations had lower immune score (ImmuneScore, $P < .05$). Classification discussion revealed that IDH1-mutant samples had relatively more CD4⁺ effector memory T cells than wild-type IDH ($P < .05$), while IDH2-mutants had relatively fewer activated dendritic cells than wild type and more common lymphoid progenitor cells than wild-type and IDH1-mutant samples (all $P < .05$). We further validated the distinct immune microenvironment

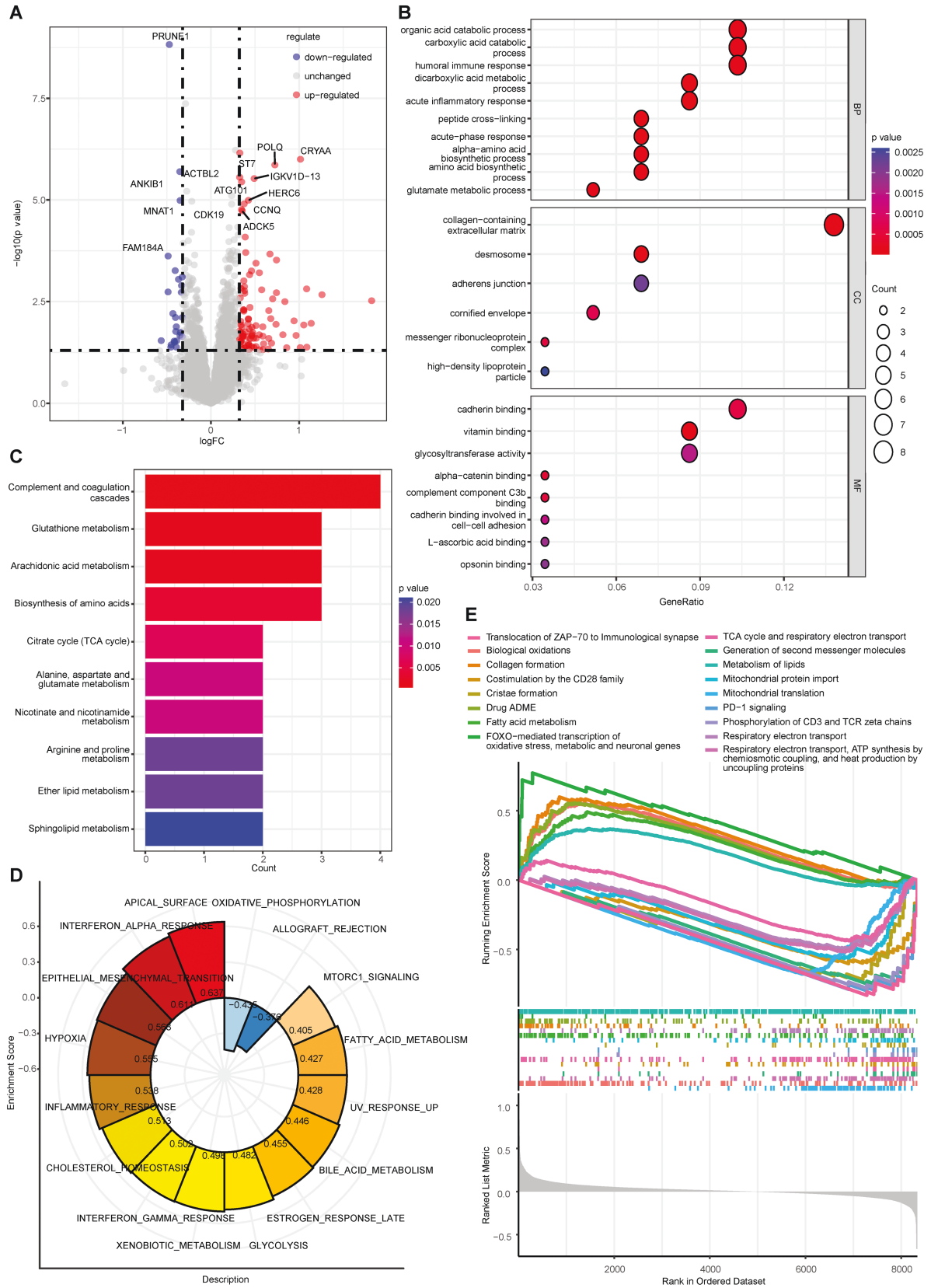

Figure 2. Differential analysis and enrichment analysis of the proteomic data between mutated IDH and wild-type IDH in Zhe cohort ($n = 57$). (A) Volcano plot of differential proteins. (B) gene ontology enrichment analysis results by bubble chart. (C) Kyoto Encyclopedia of Genes and Genomes enrichment analysis results by dotplot. (D) Gene set enrichment analysis (GSEA) analysis results using MSigdb database. (E) GSEA analysis results using ReactomePA database.

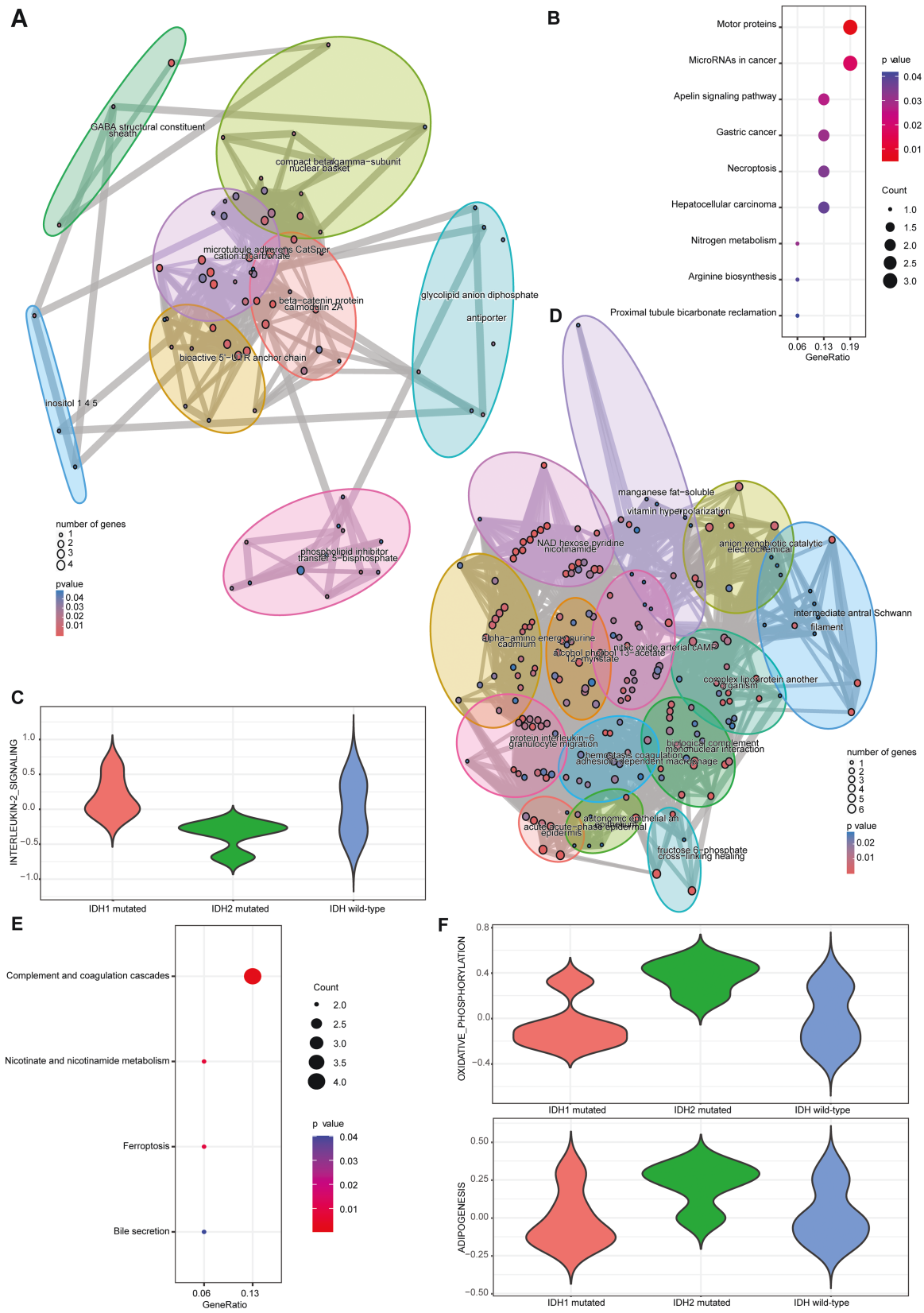

Figure 3. Enrichment analysis of the proteomic data between mutated IDH1 and mutated IDH2 in Zhe cohort (n = 57). (A) Gene ontology (GO) enrichment analysis results based on differential proteins comparing tumors with IDH1 mutations to those with IDH2 mutations and wild-type IDH. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis results based on differential proteins comparing tumors with IDH1 mutations to those with IDH2 mutations and wild-type IDH. (C) Relative enrichment score by ssGSEA comparing tumors with IDH1 mutations to those with IDH2 mutations and wild-type IDH using MSigdb database and ReactomePA database. (D) GO enrichment analysis results based on differential proteins comparing tumors with IDH2 mutations to those with IDH1 mutations and wild-type IDH. (E) KEGG enrichment analysis results based on differential proteins comparing tumors with IDH2 mutations to those with IDH1 mutations and wild-type IDH. (F) Relative enrichment score by ssGSEA comparing tumors with IDH2 mutations to those with IDH1 mutations and wild-type IDH using MSigdb database and ReactomePA database.

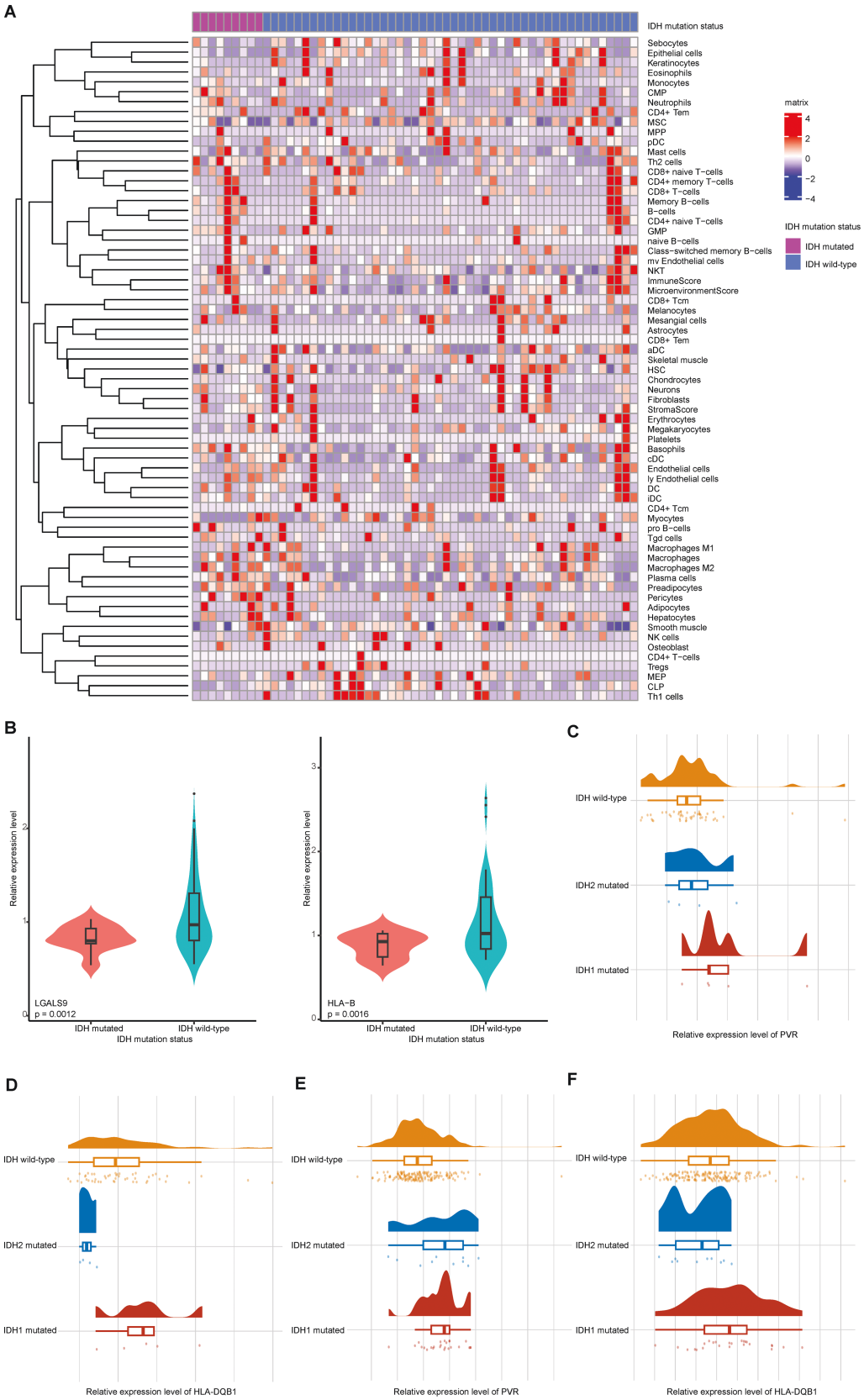

Figure 4. The immune landscape of patients. (A) Heatmap of immune components by xCell algorithm in Zhe cohort (n = 57). (B) The violin plot showed the difference in the expression of LGALS9 and HLA-B in IDH mutants and wild-type groups in Zhe cohort (n = 57). (C) The raincloud plot showed the difference in the expression of PVR in different IDH mutation status in Zhe cohort (n = 57). (D) The raincloud plot showed the difference in the expression of HLA-DQB1 in different IDH mutation status in Zhe cohort (n = 57). (E) The raincloud plot showed the difference in the expression of PVR in different IDH mutation status in Fu cohort (n = 198). (F) The raincloud plot showed the difference in the expression of HLA-DQB1 in different IDH mutation status in Fu cohort (n = 198).

profile of IDH1 and IDH2 mutations using the Fu cohort. The results of the Fu cohort found that compared with wild-type samples, IDH1-mutant samples were more enriched in CD4+ naïve T cells, conventional dendritic cells, immature dendritic cells, and NKT cells, but IDH2-mutant samples had less enriched infiltration of granulocyte-macrophage progenitors, pro B cells, common myeloid progenitors, NKT cells, and neutrophils and more enriched infiltration of CD4+ effector memory T cells, CD8+ naïve T cells, naïve B cells, and CD4+ central memory T cells (all $P < .05$). Additionally, IDH1 mutants displayed more immune cells than IDH2 mutants, including CD4+ naïve T cells, conventional dendritic cells, immature dendritic cells, memory B cells, common myeloid progenitors, and NKT cells. This indicated that samples with IDH mutations might exhibit less immune-enriched infiltration, and IDH2-mutant samples might have relatively weak enrichment of cytotoxic immune cell infiltration and relatively high enrichment of immunosuppressive cells.

In samples with IDH mutations, the enriched number of immune cells was different from that in wild-type samples, and whether the function of these immune cells was affected is unknown. Hence, we explored the differential expression level of immune checkpoints between IDH1/2 mutants and wild types. The results of the Zhe cohort showed that IDH mutants had lower expression of LGALS9 and HLA-B (Figure 4B, all $P < .05$). The expression of many immune checkpoints in IDH2 mutants was not different from that in wild type, and IDH1 mutants had higher PVR expression (Figure 4C) than wild type and higher SIRPA and HLA-DQB1 expression (Figure 4D) than IDH2 mutants (all $P < .05$). The results of the Fu cohort showed that IDH1 mutant and IDH2 mutant patients had higher PVR expression (Figure 4E) than wild type tumors (all $P < .05$). IDH1 mutants had higher HLA-DMA, HLA-DMB, and HLA-DQB1 expression (Figure 4F), and IDH2 mutants had lower CEACAM1 expression and higher CD276 and HLA-DRB4 expression than wild type (all $P < .05$). IDH1 mutants had higher CEACAM1 and HLA-DQB1 expression and lower VTCN1 expression than IDH2 mutants (all $P < .05$). These results suggested that IDH1 mutants had higher PVR expression than wild type and higher HLA-DQB1 expression than IDH2 mutants.

Proteomic subtype characteristics in IDH mutation-associated ICC

To identify different protein-based subtypes, we used a consensus clustering algorithm to perform unsupervised clustering of all proteomic data to form 4 robust clusters with 5 subtypes (Figure 5A). The fourth cluster (cluster 4) could be divided into 2 subtypes (subtypes 4 and 5). Differential analysis and GO and KEGG analysis among the subtypes were analyzed in the Zhe cohort (all $P < .05$). We found that subtype 1 was mainly enriched in DNA replication and repair (Figure 5B). Subtype 2 was primarily enriched in the regulation of glycoprotein metabolic processes and cell adhesion molecules (Figure 5C). Subtype 3 was mainly enriched in small-molecule catabolic processes and retinol metabolism (Figure 5D). Subtype 4 was mainly enriched in the regulation of plasminogen activation and complement cascades (Figure 5E). Subtype 5 was primarily enriched in mitochondrial membrane organization and apoptosis (Figure 5F). Furthermore, ssGSEA of the subtypes showed that (all subtypes $P < .05$) subtype 1 was associated with unwinding of DNA; subtype 2 was mainly enriched in the pathways related to PDGFR mutation and less enriched in interleukin-36 and interleukin-33 pathways; subtype 3 mainly associated with hormone ligand-binding receptors, formation of the active cofactor, UDP-glucuronate, and cysteine formation from homocysteine; subtype 4 was mainly associated with lactose synthesis and classical antibody-mediated complement activation; and subtype 5 was mainly associated with NLRP1 inflammasome and apoptotic cleavage of cell adhesion proteins. Therefore, subtype 1 was mainly related to DNA replication and repair functions, subtype 2 was related to cell-cell adhesion and extracellular matrix regulation, subtype 3 was related to hormones and metabolism, subtype 4 was related to complement cascades, and subtype 5 was related to the NLRP1 inflammasome pathway.

These subtype classes corresponded to the cluster classes. cluster 1 was regarded as the DNA replication and repair class, cluster 2 was regarded as the stromal regulation class, cluster 3 was regarded as the metabolism-related class, and cluster 4 was regarded as the inflammation-immune response class. Among them, tumors with IDH1 mutations were categorized into subtypes 1, 3, and 5, while tumors with IDH2 mutations were categorized into subtypes 3 and 5. Similar results were found in the Fu cohort. Consequently, patients with IDH mutations were more associated with metabolic and immune abnormalities and less involved in stromal dysregulation. Notably, some IDH1-mutant tumors belonged to the DNA replication and repair class.

Discussion

In this study, we summarized the genomic and proteomic characteristics of patients with ICC and provided a tumor landscape associated with IDH1/2 mutations through WES and proteomic profiling. Samples with IDH1/2 mutations exhibited unique molecular features, most of which were associated with cellular metabolic abnormalities, with relatively uniquely enriched DNA repair in IDH1 mutants and the secretion of biological molecules in IDH2 mutants. Inhibitory immune cells might be exhibited in IDH mutants, particularly in IDH2 mutants. Additionally, our study also provides important reference information for targeted therapy of ICC using IDH1/2 inhibitors in combination with metabolism-related drugs and immunotherapy.

TP53 and KRAS mutations in IDH-mutant tumors were less likely than in wild-type tumors, which is consistent with the previous studies.[24,25] The prevalence of IDH mutations in ICC was approximately 16%. There was no overlap between IDH1 and IDH2 mutations, and no IDH3 mutations were detected. This emphasizes the importance of detecting IDH1/2 mutations in ICC. Moreover, it has been reported that one patient developed IDH2 mutations after developing IDH1 inhibitor ivosidenib resistance.[26] This provides a theoretical basis for clinical trials using IDH2 inhibitors in the treatment of ICC. There are no studies comparing survival differences between IDH1 mutants and IDH2 mutants, and our study showed that there were no survival differences between IDH1 and IDH2 mutants and IDH wild types. However, the survival comparison between IDH mutants and IDH wild types is controversial in many studies. The results of previous systematic literature reviews and research papers showed no significant difference in survival comparisons between IDH mutants and IDH wild types, as well as no significant difference in survival comparisons between IDH1 mutants and IDH wild

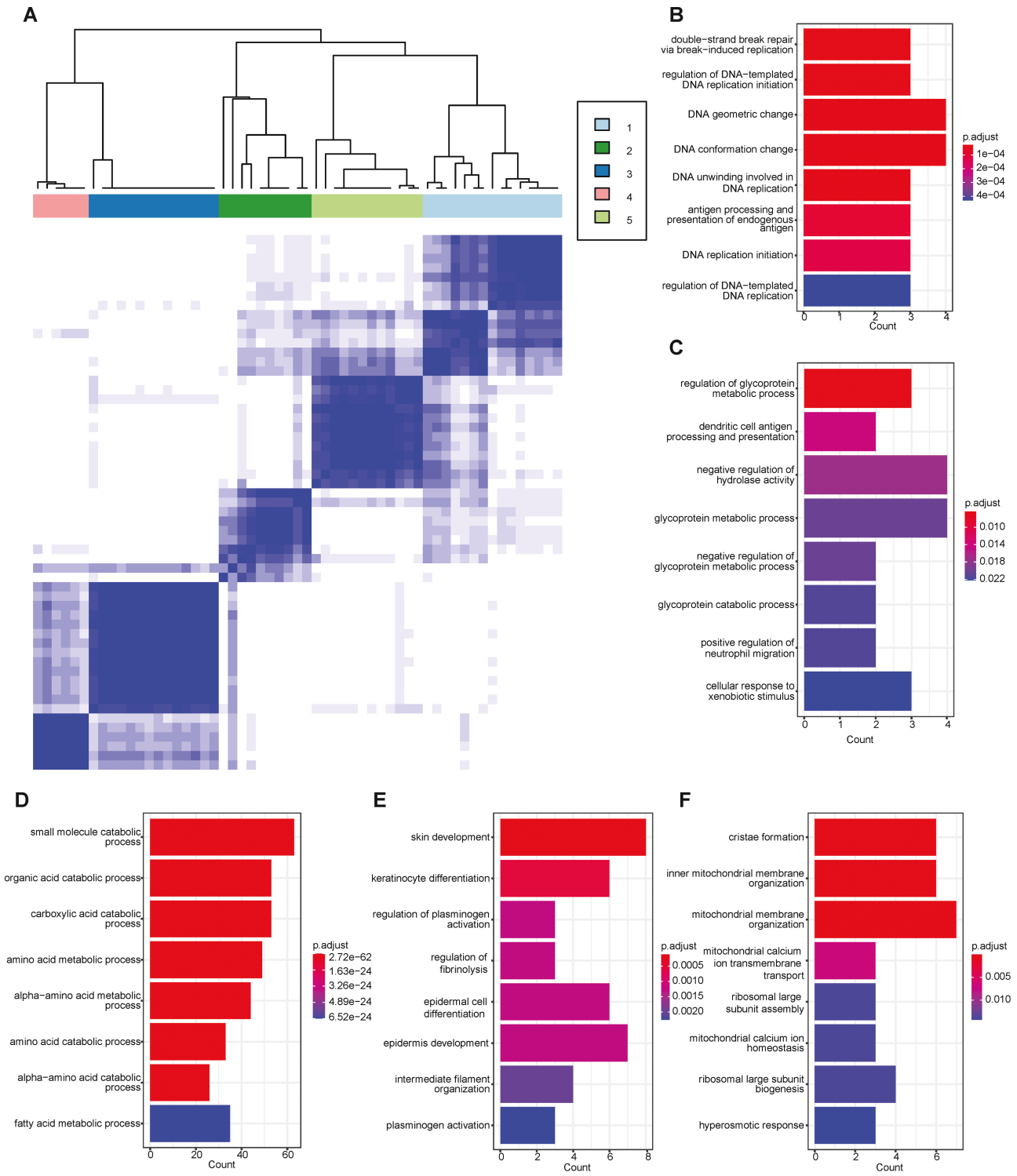

Figure 5. Proteomic subtype analysis of patients in Zhe cohort ($n = 57$). (A) Proteomic data were used to form robust 5 subtypes by consensus clustering algorithm. (B) Biological process barplot of gene ontology (GO) enrichment analysis based on differential proteins of subtype 1. (C) Biological process barplot of GO enrichment analysis based on differential proteins of subtype 2. (D) Biological process barplot of GO enrichment analysis based on differential proteins of subtype 3. (E) Biological process barplot of GO enrichment analysis based on differential proteins of subtype 4. (F) Biological process barplot of GO enrichment analysis based on differential proteins of subtype 5.

types.[10,27,28] Whereas, it has been noted in the literature that patients with mutated IDH had a relatively better prognosis relative to those with wild-type IDH.[29] It remains important to note that the number of patients with mutated IDH in the current studies on IDH1 mutations and IDH2 mutations was still relatively small, and larger sample sizes were needed to further reflect differential outcomes between survival.

IDH mutations impact relevant cellular pathways. Samples with IDH mutations showed a more significant drug interaction enrichment in the kinase pathway. Drug sensitivity tests on

IDH-mutant ICC cell lines also confirmed clear sensitivity to tyrosine kinase inhibitors.[30] The cellular metabolic reaction catalyzed by IDH is an indispensable link in the tricarboxylic acid cycle, and it mediates the production of factors involved in cellular energy metabolism and redox homeostasis. Additionally, IDH mutants acquire a novel enzymatic activity that converts α-ketoglutarate to 2-hydroxyglutarate, thereby inhibiting histone and DNA demethylases, which in turn affects epigenetics, posttranslational modification of proteins, etc.[9] These cellular metabolic activity changes associated with IDH1 and IDH2 mutations also promote tumor development and enhance tumor cell metabolism.[31] There are significant differences in molecular characterization between the IDH mutant and the wild type, and the conclusions from the Zhe cohort data are the same as those of the known studies, such as lipid metabolism, amino acid metabolism, cell-cell adhesion, and immune molecule production.[10] IDH mutations are often accompanied by a hypermethylation state[31,32] and low expression of chromatin modifiers,[33] which affect mitochondrial structure and function, glutathione metabolism, and citrate cycle in tumor cells. Our findings by GO, KEGG, GSEA, and ssGSEA enrichment analyses further indicated that IDH mutations were linked to these metabolic pathways.

Additionally, compared to IDH2 mutations, the proteomic profile associated with IDH1 mutations was enriched in DNA repair pathways. Some IDH1-mutant ICC cell lines are highly sensitive to DNA repair enzyme poly ADP-ribose polymerase (PARP) inhibitors, and experiments in vitro and in vivo have shown that IDH1 mutations make ICC cells sensitive to ionizing radiation and PARP inhibitors.[25,34] These results suggest that IDH1 inhibitors might be effective in combination with PARP inhibitors.[25,35] Furthermore, some IDH1-mutant tumors belonged to the DNA replication and repair class, while no IDH2-mutant patients were found to belong to those classes, suggesting that IDH inhibitors in combination with DNA repair-related enzyme inhibitors may benefit some patients with IDH1 mutations, but this approach may have limited benefits for patients with IDH2 mutations. Our study further found that IDH2 mutation was related to lipid metabolism, and it might be that ferroptosis therapy targeting tumor cells has some effect on tumors with IDH2 mutation. Relying on genomic and proteomic data, our results could contribute to the development of protein biomarkers for potential benefit in the clinical treatment of ICC patients with IDH mutations beyond standard chemotherapy. In the clinical management of patients with IDH1/2 mutations, it is also worth considering inhibitors targeting cellular metabolic pathways. Our analysis revealed differences in the proportion of WNT and Hippo signaling pathways among mutation-related genes in IDH1/2 mutants. The WNT pathway is closely associated with tumor epithelial-mesenchymal transition, intracellular calcium concentration, and other activities.[36] This finding validated the relationship between focal adhesion enriched in the proteomic results of IDH2 mutation. The Hippo pathway is closely related to tumor metabolic changes, including lipid metabolism.[37] It has also been verified that lipid metabolism is enriched in the proteomic results of IDH2 mutation.

Distinct immunological differences with prognostic values have been identified in many tumors and ICC. The previous studies[9,38] indicated that IDH mutants exhibited a cold tumor microenvironment, displaying less T-cell infiltration and lower cytotoxicity of T cells, consistent with our results with low immune scores. The production of D-2-hydroxyglutarate as a result of IDH mutation alters $CD8^+$ T-cell infiltration and function by undermining their lactate dehydrogenase activity.[39] IDH2 mutants showed relatively weak enrichment of cytotoxic immune cell infiltration and relatively high enrichment of immunosuppressive cells. The overall functionality of immune cells in IDH2 mutants might impede the formation of effective antitumor immune responses. Furthermore, IDH1 mutants had higher PVR and HLA-DQB1 expression, implying that it is possible that PVR and HLA-DQB1 regulation-related therapy has efficacy in patients with IDH1 mutations. One study found that IDH1 inhibitors can lead to the accumulation of $CD8^+$ T cells at the tumor site, but the expression of immune checkpoints was higher, which hampered the ability of $CD8^+$ T cells to kill tumor cells.[40] Therefore, combined and sequential therapy with IDH inhibitors and immune checkpoint inhibitors might be an area of interest.

Conclusion

Our study demonstrated the importance of comprehensive global proteomic analysis with relevant data to characterize the heterogeneity of IDH1/2 mutation-associated ICC in a reliable and clinically applicable manner. IDH1 and IDH2 mutations exhibited differential functional and immune microenvironment characteristics at the genomic and proteomic levels in ICC. In addition, this study identified candidate proteins by multiomics analysis, providing valuable resources for the development of diagnostic and prognostic biomarkers for ICC and important references for combination therapies involving IDH mutation-associated inhibitors.

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Author contributions

Conception/design: Jian Ruan, Xiaochen Zhang; data analysis and interpretation: Shuaishuai Xu, Linping Cao, Ruyin Chen; provision of study material or patients and collection and/or assembly of data: Chanqi Ye, Qiong Li, Qi Jiang, Feifei Yan, Mingyu Wan. Manuscript writing and final approval of manuscript: all authors. Shuaishuai Xu, Linping Cao, and Ruyin Chen are contributed equally.

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Conflicts of interest

The authors declare no competing interest.

Data availability

The data have been deposited at iProX (Project number: IPX0003037000).

Supplementary material

Supplementary material is available at *The Oncologist* online.

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