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Analysis

Integrative analysis of the expression profile and prognostic values of SENP gene family in hepatocellular carcinoma

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Abstract

Introduction Hepatocellular carcinoma (HCC) stands as the fourth leading cause of cancer-related deaths worldwide. SUMO-specific peptidases, known as SENPs, emerge as critical players, regulating tumorigenesis and progression of numerous cancer types. Despite this, the specific impact of SENPs in HCC remains unclear. Hence, our study aimed to reveal the immune and prognostic implications of SENPs in HCC.

Methods The gene expression of SENP in various cancers was examined using open-access databases including TCGA, GTEx, and CPTAC. In order to investigate the prognostic potential of the SENP family, Kaplan–Meier analysis was used. To clarify the underlying biological mechanisms, gene set enrichment analysis (GSEA) was carried out. cBioPortal database was used to evaluate genetic mutation profiles. For insight into the relationship between SENP genes and tumor immunity, various algorithms were used.

Results Our findings showed that SENP1, SENP2, SENP3, SENP5, SENP6, and SENP7 expression levels were significantly higher in HCC tumor tissues compared to normal tissues. In HCC patients, elevated SENP1 and SENP5 expression has been associated with tumor development and poor outcomes. Our immune infiltration patterns results also showed significant correlations between SENP5 expression and neutrophil ($cor = 0.346$, $p < 0.001$), myeloid dendritic cell ($cor = 0.491$, $p < 0.001$), macrophage ($cor = 0.465$, $p < 0.001$), and memory B cell ($cor = 0.336$, $p < 0.001$) infiltration in HCC, whereas SENP1 expression was associated with none of these infiltrations.

Conclusions The prognostic and immunogenetic value of SENP1 and SENP5 in HCC was demonstrated in this study. Therefore, these two genes have the potential to function as separate prognostic biomarkers and offer promise as immunotherapeutic targets in the fight against HCC.

Keywords Hepatocellular carcinoma · SENP family · Biomarkers · Prognostic value · Immune infiltration

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1 Introduction

One of the most common cancers worldwide, primary liver cancer will affect approximately 906,000 new cases and 830,000 fatalities in 2020. 75–85% of the incidence and mortality rates are caused by hepatocellular carcinoma (HCC) [1]. The prognosis for HCC remains poor, with a 5-year survival rate of only about 12%, despite outstanding efforts in diagnostic procedures and therapeutic approaches [2]. The reality not only poses a tremendous burden on public health but also exerts a significant economic burden on society. Potential curative interventions, including surgical resection, liver transplantation, and local radiofrequency ablation, are viable options solely for those diagnosed in the early stages [3]. Regrettably, HCC is often detected until it reaches an advanced phase, making these curative methods ineffective. Consequently, systemic pharmacological treatment emerges as the only therapeutic option for patients under this condition [4]. Despite notable advancements in cancer pharmacology, exemplified by the approval of various tyrosine kinase inhibitors (TKIs) and immunotherapy-based drugs, the efficacy of these innovations varies widely, from modest to very poor for the majority of patients [5]. Therefore, there is a pressing need for highly precise and efficient biomarkers that are crucial for accurate diagnosis and reliable prognostic assessment. These biomarkers have the potential to not only enhance prognostic accuracy but also facilitate personalized treatment strategies tailored to the specific needs of individual HCC patients, thereby improving clinical outcomes and offering new hope in the management of this disease.

Small ubiquitin-like modifiers (SUMOs) are a family of small proteins that resemble ubiquitin and go through a highly conserved process called SUMOylation during post-translational modification [6]. This modification, facilitated by SUMO proteins, influences substrate proteins and is tightly regulated through maturation and deconjugation (deSUMOylation) processes. Sentrin-specific proteases (SENPs), a group of cysteine proteases that includes SENP1, 2, 3, 5, 6, 7, and 8, control these processes [7]. Within the SENP protease family, SENP1-SENP7 serve as master regulators of SUMOylation dynamics through their conserved isopeptidase activity, whereas SENP8 displays exclusive catalytic specificity for NEDD8, a phylogenetically distinct ubiquitin-like modifier. The SUMO pathway mediates protein–protein interactions, determines subcellular localization, improves stability, and modifies the enzymatic function of target substrates, all of which are significant cellular processes [8]. SUMOylation can concurrently target multiple members within a protein group, thereby modulating specific pathway effectively [9]. For maintaining typical cellular physiology, a balance between SUMO-modified and unmodified proteins must be carefully maintained by SENP enzymes. According to mouse knockout studies, SENP1 and SENP2 are crucial for development because their absence causes embryonic lethality [10, 11]. However, due to altered SENP expression in pathological conditions, this delicate balance between SUMO-modified and unmodified proteins is upset. Numerous SENP isoforms have been linked to the emergence of a number of diseases, including atherosclerosis, thyroid cancer, colon cancer, pancreatic cancer, and prostate cancer [12–18].

In our current study, we conducted comprehensive analysis of SENP expression profiles, specifically focusing on SENP1-7, in HCC. Our finding revealed a significant overexpression of SENP1-7 in HCC. By using Kaplan–Meier Plotter analysis, prognostic relevance of SENPs has been found. According to the findings, elevated levels of SENP1 and SENP5 were strongly linked to lower rates of overall survival (OS) and recurrence-free survival (RFS) in patients with HCC. Additionally, a thorough investigation of the relationship between SENPs expression and immune cell infiltration in HCC was done as part of our research. There is an intriguing association between SENP1 expression and different immune cell subtypes in the microenvironment of HCC, including neutrophil, myeloid dendritic cell, M0 macrophage, and memory B cell. These findings demonstrated the potential of SENP1 as a promising prognostic biomarker in HCC. Our research clarifies SENP1's active participation in the immune response to HCC. This knowledge not only offers a chance for the creation of therapeutic drugs that are specifically targeted, but it also offers priceless insights into the network of immunogenic-mediated HCC.

2 Materials and methods

2.1 Gene and protein expression profile

Analysis of SENP-related gene and protein expression profiles has been carried out. The Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) datasets, which cover various tumor types and their corresponding normal samples, were the sources of the gene expression data as well as clinical information [19]. SENPs expression

between 50 normal and 374 HCC patients has been compared using the Wilcoxon rank sum test. The "ggplot2" package has been employed for data visualization of the relationship between SENPs and clinical stages/grades. Using the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN) tool, protein expression analysis of SENPs was carried out. 165 normal samples and 165 primary HCC samples from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) dataset were used in this analysis [20, 21].

2.2 Survival analysis of SENPs in HCC

The Kaplan–Meier (KM) plotter was used to conduct a survival analysis of SENPs in HCC. The "auto select best cutoff" approach was employed to assess the prognostic significance of SENPs in HCC. This method involved the computation of all feasible cutoff values, allowing the evaluation of various thresholds and the selection of the most optimal one.

2.3 Genetic alteration analysis

The cBioPortal platform integrates extensive tumor genome data from large-scale projects such as TCGA and the International Cancer Genome Consortium (ICGC). It provides access to genomic information derived from over 28,000 tumor samples, facilitating comprehensive cancer research and data analysis [22]. Epigenetic modification, gene expression profiles, and proteomic data are among the multidimensional cancer genomics data that were explored and analyzed using the cBioPortal database [22]. Therefore, the frequency and various forms of SENPs in 372 HCC samples were assessed using the cBioPortal.

2.4 Immune subtype and tumor microenvironment (TME) analysis

In this study, the R packages 'limma,' 'ggplot2,' and 'reshape2' were utilized to analyze the immune subtypes of SENPs. Immune scores, stromal scores, and estimate scores were extracted from various tumor samples. A correlation analysis was conducted to assess the relationship between SENPs expression and estimate scores across 33 TCGA tumor types, including HCC. Additionally, the CIBERSORT algorithm was employed to characterize 22 types of tumor-infiltrating immune cells (TIICs) across different cancer types. The TIMER 2.0 database was used to evaluate the correlation between specific immune cell infiltration and SENPs expression levels in HCC. To ensure accuracy, purity-adjusted Spearman's rank correlation tests were performed. The results of these analyses were visually represented using heatmaps and scatter plots.

2.5 The biological significance of SENPs expression in tumors

GSEA, or Gene Set Enrichment Analysis, was carried out. The official GSEA website (https://www.gsea-msigdb.org/gsea/downloads.jsp) provided the gene sets for the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The R-packages"limma","clusterProfiler", and"enrichplot"were used to conduct the subsequent functional analysis. Gene sets meeting the threshold of p-value < 0.05 combined with a false discovery rate (FDR) q-value < 0.25 were identified as statistically significant enriched pathways.

2.6 Correlation analysis of SENPs with immune checkpoint inhibitors (ICIs) biomarkers

TME has the potential to assess the state of the innate and adaptive immune system, providing information that will help identify reliable biomarkers for estimating the clinical effectiveness of checkpoint inhibitor therapy [23]. The co-expression of five important immune checkpoint-related genes in pan-cancer, including CD274 (PDL1), CTLA4, PDCD1 (PD-1), PDCD1LG2 (PD-L2), and LAG3, was examined using Spearman correlation analysis. The R packages"RColorBrewer,""reshape2,"and"limma"were utilized to conduct the analysis.

3 Results

3.1 Gene expression data of SENPs

The research flowchart is presented in Fig. 1. Figure 2A portrays SENPs expression in HCC tissues versus non-tumor tissues. SENP1-7 showed a significant upregulation in HCC, indicating their critical function in the development of

HCC ($p < 0.05$). The high expression patterns of SENP1-8 in paired normal tissues and HCC were also displayed in Fig. 2B. Beyond HCC, the analysis has been expanded to look at SENP expression in 33 different cancer types from the TCGA and GTEx datasets (BLCA, BRCA, CHOL, COAD, ESCA, LUAD, GBM, HNSC, KIRC, UCEC, READ, KICH, LIHC, KIRP, LUSC, THCA, PRAD, and STAD). Our findings showed that SENP1 and SENP8 as consistently highly expressed across most cancers (Fig. S1 A–G). Contrary to normal tissues, SENP6 and SENP7 showed diminished expression.

The CPTAC dataset was used to analyze SENP protein expression in addition to transcription. The findings showed that HCC tissues had higher levels of SENP1, SENP2, and SENP3 proteins than control tissues (Fig. 2C–H). The level of SENP8 protein was, however, lower in HCC tissues than in healthy liver tissues. These findings demonstrate how SENP proteins may affect the course and outcome of HCC. Analysis has also been done on the relationship between the expression of SENPs and the pathological stages of HCC patients. There is a stage-specific alterations in SENP1, SENP5, and SENP6 expression (Fig. S1H-N). Notably, the expression of these three genes increased predominately in the advanced tumor stage of HCC. These findings underscore the potential of SENPs as stage-specific biomarkers, hinting at their utility in prognostic assessments.

3.2 Prognostic value of SENPs in HCC

In order to better understand the relationship between SENPs expression and clinical outcomes in HCC, a Kaplan–Meier analysis was performed, concentrating on overall survival (OS) and recurrence-free survival (RFS). The findings from OS analysis revealed a significant correlation between elevated SENP1, SENP2, SENP3, SENP5, SENP6, and SENP7 and unfavorable outcomes among HCC patients. Interestingly, increased expression of SENP8 was associated with improved outcomes in HCC (Fig. 3A–G). Additionally, we examined the relationship between SENPs and RFS, and the results showed that higher levels of SENP1 and SENP5 expression were linked to worse RFS (Fig. 3H–N).

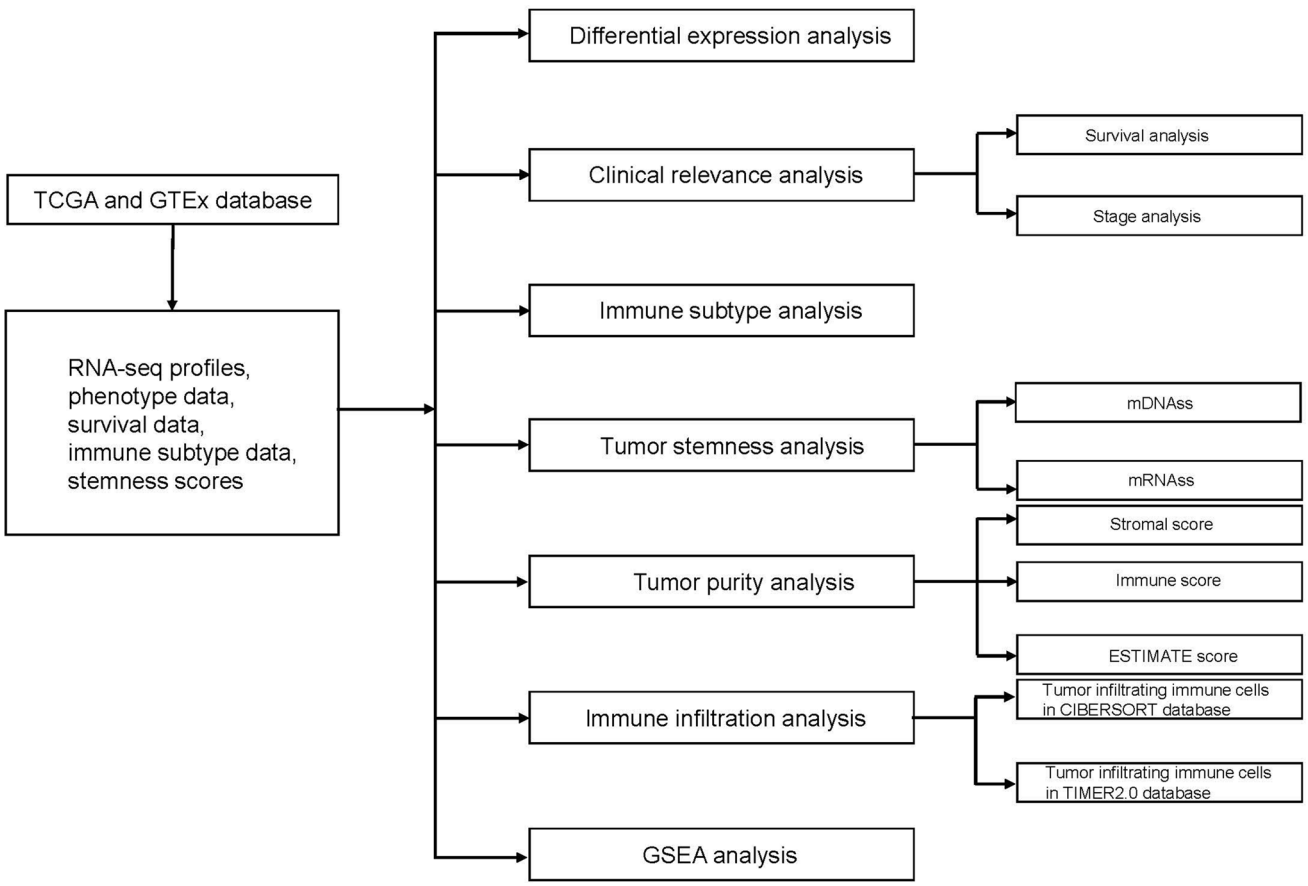


Fig. 1 The flowchart of the whole analytic process of our study

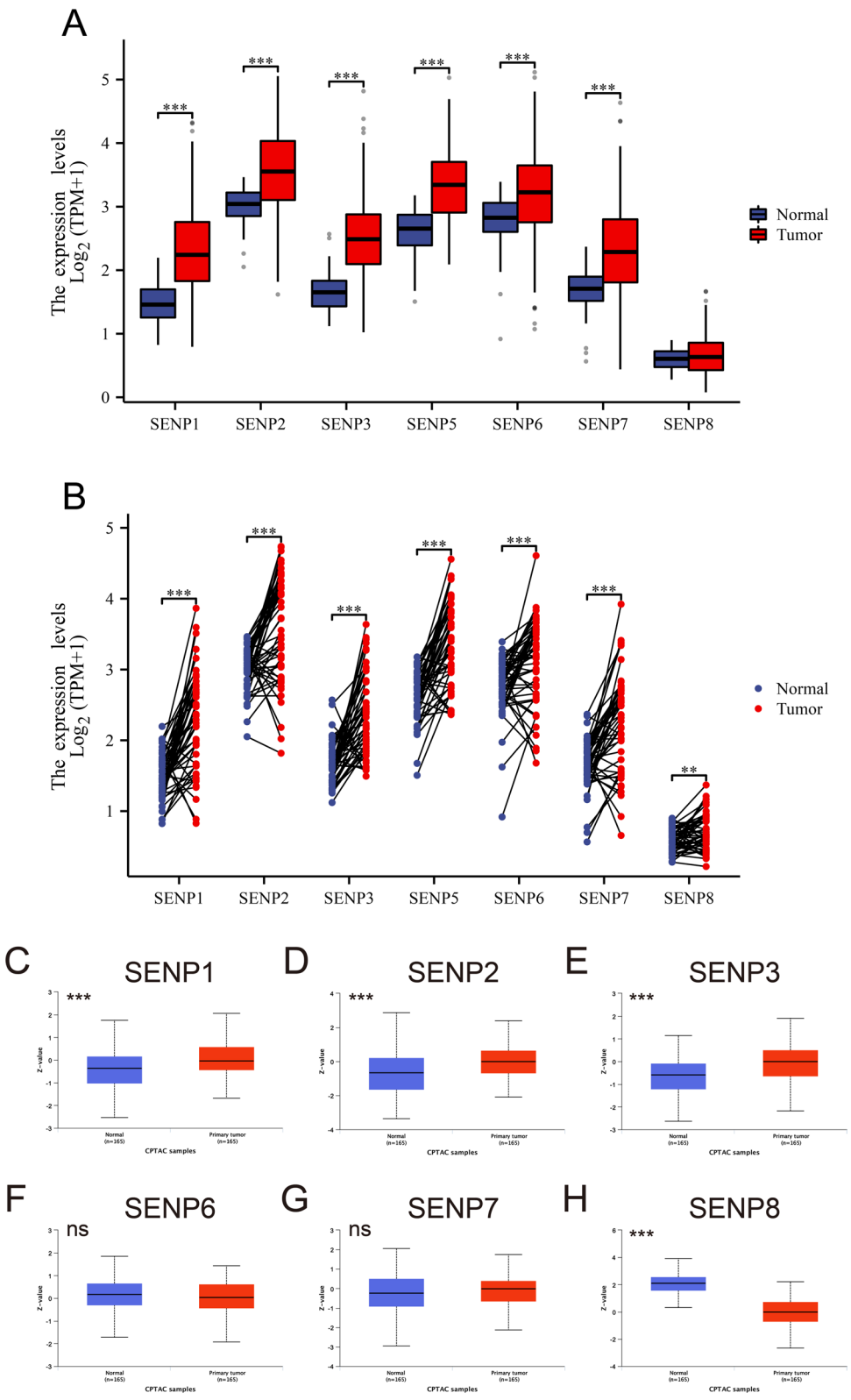


Fig. 2 **A** SENPs mRNA expression profiles in LIHC and normal tissues sourced from TCGA and GTEx database. The blue boxplots represent the normal tissues. The red boxplots represent LIHC tissues. **B** Expression levels of SENPs in LIHC and paired normal tissues from TCGA. **C–H** The expression levels of SENP gene families in LIHC. The blue boxplots represent the normal tissues. The red boxplots represent LIHC tissues

3.3 Genetic alterations in SENPs in HCC

The genetic landscape of SENPs in HCC has been studied by analyzing 372 HCC samples using the cBioPortal database. Interesting patterns of genetic alterations within SENPs have been revealed. Among the SENP family, SENP6 exhibited the highest variation rate at 4% (Fig. 4A), while SENP1 and SENP8 had the lowest rate at 0.3%. Out of 372 HCC samples, 35 patients demonstrated genetic alteration in SENPs, contributing to an overall variation rate of 10%. These alterations

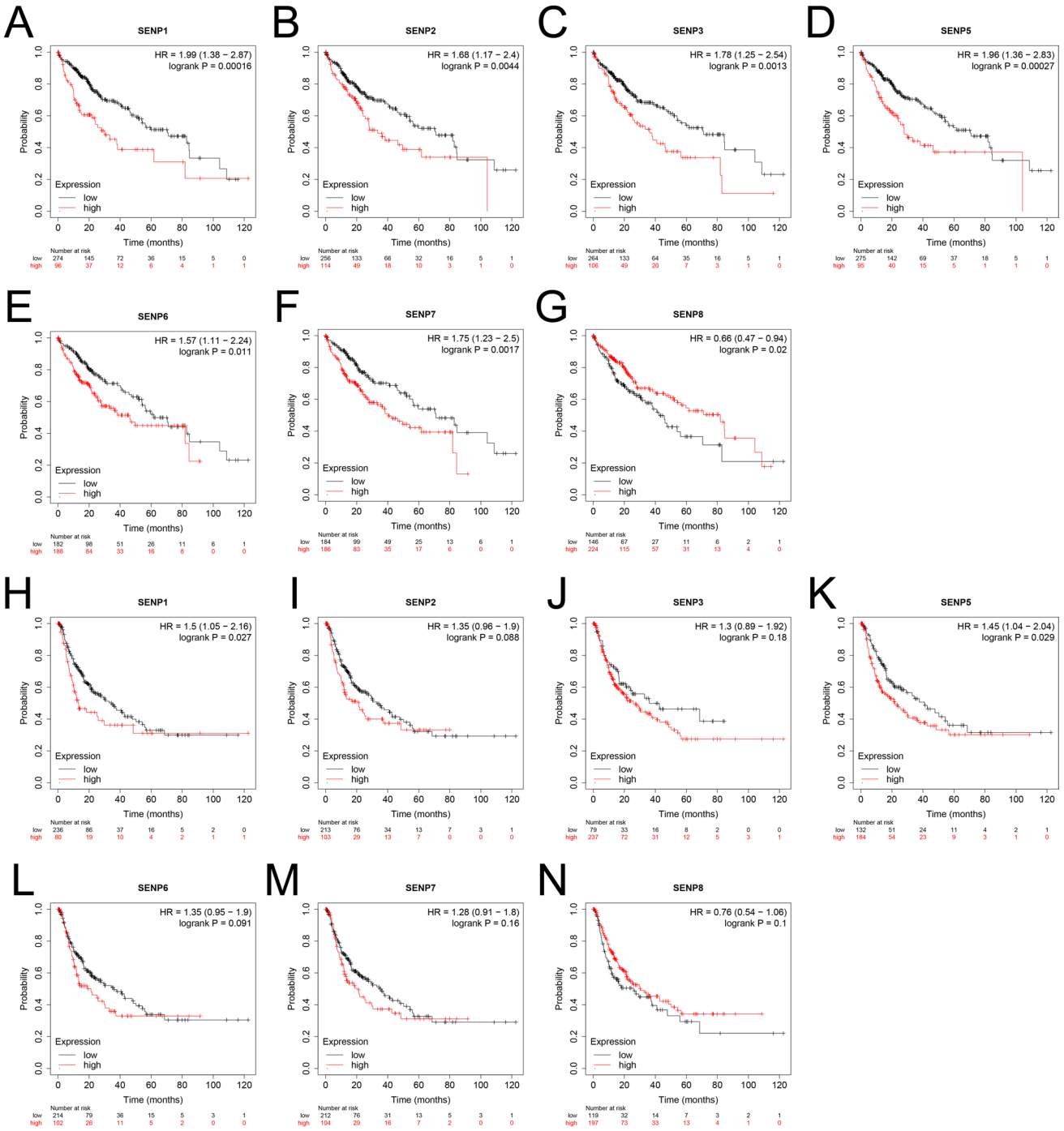


Fig. 3 **A–G** Correlation analysis between SENPs expression and OS in LIHC. The analysis was conducted through the use of KM plotter tool. Red lines indicate high expression of SENPs, and black lines indicate low expression of SENPs. **H–N** Correlation analysis between SENPs expression and RFS in LIHC

included gene mutation, amplification, and deep deletion. Deep deletion and gene amplification were predominantly in SENP1 and SENP3 (Fig. 4B).

3.4 Immune subtype and tumor microenvironment (TME) analysis of SENPs

Cancer stem cells play a key role in various tumorigenic processes, including proliferation, migration, metastasis, angiogenesis, and therapy resistance [24]. To reveal the complicated connection between SENPs expression and cellular stemness, comprehensive analysis has been conducted. According to our research, SENP2 expression positively correlated with both the RNA stemness score (RNAss) and DNA stemness score (DNAss) (Fig. 5A). In HCC, SENP3 expression was discovered to have a positive correlation with RNAss and a negative correlation with DNAss. While in HCC, SENP8 was positively correlated with DNAss and negatively correlated with RNAss. In addition, the relationship between SENP expression and DNAss and RNAss varied across different cancer types. For example, in CHOL, all SENP genes exhibited a significantly positive correlation with RNAss. In addition, SENP7 consistently showed an inverse relationship with DNAss in most malignancies (Fig. S2 A, B).

Within the TME, immune cells and stromal cells emerge as important components, serving as prognostic biomarkers for cancer patients [25]. Quantitative scores derived from ImmuneScore and StromalScore provide insights into the

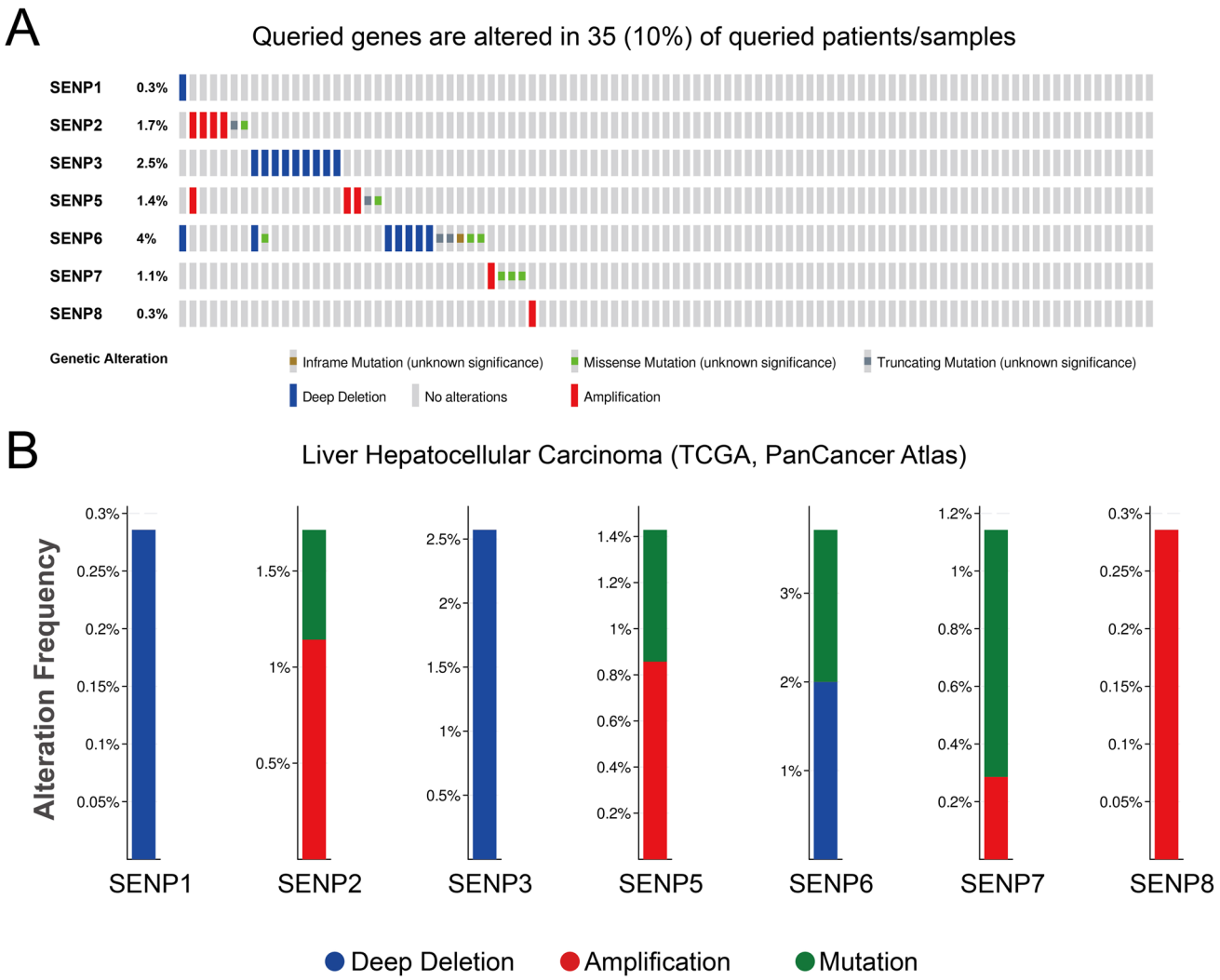


Fig. 4 Genetic mutations and correlation analysis of SENP family genes in LIHC. **A** overview of alteration rates among SENP family members in LIHC. **B** Frequency data of genetic alteration in SENP family genes in LIHC using cBioPortal

Fig. 5 **A** Correlation analysis between the expression of SENPs and RNAss, DNAss, StromalScore, ImmuneScore, and EstimateScore in LIHC. ▶ R indicates correlation value. **B** Comparative analysis of SENPs expression across immune subtypes in (**A**) LIHC and **C** pan-cancer. X axis indicates immune subtype, and Y axis indicates gene expression levels. C1, wound healing; C2, IFN-g dominant; C3, inflammatory; C4, lymphocyte depleted; C5, immunologically quiet; C6, TGF-β dominant. ** $p < 0.01$, *** $p < 0.001$

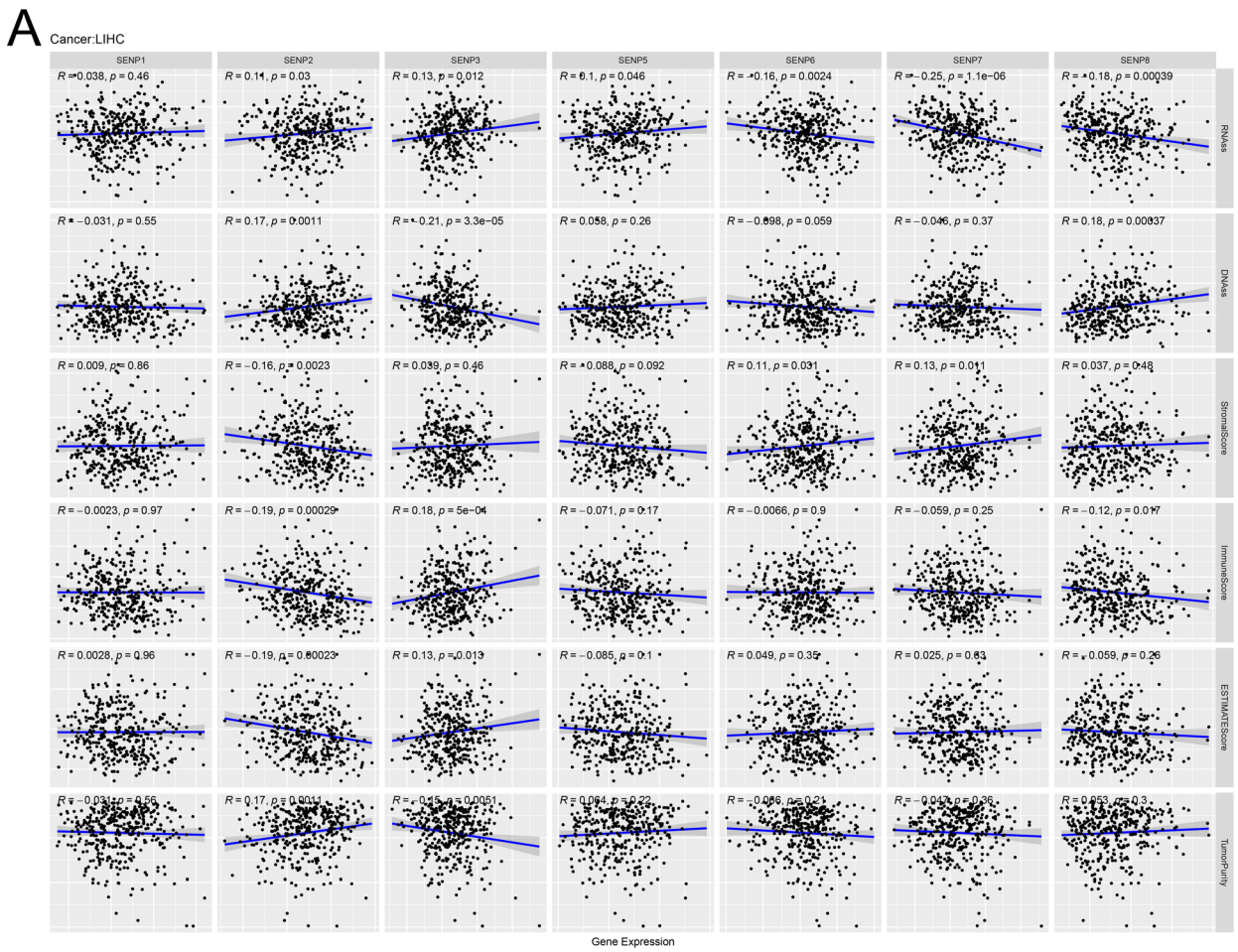
proportion of immune and stromal components, while ESTIMATEScore provides both aspects. Our analysis revealed interesting association between SENPs expression and these scores. SENP2 exhibited a significant negative correlation with ImmuneScore, StromalScore, and ESTIMAREScore in HCC. Conversely, SENP3 displayed a positive association with ImmuneScore and ESTIMATEScore. The expression of SENP6 and SENP7 was positively correlated with stromalScore. While SENP8 expression and ImmuneScore had a negative correlation. We also investigated into how these scores in pan cancer relate to the expression of SENPs. In ACC, GBM, SARC, THCA, and UCEC, all SENP genes were discovered to be specifically positively correlated with StromalScore and adversely correlated with ImmuneScore (Fig. S2 C, D). On the contrary, the expression of SENP1, SENP5, SENP7 was significantly negatively correlated with StromalScore and positively linked to ImmuneScore in OV and PAAD. Notably, all SENP genes were negatively associated with ESTIMATEScore in most types of malignances (Fig. S2E). Meantime, Fig. S2 F demonstrated that all SENP genes were negatively correlated with tumorpurity in GBM and SARC. These results demonstrated the distinctive roles of SENP family members in orchestrating the intricate immune microenvironment within divergent cancers.

The relationships between immune subtype and SENPs expression were then examined. Six categories were used to classify immune subtypes: C1 (wound healing), C2 (IFN-gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet) and C6 (TGF-b dominant) [26]. In LIHC, SENP3, SENP5, and SENP6 exhibited the most pronounced expression across all five subtypes. Particularly noteworthy was the observation that in subtype C1, SENP1, SENP3, SENP6, and SENP7 displayed the highest expression, while SENP5 was predominantly expressed in C2 (Fig. 5B). Furthermore, our result indicated significant variations in SENPs expression across these immune subtypes in pan-cancer analysis (Fig. 5C). The highest expression was seen in C5, specifically SENP2, SENP3, SENP5, SENP6, and SENP7, while the highest expression was seen in C2.

3.5 Connection between SENPs expression and immune cell infiltration in cancer

The presence and behavior of immune cells that have infiltrated the tumor stand out as significant factors affecting the development, progression, and metastasis of cancer within the TME [27]. We used the"CIBERSORT"algorithm to assess 22 immune cell types based on published data in order to better understand the relationship between the expression of SENP1 and SENP5 and immune cell infiltration in pan-cancer. According to our analysis, the majority of cancers showed a negative correlation between SENP1 expression and regulatory T cell (Treg), $CD8^+$ T cell, and activated NK cell (Fig. 6A). It is interesting to note that SENP1 expression showed a positive correlation with naive B cell in different cancer types. The complexity of immune cell interactions within the tumor environment was particularly highlighted in LIHC by the positive associations between SENP1 expression and neutrophil, myeloid dendritic cell, M0 macrophage, and memory B cell. To validate and reinforce our finding, "TIMER" algorithm has been employed, focusing specifically on LIHC (Fig. 6B–E). The outcomes from both algorithms were consistent with our initial observations, establishing a link between SENP1 expression and immune cell landscapes within the LIHC microenvironment. In addition, SENP5 expression was negatively correlated with Treg, activated NK cell, memory B cell and positively associated with naive B cell in most cancers (Fig. 7A–C).

Furthermore, coexpression analysis has been performed, revealing the interaction between SENPs expression and key immune checkpoints in LIHC (Fig. 7D–J). SENP1, SENP3, SENP5, and SENP6 exhibited positive correlation with immune checkpoints in LIHC. In addition, Fig. S3 A-G demonstrated the relationship between the expression of SENPs and more immune checkpoint molecules in pan-cancer. The potential use of the SENP family genes in immunotherapy is highlighted by these findings.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ImmuneScore
ESTIMATEScore
TumorPurity
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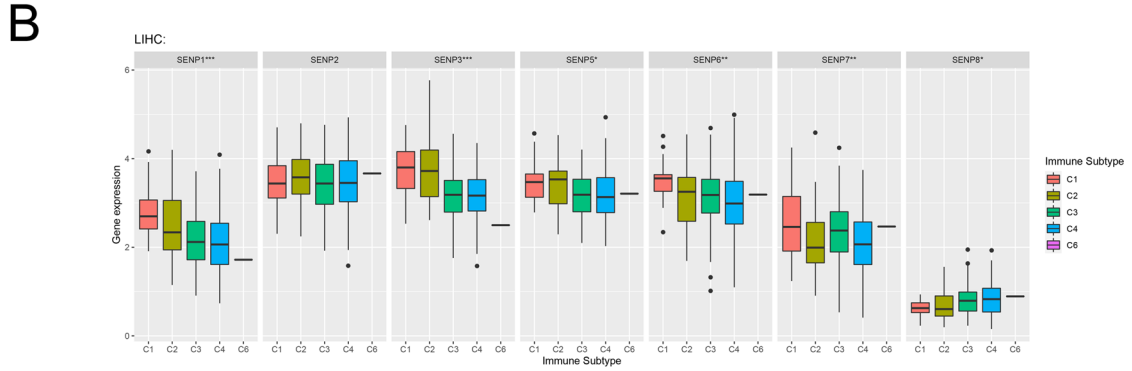
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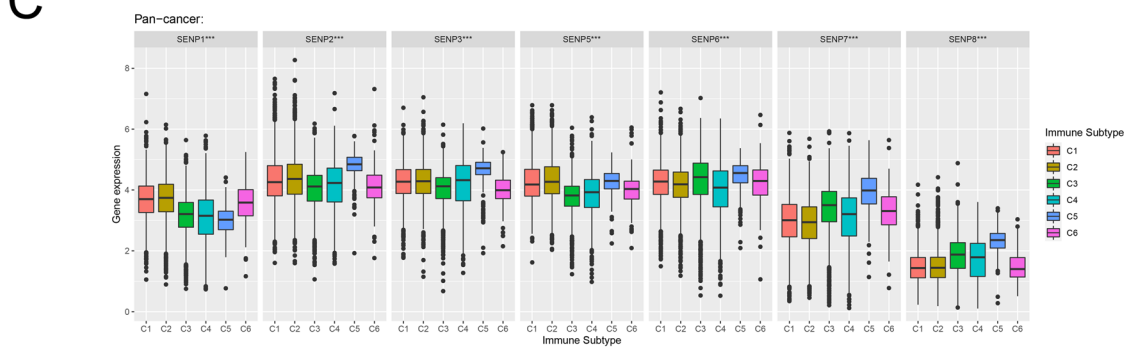
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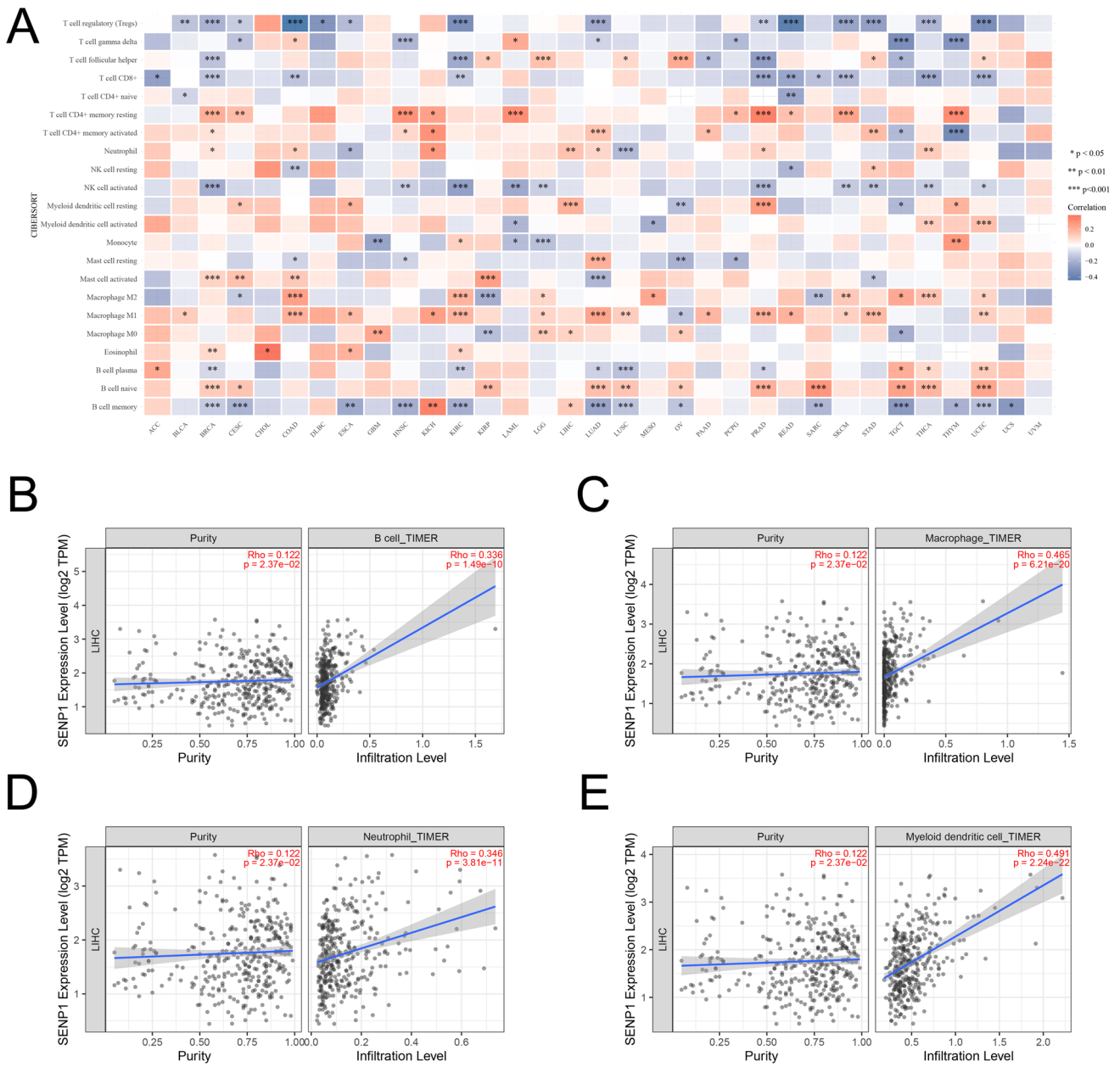


Fig. 6 Correlation analysis of SENP1 expression with immune cell infiltration analysis in LIHC. **A** The relationship between SENP1 expression level and the infiltration levels of immune-related cells. **B–E** The scatter plots of relationship between SENP1 expression and infiltration levels of immune-related cells by using TIMER2 database *p < 0.05, **p < 0.01, and ***p < 0.001

3.6 GSEA analysis of SENPs

GSEA analysis was carried out to examine the biological importance of SENPs expression in HCC (Fig. 8). According to our findings from Gene Ontology (GO) functional annotation, all SENPs exhibited a positive correlation with actin cytoskeleton in HCC. The activities of 3′–5′ exonuclease, acetyltransferase, actin binding, actin cytoskeleton, and actin cytoskeleton remodeling were revealed to be particularly concentrated in SENP1. While SENP5 was largely enriched in actin binding, actin cytoskeleton, actin filament binding, and actin filament binding rearrangement (Fig. 8A), it was also active in acetyltransferase activity. The result of KEGG analysis demonstrated that SENP1, SENP2, SENP5, SENP6, SENP7 were significantly positively associated with adherens junction. And SENP2, SENP3, SENP5, SENP6,

SENP7 were positively associated with cell cycle. In particular, it was shown that SENP1 was significantly abundant in adherens junction, apoptosis, axon guidance, and B cell receptor signaling pathway. According to Fig. 8B, SENP5 was predominantly positively correlated with adherens junction, apoptosis, cell cycle, acute myeloid leukemia, and chronic myeloid leukemia.

Additionally, we performed a correlation analysis across multiple cancer types, revealing that SENP5 and SENP2 exhibited the strongest positive correlation (correlation coefficient = 0.8; Fig. S4 C). The expression heatmap for the ten genes across 33 TCGA pan-cancer datasets is illustrated in Fig. S4 A. SENP2 showed the highest expression level in TGCT and LUSC, while SENP5 had the low expression in LUSC. As depicted in Fig. S4B, SENP6 emerged as the gene with the highest overall expression in pan-cancer, whereas SENP8 displayed the lowest expression level across the pan-cancer dataset.

4 Discussion

SUMOylation plays a vital role in cancer by protecting chromosomal integrity and regulating cell proliferation, according to newly discovered study. Within cancer tissue, increased expression of various SUMO components strongly indicates the association between activated SUMOylation and tumor proliferation [28]. Numerous cancer forms, such as ovarian, colon, and prostate cancer, have high levels of the SUMO conjugating enzyme Ubc9, which encourages cell invasion and metastasis [29, 30]. Unexpectedly, malignant malignancies have higher levels of the SUMO proteases SENP1 and SENP5, indicating the necessity of controlling SUMOylation to stop the spread of the disease and unregulated cell proliferation [31, 32].

Additionally, research using knockdown mice demonstrates the crucial involvement of SUMO E1 subunit SAE2 in promoting the formation of colon tumors, demonstrating the functional importance of SUMOylation in carcinogenesis [33]. Myc-driven cancers also require the SUMO E1 enzyme. The therapeutic importance of comprehending SUMOylation processes is shown by the relationship between decreased levels of SUMO-activating enzyme and longer metastasis-free survival in patients with Myc-dependent breast cancer [34]. Previous studies have identified SENP3 as a reliable prognostic biomarker for hepatocellular carcinoma [35]. Furthermore, SENP5 has been found to be overexpressed in HCC samples and is essential for the proliferation of HCC cells in both in vitro and in vivo models [36]. These results highlight the potential of SUMO machinery targeting for cancer treatment and call for a thorough investigation of the expression profile, prognostic implications, and possible roles of SENP family genes in HCC. Such endeavors hold the potential to uncover novel targets for inhibition and innovative therapeutic strategies, promising hope for patients with HCC.

In our study, expression patterns and clinical characteristics of SENPs have been examined in relation to HCC stages. SENP1, SENP5, and SENP6 are significant markers associated with different stages of HCC. Through survival analysis, prognostic role of the SENPs has been further evaluated, showing that SENP1 and SENP5 expressions were significantly linked to the OS and RFS in HCC patients. Moreover, our research unveiled that SENP1, SENP3, SENP6, and SENP7 exhibited increased expression in subtype C1, while SENP5 predominantly in subtype C2. The expression of SENP2 linked favorably with both RNA and DNA stability. In HCC, SENP3 expression was discovered to have a positive correlation with RNAss and a negative correlation with DNAss. While in HCC, SENP8 was favorably connected with DNAss and negatively correlated with RNAss. SENP2 exhibited a significant negative correlation with ImmuneScore, StromalScore, and ESTIMAREScore in HCC. Conversely, SENP3 displayed a positive association with ImmuneScore and ESTIMATEScore. The expression of SENP6 and SENP7 was positively linked with stromalScore. While SENP8 expression and ImmuneScore had a negative correlation. These results showed how SENPs and the dynamic TME in HCC have a complex relationship.

Foxp3-expressing regulatory T cells (Tregs) are essential for maintaining self-tolerance because they inhibit immunological responses to either self- or foreign-derived antigens [37–39]. Tregs can dramatically affect clinical outcomes in tumor by suppressing anti-tumor immunity and promoting angiogenesis, which eventually aids in the course of the illness [40]. Consequently, they pose a significant challenge to the effectiveness of cancer immunotherapy [41]. In our study, a strong correlation between the expression of important immune checkpoint genes and SENP1 and SENP5 was found. The amount of Treg infiltration was negatively correlated with these proteins. As a result of this discovery, it was shown that SENP1 and SENP5 might be useful positive indicators for estimating the prognosis of HCC patients receiving immunotherapy.

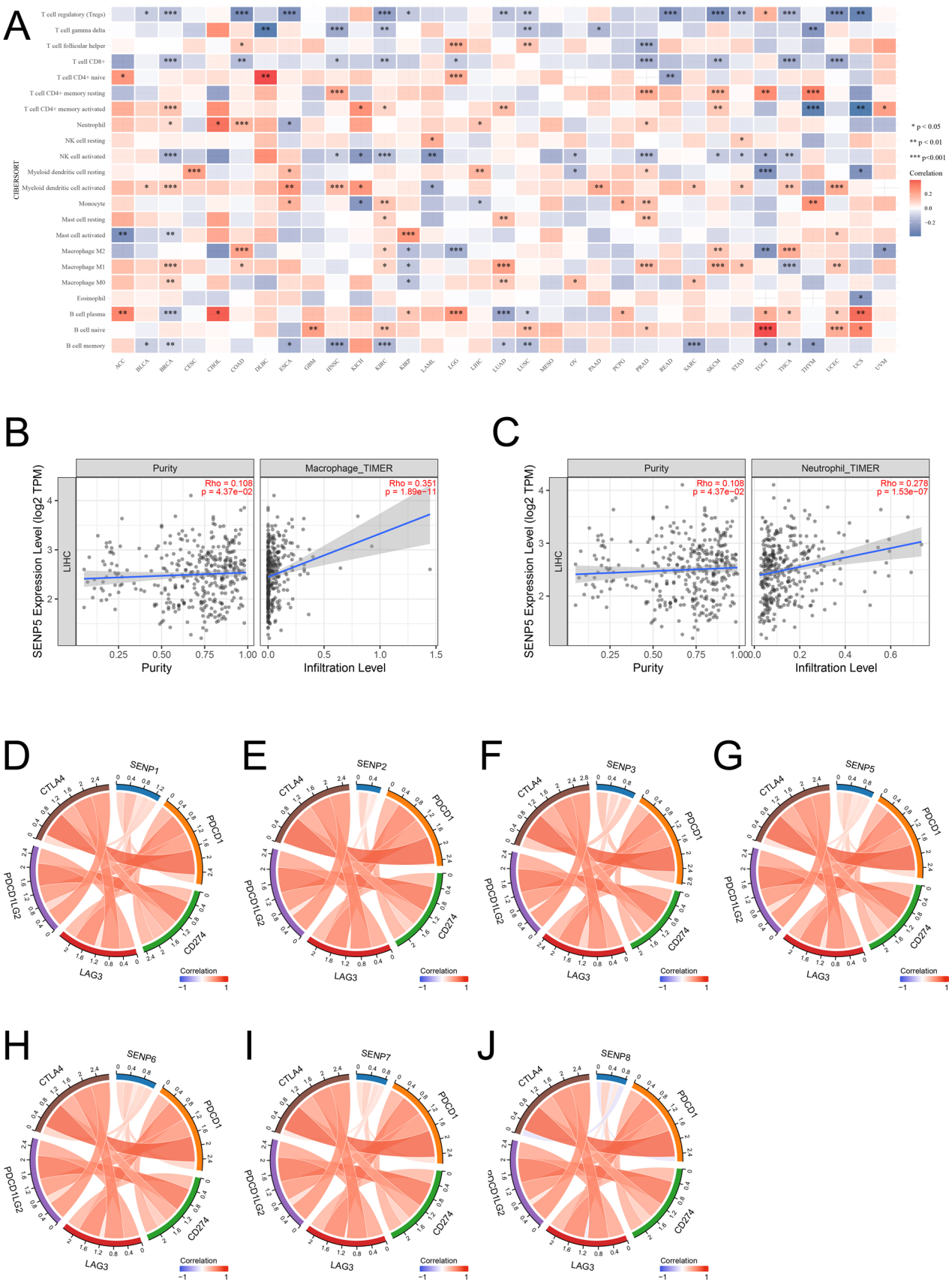
Fig. 7 Correlation analysis of SENP5 expression with immune cell infiltration analysis in LIHC. **A** The relationship between SENP5 expression level and the infiltration levels of immune-related cells. **B**, **C** The scatter plots of relationship between SENP5 expression and infiltration levels of immune-related cells by using TIMER2 database *p < 0.05, **p < 0.01, and ***p < 0.001. The correlation between five primary immune checkpoint members (PDCD1, CD274, LAG3, PDCD1LG2, CTLA4) and expression levels of **D** ELOVL1, **E** ELOVL2, **F** ELOVL3, **G** ELOVL4, **H** ELOVL5, **I** ELOVL6, **J** ELOVL7

A gene on chromosome 12q13.1 encodes SENP1, a member of the cysteine protease SENPs family and a homolog of Ulp1 in yeast [42]. This enzyme plays a crucial role in SUMO protein maturation and deSUMOylation process, maintaining the delicate equilibrium between SUMOylation and unmodified substrate levels. SENP1 exerts its influence by modulating transcription factors and coregulators, thereby regulating gene transcription. Recent studies have shown its significance in immune cell regulation, where it activates SIRT3 to facilitate T cell memory development and promotes M2 polarization of macrophages through glutamine metabolism regulation [43, 44]. SENP1 has emerged as a key player in cancer biology. Studies have demonstrated its ability to impact cancer metastasis by down-regulating matrix metalloproteinase 9 (MMP9) in prostate and pancreas cancer cells. Additionally, in prostate cancer, SENP1 controls MMP2 production through the HIF-1 signaling pathway [45]. A study on astroglioma cells demonstrated that knockdown of SENP1 resulted in the downregulation of the anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) by inhibiting the activation of the nuclear factor kappa-B (NF-κB) signaling pathway, thereby promoting cellular apoptosis [46]. Furthermore, overexpression of SENP1 was shown to facilitate the accumulation of JAK2 in the cytoplasm through deSUMOylation of JAK2, which in turn activated the JAK2/STAT3 signaling pathway, contributing to resistance to platinum-based therapies [47]. Additionally, research has implicated SENP1 in the regulation of tumor metabolism in prostate cancer cells. Specifically, SENP1 was found to induce deSUMOylation of hexokinase 2 (HK2), a key enzyme in the glycolytic pathway of tumor cells. This modification enhanced the association of HK2 with mitochondria, leading to increased glucose consumption and lactate production, thereby supporting cancer cell proliferation [48]. Enhanced VEGF synthesis, enhanced HIF-1 stabilization and transcriptional activity, and angiogenesis in endothelial cells are all caused by SENP1 in liver cancer [49, 50]. Multiple researches have consistently reported the overexpression of SENP1 in various human cancers, including colon cancer, breast cancer, and HCC tissues. Importantly, elevated SENP1 expression is linked to reduced survival rates among HCC patients [15, 51, 52]. These findings align seamlessly with our own results, indicating the critical role SENP1 plays in cancer progression and patient outcomes.

Similar to SENP3, the SENP5 protein predominantly localizes within the nucleolus and acts as a SUMO-specific protease essential for cell division [53]. Research has demonstrated that SENP5 influences mitochondrial structure and function by deSUMOylating Drp1, a protein associated with mitochondrial fission [54]. In our current study, elevated levels of SENP5 expression have been observed in osteosarcoma cells. Moreover, employing lentivirus-mediated small interfering RNA (siRNA) targeting SENP5 significantly suppressed cell growth and triggered apoptosis in osteosarcoma cells [55]. Through high-content screening and analysis of CRC patient tissue array, previous research identified SENP5 as a powerful radioresistant gene. Patients with elevated SENP5 expression exhibited greater radiation resistance [56].

Nonetheless, it is important to recognize certain limitations inherent in this study. Firstly, although we aggregated and analyzed data from multiple databases, the datasets were combined without evaluating heterogeneity, potentially impacting the robustness of our findings. Secondly, the conclusions presented here are derived solely from bioinformatic analyses, without validation through in vitro or in vivo experiments. Future work will involve conducting relevant experiments to elucidate the role of SENPs in hepatocellular carcinoma (HCC) at both cellular and molecular levels. Lastly, while this study highlights the association between SENP expression, immune cell infiltration, and patient survival in HCC, we cannot currently establish whether SENPs influence patient outcomes via immune-related pathways.

Several constraints should be acknowledged in this investigation. Primarily, the precise mechanistic roles of SENP1 and SENP5 in hepatocarcinogenesis, particularly their immunomodulatory functions, remain incompletely characterized and warrant systematic exploration through in vitro and in vivo experimental evidence. Furthermore, the prognostic signature's generalizability may be constrained by the current cohort's limited scale, necessitating expansion through multicenter collaborations and external validation cohorts to improve its discriminative performance and clinical utility."


A
T cell regulatory (Tregs)
T cell gamma delta
T cell follicular helper
T cell CD8+
T cell CD4+ naive
T cell CD4+ memory resting
T cell CD4+ memory activated
Neutrophil
NK cell resting
NK cell activated
Myeloid dendritic cell resting
Myeloid dendritic cell activated
Monocyte
Mast cell resting
Mast cell activated
Macrophage M2
Macrophage M1
Macrophage M0
Eosinophil
B cell plasma
B cell naive
B cell memory
CIBERSORT
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS UVM
* p < 0.05
** p < 0.01
*** p<0.001
Correlation
0.2
0.0
−0.2
B
Purity
Macrophage_TIMER
Rho = 0.108
p = 4.37e−02
Rho = 0.351
p = 1.89e−11
SENP5 Expression Level (log2 TPM)
LIHC
Purity
Infiltration Level
C
Purity
Neutrophil_TIMER
Rho = 0.108
p = 4.37e−02
Rho = 0.278
p = 1.53e−07
SENP5 Expression Level (log2 TPM)
LIHC
Purity
Infiltration Level
D
SENP1
E
SENP2
F
SENP3
G
SENP5
H
SENP6
I
SENP7
J
SENP8
CTLA4
PDCD1
CD274
PDCD1LG2
LAG3
Correlation
−1
1

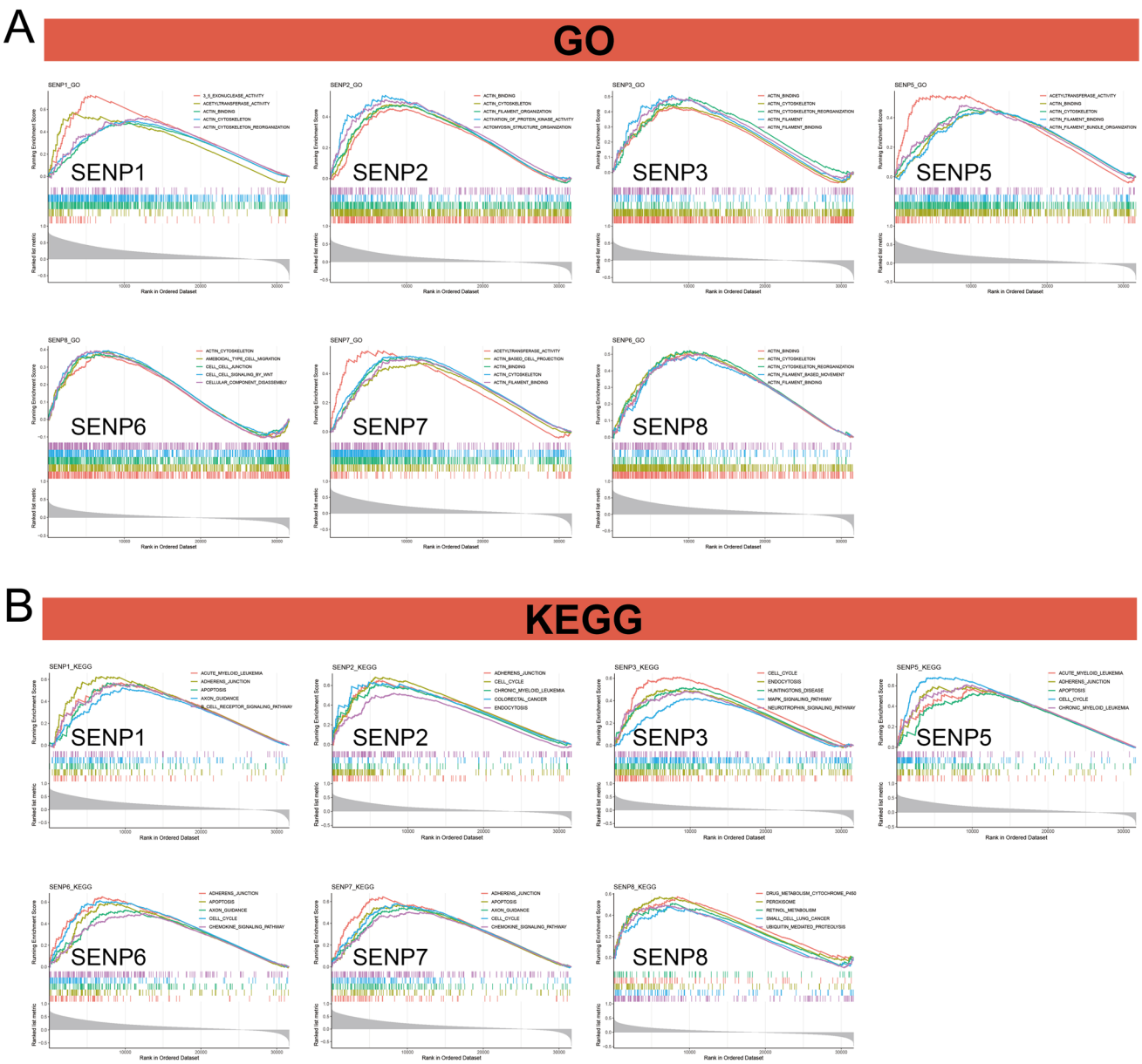


Fig. 8 Results of GSEA. **A** GO functional annotation of SENPs in LIHC. **B** KEGG pathway analysis of SENPs in LIHC. Curves of different colors show different functions or pathways regulated in different cancers. Peaks on the upward curve indicate positive regulation and peaks on the downward curve indicate negative regulation.

In conclusion, our work examined SENP expression profiles and their effects on prognosis in HCC. Our finding revealed a significant upregulation of SENP1 and SENP5 expression in HCC patients. Increased levels of SENP1 and SENP5 were correlated with unfavorable clinical outcomes in HCC cases. Furthermore, SENP1 was found to significantly correlate with neutrophil, myeloid dendritic cell, M0 macrophage, and memory B cell in the HCC by our immune infiltration research. In the HCC microenvironment, SENP5 also showed strong relationships with the neutrophil, myeloid dendritic cell, and monocyte. These results shed light on the potential roles of SENP1 and SENP5 as critical targets in HCC.

Author contributions XZ and CZ drafted the manuscript and performed the data analysis. TL participated in the data collection. All authors contributed to the article and approved the submitted version.

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Data availability The data which support the findings of our work are openly accessible from the GEO, STRING, GeneMANIA, and Cytoscape database.

Declarations

Competing interests The authors declare no competing interests.

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