Comprehensive analysis of the expression, prognosis and biological significance of FSCN family in clear cell renal cell carcinoma

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Abstract. Fascin (FSCN) is an actin-binding protein that serves a critical role in cell migration and invasion, contributing to tumor metastasis. However, there is little known about the function of *FSCN* family in kidney renal clear cell carcinoma (KIRC). The present study used the UALCAN, gene expression profiling interactive analysis, The Cancer Genome Atlas, cBioPortal, STRING and The Tumor Immune Estimation Resource databases to investigate the transcription level, genetic alteration and biological function of FSCNs in KIRC and their association with the prognosis value and immune cell infiltration in patients with KIRC. Results showed that the expression of FSCN1 and FSCN3 was markedly upregulated in patients with KIRC, while the expression of FSCN2 showed an opposite trend, which was the same as the experiments. Furthermore, the expression levels of FSCNs were associated with pathological stage, molecular subtypes and tumor grade. The expression levels of FSCNs were statistically correlated with the immune cell infiltration in KIRC. Higher expression levels of FSCN1 and FSCN3 were associated with worse overall survival (OS) and progression-free interval of patients bearing KIRC. Univariate and multivariate analysis demonstrated that FSCN2 was an independent risk factor for OS time in KIRC. Furthermore, mutations in FSCNs were significantly associated with poor OS and progression-free survival in patients with KIRC. The FSCNs were involved in pathways including focal adhesion, endocytosis, hypertrophic cardiomyopathy, regulation of actin cytoskeleton. The results indicated that FSCN2 might serve as an independent prognostic factor for OS of KIRC and that FSCN1 and FSCN3 can be used as favorable biomarkers for predicting clinical outcomes in KIRC.

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Introduction

Renal cell carcinoma (RCC) is one of the most common forms of cancer in individuals and can be classified into three types: Kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP) and malignancies of the chromophobe. KIRC, which is one of the most common forms of urinary cancer with a growing incidence (1), accounts for 70-85% of histologic subtypes of RCC, which derives from the tubule epithelium of renal parenchyma (2). Even though a number of targeted pharmaceuticals and immunosuppressives have been developed, surgical operation remained the most effective and primary method for treating this condition (3). Early-stage KIRC does not usually manifest any symptoms and 20-30% eventually progress to metastatic RCC (mRCC) (4). In recent years, there has been an increase in indolent cancers being discovered incidentally and the clinical treatment of active surveillance, robot-assisted nephron-saving surgery and minimally invasive techniques, such as thermal ablation, have become more popular. The surgery for kidney cancer at an early stage can potentially be curative, but recurrences after surgery remain common and inoperable kidney cancer at a late stage is usually fatal. It is estimated that ~40% of patients are resistant to conventional chemotherapy and radiation therapy and patients with mRCC who have experienced treatment failure have a 5-year survival rate of <20% (5). Somatic mutant genes in KIRC have been identified by whole genome sequencing and their involvement in pathogenesis and mechanisms has been explored (6). To date, the molecular pathology of renal cancer remains unclear. However, there is an urgent need to discover more ways of identifying these biomarkers in order to facilitate early detection and stop the devastating progression of KIRC. At the same time, there is a very active search for new biomarkers in the field of renal oncology that have the potential to further improve diagnosis, treatment and prognosis of RCC.

Fascin (FSCN) is an actin-binding protein of 55 kDa that is responsible for the formation and stability of microspikes, filopodia and invadopodia, which is critical for cell adhesion, motility and migration (7-9). The FSCN family contains three isoforms, namely FSCN1, FSCN2 and FSCN3, which are encoded by FSCN1, FSCN2 and FSCN3 genes, respectively (10). The actin-binding protein FSCN1 exists

in mammalian cells such as neurons, endothelial cells and mesenchymal cells, but is significantly reduced or absent in normal epithelial cells which acts as a migration factor associated with epithelial-to-mesenchymal transition (10-12). Migration and metastasis of colon cancer cells are significantly accelerated by overexpression of FSCN1 (13), while tumor metastasis and cell motility in prostate cancer are diminished when FSCN1 is knocked down in cellular models (14). FSCN2, which is expressed by retinal photoreceptor cells, serves a critical role in stabilizing stereocilia after development, is abundant in stereocilia and is developmentally regulated, appearing in inner-hair-cell stereocilia during final stages of elongation (8,15). A study has found that FSCN2 is essential for maintaining ear and eye function, is an actin cross-linking protein that is mainly localized in retinas and in the stereocilia of hair cells (16). FSCN3, which is testis specific, may function in terminal elongation of the spermatid head (17). Currently, however, little information is available on the relationships between FSCN2/3 and tumors and the role of the FSCN family in KIRC remains to be elucidated.

The current study examined the expression and functional role of FSCN1-3 in KIRC by using various public databases. Additionally, the relationship between FSCN family expression levels and clinicopathological features, prognosis, tumor immune cell infiltration and drug sensitivity was studied in patients with KIRC.

Therefore, the present study provided improved knowledge about the molecular mechanisms of KIRC to facilitate further studies.

Materials and methods

Ethics statement. The Ethics Committee of the First Affiliated Hospital of Nanchang University approved the research protocol (approval no. 12-110). All datasets were gathered from public databases with written consent.

Patient and tumor samples. There were 20 KIRC tissues and adjacent normal tissues collected from patients whose pathology was independently confirmed by two pathologists. In total, 20 matched pairs of KIRC tissues and adjacent normal kidney tissues were stored in liquid nitrogen between 2021 and 2022. The tissue samples are the same as those used in the previous article (18). Written informed consent was obtained from the patients involved.

RNA and reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted using TRIzol (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The RNA samples were stored at -80˚C until use. The extracted RNA was reverse-transcribed into cDNA using the First-Strand cDNA Synthesis kit (Qiagen, Inc.) according to the manufacturer's protocols. Each cDNA sample was added to a 20 μl reaction volume containing an appropriate primer set and SYBR green supermix. Triplicates of all samples were analyzed. The SYBR Real-Time PCR kit (Qiagen, Inc.) was used under the following conditions according to the manufacturer's protocols: 95˚C for 2 min, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 10 sec. Relative expression was normalized to GAPDH and calculated according to the $2^{-\Delta\Delta Cq}$ method (19). In the present study, the following primers were used: GAPDH forward primer GCCACATCGCTCAGACAC CAT, GAPDH reverse primer: CCCATACGACTGCAAAGA CCC, Human FSCN1 forward: GACGAGCTCTTTGCTCTG GA, Human FSCN1 reverse: TCGGTCTCCTCGTCCTGA TT, Human FSCN2 forward: TGGAGGAGAGTCACCCAC AG, Human FSCN2 reverse: TCAGGAAGGTCTCGTGGT CT, Human FSCN3 forward: GCTTCGTTCAGCCAATGG CTAC, Human FSCN3 reverse: ATCCTGCCACAGTTCCAG TGCA. The QuantiTect SYBR Green PCR kit (Qiagen, Inc.) was used to perform real-time quantitative PCR. GAPDH was used as an internal control. Experiments were replicated three times.

Gene expression profiling interactive analysis (GEPIA) dataset. The GEPIA dataset (http://gepia.cancer-pku.cn/) was used to analyze The Cancer Genome Atlas (TCGA; (https://tcga-data.nci.nih.gov/tcga/) tumors compared with TCGA normal and the genotype-tissue expression (GTEx) normal datasets and the box plots for the expression of FSCN1, FSCN2 and FSCN3 between KIRC tissues and the adjacent tissues were obtained (20). $P<0.05$ was considered to indicate a statistically significant difference.

UALCAN. The UALCAN database (http://ualcan.path.uab.edu) contains 31 types of patients with cancer with clinical and RNA-seq data. UALCAN is an interactive portal, which can be used to study the relationship between the expression of target genes in TCGA and the clinical data of patients. In the present study, correlation of the FSCNs expression with clinical pathological parameters, including individual cancer, tumor grade and KIRC subtypes, were analyzed. $P<0.05$ was considered to indicate a statistically significant difference. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

cBioPortal analysis. The c-BioPortal (https://www.cbioportal.org) is an online database for interactive exploration of multidimensional cancer genomic datasets (21). The present study analyzed the genetic alterations of FSCN1-3, which contained genomic profiles counted on mutations and putative copy-number alterations (CNA) from GISTIC 2.0 (22). OncoPrint v.3.3.1 was constructed in cBioPortal (https://www.cbioportal.org/) to directly reflect all types of changes including gene amplification, deep deletion, mRNA upregulation and mRNA downregulation in patients with KIRC. In addition, genetic alterations in FSCNs genes were correlated with OS of patients with KIRC and the log-rank test was used to perform the difference between altered group and unaltered group. Following the c-BioPortal's online instruction, 50 frequent neighbor genes of FSCNs family and the coexpression correlation of coefficient between FSCN genes were achieved.

STRING analysis. The STRING database (http://string-db.org/) provided the significant protein-protein interactions. The PPI network of FSCN1-3 and 50 frequent neighbor genes was generated using STRING (23).

Tumor immune estimation resource database (TIMER). TIMER includes >10,000 samples representing 32 types of cancer from the TCGA, which was an easy-to-operate online

tool established for systematically analyzing the abundance of immune infiltration (24). The gene module explored the relationship between members of the FSCN family and immune cell infiltration, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells in KIRC. Using the somatic copy number alterations (SCNA) module, the tumor infiltration levels was compared with different somatic copy number alterations in FSCNs.

Statistical analysis. The present study used R software (version 3.6.2; http://www.R-project.org/) to conduct the statistical analyses. Based on KIRC samples, the RNAseq data was downloaded from TCGA, which primarily included the lncRNA dataset (level 3) and clinical data for patients with RCC. Using R and the Wilcox test, the different expressions of FSCNs in KIRC were analyzed using the ggplot2 package. In order to estimate the prognosis of FSCNs, Kaplan-Meier survival analysis and Cox proportional hazards regression analysis were performed. Univariate and multifactorial Cox regression analysis were used to analyze the relationship between FSCN1-3 genes and clinicopathological parameters. Univariate analysis and multivariate analysis were used to evaluate the independent prognostic significance of FSCN1-3 mRNA expression. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were performed using the R package clusterProfiler.

Results

Transcriptional levels of different FSCN family members in patients with clear cell renal cell carcinoma. The present study first examined the mRNA levels of FSCN family members in KIRC based on RNA-seq data from TCGA KIRC cohort. As shown in Fig. 1A-C, the FSCN1 and FSCN3 expressions were significantly higher in KIRC tissue compared with normal tissue samples, whereas the FSCN2 expression was lower in cancerous tissue than in normal tissue. The mRNA transcription levels of the three FSCN members for patients with KIRC were then examined according to the GEPIA database. As shown in Fig. 1D-F, the expression level of FSCN1 mRNA in KIRC tissues was significantly higher than that in normal kidney tissues, whereas no significant difference in the expression of FSCN2 and FSCN3 was found between KIRC and non-cancerous kidney tissue. To validate this conclusion, we analyzed the FSCN1/2/3 mRNA expression in 15 pairs of KIRC samples and adjacent histologically normal tissues using real-time PCR (RT-qPCR). As shown in Fig. 1G-I, studies indicated that FSCN1/3 are highly expressed in kidney cancer, while FSCN2 is expressed at low levels in adjacent cancer tissues compared to normal tissues. On the basis of the above results, it was inferred that FSCN1 and FSCN3 transcriptional levels were significantly lower in normal tissues than in KIRC tissues compared with paired tissue samples, while the FSCN2 exhibited the opposite result.

Relationships between FSCN family expressions and clinicopathological parameters of KIRC. The present study explored the relationship between clinical and pathological parameters and the expression of FSCN1-3 based on the TCGA data (https://tcga-data.nci.nih.gov/tcga/) and UALCAN database. As shown in Fig. 2A-C, with respect to tumor stage, there was a remarkable correlation between FSCN1/3 mRNA expression level and individual cancer stages. As cancer stage increased, FSCN1 mRNA expression level increased. The highest mRNA expression of FSCN1 was found in stage IV. However, no significant difference was observed between cancer stages and FSCN2 mRNA expression. As presented in Fig. 2D-F, the mRNA expression of FSCN1 was markedly associated with tumor grade with the highest mRNA expression level expressed in grade IV, while mRNA expressions of FSCN2 and FSCN3 were not associated with tumor grade. As shown in Fig. 2G-I, the mRNA expression levels of FSCN1-2 in KIRC good risk (ccA) subtype were significantly lower compared to the KIRC poor risk (ccB) subtype, while the expression of FSCN3 showed the opposite result. Therefore, the results suggested that mRNA expressions of FSCN1-3 were significantly associated with clinicopathological parameters.

Prognostic value of mRNA expression of FSCN family members in patients with KIRC. To further explore the prognostic role of FSCN family members in patients with KIRC, survival analysis was conducted by R software according to the clinical information in TCGA database. As shown in Fig. 3A-F, the expression of mRNA of FSCN1-3 family members was significantly associated with prognosis in patients with KIRC. The results showed that higher mRNA expressions of FSCN1 (HR=1.82; 95%CI:1.32-2.50 and P<0.001) and FSCN2 (HR=1.74; 95%CI:1.25-2.42 and P=0.001) were associated with poorer OS in patients with KIRC, whereas the mRNA expression level of FSCN3 (HR=1.22; 95%CI:0.87-1.71 and P=0.248) was not associated with the OS of patients. Higher mRNA expressions of FSCN1 (HR=1.68;95%CI:1.22-2.31 and P=0.001), FSCN2 (HR=1.62; 95%CI:1.20-2.18 and P=0.002) and FSCN3 (HR=1.62; 95%CI:1.19-2.22 and P=0.003) were associated with shorter PFI. These findings indicated mRNA expressions of FSCN1-3 were found to be significantly correlated with the prognosis of patients with KIRC. Thus, FSCN 1-3 might be useful makers for predicting the overall survival of patients with KIRC.

Independent prognostic value of mRNA expression levels of FSCN1-3 in terms of OS in patients with KIRC. Following the finding that there was a significant association between FSCN1-3 mRNA levels and OS for patients with KIRC, the independent prognostic value of mRNA expression of FSCN family members for patients bearing KIRC was evaluated based on the TCGA database and prognostic data for Cox survival regression analysis (25). The univariate Cox regression analysis showed that high expression of FSCN1 (HR=1.330; 95%CI: 1.108-1.598 and P=0.002), FSCN2 (HR=1.801; 95%CI: 1.259-2.578 and P=0.001), age (HR=1.765; 95%CI: 1.298-2.398 and P<0.001), pathologic stage (HR=3.946; 95%CI: 2.872-5.423 and P<0.001) and histologic grade (HR=2.702; 95%CI: 1.918-3.807 and P<0.001) in the KIRC were significantly correlated with increased OS. Multivariate analysis showed that FSCN2 (HR=1.659; 95%CI: 1.137-2.422 and P=0.009), age (HR=1.543; 95%CI: 1.132-2.103 and P=0.006), pathologic stage (HR=3.946; 95%CI: 2.206-4.335 and P<0.001) and histologic grade (HR=1.749; 95%CI: 1.217-2.514 and P=0.003) were significant prognostic factors for overall survival (Table SI). Cox regression for OS analysis revealed that FSCN2, age, pathologic

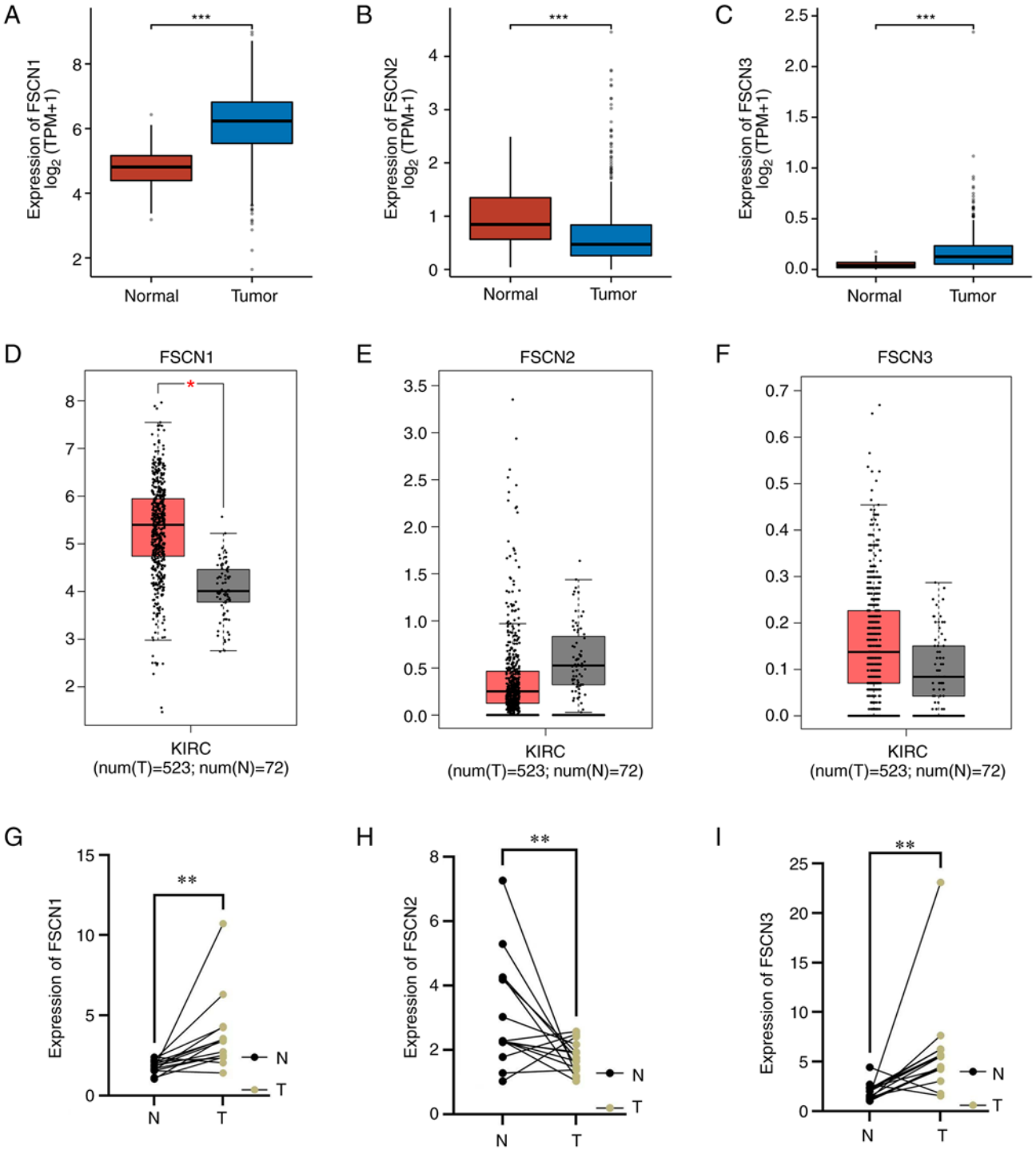

Figure 1. The FSCN1-3 expression levels in kidney cancer tissues and adjacent normal kidney tissues. Transcriptional expressions of (A) FSCN1 (B) FSCN2 and (C) FSCN3 genes were evaluated between kidney cancer tissues and adjacent normal kidney tissues based on The Cancer Genome Atlas and GTEx databases. Comparison of (D) FSCN1 (E) FSCN2 and (F) FSCN3 expression levels between ccRCC and normal kidney tissues based on GEPIA. Reverse transcription quantitative-PCR to detect the mRNA levels of (G) FSCN1 (H) FSCN2 and (I) FSCN3 in ccRCC tissues and paired-adjacent normal kidney tissues. *P>0.05, **P<0.01, ***P<0.001. ccRCC, clear cell renal cell carcinoma; FSCN, fascin; KIRC, kidney renal clear cell carcinoma; N, normal (tissues); T, tumor (tissues); TPM, Transcripts Per Million.

stage and histologic grade served as independent predictive variables in patients with KIRC.

Genetic mutations status in FSCN family members and their associations with OS and progression-free survival (PFS) of patients with KIRC. The present study analyzed the genetic alterations in FSCN family members and their associations with OS and PFS of patients with KIRC using the cBioPortal online tool to explore the potential expression pattern of FSCN1-3. To gain further insight into genetic changes that arise in KIRC, cBioPortal was used to reanalyze genomic data from 512 sequenced patients with KIRC. As shown in Fig. 4, the mutation rate of FSCN3 was the highest, at a percentage of 7% among the FSCN1-3

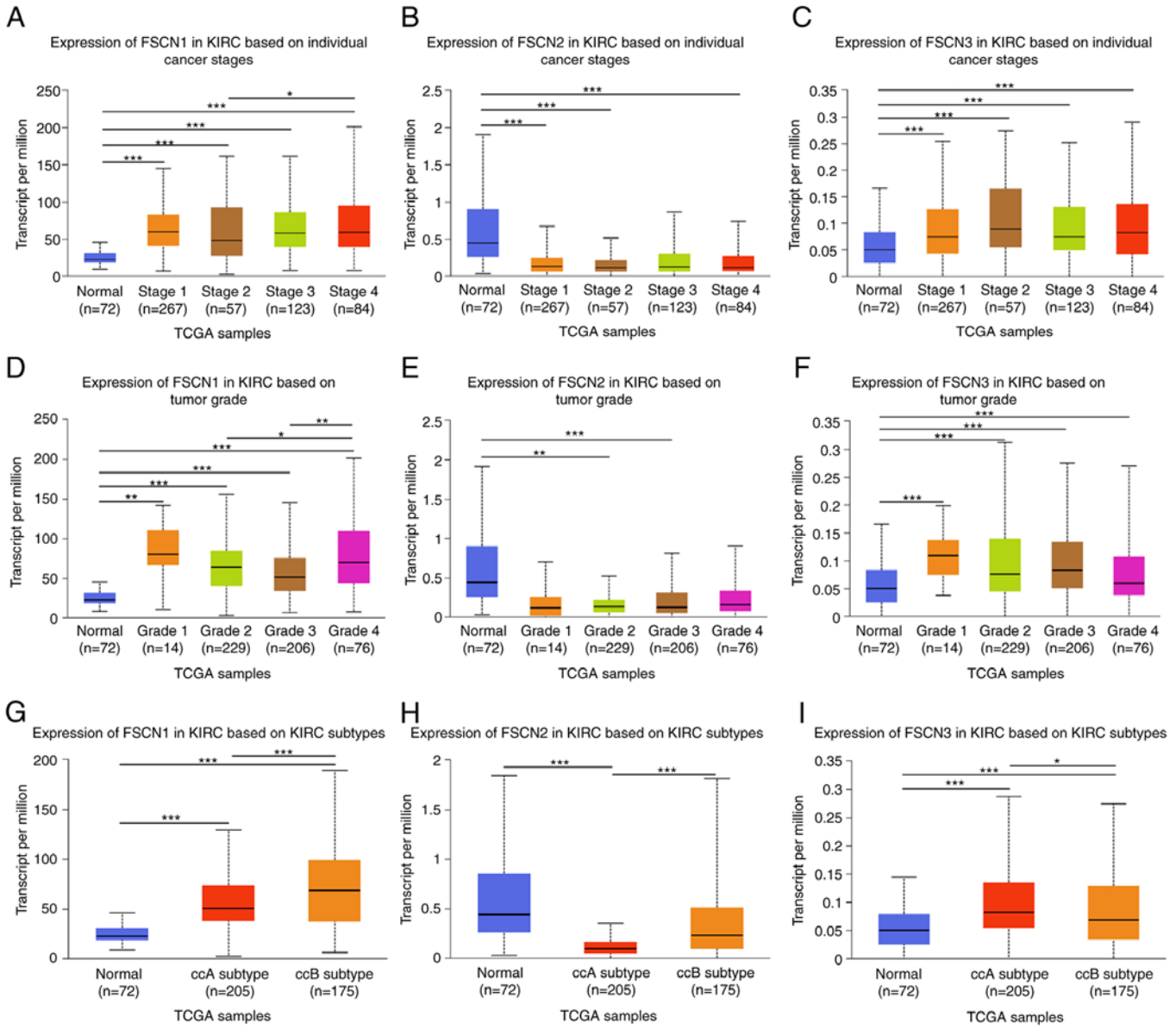

Figure 2. Relationship between the mRNA levels of FSCN1/2/3 and the clinicopathological parameters of patients with KIRC. (A-C) Correlation between mRNA level of FSCNs and individual cancer stages in patients with KIRC based on UALCAN database. (D-F) Association of mRNA expression of FSCN family members with tumor grades of patients with KIRC. (G-I) Comparison of the mRNA levels of FSCNs in KIRC subtypes between ccA subtype and ccB subtype. ns, not significant; *P<0.05; **P<0.01; ***P<0.001. FSCNs, fascins; KIRC, kidney renal clear cell carcinoma; ccA, good risk; ccB, poor risk; TCGA, The Cancer Genome Atlas.

family. The mutation rate of FSCN1 was 5%, which was twice as high as the mutation of FSCN2. Kaplan-Meier curve of patients with KIRC with (altered group) or without mutations (unaltered group) in FSCN1-3 genes showed significant difference in terms of PFS (Fig. 4C; P=2.370x10^{-4}) and PFS (Fig. 4D; P=1.558x10^{-6}). This result suggested that the poor prognosis was caused by their mutation. Additionally, the correlation between FSCN1/2/3 was calculated by analyzing their mRNA expression. The result showed that FSCN2 had positive correlations with FSCN1 and FSCN3, while no relationship was found between FSCN1 and FSCN3 (Fig. 4E).

Predicted functions and pathways of the alteration in FSCN family and the 50 most frequently altered adjacent genes in patients with KIRC. After analyzing the genetic alterations in FSCN1/2/3 and the prognostic value of patients with KIRC, the 50 neighbor genes related to the FSCN1/2/3 mutants were analyzed and an integrated network was constructed using the STRING database (https://string-db.org/). Using the cBioPortal database, the top 50 genes which were co-expressed and associated with the FSCN1-3 were identified. As shown in Fig. 5A, the actin filament organization genes including CAPZB, ITGB5, TPM2, ZYX, ARHGEF2 and PPM1F were significantly associated with FSCN1-3 mutations. GO and KEGG functional enrichment analyses were performed using the ggplot2 R package to analyze the functions of FSCN1-3 and 50 neighbor genes significantly associated with FSCN1-3 (26). As presented in Fig. 5B, biological processes such as GO: 0007015 'actin filament organization',

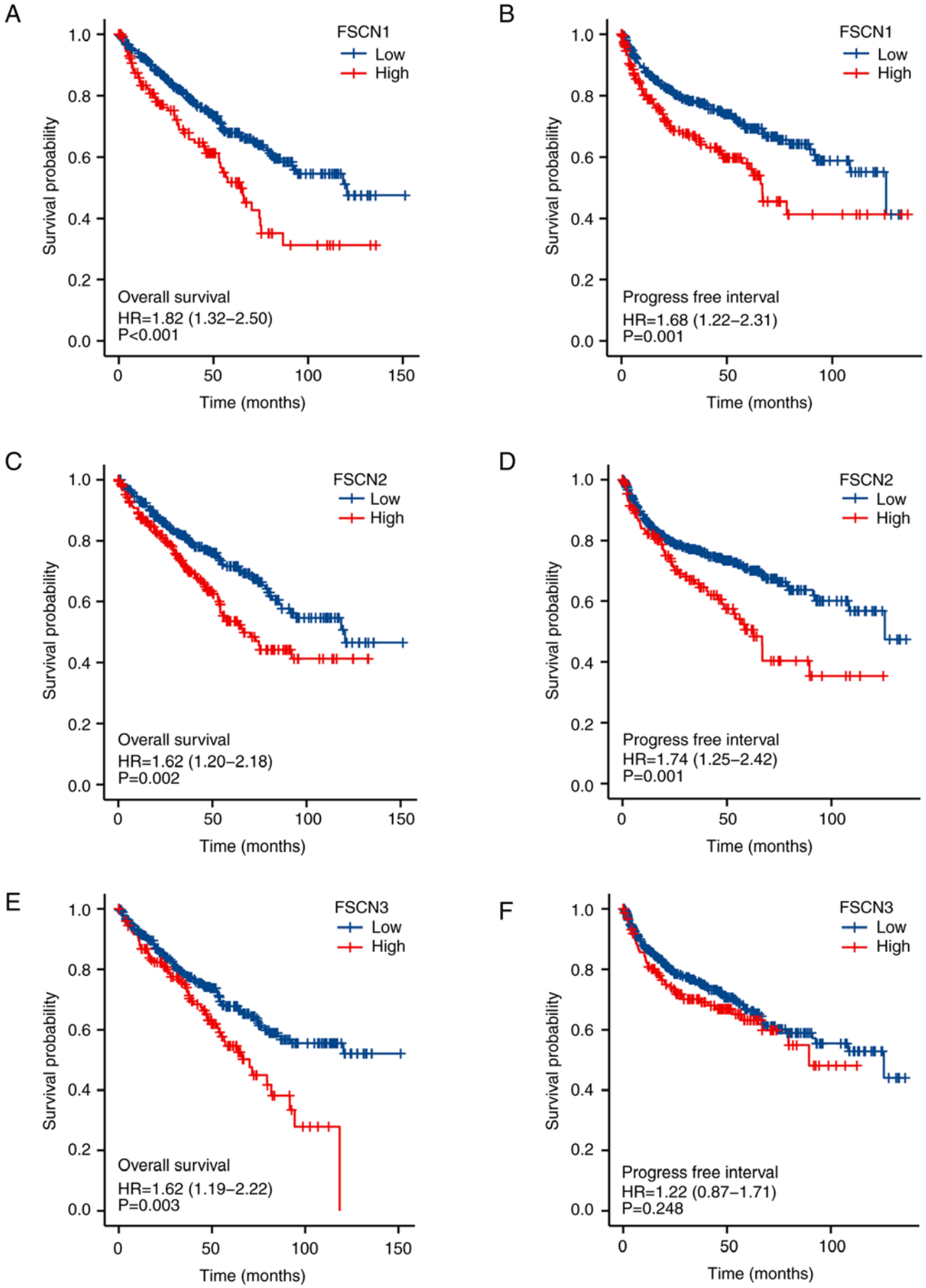

Figure 3. Evaluation of the prognostic value of FSCN mRNA expressions in KIRC. (A, C and E) The relationships between FSCNs mRNA expression levels and OS were analyzed in patients with KIRC. (B, D and F) The relationships between FSCNs mRNA expression levels and PFI of patients with KIRC were analyzed using R software. FSCN, fascin; KIRC, kidney renal clear cell carcinoma; OS, overall survival; PFI, progression-free interval; HR, hazard ratio.

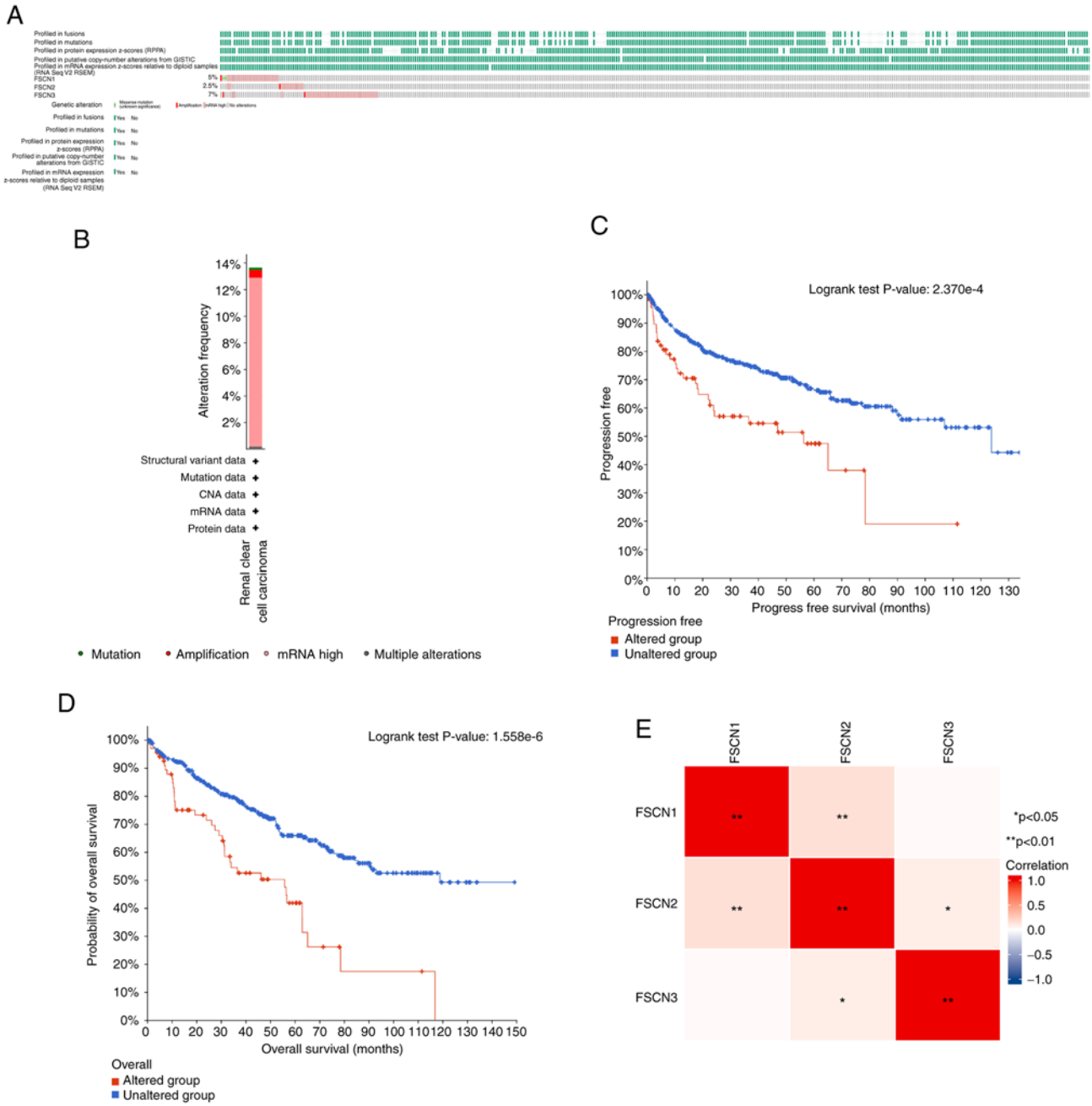

Figure 4. The mutation rate of FSCN1/2/3 in KIRC (A). Alteration frequency of FSCNs family according to the cBioPortal database (B). Genetic alterations in FSCNs family were associated with shorter (C) PFI and (D) OS of patients with KIRC. (E) Correlation between FSCN family members in KIRC by using cBioPortal. ns, not significant; *P<0.05; **P<0.01. FSCN, fascin; KIRC, kidney renal clear cell carcinoma; PFI, progression-free interval; OS, overall survival.

GO: 0051017 'actin filament bundle assembly', GO:0034329 'cell junction assembly' and GO:0034330 'cell junction organization' were significantly modulated by the FSCN1/2/3 mutations in KIRC. Cellular components, including GO:0005925 'focal adhesion', GO:0005924 'cell-substrate adherens junction', GO:0030055 'cell-substrate junction', GO:0030016 'myofibril' and GO:0043292 'contractile fiber' were significantly related to the FSCN1/2/3 alterations. Additionally, FSCN family genes mutations significantly affected molecular functions, such as GO:0003779 'actin binding', GO:0005518 'collagen binding', GO:0051015 'actin filament binding', GO:0043522 'leucine zipper domain binding' and GO:0048407 'platelet-derived growth factor binding'. In KEGG analysis, five pathways including has: 04510 'Focal adhesion', has: 04144 'Endocytosis', has: 05410 'Hypertrophic cardiomyopathy', has: 05414 'Dilated cardiomyopathy' and has: 04810 'Regulation of actin cytoskeleton' were associated with the functions of FSCN1-3 mutations in KIRC (Table SII).

Immune infiltrations analysis of the FSCN1-3 family in KIRC. Correlations between genes and immune infiltrations were estimated using TIMER. The positive connections existed between the abundance of CD4+ T cell and the expressions of all FSCN family members. The expression of FSCN1 showed a positive correlation with the abundance of CD8+ T cell,

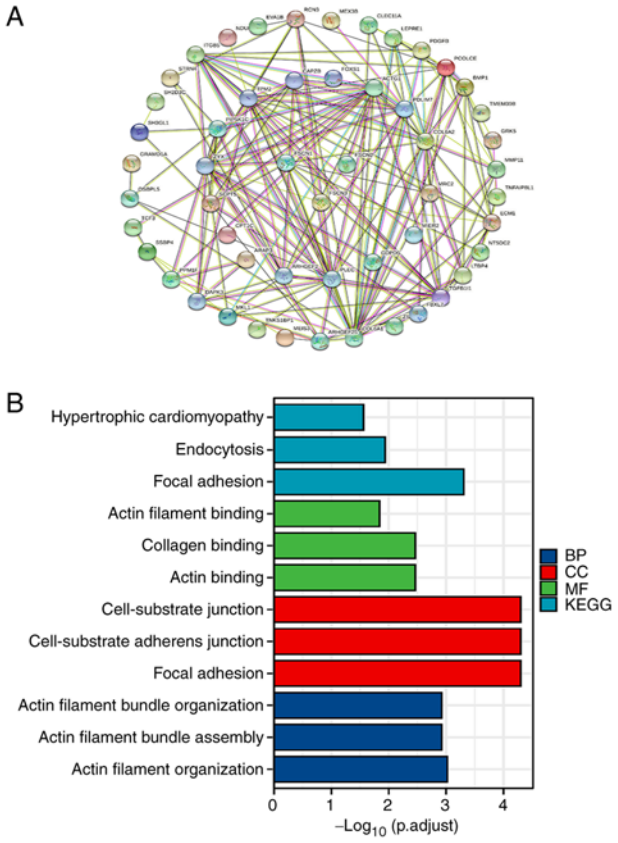

Figure 5. Predicted functions and pathways of FSCN1/2/3 and their neighboring genes in KIRC using GO and KEGG analysis. (A) PPI networks of FSCN family members and the 50 neighboring genes related to FSCNs in KIRC. (B) GO functional enrichment analysis and KEGG pathway enrichment analysis of the mutations in FSCNs and their 50 frequently altered neighbor genes in patients with KIRC. PPI, protein-protein interaction; FSCNs, fascins; KIRC, kidney renal clear cell carcinoma; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological processes; CC, cellular components; MF, molecular functions.

while FSCN2 had a negative correlation. The abundance of macrophage, neutrophil, B cell and dendritic cell positively showed significant associations with FSCN1. The expression of FSCN3 showed a positive correlation with the abundance of CD4+ T cell and neutrophil (Fig. 6A-C). Furthermore, the SCNA of FSCN1/2/3 were estimated. Results revealed the SCNA of FSCN2 significantly correlated with the infiltration levels of six immune cells composed of B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells, while that of FSCN1 was in significant connections with the infiltrating levels of B and CD4+ T cell. The SCNA of FSCN3 was only significantly associated with CD4+ T cells (Fig. 6D-F). Together, FSCN Family members were closely related to the immune infiltration in patients with KIRC.

Discussion

FSCNs cross-link filamentous actin into tightly packed parallel bundles and serve a central role in architectural maintenance and functioning of cell protrusions (8). Growing evidence suggests that FSCNs serve a critical role not only in tumorigenesis and proliferation of tumor cells, but also in tumor metastasis (27,28). However, the association between mRNA expression of distinct FSCNs family members and prognosis of patients with KIRC remains unclear. The present study systematically examined the mRNA levels, genetic alterations, functional enrichment, immune infiltration and prognostic value of FSCNs.

By considering the combined effect of mutations in multiple genes within the FSCN gene family, researchers can obtain a more comprehensive assessment of their effect on prognosis. This approach enables the capture of synergistic or cumulative effects resulting from alterations in multiple genes, which may have a greater influence on disease progression or treatment response compared to individual gene mutations. Additionally, studying mutations across the entire family can provide insights into common disrupted pathways or mechanisms, contributing to a improved understanding of the underlying biology of the disease. This knowledge can aid in the identification of potential therapeutic targets or the development of personalized treatment strategies. Furthermore, analyzing the correlation between these genes can offer insights into potential functional redundancy or compensation within the family. In cases where one family member is mutated, other members may compensate for its loss of function. By integrating correlation analysis with mutation analysis, researchers can identify patterns where mutations in one family member are associated with changes in the expression or activity of other family members. Understanding these compensatory mechanisms provides a more comprehensive view of the functional impact of mutations within the FSCN family.

FSCN-1 is an actin bundling protein that serves key functions in cell-cell interactions, adhesion and motility via regulating the function of filopodial protrusions and microfilaments (29), which are involved in the invasion and metastasis of various tumors. It has been shown that FSCN1 is mainly overexpressed in estrogen receptor-negative breast tumor tissues and positive FSCN-1 expression is associated with decreased mean tumor-free survival and overall survival (30). Furthermore, increased FSCN1 expression in nasopharyngeal carcinoma is associated with poor prognosis (31). FSCN1 is usually upregulated in a number of malignant tumors and could be considered as an oncogene since it promotes tumor cell migration and invasion (32). Among a variety of tumor types, FSCN1 is significantly associated with increased metastatic potential and more aggressive phenotypes (33-35) and by inhibiting FSCN1, tumor cells could be prevented from migrating and metastasizing (36). Knocking down FSCN1 expression can also have an anti-migration and anti-invasion effect on ovarian cancer and glioblastoma (37,38). A study found that knockdown of fascin-1 expression could suppress cell migration and invasion of non-small cell lung cancer by regulating the MAPK pathway (39). Therefore, inhibiting FSCN1 expression might be essential for the treatment of metastatic cancers. The present study detected that FSCN1 expressed higher in KIRC tissues compared with normal tissues. In addition, it was demonstrated that high expression of FSCN1 was related to shorter OS and PFI in patients with KIRC, indicating that FSCN1 acted as an oncogenic role in renal cell carcinoma and promoted the development of renal cell carcinoma. The results were similar to previous research that concluded that the increased expression of FSCN1 has been proved to be an adverse biomarker predicting poor

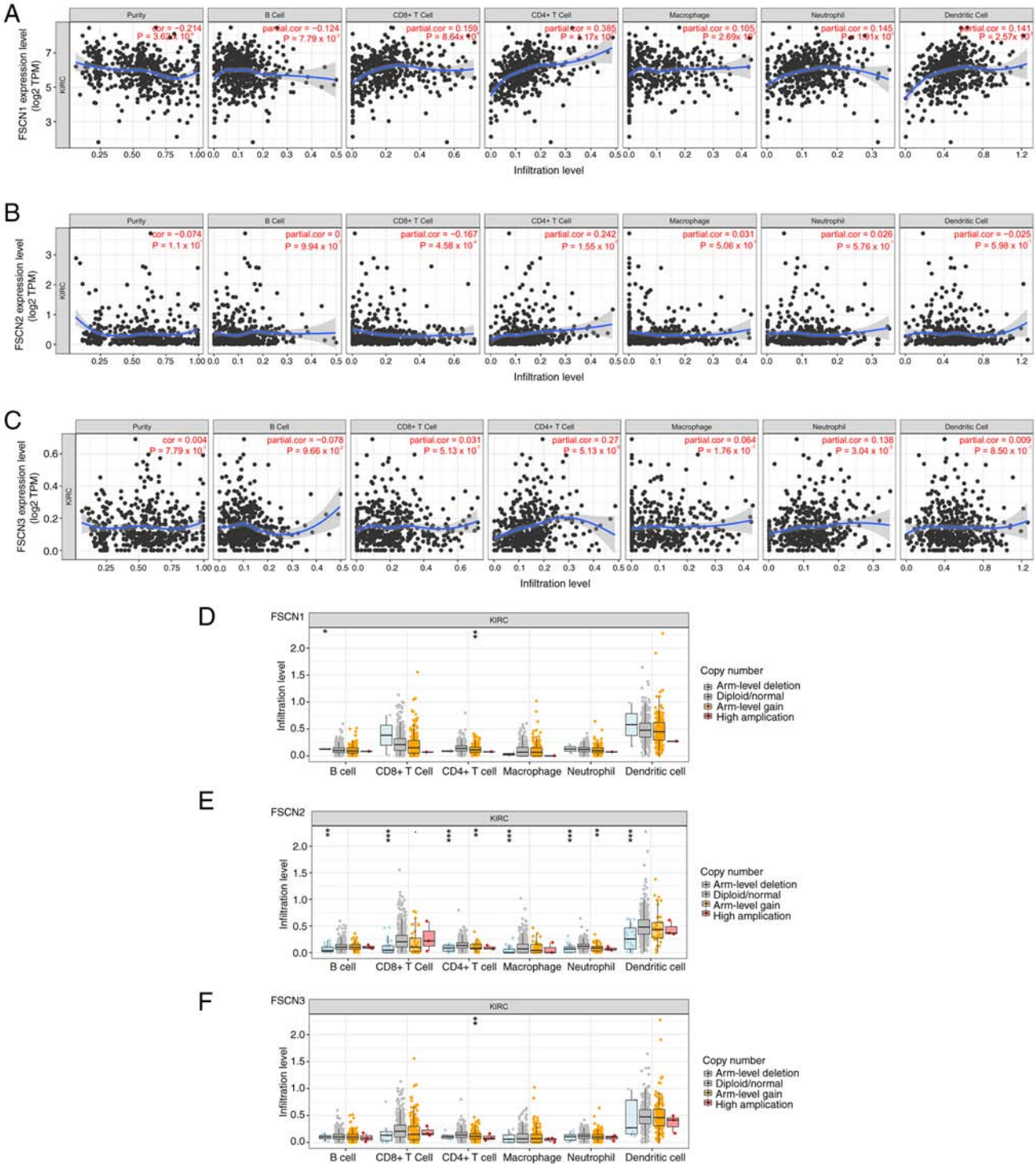

Figure 6. Analysis of the correlation between FSCN1/2/3 and immune cells. (A-C) The correlation of FSCN1/2/3 members and tumor infiltrating immune cells via TIMER and (D-F) correlation between SCAN and abundance of immune infiltrates of FSCN1/2/3 members. ns, not significant; *P<0.05; **P<0.01; ***P<0.001. FSCN, fascin; TIMER, Tumor Immune Estimation Resource; SCAN, somatic copy number alterations.

outcomes in esophageal squamous cell carcinoma (40). The present study also found that FSCN1 overexpression was significantly related to advanced individual cancer stage and tumor grade among patients with KIRC. The expression of FSCN1 was also related to immune cell infiltration in KIRC, suggesting that FSCN1 might regulate the immune response to cancer. These findings suggest that FSCN1 might be a promising prognostic and therapeutic target for patients with KIRC.

FSCN2, an actin-bundling protein, is a photoreceptor-specific protein of the fascin family that serves a significant role in maintaining ear and eye functions (16,41). Few studies have explored the relationship between FSCN2 and tumors. In the present study, the survival analyses showed that high expression of FSCN2 was significantly associated with shorter PFI and OS in KIRC. However, the expression of FSCN2 was decreased in KIRC, consistent with the results of the PCR experiment, and FSCN1 and FSCN2 showed a certain

coordinated expression pattern in the present study. This may be because of their small sample sizes and ethnic variations leading to inadequate statistical scope. These findings should be further assessed and confirmed by other studies. Multivariate analysis was conducted and high expression of FSCN2 was proved to be an independent positive prognosis indicator for OS in patients with KIRC. Further efforts are required to explore the expression of FSCN2 and how FSCN2 affects the patient survival. The TIMER analysis showed that FSCN2 is positively correlated with immune infiltration and provided strong evidences to support the high correlation between CNV and FSCN2 gene expression, indicating that abnormal expression of FSCN2 might affect the tumor cell microenvironment and regulate tumor cell behavior. FSCN2 must be further explored in order to determine how it affects patient survival.

FSCN3, a newly identified testis-specific actin-bundling protein, is specifically expressed in elongated spermatids (17). Little information is available in the literature regarding the role of fascin actin-bundling protein 3 in KIRC. In the present study, the expression level of FSCN3 was significantly increased in KIRC compared with normal tissues and the results demonstrated that patients with KIRC with high FSCN3 expression had a shorter OS time compared with those with low expression. The FSCN3 expression was positively correlated with the infiltration of immune cells including neutrophil and T cell CD4 + cell. However, this needs further study to investigate the FSCN3 gene.

The present study explored the expression and prognostic value of FSCNs in KIRC by combining public database and PCR experiments, providing an understanding of the role of FSCNs in KIRC. However, there were a few limitations to the present study. First, although enhanced expressions of FSCN1 and FSCN2 closely related to longer OS and could serve as independent favorable prognostic factors for OS in KIRC, it is necessary to conduct further studies with larger sample sizes to validate the findings of present study and explore the clinical application of FSCNs members in KIRC. Second, little information is available in the literature regarding the role of FSCN2 and FSCN3 in tumor. Additional research is necessary to further explore these potential mechanisms of FSCN2 and FSCN3.

In conclusion, present study showed that increased expression levels of FSCN1 and FSCN3 were strongly associated with shorter OS and that FSCN2 was an independent favorable prognostic factor for OS in KIRC. The FSCN1 mRNA expression was found to be significantly associated with clinical cancer stages and histologic grades in patients with KIRC. The results indicated that FSCN1 and FSCN2 could be treatment targets for KIRC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL, PZ and GC designed and directed the project. RC, BH and MJ performed bioinformatic analysis and the PCR experiment. BH and MJ confirmed the authenticity of all the raw data. YL performed the PCR experiment analysis and wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted according to the ethical principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of First Affiliated Hospital of Nanchang university (approval no. 202012-110). Written informed consent was obtained from the patients involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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