Arp2/3 Complex Subunits as Prognostic Biomarkers and Their Correlations With Immune Infiltration in Hepatocellular Carcinoma

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Abstract

Introduction: Actin-related protein 2/3 complex (Arp2/3) is a major actin nucleator containing seven subunits in humans, which has been widely reported that Arp2/3 plays an important role in promoting migration and invasion of various kinds of cancers. Nevertheless, the expression patterns and prognostic values of Arp2/3 subunits and the correlation between ARP2/3 subunits with immune infiltration in hepatocellular carcinoma remains unclear.

Methods: TCGA-FPKM dataset, Oncomine, CCLE and UCSC Xena database was used to obtained mRNA expression and clinical information of Arp2/3 subunits in HCC. The Arp2/3 subunits differential expression in HCC tissues and cell lines was analyzed by utilizing the Perl 5.30.0 and R 4.0.4 software. The protein expression data of Arp2/3 subunits was obtained from The Human Protein Atlas (HPA). The prognostic value of each individual Arp2/3 subunits was identified by R 4.0.4 software. The association between the mRNA expressions of Arp2/3 members in HCC tissues with their clinicopathologic parameters was analyzed by UALCAN. The relevance between tumor immunocyte infiltration and the Arp2/3 complex members were determined by the TIMER 2.0 platform and GEPIA database. A gene set enrichment analysis (GSEA) was performed to explore the potential mechanism of Arp2/3 complex members in the carcinogenesis of HCC.

Results The results revealed that expression of Arp2/3 family members(ACTR2,ACTR3,ARPC1A, APRC1B,ARPC2,ARPC3,ARPC4, ARPC5, ARPC5L) were up-regulated in hepatocellular carcinoma (HCC). Moreover, higher protein expressions of of ACTR3, ARPC1A, ARPC1B, ARPC2 were found in HCC tissues compared with normal liver tissues. The higher expression of of Arp2/3 family members were significantly correlated with poor survival and cancer stages in HCC patients. Multivariate analysis also demonstrated that ACTR3, ARPC2 and ARPC5 were independent prognostic biomarkers of survival in HCC patients. Meanwhile, ACTR3, ARPC2 and ARPC5 in HCC has positive significant correlations with the infiltration of immune cells. The GSEA results indicated that Arp2/3 members significantly involve in multiple cancer-related pathways by which promoted development of HCC.

Conclusions Various analysis indicated that certain Arp2/3 complex subunits were noticeably upregulated and predicted worse survival in HCC, which may be applied as promising molecular targets for diagnosis and therapy of HCC in the future.

Introduction

Liver cancer is the fourth leading cause of cancer-related mortality and rank sixth in terms of incidence rate, and the World Health Organization estimates that more than 1 million patients will die from liver cancer in 2030(1, 2).Hepatocellular carcinoma (HCC), which accounts for 75–80% of liver cancer, is the most common liver cancer with morbidity and prevalence increasing annually(3). Around 700 000 patients are new detected as HCC every year, with over half of the cases occurring in developing countries, Asian countries account for three-quarters of HCC-related deaths(4). Such a high fatality rate

mainly due to the low early diagnosis rate of HCC, rapid progress, fewer treatments for advanced cancer, especially with high heterogeneity in cancer, undefined molecular mechanisms and lacking of early prognostic indicator. Therefore it is absolutely imperative to search highly sensitive and specific prognostic markers and potential drug targets so that in favor of clarifying the molecular mechanism of HCC and consquently improving the prognosis of patients.

Actin-related protein 2/3 complex (Arp2/3) is a major actin nucleator responsible for promoting the nucleation of microfilaments then facilitate the assembly process of intracellular actin monomers into microfilaments, The actin filament form the cellular structure and promote processes involving in the formation of the cell–cell junctions, the motility of pathogens, the transport of vesicles(5). The process of actin filament nucleation also plays an important role in the formation of invasive pseudopodia in cancer cells(6). Abnormal migration and invasion are critical to tumor metastasis. Kiuchi et al(7) have proved that ARP2/3 is related to the formation of pseudopodia and the movement of bladder cancer cells. It was also reported that high expression of Arp2/3 positively correlated with the malignancy of glioma specimens and the Arp2/3 system deregulation promotes cancer progression and directly impacts on patient survival [(8, 9). Thus, ARP2/3 plays a crucially important role in the tumor invasion and metastasis.

Actin-related protein 2/3 complex family (Arp2/3) consists of seven evolutionarily conserved subunits, including two actin-associated protein Arp2 and Arp3 subunits (ACTR2 and ACTR3), and five accessory subunits ARPC1, ARPC2, ARPC3, ARPC4 and ARPC5, ARPC1 has two subtype in humans, ARPC1A and ARPC1B, the ARPC5 subunit by ARPC5 and ARPC5L(10). The center of the Arp2/3 complex was composed of ARPC2 and ARPC4 by forming C-type structure, the other subunits interact around the center structure to form stable Arp2/3 complex, ACTR2 and ACTR3 get in touch with the pointed end of new daughter filament(11). The complex comprised ARPC1B or ARPC5L subtype promote actin polymerization more efficiently than the ones containing the alternative subunits(12). Currently, many studies have shown that abnormal expression of Arp2/3 subunits are associated with cancer proliferation and invasion, including pancreatic cancer(13),gastric cancer(14)、colorectal cancer cells(15)、breast cancer(16, 17)、bladder cancer(18)、gliomas(8), lung squamous cell carcinoma (lung-SCC)(19) and head and neck squamous cell carcinoma (HNSCC)(20). However, the significance of the whole Arp2/3 subunits expression and prognostic values of HCC, has not yet to be determined. More significantly, there is little research on the correlation between the mRNA expression of Arp2/3 subunits and immune infiltration in HCC.

In this study, according to updated public resources and and multiple bioinformatic analysis, the mRNA expression and prognostic values of the Arp2/3 family members were comprehensively evaluated both in HCC tissues and HCC cell lines. Furthermore, we also investigated the potential correlation between Arp2/3 family expression and immune cell infiltration levels in HCC.

Material And Methods

Data acquisition

The mRNA expression data of Liver hepatocellular carcinoma(HCC) tissues was downloaded from Genomic Data Commons Data Portal of The Cancer Genome Atlas based on FPKM (TCGA-GDC) (https://portal.gdc.cancer.gov/), which is a is free and available reference database for cancer research covering 33 cancer types and 20,000 primary cancer samples and matched normal samples(21). The mRNA expression data of HCC cell lines was obtained from broad institute cancer cell line encyclopedia (CCLE) (https://portals.broadinstitute.org/ccle)(22). The relevant clinical information was downloaded from the UCSC Xena database (http://xena.ucsc.edu/), which included survival status, survival time (days) gender, age, histological grade, TNM stage.

Differential and correlated analysis of mRNA expression of Arp2/3 subunits

Fistly, The mRNA expression of Arp2/3 members in various cancer compared with corresponding normal tissues were analyzed by the Oncomine 4.5 database (https://www.oncomine.org/),which is an online large data-mining platform and integrated oncogene microarray database covering 715 datasets and 86733 samples(23), Students' t-test was used to assess transcriptional expression levels of Arp2/3 in cancer and normal tissues and thresholed was set as follows: p-value < 0.0001, fold change = 1.5, gene rank = 10%.Then the Arp2/3 subunits mRNA expression in HCC tissue downloaded from TCGA datebase based on FPKM. The Perl 5.30.0 software (https://www.perl.org/) was used to merge and transform date, The Arp2/3 subunits differential expression in HCC tissues compared to normal tissues was analyzed by utilizing the limma package of R 4.0.4 software (http:///www.r-project.org/). Wilcox test was applied to generate p-value and the cut-off was 0.05. The results were visualized by using the pheatmap package of R 4.0.4. To defined the facticity of the differential expression in HCC, the UALCAN(24) (http://ualcan.path.uab.edu) was selected for further verification.

Thereafter we download mRNA expression data of the Arp2/3 subunits in HCC cell lines from the Broad Institute Cancer Cell Line Encyclopedia (CCLE) (https://portals.broadinstitute.org/ ccle) ,which provides public access to genomic data, analysis and visualization for over 1100 cell lines(22). Then ggplot2 package of R 4.0.4 software was used to explore Arp2/3 subunits expression levels in HCC cell lines.

Lastly, the corrplot package of R 4.0.4 software and Pearson's correlation was used to assess whether these genes were correlated with each other, Pearson product-moment correlation coefficient (pearson's R) represent the degree of correlation between the two sunubits and the cut-off was 0.4. In addition, the significantly differential expression subunits were selected for prognostic analysis.

Differentially expressed Arp2/3 subunits at protein level

Apart from assessing the mRNA expression level of Arp2/3 members, the protein expression analysis of Arp2/3 subunits was obtained by using the data from The Human Protein Atlas (HPA ,https://www.proteinatlas.org/),which provided representative immunohistochemistry proteome analysis

based on 26941 antibodies targeting 17165 unique proteins and available for free download of nearly 20 common kinds of cancers(25). In this research, immunohistochemistry images of protein expression of Arp2/3 members were directly visualized by HPA in HCC and nomal liver tissue.

Prognostic values of Arp2/3 subunits in HCC

The prognostic values of differentially expressed Arp2/3 members in HCC were analyzed using survival and survminer package of R 4.0.4 software. First, the association between the expression of Arp2/3 subunits and Overall Survival (OS) or Progress Free Survival (PFS) was validated by Kaplan-Meier survival curves, and any differences in survival was assessed with stratified log-rank test, P value < 0 .05 was considered statistically significant.

Furthermore, the correlation between the expression of Arp2/3 subunits combining relevant clinical parameters and survival were evaluated using univariate and multivariate Cox proportional hazards regression analyses, The final prognostic results were presented with a hazard ratio (HR), 95% confidence interval (CI) and log-rank P value, P value < 0 .05 was considered statistically significant. The significantly affecting survival subunits were chosen for further analysis.

Clinicopathological analysis of Arp2/3 subunits in HCC

UALCAN(24)(http://ualcan.path.uab.edu) was used to assese the association between the mRNA expression of Arp2/3 subunits in HCC tissues with their clinicopathologic parameters, such as individual cancer stages and nodal metastasis status. The results could be obtained directly from UALCAN website basing on TACG database resource. P < 0.05 was considered statistically significant.

Immune infiltration analysis of Arp2/3 family in HCC

Tumor cells and Tumor Immune Infiltrating Cells (TIICs)interact closely related to cancer progression. To explore the connections between TIICs and Arp2/3 subunits, we used the TIMER 2.0 platform (http://timer.comp-genomics.org/), which provide comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types by using the microarray expression values for assessing the specific gene(s) associated with TIICs, including 10,897 samples from 32 kinds of cancers from the TCGA(26). In this study, the TIICs include CD4 + T-cells, CD8 + T-cells, B-cells, neutrophils and macrophages. Then the gene expression association between Arp2/3 subunits and biomarkers of subsets of TIICs was further investigated according by GEPIA databases(27)(http://gepia.cancer-pku.cn/). Both TIMER 2.0 and GEPIA databases correlation analysis was used by Spearman test and P < 0.05 was considered statistically significant. The correlation strength was evaluated by Spearman's rank correlation Rho, according to the previous studies: Rho 0.00–0.19 “very weak,” Rho 0.20–0.39 “weak,” Rho 0.40–0.59 “moderate,” Rho0.60–0.79“strong,” and Rho 0.80–1.0 “very strong(28).

Gene Set Enrichment Analysis (GSEA)

Lastly, we performed GSEA (version 4.1.0) to identify the pathways related to the differential Arp2/3 subunits expression and further to evaluate the potential biological mechanism by which differential

expression of Arp2/3 auxiliary subunits affecting the carcinogenesis of HCC patients as independent prognostic biomarkers. The c2.cp.kegg.v7.0.symbols.gmt (curated) was employed as the reference, random combination number of 1,000 permutations and false discovery rate (FDR) < 0.01 to identify the significantly enriched pathways.

Results

The mRNA expression of Arp2/3 subunits in HCC tissues and cell lines

First, the mRNA differential expression of Arp2/3 subunits in pan-cancer and the corresponding normal tissues were analyzed by using the Oncomine database. As shown in Fig. 1, overexpression of Arp2/3 subunits was observed in many kinds of cancers, including Liver cancer. Then based on the TCGA database, which collecting 374 HCC samples and 50 normal control samples, the mRNA differential expression of Arp2/3 subunits were obtained using Perl and R 4.0.4 software. We found that all of the Arp2/3 family members(ACTR2, ACTR3, ARPC1A, APRC1B, ARPC2, ARPC3, ARPC4, ARPC5, ARPC5L) were significantly up-regulated in HCC(Fig. 2). The above differential result of Arp2/3 subunits in HCC tissues was in according with the results of UALCAN databases (Fig. 3). We also used CCLE databases to probe expression of Arp2/3 subunits in HCC cell lines, the results indicated Arp2/3 subunits was expressed in 23 HCC cell lines, among them the expression of ACRT2 and ARPC3 was higher than other subunits and the ARPC5L expression level was lowest (Fig. 4). Then Pearson's correlation analysis revealed the genes expression of Arp2/3 subunits were correlated to a significant degree, such as ARPC1A and ARPC1B (Pearson' s R was 0.65), ARPC2 and APRC3 (Pearson' s R was 0.6) ARPC2 and ACTR(Pearson' s R was 0.66), ARPC3 and ARPC4 (Pearson' s R was 0.65), ARPC3 and APRC5L (Pearson' s R was 0.64), ARPC4 and ARPC5L (Pearson' s R was 0.64), ARTC2 and ARTC3 (Pearson' s R was 0.83), as shown in Fig. 5.

Protein expression of Arp2/3 subunits in HCC tissue

In addition to analyzing the mRNA expression of Arp2/3 members in HCC, we explored the protein expression condition of Arp2/3 subunits in HCC by the Human Protein Atlas (HPA).The results showed higher protein expressions of of ACTR3, ARPC1A, ARPC1B, ARPC2 were found in HCC tissues compared with normal liver tissues. while lower protein expressions of ARPC3 were observed in HCC than normal. The same expression level of ARPC5 and ARPC5L in HCC and liver tissues. Presently, there is no immunohistochemical map for ARPC4 detection in HPA. The results of Arp2/3 subunits immunohistochemistry images was shown in Fig. 6.

Prognostic values of Arp2/3 subunits in HCC

To examined the prognostic effect of Arp2/3 subunits in HCC patients, we indentified the correlation between mRNA expression and OS or PFS with log-rank test based on survival and survminer package of R 4.0.4 software. Kaplan-Meier survival curves for OS demonstrated that HCC patients with higher expression of ACTR2(p 0.001), ACTR3(p 0.001), ARPC1A(p 0.001), ARPC1B (p 0.001), ARPC2(p 0.001),

ARPC3($p = 0.008$), ARPC4 p□0.001), ARPC5(p□0.001), ARPC5L ($p = 0.004$) had worse OS than those with lower expression as shown in Fig. 7. Kaplan-Meier survival curves for PFS indicated that patients with higher expression of ACTR2(p□0.001), ACTR3(p□0.001), ARPC1A($p = 0.0058$), ARPC2($p = 0.034$), ARPC3(p□0.001), ARPC4 ($p = 0.004$), ARPC5($p = 0.002$), ARPC5L ($p = 0.005$) had shorter RFS that those with lower expression (Fig. 8). Those results indicated Arp2/3 subunits led to bad prognosis of patients with HCC.

We further explored the factors affecting survival through combining the expression of Arp2/3 subunits and clinical parameters among HCC patients, univariate Cox and multivariate Cox proportional hazards regression analyses was used. The results showed that the expression of Arp2/3 subunits (ACTR2, ACTR3, ARPC1A, ARPC2, ARPC3, ARPC4, ARPC5, ARPC5L) and clinical stage were associated with poor outcome of HCC patients. The hazard ratio (HR), 95% confidence interval (CI) and log-rank P value was shown in Table 1. Afterwards, multivariate Cox proportional hazards regression analysis found that the expression of ACTR3(HR = 1.0, 95%CI: 1.01–1.1, $p = 0.002$), ARPC2(HR = 1.0, 95%CI: 1.00–1.0, $p = 0.016$), ARPC5 (HR = 1.0, 95%CI: 1.01–1.2, $p = 0.002$)and clinical stage(HR = 1.6, 95%CI: 1.31-2.0, p□0.001) were independent prognostic biomarkers of HCC survival, as shown with forest plots in Fig. 9.

Table 1
Univariate Cox proportional hazards regression analyses of Arp2/3 members and clinical features in HCC

Parameter	Univariate analysis		
	Hazard ratio	95% CI	P value
ARPC1A	1.011946	1.004–1.020	**0.002**
ARPC1B	1.004998	1.000-1.010	**0.053**
ARPC2	1.035206	1.016–1.055	**0.3E-03**
ARPC3	1.009771	1.002–1.017	**0.011**
ARPC4	1.014924	1.005–1.025	**0.004**
ARPC5	1.040619	1.017–1.064	**0.58E-03**
ARPC5L	1.048752	1.013–1.085	**0.006**
ACTR2	1.014755	1.004–1.026	**0.010**
ACTR3	1.064911	1.025–1.106	**0.001**
Age	1.010115	0.996–1.025	0.173
Gender	1.28922	0.883–1.882	0.188
Grade	1.133154	0.881–1.457	0.330
Stage	1.679735	1.369–2.062	**6.97E-07**

Correlation analysis of mRNA expression of Arp2/3 subunits with clinicopathological features of HCC patients

we utilized UALCAN database to explore the relationship between mRNA expression of Arp2/3 members with clinicopathological parameter of HCC patients. As shown in Fig. 10, mRNA expressions of Arp2/3 subunits (ACTR2, ACTR3, ARPC1A, ARPC1B, ARPC2, ARPC3, ARPC4, ARPC5, ARPC5L) were significantly correlated with cancer stages, patients with more advanced cancer stages tended to higher mRNA expression of Arp2/3 subunits (ACTR2, ARPC1A, ARPC1B, ARPC2, ARPC3, ARPC4, ARPC5, ARPC5L). Compared with normal tissues, the mRNA expression of each subunits was remarkably higher in HCC patients. While there was no marked difference between stage4 and normal tissues in mRNA expression of ACTR2, ACTR3 and ARPC5L, that may be due to the small sample size in stage 4 (only 6 samples).

Then, we further investigated the relationship mRNA expression of Arp2/3 subunits with nodal metastasis status of HCC patients. The results showed that there was no significant relationship between mRNA expressions of Arp2/3 subunits with nodal metastasis status (Fig. 11). It may be due to the small number of patients with lymph node metastasis included by TCGA (n = 4).

Association of mRNA expression of Arp2/3 subunits with immune infiltration level in HCC

Tumor-infiltrating immune cells (TIICs) in the tumor microenvironment (TME) play crucial roles in genesis, progression, metastasis, and treatment resistance of the tumor. To probed the the correlations between Arp2/3 subunits and TIICs, we firstly explored the associations between certain prognostic biomarkers (ACTR3, ARPC2, ARPC5) and immune cells by TIMER 2.0 platform. The results showed that the expression of ARPC2 had negative correlation with the tumor purity (Rho =

-0.169, p = 1.6e-03), while the expression of ACTR3and APRC5was irrelevant with tumor purity. The expressions of ACTR3, ARPC2 and ARPC5 were both positively related with the immune infiltration cells of CD4 + T cells, CD8 + T cells, B cells, neutrophils and macrophages, the results was shown in Fig. 12.

Furthermore, we further investigated which kind of TIICs subsets was correlated with prognostic Arp2/3 subunits by analyzing the coexpression of Arp2/3 subunits and typical biomarkers of TIICs with GEPIA database. As shown **in** Table 2, the higher expression of ARPC2 and ACTR3was positively correlated with the expression of biomarkers of TIICs subsets, including B cells, CD8 + T cells, Th1 cells, Th2 cells, Th17 cells, Treg cells, neutrophils, M1 macrophages and M2 macrophages. More specifically, the ACTR3 expression in HCC was significantly correlated with STAT1 (Th1), STAT6 (Th2), STAT3 (Th17), CCR8 (Treg), CDb11(Neutrophils), PTGS2 and IRF5 (M1 macrophages). There was also significantly positively correlation between expression of ARPC2 and TICs biomakes, including STAT1 and TNF (Th1), GATA3 and STAT5A(Th2), CCR8 and TGFB1(Treg), CDb11(Neutrophils), PTGS2 and IRF5(M1 macrophages), VSIG4 and MS4A4A (M2 macrophages). The higher expression of ARPC5 was positively correlated with most biomarkers of TIICs subsets. Some of which were moderate relevancy with expression of ARPC5,

including STAT1(Th1), STAT6 and STAT5A(Th2), STAT3(Th17), CDb11(Neutrophils), IRF5(M1 macrophages). Apart from those, the correlation between ARPC2, ARPC5, ACTR3 with the other biomarkers of TIICs subsets was under weak or irrelevant. The above outcomes illuminated that Arp2/3 genes might positively modulate the infiltration and activation of TIICs in HCC.

Table 2
Correlations between Arp2/3 subunits gene expression and biomarker expression of subsets of TIICs in HCC.

Types of TIICs	Gene markers	ARPC2		ARPC5		ACRT3	
		R	P	R	P	R	P
B cell	CD19	0.36	1.2E-12	0.18	5.3E-04	0.17	0.0012
	CD79A	0.34	3.6E-11	0.088	0.092	0.17	0.0012
CD8	CD8A	0.39	9.7E-15	0.031	0.55	0.23	6.3E-06
	CD8B	0.36	6.8E-13	0.11	0.038	0.12	0.027
Th1	TBX21	0.3	4.3E-09	0.11	0.032	0.19	1.7E-04
	STAT4	0.39	6.1E-15	0.19	2.9E-04	0.26	6.6E-07
	STAT1	**0.6**	**0.3E-37**	**0.46**	**5.3E-21**	**0.59**	**3.9E-36**
	TNF	**0.48**	**7E-23**	0.23	7.1E-06	0.37	1.3E-13
	IFNG	0.34	2.7E-11	0.13	0.012	0.17	0.0014
Th2	GATA3	**0.48**	**1.4E-22**	0.19	2.3E-04	0.38	2.6E-14
	STAT6	0.29	8.6E-9	**0.48**	**2.2E-22**	**0.57**	**1.6E-33**
	IL-13	0.11	0.043	0.055	0.29	0.12	0.019
	STAT5A	**0.54**	**7.4E-30**	**0.46**	**2.6E-20**	**0.48**	**3.2E-22**
Th17	STAT3	0.37	1.7E-13	**0.48**	**4.2E-23**	**0.63**	**2.1E-42**
	IL-17A	0.052	0.32	0.089	0.089	0.14	0.007
Treg	FOXP3	0.2	1E-04	0.12	0.018	0.26	4.8E-07
	CCR8	**0.55**	**6E-30**	0.36	5.1E-13	**0.56**	**2.9E-31**
	TGFB1	**0.56**	**1.2E-31**	0.23	6.4E-06	0.29	2.2E-08
Neutrophils	CD11b	**0.52**	**2.6E-27**	**0.47**	**1.8E-21**	**0.48**	**1.1e-22**
	CCR7	0.36	5E-13	0.18	5.4E-04	0.27	1.7e-07
	CD66b	0.13	0.014	-0.0051	0.92	0.1	0.054
M1 macrophages	NOS2	0.13	0.011	0.32	3.1E-10	0.35	2.4E-12
	PTGS2	**0.47**	**6.4E-22**	0.3	4.8E-09	**0.47**	**1E-21**
	IRF5	**0.44**	**2.9E-19**	**0.51**	**5.9E-26**	**0.46**	**1.8E-20**
M2 macrophages	CD163	0.33	5.7E-11	0.23	8E-06	0.15	0.003

	VSIG4	**0.47**	**3E-21**	0.27	1.7E-7	0.33	4.2E-11
	MS4A4A	**0.46**	**1.2E-20**	0.25	1.9E-6	0.36	4.6E-13

Potential action mechanism of prognostic Arp2/3 subunits in HCC carcinogenesis

We had identified ACTR3, ARPC2 and ARPC5 was independent prognostic biomarkers affecting survival of HCC, then GSEA analysis was conducted to evaluate the potential biological mechanism by which Arp2/3 subunits lead to poor survival. According to the GSEA results, high expression of ACTR3 was positive related to 82 gene sets at FDR < 0.01, functions of which focused on regulation of antin cytoskeleton, protein ubiquitination, immune system process, genesis and progression of various tumor, leukocyte migration, DNA metabolic process, the ACTR3 overexpression was closely relevant to “JAK-STAT signaling pathway” “WNT signaling pathway” “pathway in cancer”“VEGF signaling pathway”“non small lung cancer”“pancreatic cancer” “renal cell carcinoma”, as shown in Fig. 13A. The GSEA results also indicated that high expression of ARPC2 was significantly positive related to 59 gene sets at FDR < 0.01, among them the cancer-related pathways included “WNT signaling pathway” “cell cycle” “pathway in cancer” “bladder cancer” “colorectal cancer ”“VEGF signaling pathway” “MAPK signaling pathway”, “chemokine signaling pathway”. Besides “T cell receptor signaling pathway” and “leukocyte transendothelial migration” might be associated with immune cell infiltration(Fig. 13B). 14 gene sets was significantly negative related to expression of ARPC2 at FDR < 0.01, functions of which focused on fatty metabolism, amino acid metabolism, metabolism of xenobiotics by cytochrome P450 (Fig. 13B). High expression of ARPC5 was significantly positive related to 43 gene sets at FDR < 0.01, the following pathway might involve in tumor development and pathogenesis :“MAPK signaling pathway” “non small lung cancer” “small lung cancer” “pancreatic cancer” “WNT signaling pathway” “TOLL like receptor signaling pathway” (Fig. 13C). 5 gene sets was significantly negative related to expression of ARPC2 at FDR < 0.01, including fatty metabolism, amino acid metabolism (Fig. 13C).

Discussion

Actin-related protein 2/3 complex (Arp2/3), which was first isolated from Acanthamoeba as an affinity complex for intracellular profibrin and the structure is conserved in eukaryotes, plays an important role in the formation of microfilaments and is related to cell movement. Recently, Several studies have identified the Arp2/3 subunits are upregulated in various kinds of cancer tissues or cells involving in proliferation, invasion and metastasis of cancer. It was reported coexpression of Arp2 and

WAVE2 was significantly higher in cases with high histologic grade and cases with lymph-node metastasis in adenocarcinomas of the lung and breast carcinoma(29, 30). Eeva et al (31)found ARPC1A acted as a novel regulator of cell migration and invasion in pancreatic cancer, and has been suggested to be a potential target for cancer anti-metastasis therapy. Zhang et al (14)verified that ARPC2 expression is higher in gastric cancer tissues than normal tissues and promotes gastric cancer cells proliferation and metastasis. It was reported that ARPC2 inhibitor, such as benproperine and Pimozide, inhibited the tumor

invasion and metastasis of cancer cells in animal models(32). Similarly, the significantly overexpressed of ARPC4 has also been observed in pancreatic carcinoma and gastric carcinoma and indicated that there were closely association between APRC4 expression and the tumor migration and invasion(13, 33). Furthermore, Xu et al (18)have shown that ARPC4 is necessary for proliferation, migration, invasion, and pseudopodia formation of bladder cancer cells, suggesting that ARPC4 represents a potential prognostic biomarker in those disease. ARPC5 may function as oncogenes in the development of lung squamous cell carcinoma (lung-SCC) and head and neck squamous cell carcinoma (HNSCC) and contributed to cancer cell migration and invasion which was directly regulated by miRNA(19, 20). Arp2/ 3 complex silencing mediated by siRNA led to a reduction in the migration of pancreatic cells(13). In this reseach, we identified the ARP2/3 members were significantly overexpressed in various cancers, including HCC. The differential expression of ACTR3, ARPC1A, ARPC1B, ARPC2, both in mRNA and protein level, was found in patients with HCC. Moreover, There was a significant correlation between the expression of each subunits, These results suggest that ARP2/3 subunits may serve as potential biomarkers for HCC.

Furthermore, we evaluated the whole picture of the prognostic roles of ARP2/3 subunits in HCC with Cox proportional hazards regression based on clinical parameters and ARP2/3 mRNA expression levels. We found the higher expression of ARP 2/3 subunits associated with worse OS and overexpression of ACTR2, ACTR3, ARPC1A, ARPC2, ARPC3, ARPC4, ARPC5, ARPC5L was related with shorter PFS in HCC. We also identified that the expression of ACTR2, ARPC2, ARPC5 and clinical stage were associated with poor outcome of HCC patients as independent prognostic biomarkers. In addition, mRNA expressions of Arp2/3 subunits were significantly correlated with cancer stages, patients with more advanced cancer stages tended to higher mRNA expression of Arp2/3 subunits. Previous study reported that ARPC2 was closely associated with the stage, nodal metastasis, and overall survival in breast cancer and the TGF-β/EMT pathway was involved in ARPC2-mediated carcinogenesis(16). Furthermore, ARPC2 showed significant associations with large tumor size, lymph node invasion, and high tumor stage by association analysis of 110 gastric cancer tissues, ARPC2-positive patients exhibited lower RFS and OS rates compared with ARPC2-negative patients in gastric cancer(14). In addition, ARPC5 high expression group were associated with poor overall survival compared to those in the ARPC5 low expression group, and multivariable analysis indicated that ARPC5 was an independent prognostic factor in Multiple Myeloma (MM) patients. Therefore our results are consistent with those of previous studies in other tumors(34).

Cancer immunotherapy has caused huge breakthroughs in a variety of malignancies. However, only a minority of HCC patients respond to immunotherapy (35). This is primarily due to high heterogeneity of tumor, various immune microenvironment, and lack of immune cell infiltration as well as lack of predictive markers. Therefore we further explored the association between expression of the prognostic genes and immune cell infiltration. Arp2/3 complex is critical for chemotaxis and hagocytosis and is required for macrophage integrin effects and monocyte recruitment functions (36). There is tight relationship between Arp2/3 complex and immune cells, for example, Leukocytes need adhere to cells in order to form synapses of killing infected cells, neutrophils literally squeeze their cell body during blood extravasation and efficiently migrate to the inflammatory focus(37). Moreover, cytoskeleton is crucially important to the adhesive contacts and migration in the development process of immune cells(37). Since

the Arp2/3 subunits assemble into a complex, the abnormity of one subunit is likely affect the whole complex function. It was reported that Arpc2 kockout mice caused a dramatic decrease of peripheral T cell numbers and impaired T cell homeostasis, which caused by reduction in surface TCR levels of T cell. There are higher transcription level of Arpc2 in Peripheral T cells than thymocytes, and Arp2/3 complex-promoted actin nucleation is essential for peripheral T cell homeostasis(38). In this study, we found that the expression of ARPC2, ARPC5 and ACTR3 were positively related with the immune infiltration cells of CD4 + T cells, CD8 + T cells, B cells, neutrophils and macrophages in HCC microenvironment. In addition, we discovered that the ARPC2, ACRT3 and ARPC5 expression was significantly correlated with the biomarkers of CD4 + T cells, CD8 + T cells, Neutrophils, M1 macrophages and M2 macrophages. The above results indicated Arp2/3 subunits participate in the activation and recruitment of TIICs in HCC, it played a dual role in tumor immunity which not only promoting anti-tumor immune cells infiltration but also recruiting immunosuppressive cell. Thus further research is needed to help us understand the role of Arp2/3 subunits and tumor-related immune cell functions and consequently contribute to the application of immunotherapy.

Currently, few studies were conducted on the specific mechanism by which ARP2/3 members promote tumor development and metastasis. Zhang et al(14) found that oncogenic gene including CTNND1, EZH2, BCL2L2, CDH2, VIM, and EGFR were upregulated by ARPC2, tumor suppressor gene PTEN, BAK, and CDH1 were downregulated by ARPC2. In breast cancer ARPC2 expression significantly upregulated vimentin, N-cadherin, MMP-9, ZEB1, and MMP-3 expression and also activated the TGF-β pathway to contribute to epithelial-mesenchymal transition (EMT)(16). In this study, through Gene Set Enrichment Analysis (GSEA) analysis we discover that Arp2/3 subunits may participate in the regulation of various cancer pathway, including colorectal cancer, pancreatic cancer, bladder cancer, lung cancer, renal cell carcinoma, as well as VEGF signaling pathway, MAPK signaling pathway and WNT signaling pathway. Besides, Immune-related pathways including leukocyte transendothelial migration and T cell receptor signaling pathway may lead to increasing of immune cell infiltration. Notably, we should perform a validation study regarding the exact mechanism of ACTR2, ARPC2, ARPC5 to further confirm our results identified by GSEA. Obviously, this is also the greatest limitation of this study, further research are required to verify the results before Arp2/3 complex members expression could be used routinely as a promising biomarker for risk stratification in HCC.

Conclusions

This study showed the expression profile of Arp2/3 complex members in HCC and the biological and prognostic values of the Arp2/3 subunits in HCC, which may be applied as promising molecular targets for diagnosis and therapy of HCC in the future.

Abbreviations

HCC: Hepatocellular carcinoma;

Arp2/3：Actin-related protein 2/3 complex

TCGA-FPKM: The Cancer Genome Atlas based on FPKM

HPA: The Human Protein Atlas

CCLC: the Broad Institute Cancer Cell Line Encyclopedia

OS: Overall Survival (OS)

PFS: Progress Free Survival

TIICs: Tumor cells and Tumor Immune Infiltrating Cells

GSEA: Gene Set Enrichment Analysis

KEGG: Kyoto Encyclopedia of Genes and Genomes.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials was obtained and analyzed from the current datebase and all data involved in this paper were included

Competing interests

The authors declare no competing interests.

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Authors' contributions

JB W conceived and designed the study. SL H and D L were responsible for the collection and analysis of the reseach information. LL Z：LY S and JB W critically and carefully revised this manuscript. The authors read and approved the final manuscript.

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References

1. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol. 2017;3(12):1683–91.
2. Villanueva A. Hepatocellular Carcinoma. N Engl J Med. 2019;380(15):1450–62.
3. Tan CK, Law NM, Ng HS, Machin D. Simple clinical prognostic model for hepatocellular carcinoma in developing countries and its validation. J Clin Oncol. 2003;21(12):2294–8.
4. Asia-Pacific Working Party on Prevention of Hepatocellular C. Prevention of hepatocellular carcinoma in the Asia-Pacific region: consensus statements. J Gastroenterol Hepatol. 2010;25(4):657–63.
5. Garcia-Ponce A, Citalan-Madrid AF, Velazquez-Avila M, Vargas-Robles H, Schnoor M. The role of actin-binding proteins in the control of endothelial barrier integrity. Thromb Haemost. 2015;113(1):20–36.
6. Firat-Karalar EN, Welch MD. New mechanisms and functions of actin nucleation. Curr Opin Cell Biol. 2011;23(1):4–13.
7. Kiuchi T, Nagai T, Ohashi K, Mizuno K. Measurements of spatiotemporal changes in G-actin concentration reveal its effect on stimulus-induced actin assembly and lamellipodium extension. J Cell Biol. 2011;193(2):365–80.
8. Liu Z, Yang X, Chen C, Liu B, Ren B, Wang L, et al. Expression of the Arp2/3 complex in human gliomas and its role in the migration and invasion of glioma cells. Oncol Rep. 2013;30(5):2127–36.
9. Molinie N, Gautreau A. The Arp2/3 Regulatory System and Its Deregulation in Cancer. Physiol Rev. 2018;98(1):215–38.
10. Abella JV, Galloni C, Pernier J, Barry DJ, Kjær S, Carlier MF, et al. Isoform diversity in the Arp2/3 complex determines actin filament dynamics. Nat Cell Biol. 2016;18(1):76–86.
11. Pollard TD, Beltzner CC. Structure and function of the Arp2/3 complex. Curr Opin Struct Biol. 2002;12(6):768–74.

12. Abella JV, Galloni C, Pernier J, Barry DJ, Kjaer S, Carlier MF, et al. Isoform diversity in the Arp2/3 complex determines actin filament dynamics. Nat Cell Biol. 2016;18(1):76–86.
13. Rauhala HE, Teppo S, Niemelä S, Kallioniemi A. Silencing of the ARP2/3 complex disturbs pancreatic cancer cell migration. Anticancer Res. 2013;33(1):45–52.
14. Zhang J, Liu Y, Yu CJ, Dai F, Xiong J, Li HJ, et al. Role of ARPC2 in Human Gastric Cancer. Mediators Inflamm. 2017;2017:5432818.
15. Su X, Wang S, Huo Y, Yang C. Short interfering RNA-mediated silencing of actin-related protein 2/3 complex subunit 4 inhibits the migration of SW620 human colorectal cancer cells. Oncol Lett. 2018;15(3):2847–54.
16. Cheng Z, Wei W, Wu Z, Wang J, Ding X, Sheng Y, et al. ARPC2 promotes breast cancer proliferation and metastasis. Oncol Rep. 2019;41(6):3189–200.
17. Chen P, Yue X, Xiong H, Lu X, Ji Z. RBM3 upregulates ARPC2 by binding the 3'UTR and contributes to breast cancer progression. Int J Oncol. 2019;54(4):1387–97.
18. Xu N, Qu GY, Wu YP, Lin YZ, Chen DN, Li XD, et al. ARPC4 promotes bladder cancer cell invasion and is associated with lymph node metastasis. J Cell Biochem. 2020;121(1):231–43.
19. Moriya Y, Nohata N, Kinoshita T, Mutallip M, Okamoto T, Yoshida S, et al. Tumor suppressive microRNA-133a regulates novel molecular networks in lung squamous cell carcinoma. J Hum Genet. 2012;57(1):38–45.
20. Kinoshita T, Nohata N, Watanabe-Takano H, Yoshino H, Hidaka H, Fujimura L, et al. Actin-related protein 2/3 complex subunit 5 (ARPC5) contributes to cell migration and invasion and is directly regulated by tumor-suppressive microRNA-133a in head and neck squamous cell carcinoma. Int J Oncol. 2012;40(6):1770–8.
21. Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19(1A):A68–77.
22. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483(7391):603–7.
23. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia. 2007;9(2):166–80.
24. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017;19(8):649–58.
25. Thul PJ, Akesson L, Wiking M, Mahdessian D, Geladaki A, Ait Blal H, et al. A subcellular map of the human proteome. Science. 2017;356(6340).
26. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48(W1):W509-W14.

27. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98–102.
28. Lin S, Zheng L, Lu Y, Xia Q, Zhou P, Liu Z. Comprehensive analysis on the expression levels and prognostic values of LOX family genes in kidney renal clear cell carcinoma. Cancer Med. 2020;9(22):8624–38.
29. Semba S, Iwaya K, Matsubayashi J, Serizawa H, Kataba H, Hirano T, et al. Coexpression of actin-related protein 2 and Wiskott-Aldrich syndrome family verproline-homologous protein 2 in adenocarcinoma of the lung. Clin Cancer Res. 2006;12(8):2449–54.
30. Iwaya K, Norio K, Mukai K. Coexpression of Arp2 and WAVE2 predicts poor outcome in invasive breast carcinoma. Mod Pathol. 2007;20(3):339–43.
31. Laurila E, Savinainen K, Kuuselo R, Karhu R, Kallioniemi A. Characterization of the 7q21-q22 amplicon identifies ARPC1A, a subunit of the Arp2/3 complex, as a regulator of cell migration and invasion in pancreatic cancer. Genes Chromosomes Cancer. 2009;48(4):330–9.
32. Choi J, Lee YJ, Yoon YJ, Kim CH, Park SJ, Kim SY, et al. Pimozide suppresses cancer cell migration and tumor metastasis through binding to ARPC2, a subunit of the Arp2/3 complex. Cancer Sci. 2019;110(12):3788–801.
33. Kang M, Lu S, Chong PK, Yeoh KG, Lim YP. Comparative Proteomic Profiling of Extracellular Proteins between Normal and Gastric Cancer Cells. Curr Cancer Drug Targets. 2016;16(5):442–54.
34. Xiong T, Luo Z. The Expression of Actin-Related Protein 2/3 Complex Subunit 5 (ARPC5) Expression in Multiple Myeloma and its Prognostic Significance. Med Sci Monit. 2018;24:6340–8.
35. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol. 2018;19(7):940–52.
36. Rotty JD, Brighton HE, Craig SL, Asokan SB, Cheng N, Ting JP, et al. Arp2/3 Complex Is Required for Macrophage Integrin Functions but Is Dispensable for FcR Phagocytosis and In Vivo Motility. Dev Cell. 2017;42(5):498–513.e6.
37. Tur-Gracia S, Martinez-Quiles N. Emerging functions of cytoskeletal proteins in immune diseases. J Cell Sci. 2021;134(3).
38. Zhang Y, Shen H, Liu H, Feng H, Liu Y, Zhu X, et al. Arp2/3 complex controls T cell homeostasis by maintaining surface TCR levels via regulating TCR(+) endosome trafficking. Sci Rep. 2017;7(1):8952.

Figures

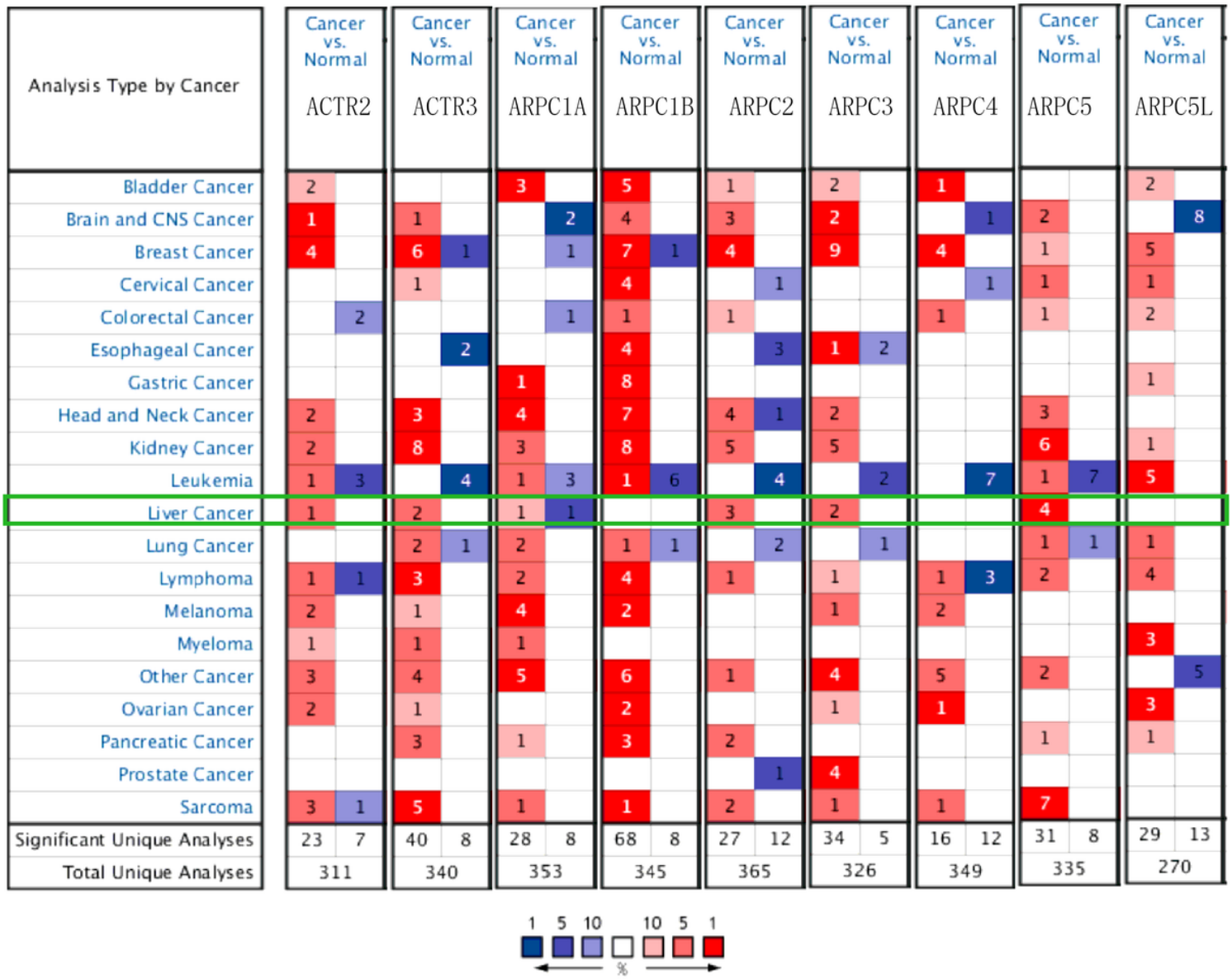

Figure 1

The mRNA expression level of Arp2/3 family members in different types of cancer (Oncomine database). The data were compared by the t-test and cut-off p-value < 0.0001,fold change= 1.5, gene rank=10%. Red stands for over-expression, blue represents down-expression.

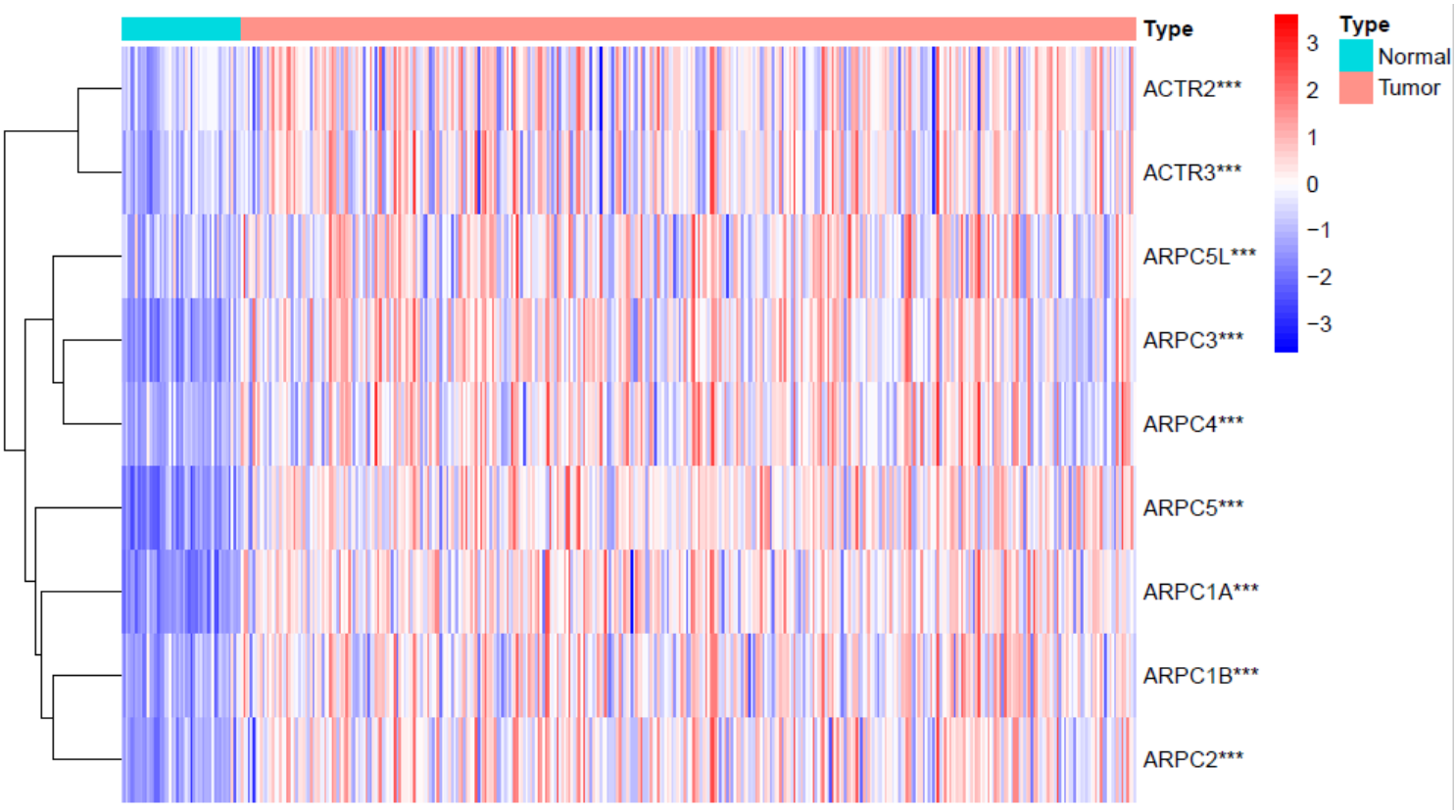

Figure 2

The expression profile level of Arp2/3 complex members in HCC tissues visualized by a heatmap. Red stands for over-expression, blue represents down-expression.

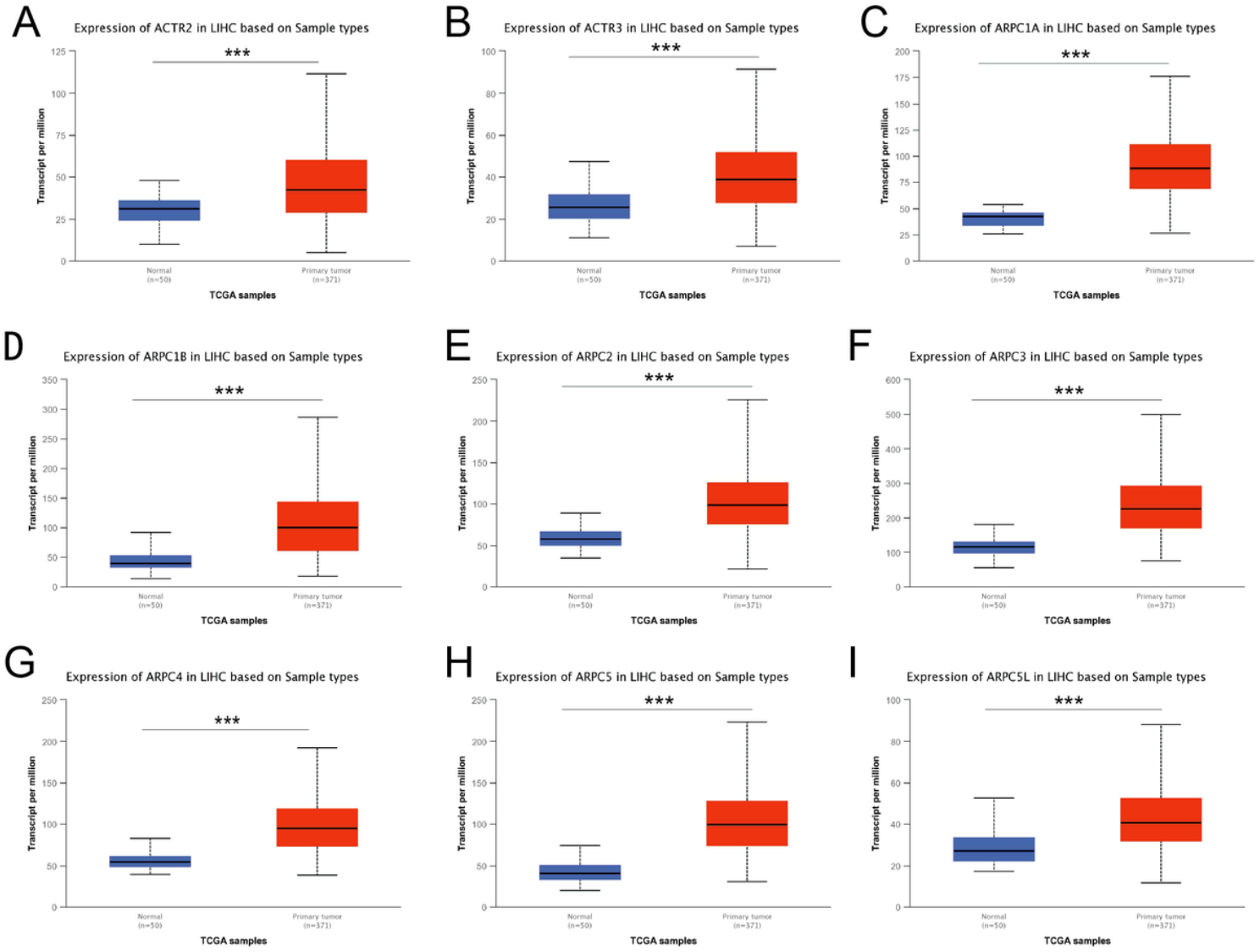

Figure 3

The relative expression of Arp2/3 complex members in normal tissues and HCC tissues basing on UALCAN database (A-I), ***stands for P < 0.001)

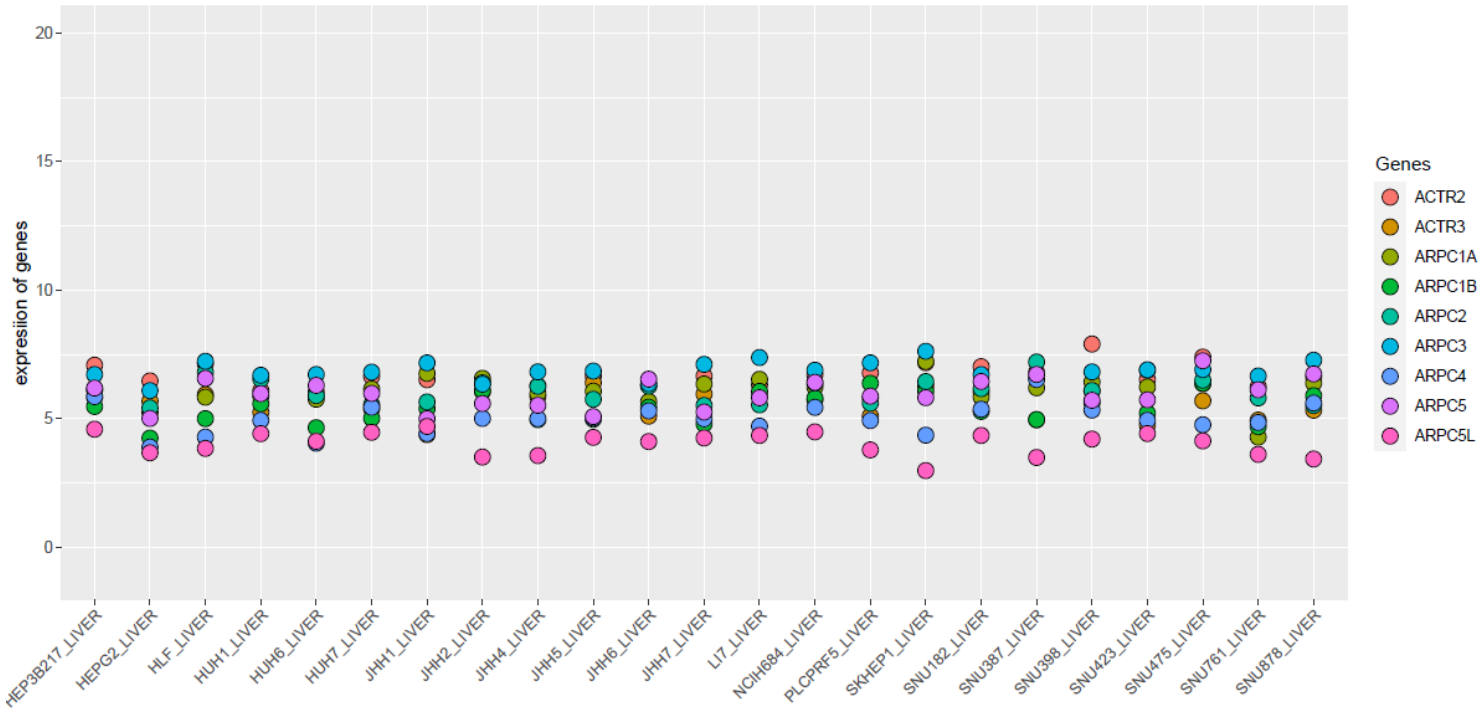

Figure 4

The expression level of Arp2/3 complex members in HCC cell lines (CCLE database)

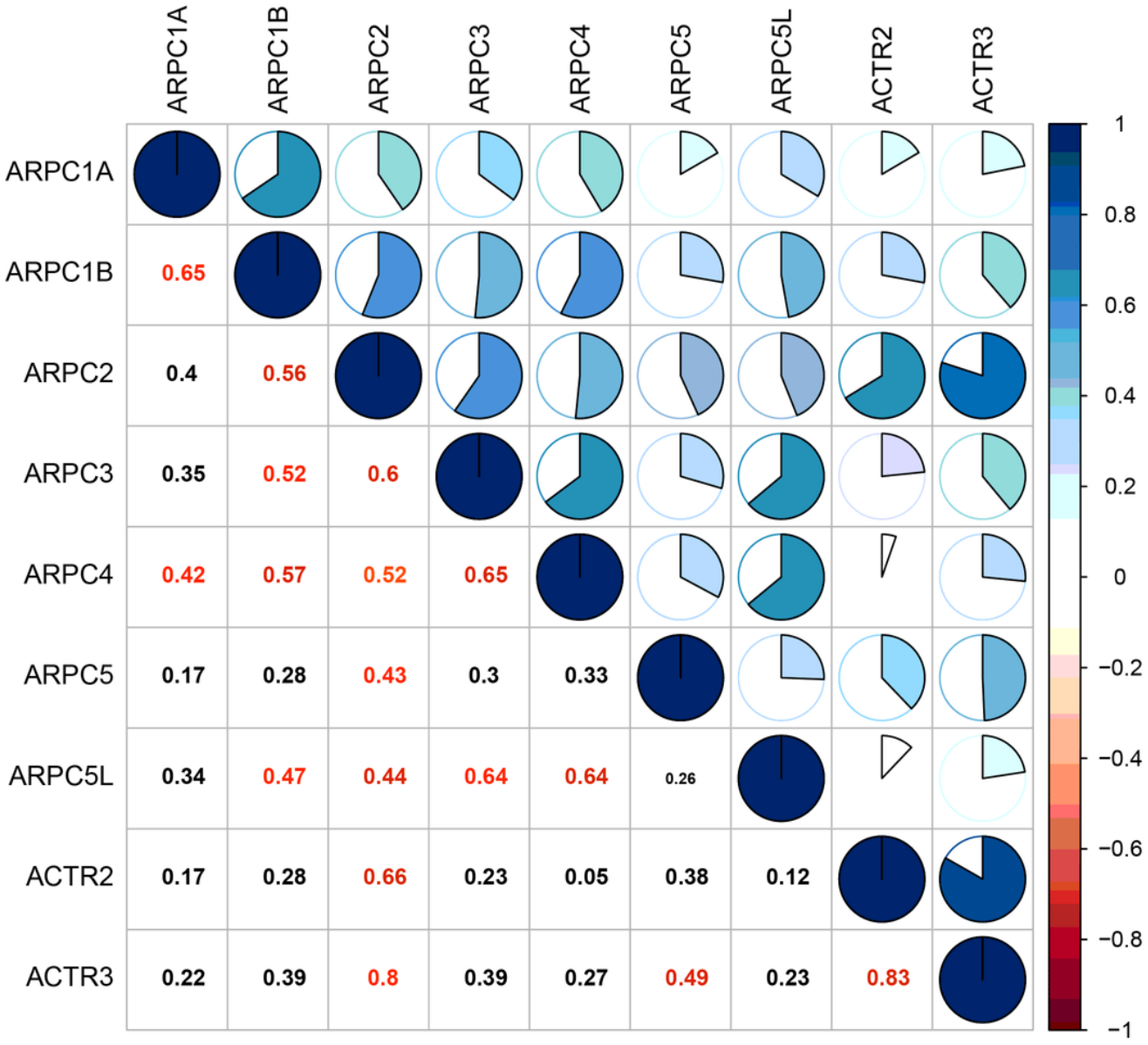

Figure 5

Correlation analysis of each Arp2/3 complex members, The date were analyzed by Pearson's correlation and pearson's R cut-off was 0.4. The red represents significant correlation between Arp2/3 members.

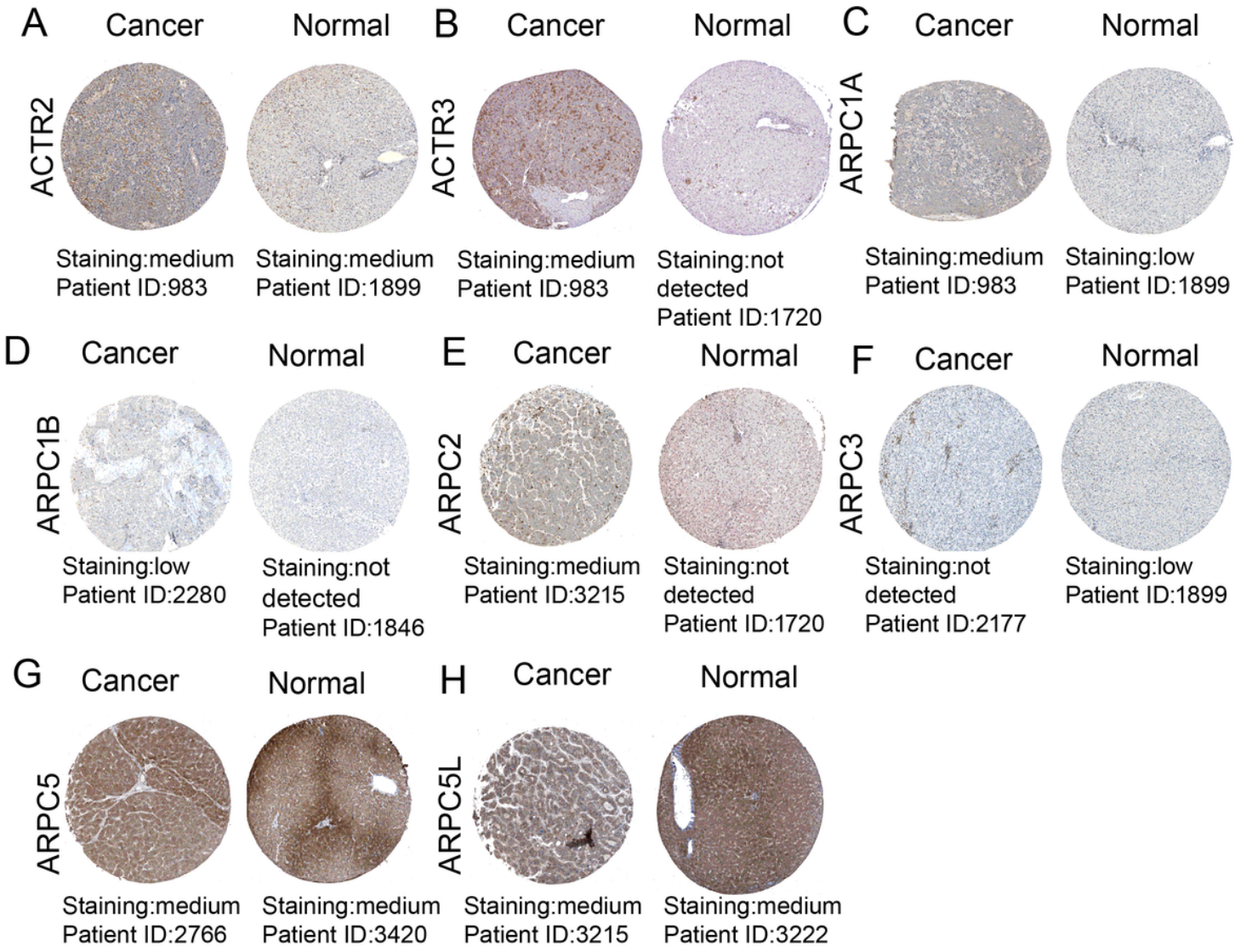

Figure 6

Representative immunohistochemistry images of Arp2/3 members (except for ARPC4,) in HCC tissues and normal liver tissues (HPA database) (A-H).

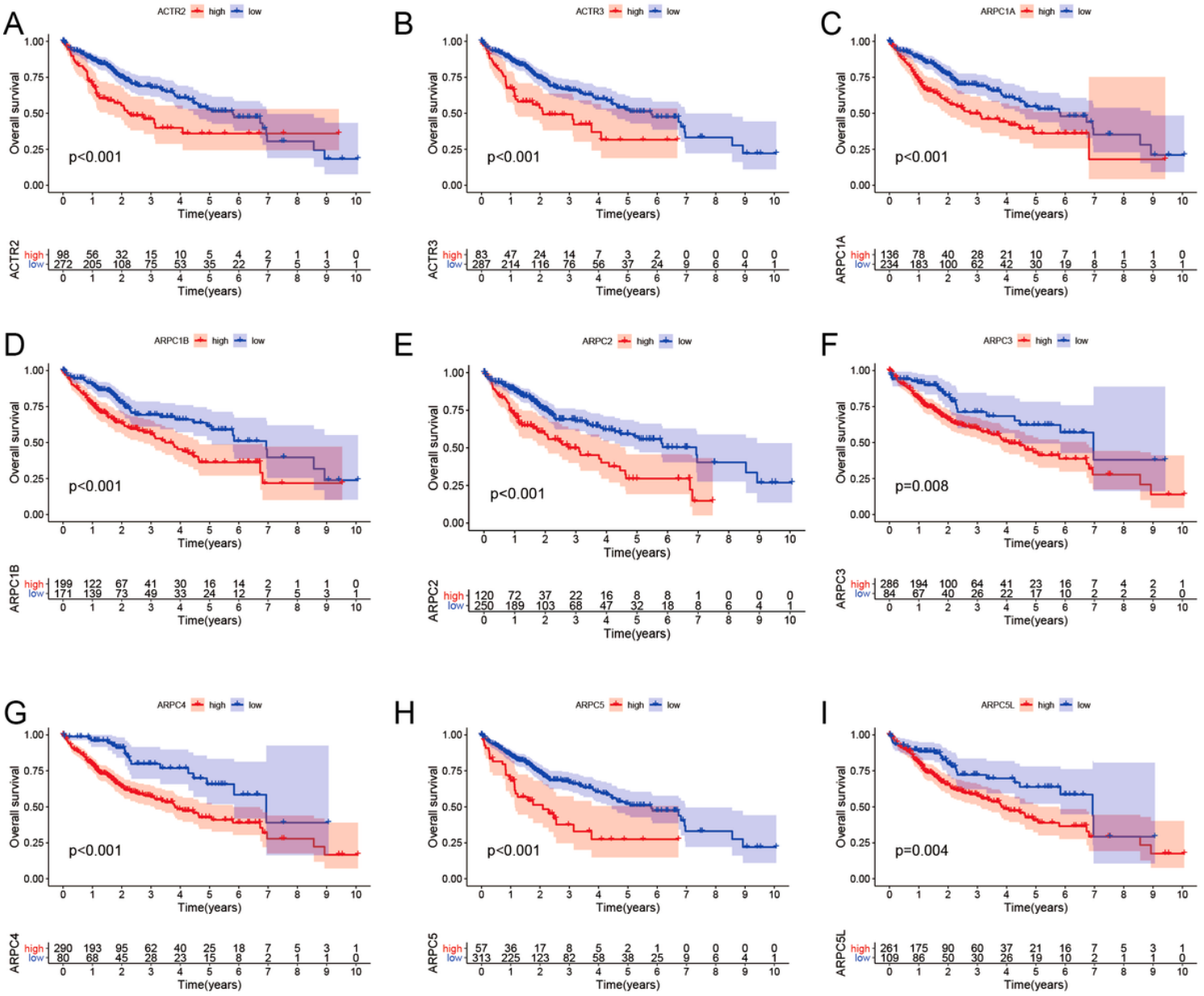

Figure 7

The impact of Arp2/3 complex members expression on overall survival (OS) of HCC patients (A-I).

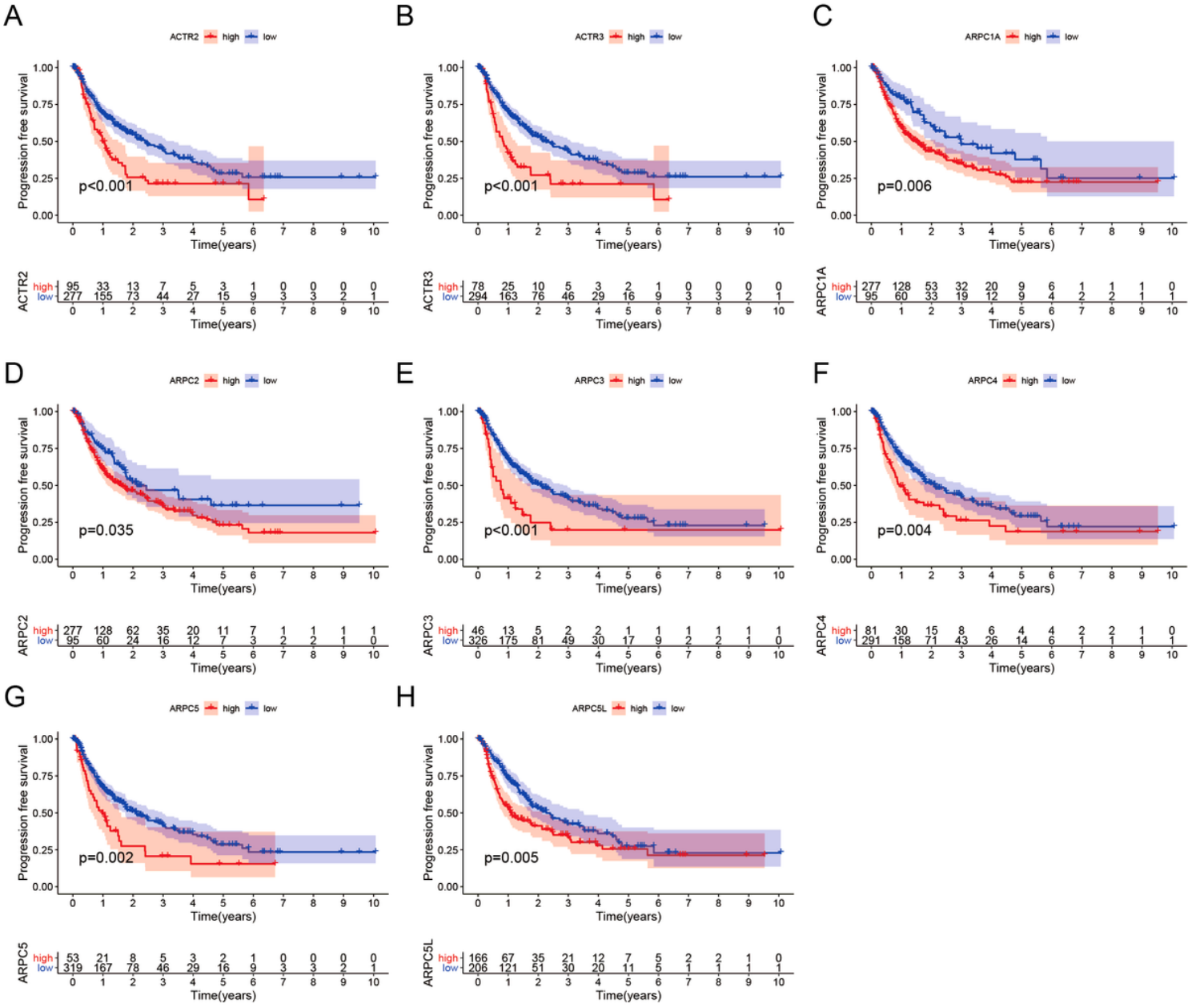

Figure 8

The impact of Arp2/3 complex members expression on progress free survival (PFS) of HCC patients (A-H).

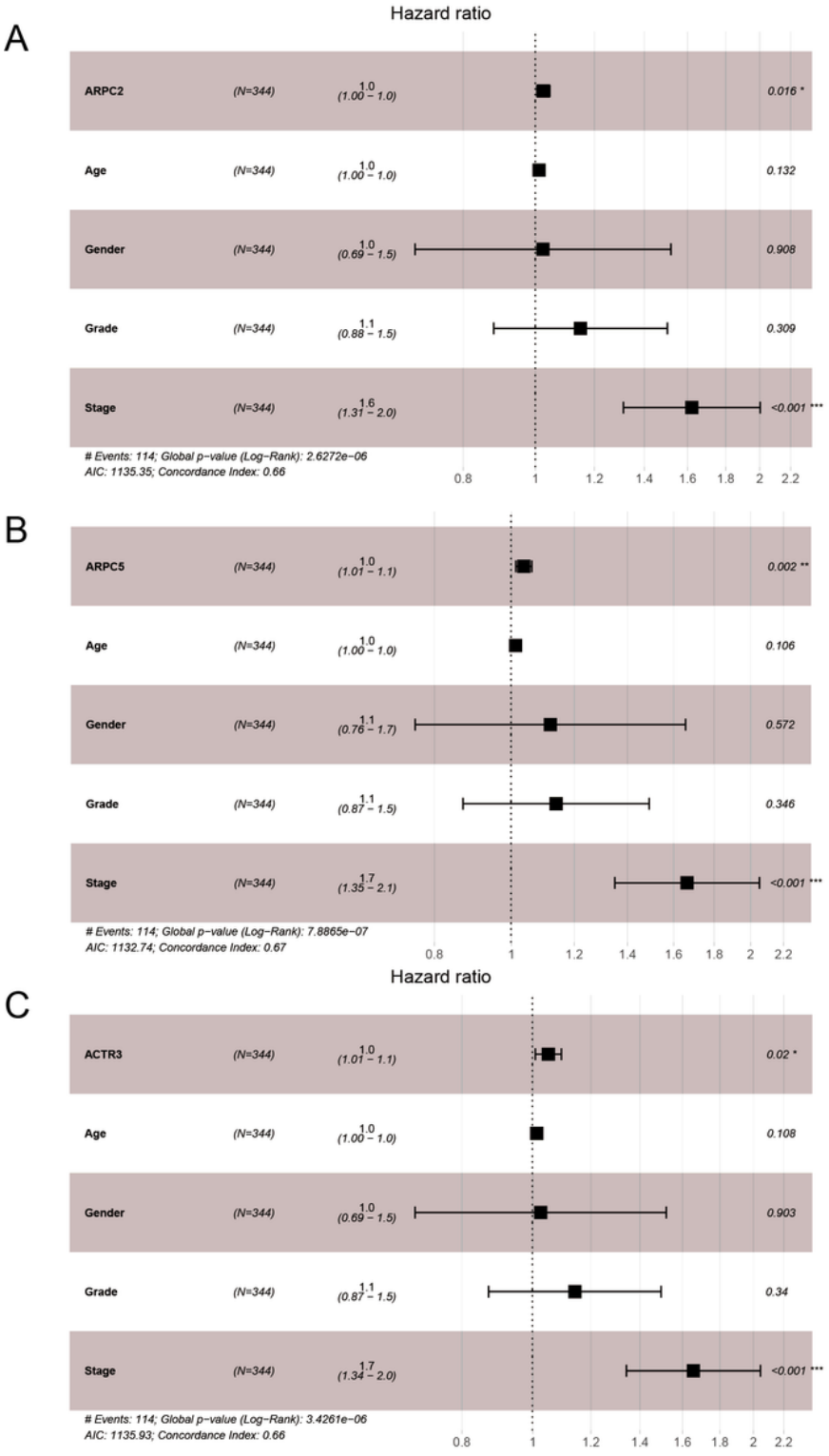

Figure 9

Forest plots of the results of multivariate Cox regression analyses of Arp2/3 members significant prognostic factors, (A) APRC2 (B)ARPC5 (C)ACTR3,*P < 0.05; ** P < 0.01; ***P < 0.001.

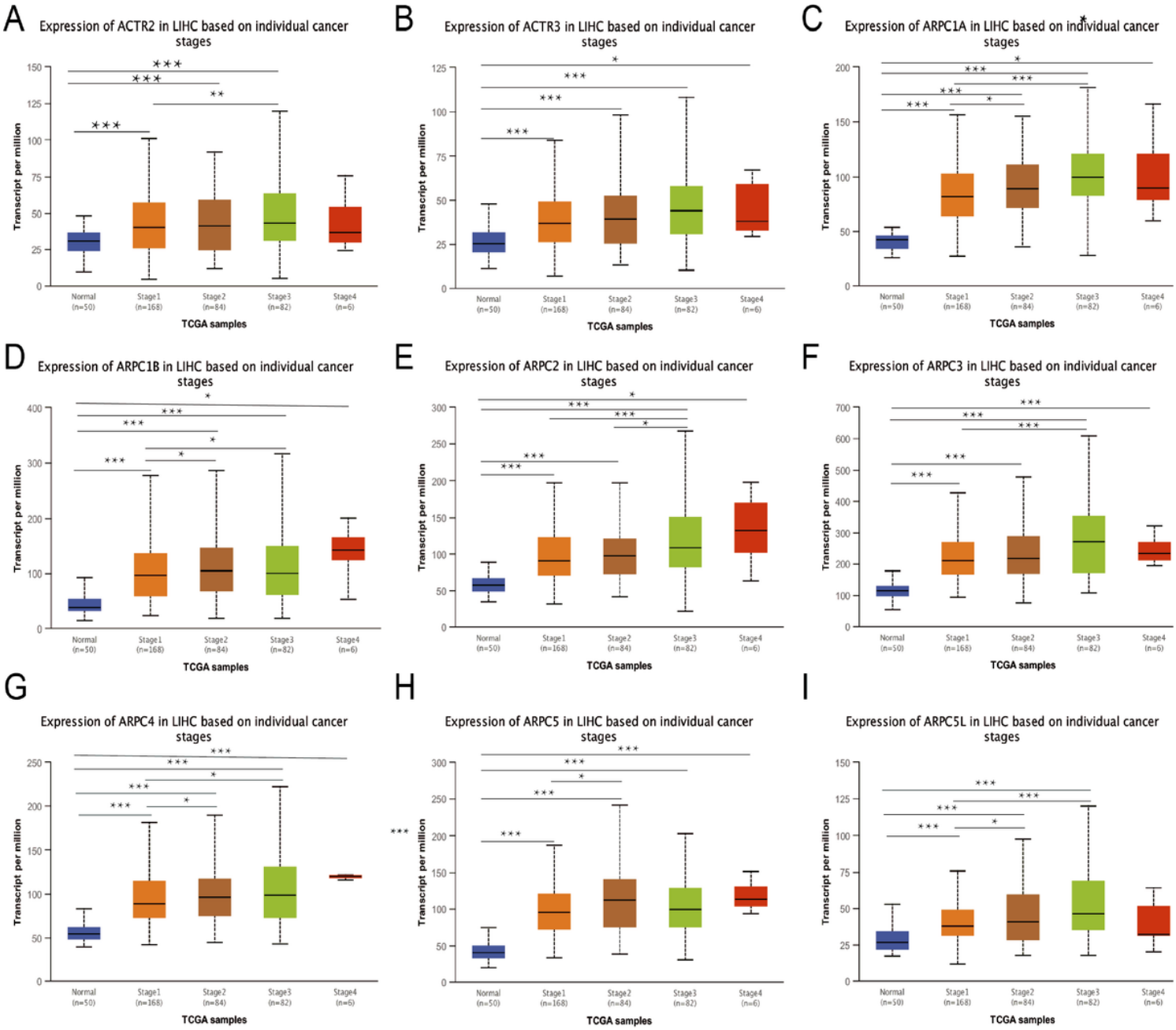

Figure 10

Association analysis of mRNA expression of Arp2/3 members with cancer stages of HCC patients. The mRNA expression of Arp2/3 members in normal individuals or in HCC patients of stages 1, 2, 3 or 4 (A-I), *P< 0.05; **P< 0.01; ***P< 0.001.

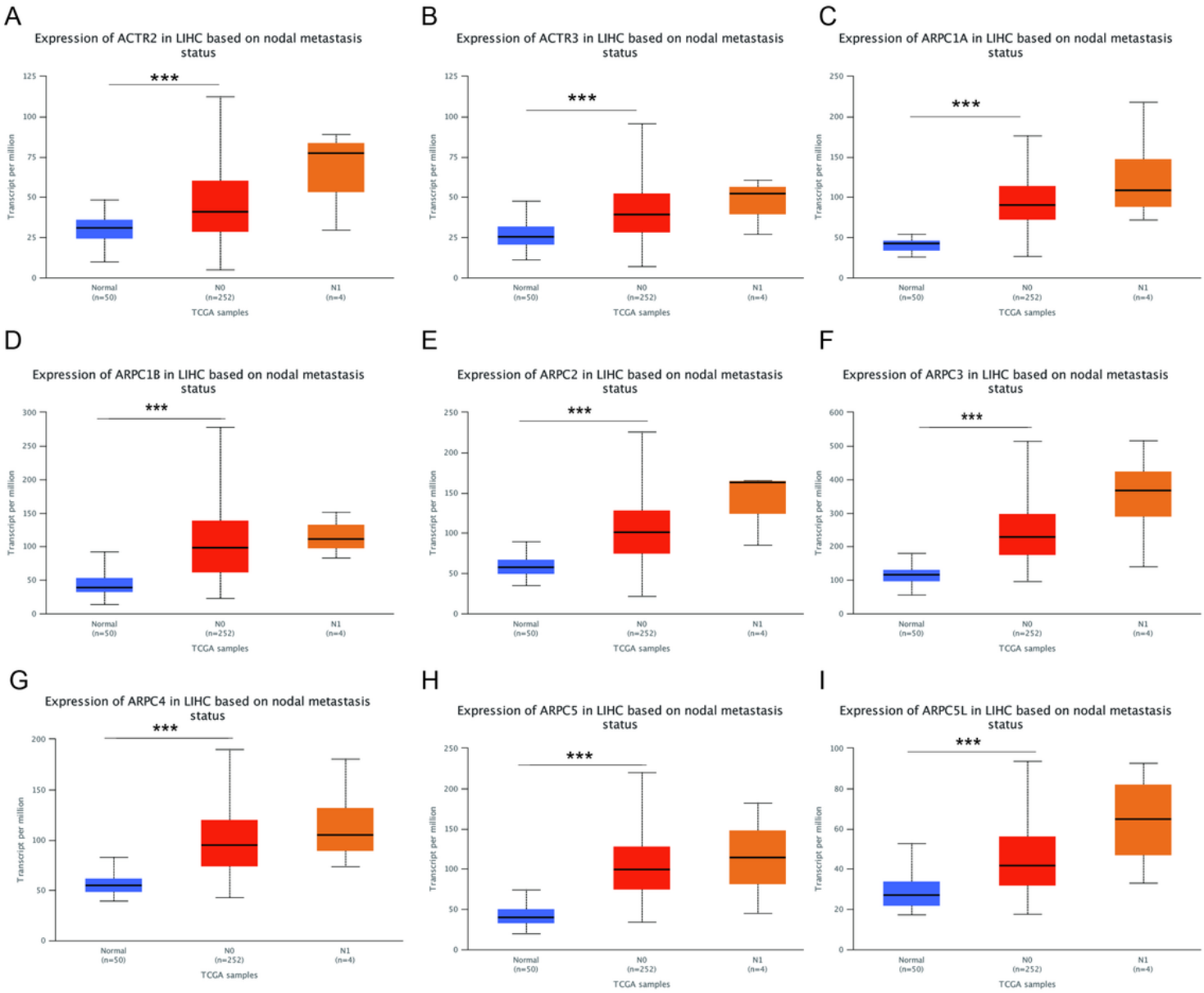

Figure 11

Association analysis of mRNA expression of Arp2/3 members with with nodal metastasis status of HCC patients. The mRNA expression of Arp2/3 members in normal individuals or in HCC patients of nodal metastasis status N0 or N1 (A-I). *P< 0.05; **P< 0.01; ***P< 0.001.

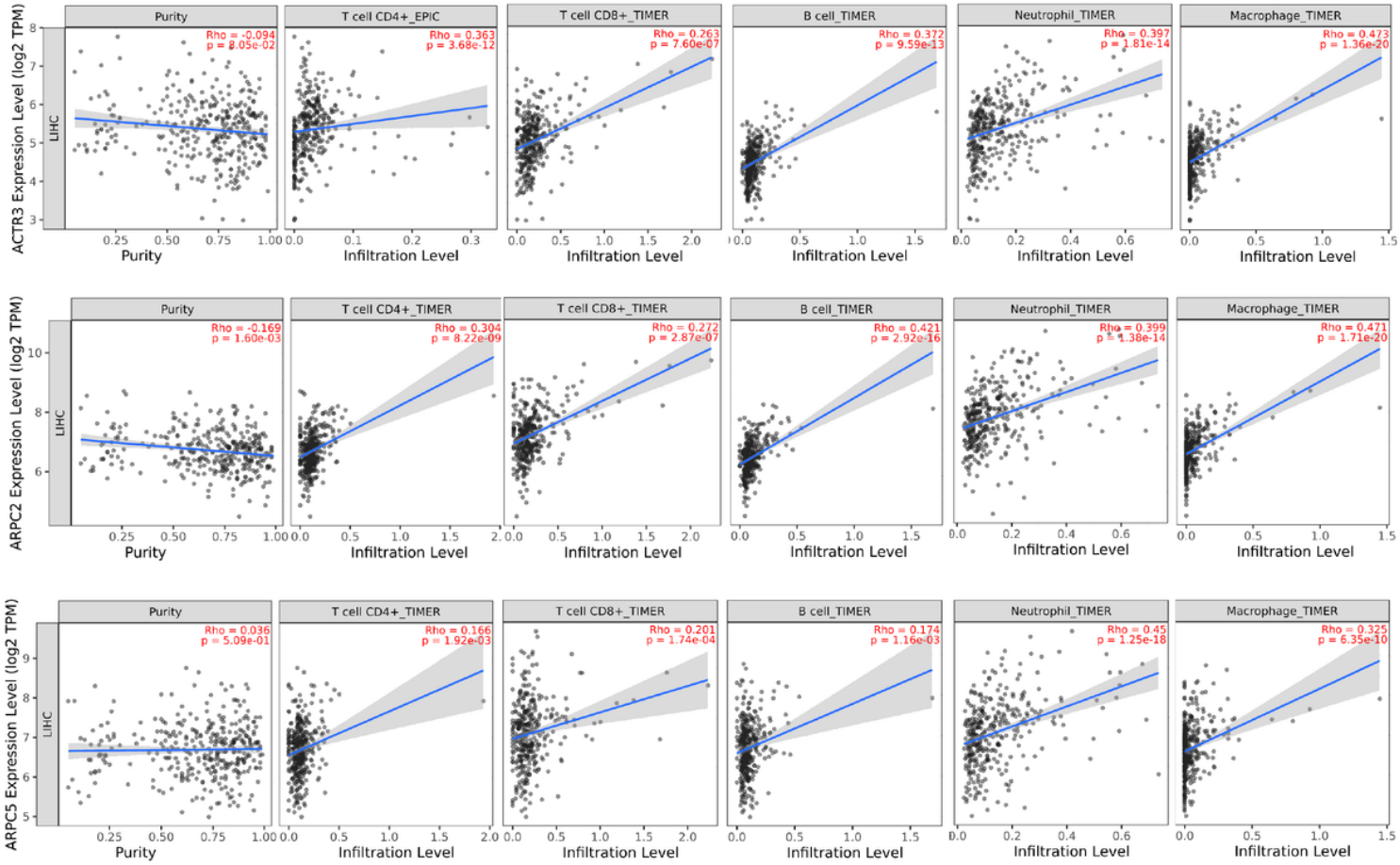

Figure 12

Correlation analysis between tumor infiltrating immune cells (TIICs:CD4+ T cells, CD8+ T cells, B cells, neutrophils, macrophages) and Arp2/3 members (A: ARPC2,B: ACTR3 C:ARPC5) in HCC. Tumor purity is shown in the panels on the left.

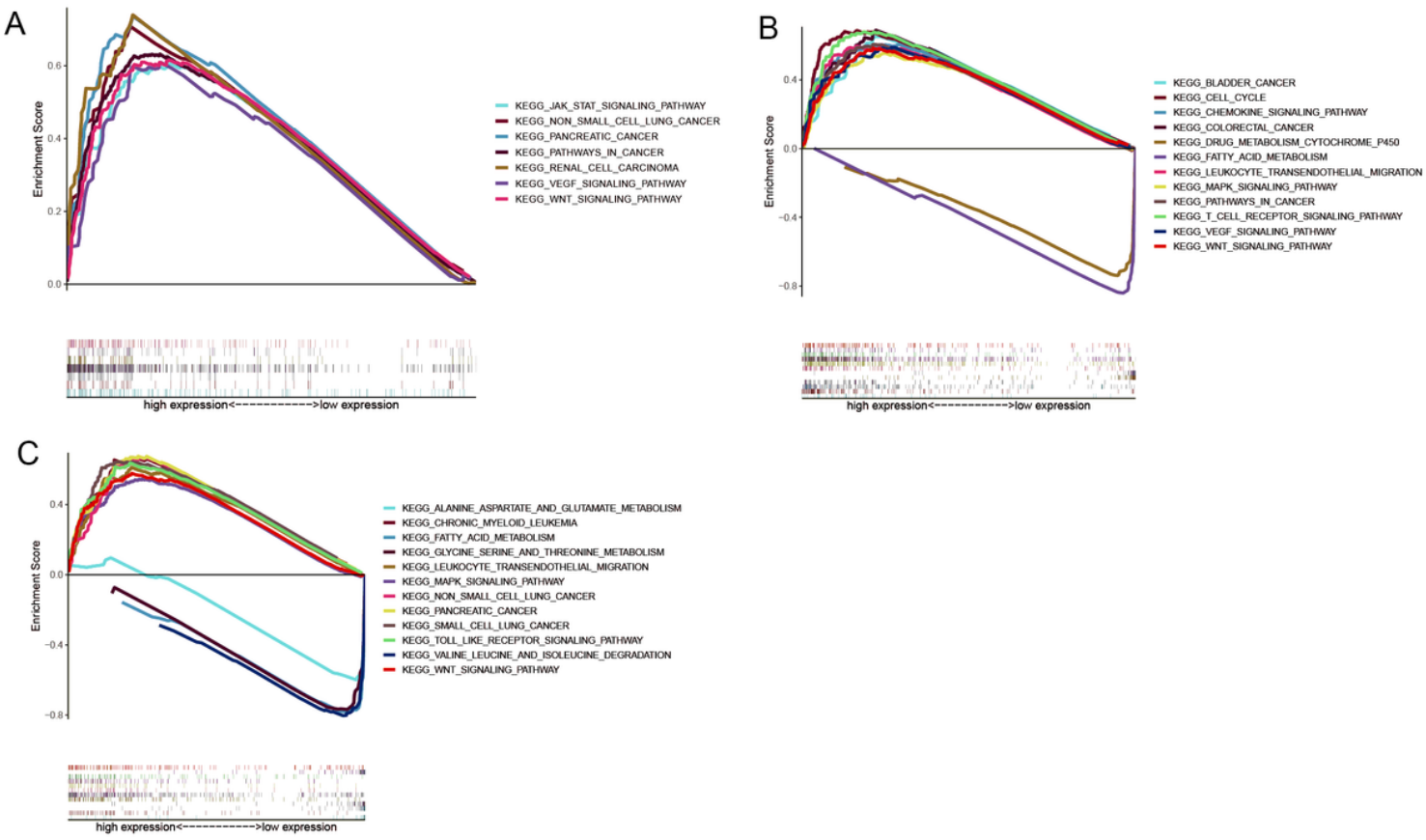

Figure 13

Gene set enrichment analysis (GSEA) of ARCT3 (A), ARPC2(B), and ARPC5(C) based on Cancer-related Kyoto Encyclopedia of Genes and Genomes (KEGG)