HUMAN BIOLOGY
2024, VOL. 94, ISSUE 1
ORIGINAL ARTICLE

OPEN ACCESS

Comprehensive Analysis of The Expression and Prognostic Value for SNRP Members in Hepatocellular Carcinoma

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ABSTRACT

Background: Heterogeneity and epigenetic modifications lead to differences among treatment strategies, management and prognosis in hepatocellular carcinomas. The family of small nuclear ribonucleoprotein polypeptides (SNRPs) plays a crucial role in tumorigenesis and progression. However, the expression profile and prognostic impact of these family members are not clear. Here, we discuss the expression levels and prognosis of SNRPs. family members.

Methods: We compared the transcript levels of each SNRPs member in pan-cancerous tissues by ONCOMINE and further analyzed the expression levels and tumor staging of these markers in hepatocellular carcinoma using UALCAN and GEPIA online databases, while assessing the prognostic value of their mRNA expression and performing functional enrichment analysis by Metascape software using Kaplan–Meier plotter database.

Results: These results showed that mRNA levels of each member of SNRP (B, D1, D2, D3, E, F, G) were significantly upregulated in hepatocellular carcinoma compared to normal tissue and were more highly expressed in patients with advanced hepatocellular carcinoma. mRNA expression of SNRPB, SNRPD1 and SNRPG was associated with poorer overall survival (OS), recurrence-free survival (RFS) and progression-free survival (PFS), which was considered to be statistically significant.

Conclusion: We systematically analyzed the mRNA expression and prognostic significance of each member of SNRPs in HCC and demonstrated the correlation, interaction network, gene alteration and functional enrichment among SNRPs members. Our data suggest that SNRPs members as oncogenes may be a potential indicator of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common of primary liver cancer, remains a high mortality cancer globally Hartke et al. (2017). HCC, which has the highest incidence in China compared to other countries, is now the third leading cause of cancer-related deaths and the fourth most common cancer in China in 2018 Feng et al. (2019), Wallace et al. (2015). Unlike other malignancies, obvious heterogeneity greatly affects the treatment and prognosis of HCC. More potential indicators need to be identified in the overall management of tumors in clinical practice.

The splicing process is accurately ensured by spliceosomes for stability and normalization Lerner et al. (1980). Smith (Sm) proteins play a decisive role in maintaining the integrity of small nuclear ribonucleic acid (snRNA) to avoid nucleases and the downstream RNA processing steps Salgado-Garrido et al. (1999). The formation of heterodimeric (SmD1-SmD2 and SmB-SmD3) or heterotrimeric (SmE-SmF-SmG) subcomplexes is one of the important mechanisms of Sm proteins Gregory Matera et al. (2014). The small nuclear ribonucleoprotein polypeptide (SNRP) B, D1, D2, D3, E, F, G genes are core components of the spliceosomal small nuclear ribonucleoproteins (snRNPs), forming a 7-membered ring/Sm-core-complex that is precursors to major and minor spliceosome Gregory Matera et al. (2014) to ensure RNA stability Salgado-Garrido et al. (1999). These complementary roles of SNRP members tumorigenesis and metastasis roles Mabonga et al. (2019) have attracted value attention.

The increasing evidence showed the indispensable role of the splicing components in the initiation, angiogenesis, apoptosis, and invasion in cancers Mabonga et al. (2019), Fackenthal et al. (2008), Pajares et al. (2007), Skotheim et al. (2007). Relevant researches Peng et al. (2020), Lei et al. (2020), Bao et al. (2020), Yi et al. (2020), 15. Quidville et al. (2013) have reported the differences and prognostic value of single SNRP

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Keywords: hepatocellular carcinoma, SNRP members, mRNA expression, prognosis.

members in different cancer types. The function of SNRPB as an oncogene served as a potential prognostic factor for HCC Peng et al. (2020). Another study

Lei et al. (2020) displayed the mRNA expression of SNRPB may be an effective therapeutic target for cervical cancer by interfering with alterations in the p53 pathway. In addition, high levels of SNRPD1 are considered as predictive biomarkers of tumorigenesis and poor prognosis in lung adenocarcinoma and ovarian cancers Bao et al. (2020), Yi et al. (2020). Even, siRNA deprivation of SNRPE or SNRPD1 drives cell death through autophagy, resulting in a marked reduction in cell viability in breast, lung, and melanoma cancer cell lines Quidville et al. (2013). In summary, current studies Peng et al. (2020), Lei et al. (2020), Bao et al. (2020), Yi et al. (2020), 15. Quidville et al described the expression of individual members of the SNRPs family in a variety of tumors; however, few studies have focused on the expression and prognostic value of the Sm core complex family (B, D1, D2, D3, E, F and G) in HCC patients.

In our study, we performed a comprehensive analysis of the expression and prognosis of core family members of seven SNRPs in HCC patients. In addition, we analyzed the interaction network, genetic alterations, and functional enrichment based on multiple datasets.

MATERIALS AND METHODS

The study has been permitted by the Institutional Review Board of Peking University International Hospital. All methods were carried out in accordance with relevant guidelines and regulations. And there is not direct human participation in these databases.

ONCOMINE Database

ONCOMINE (http://oncomine.org.cutestat.com/) is a publicly accessible online cancer database to compute gene expression signatures, clusters and gene-set modules, automatically extracting biological information from datasets Rhodes et al. (2004). We analyzed the transcriptional levels of each SNRP member and compared them among these members for patients with HCC. Then we used Duncan methods by one-way analysis of variance (ANOVA) (IBM SPSS Statistics v. 21) based on the best gene rank percentile to identify significant differences.

UALCAN

UALCAN (http://ualcan.path.uab.edu/) is a user-friendly, and interactive web resource for providing genes or miRNA analysis based on TCGA from 31 cancer types Chandrashekar et al. (2017). In our study, it was utilized to analyze the expression levels of tumor and normal tissues. Wilcoxon rank sum test was used to determine whether a significant p value or not.

The multiple comparisons by Duncan according to the high expression of SNRP members in primary tissues were described. The cross-correlation coefficients among different genes were calculated by "ggstatsplot" in R version 3.6.1 (http://www.r-pro gect.org/) using spearman correlation analysis.

GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/), is a newly web server to offer customizable functions such as tumor/normal differential expression, profiling according to cancer types or pathological stages, survival analysis, similar gene detection, correlation analysis, and dimensionality reduction analysis based on TCGA and Genotype-tissue Expression (GTE) data Tang et al. (2017). The expression of SNRP members and the tumor stages of HCC were analyzed in this study. The method using pathological stage as variable to calculate differential expression analysis is one-way ANOVA Tang et al. (2017).

Kaplan–Meier (KM) Plotter

The Kaplan-Meier Plotter (http://kmplot.com/analysis/) includes the data of patients on survival of 21 cancer types Menyhart et al. (2018). The prognostic value of mRNA expression was evaluated using the KM plotter with the hazard ratio (HR) with 95% confidence intervals (CI), log rank p-value and median overall survival (OS), relapse-free survival (RFS), progress-free survival (PFS) in lower and upper groups. We split patients by auto select best cutoff Lánczky et al. (2021). The use of false discovery rate (FDR) control of multiple testing in each member survival analysis can provide a adjust p value for drawing conclusions about statistical significance. It was considered to be significant when a adjust p value was less than 0.05. Multivariable Cox regression analysis was conducted to determine the independent predictors of OS, PFS or RFS by using R version 3.6.1.

cBioPortal

cBioPortal (http://www.cbioportal.org/) is an open web tool for interactive exploration of multiple cancer genomic datasets Gao et al. (2013). It was assessed in terms of genetic alteration frequency, the association between alterations, and survival outcome. The survival data were also recorded with respect of OS, PFS, and RFS (numbers of total and events, median survival, and p value) of each member.

Gene MANIA and STRING

Gene MANIA (http://genemania.org) is an online tool for predicting genes and gene sets Mostafavi et al. (2008). It covered 2277 association networks containing 597

million interactions mapped to 163000 genes from 9 organisms Mostafavi et al. (2008). In this study, Gene MANIA was used to describe the genes network of SNRP members and neighbouring genes. STRING (https://cn.string-db.org/) is a database that analyzed protein -protein interactions (PPI) networks Szklarczyk et al. (2017). It was applied to perform reciprocities among the PPI networks of co-expressed genes, and the species were set to Homo sapiens. The relations of expression level about the gene and protein by Cytoscape were identified. Herein, we showed 26 related genes, 47 related nodes, and 927 edges by STRING tool.

Metascape

Metascape (http://metascape.org), is an online website focusing on enrichments pathway analysis Warde-Farley et al. (2010). In this study, the pathways and enrichments were analyzed by Metascape.

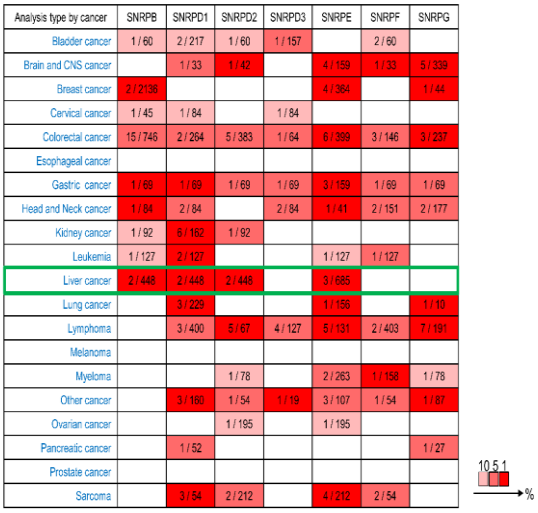
RESULTS

Differential expression of SNRP members in patients with HCC

Firstly, it was determined that genes for SNRP members are located on definite genomic sites Roessler et al. (2010), Chen et al. (2006) (Table 1). We analyzed the transcript levels of the entire cohort of 1858 assays in various cancer types using the ONCOMINE database Rhodes et al. (2004) (Fig. 1). The results showed that significant unique analyses were found in the SNRPB (26 tests), SNRPD1 (32 tests), SNRPD2 (22 tests), SNRPD3 (12 tests), SNRPE (39 tests), SNRPF (15 tests) and SNRPG (23 tests) groups, respectively. SNRPB was significantly elevated in tumor tissues, especially in bladder, cervical, colorectal, gastric, head and neck, kidney, liver and breast cancers, with 2,136 samples. In addition, the levels of each SNRP member were high in patients with gastric and colorectal cancers. Compared with other cancers, liver cancer had the large sample size and strong mRNA expression of SNRPB, SNRPD1, SNRPD2, and SNRPE. In contrast, high levels of SNRPD3 are rarely found in tumor tissue.

The results of ONCOMINE Rhodes et al. (2004) described that the transcript levels of SNRPB, SNRPD1, and SNRPD2 were significantly elevated in HCC tissues Roessler et al. (2010), Roessler et al. (2010). But SNRPD3, SNRPF, and SNRPG had no data set to study. For SNRPE mRNA levels, it was upregulated in all three datasets of the TCGA database Warde-Farley et al. (2010), Roessler et al. (2010), Chen et al. (2002), Roessler et al. (2010). It was summarized in Figure 1 and Table 2.

Figure 1: ONCOMINE analysis of statistically significant mRNA expression levels of SNRPs in different cancers.



The differences in expression levels of (up-regulation: red) the genes among different types by cancer are summarized. Cell color is determined by the best gene rank percentile for the analyses within the cell. It was as following: p-value: 0.05, fold change: 2, gene rank: 10%, data type: mRNA. The number in each cell represents the significant unique analyses and sample sizes, respectively. For example, "2/2136" in breast cancer of SNRPB means there are 2 significant unique analyses and 2136 sample sizes in total.

Table 1: The chromosomal locations of SNRP members.

SNRP familys	SNRPB	SNRPD1	SNRPD2	SNRPD3	SNRPE	SNRPF	SNRPG
Chromosomal location	20p13	18q11.2	19q13.2	22q11.23	1q32	12q32.1	2p13.3

Table 2: Differential expression analyses of SNRP family in transcription level in hepatocellular carcinoma (ONCOMINE).

	Types of cancer vs. normal	Fold change	p-value	t-test	References
SNRPB	Hepatocellular carcinoma vs. normal	2.315	4.23E-75	22.843	Roessler et al., 2010
	Hepatocellular carcinoma vs. normal	2.338	1.12E-06	5.744	Roessler et al., 2010
SNRPD1	Hepatocellular carcinoma vs. normal	3.27	2.55E-97	27.765	Roessler et al., 2010
	Hepatocellular carcinoma vs. normal	2.88	7.91E-09	7.287	Roessler et al., 2010
SNRPD2	Hepatocellular carcinoma vs. normal	2.16	4.05E-82	24.017	Roessler et al., 2010
	Hepatocellular carcinoma vs. normal	2.052	5.00E-09	7.882	Roessler et al., 2010
SNRPE	Hepatocellular carcinoma vs. normal	2.971	1.81E-103	28.959	Roessler et al., 2010
	Hepatocellular carcinoma vs. normal	2.046	1.10E-25	12.246	Chen et al., 2002

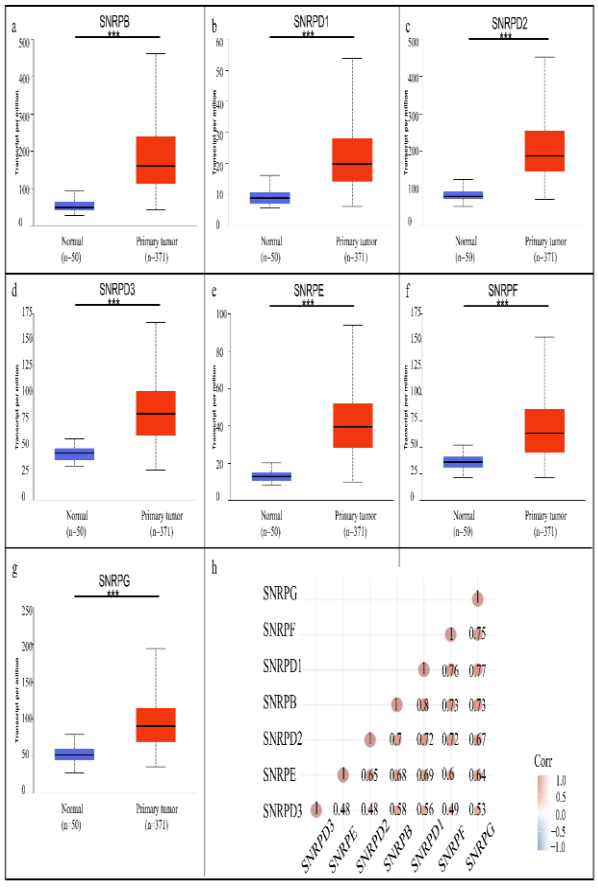
We also compared the transcript levels of SNRPs between HCC and normal tissues by using UALCAN Chandrashekar et al. (2017) (Fig. 2a-2g). We found that SNRPB, D1, D2, D3, E, F, and G were all upregulated in tumor tissues. Besides, we analyzed the correlation among different genes in HCC tissues and determined that SNRPB and SNRPD1 had the highest correlation (Fig. 2h). In conclusion, our results showed that transcriptional expression of SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, and SNRPG was overexpressed in HCC patients.

Table 3 shows multiple comparisons of significant unique analyses or median expression in HCC patients using ONCOMINE and UALCAN servers. SNRPB, SNRPD2, SNRPD3 and SNRPF; SNRPD1 and SNRPD2; SNRPD1, SNRPE and SNRPG were analyzed by best gene ranking percentile, with no differences. In addition, there was no significance between SNRPB and SNRPE; SNRPD1, SNRPD2, SNRPD3, SNRPF, and SNRPG by median expression.

Correlation between mRNA expression and tumor stages of SNRP members

We used GEPIA Tang et al. (2017) to analyze the correlation between mRNA expression and cancer stage in patients treated with HCC. Although there were significant differences between SNRPB, D1, D2, D3, F and G groups at stages I, II, III and IV, there were no differences between SNRPE groups and tumor stage (Fig. 3). That is, the mRNA expression of SNRPB, D1, D2, D3, F and G had a significant relationship with the cancer stage of the patients and appeared higher in advanced stage cancers.

Figure 2:



The mRNA expression of various SNRPs in HCC tissues and adjacent liver tissues (UALCAN) and the correlations among genes of SRNPs in HCC (GEPIA). (a-g) mRNA expressions of SNRPs were found to be over-expressed in HCC tissues compared to normal samples (B/D1/D2/D3/E/F/G). (h) the correlations of SNRPs in HCC. ***p < 0.001.

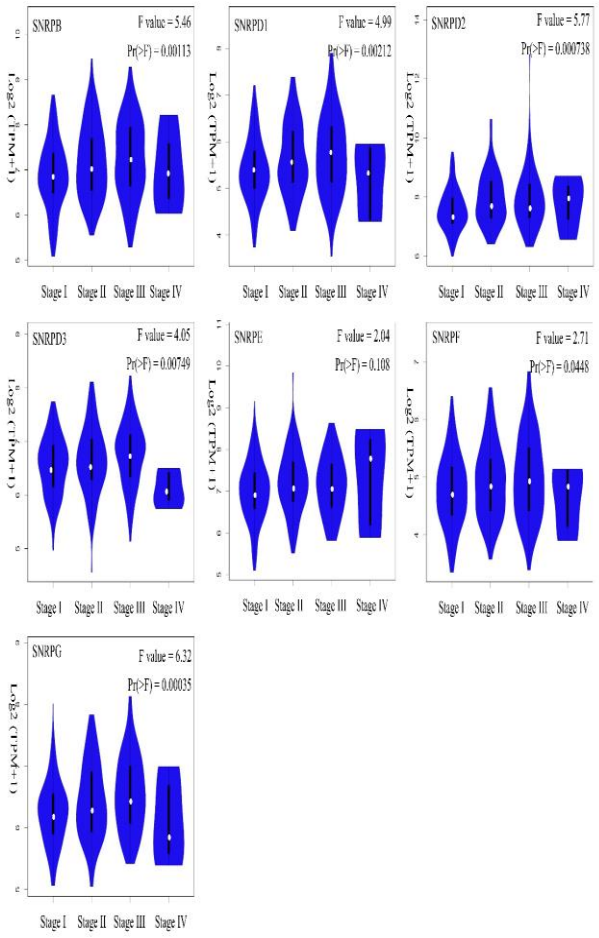
Table 3: The multiple comparisons for SNRP members about the expression levels.

SNRP members	Significant unique analyses*		High expression♯	
	N	Best gene rank	N	Median expression
SNRPB	22	4.82 ± 3.05a	90	203.73 ± 162.09a’
SNRPD1	32	2.96 ± 2.91bc	89	24.41± 18.35b’
SNRPD2	21	4.19 ± 3.08ab	89	221.16 ± 145.40a’
SNRPD3	12	5.08 ± 1.93a	92	87.90 ± 51.95b’
SNRPE	38	2.00 ± 2.35c	90	44.74 ± 31.68b’
SNRPF	16	5.13 ± 2.33a	88	73.91 ± 50.38b’
SNRPG	23	2.09 ± 2.31c	88	64.82 ± 40.17b’
F	-	6.208	-	3.799
P	-	＜0.001	-	0.007

* by ONCOMINE database; a, b, c represent post hoc by one-way ANOVA

♯ by UALCAN online server; a’, b’ represent post hoc by one-way ANOVA

Figure 3:



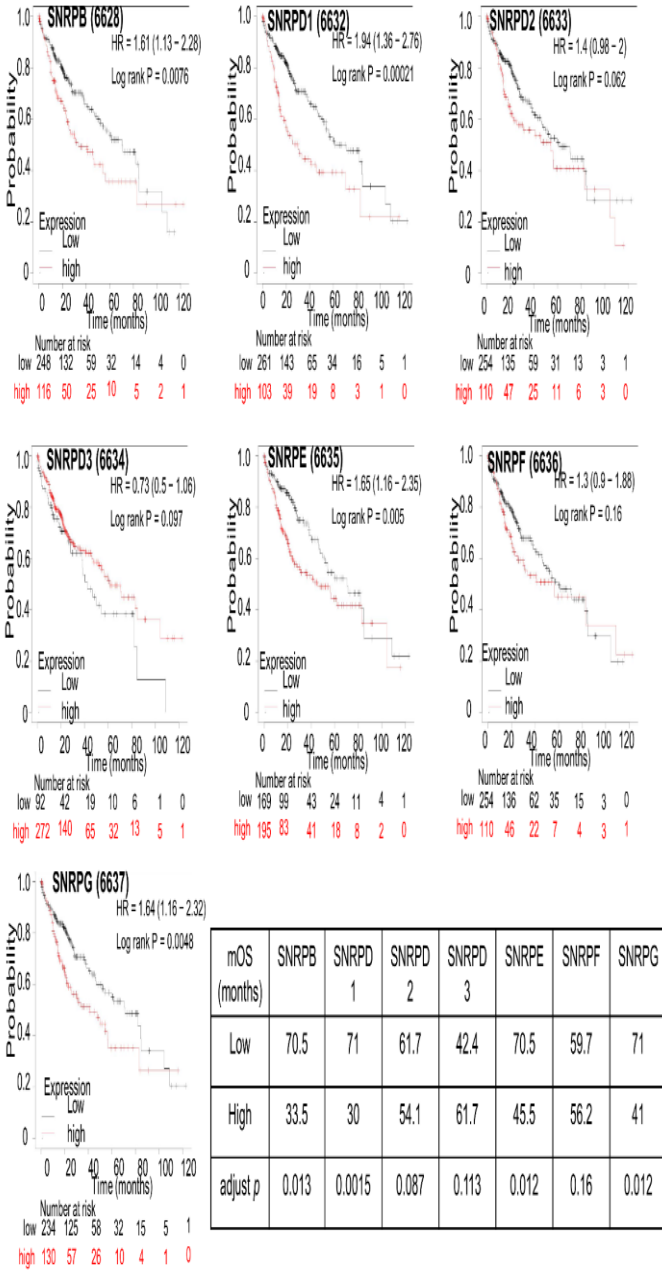
Correlation between mRNA expression and tumor stage of SNRPs in HCC patients (GEPIA). Y-axis is log2(TPM+1). The method for using pathological stage as variable for calculating differential expression analysis is one-way ANOVA. The mRNA expressions of SNRPB/D1/D2/D3/F/G were significantly in connection with patients’ cancer stages (B, D1, D2, D3, F, G), while mRNA expressions of SNRPE had not relations with patients’ cancer stages (E).

Prognostic value of SNRP members in patients undergoing HCC

Prognostic significance of mRNA expression, including OS, PFS, and RFS was under observation. It could be found that patients were classified as low (black) and high (red) risk based on their respective OS thresholds (Fig. 4). High levels of SNRPB, SNRPD1, SNRPE and SNRPG mRNA levels suggest a trend towards worse OS, but without significant differences. The cutoff values distinguishing between the high and low groups based on automatic selection of the best cutoff Lánczky et al. (2021), can be seen in Fig. S1.

Increases in SNRPB, SNRPD1, SNRPD2, and SNRPG were associated with poor PFS (Fig. S2). The relevant cutoff values can be seen in Fig. S4. Furthermore, high mRNA levels of SNRPB SNRPD1, SNRPD2, SNRPE, and SNRPG led to shorter RFS (Fig. S5), while no similar findings were found for SNRPD3, and SNRPDF. The cutoff value for each member can be showed in Fig. S7.

Figure 4:

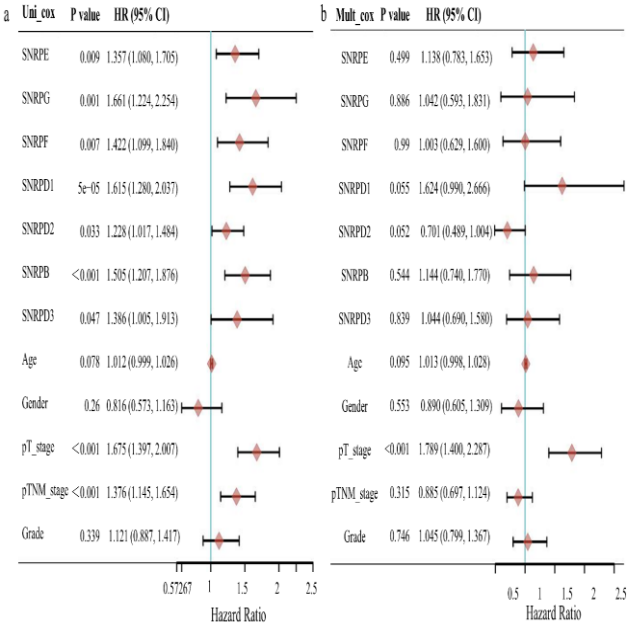


mOS (months)	SNRPB	SNRPD 1	SNRPD 2	SNRPD 3	SNRPE	SNRPF	SNRPG
Low	70.5	71	61.7	42.4	70.5	59.7	71
High	33.5	30	54.1	61.7	45.5	56.2	41
adjust *p*	0.013	0.0015	0.087	0.113	0.012	0.16	0.012

Prognostic value of mRNA expression of relative SNRPs in HCC patients (Kaplan–Meier plotter). The OS survival curves were plotted using the Kaplan–Meier plotter database at p-value of <0.05, comparing patients with high (red) and low (black) SNRPs expression in HCC and distinct median survival in OS. The correlation between prognostic significance and SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, SNRPG protein expression.

There are several known prognostic factors for HCC, such as age, gender, WHO grade, p_T stage, and p_TNM stage. It was necessary to examine whether each member could independently predict prognosis. Univariate Cox analysis presented seven genes were positively associated with survival prognosis (Fig. 5, S3, S6). Moreover, p_T and p_TNM stages were also significantly related to OS (Fig. 5a), PFS (Fig. S3a), and RFS (Fig. S6a). Subsequent multivariate Cox regression analysis indicated that SNRPD1 was significantly correlated with OS (Fig. 5b), but failed to be an independent prognostic factor for PFS (Fig. S3b) or RFS (Fig. S6b). In conclusion, high levels of SNRPB, SNRPD1 and SNRPG were associated with prognosis of OS, PFS and RFS. Details are shown in Table 4.

Figure 5: The subgroup analyses for OS in each member by KM plotter by using R version 3.6.1 (http://www.r-pro gect.org/). Univariate (a) and multivariate analysis (b) for SNRP members, with other factors such as age, gender, pT_stage, pTNM_stage and grade.



Genetic alterations and correlations of SNRP members

We analyzed genatic mutations and interactions in HCC patients by cBioPortal Gao et al. (2013) (INSERM Cancer Cell 2014 dataset, MSK Clin Cancer Res 2018 dataset, INSERM Nat Genet 2015 dataset, MSK PLOS One 2018, AMC Hepatology 2014 dataset, RIKEN Nat Genet 2012 dataset, TCGA Firehose Legacy dataset, TCGA PanCancer Atlas dataset). The results (TCGA PanCancer Atlas dataset) illustrated that the percentages of gene alterations were 0.27% mutations (1/372), 0.53% deep deletions (2/372), 7.53% amplifications

Table 4: The prognostic values of SNRP family members in liver cancer patients (Kaplan–Meier plotter).

SNRP family	OS				RFS				PFS			
	Cases	HR	95%Cl	p	Cases	HR	95%Cl	p	Cases	HR	95%Cl	p
SNRPB	364	1.61	1.13–2.28	0.0076	316	1.65	1.16-2.36	0.0048	370	1.52	1.11-2.08	0.0083
SNRPD1	364	1.94	1.36-2.76	0.00021	316	1.68	1.2-2.35	0.0022	370	1.57	1.17-2.1	0.0026
SNRPD2	364	1.4	0.98-2	0.062	316	1.53	1.1-2.13	0.01	370	1.41	1.05-1.89	0.02
SNRPD3	364	0.73	0.5-1.06	0.097	316	1.33	0.95-1.86	0.091	370	1.28	0.95-1.73	0.11
SNRPE	364	1.65	1.16-2.35	0.005	316	1.59	1.05-2.41	0.026	370	1.38	0.97-1.97	0.076
SNRPF	364	1.3	0.9-1.88	0.16	316	1.34	0.93-1.94	0.12	370	1.36	0.98-1.89	0.066
SNRPG	364	1.64	1.16-2.32	0.0048	316	1.52	1.09-2.11	0.012	370	1.45	1.08-1.94	0.013

Bold values mean p < 0.05.

(28/372) in all SNRP members, respectively (Fig. 6a). The frequency of alterations in SNRPs b was analyzed by using the AMC Hepatology 2014 dataset, TCGA Firehose Legacy dataset, and TCGA PanCancer Atlas dataset (SNRPB, 0.5%; SNRPD1, 0.3%; SNRPD2, 0.8%; SNRPD3, 0.3%; SNRPE, 5%; SNRPF, 0.2%; SNRPG, 0.2%) (Fig. 6b). Then, we further presented the survival results based on genetic alterations. Unfortunately, we did not find significance among genetic alterations, OS or PFS, respectively (p = 0.792, 0.662, Fig. 6c, 6e). However, disease-free survival (DFS) was significant in the genetically altered and unaltered groups (Fig. 6d). The reasons why genetic alterations in SNRPs and prognosis did not seem to be associated with prognosis might be related to the context of the study in the database, the material methodology and the small sample size. Table 5 summarizes the OS or DFS data for the genetically altered and unaltered groups, including the total number and number of events. Only the DFS data for SNRPE had a P value <0.05.

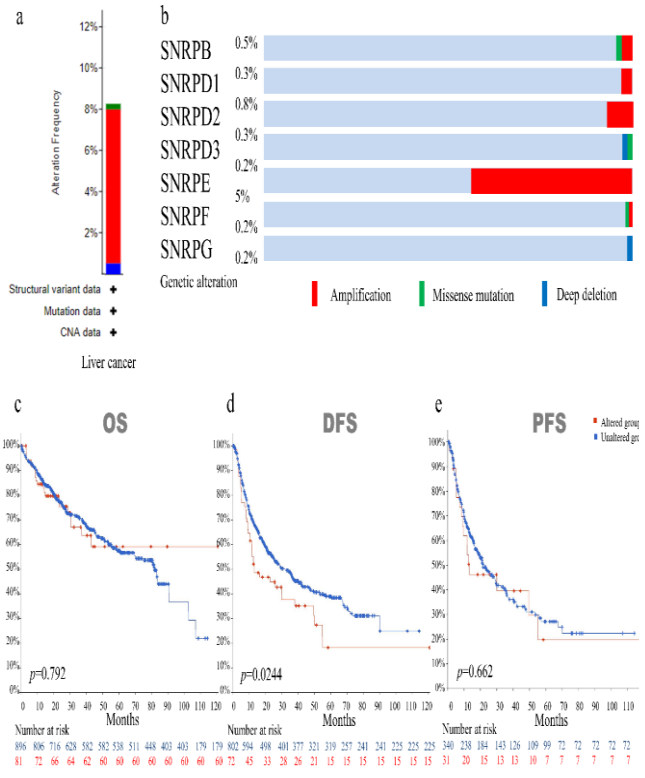


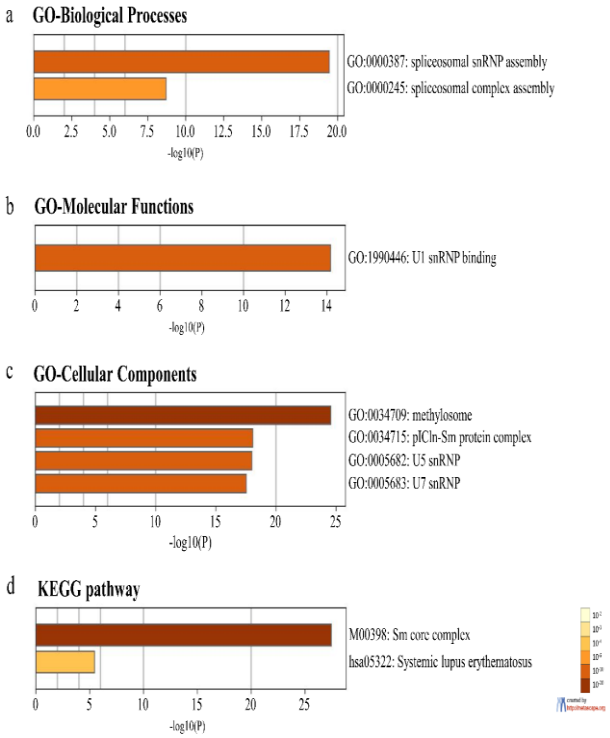
Figure 6: Alteration frequency and survival of SNRPs in HCC (cBioPortal). (a) SNRPs genetic alteration in INSERM Cancer Cell 2014 dataset, MSK Clin Cancer Res 2018 dataset,

INSERM Nat Genet 2015 dataset, MSK PLOS One 2018, AMC Hepatology 2014 dataset, RIKEN Nat Genet 2012 dataset, TCGA Firehose Legacy dataset, TCGA PanCancer Atlas dataset. (b) Alteration frequency of SNRPs based on the TCGA PanCancer Atlas dataset. (c) Kaplan–Meier plots in OS with/without all of SNRPs genetic alterations. (d) Kaplan–Meier plots in DFS with/without all of SNRPs alterations. (e) Kaplan–Meier plots in PFS with/without all of SNRPs alterations.

Functional enrichment analysis of SNRP members

To understand the function of SNRP members and their neighboring proteins, we used GO and KEGG pathways by Metascape Warde-Farley et al. (2010). The result indicated two major GO-biological processes (Fig. 7a), spliceosomal snRNP assembly (GO:0000387) and spliceosomal complex assembly (GO:0000245). U1 snRNP binding (GO:1990446) was only associated with the molecular function (Fig. 7b). The top 4 GO enrichments (Fig. 7c) were cellular components: methylosome, plCln-Sm protein complex, U5 snRNP, and U7 snRNP. The top 2 KEGG enrichments (Fig. 7d) were structural complexes: Sm core complex; pathway: systemic lupus erythematosus.

Figure 7:



The functions enrichment analysis of SNRPs and neighboring genes in HCC patients. (a) GO-Biological Processes. (b) GO-Molecular Functions. (c) GO-Cellular Components. (d) Network of KEGG enriched terms.

Interaction of correlated genes and proteins of SNRP members

We briefly illustrated the correlation of SNRP members at the genatic level by Gene MANIA online tool Mostafavi et al. (2008) (Fig. S8a). The results demonstrated SNRP members share genetic thresholds very closely. Markedly, relationships were found among SNRP members regarding the PPI network. We used STRING Szklarczyk et al. (2017) to determine the correlation of SNRP members at the protein expression level (Figure S8b).

Discussions

A growing number of studies have illustrated that SNRP members are upregulated in various types of cancer and play a vital role in cancer initiation and progression Mabonga et al. (2019). Recent studies Bao et al. (2020), Yi et al. (2020) have shown that SNRPD1 is a predictive biomarker for tumorigenesis and poor prognosis in lung and ovarian cancers. In addition, siRNA depletion of SNRPE, D1 led to a reduction of cell viability in breast, lung, and melanoma cancer cell lines Quidville et al. (2013). However, since SNRPs are the major spliceosomal precursors of the Sm core complex Gregory Matera et al. (2014), we need to study the complex as an integrator to explore the different roles in HCC tissues. We hypothesized that SNRP members might act as onco-promoters to affect the prognosis for HCC patients. Therefore, we performed a systematic analysis of the transcript levels and prognostic value of SNRP members in HCC.

We discussed that the mRNA levels of SNRPB, SNRPD1, SNRPD2, and SNRPE were upregulated in HCC tissues compared to normal tissues and illustrated high levels of SNRPD3, F and G did not present a significant disadvantage by ONCOMINE Rhodes et al. (2004). However, the high levels of expression of SNRPB, D1, D2, D3, E, F, G were found to be present in malignant tumors compared to normal tissues using UALCAN Chandrashekar et al. (2017). These two inconsistent results may be due to the diversity of the background and materials of such abundant researches.

The protein encoded by SNRPB is one of nuclear proteins found in U1, U2, U4, U6, and U5 snRNPs which affected pre-mRNA splicing and it may play an important role in snRNP combination Roessler et al. (2010). Peng NF and his colleagues Peng et al. (2020) showed that SNRPB expression was increased in HCC tissues. In our study, we found that SNRPB expression was significantly elevated in patients with advanced cancer, which is similar to the findings of Peng et al. The gene of SNRPD1 encodes for snRNP Roessler et al. (2010).

Table 5: OS and DFS for the altered and unaltered groups.

Variables	levels	Altered groups			Unaltered groups			
		Total (n)	Events (n)	Months(95%CI)	Total (n)	Events (n)	Median months	p
SNRPB	OS	5	0	NA	973	300	81.73	0.141
	DFS	5	5	38	870	417	29.36	0.192
SNRPD1	OS	4	0	NA	974	300	83.18	0.42
	DFS	4	0	NA	871	422	29.36	0.236
SNRPD2	OS	10	1	43.1	968	299	83.18	0.398
	DFS	9	3	NA	866	419	29.66	0.657
SNRPD3	OS	4	2	3.35	974	298	83.18	0.637
	DFS	NA						
SNRPE	OS	60	16	NA	917	284	83.18	0.768
	DFS	54	32	12.69	820	389	31.9	0.031
SNRPF	OS	3	0	NA	975	300	81.73	0.161
	DFS	3	1	16.4	872	421	29.66	0.539
SNRPG	OS	NA						
	DFS	NA						

OS: Overall survival;

DFS: Disease-free survival

Studies Bao et al. (2020), Yi et al. (2020) reported the use of free-scale gene co-expression networks to assess the relationship between multiple gene datasets and clinical characteristics of patients, followed by confirmation of predictors by weighted gene co-expression network analysis (WGCNA). The mentioned studies indicated that the mRNA expression of SNRPD1 and its encoded protein were highly specific and sensitive for identifying tumor lesions as one of the predictive biomarkers of tumorigenesis and poor prognosis. In our report, we found mRNA expression of SNRPD1 was upregulated in HCC tissues and led to shorter OS, RFS and PFS, and these results were similar to previous studies. Furthermore, SNRPD1 was reported systematically for the first time in HCC patients. The protein encoded by SNRPD2 and SNRPD3 also belonged to the snRNP core protein Roessler et al. (2010). It was shown to be involved in pre-mRNA splicing and snRNP biogenesis. There were few studies on SNRPD2 and SNRPD3 because SNRPD1-SNRPD2 or SNRPB-SNRPD3 preferentially form heterodimeric subcomplexes before forming Smcomplex Gregory Matera et al. (2014).

We revealed that mRNA expression of SNRPD2 and D3 was up-regulated in HCC and correlated with cancer staging. However, there was no correlation between abnormal levels of SNRPD2 and SNRPD3. This suggested that SNRPD2 and D3 were at the high levels in tumor tissues, but may not be suitable as potential prognostic indicators. As with the heterodimeric subcomplexes of SNRPD2 and SNRPD3, SNRPE, SNRPF and SNRPG could form heterotrimeric subcomplexes that cooperate with other SNRP members to form 7- member ring structure/complex and participate in the splicing process Gregory Matera et al. (2014). The current study Quidville et al. (2013) assumed that SNRPE knockdown obviously led to reduced expression in mTOR pathway and protein levels, which partly explained the SNRPE-based autophagy phenomenon. According to Blijlevens and co-workers Blijlevens et al. (2019), high levels of SNRPG protein in v various types of cancer interact positively with cancer initiation, progression and metastasis. The expression of SNRPG in different cancers can be explained by high levels of protein,

the mis-localization of unassembled or misassembled proteins Prusty et al. (2017). Thus, SNRPG might contribute to the initiation and progression of different cancers Schwer et al. (2016), Conte et al. (2002), Shi et al. (2009), Yoshitake et al. (2004), Ye et al. (2018). We found that SNRPE was highly expressed in tumor tissues by using ONCOMINE Rhodes et al. (2004), but the results of SNRPF and SNRPG groups were not similar in this study. In addition, the mRNA of SNRPE did not correlate with cancer stage and PFS. The different results compared to previous studies might be the small sample sizes or different cancer types.

In the study, we found that Gene MANIA Mostafavi et al. (2008) and STRING Szklarczyk et al. (2017) analysis revealed close co-expression between SNRP members at the gene level, while co-expression at the protein level was compactly correlated.

To investigate the correlation of gene alterations, we revealed the frequency of gene alterations in SNRPs by using cBioPortal Gao et al. (2013). We studied the functional enrichment of the SNRPs by Metascape Warde-Farley et al. (2010). Our results indicated that SNRPs members are involved in functions that may include methylosome, U1, U5, U7 snRNP binding, Sm core complex, etc., which have been studied as involved in cell cycle, signal transduction, angiogenesis, apoptosis and invasion Fackenthal et al. (2008), Pajares et al. (2007), Skotheim et al. (2007), Dutertre et al. (2010), Pettigrew et al. (2008), Srebrow et al. (2006), Venables et al. (2006), Kalnina et al. (2005), Brinkman et al. (2004). The spliceosome complex is formed by snRNP Jurica et al. (2003), Zhou et al. (2002). Each snRNP (U1, U2, U4, U6, and U5) includes an snRNA integrated with a set of Sm core complex. The Sm core complexes (B, D1, D2, D3, E, F and G) form a 7-ring core structure/complex to encapsulate RNA. All SNRP proteins have conserved Sm structural domains to help form the Sm core of the snRNPs Salgado-Garrido et al. (1999), Hermann et al. (1995) thereby determining pre-mRNA processing Wahl et al. (2009). However, further work is needed to understand the role and function of SNRP members.

There were some limitations in our research. First, all data were analyzed in our study from various online database tools, which c may be derived from various research contexts, bases and samples, and therefore further studies in larger samples are needed to demonstrate these. Then, no biological experiments, clinical specimens and cases were performed to validate the results. Next, in vitro and in vivo studies will be performed and may provide some further conclusions.

CONCLUSION

In this study, we systematically analyzed the mRNA expression and prognostic significance of SNRP members in HCC. In addition, we presented the correlations of co-expression and interaction networks, genetic alterations and function enrichment of SNRP members. Expression of SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPE, SNRPF, and SNRPG was upregulated in tumor tissues compared to normal tissues, and high levels of SNRPB, SNRPD1 and SNRPG resulted in poorer OS, RFS and PFS. In conclusion, SNRPB, SNRPD1, and SNRPG could act as the gene promoters and novel prognostic biomarkers for HCC.

Supplementary description:

In supplementary materials, we split patients by auto select best cutoff for OS, PFS, and RFS in Fig. S1, S4, S7, and the prognostic value of PFS and RFS were showed in Fig S2, S5, the subgroup analyses shown in Fig S3, S6. Interaction network of SNRPs at the gene and protein levels in HCC patients in Fig. S8.

DECLARATIONS

Ethics approval and consent to participate

All ethical approval, guidelines, and informed consent are available in each article, which is published and searchable in a public database. All methods were carried out in accordance with relevant guidelines and regulations. And there is not direct human participation in these databases.

Consent for publication

Not Applicable.

Availability of data and materials

Oncomine (http://oncomine.org.cutestat.com/); UALCAN (http://ualcan.path.uab.edu/); Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/); The Kaplan-Meier (KM) Plotter (http://kmplot.com/analysis/); cBioPortal for Cancer Genomics (http://www.cbioportal.org/); GeneMANIA (http://genemania.org); STRING (https://cn.string-db.org/); Metascape (http://metascape.org);

Competing interests

All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

Funding

Project supported by Peking University International Hospital Research Grant (No. YN2022QN12).

Authors' contribution

(I) Conception and design: Ziwei Guo

(II) Administrative support: Jun Liang

(III) Provision of study materials or patients: Ziwei Guo, Chuanhao Tang

(IV) Collection and assembly of data: Ziwei Guo, Chuanhao Tang

(V) Data analysis and interpretation: Ziwei Guo, Chuanhao Tang

(VI) Manuscript writing: All authors

(VII) Final approval of manuscript: All authors

Acknowledgements

A preprint has previously been published in research square DOI: 10.21203/rs.3.rs-633291/v1.

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