Expression Profile and Prognostic Values of SMC Family Members in HCC

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Abstract

Background: The structural maintenance of chromosome (SMC) gene family, comprising 6 members, is involved in a wide spectrum of biological functions in many types of human cancers. However, there is little research on the expression profile and prognostic values of SMC genes in hepatocellular carcinoma (HCC). Based on updated public resources and integrative bioinformatics analysis, we tried to determine the value of SMC gene expression in predicting the risk of developing HCC.

Methods and materials: The expression data of SMC family members were obtained from The Cancer Genome Atlas (TCGA). The prognostic values of SMC members and clinical features were identified. A gene set enrichment analysis (GSEA) was conducted to explore the mechanism underlying the involvement of SMC members in liver cancer. The associations between tumor immune infiltrating cells (TIICs) and the SMC family members were evaluated using the Tumor Immune Estimation Resource (TIMER) database.

Results: Our analysis demonstrated that mRNA downregulation of SMC genes was common alteration in HCC patients. SMC1A, SMC2, SMC3, SMC4, SMC6 were upregulated in HCC. Upregulation of SMC2, SMC3 and SMC4, along with clinical stage, were associated with a poor HCC prognosis based on the results of univariate and multivariate Cox proportional hazards regression analyses. SMC2, SMC3 and SMC4 are also related to tumor purity and immune infiltration levels of HCC. The GSEA results indicated that SMC members participate in multiple biological processes underlying tumorigenesis.

Conclusion: This study comprehensively analyzed the expression of SMC gene family members in patients with HCC. This can provide insights for further investigation of the SMC family members as potential targets in HCC and suggest that the use of SMC inhibitor targeting SMC2, SMC3 and SMC4 may be an effective strategy for HCC therapy.

Introduction

Liver cancer remains a global health challenge and its incidence is growing worldwide. Hepatocellular carcinoma (HCC) is the most common form of liver cancer, accounting for ~ 90% of cases[1, 2]. According to the World Health Organization's statistics in 2018, HCC ranked sixth in incidence and fourth in mortality. There are approximately 840,000 new cases and over 780,000 deaths each year[3]. In recent years, although some progress has been made through the comprehensive treatment based on surgical resection, the overall efficacy and prognosis of patients with HCC is still not ideal[4]. Therefore, the development of prognostic biomarkers and drug targets is required and will provide better prognosis and more effective personalized treatments for HCC patients.

The structural maintenance of chromosome (SMC) is composed of 6 unique members (SMC1A, SMC2, SMC3, SMC4, SMC5, SMC6). SMC proteins have been shown to be involved in DNA repair, genetic recombination, sex-chromosome dosage compensation, sister chromatid cohesion and chromosome condensation[5]. SMCs are believed to have distinct and complex roles in different tumors. Upregulation

of SMC has been frequently observed in cancers and proposed to be potential targets in therapy of diverse types of solid tumors, such as liver, colon, and lung cancer[6–8]. Meanwhile, some studies have also demonstrated that downregulation of SMC genes resulted in enhanced oncogenic properties of cancer cells. For example, Previous reports showed that knocking down the SMC2 gene could inhibit tumor growth in colorectal cancer and increase apoptosis of neuroblastoma cells[9]. However, the expression and prognostic role of SMC gene family in HCC patients remain elusive. Studying the differential expression of SMC genes in HCC provides an opportunity to advance our understanding of HCC development and to develop new therapeutic agents. In this study, based on updated public resources and integrative bioinformatics analysis, the expression profile and prognostic values of the SMC family members were comprehensively assessed.

Materials And Methods

Data Collection

The Cancer Genome Atlas (TCGA) research network, conducted by the National Cancer Institute and National Human Genome Research Institute, has molecularly analyzed over 10,000 tumor patients and 20,000 primary cancer samples across 33 cancer types. Among which, 375 cases of hepatocellular carcinoma (HCC) and the matching clinical parameters including gender, age, tumor (T) stage, node (N) stage, metastasis (M) stage were extracted (<https://portal.gdc.cancer.gov/>). Moreover, Genomic Data Commons (GDC) Data Transfer Tool was used to download high-throughput sequencing (HTSeq) Fragments Per Kilobase of transcript per Million mapped reads (FPKM) data on the SMC family.

Analysis of SMCs expression in HCC

The mRNA expression levels of SMC family were obtained from the HTSeq level 3 data on genome mRNA expression using Perl 5.26 software. The differential expression of the SMC family between HCC tissues and normal tissues was analyzed utilizing the limma package in R 3.6.0 software and visualized via heatmap package. The Human Protein Atlas database, which contains immunohistochemical and immunofluorescence expression data from different kinds of normal and cancerous tissues was used to provide us with immunohistochemical staining images of SMC family proteins in normal liver tissues and HCC tissues.

Survival Analysis of SMC Members in HCC

Two steps were used to explore the prognostic values of the SMC family members: 1) the associations between SMC members, different clinical parameters and overall survival among HCC patients were investigated using univariate Cox proportional hazards regression analyses; 2) Using multivariate Cox proportional hazards regression analysis, the independent prognostic values of the SMC members were then obtained by controlling the significant clinical parameters from step 1. All the analyses were performed using the survival package in R 3.6.0 software.

Immune cell environment analysis

The Tumor Immune Estimation Resource (TIMER) platform (<https://cistrome.shinyapps.io/timer/>), which is an integrated online tool for assessing the abundance of tumor immune infiltrating cells (TIICs) through diverse cancer types, was utilized to explore the correlations between TIICs and SMC members. Totally 6 kinds of TIICs were explored including B-cells, CD4⁺ T-cells, CD8⁺ T-cells, dendritic cells (DCs), macrophages, and neutrophils. In addition, the purity of tumors was also accurately quantified in the database.

Gene Set Enrichment Analysis (GSEA)

GSEA (version 4.0.1; <http://software.broadinstitute.org/gsea/index.jsp>) was performed to explore the potential pathways of SMC members participating in the carcinogenesis of HCC. The annotated gene set file c2.cp.kegg.v7.0.symbols.gmt (from the Msig database) was used as the reference. A random combination number of 1,000 permutations were performed and a false discovery rate (FDR) < 0.05 was used to identify the significantly enriched pathways.

Software and Statistical Analysis

Perl 5.26 software was used to analyze The HTSeq FPKM mRNA data downloaded from the TCGA database. All analyses were performed using the R software (3.5.1, www.r-project.org) loaded with different packages, the limma package for the expression of SMCs, the corrplot package for the correlation between methylation and mRNA expression of SMCs, the survival package for the analysis of prognostic values, the ggplot package for the multivariate Cox proportional hazards regression analysis. $P < 0.05$ was considered to be statistically different.

Results

mRNA Expression of SMC members in HCC

To explore the mRNA expression level of SMC family members (SMC1A, SMC2, SMC3, SMC4, SMC5, SMC6) in HCC, Perl software was used to obtain the expression data of SMCs in 375 HCC cases and 50 normal tissues derived from TCGA. Pearson's correlation of SMCs was evaluated using corrplot package to determine whether there is a correlation between these genes. Interestingly, as shown in **Fig. 1**, there is a significant correlation between SMC family members.

As shown in **Fig. 2A**, limma package was used to show the differentially expressed SMCs and pheatmap package was used to visualize the results. As shown in **Fig. 2B**, SMC1A, SMC2, SMC3, SMC4 and SMC6 were significantly upregulated in HCC tissues, whereas no difference was found in SMC5 expression between HCC and normal tissues. Moreover, the Human Protein Atlas database was used to explore the protein levels of SMCs in HCC. Next, in order to study the Protein levels of the SMC gene family in liver cancer tissues, we demonstrated the differences in the expression of SMC family proteins in HCC and normal liver tissues through the Human Protein Atlas database. As shown in **Fig. 2C**, the expressions of SMC1A, SMC2, SMC3, SMC4 and SMC6 in HCC tissues were higher than those in normal liver tissues, while there was no significant difference between SMC5 in liver cancer tissues and normal tissues.

Prognostic Values of SMC members in HCC

Subsequently, the prognostic values of SMC members for HCC were further assessed. Univariate Cox proportional hazards regression analysis was utilized to analyze the predictive ability and clinical characteristics of differentially expressed SMC members (SMC1A, SMC2, SMC3, SMC4, SMC5, SMC6). It was found that four SMC members (SMC2, SMC3, SMC4, SMC6) and one clinical feature (stage) were closely associated with poor outcome of HCC patients (hazard ratio [HR] for SMC2: 1.137 (1.051–1.230); HR for SMC3: 1.041 (1.009–1.075); HR for SMC4: 1.094 (1.038–1.152; SMC6: 1.119 (1.001-1.250)); HR for stage: 1.679 (1.368– 2.061) (**Table 1**). In order to further explore the correlation between the expression level of SMC members and the overall survival (OS) and progression-free survival (PFS) of HCC patients, we analyzed the expression profile and clinical data of HCC patients from TCGA database. The results showed that elevated expression of SMC2, SMC3, SMC4, and SMC6 in HCC patients were negatively correlated with patients' OS, while higher expression levels were associated with shorter OS (P values of 0.002, 0.001, < 0.002, and 0.014), as shown in **Fig. 3A**. Meanwhile, high expression of SMC2, SMC3, SMC4, and SMC6 was also negatively correlated with PFS, with higher expression levels associated with shorter PFS (P values of < 0.001, 0.001, < 0.001, and 0.01), as shown in **Fig 3B**. Next, multivariate Cox proportional hazards regression analysis was used to evaluate the independent prognostic values of SMC2, SMC3, SMC4 to control the influence of clinical features on the prognosis. The results, which are exhibited in forest plots (**Fig. 4**), confirmed that the expression of SMC2, SMC3, and SMC4 and a clinical parameter (stage) were independent biomarkers for the prognosis of HCC patients.

Correlations Between SMC Members and TIICs in HCC

TIICs, an important part of the complex tumor microenvironment, have been reported to be associated with the progression of many cancers. Hence, it would be meaningful to explore the association between immune infiltration and SMC members. We calculated the coefficient of SMCs and the level of immune infiltration in HCC patients to determine whether they were related. The first column in **Fig. 5** showed significant correlations between SMCs expression and tumor purity. In addition, SMC expression was notably correlated with the infiltration levels of most immune cell types in HCC. Specifically, the level of SMC2 expression positively correlated with the infiltration levels of B cells ($r=0.372$, $p=1.03e-12$), CD8+ T cells ($r=0.353$, $P=1.67e-11$), CD4+T cells ($r=0.332$, $P=2.59e-10$), macrophages ($r=0.451$, $P=1.70e-18$), neutrophils ($r=-0.484$, $P=1.12e-12$), and DCs ($r=0.468$, $P=7.03e-20$). The same thing with SMC3 and SMC4. These results indicated that 3 members of SMC gene family were related with tumor purity and immune infiltration levels in HCC.

Mechanism Underlying the Prognostic role of SMC Members in HCC

A GSEA of differentially expressed SMC members with statistical prognostic value was conducted to evaluate the potential biological mechanism by which differential expression of SMC2, SMC3, and SMC4 affects the carcinogenesis of HCC. The GSEA indicated that high expression of SMC2 was related to “cell cycle”, “oocyte meiosis”, and “ubiquitin mediated proteolysis” (**Fig. 6A**), high expression of SMC3 was

related to “cell cycle”, “ERBB signaling pathway”, “oocyte meiosis” and “ubiquitin mediated proteolysis” (**Fig. 6B**), high expression of SMC4 was related to “chronic myeloid leukemia” (**Fig. 6C**).

Discussion

Bioinformatics is a new subject that refers to the application of informatics to the field of biology and is widely used to explore the mechanisms of carcinogenesis [10][11][12]. Overexpression of SMC factors has been reported in many tumors. Increasing evidence has shown that the protein products of SMC genes not only act as transcriptional factors promoting carcinogenesis but also serve as tumor-suppressor factors, based on their aberrant expression patterns in certain organs[7, 9, 13, 14]. The availability of public genomic data provided us the opportunity to explore the expression profiles of families of genes in human cancers and their clinical practice value. This study evaluates the distinct expression and methylation profile, biological processes, and prognostic values of six SMC members expressed in HCC.

From the data, we found that the SMC family members likely contributed to carcinogenic effects in the development of HCC. Compared with normal tissues, all SMC genes with the exception of SMC5 were highly expressed in HCC cells. Moreover, the overexpression of SMC1A, SMC2, SMC3, SMC4 and SMC6 were related to the PFS of HCC patients. In this study, univariate Cox proportional hazards regression analyses were performed to analyze the prognostic values of SMC members in HCC. In fact, four SMC members were significantly associated with poor clinical outcomes in HCC (SMC2, SMC3, SMC4, SMC6). In addition, multivariate Cox proportional hazards regression analyses affirmed the prognostic value of SMC2, SMC3, SMC4 in the prediction of poor outcome in HCC patients.

The structural maintenance of chromosome 1 alpha (SMC1A) gene is located in Xp11.22-p11.21 and consists of 25 exons and 24 introns[15]. It is a member of SMC family that serves critical roles in organizing and stabilizing chromosomal segregation during mitosis, and is considered to be a component of the signaling network involved in the maintenance of genome stability[16]. SMC1A is known for its role in cell division, DNA repair and activation of cell cycle checkpoints[17, 18]. Previous studies reported that upregulated SMC1A may be associated with glioblastoma, lung cancer and colon cancer progression[19–21], but there is little information on the expression and prognosis of SMC1A in HCC. In this study, we analyzed TCGA datasets and found that SMC1A was more highly expressed in HCC tissue compared to normal tissue, and that increased expression of SMC1A is significantly associated with poor PFS.

SMC2 forms part of the condensin complex that has a central role in many aspects of chromosome biology, including the segregation of sister chromatids and compaction of chromosomes during cell division, as well as the regulation of gene expression during the interphase[22–25]. SMC2 has been found to be over-expressed in a significant number of patients with colorectal cancer, gastric cancer, lymphoma and some types of neuroblastoma[26, 27], and has been suggested as a risk biomarker in pancreatic cancers[28]. SMC3 plays an important role in DNA recombination[29] in addition to its

essential role in mitotic and meiotic chromosome segregation[30]. It is a component of the DNA damage repair mechanism[17] and is involved in microtubule-based intracellular transport[31]. SMC3 expression is elevated in a large fraction of human colon carcinoma[32]. Structural maintenance of chromosomes protein 4(SMC4) is encoded by SMC4, which is located in 3q25.33 (<https://www.ncbi.nlm.nih.gov/gene/10051>). SMC4 was found highly expressed in human primary hepatocellular carcinoma[33]. High expression of SMC4 is an independent predictor of poor survival in patients with colorectal cancer[34, 35], and is also related to the aggressiveness of glioma[36]. In the present study, it was found that the expression of SMC2, SMC3 and SMC4 in HCC were higher than in normal tissues. Both univariate and multivariate Cox proportional hazards regression analyses affirmed the prognostic value of SMC2, SMC3 and SMC4 in the prediction of poor outcome in HCC patients. Accordingly, increased expression of SMC2, SMC3 and SMC4 in HCC patients were associated with poor PFS, in agreement with the role of SMC2-4 as an oncogene.

The SMC5/6 complex, which is essential for eukaryotes, is currently the least understood in the SMC complexes. SMC5/6 plays a prominent role in DNA repair due to promoting the separation of recombinant intermediates. In addition, the role of SMC5/6 in the G2 phase of mitosis may have an impact on cell proliferation, thereby affecting cancer progression, but its function in the occurrence and development of sarcoma remains unknown[37]. In our study, it was found that there was no difference in SMC5 expression between HCC and normal tissues. SMC6 was highly expressed in HCC than in normal tissues, and the increased expression was related to poor PFS in HCC.

By using online databases, we accessed the expression and prognosis of SMC members in HCC patients. Importantly, high mRNA expression of SMC2, SMC3 and SMC4 were found to be associated with worse PFS, these findings suggested that individual SMC genes, especially SMC2, SMC3 and SMC4, are potential prognostic markers in HCC and indicated that the use of SMC inhibitor targeting SMC2, SMC3 and SMC4 may be an effective strategy for HCC therapy, but further exploration is needed.

Conclusion

This study demonstrated the expression profile of SMC family members in HCC and the biological and prognostic values of SMC family members in HCC, providing insights for further investigation of SMC members as potential targets in HCC.

Declarations

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in The Cancer Genome Atlas (TCGA) program at <https://portal.gdc.cancer.gov/>.

AUTHOR CONTRIBUTIONS

WY and QZ designed the research study and analyzed the data from public database. WY, DW, and HZ were involved in data analysis. WY was responsible for writing of manuscript. JH, LL and JZ contributed to the revised manuscript. All authors reviewed the manuscript.

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Tables

Table 1: Univariate Cox proportional hazards regression analyses of SMC members and clinical features in HCC (Bold means $P < 0.05$).

Parameter	Univariate analysis			
	HR	HR.95L	HR.95H	pvalue
SMC1A expression	1.038	0.997	1.080	0.068
SMC2 expression	1.138	1.052	1.231	0.001
SMC3 expression	1.042	1.009	1.076	0.012
SMC4 expression	1.094	1.039	1.152	0.001
SMC5 expression	1.063	0.975	1.160	0.168
SMC6 expression	1.119	1.002	1.250	0.046
Age	1.010	0.996	1.025	0.174
Gender	0.776	0.531	1.132	0.188
Grade	1.133	0.881	1.457	0.330
Stage	1.680	1.369	2.062	6.973E-07

Figures

Figure 1

Associations between SMC family members.

Figure 2

Expression profile of SMC members in HCC represented by a heatmap (A), histograms (B), and immunohistochemistry(C).

Figure 3

The prognostic value of mRNA Level of SMC factors in HCC patients. (A) The prognostic value of mRNA level of SMC factors in overall survival (OS) of HCC patients, (B) The prognostic value of mRNA level of SMC factors in progression-free survival (PFS) of HCC patients

Figure 4

Forest plots of the results of multivariate Cox regression analyses of significant prognostic factors: SMC2 (A), SMC3 (B) SMC4(C) and SMC6 (D). *stands for P < 0.05; **stands for P < 0.01.