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**Delineation of a SMARCA4-specific competing endogenous RNA network and its correlation with poor prognosis and immune cell infiltration in hepatocellular carcinoma**

competing endogenous RNA network

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## **Abstract**

### **BACKGROUND**

Hepatocellular carcinoma (HCC) is a common malignancy worldwide, and the mortality rate continues to rise each year. SMARCA4 expression has been associated with poor prognosis in various types of cancer; however, the specific mechanism of action of SMARCA4 in HCC needs to be fully elucidated.

### **AIM**

We aim to explore the specific mechanism of action of SMARCA4 in HCC.

### **METHODS**

Herein, the expression level of SMARCA4 as well as its association with HCC prognosis were evaluated using transcriptome profiling and clinical data of 18 different types of cancer collected from The Cancer Genome Atlas database. Furthermore, SMARCA4-high and -low groups were identified. Thereafter, gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed to identify the function of SMARCA4, followed by construction of a SMARCA4-specific competing endogenous RNA (ceRNA) network using starBase database. The role of SMARCA4 in immunotherapy and its association with immune cells were assessed using correlation analysis.

### **RESULTS**

It was observed that SMARCA4 was overexpressed and negatively correlated with prognosis in HCC. Further, SMARCA4 expression was positively associated with tumor mutational burden, microsatellite stability, and immunotherapy efficacy. The SNHG3/THUMP3-AS1-miR-139-5p-SMARCA4 ceRNA network was established and could be assumed to serve as a stimulatory mechanism in HCC.

### **CONCLUSION**

The findings of this study demonstrated that SMARCA4 plays a significant role in progression and immune infiltration in HCC. Moreover, a ceRNA network was detected, which was found to be correlated with poor prognosis in HCC. The findings of this study could contribute towards the identification of predictive markers for immunotherapy and a novel mechanism of action for HCC treatment.

**Key Words:** Hepatocellular carcinoma; SMARCA4; prognosis; immune infiltration

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## **INTRODUCTION**

Liver cancer is a common health concern worldwide, with a marked increase in incidence and mortality rates<sup>1</sup>. <sup>5</sup> Hepatocellular carcinoma (HCC) is, by far, the most common type of liver cancer; however, its origin is still under debate<sup>2</sup>. Presently, there are several hypotheses explaining the occurrence and development of HCC, including liver stem cells<sup>3</sup>, clone-forming cultures<sup>4</sup>, and mature liver cells<sup>5</sup>. Previous studies have confirmed that somatic mutations in mature hepatocytes are responsible for HCC<sup>6</sup>; this was further verified using high-throughput next-generation sequencing. Additionally, several mutations or genetic alterations in *CTNNB1*, *APC*, *RB1*, *CCNA2*, *PTEN*, *ARID1A*, *ARID2*, and *TP53* have been reported to promote HCC oncogenesis<sup>7</sup>. Furthermore, several pathways, including the Akt/mTOR, receptor tyrosine kinase pathway, and Wnt/ $\beta$ -catenin pathway<sup>8</sup>, were found to be involved in the progression of HCC. Based on the discovery of driver genes, several studies exemplifying the efficacy of different

inhibitors are underway<sup>8</sup>. There is an urgent need to identify additional important driver genes associated with the initiation and progression of HCC; this information would be valuable in the development of a potential specific targeted therapy in the nearer future.

Several studies have previously reported the activation of transcription of different genes through <sup>3</sup>SMARCA4, also known as BRG1, a member of the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin-remodeling complex, by manipulating the structure of chromatin<sup>9-12</sup>. David *et al* analyzed more than 363 HCC cases utilizing the whole exome sequencing technique and demonstrated that SMARCA4 was the driver gene associated with HCC<sup>1</sup>. Guerrero *et al* confirmed that the high expression of SMARCA4 was associated with poor prognosis in HCC<sup>13</sup>. Furthermore, Chen *et al* performed multi-omics analysis and illustrated that SMARCA4 promotes cell proliferation by upregulating SMAD6, by promoting the activation of HIC2 and NR4A2<sup>14</sup>. Another study performed by Wang *et al* showed that the *BRG1/RAS/C-MET* axis was involved in hepatocarcinogenesis<sup>15</sup>. The abovementioned findings demonstrated the significant role of SMARCA4 in HCC; however, only few studies have successfully elucidated the mechanism of action of SMARCA4 in the progression of HCC. In the present study, we aimed to establish a SMARCA4-related ceRNA network by mapping and analyzing the transcription profiles of SMARCA4 in HCC. This study might provide valuable insights regarding HCC occurrence and development.

## **MATERIALS AND METHODS**

### **2.1 Data sources and survival analysis**

The transcriptome profiling (fragments per kilobase million, FPKM) of 18 different types of cancer were collected from The Cancer Genome Atlas (TCGA) program database through UCBC Xena (<http://xena.ucsc.edu/>), and the miRNA isoform expression data were downloaded. Differential expression analysis was processed by R *limma* package. The survival analysis of target RNAs was performed using the R2 database and the R *survival* package.

## 2.2 Establishment of SMARCA4 related DEGs and delineation of functional enrichment analysis

After ascertaining the critical role of SMARCA4 in HCC, we further conducted differential expression analysis based on the median expression of SMARCA4 to obtain the SMARCA4-related DEGs with *limma* package. GO and KEGG enrichment of DEGs were conducted by *enrichplot* package.

## 2.3 Prediction of miRNAs and lncRNAs upstream of SMARCA4

In parallel, Upstream <sup>1</sup>miRNAs of SMARCA4 were searched by several target gene prediction programs, consisting of PITA, RNA22, miRmap, microT, miRanda, PicTar, and TargetScan from the starBase database<sup>16</sup>. The miRNA-related ncRNAs were forecasted by starBase as well (<http://starbase.sysu.edu.cn/>).

## 2.4 Correlation of SMARCA4 and immune cells and the markers

The association between the target genes and immune infiltrates along with their correlation were evaluated using TIMER2.0 (<http://timer.cistrome.org/>) and CIBERSORT (<https://cibersort.stanford.edu/>) databases. Correlation analysis were performed to elucidate the relationship between SMARCA4 and markers of immune cells.

## 2.4 Statistical analysis

The results obtained from the R2 database(<https://r2.amc.nl>) were analyzed using the signed-rank test and adjusted for P-values. The survival curves were estimated using the Kaplan-Meier method. Furthermore, Spearman's correlation was used to evaluate the relationship between the gene expression levels. The statistical significance was set at  $p < 0.05$  and the median value of the target RNAs was considered <sup>4</sup>as the cut-off value. All statistical analyses were conducted using the R software (version 4.1.2). The R packages used in this study included limma, reshape2, ggpubr, ggExtra, survival, survminer, and reshape2 whereas Cytoscape was used to establish the ceRNA network.

## RESULTS

### Pan-cancer expression levels of SMARCA4

First, The SMARCA4 mRNA expression levels, analyzed using the Wilcoxon test<sup>17</sup> and visualized by boxplots in different types of human cancer, were found to be substantially higher than in normal in most of the 18 different types of cancer (Figure 1). Furthermore, the expression levels of SMARCA4 were markedly higher in the patients with HCC, as was confirmed using the GEPIA database (Figure S1). This, in turn, demonstrated the role of SMARCA4 as an oncogenic regulator in the progression of HCC.

### **3.2 Differential expression of SMARCA4 and its prognostic value in patients with HCC**

Thereafter, Kaplan-Meier survival analysis was performed to analyze the differential expression of SMARCA4. In the present study, we failed to draw the survival curve, however, the prognostic value of SMARCA4 was verified to be related to the 1-year, 3-year, and 5-year survival rates by online R2 database. As demonstrated in Figure 2, higher expression levels of SMARCA4 were correlated with poor prognosis in patients with HCC, and a significant difference was observed. Therefore, by combining the differential expression levels and the survival curves, SMARCA4 was considered an indicator of poor prognosis in patients with HCC.

### **3.3 Identification of differentially expressed genes (DEGs) and enrichment analysis between SMARCA4<sup>high</sup> and SMARCA4<sup>low</sup> patients**

Furthermore, to figure out the DEGs correlated with SMARCA4, the patients were divided into two groups, SMARCA4<sup>high</sup> and SMARCA4<sup>low</sup>, depending on the median expression value of SMARCA4. A total of 3,540 genes were differentially expressed in HCC; among them, 573 were downregulated and 2,947 were upregulated ( $P < 0.05$ ,  $|\text{Log}_2\text{FC}| > 0.5$ ). The top 20 upregulated and downregulated genes were shown in a heatmap (Figure S2). Then, we conducted the GO and KEGG pathway enrichment analyses. Among the biological processes involved in patients with HCC, the typical GO terms used were: transcription regulator complex (GO:0005667), activation of immune response (GO:0002253), response to hypoxia (GO:0001666), and carbohydrate catabolic process (GO:0016052). Moreover, the most notable pathways identified by the

KEGG enrichment analysis included carbon metabolism (hsa01200), one carbon pool by folate (hsa00670), mismatch repair (hsa03430), and PPAR signaling pathway (hsa03320) in patients with HCC.

#### **Identification of miRNAs upstream of SMARCA4**

Aside from above, we aimed to ensure the modulation mechanism of SMARCA4. Approximately, 20 miRNAs that could potentially bind to SMARCA4 were detected (Table1). The network was visualized using the Cytoscape software<sup>18</sup> and a Spearman correlation analysis was performed to identify the negative relationship between the miRNAs and SMARCA4 (Figure3A). MiR-139-5p was found to be negatively correlated to SMARCA4 ( $R = -0.43$ ,  $P < 0.001$ ) (Figure 3B). Additionally, the expression and prognostic values of miR-139-5p were determined. As shown in Figure 3C, miR-139-5p expression was notably downregulated in HCC, and its upregulation was related to a favorable prognosis in patients with HCC (Figure 3D). The abovementioned data demonstrated the role of miR-139-5p as an upstream regulatory non-protein-coding RNA (ncRNA) of SMARCA4.

#### **3.5 Detection and analysis of long noncoding RNAs (lncRNAs) upstream of hsa-miR-139-5p**

Meanwhile, the upstream lncRNAs of miR-139-5p were predicted using an online database (<https://starbase.sysu.edu.cn/>). A total of 75 lncRNAs were identified and correlation analysis was performed to identify the upstream lncRNAs (Table2). Based on the theory of ceRNAs, lncRNAs may increase the expression levels of the target mRNAs by using different combinations of competitive interactions with miRNAs. Four lncRNAs (NUTM2A-AS1, NUTM2B-AS1, SNHG3, and THUMPD3-AS1) were found to be upregulated in patients with HCC (Figure4). Furthermore, Spearman correlation analysis was used to analyze the four lncRNAs; SNHG3 and THUMPD3-AS1 were found to be positively correlated with the expression of SMARCA4 and negatively correlated with the miR-139-5p levels (Figure 4A, B, E, F). Subsequently, the prognostic value of the two lncRNAs was evaluated in the patients with HCC. Taken together,



SNHG3 and THUMP3-AS1 might be present upstream of the miR-139-5p/SMARCA4 axis (Figure 4C, D, G, H, Figure 5).

### **3.6 Relationship between the expression levels of SMARCA4 and immune cell infiltration**

As for interpreting its role in immune-regulation, we performed a correlation analysis was to identify the relationship between the expression levels of SMARCA4 and immune cell infiltration in patients with HCC. As shown in Figure 6, the expression levels of SMARCA4 were found to be significantly correlated with all analyzed immune cells, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells, all of which were verified by the CIBERSORT approach in R packages (Figure S3).

### **3.7 Relationship between expression levels of SMARCA4 and biomarkers of immune cells**

the potential immunotherapy targets that assist in immune escape in various types of cancer include PD1/PD-L1, CTLA4, TIGIT, and LAG3 (Figure 7). At last, the relationships between the expression levels of SMARCA4 and the aforementioned coding genes were identified. The co-expression analysis confirmed the correlation of SMARCA4 with an increased expression of the immune-related genes, which was further verified by correlation analysis (Figure S4, Table 3). Therefore, the results suggested that the enhanced expression levels of SMARCA4 could regulate the immune responses, thereby leading to the progression of metastasis in patients with HCC.

## **DISCUSSION**

The gene encoding SMARCA4, a member of the SWI/SNF family, was found to be essential for embryonic development<sup>19-22</sup>. Several studies demonstrated that the loss of SMARCA4 was associated with a high risk of undifferentiated cancer, implying its anti-cancerous role<sup>10, 11, 23-26</sup>. Other studies detected the overexpression of SMARCA4 in various other types of cancer, including HCC<sup>27-30</sup>, small cell lung cancer<sup>31-34</sup>, acute leukemia<sup>35-38</sup>, and neuroblastoma<sup>39, 40</sup>, thereby exemplifying its oncogenic role. In this

study, we identified the overexpression of SMARCA4 Levels in 15 different types of cancer, excluding glioblastoma, kidney chromophobe, and kidney papillary cell carcinoma. Furthermore, the increased expression levels of SMARCA4 were associated with a poor prognosis in patients with HCC. It has been previously demonstrated that SMARCA4 can function as an oncogene in some cancers and as a tumor suppressor in other types of cancer<sup>41</sup>. The alterations in SMARCA4 were classified into two types<sup>42</sup>: Class I (truncating mutations, fusions, and homozygous deletion) and Class II (missense mutations). The loss of SMARCA4 was found to be involved in the inhibition of carcinogenesis by interacting with the Wnt protein<sup>43</sup>. Mutations in SMARCA4 have been associated with the progression of colorectal cancer by cooperating with PRMT1. Additionally, Mehta *et al* reported that the overexpression of SMARCA4 increased the activity of the ATPase subunit and could enhance SOX4-mediated PI3K/ AKT signaling in triple-negative breast cancer<sup>44</sup>. In this study, based on the GO and KEGG enrichment analyses of targeted genes related to the overexpression of SMARCA4, we found that SMARCA4 was involved in oncogenesis through PI3K, IL-17, and TGF- $\beta$  signaling, which elucidated its oncogenic role in patients with HCC.

Checkpoint inhibitors have been established as regulators of various types of cancer; however, their response rate to immunotherapy was found to be low, ranging from complete remission to super progression. Till date, Keytruda (Pembrolizumab) has been considered as a potential inhibitor of the progression death-ligand-1(PD-L1); however, its sensitivity and specificity were found to be limited. Therefore, other biomarkers are urgently required for the treatment of different types of cancer, utilizing the mechanism of action of potential inhibitors. In this study, we found that SMARCA4-related genes were enriched in the PPAR signaling pathway and identified a strong correlation among SMARCA4, CD274, and PDCD1. Furthermore, the expression levels of SMARCA4 were found to be positively correlated with immunotherapy, which were consistent with the study reported by Peng *et al*, and was further confirmed in the patients with thoracic sarcoma<sup>45</sup> and pancreatic cancer<sup>46</sup>.

ceRNAs act as key mediators in the progression of different types of cancer<sup>47</sup>. Although the ceRNA network has been established in HCC, other novel ceRNA mechanisms need to be elucidated<sup>27, 29, 48-50</sup>. To further explore the ncRNAs participating in SMARCA4-related ceRNAs, we predicted ncRNAs using the starBase database. The expression levels of ncRNAs were quantified, and correlation analysis was performed to identify the miRNAs upstream of SMARCA4. Finally, miR-139-5p was found to be a tumor-suppressive miRNA of SMARCA4. Previous studies have shown that miR-139-5p was involved in regulating the proliferation and migration of HCC cells.

Based on the ceRNA theory, downregulated miRNAs were generally accompanied by upregulated lncRNAs. Therefore, we predicted an upstream lncRNAs of miRNAs through an online starBase database and then detected SNHG3 and THUMP3-AS1 lncRNAs, which were found to be overexpressed and related to poor prognosis in patients with HCC. Thereafter, we established the SNHG3/THUMP3-AS1-miR-139-5p-SMARCA4 axis associated with the poor prognosis of patients with HCC. Utilizing the ceRNA network, Lee *et al*<sup>51</sup> reported THUMP3-AS1 as an enhancer RNA, promoting the expression of target genes, which can further lead to the progression of HCC. Meanwhile, SNHG3 lncRNAs has been reported to be involved in the hepatogenesis by regulating the levels of different miRNAs<sup>30, 52-55</sup>. However, the NHG3/THUMP3-AS1-miR-139-5p-SMARCA4 axis in HCC was constructed for the first time, and we confirmed that SMARCA4 was associated with an increased infiltration of immune cells as well as checkpoint markers. Therefore, we investigated SMARCA4-related ceRNA as a novel mechanism in HCC and found that SMARCA4 could serve as a biomarker in immunotherapy; however, this needs further validation.

All data of this study were downloaded from open database, and the results were validated using different database. Thereby, our results extrapolated the role of SMARCA4 in HCC. However, there is a major limitation of the study. we established a SMARCA4-related ceRNA and constructed the model NHG3/THUMP3-AS1-miR-139-

5p-SMARCA4 axis in HCC, which need external validation. We failed to complete the verification and validation is planned later.

In summary, we observed the overexpression of SMARCA4 in different pathways. Additionally, the overexpression of SMARCA4 was correlated to an increased immune cell infiltration and an augmented sensitivity to immunotherapy. Furthermore, a novel SMARCA4 ceRNA network (SNHG3/THUMP3-AS1-miR-139-5p-SMARCA4) was established in this study. This study could contribute towards the identification of predictive markers for immunotherapy and a novel mechanism of action for HCC treatment.

## **CONCLUSION**

Herein, we demonstrated the overexpression of SMARCA4 in patients with HCC and in several immunotherapy-related pathways. Furthermore, an increased expression of SMARCA4 was found to be positively associated with immune cell infiltration, and a SMARCA4-specific ceRNA network was established, which was found to be involved in the progression of HCC.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

Hepatocellular carcinoma (HCC) is a common malignancy worldwide, and the mortality rate continues to rise each year. SMARCA4 expression has been associated with poor prognosis in various types of cancer; however, the specific mechanism of action of SMARCA4 in HCC needs to be fully elucidated.

### ***Research motivation***

only few studies have successfully elucidated the mechanism of action of SMARCA4 in the progression of HCC. In the present study, we aimed to establish a SMARCA4-related ceRNA network by mapping and analyzing the transcription profiles of SMARCA4 in HCC.

### *Research objectives*

This study might provide valuable insights regarding HCC occurrence and development.

### *Research methods*

1. Data sources and survival analysis
2. Establishment of SMARCA4 related DEGs and delineation of functional enrichment analysis
3. Prediction of miRNAs and lncRNAs upstream of SMARCA4
4. Correlation of SMARCA4 and immune cells and the markers
5. The R packages used in this study included limma, reshape2, ggpubr, ggExtra, survival, survminer, and reshape2 whereas Cytoscape was used to establish the ceRNA network.

### *Research results*

1. Pan-cancer expression levels of SMARCA4
2. Differential expression of SMARCA4 and its prognostic value in patients with HCC
3. Identification of differentially expressed genes (DEGs) and enrichment analysis between SMARCA4<sup>high</sup> and SMARCA4<sup>low</sup> patients
4. Identification of miRNAs upstream of SMARCA4
5. Detection and analysis of long noncoding RNAs (lncRNAs) upstream of hsa-miR-139-5p
6. Relationship between the expression levels of SMARCA4 and immune cell infiltration
7. Relationship between expression levels of SMARCA4 and biomarkers of immune cells

### *Research conclusions*

Herein, we demonstrated the overexpression of SMARCA4 in patients with HCC and in several immunotherapy-related pathways. Furthermore, an increased expression of SMARCA4 was found to be positively associated with immune cell infiltration, and a SMARCA4-specific ceRNA network was established, which was found to be involved in the progression of HCC.

### *Research perspectives*

we observed the overexpression of SMARCA4 in different pathways. Additionally, the overexpression of SMARCA4 was correlated to an increased immune cell infiltration and an augmented sensitivity to immunotherapy. Furthermore, a novel SMARCA4 ceRNA network (SNHG3/THUMP3-AS1-miR-139-5p-SMARCA4) was established in this study. This study could contribute towards the identification of predictive markers for immunotherapy and a novel mechanism of action for HCC treatment.

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