Regulatory mechanism of RAD51-associated protein 1 and its upstream molecules in hepatocellular carcinoma

Jin-Wen Chai1*, Yu-Na Dong2*

1Department of Oncology, Laizhou Traditional Chinese Medicine Hospital, Laizhou 261400, China. 2Department of Gastroenterology, Laizhou People’s Hospital, Laizhou 261400, China.

*Corresponding to: Yu-Na Dong, Department of Gastroenterology, Laizhou People’s Hospital, No. 1718 Wuli Street, Laizhou 261400, China. E-mail: dongyuna2021@163.com.

Abstract

Background: The DNA damage repair mechanism plays a crucial role in the occurrence and development of hepatocellular carcinoma (HCC), and RAD51-associated protein 1 (RAD51AP1) has received increasing attention as an important protein in the homologous recombination repair pathway. However, the role of RAD51AP1 and its molecular regulatory mechanism in HCC still need further investigation. Methods: We first analysed RAD51AP1 expression, functional enrichment and prognostic value in HCC. Then, the miRWalk, miRDB, and Encyclopedia of RNA Interactomes databases were used to predict the corresponding microRNAs and long noncoding RNAs of RAD51AP1, and their expression levels and prognostic value were analysed. Results: RAD51AP1 was upregulated in the majority of cancers include HCC. The Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses revealed that RAD51AP1 was mainly involved in pathways related to the cell cycle and repair in HCC. Moreover, the expression level of RAD51AP1 was significantly correlated with T stage, pathologic stage, histologic grade and the level of alpha-fetoprotein. In addition, RAD51AP1 was an independent risk factor significantly and had a high predictive value in HCC. Based on ceRNA network, RAD51AP1 may be regulated by upstream MSC-AS1 and hsa-miR-23c to affect the HCC occurrence and development. Conclusions: High expression of RAD51AP1 plays an important biological role in the cell cycle and repair pathways, and has important diagnostic and prognostic value in HCC. Based on the regulatory mechanism of ceRNA network, we speculate that IncRNA MSC-AS1 acts on hsa-miR-23c and regulates DNA damage repair of HCC through RAD51AP1. It provides a new perspective for further study of DNA damage repair mechanism and potential related treatment of HCC.

Keywords: RAD51-associated protein 1; MSC-AS1; miR-23c; ceRNA; hepatocellular carcinoma; bioinformatics analysis
Introduction

Liver cancer is the sixth most common primary tumor and the fourth most common cause of cancer death worldwide (1), hepatocellular carcinoma (HCC) accounts for about 90% of primary liver cancers (2). Although early HCC can be cured by local ablation or surgical resection, most HCC cases in the world are in the advanced stage. Molecular targeted drugs and immune checkpoint inhibitors have been proven to be effective options for advanced HCC (3), but some patients still do not benefit from them. Therefore, looking for accurate prognostic biomarkers and treatment targets is important for improving the early diagnosis rate of HCC and developing new targeted therapy regimens.

The DNA damage repair pathway is one of the pathways of tumorigenesis, and DNA damage repair defects drive tumorigenesis. Compared with normal tissues, tumour tissues exhibit significantly more DNA damage, and a higher level of proliferation (4). RAD51-associated protein 1 (RAD51AP1) plays an important role in DNA damage repair, maintenance of replication forks and genome stability (5–8). In 2021, the Lee Zou team found that during the repair of DNA double-strand breaks, RNA molecules produced by gene transcription can form R-loop structures with homologous template DNA sequences with the assistance of RAD51AP1, promote the formation of a D-loop, and further produce a structure called a DR-loop, which ultimately improves the efficiency of homologous recombination repair (HRR) (9). This indicates that the role of RAD51AP1 in DNA damage repair is receiving increasing attention.

Liping Zhuang analysed the HCC data in Gene Expression Omnibus data sets and The Cancer Genome Atlas (TCGA) database. It was found that RAD51AP1 was significantly up-regulated in tumor tissues of HCC patients, and RAD51AP1 messenger RNA (mRNA) expression was related to the staging, intrahepatic metastasis, vascular invasion and alpha-fetoprotein level of HCC, and was a risk factor for overall survival (OS) and disease-free survival in patients with hepatocellular carcinoma (10). Muhammad Sufyan analysis of four gene chip data sets from Gene Expression Omnibus showed that RAD51AP1 was up-regulated in patients with type 2 diabetes mellitus and hepatitis C (HCV) related HCC, which may be genetically associated with HCV-related HCC and type 2 diabetes mellitus (11). Shucai Xie downloaded the hepatitis B virus related HCC expression profile from Gene Expression Omnibus. Further study found that RAD51AP1 was up-regulated in hepatitis B virus-related HCC and reduced the overall survival rate of such patients (12). Tran T N Nguyen found that HCV NSSA directly binds to RAD51AP1 and increases the protein expression level of RAD51AP1 by regulating ubiquitin-proteasome pathway. NSSA damages DNA repair by destroying the RAD51/RAD51AP1/UF1 complex and makes cells infected with HCV more sensitive to DNA damage. Silencing the expression of RAD51AP1 resulted in a decrease in HCV reproduction. This may also be the molecular mechanism of HCC induced by hepatitis C virus (13).

Although research on RAD51AP1 in HCC has increased in recent years, further studies are needed to investigate the role and molecular regulatory mechanism of RAD51AP1 in HCC.

Recently, there has been evidence that long noncoding RNAs (lncRNAs) act as competing endogenous RNAs in regulating the expression of target genes in HCC (14–16). When lncRNAs compete with miRNAs for binding to microRNAs (miRNAs), the ability of miRNAs to regulate miRNA transcription decreases. In this study, we first downloaded data from TCGA database and analysed the expression, functional enrichment, clinical correlation and survival prognosis of RAD51AP1. Then, we used the miRWalk, miRDB and Encyclopedia of RNA Interactomes (ENCORI) databases to predict RAD51AP1 corresponding miRNAs and lncRNAs, and performed the ceRNA network using ENCORI. Finally, we used HCC data downloaded from TCGA to further determine the expression and prognostic value of RAD51AP1 and predicted the regulatory relationship of upstream molecules based on the ceRNA regulatory mechanism. This might provide a new idea for the study of the regulatory mechanism of RAD51AP1 and might provide new targets and therapeutic ideas for HCC.

Materials and methods

Data collection

We obtained the expression profile of RAD51AP1 through the Prother (https://wlsh.edu.ch/prother/start/) (17), the AlphaFold DB (https://alphafold.ebi.ac.uk/) (18) and The Human Protein Atlas (https://www.proteinatlas.org/) (19).

The RNA-seq and relevant clinical data were downloaded from the TGCA (https://www.cancer.gov/tcga) (20) and the GTEx (21) data by UCSC XENA (https://xenabrowser.net/datapages/). The “ggplot2” package was used for further analysis. The detailed clinical characteristics of hepatocellular carcinoma are listed in Supplementary Table S1.

Functional enrichment analysis

A total of 20 RAD51AP1 binding proteins were obtained from the STRING website (https://string-db.org/) and a protein-protein interaction (PPi) network was constructed by setting the required interaction score (“high confidence (0.7)” (22). Cytoscape was applied for visualization of the PPi network (23). The Gene Ontology (GO) and Kroyo Encyclopedia of Genes and Genomes (REGG) pathway enrichment analyses were conducted for 20 RAD51AP1 binding proteins, and visualized in R software. The adjusted P value < 0.05 was considered to be a screening criterion the screening criterion for significantly enriched terms (24).

We used the LinkedOmics (http://www.linkedomics.org/login.php) (25) to analyse the biological significance and coexpressed genes of RAD51AP1 in HCC cohort. Differentially expressed genes associated to RAD51AP1 were selected from the TCGA HCC cohort and presented. Furthermore, the GO biological process and REGG pathways were identified by gene set enrichment analysis.

Clinical correlation analysis

Correlation analysis of RAD51AP1 expression and different clinical characteristics in HCC patients was performed using the “ggplot2” package. Kaplan-Meier plots were used to analyse the relationship between the expression of RAD51AP1 and prognosis in HCC. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of RAD51AP1 in HCC by using the “pROC” package (26). The closer the area under the curve was to 1, the better the intrinsic effectiveness of the diagnosis. Clinical factors of HCC were analysed by Cox regression analyses, and the independent prognosis of risk factors were deeply explored. The “survival” package and “ggplot2” package were used for the above COX regression analysis.

Prediction of target miRNAs of RAD51AP1

We used three online miRNA databases, miRIWalk (http://mirwalk.umm.uni-heidelberg.de/) (27), miRDB (http://mirdb.org/) (28), and ENCORI (https://starbase.sysu.edu.cn/) (29), to predict the target miRNAs of RAD51AP1, and the final miRNAs obtained were shared by the above three databases.

Prediction and construction of ceRNA networks

First, we used ENCORI to predict RAD51AP1 corresponding upstream lncRNAs. Then, we used ENCORI to obtain the intersections between the predicted lncRNAs and the target miRNAs of RAD51AP1. Finally, the “ggalluvial” R package was used to visualize the ceRNA networks constructed.

Comparison of the expression of miRNAs and lncRNAs corresponding to the target in HCC

RNA expression data from the TCGA database were used to analyse the expression of RAD51AP1-related miRNAs and the expression of RAD51AP1-related lncRNAs in HCC.

Survival prognosis analysis and correlation analysis

Submit a manuscript: https://www.tmrjournals.com/mdm
Kaplan-Meier plots were created to assess target corresponding miRNA and IncRNA expression prognosis in HCC using the survival package. We further assessed the associations between RAD51AP1 and target corresponding miRNA expression, RAD51AP1 and target corresponding IncRNA expression, and target corresponding miRNA and IncRNA expression.

Statistical methods
Statistical analysis was performed using the R software and the associated R package. Survival analysis was grouped by the minimum \( P \) value approach, and \( P \)-values < 0.05 was considered statistically significant (ns, \( P \geq 0.05; ^*, P < 0.05; ^{**}, P < 0.01; ^{***}, P < 0.001 \)).

Results
RAD51AP1 localization and expression profiles
The protein topology plots revealed that RAD51AP1 was 74 amino acids in length and located in the cell (Figure 1A). The prediction of the RAD51AP1 protein structure is shown in Figure 1B.

We used an immunofluorescence method to detect the distribution of RAD51AP1 within the nucleus, endoplasmic reticulum and microtubules of A-431, U-2 OS, and U-251 MG cells in The Human Protein Atlas database. We observed that RAD1AP1 was colocalized with nuclear marker proteins in A-431, U-2 OS, and U-251 MG cells, while RAD1AP1 did not overlap with endoplasmic reticulum or microtubules, suggesting that RAD1AP1 may have subcellular localization in the nucleus (Figure 1C). To determine the dependence of RAD51AP1 RNA expression on the cell cycle, we analysed single-cell RNA transcript expression and found that the RAD51AP1 RNA expression was related to cell cycle progression, and the RAD51AP1 RNA expression was temporally correlated with the proportion in the cell cycle G1, S and G2 phases (Figure 1D).

RAD51AP1 expression across cancers
We assessed RAD51AP1 expression from the TCGA and found that RAD51AP1 was significantly overexpressed in 15 cancer types, including bladder urothelial carcinoma, breast invasive carcinoma, cholangiocarcinoma, colon adenocarcinoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, rectum adenocarcinoma, stomach adenocarcinoma, and uterine corpus endometrial carcinoma (Figure 2A). Furthermore, we assessed TCGA tumors and GTEx samples as controls, and found that RAD51AP1 expression was overexpressed in most cancer types except acute myeloid leukemia (Figure 2B).

---

Figure 1 RAD51AP1 localization and expression profiles. (A) RAD51AP1 protein topology plots. (B) The prediction protein structure of RAD51AP1 from AlphaFold Protein Structure Database. (C) Immunofluorescence staining of RAD51AP1 from the HPA database. (D) Relationship between RAD51AP1 gene expression and cell cycle distribution. RAD51AP1, RAD51-associated protein 1; HPA, The Human Protein Atlas; ER, endoplasmic reticulum.
PPI network and functional enrichment analyses

We screened 20 RAD51AP1-binding proteins using STRING and constructed PPI networks using Cytoscape (Figure 3A). Then, we performed a functional enrichment analysis on the 20 selected proteins and displayed them with a visual network (Figure 3B). The enriched biological process terms included DNA replication, DNA-dependent DNA replication, nuclear DNA replication, and telomere maintenance via semi-conservative replication. The main enriched cellular composition terms were replication fork, nuclear replication fork, replisome, and DNA polymerase complex. The main enriched molecular function terms were catalytic activity, acting on DNA, DNA polymerase activity, DNA-directed DNA polymerase activity, and single-stranded DNA-dependent ATP-dependent DNA helicase activity (Figure 3C). KEGG pathways were mainly related to nucleotide excision repair, DNA replication, mismatch repair, Fanconi anaemia pathway, and homologous recombination (Figure 3D).

Coexpressed genes of RAD51AP1 in HCC

We used the LinkedOmics database to obtain the coexpressed genes of RAD51AP1 and analyse the coexpression network of RAD51AP1 in HCC. As shown in Figure 4A, 6859 coexpressed genes (red dots) were positively correlated with RAD51AP1, while 2783 genes (green dots) were negatively correlated with RAD51AP1.

The heatmap showing the top 50 genes positively (Figure 4B) and negatively (Figure 4C) associated with RAD51AP1 expression. We analysed the effects of the expression of the above 100 related genes on the survival of HCC patients. 49 genes had a favourable hazard ratio in the top 50 positively correlated genes. In contrast, 41 of the top 50 negatively correlated genes had unfavourable hazard ratio (Figure 4D).

KEGG pathway analysis indicated enrichment in the cell cycle, DNA replication, Fanconi anaemia pathway, homologous recombination, oocyte meiosis, mismatch repair, p53 signalling pathway, base excision repair, nucleotide excision repair, etc. (Figure 4E).

Among the GO terms, the coexpressed genes of RAD51AP1 were enriched in chromosome segregation, DNA replication, cell cycle G2/M phase transition, spindle organization, mitotic cell cycle phase transition, protein localization to chromosome, interstrand cross-link repair, double-strand break repair, cell cycle checkpoint and negative regulation of mitotic cell cycle (Figure 4F). These results suggest that RAD51AP1 has extensive effects on the cell cycle and DNA repair in HCC.

Clinical correlation analysis

We further studied and found that RAD51AP1 expression was significantly correlated with T stage (Figure 5A), pathologic stage (Figure 5B), histologic grade (Figure 5C) and the level of alpha-fetoprotein (Figure 5D) in HCC. Kaplan-Meier survival analysis revealed that HCC patients with high expression of RAD51AP1 tended to have a poor prognosis (Figure 5E, hazard ratio = 1.81, 95% confidence interval: 1.28–2.55, P = 0.001). The ROC curve showed that RAD51AP1 had a high prediction accuracy in HCC (area under the curve = 0.930) (Figure 5F). Univariate Cox regression analysis
Figure 3 PPI network and functional enrichment analyses of 20 targeted binding proteins of RAD51AP1. RAD51AP1, RAD51-associated protein 1; PPI, protein-protein interaction; BP, biological process; MF, molecular function; CC, cellular component; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 4 Coexpressed genes of RAD51AP1 in HCC. (A) The volcano plots of coexpressed genes of RAD51AP1 in HCC. (B & C) The top 100 genes correlated with RAD51AP1 in HCC. (D) The relationship between the top 100 RAD51AP1-correlated genes and the survival in HCC. (E) GO_BP and KEGG pathway analyses of RAD51AP1-correlated genes in HCC. HCC, hepatocellular carcinoma; RAD51AP1, RAD51-associated protein 1; GO, Gene Ontology; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; HR, hazard ratio.
showed that RAD51AP1, T stage and pathological stage were significantly correlated with the OS (Figure 5G). RAD51AP1 expression was an independent factor correlated with OS in multivariate Cox regression analysis (Figure 5H). This indirectly indicates the significant effect of the RAD51AP1 expression level on the OS of HCC patients.

**Prediction of target miRNAs of RAD51AP1**

In the ceRNA regulatory network, miRNAs induce gene silencing or down-regulate gene expression by binding to the 3 UTRs of downstream mRNAs [30, 31]. In order to predict the target miRNAs of RAD51AP1, three online databases related to miRNA (miRWalk, miRD8 and ENCORI) were used. Finally, 9 miRNAs were predicted in at least 3 databases (Figure 6).

**Prediction and construction of ceRNA networks**

We used ENCORI to predict 10 upstream lncRNAs corresponding to RAD51AP1 (Table 1). Meanwhile, 9 target miRNAs interacting with RAD51AP1 were predicted. According to the predicted regulation relationship between miRNAs and lncRNAs, we finally obtained 7 corresponding upstream LncRNAs and 7 target miRNAs. The regulatory network is shown in Figure 7.
Figure 6 Target miRNAs of RAD51AP1. (A) Venn diagram of 3 miRNAs datasets. (B) Nine miRNAs were obtained from least 3 datasets. ENCORI, Encyclopedia of RNA Interactomes; RAD51AP1, RAD51-associated protein 1; miRNAs, microRNAs.

<table>
<thead>
<tr>
<th>IncRNA ID</th>
<th>IncRNA name</th>
<th>IncRNA type</th>
<th>P-value</th>
<th>False discovery rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENSG00000250874</td>
<td>AC010595.1</td>
<td>lincRNA</td>
<td>3.21E-05</td>
<td>7.32E-05</td>
</tr>
<tr>
<td>ENSG00000269925</td>
<td>Z98884.2</td>
<td>sense_intronic</td>
<td>3.35E-05</td>
<td>7.54E-05</td>
</tr>
<tr>
<td>ENSG00000277687</td>
<td>AL139407.1</td>
<td>sense_intronic</td>
<td>1.50E-04</td>
<td>2.40E-04</td>
</tr>
<tr>
<td>ENSG00000279066</td>
<td>HEXDC-IT1</td>
<td>sense_intronic</td>
<td>1.92E-04</td>
<td>2.90E-04</td>
</tr>
<tr>
<td>ENSG00000274001</td>
<td>AL512506.1</td>
<td>sense_intronic</td>
<td>2.00E-04</td>
<td>2.99E-04</td>
</tr>
<tr>
<td>ENSG00000228293</td>
<td>AL049712.1</td>
<td>antisense</td>
<td>2.55E-04</td>
<td>3.63E-04</td>
</tr>
<tr>
<td>ENSG00000267152</td>
<td>AC093227.1</td>
<td>lincRNA</td>
<td>1.07E-03</td>
<td>1.16E-03</td>
</tr>
<tr>
<td>ENSG00000235531</td>
<td>MASC-AS1</td>
<td>antisense</td>
<td>1.14E-03</td>
<td>1.21E-03</td>
</tr>
<tr>
<td>ENSG00000273301</td>
<td>AC016717.2</td>
<td>lincRNA</td>
<td>1.43E-03</td>
<td>1.47E-03</td>
</tr>
<tr>
<td>ENSG00000228223</td>
<td>HCG11</td>
<td>lincRNA</td>
<td>1.65E-03</td>
<td>1.68E-03</td>
</tr>
</tbody>
</table>

IncRNA, long noncoding RNA; RAD51AP1, RAD51-associated protein 1.

Figure 7 Sankey diagram of ceRNA networks. RAD51AP1, RAD51-associated protein 1; mRNA, messenger RNA; miRNA, microRNA; IncRNA, long noncoding RNA.
Comparison of target miRNA and corresponding upstream lncRNA expression in HCC

We investigated the expression of corresponding upstream lncRNAs and target miRNAs in HCC tumours and normal tissues by using HCC data downloaded from the TCGA (Figure 8). The expression of miRNAs, including hsa-miR-23c, hsa-miR-383-5p, and hsa-miR-873-5p, was significantly decreased in HCC tumours. The expression of lncRNAs, including MSC-AS1, AC016717.2, AC010595.1, AC093227.1, 298884.2, and AL139407.1, was significantly increased in HCC tumours.

Prognostic analysis of target miRNAs and corresponding upstream lncRNAs in HCC

To better understand the prognostic value of the above miRNAs and lncRNAs in HCC, we explored the relationship between the expression of miRNA/lncRNA and OS using the “survival” package (Figure 9). For miRNA in HCC patients, hsa-miR-23c was upregulated in normal tissue and significantly correlated with good OS, but hsa-miR-18a-5p was upregulated in HCC tissue and correlated with poor OS. Among lncRNAs, MSC-AS1 and AC093227.1 were highly expressed in tumor tissue and significantly correlated with poor OS in HCC patients. Hsa-miR-4735-3p, hsa-miR-513b-5p, hsa-miR-518f-5p, hsa-miR-873-5p, AC016717.2 and AC010595.1 presented very low expression in more than half of the samples, so patients could not be divided into two groups for these variables.

Correlation analysis

According to the regulatory mechanism of competing endogenous RNAs, we predicted the regulatory molecules upstream of RAD51AP1. RAD51AP1 was highly expressed in tumour tissue and correlated with poor OS in HCC. Hsa-miR-23c was highly expressed in paracancerous tissue and correlated with good OS in HCC patients. MSC-AS1 was associated with hsa-miR-23c through the ceRNA network and highly expressed in tumour tissue, and significantly correlated with poor OS. The correlation analysis is shown in Figure 10.

Therefore, we speculate that MSC-AS1, hsa-miR-23c and RAD51AP1 might affect the occurrence and development of HCC through the complex ceRNA regulatory network.

Figure 8 Expression of corresponding target miRNAs and lncRNAs in HCC. (A–G) RAD51AP1-related target miRNAs. (H–N) RAD51AP1-related target lncRNAs. RAD51AP1, RAD51-associated protein 1; miRNAs, microRNAs; lncRNAs, long noncoding RNAs; HCC, hepatocellular carcinoma.

Submit a manuscript: https://www.tmrjournals.com/mdm
ARTICLE

Medical Data Mining 2023;6(4):24. https://doi.org/10.53388/MDM202306024

Submit a manuscript: https://www.tmjrournals.com/mdm

Discussion

The regenerative capacity of the liver is closely related to the DNA repair process [32, 33]. When this balance is dysregulated, the risk of genome instability related to DNA repair-related pathways, such as HRR, mismatch repair, and nonhomologous end join, is increased, leading to the occurrence of HCC [34, 35]. As a key protein downstream of the HRR pathway, RAD51AP1 is located in the nucleus and maintains a high level throughout the S and G2 phases [36, 37]. Recent studies have confirmed that RAD51AP1 is highly expressed in some primary tumors, including HCC [10], esophageal cancer [38, 39], pancreatic cancer [40], breast cancer [41], non-small cell lung cancer [42], human glioblastomas [43], and ovarian cancer [44–46]. In this study, we found that RAD51AP1 was upregulated in the majority of cancers excepted acute myeloid leukemia. We performed enrichment analysis of GO and KEGG pathway for 20 targeted binding proteins to predict the biological function of RAD51AP1. GO enrichment analysis revealed that RAD51AP1 mainly participate in the basic biological process of DNA replication, the cellular components mainly included replication forks, and the molecular function was the catalysis of DNA polymerase on DNA. KEGG enrichment analysis showed RAD51AP1 was involved in nucleotide excision repair, DNA replication, mismatch repair, the Fanconi anaemia pathway, and homologous recombination.

HCC is a special type of cancer that varies significantly from other tumors. We analysed the coexpressed genes of RAD51AP1 in HCC and found that both positively and negatively correlated genes were significantly associated with the survival. In addition, the GO and KEGG pathways of these coexpressed genes were mainly concentrated in pathways related to the cell cycle and repair in HCC. Moreover, we found that the expression level of RAD51AP1 was significantly

Figure 9 Associations between miRNA/IncRNA expression and OS in HCC. (A) Hsa-miR-18a-5p. (B) Hsa-miR-23c. (C) Hsa-miR-383-5p. (D) AC093227.1. (E) AL139407.1. (F) Z98884.2. (G) MSC-AS1. (H) HCG11. miRNA, microRNA; IncRNA, long noncoding RNA; OS, overall survival; HCC, hepatocellular carcinoma; HR, hazard ratio.

Figure 10 Correlation analysis among MSC-AS1, hsa-miR-23c and RAD51AP1. RAD51AP1, RAD51-associated protein 1.
correlated with T stage, pathologic stage, histologic grade and the level of alpha-fetoprotein. In addition, RAD51AP1 was an independent risk factor significantly and had a high predictive value in HCC. All these results indicate that RAD51AP1 has important diagnostic and prognostic value in HCC.

To elucidate the upstream regulatory mechanism of RAD51AP1 in HCC, we constructed ceRNA networks. In this study, we used the mirWalk, miRDB and ENCORI databases to predict miRNAs and lncRNAs associated with RAD51AP1. The expression and survival curves were separately analysed according to the HCC data in TCGA. Fortunately, we found that hsa-miR-23c was upregulated in normal tissue and significantly correlated with a good prognosis, and MSC-AS1 was highly expressed in tumor tissues and significantly correlated with a poor prognosis. There was a negative correlation between the expression of MSC-AS1 and hsa-miR-23c in HCC tissues. Recently, the role of lncRNA MSC-AS1 has been investigated in HCC. Xiaoni Kou showed that MSC-AS1 expression was upregulated in HCC tissues, and the downregulation of MSC-AS1 expression could inhibit the progression of HCC [47]. Cong Cao pointed out that knockdown of MSC-AS1 gene stagnated the cell cycle of HCC cells in G1 phase, inhibited the proliferation of HCC cells and induced apoptosis [48]. Different bioinformatics analyses have shown that MSC-AS1 has prognostic value in HCC [49–51]. After reviewing previous articles, we found that miR-23c can inhibit the progression of HCC. Shuai Xue found that IncRNA ZEB1-AS1 could inhibit the progression of HCC through miR-23c, while inhibition of miR-23c could restore the inhibitory effect of ZEB1-AS1 gene knockout on the proliferation and invasion of HCC cells [52]. Wenchuan Li suggested that miR-23c could suppress HCC cell growth by attenuating ERBB2IP, and IncRNA KTN1-AS1 could promote HCC tumor growth through the miR-23c/ERBB2IP axis [53, 54]. Hence, we speculated that upstream lncRNA MSC-AS1 upregulates the expression of RAD51AP1 by inhibiting hsa-miR-23c in HCC, thus promoting the process of hepatoma cell division. Down-regulation or silencing of MSC-AS1 expression can inhibit the proliferation of HCC cells, and the MSC-AS1/hsa-miR-23c axis modulates DNA damage repair in HCC via RAD51AP1. However, the expression and regulation mechanism of RAD51AP1 are complex, and our discovery is only one of them. In conclusion, the study of the role of RAD51AP1 and the regulation mechanism of upstream molecules in HCC is of great significance for the diagnosis, prognosis and pathogenesis of HCC, and provides a new idea for the individualized treatment, efficacy monitoring and prognosis of HCC.

Although the above approaches reveal an association between RAD51AP1 and its related molecules in HCC, there are still some limitations to this study. First, although there is a large amount of high-throughput data stored in the TCGA database, the number of samples related to HCC is still insufficient. Second, although downregulation of MSC-AS1 expression can inhibit the progression of HCC, the changes of hsa-miR-23c and RAD51AP1 expression still need to be further verified in animal experiments or cell experiments. Therefore, it is necessary to conduct further research to address the limitations of the above conclusions.

Conclusion

In our study, we determined the expression and biological function of RAD51AP1 and its value for the diagnosis and prediction of prognosis in HCC. Based on the regulatory mechanism of ceRNA networks, the target miRNA of RAD51AP1 and the corresponding upstream lncRNA were predicted by a bioinformatics approach, which provides new insight for further study of the DNA damage repair mechanism and potential related treatments in HCC.

References


