A functional variant in the RAD51 3' UTR is associated with survival of hepatocellular carcinoma patients

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Research article

Abstract

The *RAD51* gene plays an important role in DNA repair by homologous recombination, and is involved in the development and progression of multiple cancers. While single nucleotide polymorphisms in *RAD51* have been previously described to impact patient prognosis, it is still unclear whether this is also true for hepatocellular carcinoma (HCC). This study therefore aimed to identify genetic variants in *RAD51* and determine the effect of these variants on the survival of patients with HCC. In this study, we performed genotyping assays for *RAD51* polymorphisms in a cohort of 368 patients with HCC who had undergone radical surgery resection. Using multivariate cox proportional hazards model and Kaplan-Meier analyses with log-rank tests, we compared the survival of patients with HCC according to *RAD51* SNP genotypes. We performed expression quantitative trait loci (eQTL) analysis to investigate correlations between SNP rs12593359 and *RAD51* mRNA expression levels from the genotype-tissue expression portal. We identified one potential functional variant, rs12593359, located in a microRNA (miRNA) binding site in the *RAD51* 3' untranslated region, to be an independent predictor of overall survival of patients with HCC in the dominant model. Patients carrying GT/TT genotypes had a significantly increased risk of death when compared with those carrying GG genotype (adjusted hazard ratio = 1.34, 95% confidence interval = 1.02–1.76, P = 0.035). Kaplan-Meier curve analysis showed a markedly shorter survival time for patients with HCC carrying GT/TT genotypes of SNP rs12593359 (19.0 months vs. 36.0 months; $P_{log-rank}$ = 0.012). Notably, this effect was particularly pronounced in several subgroups of patients (e.g., males, Hepatitis B virus-positive patients, patients with a single tumor nodule, patients with alpha-fetoprotein (AFP) < 400 ng/ml, or patients who were cancer embolus-free). Additional expression analysis of quantitative trait loci showed that the rs12593359 was significantly associated with *RAD51* mRNA expression levels in 483 cell-cultured fibroblasts (P = 1.1 × 10^{-4}). These findings provide evidence that *RAD51* rs12593359 is associated with HCC survival and may serve as a promising predictor of survival in patients with HCC.

Introduction

Liver cancer was the fourth leading cause of cancer-related deaths worldwide in 2018, with approximately 782,000 deaths occurring annually [1]. Hepatocellular carcinoma (HCC) accounts for 75–85% of all primary liver cancer cases [2]. Since more than 50% of HCC cases are diagnosed in the late stages of the disease, HCC prognosis has only marginally improved over the time, with a 5-year survival rate of just 18.1%, making HCC the second most lethal tumor after pancreatic cancer (which has an 8.5% 5-year survival rate) [3]. Therefore, it is imperative to improve our ability to predict which patients are likely to have a poor survival outcome. Clinically, the known characteristics of HCC such as tumor size, serum alpha-fetoprotein (AFP) levels, and Barcelona Clinic Liver Cancer (BCLC) stage are commonly used to predict the prognosis of patients with HCC [4, 5]. However, these histopathological features of primary tumors do not provide sufficient information for assessing tumor malignancy. The response of individuals to treatments such as hepatectomy is heterogeneous, suggesting that genetic factors may also play an important role in the prognosis of this cancer and investigating such molecular mechanisms could reveal promising prognostic or predictive factors for HCC [6–8].

Homologous recombination (HR) plays an important role in tumorigenesis and the proteins involved in HR protect chromosomes against endogenous damage affecting both DNA strands, such as double-strand breaks [9]. They maintain genomic stability by supporting DNA replication and repairing DNA damage, which helps cells to avoid gene mutations [10]. *RAD51* is one of the most important human HR genes. It is located on chromosome 15 at position q15.1 and encodes a product of 339 amino acids [11]. *RAD51* performs an irreplaceable function by forming nucleoprotein filaments in single-stranded DNA, which mediates homologous pairing, and strand exchange reactions between single- and double-stranded DNA during the repair process [12].

Several studies have provided evidence demonstrating that abnormal *RAD51* expression is associated with poorer outcomes in several tumor types [13−16]. For example, *RAD51* overexpression was shown to correlate with malignant phenotypes of colorectal cancer and may predict poor prognosis for these patients [17]. Elevated expression of *RAD51* has been associated with decreased survival of patients with non-small-cell lung cancer, which may be due to enhanced tumor cell survival, anti-apoptotic activity, and chemo-/radioresistance [18]. In vitro studies have demonstrated that the abnormal increase of *RAD51* expression favors tumor progression by inhibiting caspase-3-mediated apoptosis, as well as by interfering with other intracellular pathways, including p53, p21 and Bcl-2, which may cause improper repair of damaged DNA or further amplify the damage of sites in a recombinant DNA strand [19]. In addition, a previous study showed that miR-103 was able to dramatically enhance the chemosensitivity of U2OS osteosarcoma cells by specifically targeting the 3' untranslated region (3' UTR) of *RAD51* to reduce its expression [20]. Recently, many reports have also demonstrated that *RAD51* polymorphisms, located in potential functional sites, could modulate its expression and function, thereby affecting cancer development and progression [21−23].

MicroRNAs (miRNA) are a class of evolutionarily conserved small noncoding RNAs that are known to regulate gene expression by binding to the 3' UTR of target mRNAs, leading to mRNA cleavage and/or translational repression [24]. MiRNAs are proposed to regulate the expression of more than 30% of the protein-coding genes in the human genome [25]. However, the binding process can be influenced by SNPs within miRNA binding sites, and disrupting miRNA-mRNA interactions may result in the deregulation of target gene expression, especially with respect to certain oncogenes, tumor suppressor genes, or genes involved in oncogenic pathways [26]. A variety of molecular epidemiological studies have shown that SNPs located in miRNA-binding sites in DNA repair genes, including *RAD51*, were associated with the risk and prognosis of lung cancer, breast cancer, and head and neck carcinoma [27−29]. Nevertheless, the associations between genetic variants in *RAD51* and the survival of patients with HCC are still unclear.

In the present study, to test the hypothesis that genetic variants in the predicted miRNA binding sites of the *RAD51* gene are associated with the survival of patients with HCC, we first performed bioinformatic prediction for *RAD51* gene SNPs with a minor allele frequency (MAF) of ≥ 0.05 in the Chinese Han population. We selected one SNP, rs12593359, located in the 3' UTR of the *RAD51* gene, for further analysis as the location of this polymorphism has the potential to influence miRNAs binding activity.

Then, we analyzed data from a cohort of 368 patients with HCC to determine the effects of this SNP on overall survival, and assessed whether the *RAD51* rs12593359 polymorphism could be a potential biomarker to predict HCC prognosis.

Materials And Methods

Study population

A total of 368 patients with newly diagnosed and pathologically confirmed HCC were recruited between July 2007 and December 2013 from Guangxi Medical University cancer hospital. All patients had undergone radical surgery resection and their files contained complete clinical information. Detailed clinical information was collected through medical chart review or by consultation with treating physicians, and included age at diagnosis, sex, smoking status, drinking status, hepatitis B virus infection, tumor size, number of tumors, BCLC staging, AFP level, and cancer embolus status. All patients were followed up to collect data via telephone or hospital visits after surgery until patients died or up to the date of the last follow-up in June 2019. Peripheral blood sample (5 mL) was collected in a vacuum EDTA anticoagulant tube from each study participant. The research protocol was approved by the Ethical Committee Review Board of Guangxi Medical University, and written informed consent was obtained from each of the participants.

SNP selection and genotyping

We used the National Institute of Environmental Health Sciences (NIEHS) SNP database (https://snpinfo.niehs.nih.gov/) to identify functional *RAD51* SNPs using the following criteria: (1) SNPs located in the 3' -UTR of *RAD51* in potential miRNA target sites; (2) minor allele frequency (MAF) in Chinese Han population > 0.05; and (3) low linkage disequilibrium using an r^2 threshold of < 0.8 for each other. Ultimately, only one SNP (rs12593359, located in the miR-129-3p binding site in the *RAD51* 3' UTR) fulfilled all of these criteria. Genomic information regarding *RAD51* rs12593359 and the linkage disequilibrium plot are shown in Table 1S and Figure 1S.

For genotyping, we extracted genomic DNA by using a standard phenol/chloroform extraction method and stored at -80°C. Then, we performed the genotyping using the Agena MassARRAY system (Agena, San Diego, CA, USA) according to the manufacturer's instructions. The primers for PCR were designed using the Assay Designer software package of the Sequenom system (San Diego, CA, USA). The primers used for *RAD51* rs12593359 were: F: 5'-ACGTTGGATGTAGGTTTGGCACAAGACTCC-3' and R: 5'-ACGTTGGATGCAGTAAAACTCTCAAGCAGG-3'. The genotyping results were analyzed using the MassARRAY Typer software version 4.0.

Statistical analysis

Overall survival (OS) was defined as the interval between the date of surgery and the date of death, or the date of the last follow-up. We used the Cox proportional hazards regression model to evaluate the effect of genotypes and clinicopathological variables on OS, calculated as hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). All HRs were adjusted for age at diagnosis, sex, smoking status, drinking status, hepatitis B virus infection, tumor size, number of tumors, BCLC staging, AFP level, and cancer embolus status. We performed a stepwise conditional logistic regression analysis to explore the best model for predicting survival outcome. Kaplan-Meier curves and log-rank tests were also used to assess differences in OS.

We performed expression quantitative trait loci (eQTL) analysis to investigate correlations between SNP rs12593359 and *RAD51* mRNA expression levels, which were obtained from the genotype-tissue expression (GTEx) Portal (http://www.gtexportal.org/home/). The differences in *RAD51* mRNA expression between HCC and normal tissues and associations between *RAD51* expression levels and HCC overall survival were examined using data from the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/index.html), including data from 369 HCC tumor tissues, 50 normal liver tissues in The Cancer Genome Atlas (TCGA) dataset, and 110 normal liver tissues in the GTEx dataset. Finally, to provide additional supporting evidence, we also used the online Kaplan-Meier plotter tool (http://kmplot.com/analysis/) to assess the association between *RAD51* expression levels and survival of liver cancer patients.

All statistical analyses were performed using the SPSS software, version 18.0 (SPSS Institute, Chicago, IL, USA) or GraphPad Prism, version 6.0 (GraphPad Software Inc., San Diego, CA, USA). All statistical tests were two-sided and $P < 0.05$ was considered to be statistically significant.

Results

Distribution of patient characteristics and prognosis analyses

All patient characteristics and clinical data are summarized in Table 1. The patients included 334 men (90.8%) and 34 women (9.2%) with a median age of 50 years (range: 22–82 years). Among this cohort, 62.5% (230) were non-drinkers and 58.4% (215) were non-smokers, while 84.0% (309) were hepatitis B surface antigen (HBsAg) positive. The patient cohort comprised 238 (64.7%) patients with a tumor size of ≥ 5 cm, 77 (20.9%) with multiple tumor nodules (≥ 2), 105 (28.5%) with cancer embolus, and 151 (41.0%) with significantly increased serum alpha-fetoprotein (AFP) levels (≥ 400 ng/mL). The percentage of patients with Barcelona clinic liver cancer (BCLC) stage A/B and stage C was 55.4% (204) and 44.6% (164), respectively.

To determine whether there were any confounding factors influencing patient deaths or survival time, we performed a Cox proportional hazards regression analysis to assess whether there were any associations between OS and clinicopathological characteristics. Multivariate analyses showed that large tumor size

(≥ 5 cm) (adjusted HR = 1.83, 95% CI = 1.32−2.53; P < 0.001), advanced BCLC stage C (adjusted HR = 2.17, 95% CI = 1.59−2.96; P < 0.001), and cancer embolus (adjusted HR = 1.54, 95% CI = 1.13−2.11; P = 0.006) predicted poor overall survival of patients with HCC. There was no association between HCC outcome and age (P = 0.470), gender (P = 0.671), smoking status (P = 0.702), drinking status (P = 0.226), HBV infection status (P = 0.801), number of tumor nodules (P = 0.125), or AFP levels (P = 0.378) (Table 1).

Association between *RAD51* rs12593359 and clinical outcome in HCC patients

The effect of *RAD51* rs12593359 on HCC prognosis is shown in Table 2. We observed that the dominant genetic model of the SNP rs12593359 had a significant correlation with HCC prognosis. Compared to the rs12593359 GG genotype, the GT/TT genotype conferred a significant increase in the risk of death in multivariate Cox proportional hazards model adjusted for all variables (adjusted HR = 1.34, 95% CI = 1.02−1.76; P = 0.035). Kaplan-Meier curve analysis showed a markedly shorter survival time in HCC patients with GT/TT genotypes of SNP rs12593359 compared with that of patients carrying a GG genotype (19.0 months vs. 36.0 months; $P_{\text{log-rank}}$ = 0.012) (Figure 1a).

Stepwise regression analysis of HCC prognosis

To determine the independent prognostic factors associated with HCC OS, we performed a stepwise multivariate Cox proportional hazards regression analysis using covariates listed in Table 1 and the *RAD51* SNP rs12593359. The results demonstrated that the survival of patients with HCC had a relationship with BCLC stage (A/B vs. C), tumor size (< 5 cm vs. ≥ 5 cm), cancer embolus (no vs. yes), and rs12593359 genotypes (GG vs. TT/TG) (Table 3).

Stratified analysis of association between SNP rs12593359 with OS by HCC patient characteristics

We further analyzed the effect of the SNP rs12593359 on OS in HCC patients stratified by clinical characteristics. As shown in Table 4, the significant increase in risk of death conferred by the SNP rs12593359 was observed in males (adjusted HR = 1.40, 95% CI = 1.05−1.87; P = 0.021), but not in females (P = 0.707); in HBV-positive patients (adjusted HR = 1.37, 95% CI = 1.01−1.85; P = 0.042), but not in HBV-negative patients (P = 0.901); in those with a single tumor nodule (adjusted HR = 1.51, 95% CI = 1.11−2.06; P = 0.009), but not in those with multiple tumor nodules (P = 0.940); in those with AFP levels < 400ng/mL (adjusted HR = 1.58, 95% CI = 1.10−2.29; P = 0.014), but not in those with levels ≥ 400ng/mL (P = 0.451); and in patients who were cancer embolus-free (adjusted HR = 1.55, 95% CI = 1.10−2.20; P =

0.014), but not in those with a cancer embolus (P = 0.461). No significant association between SNP rs12593359 and HCC OS existed in other subgroups (P > 0.05).

As indicated in Figure 1b-f, log-rank tests indicated that there was a significant difference in overall survival between patients with TT/TG genotypes and those with the GG genotype of SNP rs12593359 in the above-mentioned subgroups.

Correlation between rs12593359 genotypes and the mRNA expression level of *RAD51*

We performed the eQTL analysis to correlate the rs12593359 genotypes and mRNA expression levels of *RAD51* using data of the 483 cell-cultured fibroblasts from the GTEx database. The rs12593359 GG genotype was significantly associated with lower expression levels of *RAD51* mRNA than the TT genotype, but not TG genotype (P = 1.1×10^{-4}, Figure 2).

Association of *RAD51* mRNA expression levels with survival of patients with HCC

By using the GEPIA database, we evaluated mRNA expressions levels of *RAD51* in 371 HCC and 160 normal tissue samples. HCC tissues exhibited a significantly higher expression of *RAD51* as compared to that of the normal tissues (P < 0.001, Figure 3), and patients with higher *RAD51* mRNA expression in tumor tissues showed a poorer overall survival (HR=1.6, $P_{\text{log-rank}}$= 0.0054, Figure 4). Furthermore, we also found that a higher expression level of *RAD51* was associated with poorer survival in 364 liver cancer patients from the online tool Kaplan-Meier plotter (P = 0.00097, Figure 2S).

Discussion

In the present study, we evaluated the prognostic value of the *RAD51* SNP rs12593359, in a cohort of 368 patients with HCC in a population of Southern China. The most important finding was that SNP rs12593359, located in a miRNA binding site in the *RAD51* 3' UTR, was significantly associated with HCC OS, and this effect was particularly pronounced in several subgroups of HCC patients (males, HBV positive patients, patients with a single tumor nodule, patients with AFP < 400 ng/ml, or patients with no cancer embolus). Furthermore, we observed that the genotypes of SNP rs12593359 was significantly associated with *RAD51* mRNA expression levels in 483 cell-cultured fibroblasts.

RAD51 encodes a homolog of the *E. coli* RecA protein that catalyzes DNA repair via homologous recombination, an evolutionarily conserved mechanism for the repair of DNA damage and the generation of genetic diversity [30]. *RAD51* expression is tightly controlled in normal cells, as it promotes strand exchange between unimpaired and impaired homologous DNA fragments, insuring a highly reproducible

quality of DNA repair. Even slight changes in *RAD51* gene expression may lead to DNA instability [31]. Indeed, overexpression of *RAD51* is detected in multiple human tumor cells and correlates with poor prognosis [32-35]. In this study, we evaluated the prognostic value of *RAD51* in patients with HCC using the GEPIA database. Consistent with studies in several other tumors, we found that *RAD51* mRNA levels were higher in HCC tissues as compared to that of normal tissues, and the higher expression levels were associated with poorer survival. These findings suggest that *RAD51* may serve as an independent prognostic marker of survival in HCC patients, and raises the question of why certain individuals experience elevated expression of *RAD51* and eventually develop HCC with a poor prognosis.

Accumulating evidence has suggested that miRNAs actively take part in the regulation of the DNA damage/repair network by affecting the expression of DNA repair genes such as *RAD51*, ultimately regulating their biological functions [36, 37]. Polymorphisms in the predicted miRNA target sites of these genes have been shown to be strongly involved in regulating the expression of the mRNAs, either by providing mutated binding sites which alter miRNA-mRNA interactions, or by forming hairpin loop structures which stabilize and thus slow down mRNA degradation [38, 39]. Our functional prediction analysis indicated that the rs12593359 SNP in the 3-UTR region of *RAD51* was located within a predicted miRNA binding site for hsa-miR-129-3p (http://snpinfo.niehs.nih.gov/snpfunc.htm). The hsa-miR-129-3p may act as an important tumor suppressor that could inhibit tumor development and progression in several cancers [40-42]. Cui et al. reported that hsa-miR-129-3p was downregulated in HCC tissues, and patients with low hsa-miR-129-3p levels had a poorer prognosis than those with higher levels of this miRNA. However, re-expression of hsa-miR-129-3p was able to reverse epithelial-mesenchymal transition, and inhibit invasion and metastasis of HCC cells through targeting of the Aurora-A kinase [43].

In our study, we found that the *RAD51* SNP rs12593359 is an independent predictor of HCC OS, and that patients with HCC carrying GT or TT genotypes had a significantly increased risk of death when compared with those carrying GG genotypes. In subsequent genotype−mRNA expression correlation analysis, the TT genotype was significantly associated with increased mRNA levels of *RAD51* in cell-cultured fibroblasts. Chen et al. demonstrated that rs12593359 may be a putative variant mediating post-transcriptional regulation of the *RAD51* gene, and the rs12593359 GT or TT genotype was found to yield significantly higher *RAD51* mRNA levels compared with the GG genotype in lymphoblastoid cell lines [44]. Based on these results, together with our own findings, we speculate that the functional SNP rs12593359 in the *RAD51* 3' UTR may disrupt the binding ability of has-miR-129-3p, thus inhibiting its regulation of target gene expression. At the same time, such a change might lead to increased expression of *RAD51*, thus modifying the overall survival of HCC patients. However, the mechanisms underlying this phenomenon need to be studied further. Such findings would help us to better assess the prognosis of patients with HCC, and develop a timely and individualized treatment plan, or even provide new ideas for targeted drugs.

It is important to acknowledge the limitations of the present study. Firstly, our sample was not large enough to detect minimal associations, which may have limited the statistical power of our analyses.

Secondly, whether these findings (identified in a population of Southern China) can be applied at a more general level needs to be further validated in other district and ethnic populations. Thirdly, we did not determine the expression of *RAD51* in our own HCC cohort, and assess its relationship with the rs12593359 genotypes and the survival of our patients. Finally, no direct functional investigations were conducted to provide mechanistic insights into this SNP, which will be an important topic of investigation in future studies.

In conclusion, our results suggest that the *RAD51* 3'-UTR polymorphism rs12593359 may be functional and may affect the survival of patients with HCC by altering *RAD51* expression. Further studies using a larger population and functional exploration of the underlying molecular mechanisms are warranted to confirm these findings.

Declarations

Ethics approval and consent to participate

This study was approved by the ethical committee of Guangxi Medical University Cancer Hospital and was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent after a detailed explanation.

Author Contributions

Moqin Qiu and Yingchun Liu performed the statistical analysis and interpreted data and wrote the manuscript. Hongping Yu and Xianguo Zhou made substantial contributions to the study's conception and design. Qiuling Lin, Zihan Zhou, Yanji Jiang, and Zihan Zhou performed the genetic examinations of the blood samples and collected the data. Qiuping Wen, Xiumei Liang, and Rongrui Huo performed the follow-up survey. All authors participated in the drafting of the article or revising it critically for important intellectual content, and they provided final approval of the version to be published.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* CA Cancer J Clin, 2018. **68**(6): p. 394-424.

2. Villanueva, A., *Hepatocellular Carcinoma.* N Engl J Med, 2019. **380**(15): p. 1450-1462.

3. Jemal, A., et al., *Annual Report to the Nation on the Status of Cancer, 1975-2014, Featuring Survival.* J Natl Cancer Inst, 2017. **109**(9).

4. Wang, J.H., et al., *The efficacy of treatment schedules according to Barcelona Clinic Liver Cancer staging for hepatocellular carcinoma - Survival analysis of 3892 patients.* Eur J Cancer, 2008. **44**(7): p. 1000-6.

5. Lv, Y., et al., *High preoparative levels of serum periostin are associated with poor prognosis in patients with hepatocellular carcinoma after hepatectomy.* Eur J Surg Oncol, 2013. **39**(10): p. 1129-35.

6. Zhu, X., et al., *Rs2303428 of MSH2 Is Associated with Hepatocellular Carcinoma Prognosis in a Chinese Population.* DNA Cell Biol, 2018. **37**(7): p. 634-641.

7. Liu, S., et al., *Genetic variants of cell cycle pathway genes predict disease-free survival of hepatocellular carcinoma.* Cancer Med, 2017. **6**(7): p. 1512-1522.

8. Pan, D., et al., *Role of cytokine gene polymorphisms on prognosis in hepatocellular carcinoma after radical surgery resection.* Gene, 2014. **544**(1): p. 32-40.

9. Gachechiladze, M., et al., *RAD51 as a potential surrogate marker for DNA repair capacity in solid malignancies.* Int J Cancer, 2017. **141**(7): p. 1286-1294.

10. Moynahan, M.E. and M. Jasin, *Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis.* Nat Rev Mol Cell Biol, 2010. **11**(3): p. 196-207.

11. Shinohara, A., et al., *Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA.* Nat Genet, 1993. **4**(3): p. 239-43.

12. Baumann, P. and S.C. West, *Role of the human RAD51 protein in homologous recombination and double-stranded-break repair.* Trends Biochem Sci, 1998. **23**(7): p. 247-51.

13. Li, Y., et al., *Elevated expression of Rad51 is correlated with decreased survival in resectable esophageal squamous cell carcinoma.* J Surg Oncol, 2011. **104**(6): p. 617-22.

14. Tennstedt, P., et al., *RAD51 overexpression is a negative prognostic marker for colorectal adenocarcinoma.* Int J Cancer, 2013. **132**(9): p. 2118-26.

15. Soderlund, K., et al., *The BRCA1/BRCA2/Rad51 complex is a prognostic and predictive factor in early breast cancer.* Radiother Oncol, 2007. **84**(3): p. 242-51.

16. Sarwar, R., et al., *Upregulation of RAD51 expression is associated with progression of thyroid carcinoma.* Exp Mol Pathol, 2017. **102**(3): p. 446-454.

17. Li, Y., et al., *Overexpression of Rad51 Predicts Poor Prognosis in Colorectal Cancer: Our Experience with 54 Patients.* PLoS One, 2017. **12**(1): p. e0167868.

18. Qiao, G.B., et al., *High-level expression of Rad51 is an independent prognostic marker of survival in non-small-cell lung cancer patients.* Br J Cancer, 2005. **93**(1): p. 137-43.

19. Raderschall, E., et al., *Formation of higher-order nuclear Rad51 structures is functionally linked to p21 expression and protection from DNA damage-induced apoptosis.* J Cell Sci, 2002. **115**(Pt 1): p. 153-64.

20. Huang, J.W., et al., *Systematic screen identifies miRNAs that target RAD51 and RAD51D to enhance chemosensitivity.* Mol Cancer Res, 2013. **11**(12): p. 1564-73.

21. Costa, S., et al., *XRCC1 Arg399Gln and RAD51 5'UTR G135C polymorphisms and their outcome in tumor aggressiveness and survival of Portuguese breast cancer patients.* Breast Cancer Res Treat, 2008. **109**(1): p. 183-5.

22. Zhou, J., et al., *Genetic polymorphisms of DNA repair pathways influence the response to chemotherapy and overall survival of gastric cancer.* Tumour Biol, 2015. **36**(4): p. 3017-23.

23. Ding, C., et al., *Genetic variability of DNA repair mechanisms influences treatment outcome of gastric cancer.* Oncol Lett, 2015. **10**(4): p. 1997-2002.

24. Fabbri, M., C.M. Croce, and G.A. Calin, *MicroRNAs.* Cancer J, 2008. **14**(1): p. 1-6.

25. Lewis, B.P., C.B. Burge, and D.P. Bartel, *Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets.* Cell, 2005. **120**(1): p. 15-20.

26. Kertesz, M., et al., *The role of site accessibility in microRNA target recognition.* Nat Genet, 2007. **39**(10): p. 1278-84.

27. Zhu, L., et al., *Genetic variants in microRNA-binding sites of DNA repair genes as predictors of recurrence in patients with squamous cell carcinoma of the oropharynx.* Int J Cancer, 2017. **141**(7): p. 1355-1364.

28. Cao, J., et al., *MiRNA-binding site functional polymorphisms in DNA repair genes RAD51, RAD52, and XRCC2 and breast cancer risk in Chinese population.* Tumour Biol, 2016.

29. Matakidou, A., et al., *Genetic variation in the DNA repair genes is predictive of outcome in lung cancer.* Hum Mol Genet, 2007. **16**(19): p. 2333-40.

30. Richardson, C., *RAD51, genomic stability, and tumorigenesis.* Cancer Lett, 2005. **218**(2): p. 127-39.

31. Tambini, C.E., et al., *The importance of XRCC2 in RAD51-related DNA damage repair.* DNA Repair (Amst), 2010. **9**(5): p. 517-25.

32. Welsh, J.W., et al., *Rad51 protein expression and survival in patients with glioblastoma multiforme.* Int J Radiat Oncol Biol Phys, 2009. **74**(4): p. 1251-5.

33. Raderschall, E., et al., *Elevated levels of Rad51 recombination protein in tumor cells.* Cancer Res, 2002. **62**(1): p. 219-25.

34. Xu, Y., et al., *Overexpression of Rad51 predicts poor prognosis and silencing of Rad51 increases chemo-sensitivity to doxorubicin in neuroblastoma.* Am J Transl Res, 2019. **11**(9): p. 5788-5799.

35. Hu, J., et al., *High expression of RAD51 promotes DNA damage repair and survival in KRAS-mutant lung cancer cells.* BMB Rep, 2019. **52**(2): p. 151-156.

36. Liu, G., F. Xue, and W. Zhang, *miR-506: a regulator of chemo-sensitivity through suppression of the RAD51-homologous recombination axis.* Chin J Cancer, 2015. **34**(11): p. 485-7.

37. Chen, S., et al., *MiR-34s negatively regulate homologous recombination through targeting RAD51.* Arch Biochem Biophys, 2019. **666**: p. 73-82.

38. Bartel, D.P., *MicroRNAs: genomics, biogenesis, mechanism, and function.* Cell, 2004. **116**(2): p. 281-97.

39. Yu, Z., et al., *Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers.* Nucleic Acids Res, 2007. **35**(13): p. 4535-41.

40. Zhang, M., et al., *miR-129-3p inhibits NHEJ pathway by targeting SAE1 and represses gastric cancer progression.* Int J Clin Exp Pathol, 2019. **12**(5): p. 1539-1547.

41. Fang, D.Z., et al., *MicroRNA-129-3p suppresses tumor growth by targeting E2F5 in glioblastoma.* Eur Rev Med Pharmacol Sci, 2018. **22**(4): p. 1044-1050.

42. Jia, Y., Y. Gao, and J. Dou, *Effects of miR-129-3p on biological functions of prostate cancer cells through targeted regulation of Smad3.* Oncol Lett, 2020. **19**(2): p. 1195-1202.

43. Cui, S., et al., *Methylation-associated silencing of microRNA-129-3p promotes epithelial-mesenchymal transition, invasion and metastasis of hepatocelluar cancer by targeting Aurora-A.* Oncotarget, 2016. **7**(47): p. 78009-78028.

44. Chen, F., H. Zhang, and F. Pu, *Association between a functional variant in RAD51 gene's 3' untranslated region and its mRNA expression in lymphoblastoid cell lines.* Springerplus, 2016. **5**(1): p. 1688.

Tables

Table 1. Characteristics and clinical features of patients

Variables	Patients n(%)	Deaths n(%)	Adjusted HR (95% CI)	P-value [a]
All subjects	368(100)	215(100)		
Age				
<50	177(48.1)	97(45.1)	1	
≥50	191(51.9)	118(54.9)	1.11(0.84-1.47)	0.470
Sex				
Females	34(9.2)	23(10.7)	1	
Males	334(90.8)	192(89.3)	1.11(0.70-1.76)	0.671
Smoking status				
Never	215(58.4)	130(60.5)	1	
Ever	153(41.6)	85(39.5)	0.92(0.61-1.40)	0.702
Drinking status				
Never	230(62.5)	141(65.6)	1	
Ever	138(37.5)	74(34.4)	0.77(0.51-1.18)	0.226
HBV infection status				
🞏-🞏	59(16.0)	36(16.7)	1	
🞏+🞏	309(84.0)	183(83.3)	0.95(0.66-1.38)	0.801
Tumor size (cm)				
<5	130(35.3)	58(27.0)	1	
≥5	238(64.7)	157(73.0)	**1.83(1.32-2.52)**	**< 0.001**
Tumor number				
Single	291(79.1)	167(77.7)	1	
Multiple	77(20.9)	47(22.3)	1.30(0.93-1.83)	0.125
BCLC stage				
A/B	204(55.4)	105(48.8)	1	
C/D	164(44.6)	110(51.2)	**2.17(1.59-2.96)**	**< 0.001**
AFP level (ng/mL)				
< 400	217(59.0)	119(55.3)	1	
≥400	151(41.0)	96(44.7)	1.14(0.85-1.52)	0.378

Cancer embolus				
No	263(71.5)	138(64.2)	1	
Yes	105(28.5)	77(35.8)	**1.54(1.13-2.11)**	**0.006**

[a] Multivariate Cox regression analyses were adjusted for all factors listed in Table 1.

Bold values are statistically significant.

Table 2. Genotypes of *RAD51* rs12593359 and HCC patients' survival

Genotype	Patients n(%)	Deaths n(%)	Adjusted HR (95% CI)	P [a]
rs12593359				
GG	218(59.2)	118(54.9)	1	
TG	127(34.5)	82(38.1)	1.31(0.98-1.75)	0.065
TT	23(6.3)	15(7.0)	1.51(0.87-2.62)	0.143
GT/TT	150(40.8)	97(35.1)	**1.34(1.02-1.76)**	**0.035**

[a] Multivariate Cox regression analyses were adjusted for all factors listed in Table 1.

Bold values are statistically significant.

Table 3. Predictors of overall survival in HCC patients obtained from stepwise multivariate cox regression analysis of selected variables

Selected variables[a]	β	SE	HR	95% CI	*P*-value [a]
BCLC stage (A/B vs. C)	0.760	0.154	2.14	1.58-2.89	< 0.001
Tumor size (< 5 cm vs. ≥ 5 cm)	0.630	0.160	1.88	1.37-2.57	< 0.001
Cancer embolus (no vs. yes)	0.438	0.155	1.55	1.15-2.10	0.005
rs12593359 (GG vs. GT/TT)	0.287	0.138	1.33	1.02-1.75	0.037

[a] Age, sex, smoking, drinking, HBV infection, tumor size, tumor number, BCLC stage, AFP, cancer embolus, and rs12593359 genotypes were included in the stepwise multivariate Cox proportional hazards regression analysis.

Table 4. Stratification analyses of rs12593359 genotypes associated with HCC patients' survival

variables	rs12593359 (patients/deaths)		Adjust HR (95%CI)	P [a]
	GG	GT/TT		
Age				
<50	100//52	77/45	1.30(0.85-1.98)	0.222
≥50	118/66	73/52	1.43(0.99-2.07)	0.058
Sex				
Females	21/14	13/9	1.21(0.43-3.39)	0.707
Males	197/104	137/88	**1.40(1.05-1.87)**	**0.021**
Smoking				
Never	132/77	83/53	1.16(0.80-1.67)	0.435
Ever	86/41	67/44	1.53(0.97-2.41)	0.067
Drinking				
Never	143/82	87/59	1.25(0.88-1.77)	0.207
Ever	75/36	63/38	1.32(0.82-2.13)	0.253
HBV infection				
▯-▯	35/21	24/15	0.95(0.45-2.03)	0.901
▯+▯	183/97	126/82	**1.37(1.01-1.85)**	**0.042**
Tumor size (cm)				
<5	81/33	49/25	1.14(0.65-2.00)	0.649
≥5	137/85	101/72	1.35(0.98-1.86)	0.070
Tumor number				
Single	175/92	116/75	**1.51(1.11-2.06)**	**0.009**
Multiple	43/26	34/22	0.97(0.49-1.95)	0.940
BCLC stage				
A/B	126/61	78/44	1.39(0.92-2.11)	0.116
C	92/57	72/53	1.43(0.97-2.11)	0.073
AFP level (ng/mL)				
< 400	128/61	89/58	**1.58(1.10-2.29)**	**0.014**
≥400	90/57	61/39	1.18(0.77-1.80)	0.451

Cancer embolus				
No	159/77	104/61	**1.55(1.10-2.20)**	**0.014**
Yes	59/41	46/36	1.20(0.74-1.93)	0.461

[a] Multivariate Cox regression analyses were adjusted for all factors listed in Table 1.

Bold values are statistically significant.