

Prognostic role of miR-760 in hepatocellular carcinoma

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Abstract. Previous studies have demonstrated that microRNA (miR)-760 serves an important role in various cancer types. However, to the best of our knowledge, its role in hepatocellular carcinoma (HCC) has not been fully elucidated. The current study investigated the prognostic role of miR-760 in HCC by using the Kaplan-Meier plotter database. The current data indicated that low expression of miR-760 was associated with higher overall survival (OS) for all patients with HCC from both the RNA-seq [hazard ratio (HR)=2.04; 95% confidence interval (CI)=1.44-2.89; P=4.9x10⁻⁵] and the non-commercial spotted microarray (HR=1.71; CI=1.05-2.76; P=0.028). In the RNA-seq platform, a lower expression of miR-760 was strongly associated with improved OS in male patients with HCC, but not in female patients with HCC. Additionally, low expression of miR-760 was associated with improved OS in patients with stage I, II and III HCC, and was associated with improved OS in Asian and Caucasian patients. The current results indicated that miR-760 serves as an oncogene for HCC and high expression of miR-760 is significantly associated with tumor progression and poor prognosis in patients with HCC.

Introduction

Hepatocellular carcinoma (HCC), the most common type of liver cancer in adults, occurs more frequently in males compared with females (1). The incidence of HCC is

increasing worldwide due to an increased rate of hepatitis virus infection (1). Chronic liver inflammation, due to hepatitis infection or exposure to toxins, is thought to be the leading cause of HCC (2,3). Many previous studies suggest that microRNAs (miRNAs/miRs), small non-coding RNA molecules, ~22 nucleotides in length (4), serve an essential role in the progression of HCC (4-7). Notably, recent studies of serum from patients with liver diseases have indicated that circulating miRNAs are a novel class of biomarkers of HCC and other liver diseases (6,7). These studies suggest serum miRNAs may be potential biomarkers for HCC. Therefore, it is critical to identify novel miRNA biomarkers to help predict the progression of HCC.

Human miR-760 is involved in the pathogenesis of various human diseases and its expression varies dramatically in numerous pathological conditions. In patients with oral submucous fibrosis (OSF) the expression of miR-760 is decreased in OSF tissue compared with normal tissue (8). In T cells of patients with rheumatoid arthritis the expression of miR-760 is increased compared with that in T cells from healthy individuals (9). In addition, miR-760 has been identified as a novel biomarker to predict the progression of various cancer types, including gastric cancer (GC) (10), breast cancer (11-13), colorectal cancer (CRC) (14), colon cancer (CC) and ovarian cancer (OC) (15). Based on an RNA-seq analysis of bone marrow cells from patients with GC, the expression of miR-760 in bone marrow and tumor tissue is significantly lower in stage IV patients in comparison with stage I patients (10). A higher miR-760 expression was also associated with better overall survival (OS) in patients with GC (10). This may be due to the direct interaction of miR-760 with histone mRNA, resulting in the decreased expression of histone cluster 1 H3 family member D (10). Furthermore, an *in vitro* study indicated that miR-760 regulates breast cancer stem cell metastasis and gene expression by targeting Nanog homeobox dependent pathways (16). miR-760 is also significantly downregulated in breast cancer cells that exhibit chemoresistance (12). By contrast, overexpression of miR-760 induces the resistance of human breast cancer cells to doxorubicin (11). In human CC cells and replicatively senescent human lung fibroblast cells, miR-760 expression is upregulated and regulates protein kinase CK2 α subunit expression (17). In Egyptian patients with CRC, miR-760 is significantly downregulated in CRC tissue compared with healthy tissue and the expression of miR-760 is inversely associated with

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Abbreviations: HCC, hepatocellular carcinoma; HR, hazard ratio; KM, Kaplan-Meier; OS, overall survival; miR, microRNA; OSF, oral submucous fibrosis; GC, gastric cancer; CRC, colorectal cancer; CC, colon cancer; OC, ovarian cancer; PHLPP2, PH domain and leucine rich repeat protein phosphatase 2; NSCLC, non-small cell lung cancer

Key words: microRNA-760, hepatocellular carcinoma, Kaplan-Meier plotter, prognostic role

advanced CRC stages (18). Previously, miR-760 has been detected in the plasma of patients with CRC and further investigation demonstrated that plasma miR-760 may be a potential novel biomarker for the early stages of CRC (14). A systematic review and meta-analysis in gastrointestinal cancer types has also indicated that miR-760 is downregulated and associated with poor prognosis (19). By contrast, a quantitative polymerase chain reaction assay for miR-760 in OC tissues revealed a markedly higher expression of miR-760 in tumor tissue compared with healthy tissue. Additionally, this assay demonstrated that miR-760 directly regulates the expression of PH domain and leucine rich repeat protein phosphatase 2 (PHLPP2), which subsequently results in tumor cell proliferation (15). The low expression of miR-760 is strongly associated with a better phenotype and prognosis for patients with OC, suggesting that miR-760 serves an oncogenic role in human OC progression (15). However, to the best of our knowledge, the role of miR-760 in patients with HCC has not been studied.

The Kaplan-Meier (KM) plotter miRpower analysis tool has been reported to analyze the prognostic role of miRNAs in breast cancer (20). The current study used the KM plotter database to investigate the prognostic role of miR-760 expression in HCC patients.

Materials and methods

To analyze the prognostic role of miRNA expression in HCC, the current study used the KM plotter database and miRpower analysis tool to study the association of miR-760 expression with OS in different analysis selections (http://kmplot.com/analysis/index.php?p=service&cancer=liver_mirna). The KM plotter database has combined the published miRNA expression, OS and clinical data from The Cancer Genome Atlas (<http://cancergenome.nih.gov>), Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>), European Genome-Phenome Archive (<https://www.ebi.ac.uk/ega/home>) and PubMed (<http://www.pubmed.com>) (21). The database included the miRNA expression and OS rates (with a 10 year follow-up) of 614 patients with HCC.

The KM plotter analysis tool was used to obtain Kaplan-Meier plots for the OS rate of patients with HCC. hsa-miR-760 was entered into the database and the number-at-risk was displayed under the main panel of the OS. Based on the expression of miR-760, patients were divided into two groups. Patients with miR-760 expression higher than the median were pooled into the group with high expression, while the patients with miR-760 expression lower than the median were pooled into the group with low expression. Other statistical outcomes calculated from the database, including hazard ratio (HR), 95% confidence intervals and log rank P-values, were also included in the figures and table in the current study. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The current study used the KM plotter database and analysis tool to determine the prognostic value of miR-760 for HCC. Survival curves were generated for patients with HCC from the RNA-seq platform ($n=372$; Fig. 1A) and from

the non-commercial spotted microarray ($n=164$; Fig. 1B). Compared with high expression, low expression of miR-760 was associated with higher OS for all patients with HCC from both platforms (Fig. 1). In the RNA-seq platform, the HR for miR-760 was 2.04 [95% confidence interval (CI)=1.44-2.89] and $P=4.9 \times 10^{-5}$ (Fig. 1A), and in the non-commercial spotted platform the HR for miR-760 was 1.71 (95% CI=1.05-2.76) and $P=0.028$ (Fig. 1B).

Subsequently, the current study analyzed the expression of miR-760 in patients with HCC of different sex (Table I and Fig. 2), different clinical stages (Table I and Fig. 3) and different ethnicities (Table I and Fig. 4) using the data from the RNA-Seq platform. As demonstrated in Fig. 2 and Table I, compared with high miR-760 expression, low expression of miR-760 was associated with a higher OS in male patients with HCC [hazard ratio (HR)=2.36 (1.51-3.67), $P=9.2 \times 10^{-5}$; Fig. 2A], but not in female patients with HCC [HR=1.58 (CI=0.89-2.82), $P=0.11$; Fig. 2B]. As demonstrated in Fig. 3 and Table I, compared with high miR-760 expression, low expression of miR-760 was associated with a higher OS in stage I patients [HR=2.19 (CI=1.18-4.05), $P=0.01$; Fig. 3A], stage II patients [HR=3.44 (CI=1.49-7.93), $P=0.002$; Fig. 3B] and stage III patients [HR=2.29 (CI=1.18-4.44), $P=0.01$; Fig. 3C]. Stage IV patients was excluded due to a low population. Additionally, compared with high expression, low miR-760 expression was associated with a higher OS in Asian patients and Caucasian patients (Fig. 4 and Table I). As demonstrated in Fig. 4 and Table I, in the 393 patients with complete ethnicity information (29 patients were excluded in this study due to the missing of ethnicity information), the patients with low miR-760 expression was marginally associated with higher OS in Caucasian patients [HR=1.99 (CI=1.05-3.8), $P=0.032$; Fig. 4A] and was strongly associated with higher OS in Asian patients [HR=3.44 (CI=1.82-6.51), $P=5.3 \times 10^{-5}$; Fig. 4B].

Discussion

As the primary cancer of the liver, HCC is one of the most prevalent cancer types and affects more than 5×10^5 individuals globally every year (22). HCC is associated with a poor prognosis rate; the five-year survival rate of patients with $<10\%$ (23). This may be due to various risk factors and limited approaches for early diagnosis (22,23). Liver transplantation and resection are commonly used as the most effective clinical therapies for HCC worldwide (24). However, the worldwide incidence rate of HCC and HCC-associated cases of mortality are very high, particularly in developing countries, including China and countries in Africa (2). Previously, it has been identified that various protein-coding genes serve indispensable roles in the onset and development of HCC in humans (25). HCC is commonly not detected until it is in the advanced stage (2,23,24), therefore identifying more sensitive biomarkers for early stage HCC may be beneficial for earlier diagnosis.

Recently, many fundamental and clinical studies have identified that miRNAs, including miR-21, -122, -221/222, -520 and -657, are involved in the progression of HCC (5,26-28). These studies may assist in the identification of novel biomarkers for early stage HCC and may also be beneficial in the development

Table I. Association of miR-760 expression and patients with HCC with different clinical variables.

Clinical variables	Cases, n	HR	95% CI	P-value
Sex				
Male	253	2.36	1.51-3.67	<0.001
Female	119	1.58	0.89-2.82	0.115
Clinical stage				
I	172	2.19	1.18-4.05	0.010
II	86	3.44	1.49-7.93	0.002
III	85	2.29	1.18-4.44	0.011
Ethnicity				
Caucasian	182	1.99	1.05-3.80	0.032
Asian	161	3.44	1.82-6.51	<0.001

HR, hazard ratio; CI, confidence interval.

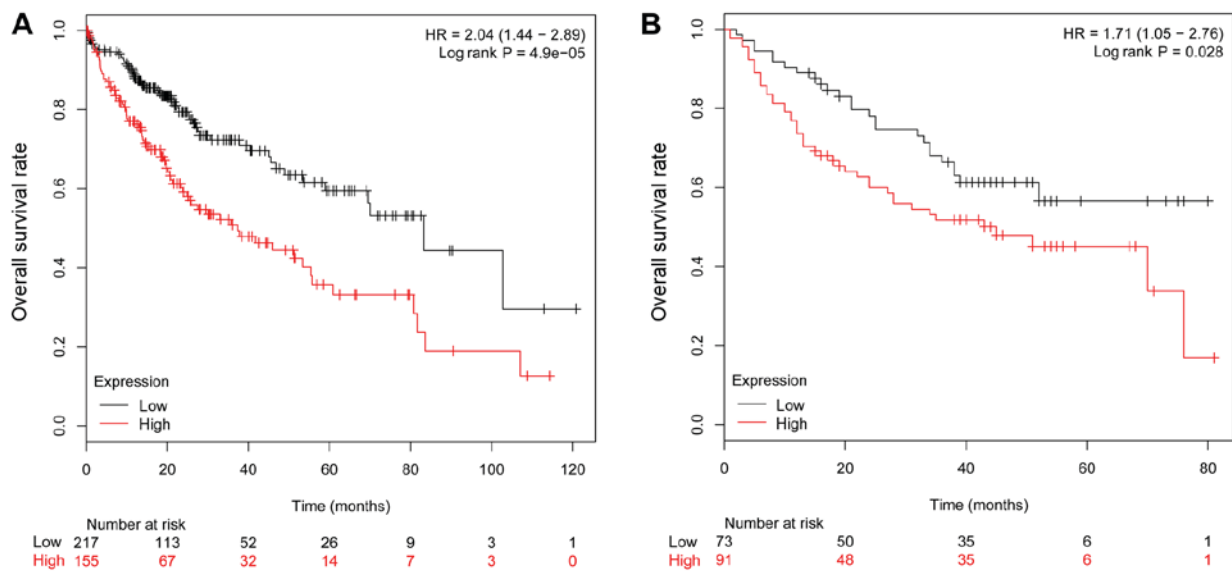


Figure 1. Prognostic value of miRNA-760 in patients with HCC. Survival curves are plotted for all patients with HCC in different platforms. (A) RNA-seq platform (n=372). (B) Non-commercial spotted platform (n=164). HR, hazard ratio; HCC, hepatocellular carcinoma; miRNA, microRNA.

of precision therapy for patients with HCC. Notably, miRNAs have also been detected in the serum of patients with HCC (29,30). Thus, monitoring serum miRNA profiles may be a convenient approach to identify early changes in the pathological functions of the liver during the onset and development of HCC.

The current study investigated the clinical value of miR-760 in predicting the development of HCC and the OS rate. The current data indicated that low expression of miR-760, compared with high expression, was associated with higher OS for patients with HCC and suggested that miR-760 may be a novel biomarker for HCC that serves as an oncogene in HCC development. In the development of non-small cell lung cancer (NSCLC), miR-760 suppresses the proliferation and metastasis of NSCLC by directly decreasing the expression of the proto-oncogene, tyrosine-protein kinase ROS (31). Furthermore, in human CRC, miR-760 attenuates tumor

progression by targeting the basic leucine zipper transcriptional factor ATF-like 3 dependent signaling pathways (32). Notably, miR-760 has been demonstrated to serve different roles in the development of OC. The expression of miR-760 is increased in the tumor tissue of patients with OC compared with that in healthy tissue from patients with OC and miR-670 promotes proliferation of OC cells via the downregulation of PHLPP2 expression (15). Similar to OC development, miR-760 may negatively regulate the expression of cancer suppressing genes, including PHLPP2, to promote the progression of HCC. This potential function of miR-760 requires further investigation to provide a better understanding of the mechanistic details involved. The current detailed analysis of miR-760 expression in different sexes and ethnicities suggested that miR-760 would be more sensitive for predicting prognosis in male patients compared with female patients and more sensitive in Asian patients compared with Caucasian patients. Notably,

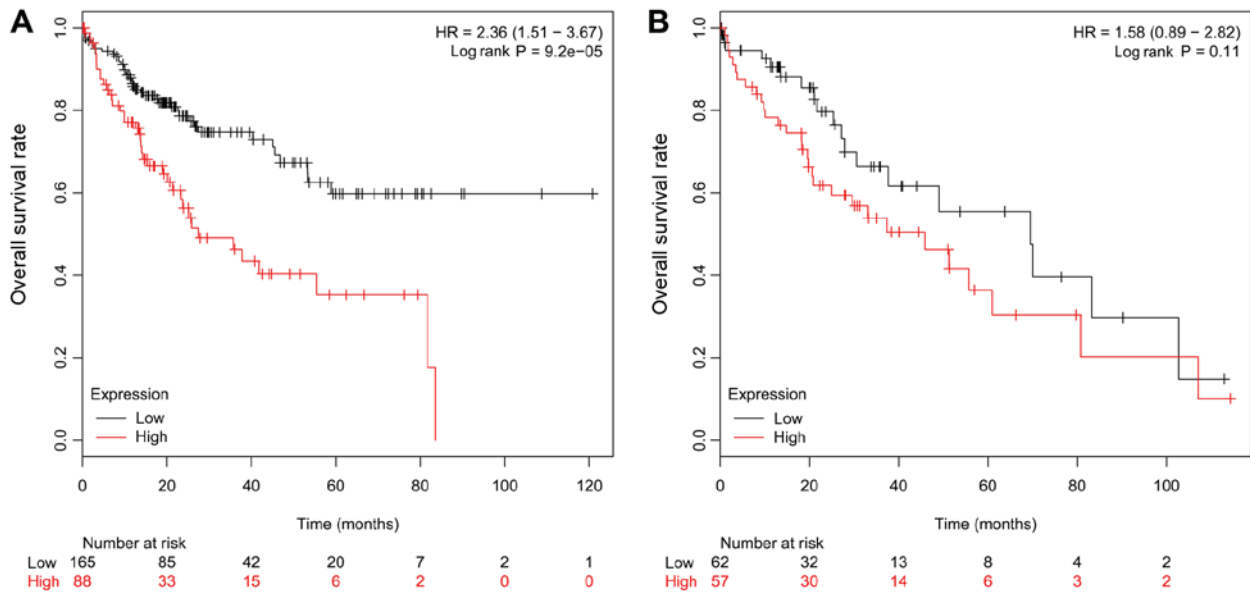


Figure 2. Prognostic value of miRNA-760 expression in patients with HCC of different sex. Survival curves are plotted for all patients with HCC of different sex. (A) Male (n=253). (B) Female (n=119). HR, hazard ratio; HCC, hepatocellular carcinoma; miRNA, microRNA.

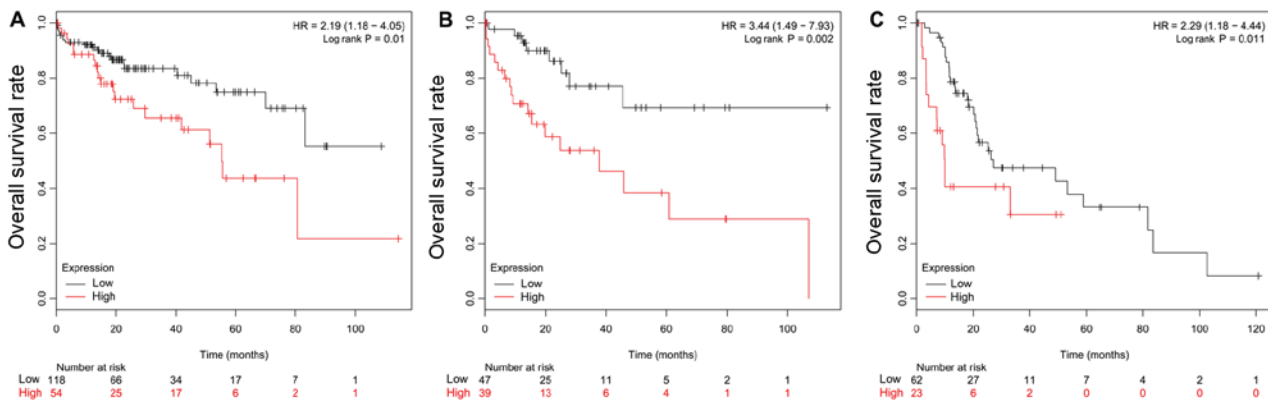


Figure 3. Prognostic value of miRNA-760 in patients with HCC at different stages. Survival curves are plotted for all patients with HCC at different stages. (A) Stage I (n=172). (B) Stage II (n=86). (C) Stage III (n=85). HR, hazard ratio; HCC, hepatocellular carcinoma; miRNA, microRNA.

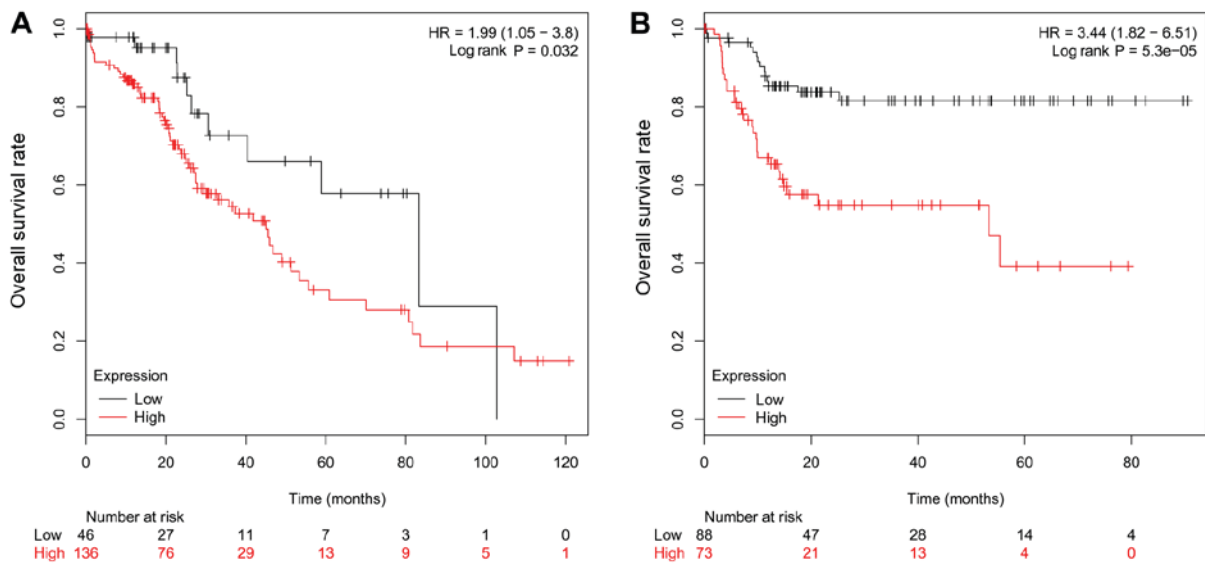


Figure 4. Prognostic value of miRNA-760 expression in patients with HCC of different ethnicity. Survival curves are plotted for patients with HCC of different ethnicity. (A) Caucasian patients (n=182). (B) Asian patients (n=161). HR, hazard ratio; HCC, hepatocellular carcinoma; miRNA, microRNA.

compared with high miR-760 expression, low expression of miR-760 was strongly associated with a higher OS in patients with HCC in stage I, II and III.

In conclusion, the current study demonstrated the distinct prognostic role of miR-760 for HCC. Compared with high expression, low miR-760 expression was associated with better OS for all patients with HCC, particularly for male patients and Asian patients. Therefore, the current results suggest that miR-760 may be a novel biomarker for patients with HCC both in the early and advanced stages. Furthermore, by targeting miR-760, a novel approach for earlier diagnosis of HCC may be achieved, which is essential to improve the survival rate of patients with HCC.

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Availability of data and materials

All data generated or analyzed during the present study are included in this article.

Authors' contributions

DS, JL, CH, QZ, XW, ZZ and SH designed the experiments and collected the data; DS, JL, ZZ and SH contributed to the data analysis and wrote the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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