RESEARCH ARTICLE / *Araştırma Makalesi*

Mining Database for The Clinical Significance, Prognostic Value and Expression of Mir-4746 In Hepatocellular Carcinoma

Hepatosellüler Karsinomda Mir-4746'nın Klinik Önemi, Kestirim Değeri ve İfadesi İçin Veri Tabanı Madenciliği

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Abstract

Introduction	MicroRNAs (miRNAs) are key regulators in the progression and development of hepatocellular carcinoma (HCC). In recent study the miR-4746 was found to be overexpressed in HCC, however, differential expression pattern and clinicopathological significance of miR-4746 in HCC remains unclear. In current study we aimed to evaluate expression profile, clinicopathological role and prognostic value of miR-4746 by using computational approaches.
Materials and Methods	The expression profile of miR-4746 in various human cancers was determined using the dbDEMC database. Also, we used ENCORI/Starbase v2 and UALCAN databases to analyze miR-4746 expression level in HCC. Moreover, we investigated clinicopathological function of miR-4746 by using UALCAN database. Finally, survival analysis was performed to determine prognostic significance of miR-4746 in HCC by Kaplan-Meier plotter and ENCORI/Starbase v2 databases.
Results	The miR-4746 had differential expression patterns in various human cancers and was significantly upregulated in HCC tissues compared with normal samples. Clinicopathological analysis revealed that, miR-4746 was differentially expressed in different clinical parameters including cancer stage, tumor grade, nodal metastasis status, TP53 mutation status, and patient's age. In addition, high expression of miR-4746 was significantly correlated with poor prognosis in HCC.
Conclusion	Our findings indicated that miR-4746 might be as an oncogenic miRNA which were correlated with poor prognosis and worse clinicopathological outcomes. Furthermore, miR-4746 might have an important role in tumorigenesis of HCC and it might serve as potential prognostic biomarker.
Keywords	miR-4746; hepatocellular carcinoma; miRNA; bioinformatics; prognosis

Öz

Amaç	*MikroRNA'lar, hepatosellüler karsinomun (HCC) gelişiminde ve ilerlemesinde anahtar düzenleyicilerdir. Yakın tarihli bir çalışmada, miR-4746'nın HCC'de aşırı ifade edildiği bulunmuş olsa da miR-4746'nın HCC'deki farklı seviyelerde ifade edilmesi ve klinikopatolojik önemi belirsizliğini korumaktadır. Bu çalışmada miR-4746'nın gen ifade özelliğini, klinikopatolojik rolünü ve kestirim değerini hesaplamalı yaklaşımlar kullanarak değerlendirmeyi amaçladık.*
Yöntem ve Gereçler	*Çeşitli insan kanserlerinde miR-4746'nın gen ifade özelliği dbDEMC veri tabanı kullanılarak belirlendi. Ayrıca, HCC'de miR-4746 gen ifade seviyesini analiz etmek için ENCORI/Starbase v2 ve UALCAN veri tabanlarını kullandık. Ayrıca UALCAN veri tabanını kullanarak miR-4746'nın klinikopatolojik işlevini araştırdık. Son olarak, miR-4746'nın HCC'deki prognoz özelliğini belirleyebilmek için Kaplan-Meier plotter ve ENCORI/Starbase v2 veri tabanları aracılığıyla sağ kalım analizi ile gerçekleştirdik.*
Bulgular	*miR-4746, çeşitli insan kanserlerinde farklı gen ifade değerlerine sahipti ve normal örneklere kıyasla HCC dokularında önemli ölçüde arttığı gözlemlenmiştir. Klinikopatolojik analiz, miR-4746'nın kanser evresi, tümör derecesi, lenf bezi metastaz durumu, TP53 mutasyon durumu ve hastanın yaşı dahil olmak üzere farklı klinik parametrelerde farklı gen ifade seviyelerine sahip olduğunu koydu. Ek olarak, miR-4746'nın yüksek ifadesi, HCC'de kötü prognoz ile önemli ölçüde ilişkili olduğu bulunmuştur.*
Sonuç	*Bulgularımız, miR-4746'nın kötü kestirim ve çok kötü klinikopatolojik çıktılarla ilişkilendirilen onkojenik bir miRNA olabileceğini gösterdi. Ayrıca miR-4746, HCC'nin tümorogenezinde önemli bir role sahip olabilir ve potansiyel prognostik biyobelirteç olarak hizmet edebilir.*
Anahtar Kelimeler	*miR-4746; hepatosellüler karsinom; miRNA; biyoinformatik; kestirim*

INTRODUCTION

Hepatocellular carcinoma (HCC) has high mortality rates and its incidence is increasing steadily in the developed countries such as Europe, Australia and North America[1]. HCC is mainly induced by chronic liver inflammation mainly due to infection of hepatitis viruses (Hepatitis B and C). In addition, excessive alcohol intake, aflatoxin, obesity and diabetes are the risk factors for HCC[2].

The microRNAs (miRNAs) are short non-coding RNA molecules which are valuable gene regulators involved many cancer related processes such as cell cycle, cell differentiation, apoptosis and tumorigenesis[3]. miRNAs can regulate the wide range of gene expression by binding 3'UTR regions of their target genes[4]. Therefore, overexpression or downregulation of miRNAs generally have been associated with many human pathologies such as cancer, cardiovascular, neurological, metabolic, and developmental diseases[5-7]. In previous studies, increasing evidence have suggested that many miRNAs differentially expressed in HCC. In their study Liu et. al[8], determined five significantly differentially expressed miRNAs in HCC samples. The hsa-miR-4746-5p is one of these miRNAs which is upregulated in HCC samples[8]. On the other hand, Ren et al., have showed that miR-4746 was downregulated in colorectal cancer (CRC) and inhibits CRC growth[9]. However, the prognostic and clinicopathological roles of miR-4746 in many cancers including HCC have not been reported yet in literature.

In this study, we determined differential expression of miR-4746 in various human cancers the data obtained from miRNA-microarray or miRNA-seq platforms. Next, we identified expression level of miR-4746 in HCC and liver tissues by using computational approaches. Additionally, we analyzed the prognostic and clinicopathological role of miR-4746 via using bioinformatics tools.

MATERIAL and METHODS

Ethics Committee Approval

Ethics statement is not applicable to our study as this study only uses publicly available data.

Differential expression analysis of miR-4746

Differentially Expressed miRNAs in Human Cancers (dbDEMC) (https://www.biosino.org/dbDEMC/index) database is an online tool for detection of differentially expressed miRNAs based on microarray or miRNA-seq platforms[10]. We performed differential expression analysis of miR-4746 in various human cancers by using dbDEMC. Next, we used the Encyclopedia of RNA Interactomes (ENCORI/Starbase v2, https://starbase.sysu.edu.cn) database to analyze miR-4746 expression level in HCC datasets obtained from The Cancer Genome Atlas (TCGA) data.

UALCAN database analysis

The University of Alabama at Birmingham Cancer data analysis portal (UALCAN) is an online web tool which provides to access OMICS data and evaluate multiple gene expression[11]. By using miRNA expression analysis module of UALCAN database, we determined expression level of miR-4746 in 369 HCC samples and 49 normal samples. Besides, we analyzed association between the expression level of miR-4746 and various clinicopathological characteristics of HCC patients including individual cancer stage, tumor grade, nodal metastasis status (N0 and N1), TP53 mutation status, patient's race and patient's age.

Survival analysis of miR-4746

To further evaluate prognostic significance of miR-4746 in HCC patients, we used Kaplan-Meier plotter (KMplot, https://kmplot.com/analysis/) web tool. KMplot is an integrated web tool to analyze correlation between gene expression and survival rates in various tumor types based on TCGA, European Genome-Phenome Archive (EGA) and Gene Expression Omnibus (GEO) databases[12]. In addition, we confirmed KMplot survival analysis by using the ENCORI/Starbase v2 database.

RESULTS

The types of cancer acronyms analyzed in this study are as follows: adrenocortical cancer (ADCA), biliary tract cancer/cholangiocarcinoma (BTCA), bladder cancer (BLCA), BNCA BRCA cervical cancer/cervical squamous cell carcinoma (CECA), chordoma (CHOR), colon cancer (COAD), colorectal cancer (CLCA), endometrial cancer/uterine corpus endometrial carcinoma (ENCA), esophageal cancer/ esophageal carcinoma (ESCA), gallbladder carcinoma (GBCA), gastric cancer/stomach adenocarcinoma (GSCA), gastrointestinal stromal tumor (GAST), head and neck cancer/head and neck squamous cell carcinoma (HNSC), hemangioma (HEGI), hepatocellular carcinoma (LIHC), kidney cancer/kidney chromophobe cancer (KDCA), larynx cancer (LNCA), leukemia (LEUK), liver cancer (LICA), lung cancer/lung squamous cell carcinoma (LUCA), lymphoma (LYMP), melanoma (MELA), mesothelioma (MESO), NSCA (nasopharyngeal cancer), neuroendocrine neoplasia (NDCA), oral squamous cell carcinoma (OSCA), oropharyngeal squamous cell carcinoma (OPSC), ovarian cancer (OVCA), prostate cancer (PCNA), prostate cancer/prostate adenocarcinoma (PRCA), retinoblastoma (RETI), sarcoma (SCRA), skin cancer (SKCA), small intestinal neuroendocrine tumor (SINT), testicular cancer (TECA), thyroid cancer/thyroid carcinoma (THCA), tonsil cancer (TOCA), uterus cancer (UTCA).

According to differential expression analysis of miR-4746 we found that miR-4746 was significantly upregulated in ADCA, BTCA, BLCA, BRCA, CECA, ENCA, ESCA, GBCA, GSCA, HNSC, LIHC, KDCA, LUCA, PRCA and THCA meanwhile it was significantly downregulated in PCNA (Fig 1a).

To identify expression level of miR-4746 in normal liver tissues and HCC tissues, we performed ENCORI/Starbase v2 database analysis. Our results showed that miR-4746 significantly upregulated in HCC datasets (n=370) compared with normal datasets (n=50) (Fig 1b). We also confirmed miR-4746 expression level in HCC and normal tissues by using UALCAN database results. UALCAN database analysis also confirmed that, miR-4746 was significantly upregulated in HCC samples (n=369) compared with normal samples (n=49) ($p<0.01$) (Fig 1c).

The analysis of miR-4746 expression profile based on individual cancer stages showed that miR-4746 was significantly upregulated in stage 1 (n=171) ($p<0.001$), stage 2 (n=85) ($p<0.001$), stage 3 (n=85) ($p<0.001$) HCC samples compared with normal (n=49) liver samples. Also, miR-4746 had high expression level in stage 4 (n=5) of HCC samples compared with normal samples but it was not statistically significant. In addition, miR-4746 was significantly upregulated in stage 2 ($p<0.05$) and stage 3 ($p<0.01$) compared with stage 1 (Fig 2a).

Next, we analyzed miR-4746 expression profile based on HCC tumor grade. Our results demonstrated that miR-4746 was significantly upregulated in all grades of HCC samples compared with normal samples (grade 1 (n=55) ($p<0.001$), grade 2 (n=173) ($p<0.001$), grade 3 (n=124) ($p<0.001$), grade 4 (n=13) ($p<0.001$)). Furthermore, miR-4746 was differentially upregulated in grade 3 compared with grade 1 ($p<0.05$) and grade 2 ($p<0.01$) (Fig 2b).

In addition, we evaluated association between nodal metastasis status and expression level of miR-4746. According to our results, miR-4746 was remarkably upregulated in N0 (n=253) compared with normal samples (n=49) ($p<0.001$), however, there was no significant difference in expression level of miR-4746 in each nodal metastasis status (N1 (n=4) and N0 (n=253)) of HCC (Fig 2c).

TP53 mutation status analysis showed that miR-4746 is remarkably upregulated in TP53 mutant (n=107) and TP53 non-mutant (n=260) samples compared with normal samples (n=49) ($p<0.001$). TP53 mutant and non-mutant were expressed differentially and TP53 mutant samples had higher expression level compared with TP53 non-mutant

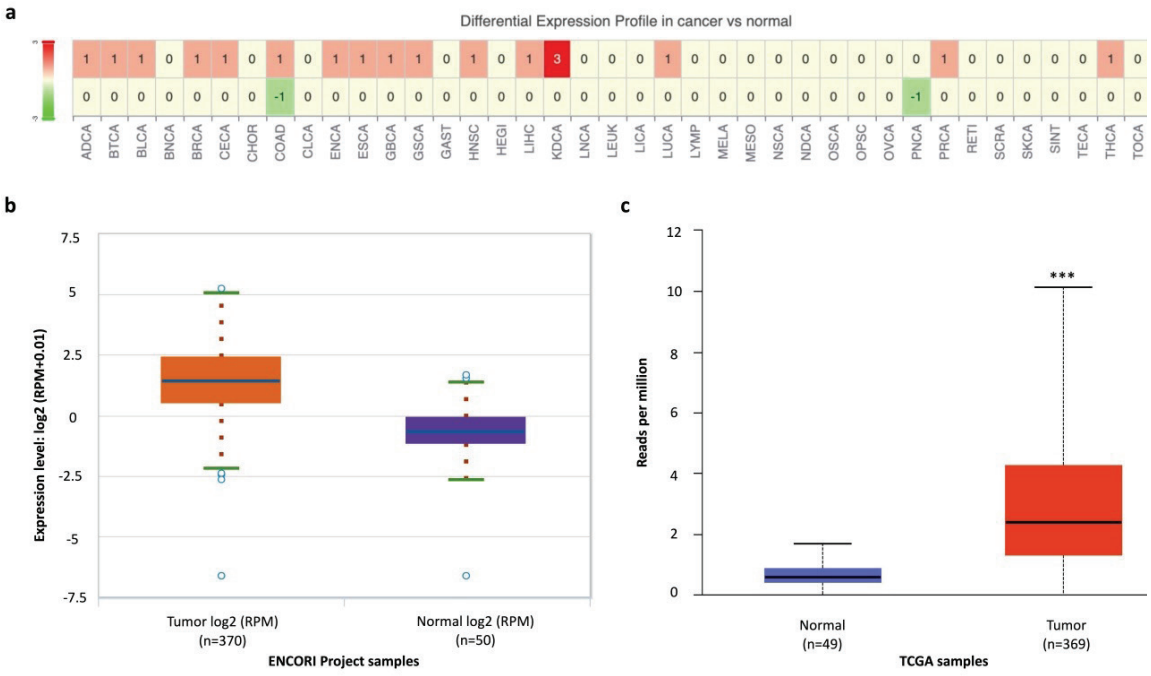

Figure 1. miR-4746 was significantly upregulated in HCC. a. Differential expression profile of miR-4746 in various human cancers and normal tissues. Red boxes indicate high expression, green boxes indicate low expression. b. The analysis of ENCORI/Starbase v2 database showed the upregulation of miR-4746 in HCC tissues. c. The UALCAN database expression analysis showed the upregulation of miR-4746 in HCC samples.

samples and the difference was significant (p<0.001) (Fig 2d).

Patient's race-based expression level analysis was demonstrated that miR-4746 significantly upregulated in Caucasian (n=179) (p<0.001), African-American (n=17) (p<0.01), Asian (n=161) (p<0.001) compared with normal samples (n=49). However, there was no significant difference in expression level of miR-4746 between each race (Fig 2e).

Finally, we evaluated miR-4746 expression profile based on patient's age. According to our results, miR-4746 was significantly upregulated in 21-40 (n=27) (p<0.001), 41-60 (n=145) (p<0.001), 61-80 (n=180) (p<0.001), 81-100 (n=10) (p<0.05) years of age groups of the patients compared with normal healthy samples (n=49). In addition, we found that miR-4746 expression level was significantly lower in 81-100 years of age group of HCC patients compared with 21-40 (p<0.01), 41-60 (p<0.001) and 61-80 (p<0.01) age groups (Fig 2f).

Kaplan-Meier plotter analysis indicated that the high expression level of miR-4746 was significantly correlated with poor overall survival rates in HCC patients (Fig 3a). We also evaluated prognostic significance of miR-4746 in HCC patients by using ENCORI/Starbase v2 database analysis. The results of ENCORI/Starbase v2 analysis were consistent with our Kaplan-Meier plotter analysis results (Fig 3b). These results have suggested that miR-4746 may function as an oncogenic miRNA and serve as a prognostic biomarker.

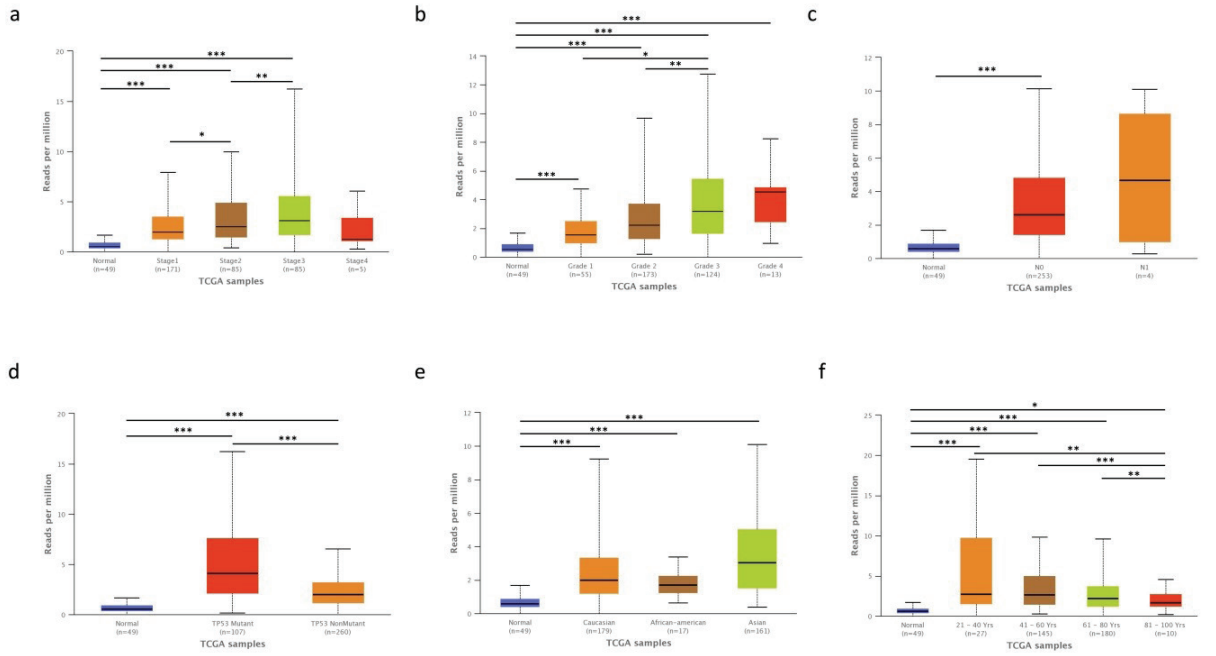

Figure 2. Expression level of miR-4746 in various clinicopathological parameters of HCC patients. Expression of miR-4746 in HCC based on a) individual cancer stages, b) tumor grade, c) nodal metastasis status, d) TP53 mutation status, e) patient's race, and f) patient's age.

DISCUSSION

Dysregulation of ncRNAs has been reported in numerous cancers including HCC[5-7,13,14]. One of the most studied class of ncRNAs, miRNAs, has been linked to orchestration of cell cycle progression, metastasis, apoptosis, cellular differentiation, and tumorigenesis[3]. Also, in many studies it has been suggested that differentially expression of miRNAs serve as a prognostic and diagnostic biomarker in human cancers[15]. In recent study, Liu et al., determined 10 (5 downregulated and 5 upregulated) differentially expressed miRNAs including miR-4746 in HCC by using multi-omics data and various bioinformatic approaches[8]. miR-9[14], miR-21[16] and miR-221[17] has been identified as prognostically significant miRNAs in HCC. However, the role of miR-4746 has not been reported before in HCC-related studies.

In this study, first we used dbDEMC database to evaluate differential expression of miR-4746 in 40 types of human cancers. Our results indicated that miR-4746 overexpressed in 15 types of human cancers including HCC, however, it was downregulated in PNCA (Fig 1a). Thus, we speculate that miR-4746 acts as an oncogenic miRNA and it may have an important regulatory role in expression level of tumor suppressor genes. On the other hand, in their study Ren et al., reported that miR-4746 significantly downregulated in CRC, and miR-4746 regulates expression level of CCND1 by degrading of CCND1 mRNA8. The group of studies have revealed that some miRNAs may have bidirectional functions in cancer cells[18]. Taken together, these two studies[6-8] it can be predicted miR-4746 may also be a potential miRNA with bidirectional function in human cancers.

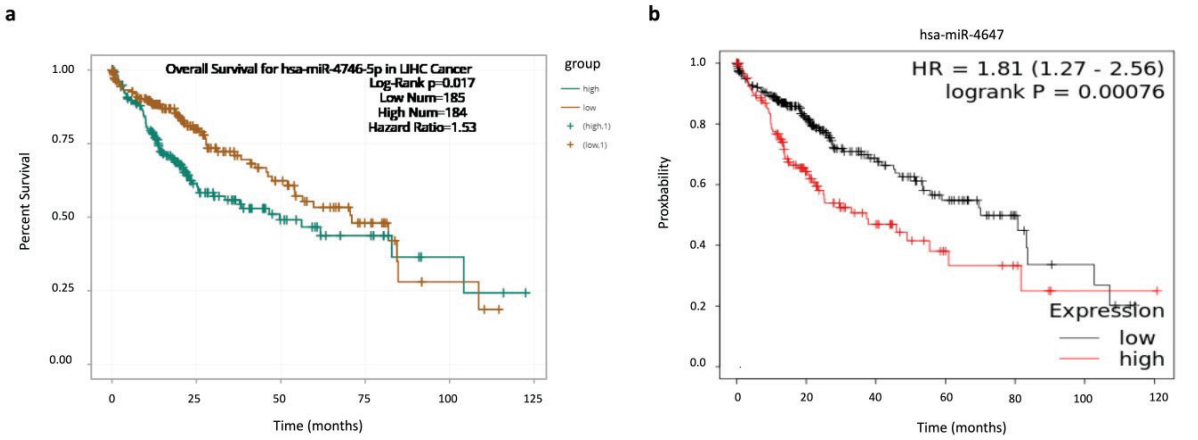

Figure 3. High expression level of miR-4746 was significantly associated with poor overall survival rates of HCC patients. The Kaplan-Meier (a) and ENCORI/Starbase v2 database, (b) survival curves for overall survival analysis between high expression and low expression of miR-4746 in HCC patients. HR, hazard ratio, Num, number.

To determine expression level of miR-4746 we used UALCAN and ENCORI databases. Our UALCAN and ENCORI database analysis demonstrated that miR-4746 upregulated in HCC tissues compared with normal tissues (Fig 1b-c).

Increasing evidence supports that there is a strong association between miRNA expression and various clinicopathological parameters in many of cancer patients. Therefore, great number of miRNAs have been demonstrated as potential candidates for prognostic and diagnostic biomarkers in cancer related studies[19]. According to our UALCAN database analysis was revealed that for the first time miR-4746 differentially expressed in clinicopathological parameters of HCC, including individual cancer stage, tumor grade, nodal metastasis status, TP53 mutation status, and patient's age (Fig 2). Furthermore, miR-4746 was upregulated among patients with HCC, however it has not been differentially expressed in different patient's race (Fig 2e).

To evaluate prognostic significance of miR-4746 expression level in HCC we used ENCORI and KM plotter databases. According to our results upregulation of miR-4746 positively correlated with poor overall survival rates (Fig 3). These findings support oncogenic function of miR-4746 in HCC within poor prognosis. However, more solid experiments are required to identify the exact role of miR-4746 in molecular mechanisms in HCC progression.

In conclusion, our current findings revealed that for the first time miR-4746 might act as an oncogenic miRNA that plays a crucial role in clinicopathological features of HCC. Moreover, the results exhibited that upregulated miR-4746 might serve as a valuable prognostic biomarker for HCC.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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