Original Article

Down-regulation of miR-26a-5p and its regulatory roles in hepatocellular carcinoma: an in silico analysis

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Abstract: To investigate the clinical potential of miR-26a-5p in hepatocellular carcinoma (HCC) and further reveal the latent molecular mechanism. The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases were used to extract miR-26a-5p expression and its clinical potential. Afterwards a comprehensive meta-analysis was conducted with data from TCGA, GEO and literature. With regards of bioinformatics analysis, we obtained potential target genes via the intersection of literatures, 12 online databases, differentially expressed genes (DEGs) in TCGA and natural language processing (NLP) analysis. The predicted target genes were subsequently analyzed by Gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and protein-protein interaction (PPI) analysis. In TCGA data, miR-26a-5p indicated lower expression in HCC tissues than adjacent benign liver tissues (P<0.001). Significant correlation between down-regulation of miR-26a-5p and male (P=0.002) was also discovered. The area under curve (AUC) in receiver operating characteristic (ROC) of miR-26a-5p was 0.828 (95% CI: 0.774-0.883, P<0.001) to diagnose HCC. Down-regulated miR-26a-5p was also represented in pooled meta-analysis (SMD=-0.62, 95% CI: -0.98, -0.26) with GEO and TCGA data together. The most prevailing GO terms of potential targets of miR-26a-5p were: response to steroid hormone stimulus, response to estrogen stimulus, extracellular space, and AT DNA binding. The KEGG pathway analysis mainly indicated focal adhesion and pathways in cancer, and ESR1 was highlighted by PPI analysis. Down-regulated miR-26a-5p may be crucial in tumorigenesis and progression, possessing potential diagnostic value in HCC. MiR-26a-5p exerted modulated functions via multiple target genes and pathways, which requires further validation.

Keywords: miR-26a-5p, hepatocellular carcinoma, TCGA, GEO, target gene

Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor accompanied by high mortality rate mortality rate globally [1-5]. There were roughly 394,770 patients newly diagnosed with liver cancer in 2012 [6], and the annual incidence has maintained the tendency of increasing. One of the main reasons for the lethality of HCC is the lack of specific biomarkers for early diagnosis. Upon confirming diagnosis, HCC patients are generally observed with invasive and advanced tumors, in whom distant metastases are frequently presented [7-11]. Up until now, surgery is regarded as the most effective therapeutic approach. Nevertheless, only about 10% to 20% of patients with HCC are enable to undergo surgical intervention nowadays [12-15]. Besides, it is highly possible that patients who received surgical treatment may relapse afterwards [16-18]. Although recent advancements in functional genomics have increased our knowledge of HCC tremendously [19], our understanding of the molecular mechanisms leading to the disease still remains highly fragmentary.

MicroRNAs (miRNAs) are 22-nucleotide-long non-coding RNAs as potential diagnostic and prognostic biomarkers for cancer detection and staging [20-25], who also exert significant functions in gene transcript stability, translation efficiency [26] and cancer metabolism [27]. Both the functions of tumor suppressor and onco-
gene can be exerted by miRNAs, including proliferation, apoptosis, metastasis and relapse [28-33]. As a new type of genomic information, the aberrant expression of miRNA can offer insights for discovering novel pathways of carcinogenesis and gaining opportunity for the discovery of biomarkers and therapeutic targets, including HCC [34-38].

MiR-26a-5p has been reported to be differentially expressed in cancers, such as non-small cell lung cancer, prostate cancer and oral cancer [39-41]. Particularly, we have found that miR-26a-5p had low expression level in HCC, but the number of patients involved was limited [42]. Studies have partially investigated the clinical value of miR-26a-5p including early detection and promising therapeutic target of HCC. However, the existing research concerning clinical application of miR-26a-5p basically collected data without excavating the public databases. The mechanisms of miR-26a-5p in HCC have also been discussed in a few studies. For instance, Wang G et al [43] indicated that miR-26a-5p inhibited the growth of tumor cells by down-regulating EZH2 expression. Yang X et al [44] identified that miR-26a-5p coordinately inhibited angiogenesis of HCC by HGF-cMet signaling. Furthermore, Zhuang C et al [45] estimated that miR-26a-5p could cooperate with AFP and perform as a promising biomarker of HCC. Nevertheless, systematic target prediction and bioinformatics analysis about miR-26a-5p are yet to be carried out.

Considering the drawbacks in previous studies, we were interested in the expression profile in The Cancer Genome Atlas (TCGA), which provides insights into cancer at genetic level by genome sequencing and bioinformatics [46-48]. Furthermore, we also correlated miR-26a-5p in Gene Expression Omnibus (GEO) microarray. GEO is an international database consisting of microarray, next-generation sequence and other genomic datasets [49-52]. To further reveal the molecular mechanism and regulatory network of miR-26a-5p in HCC, we performed bioinformatics analysis and natural language processing (NLP) [53-55] to gather the possible target genes and potential pathways of miR-26a-5p. Protein-protein interaction (PPI) network was then conducted to reveal the correlation among miR-26a-5p-related protein molecules.

**Materials and methods**

**Sequencing data of TCGA and microarray data analysis of GEO database**

The analysis consisting of 353 patients with HCC and 50 adjacent liver tissues attained data from TCGA liver hepatocellular carcinoma (LIHC). The level 3 microRNA expression profile of the related patients were downloaded from TCGA miRNA-seq profiles. Both the miRNA profiles data and clinical data of LIHC are available and for free [56, 57]. The expression level of miR-26a-5p was represented after being calculated by log2.

Additionally, another data source of the miR-26a-5p expression was GEO of National Center for Biotechnology Information (NCBI). The gene expression data series of normal liver tissues and HCC tissues were attained for further analysis. Peritumoral tissues were not chosen in order to keep the accuracy of our study. Microarrays of other species and experimental cell lines were also excluded for being unrelated to our study. After confirmation of miRNA expression in the microarray, miR-26a-5p expression was further selected. Once validated, significance analysis of microarrays (SAM) was then conducted to calculate the clinical role of miR-26a-5p in HCC.

**Identification and eligibility of relevant studies**

Apart from the excavation of public databases, we attempted to conduct a meta-analysis based upon existing literature according to a predetermined written protocol. Aiming to further testify the relationship between the expression level of miR-26a-5p and HCC, We searched for studies in databases of PubMed, Wiley Online Library, Web of Science, Science Direct, Cochrane Central Register of Controlled Trials, Google Scholar, EMBASE, Ovid, LILACS, Chinese CNKI, Chong Qing VIP, Wan Fang, China Biology Medicine disc. Since the previous name of miR-26a-5p was miR-26a and there are several synonyms of miR-26a-5p available, the following searching key words were applied: “miR-26a OR miRNA-26a OR microRNA-26a OR mir 26a OR miRNA26a OR microRNA26a OR “mir 26a” OR “miRNA 26a” OR “microRNA 26a” OR mir-26a-5p OR miRNA-26a-5p OR microRNA-26a-5p”, “malignan* OR cancer OR tumor OR tumour OR neoplas* OR carcinoma” and “hepa-
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Figure 1. Flowchart shows the schematic diagram of the miR-26a-5p.

tocellular OR liver OR hepatic OR HCC”. Qualified studies were included when satisfying the following criteria: 1) the studies measured miR-26a-5p expression in the HCC; 2) the results of these expression profiles were represented in the form of “mean ± standard deviation”.

Collection of prospective target genes of miR-26a-5p via bioinformatics approaches and NLP

To obtain a better picture of the potential mechanism of miR-26a-5p in the progression of HCC, the validated target genes of miR-26a-5p were collected by screening literatures from aforementioned 13 databases. The same searching key words were applied as mentioned in the meta-analysis: “(miR-26a OR miRNA-26a OR microRNA-26a OR miR26a OR miRNA26a OR microRNA26a OR “miR 26a” OR “miRNA 26a” OR “microRNA 26a” OR miR-26a-5p OR microRNA-26a-5p) and (target*).” Concurrently, to further acquire a more complete image of potential target genes of miR-26a-5p, we predicted the possible targets via 12 online methods, including miRWalk2.0, MirTarBase, TarBase, Targetminer, polymiRTS, RNA22, microRNA, org, Pita, mirNAMAP, Targetscan, miRDB, and Pictar-vert. Genes appearing more than eight times were selected as the “predicted targets” of miR-26a-5p.

Bioinformatics analyses with gene ontology (GO) and pathway enrichment

The identified target genes were analyzed by Gene ontology (GO), which was completed by DAVID tool (https://david.ncifcrf.gov/). Further, the crucial signaling pathways of the target genes were explored via the database of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. P-value <0.05 was of significance in GO analysis and KEGG pathway analysis.

The hub genes were uploaded to Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 9.1 online tool (http://string-db.org/) in order to construct PPI network. The STRING software, collaboratively launched by European Molecular Biology Laboratory (EMBL), Swiss Institute of Bioinformatics (SIB) and University of Zurich (UZH), is a database containing all known and predicted protein interactions. The interactions include direct (physical) and indirect (functional) associations as derived from four sources, comprised of literature curation, genome analysis and prediction, high-throughput experimentations as well as co-expression researches. The PPI network was
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visualized by Cytoscape software (version 3.3.3).

**Statistical analysis**

All statistical analyses were carried out using SPSS version 22.0 (Armonk, NY, USA) except for meta-analysis. The expression levels were presented as mean ± standard deviation. Receiver operator characteristic curve (ROC) was applied to evaluate the diagnostic value. P-value <0.05 was considered of statistical significance. Cox regression was applied to test the relationship between miR-26a-5p expression and overall survival (OS). Besides, the equality for survival distributions in different groups of miR-26a (high expression vs. low expression) was tested by the Kaplan-Meier, and log rank P-value <0.05 was considered to be significant. Student’s t test was applied to identify the difference of miR-26a between the HCC and adjacent non-cancerous hepatic tissues. The correlation between miR-26a and clinicopathological parameters were validated by Spearman’s rank correlation coefficient. All above applied SPSS version 22.0 (Armonk, NY, USA) to analyze.

In the aspect of meta-analysis, STATA 12.0 (College Station, Texas, USA) was used. Standardized mean difference (SMD) and 95% confidence interval (CI) were computed and displayed in forest plot. The heterogeneity was appraised by chi square test and $I^2$. Heterogeneity was verified with P value <0.05 or $I^2$>50%, decided by which fixed effect model or random effect model was selected to estimate pooled effect size. Publication bias was assessed by egger’s test.

The process design of our study was represented in a flowchart (Figure 1).

**Results**

**Clinical value of miR-26a in HCC of TCGA data**

According to analysis of 353 patients with HCC and 50 adjacent normal liver tissues, we identified the expression of miR-26a-5p in HCC was 13.177 ± 0.928, significantly lower than that in...
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adjacent normal liver tissues (14.357±0.767, P<0.001, Figure 2). Apparent higher expression level was also discovered in female (13.395±0.954) than male (13.074±0.899, P=0.002, Figure 2). However, there was no significant correlation observed between miR-26a expression and clinicopathological parameters such as age, differentiation, clinical TNM stage, vascular invasion and alcohol. In addition, the area under curve (AUC) of miR-26a-5p was 0.828 (95% CI: 0.774-0.883, P<0.001) in ROC curve, indicating noteworthy diagnostic value between HCC tissues and adjacent normal liver tissues in these 9 data sets were carried out by t test (Figure 5). There were 4 data sets showing apparent lower expression level of miR-26a-5p in HCC tissues (GSE6857, GSE21362, GSE22058, GSE41874, all P<0.05), while the others revealed no statistically significant correlation. Thus, in order to estimate the overall efficiency, we conducted a meta-analysis through SMD method. The result showed that the combination of SMD from 9 datasets was -0.51 (95% CI: -0.86, -0.16) with random effects

Figure 3. Receiver operating characteristic (ROC) curve and Kaplan-Meier survival curve analysis of miR-26a-5p. Area under curve (AUC) was 0.828 (81.87% Sensitivity, 72% specificity). Cutoff value was 14.02. Positive predictive value was 88.92% and negative predictive value was 36%, respectively. Accuracy was 80.65%. Expression level of miR-26a-5p showed no significance in HCC prognosis (Log rank, P=0.718).

Exclusion based on following criteria: No Homo sapiens; No miRNA profiles; No available data for meta-analysis

Figure 4. Flow diagram shows details of study selection process.

Expression of miR-26a-5p in HCC of GEO data

After screening microarrays and excluding inapplicable data, we selected a total of 9 data sets, comprising 540 disease samples and 536 control samples, for further analysis (Figure 4; Table 1). Expression differences between HCC tissues and adjacent normal liver tissues in these 9 data sets were carried out by t test (Figure 5). There were 4 data sets showing apparent lower expression level of miR-26a-5p in HCC tissues (GSE6857, GSE21362, GSE22058, GSE41874, all P<0.05), while the others revealed no statistically significant correlation. Thus, in order to estimate the overall efficiency, we conducted a meta-analysis through SMD method. The result showed that the combination of SMD from 9 datasets was -0.51 (95% CI: -0.86, -0.16) with random effects
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The expression of miR-26a-5p in HCC tissue compared with normal tissue with Gene Expression Omnibus (GEO) data. Error bars represented SD, and the P value was analyzed by independent T test.

Table 1. The summary of samples series of GEO database and TCGA

<table>
<thead>
<tr>
<th>ID</th>
<th>Patient’s nationality</th>
<th>Platforms</th>
<th>HCC (n=893)</th>
<th>Control (n=586)</th>
<th>Total (n=1479)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSE6857</td>
<td>China (2007)</td>
<td>GPL4700</td>
<td>241</td>
<td>241</td>
<td>482</td>
<td>12.687 ± 0.998 13.128 ± 0.846</td>
</tr>
<tr>
<td>GSE22058</td>
<td>China (2010)</td>
<td>GPL10457</td>
<td>96</td>
<td>96</td>
<td>192</td>
<td>2.054 ± 0.146 2.1985 ± 0.086</td>
</tr>
<tr>
<td>GSE10694</td>
<td>China (2008)</td>
<td>GPL6542</td>
<td>78</td>
<td>88</td>
<td>166</td>
<td>14.693 ± 1.222 14.8131 ± 1.246</td>
</tr>
<tr>
<td>GSE21362</td>
<td>Japan (2011)</td>
<td>GPL10312</td>
<td>73</td>
<td>73</td>
<td>146</td>
<td>12.356 ± 0.528 12.680 ± 0.334</td>
</tr>
<tr>
<td>GSE40744</td>
<td>USA (2013)</td>
<td>GPL14613</td>
<td>26</td>
<td>18</td>
<td>44</td>
<td>13.738 ± 0.236 13.766 ± 0.230</td>
</tr>
<tr>
<td>GSE41874</td>
<td>Japan (2013)</td>
<td>GPL7722</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>6.989 ± 0.158 1.674 ± 0.093</td>
</tr>
<tr>
<td>GSE54751</td>
<td>USA (2014)</td>
<td>GPL18262</td>
<td>13</td>
<td>7</td>
<td>20</td>
<td>0.207 ± 0.122 0.311 ± 0.213</td>
</tr>
<tr>
<td>GSE69580</td>
<td>China (2015)</td>
<td>GPL10850</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>5.718 ± 1.014 5.655 ± 0.575</td>
</tr>
<tr>
<td>GSE57555</td>
<td>Japan (2015)</td>
<td>GPL18044</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>0.087 ± 0.077 0.085 ± 0.048</td>
</tr>
<tr>
<td>TCGA</td>
<td>USA</td>
<td></td>
<td>353</td>
<td>50</td>
<td>403</td>
<td>13.75 ± 0.928 14.362 ± 0.774</td>
</tr>
</tbody>
</table>

Figure 5. The expression of miR-26a-5p in HCC tissue compared with normal tissue with Gene Expression Omnibus (GEO) data. Error bars represented SD, and the P value was analyzed by independent T test.

The pooled meta-analysis with all datasets involved

Since we retrieved no literature providing the expression of miR-26a-5p in HCC, we conducted meta-analysis with data from TCGA and GEO only (Table 1). The pooled SMD of GEO and TCGA was -0.62 (95% CI: -0.98, -0.26) (Figure 7). The heterogeneity was evident ($I^2=84.5\%$, $P<0.001$), due to which a random effects model was then applied. The sensitivity analysis showed that heterogeneity has no significant change with excluding each study (Figure 8).
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Figure 6. Forest plots of combined analysis of miR-26a-5p expression of GEO data with standardized mean difference (SMD). Squares and horizontal lines related to SMD of each data and 95% CIs, respectively. The area of the squares related to the weight, and the diamond stands for the combined SMD and 95% CIs.

Figure 7. Forest plots of combined analysis of miR-26a-5p expression of TCGA and GEO data with SMD. Squares and horizontal lines related to SMD of each data and 95% CIs, respectively. The area of the squares related to the weight, and the diamond stands for the combined SMD and 95% CIs.
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And no significant publication bias was suggested in the funnel plot (P = 0.804) (Figure 9).

### Predicting target genes, NLP and DEGs of TCGA

To comprehend the function of miR-26a-5p and thus better understand the mechanism of miR-26a-5p in HCC, 12 online prediction software were used to obtain predicted target genes of miR-26a-5p. Genes appearing over 8 times in the prediction of 12 software were chosen for further study. Finally, 679 genes were identified as predicted targets. Subsequently, a total of 4,159 DEGs of TCGA was identified, including 3,156 up-regulated ones and 1,003 down-regulated ones. Afterwards, the abstracts or titles of 64,577 studies were included through data and text mining tools in the process of NLP, and 1,800 HCC-related genes were subsequently identified as we previously reported [58, 59]. Furthermore, we attained the potential hub target genes of miR-26a-5p in HCC, combining the predicted target genes, NLP-related genes and DEGs of TCGA (Figure 10). Altogether, 31 overlapping genes (GALNT10, PFKFB2, SULF1, PDGFRα, TERT, COL1A2, EZH2, INSC, ABCC4, LEPF1, SLC38A2, PLAG1, KIF18A, PCDH9, CDC6, EPHA7, PTGS2, ESR1, SLC7A11, LOX, ADM, HGF, SATB1, EPHA2, SOCS6, CDC22, HMGA2, ATAD2, CA2, CCND2, HMGA1) which play important roles in HCC proceeding were selected for gene-annotation enrichment analysis and KEGG pathway annotation, basically representing the molecular mechanism of miR-26a-5p in HCC.

To further explore the biological function of target genes, the 31 genes were analyzed by the GO enrichment analysis and KEGG pathway analysis (Figure 11). The results of enrichment analysis showed that the significantly enriched GO terms for biological process were response to steroid hormone stimulus (P-value = 2.74E-06) and response to estrogen stimulus (P-value = 7.01E-05). Moreover, the extracellular space (P = 0.032) was the significantly enriched GO term for cellular component. Notably, the significantly enriched GO term for molecular functions was AT DNA binding (P = 0.006). Furthermore,
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The KEGG pathway enrichment analysis indicated that focal adhesion (P-value =0.010) (CCND2, COL1A2, PDGFR, HGF) and pathways in cancer (P-value =0.037) (PTGS2, PDGFR, LEF1, HGF) were significantly enriched. Afterwards, PPI and Cytoscape illustrated the interaction among hub genes, and implied that ESR1 might be crucial to the entire network (Figure 12).

Discussion

In our study, we observed obvious lower expression of miR-26a-5p in HCC tissues than in non-cancerous tissues based on data from TCGA and GEO database. Notable diagnostic value was discovered for miR-26a-5p to detect HCC. Meanwhile, lower miR-26a-5p expression was observed in male HCC patients. In GO analysis, the predicted 31 hub genes showed significant enrichment in response to steroid hormone stimulus, response to estrogen stimulus, the extracellular space and AT DNA binding. As for KEGG pathway analysis, focal adhesion and pathways in cancer were highlighted. ESR1 signified pivotal function in the PPI analysis.

MiR-26a-5p has been extensively concentrated by researchers worldwide, especially its regulatory roles in cancer. Generally regarded as a tumor suppressor in several solid cancers, miR-26a-5p indicated low expression in prostate cancer [40], breast cancer [60], gastric cancer [61], bladder cancer [62], esophageal squamous cell cancer [63], pancreatic cancer [64], nasopharyngeal carcinoma [65], etc. However, in lung cancer [66], glioma [67], cholangiocarcinoma [68] and chronic lymphocytic leukemia [69], miR-26a-5p was up-regulated, exerting its function as an oncogene. Thus we deduced that miR-26a-5p might have specificity in different malignancies, and its function in HCC was still waited to be further discovered.

The potential for clinical application of miR-26a-5p in HCC has been on heated debate. With regard to diagnostic value of miR-26a-5p, Zhuang et al [45] presented that the expression level of miR-26a-5p might have the potential to distinguish HCC patients from chronic hepatitis patients and healthy controls based on relatively high AUC of 0.754. Zhou et al [70] and Tan et al [71] set two miRNA panels both including miR-26a-5p to assess their function in HBV-related HCC detection, receiving impressive diagnostic efficiencies. In our study, data from TCGA indicated promising significance for the detection of HCC (AUC=0.828). Furthermore, the SMD of miR-26a-5p from GEO datasets was -0.51 (95% CI: -0.86, -0.16), which could also validate its diagnostic effects. Thus, the analyses above jointly suggested the diagnostic potential of miR-26a-5p in HCC. Since there was no study offering specific miR-26a-5p expression according to our search, more pathological samples and normal liver tissues instead of adjacent benign tissues should be collected for further confirmation.

As for prognosis, our survival analysis implied no statistical significance. No association was found between miR-26a and clinicopathological features indicating HCC progression as well. For the purpose of analyzing the prognostic value of miR-26a-5p, we attempted to carry out meta-analysis with existing literature included. However, the information that we collected was not sufficient for a meta-analysis. Ji et al [72] reported that miR-26a-5p showed significant prognostic value in HCC when performing both univariate and multivariate analyses. Nevertheless, this study only recruited 455 patients in total from China. Hence, we expected to study the prognostic value of miR-26a-5p based upon a larger sample in the future.

In terms of therapeutic effects of miR-26a-5p, Kota et al [73] demonstrated the curative role of miR-26a-5p in HCC by delivering miR-26a-5p
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They discovered that therapeutic delivery of miR-26a-5p could not only inhibit tumorigenesis and cancer cell proliferation, but also induce tumor apoptosis. Another research in vivo was conducted by Yang et al. [42], animals that received HCC cells with constant expression of miR-26a-5p manifested slighter signs of tumor growth and metastasis. MiR-26a-5p may act as a biomarker worth investigation for therapeutic use in HCC.

Therefore, we inferred that the underlying mechanism of its clinical value in HCC might associate with various target genes. The validated targets of miR-26a-5p in HCC, which we acquired from literature, were EZH2 [74], HGF [44], IL-6 [42], ITGA5 [75], PIK3C2α [76] and FUT8 [77]. Targeting these genes, miR-26a-5p functioned in HCC as a tumor suppressor by inhibiting tumor growth, proliferation, migration, angiogenesis, inducing anoikis, etc. Nevertheless, miRNAs are involved in post-transcriptional regulation of gene expression in almost every biological process [78, 79], which suggests
that there are more target genes of miR-26a-5p worthy of prediction. So an integrated prediction obtained the intersection of online databases, literature search, NLP analysis and DEGs in TCGA was carried out. The data sources of NLP and DEGs in TCGA were intended to select genes that particularly expressed in HCC.

The majority of existing studies focused on the mechanism of miR-26a-5p in HCC, which mainly discussed several molecular pathways dispersedly. Moreover, Thurnherr et al [80] once performed target prediction of a group of miRNA (miR-26a-5p included) and then carried out KEGG pathway analysis. However, only the predicted targets in two online databases were gathered and KEGG pathway analysis was not sufficient to cover the comprehensive biological mechanism. Systematic in silico study is still needed to independently elucidate the mechanism of miR-26a-5p in HCC.

In the current GO analysis with 31 prospective target genes of miR-26a-5p, we discovered the most enriched biological process was response to estrogen stimulus. Studies [81] have identified that estrogen functioned as a protective factor in HCC. Xu et al [82] specifically investigated the mechanism by studying in vivo and in vitro, demonstrating that estrogen could suppress tumor invasion, progression, and induce apoptosis. Both indicated that miR-26a-5p might correlate with estrogen in regulation of HCC. PPI network analysis afterwards further verified the modulation of miR-26a-5p via steroid hormone receptors, in which estrogen receptors 1 (ESR1) was selected by PPI as pivot of the whole network. ESR1, a gene that could code estrogen receptor alpha (ERα), was concentrated by researchers as a potential tumor suppressor in HCC as well. Hishida et al [83] observed significant down-regulation of ESR1 in HCC tissues and its association with malignant indications of HCC by triple-combination array analysis. Reduced ESR1 expression was detected as well by Dai et al [84]. Meanwhile, we also observed significant lower expression level of miR-26a-5p in male during TCGA data analysis. As a result, it is quite likely that miR-26a-5p acted as a tumor suppressor in HCC by targeting ESR1. Besides, KEGG pathway analysis indicated abundance in focal adhesion and cell cycle, suggesting that miR-26a-5p might play important role in the progression of HCC.

There were still some inevitable limitations in our study. Firstly, considering that TCGA and GEO provide sequencing and microarray data respectively, practical studies comprising of RT-qPCR and fluorescence in situ hybridization (FISH) should be conducted to validate their data. As for the high heterogeneity existing in current analysis, we discovered no improvement after removing one microarray dataset. It was inferred that different mechanisms for HCC progression in various human species and diverse methods to cope with raw data might contribute to the heterogeneity. Size of the sample should also be broadened to uplift the efficiency of the analysis. Moreover, we studied the aberrant expression of miR-26a-5p in tissue samples in current research. However, detection of miR-26a-5p in serum instead of in hepatic tissues possesses greater value for clinical application. As a result, we intend to continue verifying the clinical value of miR-26a-5p by investigating blood samples. With regard to predicted targets, studies in vivo and in vitro were demanded to confirm the targets acquired in bioinformatics analysis.

To conclude, our study suggests that the value of miR-26a-5p for tumorigenesis and diagnosis in HCC deserve further excavating, due to its role of tumor suppressor and multiple regulatory mechanisms. More relative researches are expected to be carried out to complete and confirm our results.

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Disclosure of conflict of interest

None.

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