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 $\log_2$.

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-
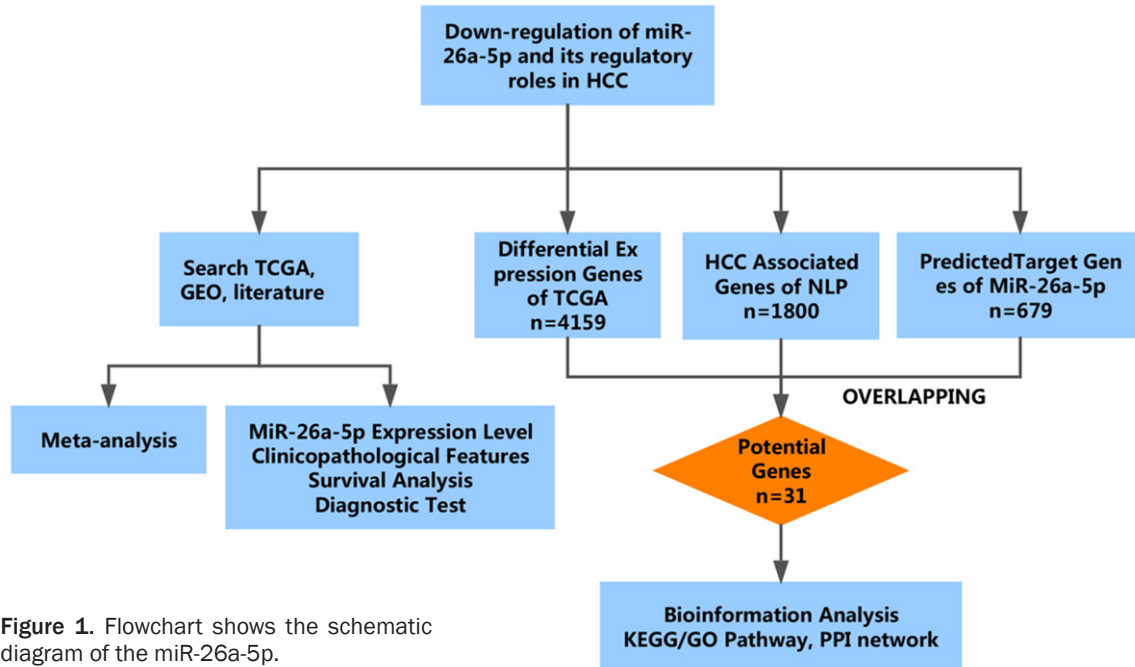
Figure 1. Flowchart shows the schematic diagram of the miR-26a-5p.

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 miRNA-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, mirRNAMAP, 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
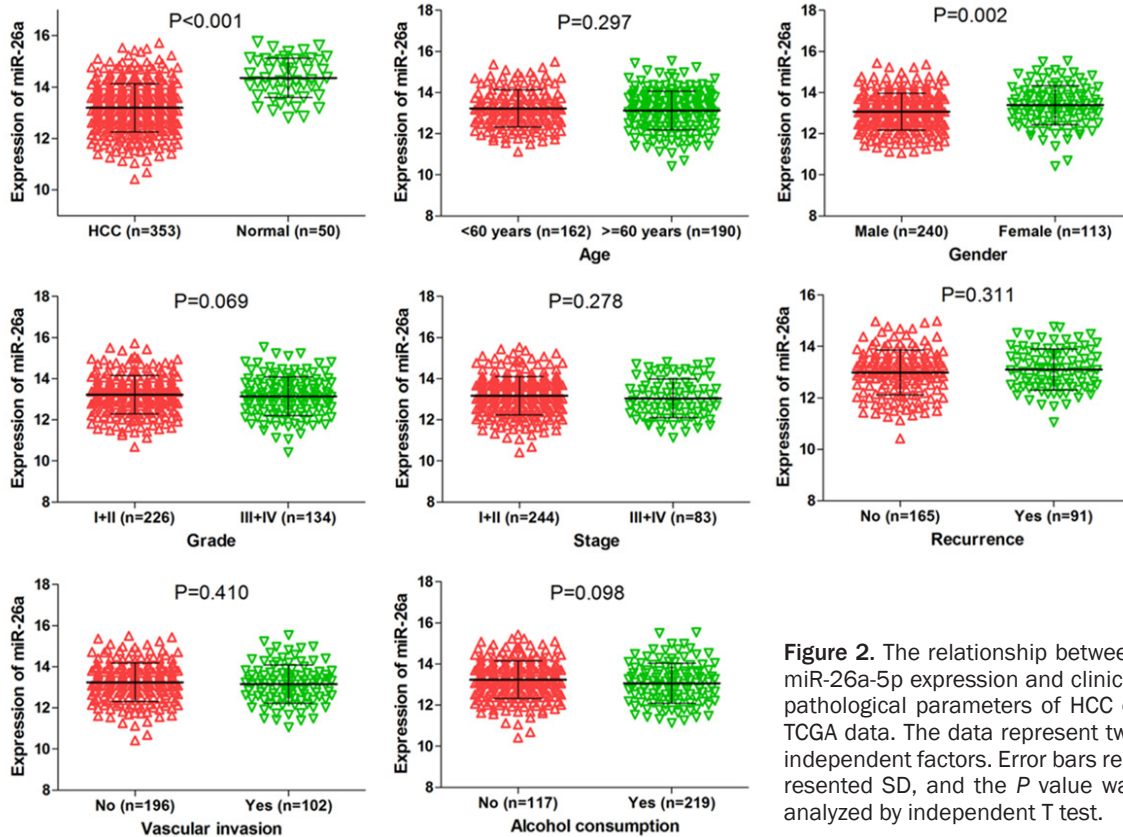
Figure 2. The relationship between miR-26a-5p expression and clinico-pathological parameters of HCC of TCGA data. The data represent two independent factors. Error bars represented SD, and the *P* value was analyzed by independent T test.

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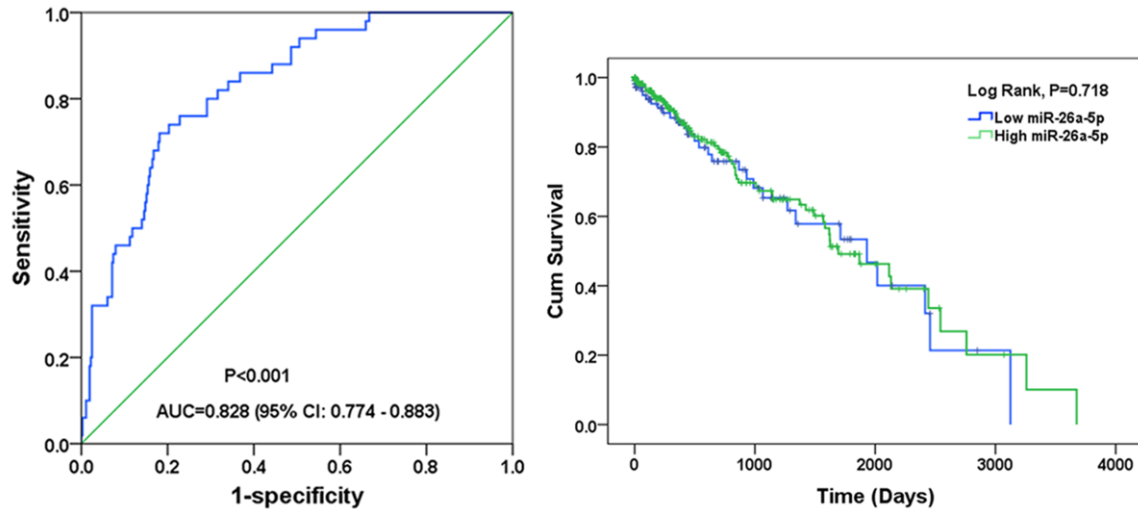
Figure 3. Receiver operating characteristic (ROC) curve and Kaplan-Meier survival curve analysis of miR-26a-5p. Area under curve (AUC) was 0.828 (81.87% Sensitivity, 72% specificity). Cutoff value was 14.02. Positive predictive value was 88.92% and negative predictive value was 36%, respectively. Accuracy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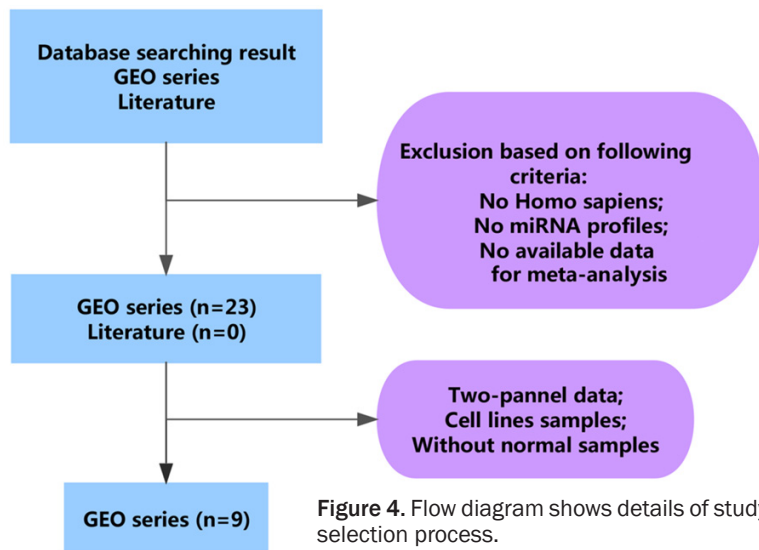

Figure 4. Flow diagram shows details of study selection process.

(**Figure 3**). The median expression of miR-26a-5p was set as cut-off value. However, the expression level of miR-26a-5p represented no significant association with survival (log rank, P=0.718, **Figure 3**).

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

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

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

| ID	Patient's nationality	Platforms	HCC (n=893)	Control (n=586)	Total (n=1479)	Mean ± SD	
						HCC	Normal
GSE6857	China (2007)	GPL4700	241	241	482	12.687±0.998	13.128±0.846
GSE22058	China (2010)	GPL10457	96	96	192	2.054±0.146	2.1985±0.086
GSE10694	China (2008)	GPL6542	78	88	166	14.693±1.222	14.813±1.246
GSE21362	Japan (2011)	GPL10312	73	73	146	12.356±0.528	12.680±0.334
GSE40744	USA (2013)	GPL14613	26	18	44	13.738±0.236	13.766±0.230
GSE41874	Japan (2013)	GPL7722	3	3	6	0.698±0.158	1.674±0.093
GSE54751	USA (2014)	GPL18262	13	7	20	0.207±0.122	0.311±0.213
GSE69580	China (2015)	GPL10850	5	5	10	5.718±1.014	5.655±0.575
GSE57555	Japan (2015)	GPL18044	5	5	10	0.087±0.077	0.085±0.048
TCGA	USA		353	50	403	13.175±0.928	14.362±0.774
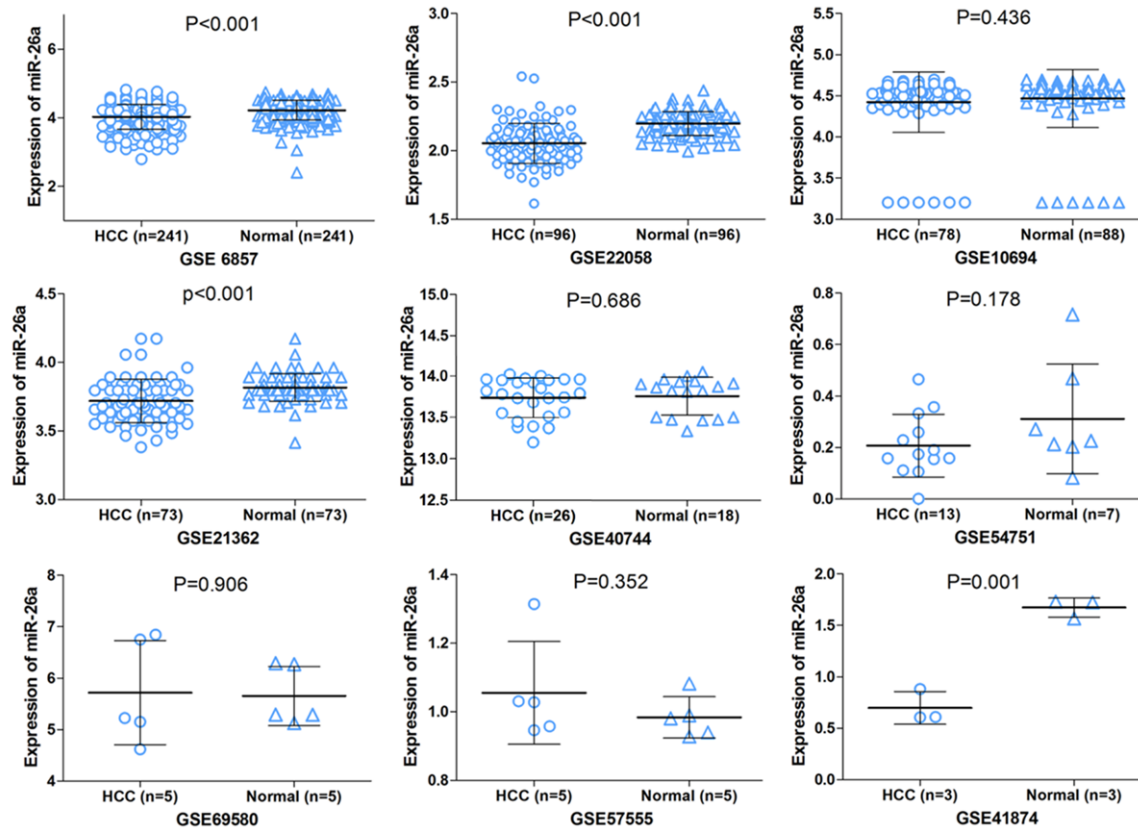
Figure 5. The expression of miR-26a-5p in HCC tissue compared with normal tissue with Gene Expression Omnibus (GEO) data. Error bars represented SD, and the *P* value was analyzed by independent T test.

analysis because of high heterogeneity (I^2= 73.6%, P<0.001) (**Figure 6**).

The pooled meta-analysis with all datasets involved

Since we retrieved no literature providing the expression of miR-26a-5p in HCC, we conduct-ed 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**).
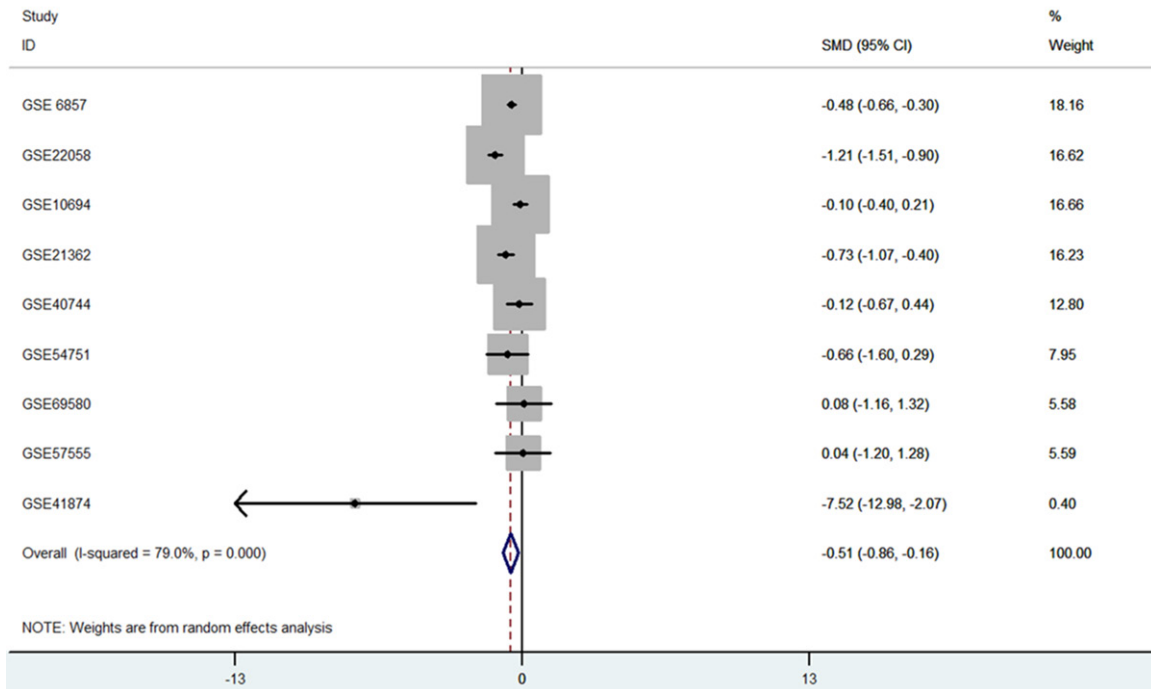
Figure 6. Forest plots of combined analysis of miR-26a-5p expression of GEO data with standardized mean difference (SMD). Squares and horizontal lines related to SMD of each data and 95% CIs, respectively. The area of the squares related to the weight, and the diamond stands for the combined SMD and 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.
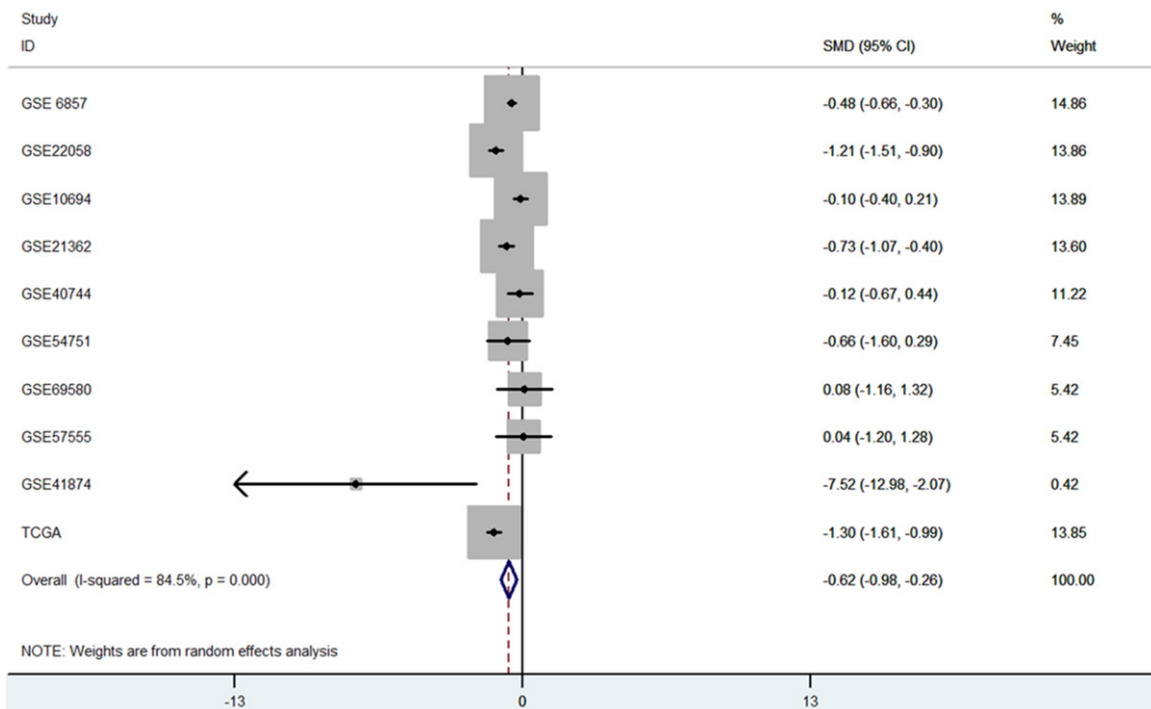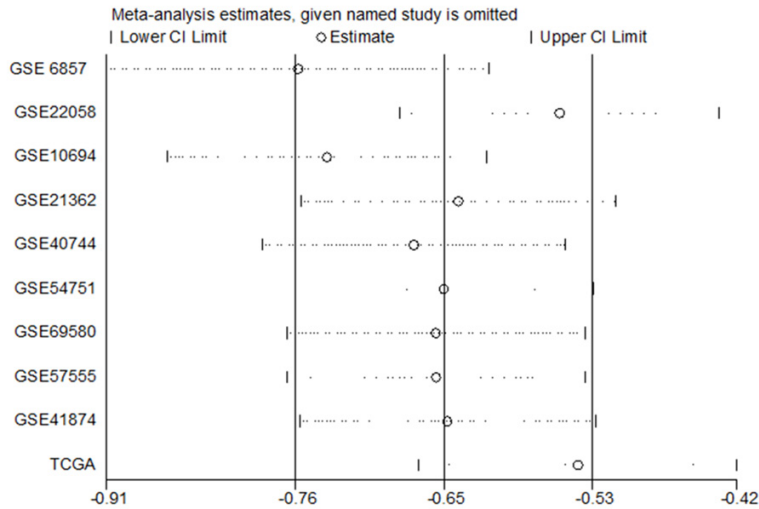
Figure 8. Sensitivity analysis of the effect of individual studies with miR-26a-5p expression results.
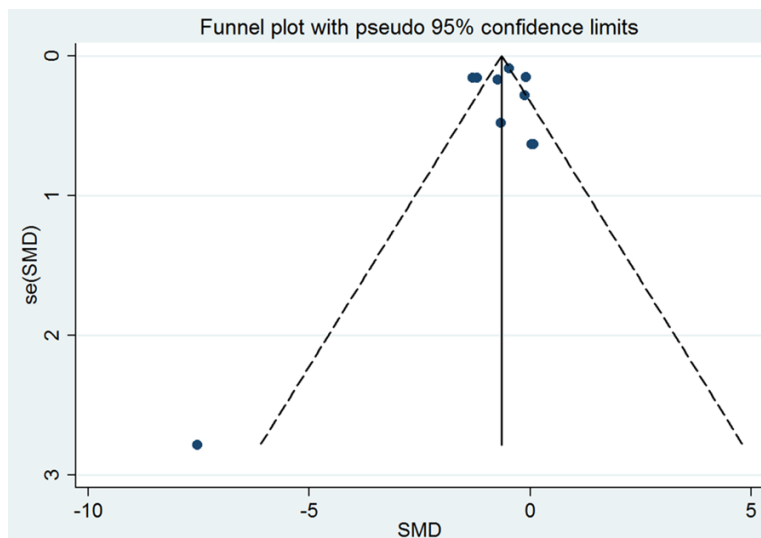
Figure 9. Egger's and Begg's funnel plot of miR-26a-5p expression with SMD.

as predicted targets. Subsequently, a total of 4,159 DEGs of TCGA was identified, including 3156 up-regulated ones and 1003 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, PDGFRA, TET1, COL1A2, EZH2, INSC, ABCC4, LEF1, SLC38A2, PLAG1, KIF18A, PCDH9, CDC6, EPHA7, PTGS2, ESR1, SLC7A11, LOX, ADM, HGF, SATB1, EPHA2, SOCS6, DCDC2, 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.

Enrichment analysis and bioinformation of target genes

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

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=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,
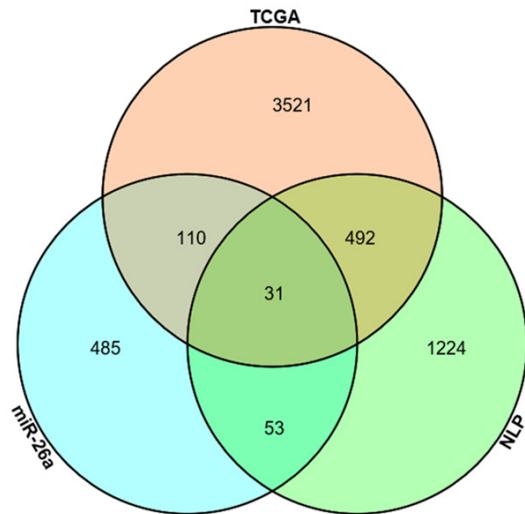
Figure 10. Venn diagram showed the intersection genes of TCGA, NLP, and predicting target genes.

the KEGG pathway enrichment analysis indicated that *focal adhesion* (*P*-value =0.010) (CCND2, COL1A2, PDGFRA, HGF) and *pathways in cancer* (*P*-value =0.037) (PTGS2, PDGFRA, 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 squa-

mous 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
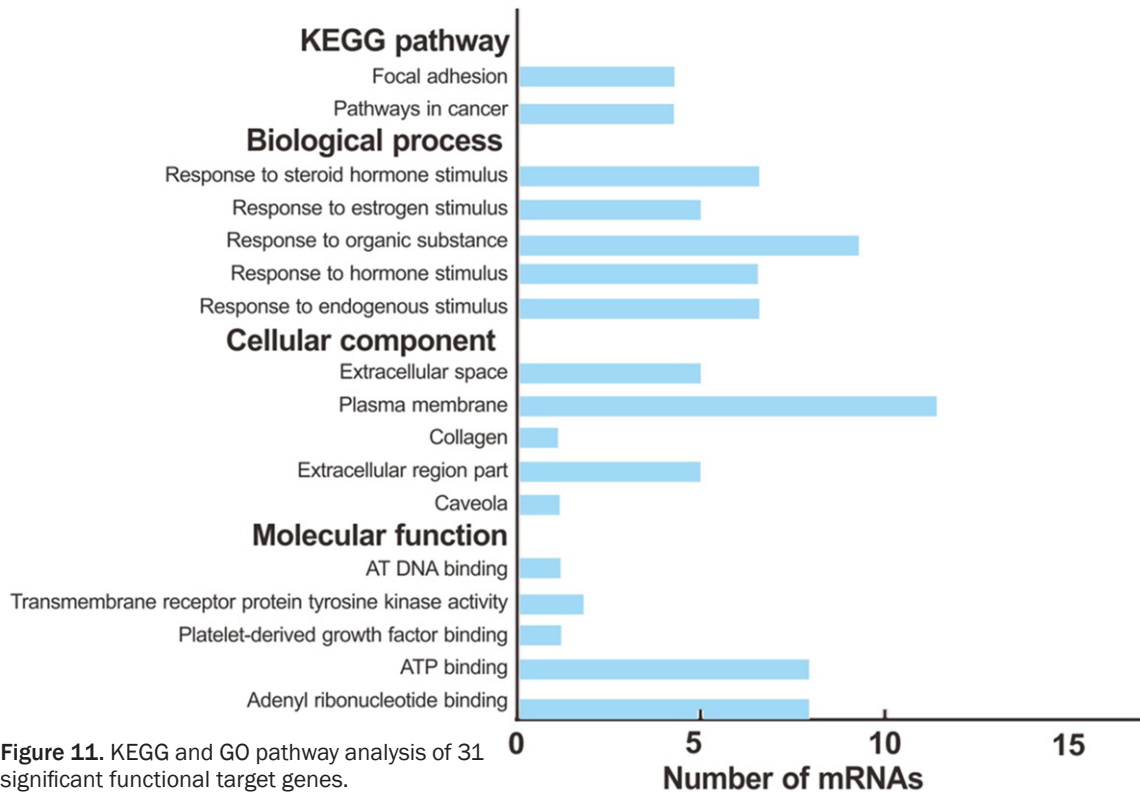
Figure 11. KEGG and GO pathway analysis of 31 significant functional target genes.
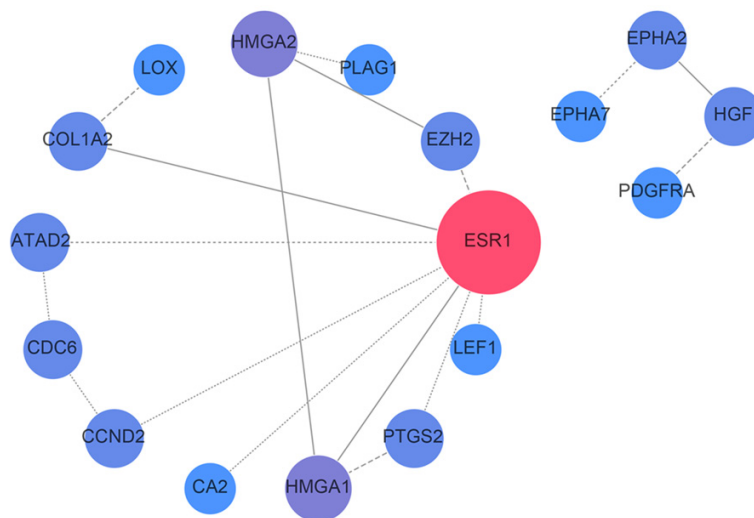

Figure 12. The protein-protein interaction (PPI) network of 31 target genes. Nodes represent target genes; the size and color represent the connectivity; the solid and dashed lines, which represent interaction with proportional combined score.

[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

into mice model. 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

that there are more target genes of miR-26a-5p worthy of prediction. So an integrated prediction obtaining 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, which was strongly connected to cell motility

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.

Acknowledgements

Disclosure of conflict of interest

None.

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References

[1] Zuo D, Chen L, Liu X, Wang X, Xi Q, Luo Y, Zhang N and Guo H. Combination of miR-125b and miR-27a enhances sensitivity and specificity of AFP-based diagnosis of hepatocellular carcinoma. Tumour Biol 2016; 37: 6539-6549.

[2] Peng C, Guo Z, Wu X and Zhang XL. A polymorphism at the microRNA binding site in the 3' untranslated region of RYR3 is associated with outcome in hepatocellular carcinoma. Onco Targets Ther 2015; 8: 2075-2079.

[3] Zhang S, Yue M, Shu R, Cheng H and Hu P. Recent advances in the management of hepatocellular carcinoma. J BUON 2016; 21: 307-311.

[4] Zhu RX, Seto WK, Lai CL and Yuen MF. Epidemiology of hepatocellular carcinoma in the Asia-Pacific region. Gut Liver 2016; 10: 332-339.

[5] Liu XH, Dai ZM, Kang HF, Lin S, Ma XB, Wang M, Liu K, Dai C, Wang XJ and Dai ZJ. Association of IL-23R polymorphisms (rs6682925, rs10889677, rs1884444) with cancer risk: a PRISMA-compliant meta-analysis. Medicine (Baltimore) 2015; 94: e2361.

[6] Tang ZY, Ye SL, Liu YK, Qin LX, Sun HC, Ye QH, Wang L, Zhou J, Qiu SJ, Li Y, Ji XN, Liu H, Xia JL, Wu ZQ, Fan J, Ma ZC, Zhou XD, Lin ZY and Liu KD. A decade's studies on metastasis of hepatocellular carcinoma. J Cancer Res Clin Oncol 2004; 130: 187-196.

[7] Li JJ, Luo J, Lu JN, Liang XN, Luo YH, Liu YR, Yang J, Ding H, Qin GH, Yang LH, Dang YW, Yang H and Chen G. Relationship between TRAF6 and deterioration of HCC: an immunohistochemical and in vitro study. Cancer Cell Int 2016; 16: 76.

[8] Kim J, Won R, Ban G, Ju MH, Cho KS, Young Han S, Jeong JS and Lee SW. Targeted regression of hepatocellular carcinoma by cancer-specific RNA replacement through MicroRNA regulation. Sci Rep 2015; 5: 12315.

[9] Wang M, Zhang J, Tong L, Ma X and Qiu X. MiR-195 is a key negative regulator of hepatocellular carcinoma metastasis by targeting FGF2 and VEGFA. Int J Clin Exp Pathol 2015; 8: 14110-14120.

[10] Jin Y, Ai J and Shi J. Lung microenvironment promotes the metastasis of human hepatocellular carcinoma cells to the lungs. Int J Clin Exp Med 2015; 8: 9911-9917.

[11] Wang X, Lan H, Shen T, Gu P, Guo F, Lin X and Jin K. Perineural invasion: a potential reason of hepatocellular carcinoma bone metastasis. Int J Clin Exp Med 2015; 8: 5839-5846.

[12] Fornari F, Ferracin M, Trere D, Milazzo M, Marinelli S, Galassi M, Venerandi L, Pollutri D, Patrizi C, Borghi A, Foschi FG, Stefanini GF, Negrini M, Bolondi L and Gramantieri L. Circulating microRNAs, miR-939, miR-595, miR-519d and miR-494, identify cirrhotic patients with HCC. PLoS One 2015; 10: e0141448.

[13] Tang G, Shen X, Lv K, Wu Y, Bi J and Shen Q. Different normalization strategies might cause inconsistent variation in circulating microRNAs in patients with hepatocellular carcinoma. Med Sci Monit 2015; 21: 617-624.

[14] You XM, Mo XS, Ma L, Zhong JH, Qin HG, Lu Z, Xiang BD, Wu FX, Zhao XH, Tang J, Pang YH, Chen J and Li LQ. Randomized clinical trial comparing efficacy of Simo decoction and acupuncture or chewing gum alone on postoperative ileus in patients with hepatocellular carcinoma after hepatectomy. Medicine (Baltimore) 2015; 94: e1968.

[15] Iezzi R, Pompili M, Posa A, Coppola G, Gasbarrini A and Bonomo L. Combined locoregional treatment of patients with hepatocellular carcinoma: state of the art. World J Gastroenterol 2016; 22: 1935-1942.

[16] Zhao Y, Fang Z, Luo J, Liu Q, Xu G, Pan H, Wei W and Yan Z. Evaluation of extrahepatic collateral arteries in hepatocellular carcinoma in three independent groups in a single center. Exp Ther Med 2015; 10: 2366-2374.

[17] Li T, Zhao S, Song B, Wei Z, Lu G, Zhou J and Huo T. Effects of transforming growth factor beta-1 infected human bone marrow mesenchymal stem cells on high- and low-metastatic potential hepatocellular carcinoma. Eur J Med Res 2015; 20: 56.

[18] Zhang B, Liu K, Zhang J, Dong L, Jin Z, Zhang X, Xue F and He J. Glutamine synthetase predicts adjuvant TACE response in hepatocellular carcinoma. Int J Clin Exp Med 2015; 8: 20722-20731.

[19] Hoshida Y, Toffanin S, Lachenmayer A, Villanueva A, Minguez B and Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. Semin Liver Dis 2010; 30: 35-51.

[20] Liao Q, Han P, Huang Y, Wu Z, Chen Q, Li S, Ye J and Wu X. Potential role of circulating microRNA-21 for hepatocellular carcinoma diagnosis: a meta-analysis. PLoS One 2015; 10: e0130677.

[21] Liu Y, Ren F, Luo Y, Rong M, Chen G and Dang Y. Down-regulation of MiR-193a-3p dictates deterioration of HCC: a clinical real-time qRT-PCR study. Med Sci Monit 2015; 21: 2352-2360.

[22] He R, Yang L, Lin X, Chen X, Lin X, Wei F, Liang X, Luo Y, Wu Y, Gan T, Dang Y and Chen G. MiR-30a-5p suppresses cell growth and enhances apoptosis of hepatocellular carcinoma cells via targeting AEG-1. Int J Clin Exp Pathol 2015; 8: 15632-15641.

[23] Chen ZY, Chen X and Wang ZX. The role of microRNA-196a in tumorigenesis, tumor progression, and prognosis. Tumour Biol 2016; [Epub ahead of print].

[24] Lyra-Gonzalez I, Flores-Fong LE, Gonzalez-Garcia I, Medina-Preciado D and Armendariz-Borunda J. MicroRNAs dysregulation in hepatocellular carcinoma: insights in genomic medicine. World J Hepatol 2015; 7: 1530-1540.

[25] Hung CH, Chiu YC, Chen CH and Hu TH. MicroRNAs in hepatocellular carcinoma: carcinogenesis, progression, and therapeutic target. Biomed Res Int 2014; 2014: 486407.

[26] He L and Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-531.

[27] Burchard J, Zhang C, Liu AM, Poon RT, Lee NP, Wong KF, Sham PC, Lam BY, Ferguson MD, Tokiwa G, Smith R, Leeson B, Beard R, Lamb JR, Lim L, Mao M, Dai H and Luk JM. microRNA-122 as a regulator of mitochondrial metabolic gene network in hepatocellular carcinoma. Mol Syst Biol 2010; 6: 402.

[28] Liu Y, Ren F, Rong M, Luo Y, Dang Y and Chen G. Association between underexpression of microrna-203 and clinicopathological significance in hepatocellular carcinoma tissues. Cancer Cell Int 2015; 15: 62.

[29] Tu H, Wei G, Cai Q, Chen X, Sun Z, Cheng C, Zhang L, Feng Y, Zhou H, Zhou B and Zeng T. MicroRNA-212 inhibits hepatocellular carcinoma cell proliferation and induces apoptosis by targeting FOXA1. Onco Targets Ther 2015; 8: 2227-2235.

[30] Luo X, Yang S, Zhou C, Pan F, Li Q and Ma S. MicroRNA-328 enhances cellular motility through posttranscriptional regulation of PTPRJ in human hepatocellular carcinoma. Onco Targets Ther 2015; 8: 3159-3167.

[31] Zhao Y, Li F, Zhang X, Liu A, Qi J, Cui H and Zhao P. MicroRNA-194 acts as a prognostic marker and inhibits proliferation in hepatocellular carcinoma by targeting MAP4K4. Int J Clin Exp Pathol 2015; 8: 12446-12454.

[32] Zhong C, Li MY, Chen ZY, Cheng HK, Hu ML, Ruan YL and Guo RP. MicroRNA-200a inhibits epithelial-mesenchymal transition in human hepatocellular carcinoma cell line. Int J Clin Exp Pathol 2015; 8: 9922-9931.

[33] Zhao L and Wang W. miR-125b suppresses the proliferation of hepatocellular carcinoma cells by targeting Sirtuin7. Int J Clin Exp Med 2015; 8: 18469-18475.

[34] Chen L, Chu F, Cao Y, Shao J and Wang F. Serum miR-182 and miR-331-3p as diagnostic and prognostic markers in patients with hepatocellular carcinoma. Tumour Biol 2015; 36: 7439-7447.

[35] Jiang L, Li X, Cheng Q and Zhang BH. Plasma microRNA might as a potential biomarker for hepatocellular carcinoma and chronic liver disease screening. Tumour Biol 2015; 36: 7167-7174.

[36] Zhang X, Tang W, Li R, He R, Gan T, Luo Y, Chen G and Rong M. Downregulation of microRNA-132 indicates progression in hepatocellular carcinoma. Exp Ther Med 2016; 12: 2095-2101.

[37] Zhang JG, Shi Y, Hong DF, Song M, Huang D, Wang CY and Zhao G. MiR-148b suppresses cell proliferation and invasion in hepatocellular carcinoma by targeting WNT1/beta-catenin pathway. Sci Rep 2015; 5: 8087.

[38] Chen Y, Chen J, Liu Y, Li S and Huang P. Plasma miR-15b-5p, miR-338-5p, and miR-764 as biomarkers for hepatocellular carcinoma. Med Sci Monit 2015; 21: 1864-1871.

[39] Leidinger P, Brefort T, Backes C, Krapp M, Galata V, Beier M, Kohlhaas J, Huwer H, Meese E and Keller A. High-throughput qRT-PCR validation of blood microRNAs in non-small cell lung cancer. Oncotarget 2016; 7: 4611-4623.

[40] Zhao S, Ye X, Xiao L, Lian X, Feng Y, Li F and Li L. MiR-26a inhibits prostate cancer progression by repression of Wnt5a. Tumour Biol 2014; 35: 9725-9733.

[41] Jia LF, Wei SB, Gan YH, Guo Y, Gong K, Mitchelson K, Cheng J and Yu GY. Expression, regulation and roles of miR-26a and MEG3 in tongue squamous cell carcinoma. Int J Cancer 2014; 135: 2282-2293.

[42] Yang X, Liang L, Zhang XF, Jia HL, Qin Y, Zhu XC, Gao XM, Qiao P, Zheng Y, Sheng YY, Wei JW, Zhou HJ, Ren N, Ye QH, Dong QZ and Qin LX. MicroRNA-26a suppresses tumor growth and metastasis of human hepatocellular carcinoma by targeting interleukin-6-Stat3 pathway. Hepatology 2013; 58: 158-170.

[43] Wang G, Sun Y, He Y, Ji C, Hu B and Sun Y. miR-26a promoted by interferon-alpha inhibits hepatocellular carcinoma proliferation and migration by blocking EZH2. Genet Test Mol Biomarkers 2015; 19: 30-36.

[44] Yang X, Zhang XF, Lu X, Jia HL, Liang L, Dong QZ, Ye QH and Qin LX. MicroRNA-26a suppresses angiogenesis in human hepatocellular carcinoma by targeting hepatocyte growth factor-cMet pathway. Hepatology 2014; 59: 1874-1885.

[45] Zhuang C, Jiang W, Huang D, Xu L, Yang Q, Zheng L, Wang X and Hu L. Serum miR-21, miR-26a and miR-101 as potential biomarkers of hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2016; 40: 386-396.

[46] Lin S, Yin YA, Jiang X, Sahni N and Yi S. Multi-OMICs and genome editing perspectives on liver cancer signaling networks. Biomed Res Int 2016; 2016: 6186281.

[47] Klonowska K, Czubak K, Wojciechowska M, Handschuh L, Zmienko A, Figlerowicz M, Dams-Kozlowska H and Kozlowski P. Oncogenomic portals for the visualization and analysis of genome-wide cancer data. Oncotarget 2016; 7: 176-192.

[48] Shukla HD, Mahmood J and Vujaskovic Z. Integrated proteo-genomic approach for early diagnosis and prognosis of cancer. Cancer Lett 2015; 369: 28-36.

[49] Wang Z, Monteiro CD, Jagodnik KM, Fernandez NF, Gundersen GW, Rouillard AD, Jenkins SL, Feldmann AS, Hu KS, McDermott MG, Duan Q, Clark NR, Jones MR, Kou Y, Goff T, Woodland H, Amaral FM, Szeto GL, Fuchs O, Schussler-Fiorenza Rose SM, Sharma S, Schwartz U, Bausela XB, Szymkiewicz M, Maroulis V, Salykin A, Barra CM, Kruth CD, Bongio NJ, Mathur V, Todoric RD, Rubin UE, Malatras A, Fulp CT, Galindo JA, Motiejunaite R, Juschke C, Dishuck PC, Lahl K, Jafari M, Aibar S, Zaravinos A, Steenhuizen LH, Allison LR, Gamallo P, de Andres Segura F, Dae Devlin T, Perez-Garcia V and Ma'ayan A. Extraction and analysis of signatures from the gene expression omnibus by the crowd. Nat Commun 2016; 7: 12846.

[50] Clough E and Barrett T. The gene expression omnibus database. Methods Mol Biol 2016; 1418: 93-110.

[51] Yang Z, Lu Y, Xu Q, Tang B, Park CK and Chen X. HULC and H19 played different roles in overall and disease-free survival from hepatocellular carcinoma after curative hepatectomy: a preliminary analysis from gene expression omnibus. Dis Markers 2015; 2015: 191029.

[52] Hasan AN, Ahmad MW, Madar IH, Grace BL and Hasan TN. An in silico analytical study of lung cancer and smokers datasets from gene expression omnibus (GEO) for prediction of differentially expressed genes. Bioinformation 2015; 11: 229-235.

[53] Patel TA, Puppala M, Ogunti RO, Ensor JE, He T, Shewale JB, Ankerst DP, Kaklamani VG, Rodriguez AA, Wong ST and Chang JC. Correlating mammographic and pathologic findings in clinical decision support using natural language processing and data mining methods. Cancer 2017; 123: 114-121.

[54] Yim WW, Yetisgen M, Harris WP and Kwan SW. Natural language processing in oncology: a review. JAMA Oncol 2016; 2: 797-804.

[55] Wieneke AE, Bowles EJ, Cronkite D, Wernli KJ, Gao H, Carrell D and Buist DS. Validation of natural language processing to extract breast cancer pathology procedures and results. J Pathol Inform 2015; 6: 38.

[56] Zhang J, Fan D, Jian Z, Chen GG and Lai PB. Cancer specific long noncoding RNAs show differential expression patterns and competing endogenous RNA potential in hepatocellular carcinoma. PLoS One 2015; 10: e0141042.

[57] Zhang J, Chong CC, Chen GG and Lai PB. A seven-microrna expression signature predicts survival in hepatocellular carcinoma. PLoS One 2015; 10: e0128628.

[58] Huang WT, Wang HL, Yang H, Ren FH, Luo YH, Huang CQ, Liang YY, Liang HW, Chen G and Dang YW. Lower expressed miR-198 and its potential targets in hepatocellular carcinoma: a clinicopathological and in silico study. Onco Targets Ther 2016; 9: 5163-5180.

[59] Zhang X, Tang W, Chen G, Ren F, Liang H, Dang Y and Rong M. An encapsulation of gene signatures for hepatocellular carcinoma, MicroRNA-132 predicted target genes and the corresponding overlaps. PLoS One 2016; 11: e0159498.

[60] Gao J, Li L, Wu M, Liu M, Xie X, Guo J, Tang H and Xie X. MiR-26a inhibits proliferation and migration of breast cancer through repression of MCL-1. PLoS One 2013; 8: e65138.

[61] Deng M, Tang HL, Lu XH, Liu MY, Lu XM, Gu YX, Liu JF and He ZM. miR-26a suppresses tumor growth and metastasis by targeting FGF9 in gastric cancer. PLoS One 2013; 8: e72662.

[62] Lin Y, Chen H, Hu Z, Mao Y, Xu X, Zhu Y, Xu X, Wu J, Li S, Mao Q, Zheng X and Xie L. miR-26a inhibits proliferation and motility in bladder cancer by targeting HMGA1. FEBS Lett 2013; 587: 2467-2473.

[63] Shao Y, Li P, Zhu ST, Yue JP, Ji XJ, Ma D, Wang L, Wang YJ, Zong Y, Wu YD and Zhang ST. MiR-26a and miR-144 inhibit proliferation and metastasis of esophageal squamous cell cancer by inhibiting cyclooxygenase-2. Oncotarget 2016; 7: 15173-15186.

[64] Deng J, He M, Chen L, Chen C, Zheng J and Cai Z. The loss of miR-26a-mediated post-transcriptional regulation of cyclin E2 in pancreatic cancer cell proliferation and decreased patient survival. PLoS One 2013; 8: e76450.

[65] Lu J, He ML, Wang L, Chen Y, Liu X, Dong Q, Chen YC, Peng Y, Yao KT, Kung HF and Li XP. MiR-26a inhibits cell growth and tumorigenesis of nasopharyngeal carcinoma through repression of EZH2. Cancer Res 2011; 71: 225-233.

[66] Liu B, Wu X, Liu B, Wang C, Liu Y, Zhou Q and Xu K. MiR-26a enhances metastasis potential of lung cancer cells via AKT pathway by target-

ing PTEN. Biochim Biophys Acta 2012; 1822: 1692-1704.

[67] Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, Sohn-Lee C, le Sage C, Agami R, Tuschl T and Holland EC. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev 2009; 23: 1327-1337.

[68] Wang LJ, Zhang KL, Zhang N, Ma XW, Yan SW, Cao DH and Shi SJ. Serum miR-26a as a diagnostic and prognostic biomarker in cholangiocarcinoma. Oncotarget 2015; 6: 18631-18640.

[69] Zou ZJ, Fan L, Wang L, Xu J, Zhang R, Tian T, Li JY and Xu W. miR-26a and miR-214 down-regulate expression of the PTEN gene in chronic lymphocytic leukemia, but not PTEN mutation or promoter methylation. Oncotarget 2015; 6: 1276-1285.

[70] Zhou J, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang JF, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H and Fan J. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol 2011; 29: 4781-4788.

[71] Tan Y, Ge G, Pan T, Wen D, Chen L, Yu X, Zhou X and Gan J. A serum microRNA panel as potential biomarkers for hepatocellular carcinoma related with hepatitis B virus. PLoS One 2014; 9: e107986.

[72] Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Ambs S, Chen Y, Meltzer PS, Croce CM, Qin LX, Man K, Lo CM, Lee J, Ng IO, Fan J, Tang ZY, Sun HC and Wang XW. MicroRNA expression, survival, and response to interferon in liver cancer. N Engl J Med 2009; 361: 1437-1447.

[73] Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR and Mendell JT. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell 2009; 137: 1005-1017.

[74] Zhuang C, Wang P, Huang D, Xu L, Wang X, Wang L and Hu L. A double-negative feedback loop between EZH2 and miR-26a regulates tumor cell growth in hepatocellular carcinoma. Int J Oncol 2016; 48: 1195-1204.

[75] Zhang X, Cheng SL, Bian K, Wang L, Zhang X, Yan B, Jia LT, Zhao J, Gammoh N, Yang AG and Zhang R. MicroRNA-26a promotes anoikis in human hepatocellular carcinoma cells by targeting alpha5 integrin. Oncotarget 2015; 6: 2277-2289.

[76] Chai ZT, Kong J, Zhu XD, Zhang YY, Lu L, Zhou JM, Wang LR, Zhang KZ, Zhang QB, Ao JY, Wang M, Wu WZ, Wang L, Tang ZY and Sun HC. MicroRNA-26a inhibits angiogenesis by down-regulating VEGFA through the PIK3C2alpha/Akt/HIF-1alpha pathway in hepatocellular carcinoma. PLoS One 2013; 8: e77957.

[77] Cheng L, Gao S, Song X, Dong W, Zhou H, Zhao L and Jia L. Comprehensive N-glycan profiles of hepatocellular carcinoma reveal association of fucosylation with tumor progression and regulation of FUT8 by microRNAs. Oncotarget 2016; 7: 61199-61214.

[78] Zhang W, Liu K, Liu S, Ji B, Wang Y and Liu Y. MicroRNA-133a functions as a tumor suppressor by targeting IGF-1R in hepatocellular carcinoma. Tumour Biol 2015; 36: 9779-9788.

[79] Zhang Y, Huang W, Ran Y, Xiong Y, Zhong Z, Fan X, Wang Z and Ye Q. miR-582-5p inhibits proliferation of hepatocellular carcinoma by targeting CDK1 and AKT3. Tumour Biol 2015; 36: 8309-8316.

[80] Thurnherr T, Mah WC, Lei Z, Jin Y, Rozen SG and Lee CG. Differentially expressed miRNAs in hepatocellular carcinoma target genes in the genetic information processing and metabolism pathways. Sci Rep 2016; 6: 20065.

[81] Ruggieri A, Barbati C and Malorni W. Cellular and molecular mechanisms involved in hepatocellular carcinoma gender disparity. Int J Cancer 2010; 127: 499-504.

[82] Xu H, Wei Y, Zhang Y, Xu Y, Li F, Liu J, Zhang W, Han X, Tan R and Shen P. Oestrogen attenuates tumour progression in hepatocellular carcinoma. J Pathol 2012; 228: 216-229.

[83] Hishida M, Nomoto S, Inokawa Y, Hayashi M, Kanda M, Okamura Y, Nishikawa Y, Tanaka C, Kobayashi D, Yamada S, Nakayama G, Fujii T, Sugimoto H, Koike M, Fujiwara M, Takeda S and Kodera Y. Estrogen receptor 1 gene as a tumor suppressor gene in hepatocellular carcinoma detected by triple-combination array analysis. Int J Oncol 2013; 43: 88-94.

[84] Dai B, Geng L, Yu Y, Sui C, Xie F, Shen W, Zheng T and Yang J. Methylation patterns of estrogen receptor alpha promoter correlate with estrogen receptor alpha expression and clinicopathological factors in hepatocellular carcinoma. Exp Biol Med (Maywood) 2014; 239: 883-890.