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The clinical significance of endothelin receptor type B in hepatocellular carcinoma and its potential molecular mechanism

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ABSTRACT

Objective: To explore the clinical significance and potential molecular mechanism of endothelin receptor type B (EDNRB) in hepatocellular carcinoma (HCC).

Methods: Immunohistochemistry was used to detect EDNRB protein expression level in 67 HCC paraffin embedded tissues and adjacent tissues. Correlations between EDNRB expression level and clinicopathologic parameters were analyzed in our study. The expression level and clinical significance of EDNRB in HCC were also evaluated from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) database. The cBioPortal for Cancer Genomics was employed to analyze the EDNRB related genes, and Gene Ontology (GO) annotation, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Protein-Protein Interaction (PPI) network were conducted for those EDNRB related genes.

Results: Lower expression level of EDNRB in HCC was verified by immunohistochemistry than adjacent tissues ($P < 0.0001$). The expression level of EDNRB in HCC tissues was lower than normal control liver tissues based on TCGA and GEO data (standard mean difference [SMD] = −1.48, 95% [confidence interval] CI: −1.63−(−1.33), $P_{\text{heterogeneity}} = 0.116$, $I^2 = 32.4\%$). Kaplan-Meier analysis showed that HCC patients with lower EDNRB expression were more prone to poor prognosis ($P = .0041$). The top terms of GO annotation in biological process, cellular component and molecular function were vasculature development, actin filament and transmembrane receptor protein kinase activity, respectively. The KEGG pathway enrichment analysis confirmed that EDNRB related genes mainly participated in Vascular smooth muscle contraction, cGMP-PKG signaling pathway and Focal adhesion pathways. The result of PPI network construction showed that KDR, VEGFC, FLT1, CDH5 and ADCY4 were possible to become the core genes of EDNRB related genes, which need further experiments to confirm.

Conclusion: Our study provides novel findings and insights on the molecular pathogenesis of HCC from EDNRB view.

1. Introduction

Hepatocellular carcinoma (HCC) is a malignancy that originates from the liver, with its morbidity rate ranking fourth in all malignancies and the mortality rate ranking third. Guangxi is a region with high morbidity rate of HCC (National Health Commission of the People's Republic of China, 2017; Fu and Wang, 2018; Li et al., 2018a; Ozakyol, 2017; Sun et al., 2018). Multiple factors contributed to the initiation of HCC, including environmental influences and the genetic susceptibility. The risk factors in the environment involved the infection of viral hepatitis, intake of aflatoxin, alcohol addiction and the use of oral contraceptive (Brandi et al., 2017; Xu et al., 2017; Zheng et al., 2017). Currently, the treatment for HCC mainly depends on the operation, with assistance of combined therapies. However, the treatment usually seemed unsatisfactory due to undiagnosed initiation, advanced stages when diagnosed, high recurrence rate, drug resistance, etc. (Ayuso et al., 2018; Crocetti et al., 2017; Foerster et al., 2018; Katsura et al., 2017; Kim et al., 2017; Mao et al., 2018; Reig et al., 2018; Shiina et al.,

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Table 1
The clinicopathologic features of the 67 cases of HCC patients.

Clinicopathologic parameters		N
Sex	Male	56 (83.6%)
	Female	11 (16.4%)
Age	< 60	54 (80.6%)
	≥60	13 (19.4%)
Grading	I-II	39 (58.2%)
	III-IV	28 (41.8%)
Tumor size (cm)	≥5	45 (67.2%)
	< 5	22 (32.8%)
Tumor nodule	Single	56 (83.6%)
	Multiple	11 (16.4%)
Cirrhosis	Yes	36 (53.7%)
	No	31 (46.3%)
Portal vein tumor thrombus (PVTT)	Yes	7 (10.4%)
	No	60 (89.6%)
Vascular invasion	Yes	25 (37.3%)
	No	42 (62.7%)
The infiltration of Glisson's capsule	Yes	16 (23.9%)
	No	51 (76.1%)
AFP (ng/ml)	≥400	27 (40.3%)
	< 400	39 (58.2%)
nm23	Positive	64 (95.5%)
	Negative	3 (4.5%)
P53	Positive	52 (77.6%)
	Negative	15 (22.4%)
P21	Positive	8 (11.9%)
	Negative	59 (88.1%)
VEGF	Positive	33 (49.3%)
	Negative	33 (49.3%)
Ki-67	High	37 (55.2%)
	Low	27 (40.3%)
CD34	High	34 (50.7%)
	Low	12 (17.9%)
HBV infection	Yes	56 (83.6%)
	No	11 (16.4%)
HCV infection	Yes	2 (3%)
	No	63 (94%)
Child-Pugh class	A	39 (58.2%)
	B	5 (7.5%)
BCLC stage	0	2 (3%)
	A	21 (31.3%)
	B	18 (26.9%)
	C	3 (4.5%)

Note: HCC, hepatocellular carcinoma; AFP, α-fetoprotein; nm23, Non-metastasis 23; VEGF, vascular endothelial growth factor; HBV, hepatitis B virus; HCV, hepatitis C virus; BCLC, Barcelona Clinic Liver Cancer.

2018; Wu et al., 2018; Xu et al., 2018). The trend of precision medicine has marked the start of using molecularly targeted therapy against malignant tumors, and the discovery of novel molecular targets with the diagnostic and prognostic value has laid the foundation for targeted therapy (Amicone and Marchetti, 2018; Chen et al., 2018; Dhanasekaran et al., 2018; Li et al., 2018c; Song et al., 2018; Yao et al., 2018).

The endothelin receptor type B (EDNRB) belongs to the family of G protein-coupled receptors, which functions as a vital regulatory factor in signal transduction in cells, locating on human chromosome 13q22.3 (Ayala-Valdovinos et al., 2016; Bregar et al., 2018; Morimoto et al., 2018; Widowati et al., 2016). It has been confirmed that EDNRB exhibited high level of methylation and reduced expression of mRNA in tumors like nasopharyngeal cancer (Lo et al., 2002; Xu et al., 2016; Zhou et al., 2007), esophageal squamous cell carcinoma (Zhao et al., 2009), oral squamous cell carcinoma (Viet et al., 2011), leukemia (Hsiao et al., 2008), gastric cancer (Tao et al., 2012) and colorectal cancer (Chen et al., 2013; Mousavi Ardehaie et al., 2017). Furthermore, EDNRB also participated in the initiation and development of malignant tumors. Mou et al. found that the mRNA expression and protein level of EDNRB were downregulated in hepatoma cell line and HCC tissues, which could inhibit the metastasis and invasion of HCC cells SMMC-7721 and Huh7 (Mu, 2017). Nonetheless, further researches are required in the clinical significance of EDNRB and molecular mechanism in HCC.

In this study, immunohistochemistry (IHC), The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) database were employed to investigate the relationships between EDNRB expression and the clinical parameters and the prognosis. In addition, we attempted to explore the potential molecular mechanism of EDNRB in the initiation and development of HCC, which would provide novel insights on the diagnosis and treatment of HCC.

2. Materials and methods

2.1. Tissue samples

Sixty-seven cases of HCC paraffin embedded tissues and adjacent tissues were collected from the Pathology Department of The First Affiliated Hospital of Guangxi Medical University between Jan.1, 2015 and May 1, 2016. The clinicopathologic features of the 67 patients, who had been pathologically diagnosed with HCC, were summarized as follows (Table 1).

2.2. Reagents

Anti-Endothelin B Receptor antibody (ab117529, Rabbit polyclonal to Endothelin B Receptor), purchased from Abcam, was treated with a 1:2000 dilution. Anti-mouse/rabbit secondary antibody (D-3004-15) was used directly with no dilution, which was purchased from Shanghai Long Island Antibody Diagnostica Inc.

2.3. IHC

The paraffin-embedded tissues were sliced into 4 μm sections and incubated at 65 °C overnight. The xylene was used as the paraffin solvent, and dehydration was completed by ethanol solutions of increasing alcohol concentration until 100%. Ethylenediamine tetraacetic acid buffer solution was applied to recover antigens, and the endogenous peroxidase was blocked by 3% H_2O_2. The dry towel was used to absorb the excess water surrounding the sections. A volume of 70 μl EDNRB, as the primary antibody, was applied evenly on the tissues in the sections, and the amount could be adjusted according to the size of tissues, then the sections were incubated at 37 °C for one hour. After being washed and soaked in phosphate-buffered saline (PBS), the sections were treated with the secondary antibody and incubated at room temperature for half an hour. Subsequently, the sections were again washed and soaked in PBS, and diaminobenzidine staining was carried out for 2–5 min. Eventually, following the re-staining by hematoxylin, the sections were dehydrated and later mounted with neutral balsam. The positive tissues were used as positive controls, and PBS, instead of the primary antibody, acted as the normal control.

2.4. Interpretation of IHC results

EDNRB was expressed in the cytoplasm. The scores were calculated based on the staining intensity and the percentage of the positive cells. The scoring was as follows: A. The staining intensity: 0 (no staining), 1 (light staining), 2 (moderate staining), 3 (strong staining); B. The percentage of the positive cells: 0 (< 5%), 1 (5%–25%), 2 (26%–50%), 3 (51%–75%), 4 (76%–100%).

The total score of IHC staining = staining intensity * percentage of positive cells. The EDNRB with the total score more than or equal to 6 was classified into the positive group, while that with score < 6 was categorized into the negative one.

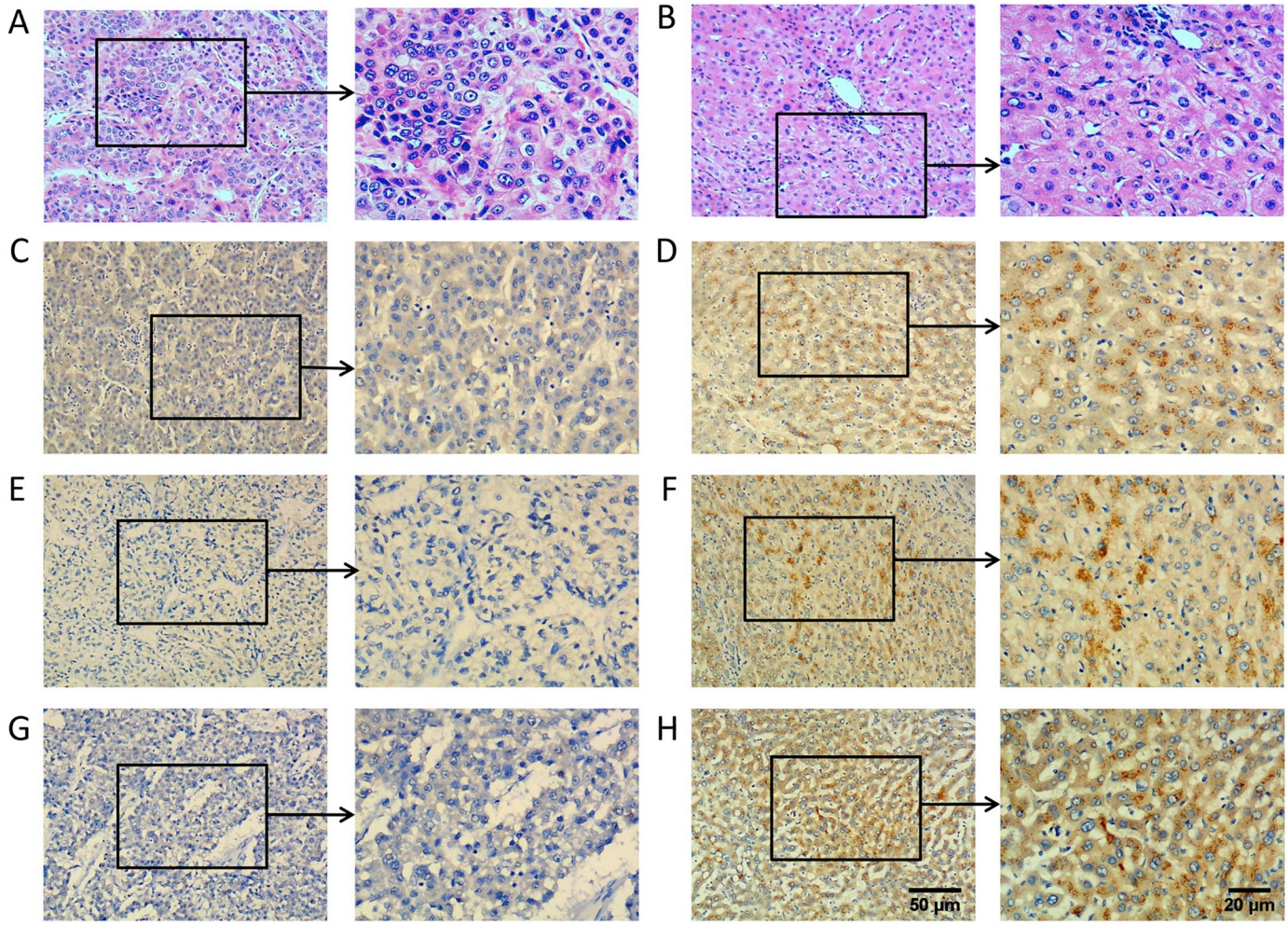

Fig. 1. The expression levels of endothelin receptor type B (EDNRB) in hepatocellular carcinoma (HCC) tissues and corresponding noncancerous tissues. A: Hematoxylin (HE) staining of HCC tissues (×200, ×400). B: HE staining of noncancerous tissues (×200, ×400). C: The expression level of EDNRB in Grade I HCC tissues (×200, ×400). D: The expression level of EDNRB in corresponding noncancerous tissues of Grade I (×200, ×400). E: The expression level of EDNRB in Grade II HCC tissues (×200, ×400). F: The expression level of EDNRB in corresponding noncancerous tissues of Grade II (×200, ×400). G: The expression level of EDNRB in Grade III HCC tissues (×200, ×400). H: The expression level of EDNRB in corresponding noncancerous tissues of Grade III (×200, ×400).

2.5. Collection of the RNA sequencing data in TCGA database

From the TCGA database (https://cancergenome.nih.gov/), we downloaded the expression of mRNA in HCC and the clinicopathologic parameters, and selected the data concerning EDNRB, which involved 371 cases of HCC tissues and 50 cases of normal liver tissues as controls (Danaher et al., 2018; Hutter and Zenklusen, 2018).

Kaplan-Meier plotter database (http://kmplot.com/analysis/index.php) was dealt with PostgreSQL, in which gene expression and clinical data could be acquired and the prognostic value of specific genes could be analyzed by Kaplan-Meier estimator(Lanczky et al., 2016). Therefore, we used Kaplan-Meier plotter database to clarify the prognostic capability of EDNRB in HCC.

2.6. Collection of the microarray data in GEO

We retrieved the mRNA microarray data regarding HCC in the GEO database (https://www.ncbi.nlm.nih.gov/geo/) until April 27, 2018 (Gao et al., 2018). The search strategy was (hepatocellular OR liver OR hepatic) AND (mRNA OR gene). For these mRNA microarrays the inclusion criteria were as follows: (1) the cancer tissues were diagnosed with HCC; (2) each microarray contained HCC tissues and the normal controls; (3) the expression profiling data of EDNRB was provided; (4) the species was *Homo sapiens*. The exclusion criteria were as follows: (1) no expression profiling data of EDNRB was provided; (2) no normal controls were contained in the cases; (3) the species were animals.

2.7. EDNRB related genes

The cBioPortal for Cancer Genomics (http://www.cbioportal.org/) was employed to analyze the EDNRB related genes, and fuctional annotation and pathway enrichment analysis were conducted for those EDNRB related genes of which Spearman correlation coefficient was more than or equal to 5 (Gao et al., 2013).

2.8. Fuctional annotation, pathway enrichment analysis and protein-protein interaction (PPI) network construction

The DAVID database (https://david.ncifcrf.gov/) was used for the fuctional annotation of EDNRB related genes and pathway enrichment

Table 2
The relationships of EDNRB expression with the clinicopathologic parameters by interpretation of the immunohistochemistry.

Clinicopathologic parameters		EDNRB expression		*P* value	Statistical tests
		Cases of high expression	Cases of low expression		
Sex	Male	8	48		
	Female	0	11	0.408	Y
Age	< 60	6	48		
	≥60	2	11	1	Y
Grading	I-II	7	32		
	III-IV	1	27	0.159	Y
Tumor size (cm)	≥5	3	42		
	< 5	5	17	0.133	Y
Tumor nodule	Single	8	48		
	Multiple	0	11	0.408	Y
Cirrhosis	Yes	3	33		
	No	5	26	0.546	Y
Portal vein tumor thrombus (PVTT)	Yes	0	7		
	No	8	52	0.586	F
Vascular invasion	Yes	2	23		
	No	6	36	0.706	Y
The infiltration of Glisson's capsule	Yes	2	14		
	No	6	45	1	Y
AFP (ng/ml)	≥400	0	27		
	< 400	7	32	0.055	Y
nm23	Positive	8	56		
	Negative	0	3	1	F
P53	Positive	5	47		
	Negative	3	12	0.522	Y
P21	Positive	0	8		
	Negative	8	51	0.582	F
VEGF	Positive	4	29		
	Negative	4	29	1	Y
Ki-67	High	3	34		
	Low	5	22	0.389	Y
CD34	High	7	27		
	Low	0	12	0.215	Y
HBV infection	Yes	6	50		
	No	2	9	0.85	Y
HCV infection	Yes	1	1		
	No	7	56	0.233	F
Child-Pugh class	A	7	32		
	B	0	5	0.574	F
BCLC stage	0	0	2		
	A	4	17		
	B	3	15		
	C	0	3	0.781	K

Note: EDNRB, endothelin receptor type B; AFP, α-fetoprotein; nm23, Non-metastasis 23; VEGF, vascular endothelial growth factor; HBV, hepatitis B virus; HCV, hepatitis C virus; BCLC, Barcelona Clinic Liver Cancer; Y, Yates's correction; F, Fisher's exact test: K, Kruskal–Wallis test; *$P < .05$.

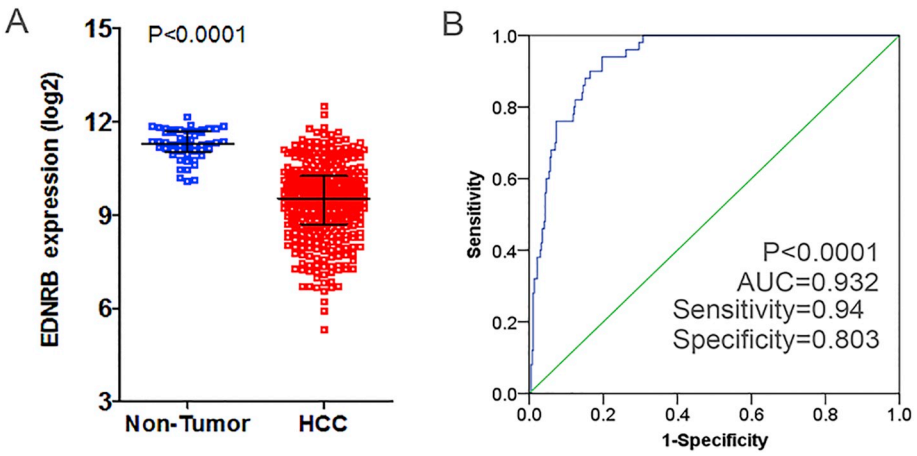

Fig. 2. The expression level and receiver operating characteristic (ROC) curve analysis of endothelin receptor type B (EDNRB) in hepatocellular carcinoma (HCC) based on TCGA database. A: EDNRB expression level in HCC was lower than in normal liver tissues; B: ROC curve analysis of EDNRB for discriminating HCC from normal liver tissues.

Table 3
The relationships of EDNRB expression with the clinicopathologic parameters in TCGA.

Clinicopathologic parameters	Number of cases	EDNRB expression (mean ± SD)	P value
Tissues			
HCC	371	9.44 ± 1.20	
Normal controls	50	11.27 ± 0.49	< 0.0001
Sex			
Male	250	9.51 ± 1.16	
Female	121	9.31 ± 1.26	0.139
Age			
< 60	169	9.53 ± 1.19	
≥60	201	9.38 ± 1.20	0.234
Tumor grading			
I~II	232	9.58 ± 1.13	
III~IV	134	9.20 ± 1.28	0.004*
Stage			
I~II	257	9.46 ± 1.20	
III~IV	87	9.30 ± 1.18	0.283
T			
TX	1	10.38	
T1	181	9.56 ± 1.19	
T2–4	187	9.30 ± 1.18	0.082
N			
NX	114	9.42 ± 1.27	
N0	252	9.45 ± 1.15	
N1	4	9.05 ± 1.86	0.788
M			
MX	101	9.50 ± 1.22	
M0	266	9.42 ± 1.20	
M1	4	9.27 ± 0.52	0.823
Vascular invasion			
Yes	109	9.39 ± 1.31	
No	206	9.53 ± 1.13	0.322
Cirrhosis			
Yes	6	9.22 ± 0.78	
No	346	9.46 ± 1.21	0.636
Alcohol addiction			
Yes	119	9.24 ± 1.25	
No	233	9.56 ± 1.17	0.018*
Smoking			
Yes	17	9.45 ± 0.99	
No	335	9.45 ± 1.22	0.994
HBV infection			
Yes	104	9.41 ± 1.19	
No	248	9.47 ± 1.22	0.683
HCV infection			
Yes	56	9.39 ± 1.05	
No	296	9.46 ± 1.24	0.686

Note: HCC, hepatocellular carcinoma; EDNRB, endothelin receptor type B; TCGA, The Cancer Genome Atlas; HBV, hepatitis B virus; HCV, hepatitis C virus; *P < .05.

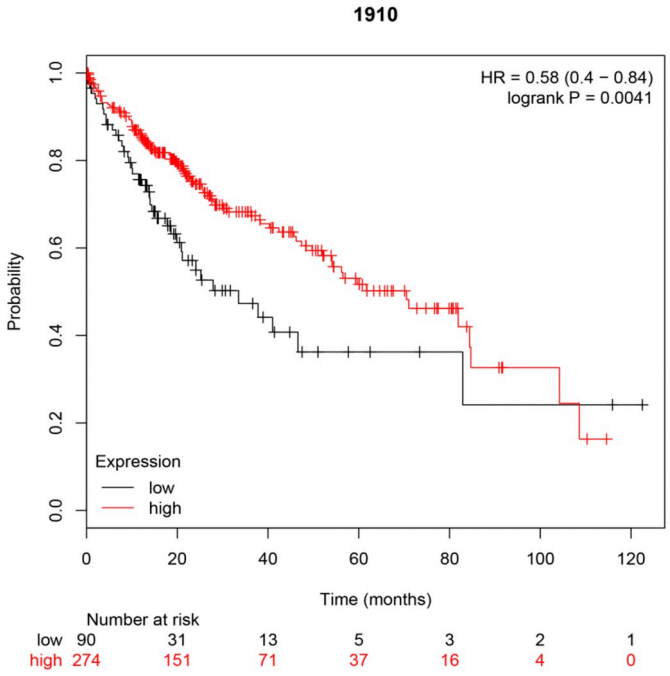

Fig. 3. The Kaplan-Meier survival curve of endothelin receptor type B in hepatocellular carcinoma.

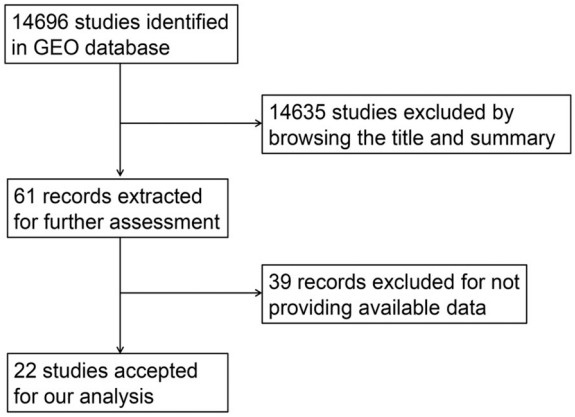

Fig. 4. Flow chart of study selection for endothelin receptor type B microarray data based on GEO datasets.

analysis, including the Gene Ontology (OG) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (Li et al., 2018b; Liu et al., 2018).

The Search Tool for the Retrieval of Interacting Genes (STRING) database was applied for the analysis of PPI. The EDNRB related genes were input into the STRING, and the confidence score > 0.7 was set, and therefore, the PPI network was generated. In the PPI network, the node represented the protein, and the line represented the interactions between proteins. The more a protein interacted with others, the more likely it would become the core gene (Li et al., 2018b).

2.9. Statistical analysis

The Wilcoxon signed-rank test was employed to detect the differential expression of EDNRB in the tumor and the adjacent tissues. The Yates's correction test or the Fisher's exact test was used to calculate the enumerated data like the relationships between the EDNRB expression and the clinicopathologic parameters, and the Kruskal–Wallis test was applied to examine the ranked data.

The Student's *t*-test was used to investigate the differential expression of EDNRB in the tumor and adjacent tissues in TCGA. The GraphPad Prism Version 5.0 was applied to draw the scatter diagram. Also, we took advantage of receiver operating characteristic (ROC) curve to assess the capability of EDNRB to distinguish the tumor tissues from the non-tumor ones, and area under the curve (AUC) was used to

Table 4
The basic features of the 22 microarrays of EDNRB expression profiling included in this study.

Data	Platform	Country/Region	Citation	Number of samples		EDNRB expression (mean ± SD)	
				HCC	Normal control	HCC	Normal control
GSE6764	GPL570	USA	Wurmbach et al. (2007)	35	10	8.019 ± 0.954	9.412 ± 0.315
GSE12941	GPL5175	Japan	Satow et al. (2010)	10	10	6.955 ± 0.594	7.919 ± 0.317
GSE14323	GPL571, GPL96	USA	Mas et al. (2009)	19	38	8.599 ± 1.133	8.292 ± 0.594
GSE14520	GPL571, GPL3921	USA	Roessler et al. (2010)	247	239	5.716 ± 0.956	7.026 ± 0.828
GSE17856	GPL6480	USA	Tsuchiya et al. (2010)	43	44	0.885 ± 0.149	1.026 ± 0.087
GSE22405	GPL10553	USA	None	24	24	4.597 ± 0.314	4.788 ± 0.343
GSE25097	GPL10687	USA	Tung et al. (2011)	268	243	1.436 ± 0.908	3.846 ± 1.146
GSE36376	GPL10558	Korea	Lim et al. (2013)	240	193	7.331 ± 0.544	7.442 ± 0.405
GSE39791	GPL10558	USA	Kim et al. (2014)	72	72	7.125 ± 0.262	7.387 ± 0.385
GSE45436	GPL570	Taiwan	None	95	39	6.663 ± 1.092	8.084 ± 0.540
GSE46408	GPL4133	Taiwan	None	6	6	9.089 ± 1.202	9.284 ± 0.740
GSE50579	GPL14550	Germany	Neumann et al. (2012)	67	10	6.512 ± 0.977	7.240 ± 0.485
GSE55092	GPL570	USA	Melis et al. (2014)	39	81	8.460 ± 0.924	9.477 ± 0.806
GSE57555	GPL18044, GPL16699	Japan	Murakami et al. (2015)	5	16	0.909 ± 0.013	0.927 ± 0.021
GSE57957	GPL10558	Singapore	Mah et al. (2014)	39	39	8.453 ± 0.399	8.710 ± 0.436
GSE59259	GPL18451	Italy	Udali et al. (2015)	8	8	10.595 ± 0.691	11.768 ± 0.222
GSE60502	GPL96	Taiwan	Wang et al. (2014)	18	18	8.437 ± 0.826	9.763 ± 0.522
GSE62232	GPL570	France	Schulze et al. (2015)	81	10	7.481 ± 1.011	8.952 ± 0.272
GSE64041	GPL6244	Switzerland	Makowska et al. (2016)	60	60	8.609 ± 0.654	9.470 ± 0.489
GSE74656	GPL16043	China	None	5	5	7.947 ± 0.475	9.141 ± 0.254
GSE76427	GPL10558	Singapore	Grinchuk et al. (2018)	115	52	7.887 ± 0.512	8.133 ± 0.366
GSE84005	GPL5175	China	None	38	38	7.407 ± 0.870	8.770 ± 0.434

Note: HCC, hepatocellular carcinoma; EDNRB, endothelin receptor type B; USA, United States of America; SMD, standard mean difference.

evaluate its accuracy. SPSS 22.0 was utilized to draw the ROC curves. In addition, Stata Version 12.0 was employed to draw the summary ROC (SROC) curves to analyze the ability of EDNRB to differentiate the tumor tissues from the normal ones.

The standard mean difference (SMD) and 95% confidence interval (CI) was applied to compare the expression of EDNRB in HCC and in normal liver tissues. The heterogeneity of the meta results was represented by the chi-squared test or I^2. If the $P < .05$ or $I^2 > 50\%$, the heterogeneity existed in the meta results, and thus the random effect model was applied. When $P > .05$ or $I^2 < 50\%$, we failed to detect remarkable heterogeneity in the results, so fixed effect model could be used. Stata Version 12.0 would be applied to draw the forest plots.

3. Results

3.1. The clinical significance of EDNRB in HCC by IHC

Of the 67 cases of HCC tissues, EDNRB was positively expressed in 8 cases (11.9%), while in the 67 cases of adjacent tissues, the rate of positive expression reached 73.1% (49/67). We applied the Wilcoxon test to compare the IHC staining scores of EDNRB in HCC tissues as well as in the adjacent tissues, finding the statistical significance existed in the differences ($P < .0001$), which indicated that the lower expression of EDNRB was detected in HCC tissues rather than in the adjacent tissues (Fig. 1A-F). By analyzing the associations between the EDNRB expression and the clinicopathologic parameters, it was revealed that no obvious correlations existed between these two factors ($P > 0.05$). However, the EDNRB expression seemed associated with the α-fetoprotein (AFP) of the blood test ($P = .055$), which required large samples for confirmation (Table 2).

3.2. The clinical significance of EDNRB in HCC in TCGA

From TCGA database, we researchers downloaded the expression profiling of EDNRB, and then compared the 371 cases of HCC tissues with 50 cases of normal tissues, discovering that the EDNRB expression was relatively lower in HCC tissues (9.44 ± 1.20) than in the normal liver tissues (11.27 ± 0.49), with statistical significance ($P < .0001$) (Fig. 2A). The AUC of the EDNRB expression to distinguish the tumor tissues from the non-tumor ones was 0.932 (95%CI: 0.905–0.959, $P < .0001$), and the sensitivity and specificity was 0.94 and 0.803, respectively (Fig. 2B). It was also demonstrated that the expression level of EDNRB in HCC was closely connected with the grading and the alcohol addiction ($P < .05$) (Table 3). The analysis by the Kaplan-Meier estimator showed that HCC patients with lower EDNRB expression were more prone to poor prognosis, with statistical significance ($P = .0041$) (Fig. 3).

3.3. The analysis of the EDNRB expression in HCC in TCGA and GEO by meta-analysis

After the retrieval in the GEO database, a total of 22 microarrays of EDNRB expression profiling were included in our study. The retrieval process was listed in Fig. 4, and the features of these 22 microarrays were summarized in Table 4. Of the 22 microarrays, 18 showed that EDNRB was more lowly expressed in HCC tissues than in the normal liver tissues (Fig. 5, Fig. 6). Four microarrays (GSE14323, GSE22405, GSE46408 and GSE57555) displayed that no statistical significance was found in the expression of EDNRB in HCC tissues and the normal tissues (Fig. 5C, F, K, Fig. 6B). The ROC curves of the 22 microarrays were shown in Figs. 7 & 8. In order to comprehensively and systematically explore the EDNRB expression, we combined the microarrays in TCGA and GEO and performed Meta-analysis, including 1905 cases of HCC tissues and 1305 cases of normal liver tissues. The Meta-analysis

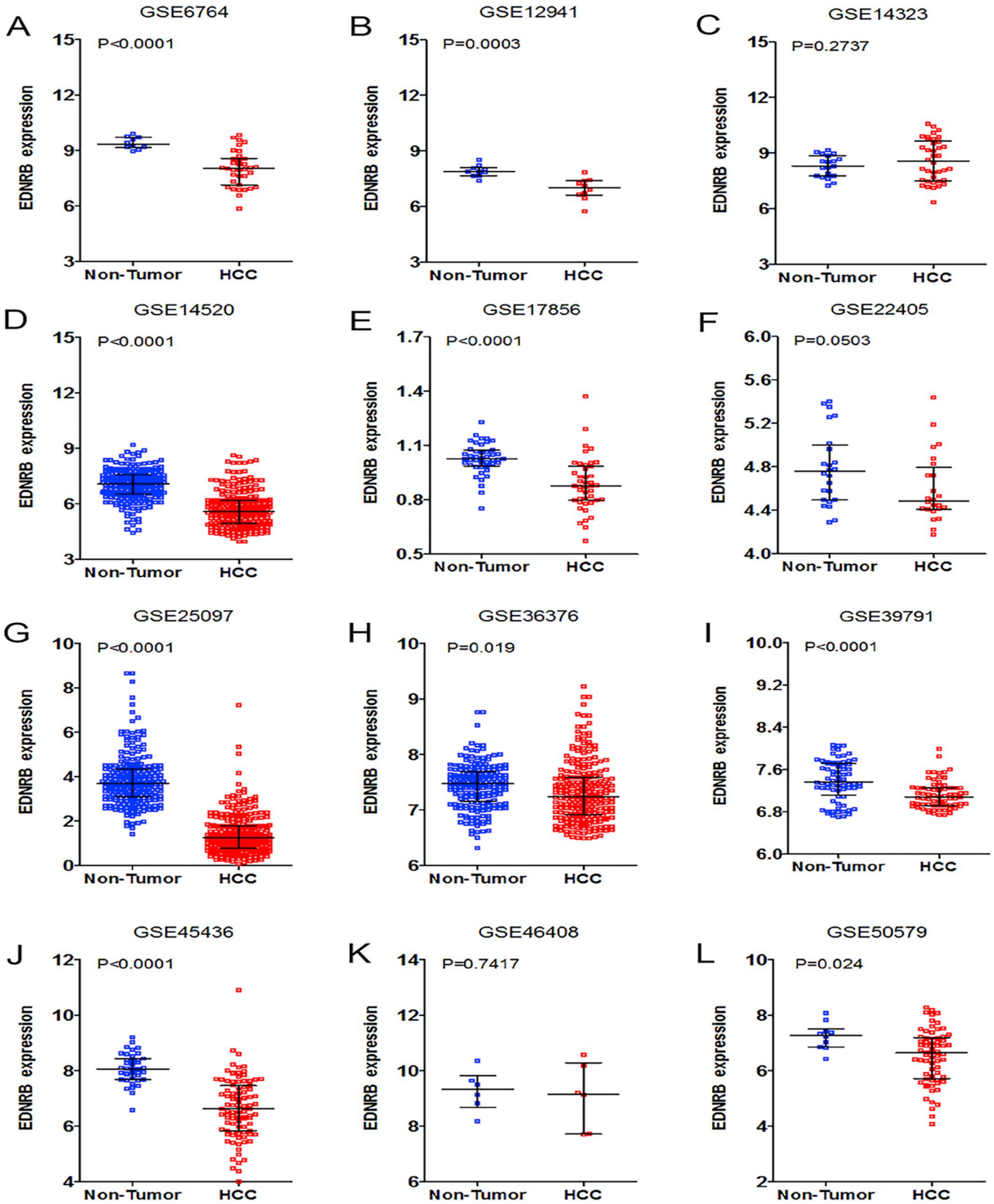

Fig. 5. The expression data of endothelin receptor type B (EDNRB) in hepatocellular carcinoma (HCC) in 12 microarrays from GEO datasets. A: the expression level of EDNRB from GSE6764. B: the expression level of EDNRB from GSE12941. C: the expression level of EDNRB from GSE14323. D: the expression level of EDNRB from GSE14520. E: the expression level of EDNRB from GSE17856. F: the expression level of EDNRB from GSE22405. G: the expression level of EDNRB from GSE25097. H: the expression level of EDNRB from GSE36376. I: the expression level of EDNRB from GSE39791. J: the expression level of EDNRB from GSE45436. K: the expression level of EDNRB from GSE46408. L: the expression level of EDNRB from GSE50579.

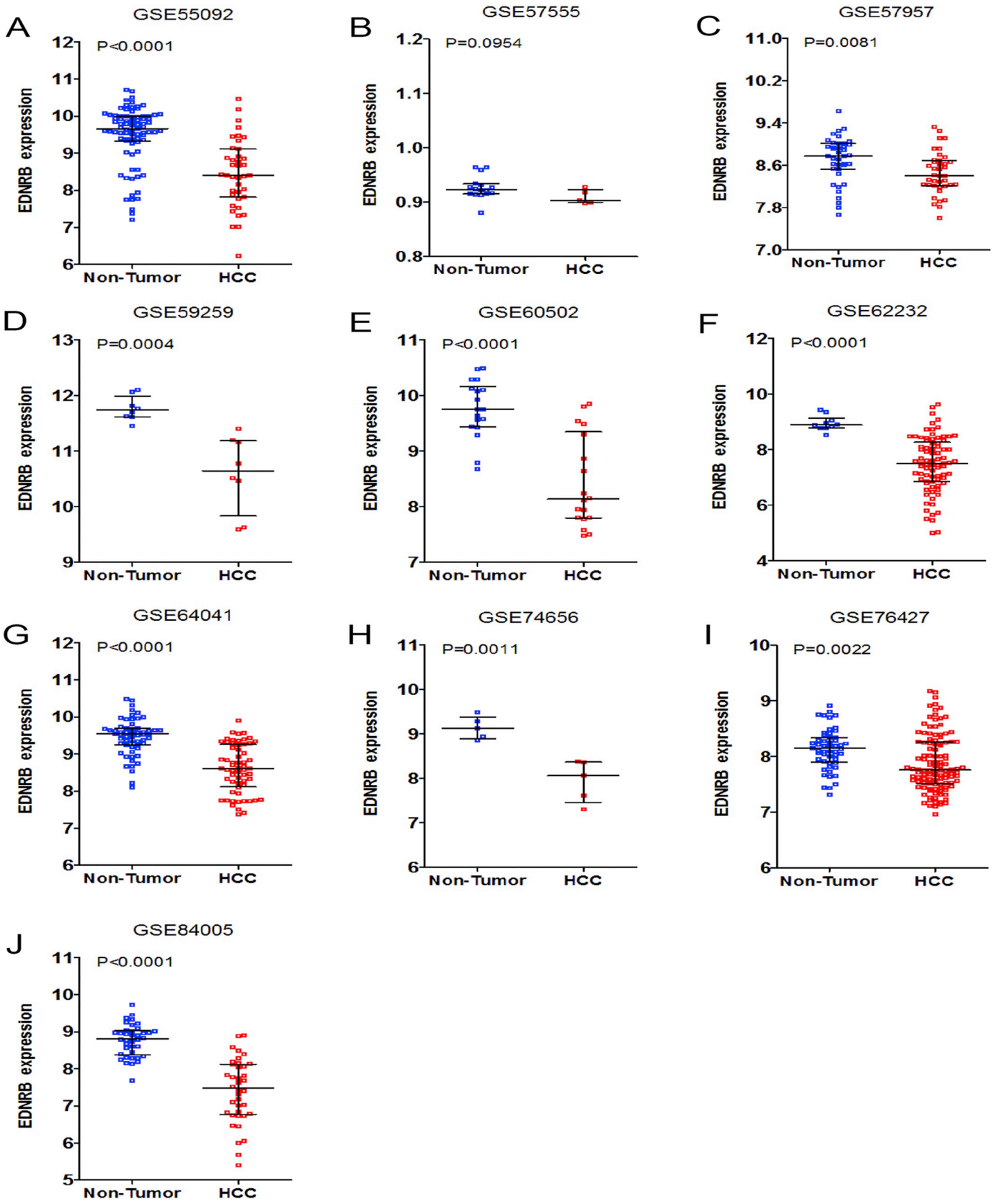

Fig. 6. The expression data of endothelin receptor type B (EDNRB) in hepatocellular carcinoma in 10 microarrays from GEO datasets. A: the expression level of EDNRB from GSE55092. B: the expression level of EDNRB from GSE57555. C: the expression level of EDNRB from GSE57957. D: the expression level of EDNRB from GSE59259. E: the expression level of EDNRB from GSE60502. F: the expression level of EDNRB from GSE62232. G: the expression level of EDNRB from GSE64041. H: the expression level of EDNRB from GSE74656. I: the expression level of EDNRB from GSE76427. J: the expression level of EDNRB from GSE84005.

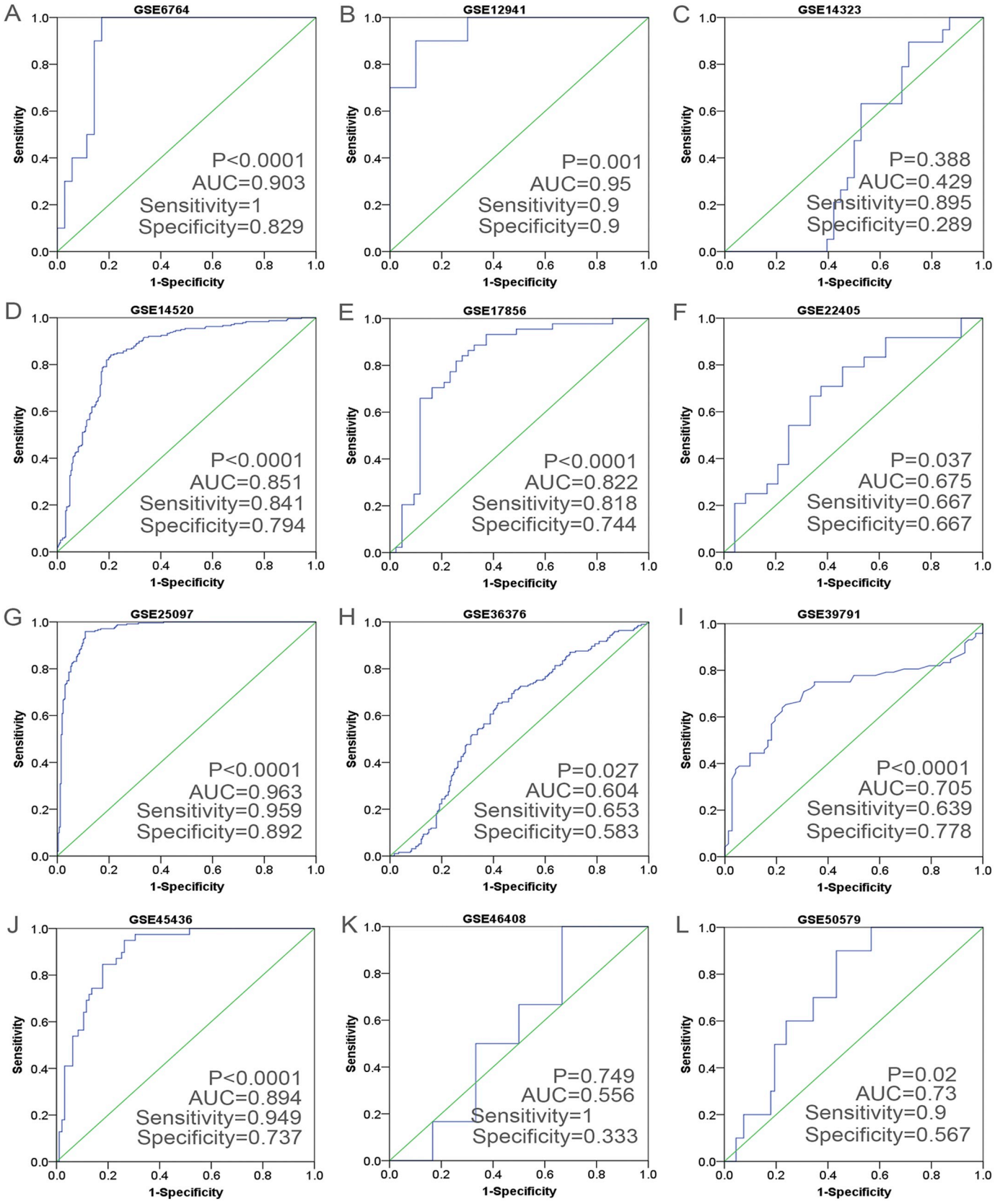

Fig. 7. The receiver operating characteristic (ROC) curves of endothelin receptor type B (EDNRB) in hepatocellular carcinoma in 12 microarrays from GEO datasets. A: the ROC curve of EDNRB from GSE6764. B: the ROC curve of EDNRB from GSE12941. C: the ROC curve of EDNRB from GSE14323. D: the ROC curve of EDNRB from GSE14520. E: the ROC curve of EDNRB from GSE17856. F: the ROC curve of EDNRB from GSE22405. G: the ROC curve of EDNRB from GSE25097. H: the ROC curve of EDNRB from GSE36376. I: the ROC curve of EDNRB from GSE39791. J: the ROC curve of EDNRB from GSE45436. K: the ROC curve of EDNRB from GSE46408. L: the ROC curve of EDNRB from GSE50579.

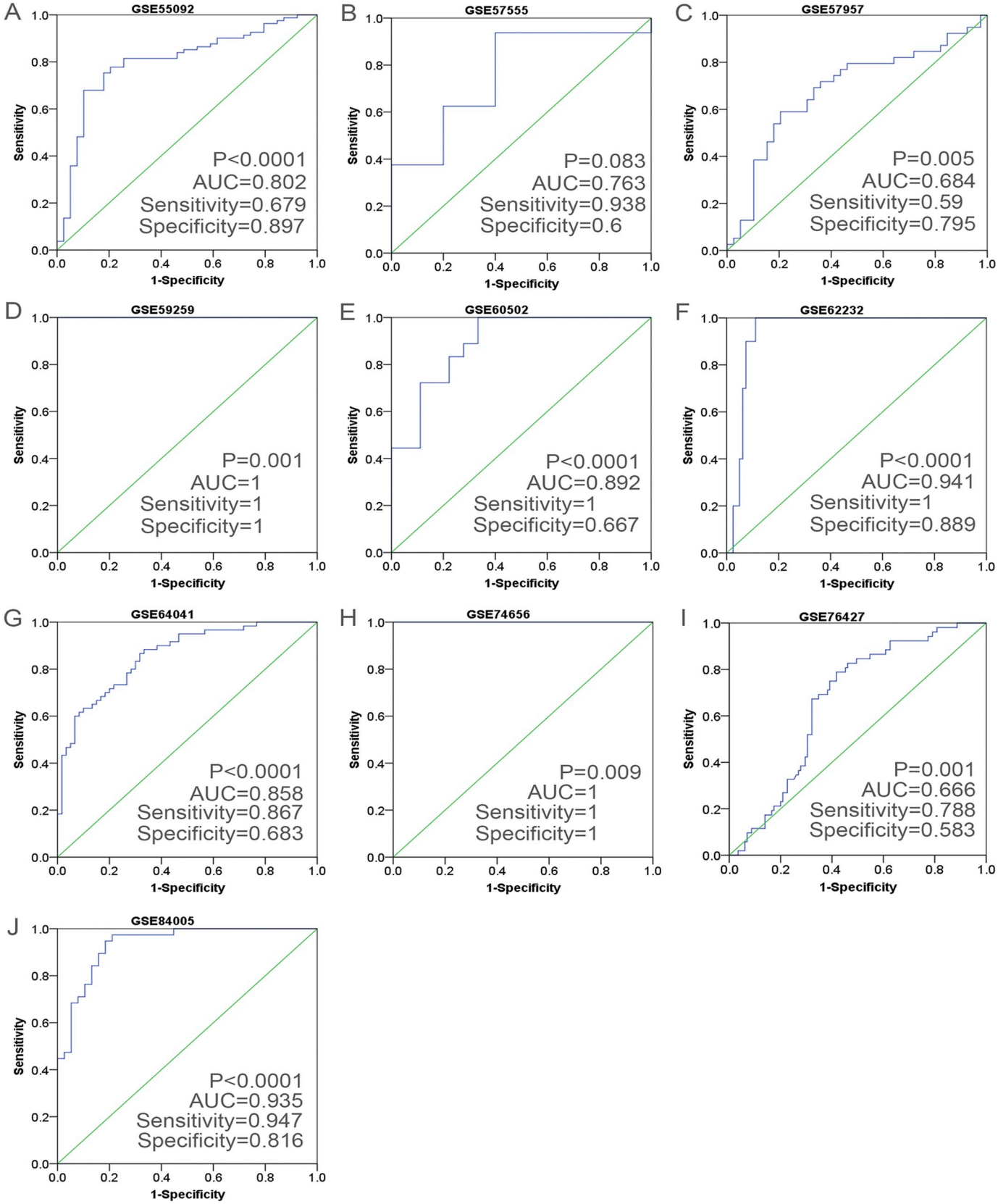

Fig. 8. The receiver operating characteristic (ROC) curves of endothelin receptor type B (EDNRB) in hepatocellular carcinoma in 10 microarrays from GEO datasets. A: the ROC curve of EDNRB from GSE55092. B: the ROC curve of EDNRB from GSE57555. C: the ROC curve of EDNRB from GSE57957. D: the ROC curve of EDNRB from GSE59259. E: the ROC curve of EDNRB from GSE60502. F: the ROC curve of EDNRB from GSE62232. G: the ROC curve of EDNRB from GSE64041. H: the ROC curve of EDNRB from GSE74656. I: the ROC curve of EDNRB from GSE76427. J: the ROC curve of EDNRB from GSE84005.

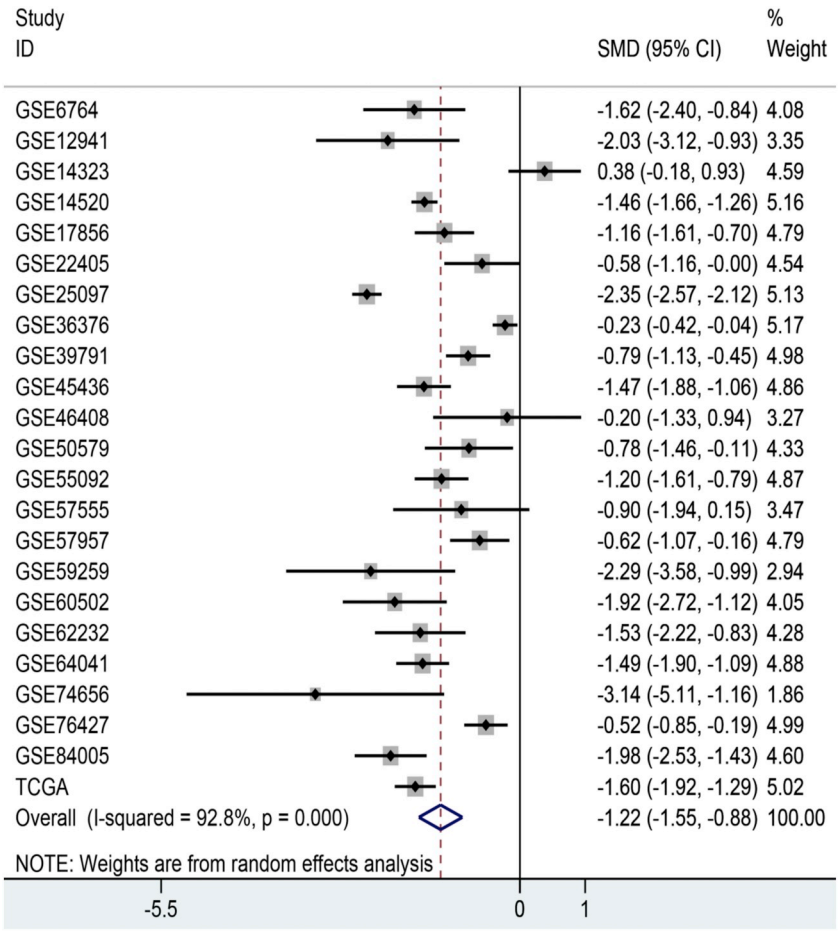

Fig. 9. Forest plot of studies evaluating standard mean difference of endothelin receptor type B expression between hepatocellular carcinoma group and non-tumor group based on TCGA and GEO databases.

revealed SMD = −1.22 (95%CI: −1.55−(−0.88)), which confirmed that the expression of EDNRB was lower in HCC tissues than in normal controls (Fig. 9), due to SMD < 0 and 95%CI not containing 0. The examination of heterogeneity demonstrated the existence of heterogeneity in the Meta-analysis ($P_{heterogeneity} < 0.0001$, $I^2 = 92.8\%$; Fig. 9); hence we preferred the random effect model. The sensitivity analysis showed that after the removal of 9 microarrays (GSE14323, GSE14520, GSE22405, GSE25097, GSE36376, GSE39791, GSE46408, GSE57957 and GSE76427), SMD = −1.48 (95%CI: −1.63−(−1.33)), and no obvious heterogeneity was discovered ($P_{heterogeneity} = 0.116$, $I^2 = 32.4\%$; Fig. 10A, B). No publication bias was found in the Meta-analysis of EDNRB expression, owing to the $P = .791$ by Egger's test, $P = .635$ by Begg's test and the symmetrical distribution in the funnel plot (Fig. 11).

We used SROC curves to calculate the AUC and 95%CI in order to evaluate the capacity of EDNRB expression to differentiate the tumor tissues from the non-tumor ones. As Fig. 12 showed, the AUC of the EDNRB expression was 0.88 (95%CI: 0.85–0.91), and the corresponding sensitivity and specificity was 0.75 (95%CI: 0.69–0.81) and 0.88 (95%CI: 0.82–0.93), respectively.

3.4. EDNRB related genes

By the cBioPortal for Cancer Genomics, we selected the EDNRB related genes whose absolute value of the Spearman correlation coefficient was more than or equal to 5. Finally, a total of 196 EDNRB related genes were acquired (Table 5).

3.5. The fuctional annotation of EDNRB related genes, pathway enrichment analysis and PPI network construction

GO annotation and KEGG pathway enrichment analysis by DAVID database were carried out to enquire into the functions of EDNRB related genes and their molecular mechanism, the top ten results were listed in Table 6. The GO analysis unveiled that in the biological process the EDNRB related genes mainly participated in vasculature development, blood vessel development, cardiovascular system development, etc. In the category of cellular component, EDNRB related genes were largely enriched in actin filament, network-forming collagen trimer and collagen network. In the molecular function, EDNRB genes were found to take part in the molecular processes like transmembrane receptor protein kinase activity, growth factor binding, transmembrane receptor protein tyrosine kinase activity, etc. KEGG analysis revealed that EDNRB related genes were mostly involved in the pathways of Vascular

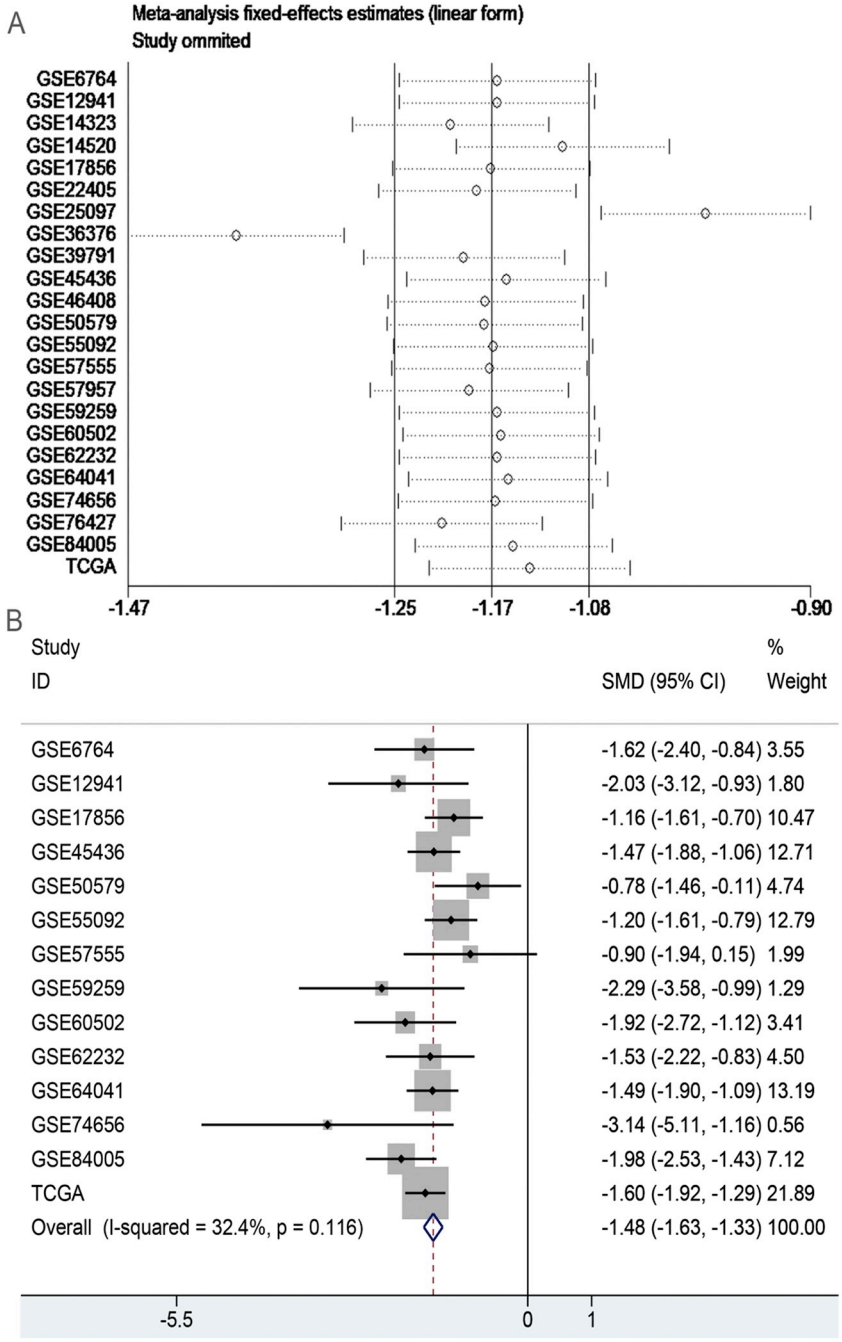

Fig. 10. Sensitivity analysis and the forest plot after sensitivity analysis. A: sensitivity analysis of Meta-analysis of the expression level of endothelin receptor type B (EDNRB) in hepatocellular carcinoma (HCC) group and in non-tumor group based on TCGA and GEO databases. B: forest plot of EDNRB expression between HCC group and non-tumor group after removing the study of GSE14323, GSE14520, GSE22405, GSE25097, GSE36376, GSE39791, GSE46408, GSE57957 and GSE76427.

smooth muscle contraction, cGMP-PKG signaling pathway, Focal adhesion, etc. By STRING database, we constructed the PPI network of 196 EDNRB-related genes (Fig. 13).

4. Discussion

The endothelin receptor type B (EDNRB), a member of the G protein-coupled receptors, was located on human chromosome 13q22.3 (Ayala-Valdovinos et al., 2016; Bregar et al., 2018; Lo et al., 2002; Zhang and Sui, 2014). Previous studies demonstrated that the abnormal methylation of EDNRB and its aberrant expression of mRNA were detected in various malignancies, and also EDNRB was found to participate in the onset and development of malignant tumors (Chen et al., 2013; Mousavi Ardehaie et al., 2017; Schussel et al., 2013; Xu et al., 2016). For instance, in the nasopharyngeal cancer, Lo et al. discovered the higher methylation of EDNRB in the tumor tissues rather than in normal nasopharynx tissues (Lo et al., 2002). Zhou et al. also confirmed that higher level of methylation was observed in the tissues of nasopharyngeal cancer than in the tissues of chronic nasopharyngitis, and the downregulated expression of EDNRB was detected in the tissues of nasopharyngeal carcinoma and the nasopharyngeal carcinoma cell line (Zhou et al., 2007).In esophageal squamous cell carcinoma, Zhao et al. found that the mRNA of EDNRB was more remarkably lowly expressed in tumor tissues than in normal controls; in addition, increased

EDNRB methylation was detected in tumor tissues (Zhao et al., 2009). In oral squamous cell carcinoma, Viet et al. suggested that high methylation of EDNRB was associated with the pain caused by the cancer (Viet et al., 2011). Hsiao et al. demonstrated that patients with leukemia tended to have the highly methylated EDNRB promoter (Hsiao et al., 2008).In gastric cancer, the result of Tao et al. showed that the increased methylation of EDNRB was closely related with the infiltration and metastasis of the gastric carcinoma, indicating that EDNRB might play a vital part in the pathogenesis of the gastric cancer (Tao et al., 2012). In colorectal cancer, research by Chen et al. suggested that the highly methylated promoter of EDNRB downregulated the its mRNA expression and took part in the initiation and progression of the colorectal carcinoma (Chen et al., 2013). Besides, Mousavi et al. confirmed that the aberrant methylation of EDNRB could be used as the potential diagnostic marker of the colorectal cancer (Mousavi Ardehaie et al., 2017). Despite the abovementioned studies, we researchers were still less informed of the clinical significance of EDNRB in HCC and the molecular mechanism.

In this study, we applied IHC to investigate the expression of EDNRB in the 67 cases of HCC tissues and the corresponding controls, discovering that EDNRB was more lowly expressed in HCC tissues than in the adjacent ones. Also, we found no relationship was established between the expression of EDNRB and the clinicopathologic parameters of patients; however, the expression of EDNRB seemed associated with the amount of AFP in HCC patients, which required larger samples for validation. Subsequently, the analysis by TCGA databases showed that lower expression of EDNRB was detected in HCC tissues than in the normal liver tissues. The relationship analysis revealed that the expression of EDNRB correlated with the tumor grading and alcohol addiction. By the Kaplan-Meier estimator, it was uncovered that the HCC patients who had lowly expressed EDNRB was more likely to suffer bad prognosis. For a more comprehensive understanding of the expression of EDNRB, we conducted Meta-analysis of each microarray in TCGA and GEO after combination, demonstrating that HCC tissues exhibited lower expression of EDNRB than the normal liver tissues did.

The results above confirmed the abnormal expression of EDNRB in HCC and its potential clinical significance. In order to explore its potential molecular mechanism in HCC, for the EDNRB related genes, we performed GO annotation and pathway enrichment analysis, finding that the top terms of GO annotation in biological process, cellular component and molecular function were vasculature development, actin filament and transmembrane receptor protein kinase activity, respectively. The KEGG pathway enrichment analysis confirmed that EDNRB related genes mainly participated in Vascular smooth muscle contraction, cGMP-PKG signaling pathway and Focal adhesion pathways. The PPI network construction showed that KDR, VEGFC, FLT1, CDH5 and ADCY4 were possible to become the core genes of EDNRB related genes, which need further experiments to confirm.

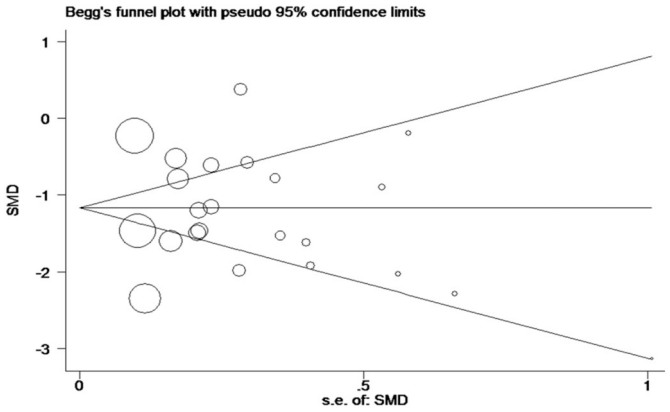

Fig. 11. Funnel plot for publication bias test after Meta-analysis of the expression level of endothelin receptor type B based on TCGA and GEO databases.

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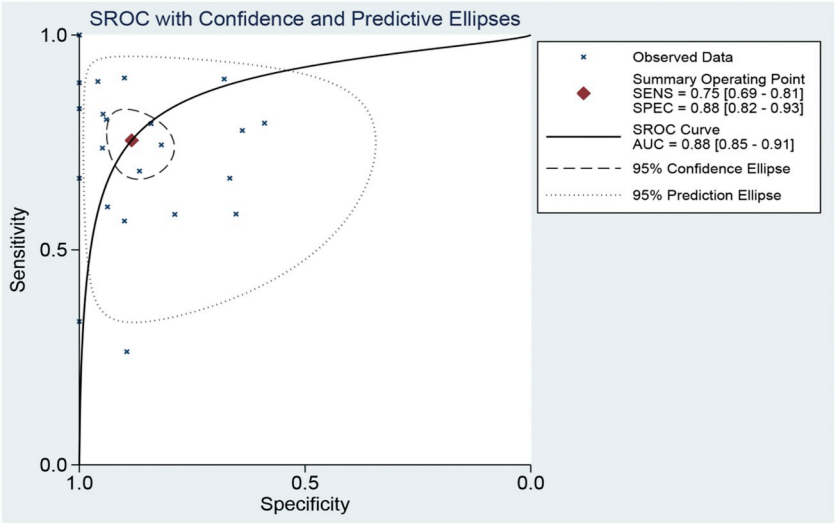

Fig. 12. The summary receiver operating characteristic curves analysis of endothelin receptor type B for discriminating hepatocellular carcinoma from normal liver tissues based on TCGA and GEO databases.

Table 5
196 EDNRB related genes.

Gene names						
CDH5	EFNB2	NID2	CCDC85A	EPHA3	PGM5	KLHL20
PTPRB	TMEM204	LHFPL6	ADAMTS1	MYOCD	IL33	GPR4
ST6GALNAC3	CDC42EP3	TMEM233	MEIS3P1	DOCK9	LAMA2	PLAT
ADGRF5	SOX7	ITGA9	ARHGEF15	SLCO2A1	FBN1	SOX17
CALCRL	FAM107A	SYNPO2	SH2D3C	NEXN	SPARCL1	TLCD1
LDB2	ADGRL4	ZNF462	GUCY1A2	USHBP1	KLF9	MTMR6
ERG	ZNF521	ARL15	PCDH12	LRRC70	PDE7B	TSEN54
CYYR1	ARHGAP31	FILIP1L	BMX	C7	PLCL1	TMEM100
MYCT1	EPHA4	TCF21	MMRN1	PRKCH	NOS3	PODXL
KDR	RHOJ	CD93	JAM3	RGCC	IRAK3	FRMD3
TEK	RGS5	JAM2	CALHM5	FGD5	OMD	HEPH
S1PR1	TAL1	RUNX1T1	CCM2L	FXYD6	HCG11	CASQ2
FAM198B	EMP1	GJD3	ZEB1	NPR1	CALD1	DLC1
APOLD1	GIMAP8	TIMP3	PALM2-AKAP2	EXOC3L2	DAAM2	PRR16
PDE2A	MEF2C	MAP1B	F2R	ADCY4	AQP1	ZEB2
ETS1	PLEKHG1	PDE10A	ARHGAP6	COL12A1	COL4A1	LINC00924
SHROOM4	PRKG1	BCL6B	SH3BP5	CACNA1C	SYTL2	ZNF423
EMCN	SNRK	CD34	MYLK	RASGRF2	IGFBP5	TMEM47
MMRN2	SPRY1	FMO2	IGFBP7	IL1RL1	FSTL1	NHSL2
HECW2	RBMS3	SASH1	ITPRIP	GJA1	FHL1	TSHZ3
ZFPM2	CLEC14A	TM4SF18	SCARF1	NRP2	PCDH17	SLIT3
NOTCH4	ESAM	THBD	EPAS1	VEGFC	ARHGAP23	TSPAN18
TCF4	ADAMTSL1	ELK3	GALNT15	KL	LMOD1	GJA4
FLT4	PEAR1	HDGFL3	AOC3	SRL	LRRC32	ELN
FILIP1	PDGFD	PCDHGB7	SPARC	PPP1R12B	ARFGAP1	ZDHHC15
FLT1	CAVIN2	CDH13	KLHL4	HSPA12B	ENG	PLVAP
TIE1	GIMAP6	TGFBR2	AKAP2	COL8A1	C3ORF70	GIMAP7
CLEC1A	ZNF366	STEAP4	ROBO4	ADRA2B	RGMA	MRVI1

Note: EDNRB, endothelin receptor type B.

Table 6
The GO annotation and KEGG pathway enrichment analysis of the 196 EDNRB related genes.

ID	Term	Count	%	P Value	Genes
Biological process					
GO:0001944	Vasculature development	49	25.5	2.03E-29	RHOJ, NRP2, MEF2C, EMCN, GJA1, ELK3, AQP1, GJA4, GPR4, CDH5, etc.
GO:0001568	Blood vessel development	46	24	2.17E-27	NRP2, MEF2C, EMCN, GJA1, ELK3, AQP1, GJA4, GPR4, CDH5, MMRN2, etc.
GO:0072358	Cardiovascular system development	56	29.2	2.83E-27	NRP2, DLC1, MEF2C, RHOJ, GJA1, AQP1, GJA4, MMRN2, S1PR1, MYOCD, etc.
GO:0072359	Circulatory system development	56	29.2	2.83E-27	NRP2, DLC1, MEF2C, RHOJ, GJA1, AQP1, GJA4, MMRN2, S1PR1, MYOCD, etc.
GO:0048514	Blood vessel morphogenesis	42	21.9	5.03E-26	NRP2, EMCN, GJA1, ELK3, AQP1, GPR4, MMRN2, TAL1, TCF21, S1PR1, etc.
GO:0001525	Angiogenesis	38	19.8	1.52E-24	NRP2, EMCN, ELK3, AQP1, GPR4, MMRN2, TAL1, TCF21, S1PR1, TEK, etc.
GO:0048646	Anatomical structure formation involved in morphogenesis	49	25.5	1.25E-17	NRP2, DLC1, MEF2C, EMCN, SOX7, ELK3, AQP1, GPR4, MMRN2, TAL1, etc.
GO:0009653	Anatomical structure morphogenesis	74	38.5	2.59E-17	RHOJ, MEF2C, NRP2, DLC1, GJA1, ZEB1, AQP1, MMRN2, SPRY1, S1PR1, etc.
GO:0051239	Regulation of multicellular organismal process	70	36.5	1.36E-14	MEF2C, NRP2, GJA1, ZEB1, AQP1, PRKG1, MMRN2, SPRY1, S1PR1, MYOCD, etc.
GO:1901342	Regulation of vasculature development	22	11.5	8.12E-14	SASH1, FLT1, TGFBR2, EFNB2, NPR1, SPARC, AQP1, GPR4, KDR, MMRN2, etc.
Cellular component					
GO:0005886	Plasma membrane	89	46.4	2.97E-09	RHOJ, DLC1, NRP2, STEAP4, ADCY4, PEAR1, ADGRF5, GJA1, AQP1, PRKG1, etc.
GO:0071944	Cell periphery	90	46.9	3.91E-09	RHOJ, DLC1, NRP2, STEAP4, ADCY4, PEAR1, ADGRF5, GJA1, AQP1, PRKG1, etc.
GO:0031012	Extracellular matrix	21	10.9	3.07E-07	PLAT, COL4A1, ADAMTSL1, SPARCL1, IL1RL1, IGFBP7, FBN1, ELN, SPARC, NID2, etc.
GO:0044459	Plasma membrane part	53	27.6	4.10E-07	DLC1, STEAP4, PEAR1, GJA1, GJA4, AQP1, S1PR1, NOS3, TIE1, CALCRL, etc.
GO:0044420	Extracellular matrix component	11	5.7	5.71E-07	LAMA2, COL4A1, ELN, FBN1, COL12A1, ADAMTS1, NID2, SPARC, COL8A1, TIMP3, etc.
GO:0005578	Proteinaceous extracellular matrix	16	8.3	3.12E-06	COL4A1, ADAMTSL1, SPARCL1, IL1RL1, FBN1, ELN, SPARC, NID2, TIMP3, MMRN2, etc.
GO:0005604	Basement membrane	9	4.7	4.53E-06	LAMA2, COL4A1, FBN1, ADAMTS1, NID2, SPARC, COL8A1, TIMP3, MMRN2
GO:0009986	Cell surface	23	12	6.91E-06	PLAT, EMCN, GJD3, IL1RL1, TGFBR2, ADGRF5, PLVAP, SPARC, CDH5, RGMA, etc.
GO:0031226	Intrinsic component of plasma membrane	35	18.2	5.33E-05	C7, STEAP4, LRRC32, GJA1, AQP1, GJA4, GPR4, SLCO2A1, S1PR1, TEK, etc.
GO:0005887	Integral component of plasma membrane	34	17.7	5.80E-05	C7, STEAP4, LRRC32, GJA1, AQP1, GJA4, GPR4, SLCO2A1, TEK, TIE1, etc.
Molecular function					
GO:0019199	Transmembrane receptor protein kinase activity	10	5.2	9.88E-08	NRP2, EPHA4, FLT1, FLT4, TGFBR2, TEK, TIE1, ENG, KDR, EPHA3
GO:0019838	Growth factor binding	11	5.7	4.82E-07	NRP2, COL4A1, FLT1, KL, FLT4, IGFBP7, TGFBR2, TEK, ENG, KDR, etc.
GO:0004714	Transmembrane receptor protein tyrosine kinase activity	8	4.2	3.12E-06	NRP2, EPHA4, FLT1, FLT4, TEK, TIE1, KDR, EPHA3
GO:0005021	Vascular endothelial growth factor-activated receptor activity	4	2.1	3.06E-05	NRP2, FLT1, FLT4, KDR
GO:0005515	Protein binding	128	66.7	1.39E-04	MEF2C, DLC1, ADCY4, PRR16, FSTL1, KLHL4, AQP1, MMRN1, PRKG1, MMRN2, etc.
GO:0005509	Calcium ion binding	19	9.9	2.15E-04	SPARCL1, FBN1, PCDHGB7, PCDH12, FSTL1, SPARC, NID2, PCDH17, MMRN1, CDH5, etc.
GO:0004713	Protein tyrosine kinase activity	9	4.7	3.80E-04	NRP2, EPHA4, FLT1, FLT4, TEK, BMX, TIE1, KDR, EPHA3
GO:0005488	Binding	155	80.7	4.16E-04	DLC1, MEF2C, ADCY4, PRR16, FSTL1, KLHL4, PRKG1, MMRN1, AQP1, MMRN2, etc.
GO:0004672	Protein kinase activity	17	8.9	6.24E-04	NRP2, FLT1, FLT4, TGFBR2, BMX, PRKCH, NPR1, PRKG1, KDR, EPHA3, etc.
GO:0016773	Phosphotransferase activity, alcohol group as acceptor	18	9.4	0.00164	NRP2, FLT1, KL, FLT4, TGFBR2, BMX, PRKCH, NPR1, PRKG1, KDR, etc.
KEGG pathway					
hsa04270	Vascular smooth muscle contraction	11	5.7	2.27E-07	ADCY4, CALD1, PPP1R12B, MRVI1, GUCY1A2, PRKCH, NPR1, CALCRL, PRKG1, CACNA1C, etc.
hsa04022	cGMP-PKG signaling pathway	11	5.7	4.89E-06	MEF2C, ADCY4, PDE2A, MRVI1, GUCY1A2, NPR1, NOS3, ADRA2B, PRKG1, CACNA1C, etc.
hsa04510	Focal adhesion	10	5.2	1.88E-04	LAMA2, VEGFC, ITGA9, COL4A1, FLT1, FLT4, PPP1R12B, PDGFD, MYLK, KDR
hsa04921	Oxytocin signaling pathway	8	4.2	9.40E-04	MEF2C, ADCY4, PPP1R12B, GUCY1A2, NPR1, NOS3, CACNA1C, MYLK
hsa04151	PI3K-Akt signaling pathway	11	5.7	0.00206	LAMA2, VEGFC, ITGA9, COL4A1, FLT1, FLT4, TEK, NOS3, PDGFD, KDR, etc.
hsa04015	Rap1 signaling pathway	8	4.2	0.00473	VEGFC, ADCY4, FLT1, FLT4, TEK, PDGFD, KDR, F2R
hsa04014	Ras signaling pathway	8	4.2	0.00702	VEGFC, FLT1, RASGRF2, ETS1, FLT4, TEK, PDGFD, KDR
hsa04611	Platelet activation	6	3.1	0.00937	ADCY4, GUCY1A2, NOS3, PRKG1, MYLK, F2R
hsa04540	Gap junction	5	2.6	0.01132	ADCY4, GUCY1A2, GJA1, PDGFD, PRKG1
hsa04514	Cell adhesion molecules (CAMs)	6	3.1	0.01339	ITGA9, CD34, ESAM, JAM2, JAM3, CDH5

Note: EDNRB, endothelin receptor type B; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

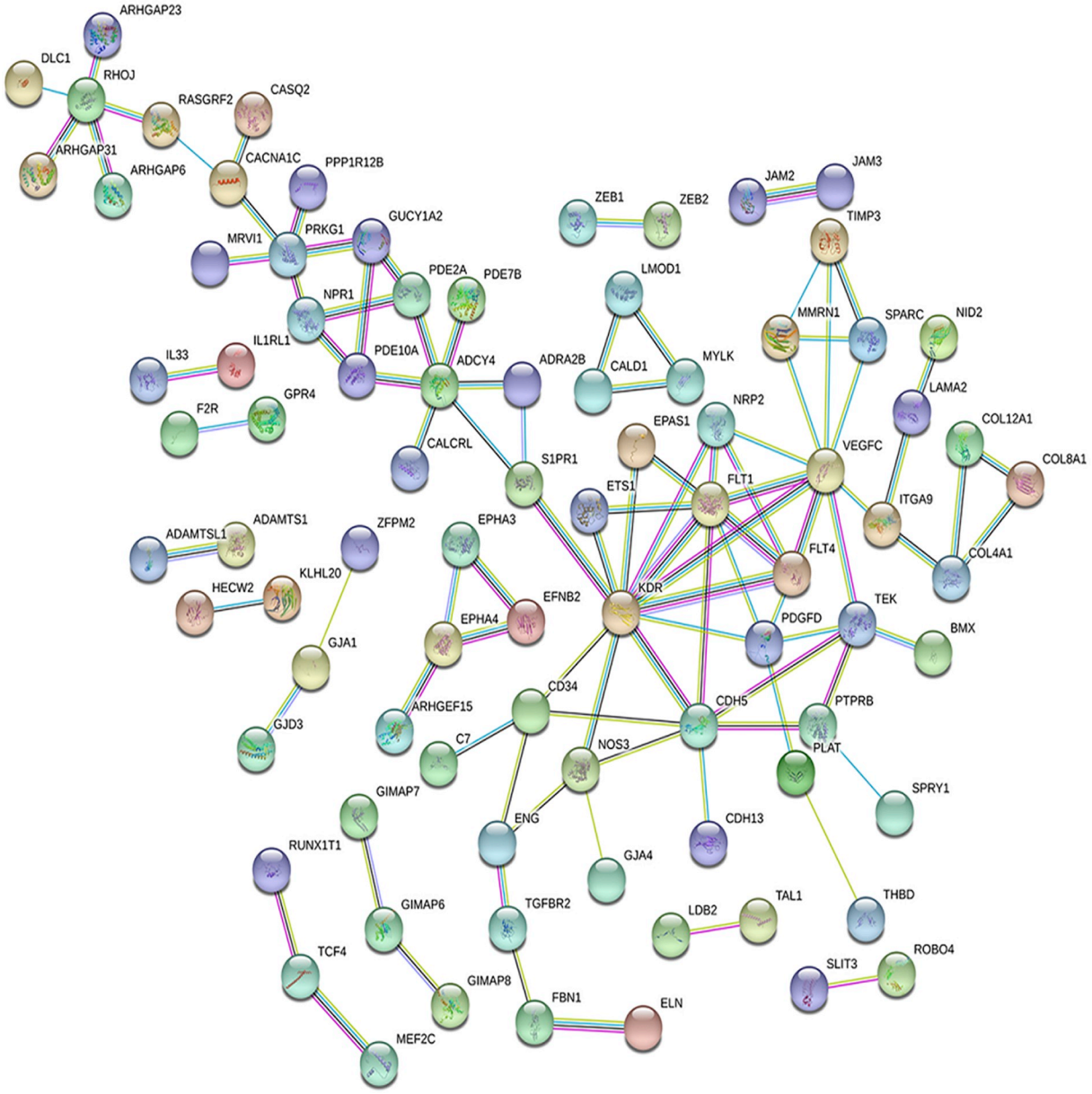

Fig. 13. Protein-Protein Interaction network of the 196 correlated genes of endothelin receptor type B constructed by Search Tool for the Retrieval of Interacting Genes online database, nodes represent proteins and edges represent protein-protein associations.

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Conflict of interest

The authors declare no conflict of interest.

References

Amicone, L., Marchetti, A., 2018. Microenvironment and tumor cells: two for new molecular therapies of hepatocellular carcinoma. Transl. Gastroenterol. Hepatol. 3, 24.

Ayala-Valdovinos, M.A., Galindo-Garcia, J., Sanchez-Chipres, D., Duifhuis-Rivera, T., 2016. New test for endothelin receptor type B (EDNRB) mutation genotyping in horses. Mol. Cell. Probes 30, 182–184.

Ayuso, C., Rimola, J., Vilana, R., Burrel, M., Darnell, A., Garcia-Criado, A., et al., 2018. Diagnosis and staging of hepatocellular carcinoma (HCC): current guidelines. Eur. J. Radiol. 101, 72–81.

Brandi, G., De Lorenzo, S., Candela, M., Pantaleo, M.A., Bellentani, S., Tovoli, F., et al., 2017. Microbiota, NASH, HCC and the potential role of probiotics. Carcinogenesis 38, 231–240.

Bregar, D., Cilensek, I., Mankoc, S., Reschner, A., Petrovic, D., Globocnik Petrovic, M., 2018. The joint effect of the endothelin receptor B gene (EDNRB) polymorphism rs10507875 and nitric oxide synthase 3 gene (NOS3) polymorphism rs869109213 in Slovenian patients with type 2 diabetes mellitus and diabetic retinopathy. Bosn J. Basic Med. Sci. 18, 80–86.

Chen, C., Wang, L., Liao, Q., Huang, Y., Ye, H., Chen, F., et al., 2013. Hypermethylation of EDNRB promoter contributes to the risk of colorectal cancer. Diagn. Pathol. 8, 199.

Chen, L., Guo, P., He, Y., Chen, Z., Chen, L., Luo, Y., et al., 2018. HCC-derived exosomes elicit HCC progression and recurrence by epithelial-mesenchymal transition through MAPK/ERK signalling pathway. Cell Death Dis. 9, 513.

Crocetti, L., Bargellini, I., Cioni, R., 2017. Loco-regional treatment of HCC: current status. Clin. Radiol. 72, 626–635.

Danaher, P., Warren, S., Lu, R., Samayoa, J., Sullivan, A., Pekker, I., et al., 2018. Pan-cancer adaptive immune resistance as defined by the tumor inflammation signature (TIS): results from The Cancer Genome Atlas (TCGA). J. Immunother. Cancer. 6, 63.

Dhanasekaran, R., Gabay-Ryan, M., Baylot, V., Lai, I., Mosley, A., Huang, X., et al., 2018. Anti-miR-17 therapy delays tumorigenesis in MYC-driven hepatocellular carcinoma (HCC). Oncotarget 9, 5517–5528.

Foerster, F., Mittler, J., Darstein, F., Heise, M., Marquardt, J.U., Worns, M.A., et al., 2018. Recipient liver function before liver transplantation influences post-transplantation survival in patients with HCC. Eur. J. Intern. Med. https://doi.org/10.1016/j.ejim.2018.05.024.

Fu, J., Wang, H., 2018. Precision diagnosis and treatment of liver cancer in China. Cancer Lett. 412, 283–288.

Gao, J., Aksoy, B.A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S.O., et al., 2013. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 6, l1. https://doi.org/10.1126/scisignal.200408.

Gao, L., Zhang, L.J., Li, S.H., Wei, L.L., Luo, B., He, R.Q., et al., 2018. Role of miR-452-5p in the tumorigenesis of prostate cancer: a study based on the Cancer Genome Atl (TCGA), Gene Expression Omnibus (GEO), and bioinformatics analysis. Pathol. Res. Pract. 214, 732–749.

Hsiao, P.C., Liu, M.C., Chen, L.M., Tsai, C.Y., Wang, Y.T., Chen, J., et al., 2008. Promoter methylation of p16 and EDNRB gene in leukemia patients in Taiwan. Chin. J. Physiol. 51, 27–31.

Hutter, C., Zenklusen, J.C., 2018. The Cancer Genome Atlas: creating lasting value beyond its data. Cell 173, 283–285.

Katsura, Y., Takeda, Y., Ohmura, Y., Sakamoto, T., Kawai, K., Inatome, J., et al., 2017. Evaluation of the occurrence of surgical site infection(SSI)after hepatectomy in elderly patients with hepatocellular carcinoma(HCC). Gan. To Kagaku Ryoho. 44, 1668–1670.

Kim, D.W., Talati, C., Kim, R., 2017. Hepatocellular carcinoma (HCC): beyond sorafenib-chemotherapy. J. Gastrointest. Oncol. 8, 256–265.

Lanczky, A., Nagy, A., Bottai, G., Munkacsy, G., Szabo, A., Santarpia, L., et al., 2016. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. Breast Cancer Res. Treat. 160, 439–446.

Li, J., Huang, L., Yan, J., Qiu, M., Yan, Y., 2018a. Liver resection for hepatocellular carcinoma: personal experiences in a series of 1330 consecutive cases in China. ANZ J. Surg. https://doi.org/10.1111/ans.14381.

Li, T., Gao, X., Han, L., Yu, J., Li, H., 2018b. Identification of hub genes with prognostic values in gastric cancer by bioinformatics analysis. World J. Surg. Oncol. 16, 114.

Li, T., Zhu, Y.Y., Ji, Y., Zhou, S., 2018c. Interfering RNA with multi-targets for efficient gene suppression in HCC cells. Int. J. Mol. Med. 41, 3604–3610.

Liu, N., Jiang, Y.Y., Huang, T.T., Hou, J.C., Liu, J.X., 2018. A network pharmacology approach to explore mechanisms of Buyang Huanwu decoction for treatment of cerebral infarction. Zhongguo Zhong Yao Za Zhi. 43, 2190–2198.

Lo, K.W., Tsang, Y.S., Kwong, J., To, K.F., Teo, P.M., Huang, D.P., 2002. Promoter hypermethylation of the EDNRB gene in nasopharyngeal carcinoma. Int. J. Cancer 98, 651–655.

Mao, J., Yang, H., Cui, T., Pan, P., Kabir, N., Chen, D., et al., 2018. Combined treatment with sorafenib and silibinin synergistically targets both HCC cells and cancer stem cells by enhanced inhibition of the phosphorylation of STAT3/ERK/AKT. Eur. J. Pharmacol. 832, 39–49.

Morimoto, N., Mutai, H., Namba, K., Kaneko, H., Kosaki, R., Matsunaga, T., 2018. Homozygous EDNRB mutation in a patient with Waardenburg syndrome type 1. Auris Nasus Larynx 45, 222–226.

Mousavi Ardehaie, R., Hashemzadeh, S., Behrouz Sharif, S., Ghojazadeh, M., Teimoori-Toolabi, L., Sakhinia, E., 2017. Aberrant methylated EDNRB can act as a potential diagnostic biomarker in sporadic colorectal cancer while KISS1 is controversial. Bioengineered 8, 555–564.

Mu, T., 2017. Study of the Inhibition of the Migration and Invasion of Hepatocellular Carcinoma Cells caused by EDNRB Gene and Identification and Interaction Analysis of Key Genes and microRNA in Hepatocellular Carcinoma. Chongqing Medical University Master's Thesis (In Chinese).

National Health Commission of the People's Republic of China, 2017. Primary liver cancer diagnosis and treatment specification (2017 edition). Chin. J. Prac. Surg. 37, 705–720 In Chinese.

Ozakyol, A., 2017. Global epidemiology of hepatocellular carcinoma (HCC epidemiology). J. Gastrointest. Cancer. https://doi.org/10.1007/s12029-017-9959-0.

Reig, M., da Fonseca, L.G., Faivre, S., 2018. New trials and results in systemic treatment of HCC. J. Hepatol. 69, 525–533.

Schussel, J., Zhou, X.C., Zhang, Z., Pattani, K., Bermudez, F., Jean-Charles, G., et al., 2013. EDNRB and DCC salivary rinse hypermethylation has a similar performance as expert clinical examination in discrimination of oral cancer/dysplasia versus benign lesions. Clin. Cancer Res. 19, 3268–3275.

Shiina, S., Sato, K., Tateishi, R., Shimizu, M., Ohama, H., Hatanaka, T., et al., 2018. Percutaneous ablation for hepatocellular carcinoma: comparison of various ablation techniques and surgery. Can. J. Gastroenterol. Hepatol. https://doi.org/10.1177/1534735418775819.

Song, X., Tan, S., Wu, Z., Xu, L., Wang, Z., Xu, Y., et al., 2018. HBV suppresses ZHX2 expression to promote proliferation of HCC through miR-155 activation. Int. J. Cancer. https://doi.org/10.1002/ijc.3159.

Sun, L., Fahey, P., Zhu, X., Ng, W., Chen, Z.P., Qiu, Y., et al., 2018. A cohort study to examine the use of Chinese herbal medicine in combination with conventional therapies for patients with hepatocellular carcinoma in China. Integr. Cancer Ther. https://doi.org/10.1155/2018/4756147.

Tao, K., Wu, C., Wu, K., Li, W., Han, G., Shuai, X., et al., 2012. Quantitative analysis of promoter methylation of the EDNRB gene in gastric cancer. Med. Oncol. 29, 107–112.

Viet, C.T., Ye, Y., Dang, D., Lam, D.K., Achdjian, S., Zhang, J., et al., 2011. Re-expression of the methylated EDNRB gene in oral squamous cell carcinoma attenuates cancer-induced pain. Pain 152, 2323–2332.

Widowati, T., Melhem, S., Patria, S.Y., de Graaf, B.M., Sinke, R.J., Viel, M., et al., 2016. RET and EDNRB mutation screening in patients with Hirschsprung disease: functional studies and its implications for genetic counseling. Eur. J. Hum. Genet. 24, 823–829.

Wu, Z.F., Wang, Y., Yang, P., Hou, J.Z., Zhang, J.Y., Hu, Y., et al., 2018. Toll-like receptor 4 and its associated proteins as prognostic factors for HCC treated by post-radiotherapy surgery. Oncol. Lett. 15, 9599–9608.

Xu, C., Xiao, H.W., Zhang, L.H., Wu, P., Fang, Y., Li, D.W., et al., 2016. Detection and screen of pulmonary metastasis-related signature genes in nasopharyngeal carcinoma. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 51, 891–895.

Xu, W., Yu, J., Wong, V.W., 2017. Mechanism and prediction of HCC development in HBV infection. Best Pract. Res. Clin. Gastroenterol. 31, 291–298.

Xu, X., Chen, Y., Zhang, R., Miao, X., Chen, X., 2018. Activation of anti-tumor immune response by ablation of HCC with nanosecond pulsed electric field. J. Clin. Transl. Hepatol. 6, 85–88.

Yao, Z., Xiong, Z., Li, R., Liang, H., Jia, C., Deng, M., 2018. Long non-coding RNA NRON is downregulated in HCC and suppresses tumour cell proliferation and metastasis. Biomed. Pharmacother. 104, 102–109.

Zhang, L., Sui, R., 2014. Effect of SNP polymorphisms of EDN1, EDNRA, and EDNRB gene on ischemic stroke. Cell Biochem. Biophys. 70, 233–239.

Zhao, B.J., Sun, D.G., Zhang, M., Tan, S.N., Ma, X., 2009. Identification of aberrant promoter methylation of EDNRB gene in esophageal squamous cell carcinoma. Dis. Esophagus 22, 55–61.

Zheng, B., Zhu, Y.J., Wang, H.Y., Chen, L., 2017. Gender disparity in hepatocellular carcinoma (HCC): multiple underlying mechanisms. Sci. China Life Sci. 60, 575–584.

Zhou, L., Feng, X., Shan, W., Zhou, W., Liu, W., Wang, L., et al., 2007. Epigenetic and genetic alterations of the EDNRB gene in nasopharyngeal carcinoma. Oncology 72, 357–363.