ORIGINAL RESEARCH

Prognostic value of Notch-1 expression in hepatocellular carcinoma: a meta-analysis

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Abstract: Association of Notch-1 expression with prognosis of patients with hepatocellular carcinoma (HCC) remains controversial. We conducted a meta-analysis to reevaluate the association of Notch-1 expression with clinicopathological characteristics and prognosis of HCC. PubMed, Embase, Web of Science, and China National Knowledge Infrastructure were searched to look for relevant studies. The association between Notch-1 expression and clinico-pathological parameters and overall survival (OS) was then reassessed using the meta-analysis for odds ratio (OR) or hazard ratio (HR) and 95% confidence interval (CI). A total of seven studies, including 810 HCC patients, were eligible for the meta-analysis. Our data showed that high Notch-1 expression was able to predict poor OS (HR 1.50, 95% CI 1.17–1.83, P=0.0001). The pooled OR showed that high Notch-1 expression was significantly associated with tumor metastasis (OR 0.37, 95% CI 0.16–0.86, P=0.02) and tumor size >5 cm (OR 0.48, 95% CI 0.26–0.88, P=0.02). In contrast, there was no association between high Notch-1 expression and tumor differentiation, late TNM stage, tumor number, and portal vein invasion of HCC. In conclusion, Notch-1 overexpression might predict poorer survival and more aggressive behavior in patients with HCC.

Keywords: hepatocellular carcinoma, Notch-1, prognosis, clinicopathological features, meta-analysis

Introduction

Liver cancer is a lethal and aggressive neoplasm representing the fifth most commonly diagnosed malignancy in men and the ninth in women globally; it is also a leading cause of cancer-related death.[1,2] Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma account for the majority of primary liver malignancies, in which HCC represents the major histological subtype (>80% of primary liver cancers).[3] HCC is well characterized as a highly refractory malignancy associated with rapid tumor progression and metastasis, resulting in a 5-year survival rate as low as 16%.[4] Therefore, efforts to identify new additional prognostic and predictive markers should be made to improve individual treatment strategies and prognosis in HCC.

Accumulated evidence shows that the Notch signaling pathways play a significant role in HCC progression, and could be used as therapeutic targets for the patients. A variety of potential therapies exist to modulate Notch signaling in HCC, including neutralizing antibodies, siRNAs, and miRNA therapies, which target specific Notch tumorigenic subunits.[5] Indeed, the Notch signaling is a highly evolutionarily conserved pathway that regulates cell fate, proliferation, apoptosis, differentiation, and survival, and dysregulation of this pathway is the basis of different diseases, including cancer.[6] However, mounting evidence indicates that the Notch signaling pathway can exert oncogenic or tumor-suppressive action in various cancers.[7] For example,

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the oncogenic potential of the Notch activation is implicated in breast, colorectal, and melanoma cancers. On the other hand, it can also be tumor suppressive in HCC, as well as in head and neck squamous cell carcinoma.[8] Furthermore, the Notch pathway consists of four transmembrane receptors (Notch-1, -2, -3, and -4) and five ligands that include the Jagged family (Jagged1 and Jagged2) and Delta-like family (DLL1, 3, and 4) in mammals.[9] When these ligands bind to Notch receptors, a γ-secretase complex then mediates the transmembrane domain cleavage of the Notch receptor and the Notch intracellular domain (NICD). The released NICD then translocates into the nucleus and functions as a transcriptional coactivator to drive the expression of target genes.[6] As an important receptor of Notch family, Notch-1 was first discovered through its involvement in T-cell acute lymphoblastic leukemia and T-cell leukemogenesis. To date, numerous studies have shown that high Notch-1 expression is associated with progression and prognosis of various tumors, including breast cancer,[10,11] non-small-cell lung cancer,[12,13] esophageal cancer,[14] colorectal cancer,[15,16] and ovarian cancer.[17]

Although Notch-1 is implicated in clinicopathological characteristics and prognosis of HCC, other studies[18,19] did report conflicting results. Therefore, it remains unknown whether this discrepancy is caused by limited sample sizes or genuine heterogeneity. In this study, a meta-analysis was performed to assess the relationship between Notch-1 expression and clinicopathological parameters and clinical outcomes of HCC.

Materials and methods
Literature search strategy
This meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[20] The following electronic databases were systematically searched: PubMed, Embase, Web of Science, and China National Knowledge Infrastructure. The search was conducted on June 1, 2015 and was limited to papers published in English and Chinese. Studies were selected using the combinations of the following search terms: (Notch OR Notch-1 OR Notch 1) AND (hepatocellular carcinoma OR hepatoma OR HCC OR liver cancer) AND (prognosis OR prognostic OR outcome OR mortality OR survival). To obtain some unavailable data from the eligible articles, we contacted some of the authors. The bibliographies of articles were also manually examined to identify additional studies. Two authors conducted the search and assessed the eligibility of studies independently. Any disagreements were resolved by iteration, discussion, and consensus between the two reviewers.

Study selection
The inclusion criteria were as follows: 1) cancer patients who were pathologically confirmed; 2) Notch-1 expression was evaluated in HCC tissues; 3) Notch-1 expression was examined by immunohistochemistry (IHC) or polymerase chain reaction (PCR); 4) studies analyzed the association of Notch-1 expression with HCC clinicopathological parameters or prognosis; 5) studies of prognosis association provided sufficient information to estimate hazard ratio (HR) for overall survival (OS) or disease-free survival (DFS) and 95% confidence interval (CI); 6) sample size was more than 20 cases; 7) if there were multiple articles overlapping the same cohorts, only the most complete article was included. The exclusion criteria were as follows: 1) publication was of non-research articles; 2) studies had duplicate data or lack of key information for further analysis; 3) studies were based on animal or human cell lines.

Data extraction
All eligible publications were reviewed, and then data were extracted by two independent authors (Min-Cong Wang and Qing Li). The extracted data were then summarized in a consistent manner to prevent bias. The following information was extracted: name of the first author, year of publication, study population characteristics (patient number, age, sex), clinicopathological parameters (tumor size, metastasis, vein invasion, stage, differentiation), follow-up data (OS and DFS, Notch-1 assessment method, and cutoff value of Notch-1. HR was first extracted and synthesized from multivariable analysis where available. If such information was missing, we estimated the HR from Kaplan–Meier curves using the methods reported by Tierney et al.[21]

Qualitative assessment
Li Jing and Qing Li independently assessed the quality of each of the available studies using the Newcastle–Ottawa Quality Assessment Scale (NOS) with our reasonable modifications (Table S1).[22] This scale uses a star system (a score of 0–9) to indicate the quality of each study (Table 1). Studies labeled with six or more stars were considered to be of high quality.

Statistical analysis
All statistical analyses were carried out by Stata 12.0 (Stata Corporation, College Station, TX, USA) and Review

Table 1 Quality of the included studies based on the Newcastle–Ottawa scale

Studies	Selection				Comparability	Outcome			Total score
	Representativeness of the exposed cohort	Selection of the nonexposed cohort	Assessment of exposure	Outcome not present at start of study		Assessment of outcome	Follow-up long enough for outcomes	Adequacy of follow-up	
Zhou et al[18]	—	—	—	—	0	—	—	0	6
Yu et al[26]	—	—	—	—	0	—	—	0	6
Zhang[19]	—	—	—	0	—	0	—	0	5
Ahn et al[30]	—	—	—	—	—	—	—	0	7
Zhang et al[27]	—	—	—	0	0	—	—	0	5
Zhang et al[28]	—	—	—	—	0	—	0	0	5
Pan et al[29]	—	—	—	—	0	—	0	0	5

Notes: *A study can be awarded a maximum of one point for each numbered item within the "Selection" and "Outcome" categories, and a maximum of two points can be given for "Comparability".

Manager 5.2 (Cochrane Collaboration, London, UK). Data on prognostic ability of Notch-1 expression predicting OS were pooled across studies. When these data were not directly provided in the eligible articles, we calculated the HR and its 95% CI from Kaplan–Meier survival curve using Engauge Digitizer version 4.1 (free software downloaded from http://sourceforge.net). The estimated odds ratio (OR) was used to summarize the relationship between Notch-1 expression and the clinicopathological features of patients. In the present study, a combined HR >1 implied a worse prognosis in the group with high Notch-1 expression, while an OR <1 indicated a higher probability of tumor progression in HCC with Notch-1 overexpression. There was no overlap of the 95% CI with 1, with 1 indicating a statistical significance. In the course of data pooling, statistical heterogeneity was performed by using chi-square-based Q-test. The I^2 value indicated the degree of heterogeneity. A P-value <0.10 and/or $I^2 > 50\%$ was considered significant heterogeneity, and a random-effects model (REM) was used. Otherwise, a fixed-effects model was used.[23] Publication bias was assessed by Egger's and Begg's funnel plot test.[24,25]

Results

Characteristics of studied populations

As shown in Figure 1, 125 records of the association of Notch-1 with HCC were identified via database searching. However, 108 studies were excluded because of duplicate reports, studies irrelevant to our aim, or studies without clinical specimens. Of the remaining 17 studies identified for further evaluation, ten were then excluded after full-text assessment due to insufficient data and duplicated cohort of patients. Eventually, seven studies were identified for the final meta-analysis.[18,19,26–30] The major characteristics of the seven studies are summarized in Table 2. Specifically, they included a total of 810 patients from People's Republic of China and Korea with 585 males and 225 females. IHC and real-time PCR were used to detect Notch-1 expression in HCC specimens. The patients' age ranged from 48.5 to 66.7 years. Six studies defined the cutoff value of Notch-1 expression by combining the intensity and percentage, whereas only one study used the staining percentage of Notch-1 expression. DFS was estimated in one study, and OS was presented in four studies. All seven eligible studies evaluated the association of Notch-1 expression with tumor pathological features. HR and 95% CI were directly obtained from four studies, and for the remaining study, they were extrapolated from Kaplan–Meier curves. According to NOS
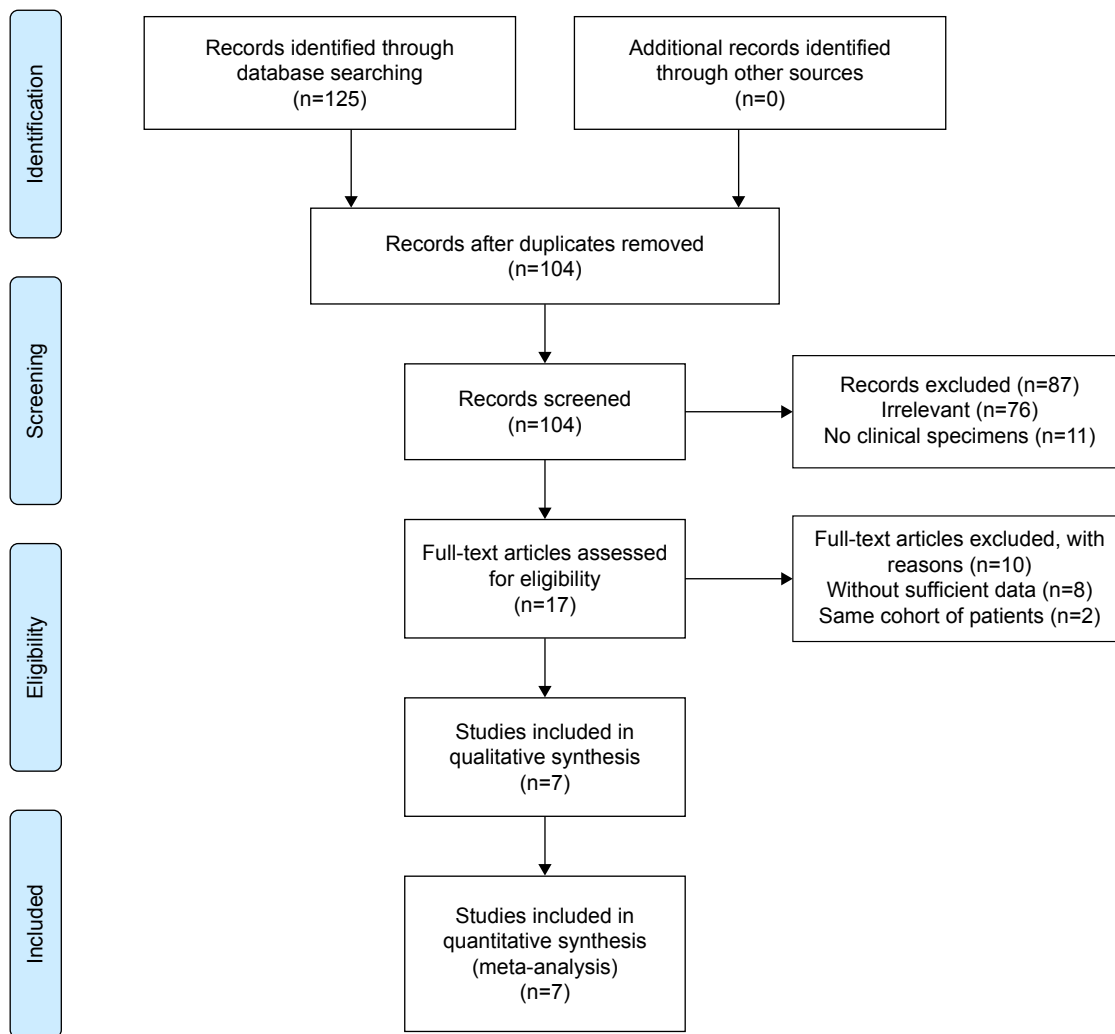
Figure 1 Flow diagram showing study selection procedure.

quality assessment, the scores of included studies ranged from 5 to 7.

Notch-1 expression and OS of HCC patients

Four of the seven studies had estimated association of Notch-1 expression with OS. As shown in Figure 2, pooled data from all these studies suggested that high Notch-1 expression was significantly associated with poor OS. The pooled HR was 1.50 (95% CI 1.17–1.83, $P=0.0001$), indicating that higher Notch-1 expression predicted worse prognosis in HCC. No significant heterogeneity was observed ($I^2=0.0\%$, $P=0.579$), so the fixed-effects model was used for further data analysis.

Correlation of Notch-1 expression and clinicopathological features

Six studies reported the association of TNM stage with Notch-1 expression. The data were significantly heterogeneous ($P<0.00001$, $I^2=88\%$). Thus, an REM was used. The pooled OR revealed that high Notch-1 expression was not associated with tumor stages (OR 0.31, 95% CI 0.08–1.15, $P=0.08$; Figure 3A). Three studies investigated the relationship between Notch-1 expression and tumor number.[18,26,27] Their pooled analysis showed that high Notch-1 expression was not connected to tumor number (OR 0.75, 95% CI 0.48–1.16, $P=0.19$; Figure 3B), and no heterogeneity was found among these studies ($P=0.47$, $I^2=0\%$). All studies described Notch-1 expression according to tumor size. Heterogeneity was observed among these studies ($P=0.001$, $I^2=72\%$). The results showed that Notch-1 overexpression was associated with larger tumor (size >5 cm: OR 0.48, 95% CI 0.26–0.88, $P=0.02$ using REM; Figure 3C).

Four studies investigated association of Notch-1 expression with HCC metastasis[18,19,26,28] and showed that high Notch-1 expression was significantly associated with tumor metastasis, with a pooled OR estimate of 0.37 (95% CI

Table 2 Baseline characteristics of the studies in the meta-analysis

Studies	Year	Country	Tumor type	Sample size (male/female)	Mean age (years)	Vein invasion (yes/no)	Stage (I–II/III–IV)
Zhou et al[18]	2012	People's Republic of China	HCC	74/46	48.5	26/94	32/88
Yu et al[26]	2014	People's Republic of China	HCC	70/62	50.3	70/62	49/83
Zhang[19]	2010	People's Republic of China	HCC	33/7	NA	NA	31/9
Ahn et al[30]	2013	Korea	HCC	237/51	52.6	13/275	225/63
Zhang et al[27]	2013	People's Republic of China	HCC	74/36	66.7	26/84	60/50
Zhang et al[28]	2012	People's Republic of China	HCC	65/13	NA	NA	35/43
Pan et al[29]	2014	People's Republic of China	HCC	32/10	NA	NA	38/4

Studies	Multivariate analysis	Tumor size (<5 cm/>5 cm)	Analysis method	Evaluation method	Metastasis (yes/no)	Tumor number (single/multiple)	Differentiation (W/M+P)
Zhou et al[18]	Yes	51/69	IHC	CS	38/82	87/33	41/79
Yu et al[26]	Yes	52/80	IHC	CS	68/64	84/48	29/103
Zhang[19]	Yes	10/30	RT-PCR	CS	27/13	NA	9/31
Ahn et al[30]	Yes	190/98	IHC	CS	NA	NA	NA
Zhang et al[27]	Yes	53/57	IHC	CS	NA	57/53	24/86
Zhang et al[28]	No	45/33	IHC	CS	28/50	NA	45/33
Pan et al[29]	No	31/11	IHC	Percentage of positive cells	NA	NA	4/38

Studies	Follow-up (months)	Outcome indexes	Hazard ratio (95% CI)	Notch-1 (H/L)	"High" Notch-1 cutoff level	Notch-1 staining	Study quality[#] (points)
Zhou et al[18]	60	OS	2.09 (1.26–3.45)	64/56	≥5	Cytoplasm membrane	6/9
Yu et al[26]	36	OS	1.39 (1.07–1.81)	72/60	≥5	Cytoplasm membrane	6/9
Zhang[19]	31	OS	1.28 (0.42–4.68)*	20/20	NA	NA	5/9
Ahn et al[30]	97.1	DFS	1.40 (1.03–1.89)	145/143	≥4	Cytoplasm membrane	7/9
Zhang et al[27]	NA	OS	1.88 (1.09–3.26)	48/62	≥5	Cytoplasm membrane	5/9
Zhang et al[28]	NA	NA	NA	52/26	≥3	Cytoplasm	5/9
Pan et al[29]	NA	NA	NA	24/18	>10%	Cytoplasm membrane nucleus	5/9

Notes: *Estimated by survival curves. #Study quality was judged based on the Newcastle–Ottawa Scale (range 1–9).
Abbreviations: HCC, hepatocellular carcinoma; NA, not available; W, well differentiation; M+P, moderate and poor differentiation; IHC, immunohistochemistry; CS, complex scoring; RT-PCR, real-time polymerase chain reaction; H, high expression; L, low expression; OS, overall survival; DFS, disease-free survival.
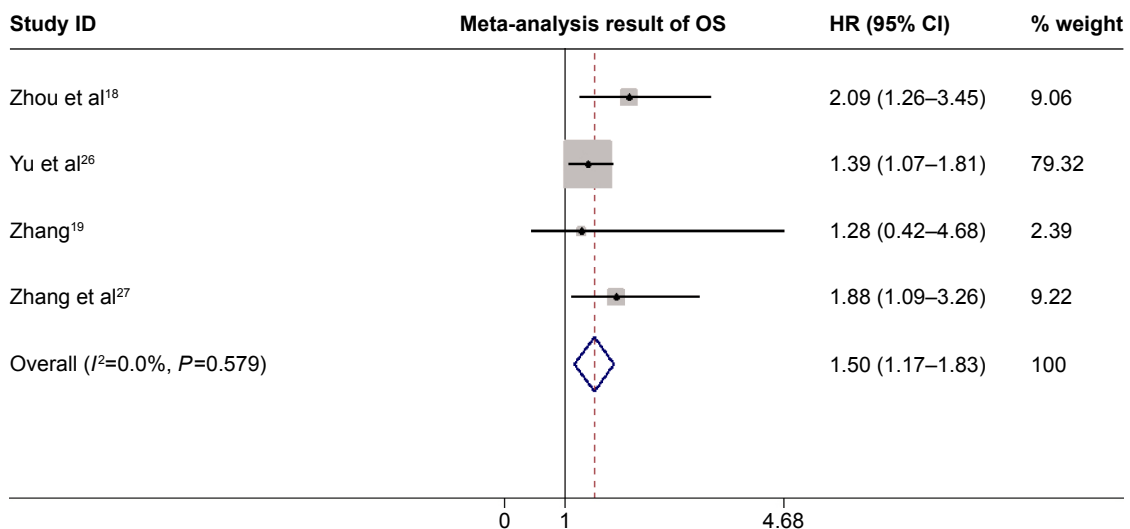

Figure 2 Forest plot of HR for OS of patients with HCC.
Notes: The squares and horizontal lines represent HR and 95% CI. The diamonds represent the pooled HR and 95% CI. The solid vertical line is at the null value.
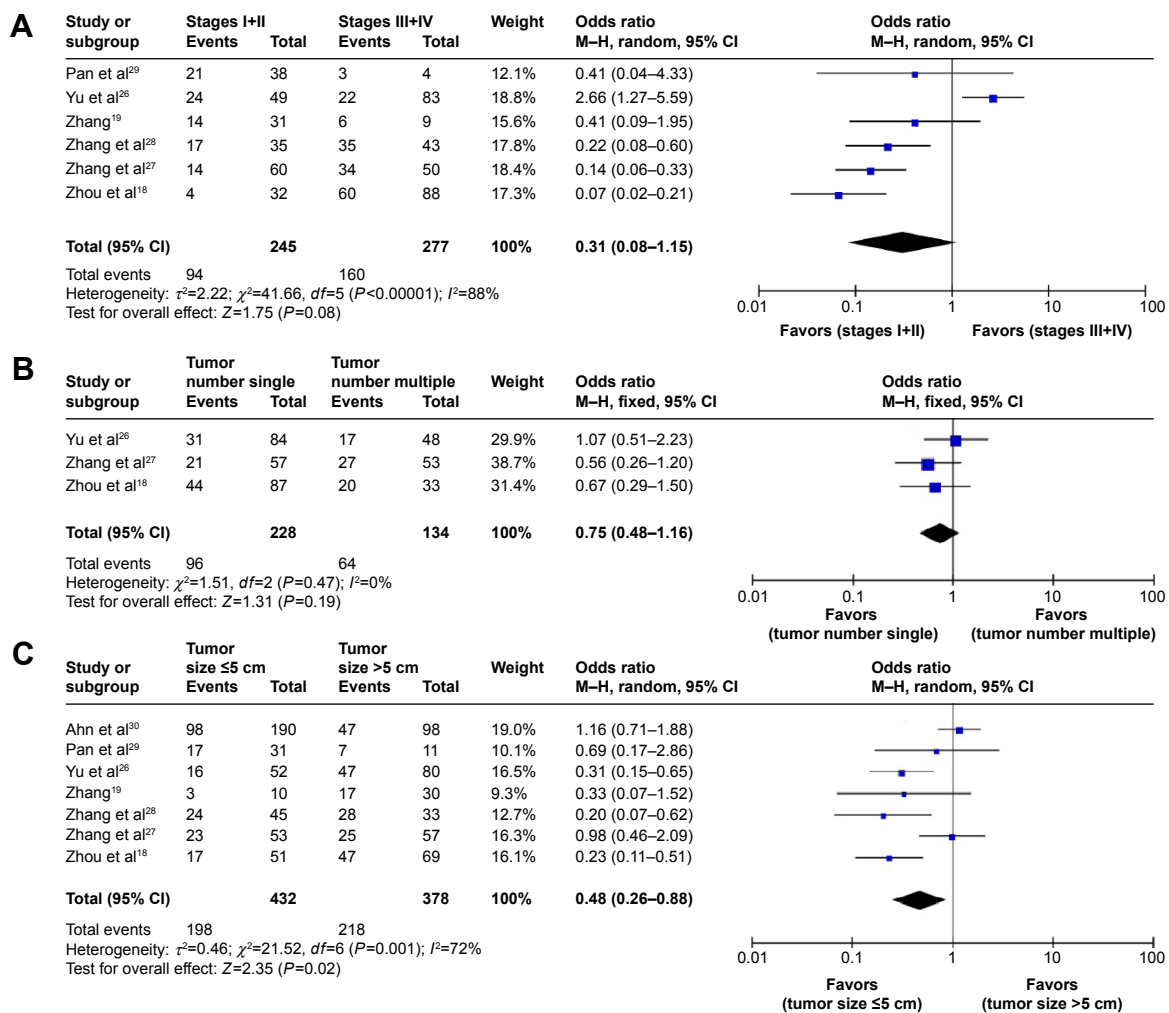**Abbreviations:** HR, hazard ratio; OS, overall survival; HCC, hepatocellular carcinoma; CI, confidence interval.

Figure 3 Association of Notch-1 expression with clinicopathological parameters.

Notes: (**A**) The relationship between high Notch-1 expression and tumor TNM stage. High Notch-1 expression was not associated with TNM stages. (**B**) The association between high Notch-1 expression and tumor number. High Notch-1 expression was not associated with tumor number. (**C**) High Notch-1 expression tended to be associated with tumor size of >5 cm.

Abbreviations: TNM, Tumor, Nodes, and Metastases; CI, confidence interval; M–H, Mantel-Haenszel.

0.16–0.86, P=0.02 using REM; Figure 4A). Four studies assessed the association of Notch-1 expression and portal vein invasion.[18,26,27,30] The combined OR revealed no significant association between high Notch-1 expression and the presence of portal vein invasion (OR 0.46, 95% CI 0.19–1.11, P=0.08; Figure 4B). Remarkable heterogeneity was found among these studies. Five studies investigated Notch-1 expression and tumor differentiation, and the pooled OR revealed that high Notch-1 expression was not associated with tumor differentiation (OR 0.68, 95% CI 0.23–2.01, P=0.48 using REM; Figure 4C).

Sensitivity analysis

Sensitivity analysis was conducted to gauge the stability of the result. As shown in Table 3, when individual studies were removed sequentially, the pooled HR for OS was not significantly altered, suggesting stability of our results.

Publication bias

The reliability of results was evaluated by publication bias estimation. As shown in Figure 5, the symmetrical funnel plots revealed no evidence of publication bias for pooled OS. Furthermore, we also performed Begg's and Egger's tests for more precise assessment (Egger's test, P=0.556; Begg's test, P=1.000), which also implied no publication bias.

Discussion

In recent years, because of the high levels of progression and metastasis of HCC resulting in a dismal prognosis, numerous studies have focused on the underlying molecular mechanism in HCC metastasis. Increasing evidence proves that biomarkers can be helpful in predicting prognosis and guiding surveillance in HCC. However, such useful biomarkers have not been well identified. From a clinical perspective, therefore, it is urgent to identify new additional prognostic
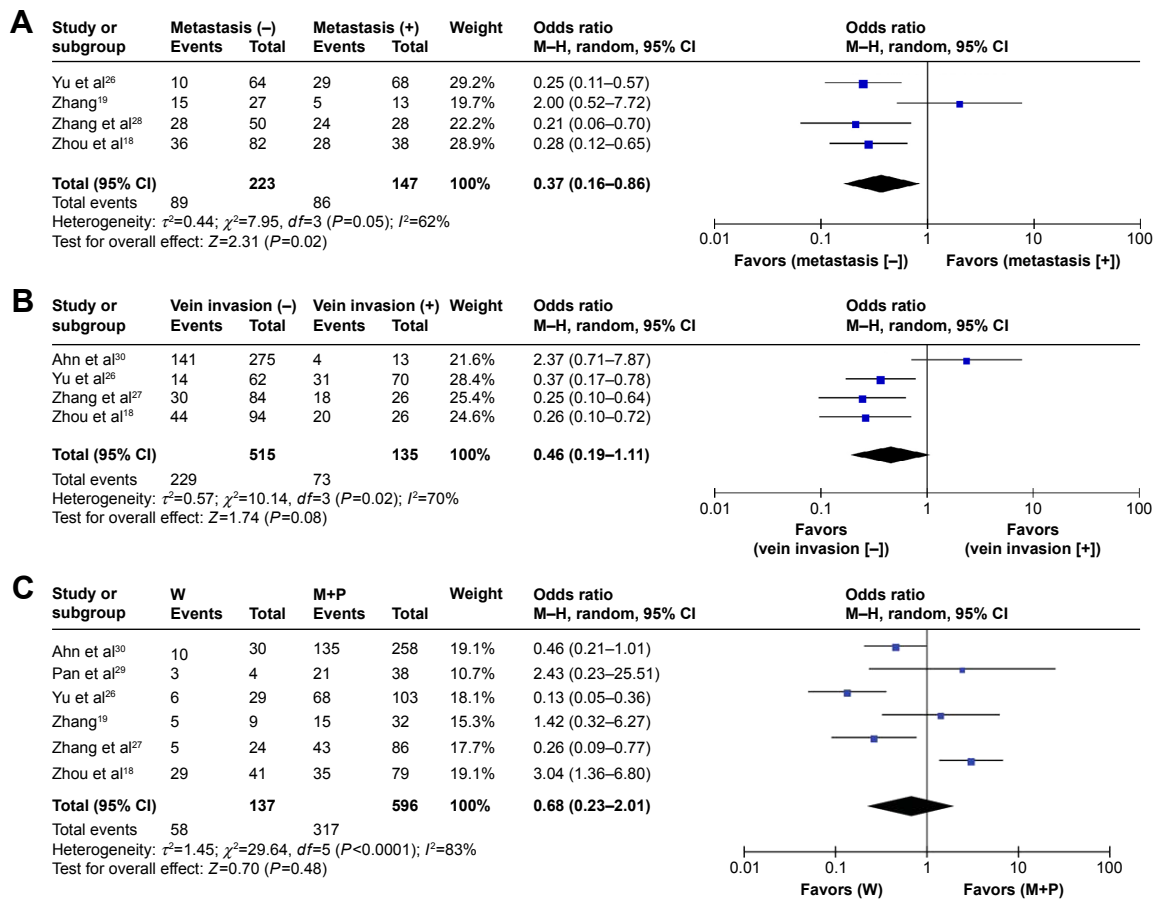
Figure 4 Association of Notch-1 expression with clinicopathological parameters.
Notes: (**A**) High Notch-1 expression was significantly associated with tumor metastasis. (**B**) There was no significant association between high Notch-1 expression and portal vein invasion. (**C**) High Notch-1 expression was not associated with tumor differentiation.
Abbreviations: CI, confidence interval; M–H, Mantel-Haenszel; M+P, moderate and poor differentiation; W, well differentiation.

and predictive markers to improve individual treatment strategies and prognosis. In this study, we introduced a potential candidate biomarker, Notch-1, for prediction of prognosis of HCC.

This study, to our knowledge, is the first meta-analysis to systematically determine the association of Notch-1 expression with clinicopathological features and prognosis of HCC. We first assessed the relationship between Notch-1 expression and OS. The pooled data indicated that high Notch-1 expression significantly predicted poor OS. Indeed, accumulating evidence indicates that the Notch signaling is involved in the initiation and progression of HCC and associated with poor clinical outcomes.[5] Notch-1, as a key receptor of the

Notch signaling, has been reported to regulate liver cancer cell growth and invasion.[31] These may partially explain the aggressive progression and dismal prognosis of HCC with high Notch-1 expression.
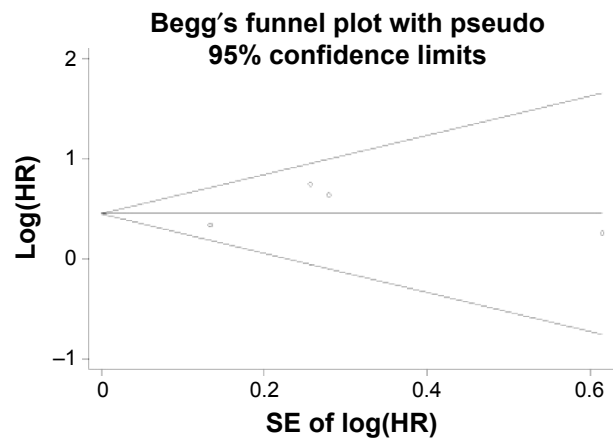

Figure 5 Publication bias analysis.
Notes: Begg's funnel plot of publication bias of HR for OS in the meta-analysis. There was no significant evidence of publication bias observed in this meta-analysis.
Abbreviations: HR, hazard ratio; OS, overall survival; SE, standard error.

Table 3 Sensitivity analysis for OS

Outcome	Study omitted	Resulting HR (95% CI)
OS	Zhou et al[18]	1.43 (1.09–1.78)
OS	Yu et al[26]	1.90 (1.17–2.62)
OS	Zhang[19]	1.50 (1.17–1.83)
OS	Zhang et al[27]	1.45 (1.11–1.80)

Abbreviations: OS, overall survival; HR, hazard ratio; CI, confidence interval.

In this meta-analysis, we also investigated the pooled association between Notch-1 expression and clinicopathological features. The pooled data indicated that higher Notch-1 expression was positively correlated with tumor metastasis of HCC. The results of meta-analysis supported the hypothesis that Notch-1 overexpression might contribute to malignant progression of HCC, which subsequently leads to a poorer prognosis.

Although we conducted a systematical and comprehensive analysis, certain limitations exist and some results need to be cautiously interpreted. We estimated the HR for OS from Kaplan–Meier curves in one original study.[19] These data might be less reliable than direct analysis from the original paper. The variations of the baseline characteristics of patients might have caused inherent heterogeneity within studies, affecting the interpretation of results. The data sets and total sample size are relatively limited, which might have impacted the validity of our analysis. The difference of cutoff value for judging high Notch-1 expression and criteria used to diagnose the stage and grade of HCC may have led to between-study heterogeneity. Other factors, such as the type of primary antibody and dilution, and interobserver variation, may have led to the heterogeneity of IHC studies. Although random-effects modeling and sensitivity analyses were conducted to address this heterogeneity, these statistical methods may not be sufficient. The included studies are all not of the highest quality, so the pooled results of this meta-analysis may have been affected. We assessed the publication bias and did not find significant deviation, but it is worth considering that positive results are the ones that tend to be published; thus, the association between high Notch-1 expression and poor outcome of patients with HCC might have exaggerated our evaluation.

Conclusion

In summary, although certain limitations exist, the results of current study showed that higher Notch-1 expression was associated with poorer prognosis in terms of OS of HCC. Notch-1 could be used as a useful biomarker for prediction of tumor metastasis. However, a future larger prospective study may be needed to validate our current data.

Acknowledgment

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Newcastle–Ottawa quality assessment scale

Selection

1) Representativeness of the exposed cohort
   a) Truly representative of the average HCC patients in the community
   b) Somewhat representative of the average HCC patients in the community
   c) Selected group of users (eg, nurses, volunteers)
   d) No description of the derivation of the cohort

2) Selection of the nonexposed cohort
   a) Drawn from the same community as the exposed cohort
   b) Drawn from a different source
   c) No description of the derivation of the nonexposed cohort

3) Ascertainment of exposure (proof of HCC and Notch-1 measurement)
   a) Secure record (eg, surgical records)
   b) Structured interview
   c) Written self-report
   d) No description

4) Demonstration that outcome of interest was not present at start of study
   a) Yes
   b) No

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) Study controls for recurrence or metastasis
   b) Study controls for any additional factor (age, sex, grade, tumor number, etc)

Outcome

1) Assessment of outcome
   a) Independent blind assessment
   b) Record linkage
   c) Self-report
   d) No description

2) Was follow-up long enough for outcomes to occur
   a) Yes (3 years)
   b) No

3) Adequacy of follow-up of cohorts
   a) Complete follow-up – all subjects accounted for
   b) Subjects lost to follow-up unlikely to introduce bias – small number lost – >25% follow-up, or description provided of those lost
   c) Follow-up rate <75% and no description of those lost
   d) No statement

Notes: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.
Abbreviation: HCC, hepatocellular carcinoma.