

Review Article

Meta analysis for association of TRAIL DR4/TRAIL-R1 and DR5/TRAIL-R2 with hepatocellular carcinoma prognosis

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Abstract: TRAIL may have therapeutic potential in the treatment of human HCC by combining with its death receptors. To study the relationship between TRAIL Death Receptors and the prognosis of hepatocellular carcinoma, we performed a meta-analysis to study the expression of TRAIL DR4/TRAIL-R1, DR5/TRAIL-R2 and various clinicopathological characteristics of HCC. Publications were searched using PubMed, Medline, Embase and the Chinese National Knowledge Infrastructure (CNKI) for studying about the association between TRAIL DR4/TRAIL-R1, DR5/TRAIL-R2 and hepatocellular carcinoma up to October 2015. Totally, seven studies with 423 patients were included into this meta-analysis. Our results revealed that the higher expression of DRs (DR4/TRAIL-R1 and DR5/TRAIL-R2) in the liver tissues of HCC patients may tend to be associated with the well differentiation (pooled OR=0.15, 95% CI=0.06-0.38, Z=3.98, P<0.0001; pooled OR=0.30, 95% CI=0.13-0.70, Z=2.80, P=0.005, respectively); In addition, lower expression of DRs (DR4/TRAIL-R1 and DR5/TRAIL-R2) tended to be associated with advanced tumor TNM stage (pooled OR=4.81, 95% CI=1.68-13.80, Z=2.93, P=0.003; pooled OR=10.49, 95% CI=2.65-41.47, Z=3.35, P=0.0008, respectively); and the relationship between the DR5 and portal vein thrombus of HCC indicated higher DR5 expression was lower in patients with portal vein thrombus than in those without it (pooled OR=0.33, 95% CI=0.12-0.90, Z=2.17, P=0.03). Overall, the results of this study indicated the lower expression of TRAIL Death Receptors tended to indicate a bigger possibility of poor differentiation, advanced TNM stage, and the present of portal vein thrombus, which suggested the lower expression of TRAIL DR4/TRAIL-R1 and DR5/TRAIL-R2 may be related to malignant progression and a poorer prognosis of liver cancer.

Keywords: Hepatocellular carcinoma, TRAIL death receptors, meta-analysis

Introduction

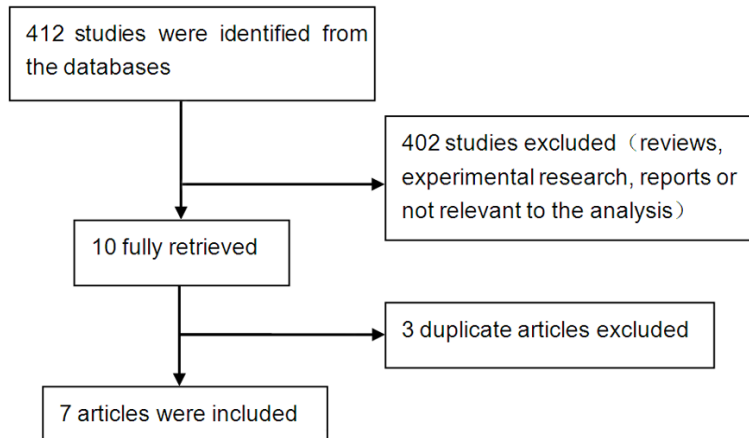
Hepatocellular carcinoma (HCC) is the sixth most common cancer, which is attributed to the third cause of cancer-related mortality in overall global [1]. Prognosis of hepatocellular carcinoma is very poor with a just 14% five-year survival rate because of the rapid process and high malignancy [2, 3]. Lacking of novel therapeutic agents and strategies, HCC is frequently found in late stages. Biological targeted therapy is currently the main objective to improve the efficacy of cancer treatment, targeting the TRAIL receptors, fully realized the potential of TRAIL-induced apoptosis inhibiting tumor progression. Nowadays, using the sorafenib (tyrosine kinase inhibitor) can improve clinical course of HCC patients [4]. The success

may stimulate intensive researches to reveal other different signaling pathways to provide an important method to treat HCC.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) was first discovered and successfully cloned by Wiley in 1995 [5]. TRAIL belonging to the TNF ligand super family, is a better described mechanism of tumor surveillance, with its receptors on the surface of cancer cells. The chemotherapeutic agents can strong TRAIL-induced apoptosis in human hepatocellular carcinoma, prompting TRAIL may have therapeutic potential in the treatment of human HCC [6]. Totally, there are five types of TRAIL receptors. But TRAIL can regulate and activate a series of signal routes ultimately inducing apoptosis through binding to the TRAIL DR4/TRAIL-R1 and DR5/TRAIL-R2 [7]. In the

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Table 1. The studied selected process and included papers



Data extraction and quality assessment

Two independent investigators (L.Z and D.H) extracted all data from eligible studies to minimize the bias, and a third author (XJC) resolved disagreements through discussion. The following were documented from the original articles: the first author's name, the publication year, country, ethnicity, detection methods, number of patients and quality score.

study, we studied the expression of TRAIL Death Receptors and its association with HCC.

Methods

Search strategy

The publications were searched in Medline, Embase, PubMed and the Chinese National Knowledge Infrastructure (CNKI) for assessing the expression of TRAIL DR4/TRAIL-R1, DR5/TRAIL-R2 in liver tissues and the connection with the clinicopathological parameters of HCC (up until October 2015), using the following search terms: ("hepatocellular carcinoma" OR "liver cancer" OR "liver cell carcinoma" or "HCC") AND ("TRAIL death receptors").

Study selection

For inclusion into the meta-analysis, studies had to meet the criteria as the following: (1) original articles directly explored the expression of TRAIL death receptors in HCC and clinicopathological characteristics of HCC combined with TRAIL death receptors; (2) used immunohistochemistry (IHC), RT-PCR or Western blot to examine the expression of TRAIL DRs in HCC tissues; (3) sufficient information was reported to estimate an odds ratio (OR), hazard ratios (HR) and 95% confidence interval (CI).

Studies were excluded as the following: (1) case reports, reviews, letters and editorial articles were refused in the analysis; (2) articles without sufficient data were also excluded (**Table 1**).

The data about the relationship between the HCC and clinicopathological characteristics (tumor differentiation, tumor TNM stage, AFP, portal vein thrombus, tumor size and tumor metastasis) were extracted too. The quality of all selected studies in our analysis was assessed based on the Newcastle-Ottawa Scale (NOS). To guarantee the effectiveness, each study was judged by eight questions with a maximum score of 9, and studies with scores below 6 were considered as low-quality. On the contrary, studies with scores equal or above 6 were considered as high-quality (**Tables 2 and 3**).

Statistical analysis

All analysis was performed by Review Manager 5.2. To estimate the relationship between TRAIL Death Receptors expression and the conditions of HCC, OR and 95% CI were combined and calculated to provide the effective value for the quantitative aggregation of the results. In the process, we used Cochran Q and I^2 statistic to assess the heterogeneity. All data was calculated with a fixed or random effect model, which depended on heterogeneity [8] (a fixed effect model for $I^2 < 50\%$, a random effect model for $I^2 > 50\%$), statistical significance from different studies was defined as a *P* value less than 0.05. The publication bias was assessed by Begg's test and Egger's test.

Results

Study selection and characteristics

Initially we retrieved 412 studies from PubMed, Embase and the Chinese National Knowledge

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Table 2. Basic information about seven studies

Studies	Country	Ethnicity	Study design	Method	N (cancer)	N (para-cancer)	Quality score
He 2003	China	Asian	Case-control	IHC	60	NR	7
Xiao 2005	China	Asian	Case-control	IHC	100	NR	6
Lydia 2010	Germany	Caucasian	Case-control	IHC	157	157	6
Li 2011	China	Asian	Case-control	IHC	30	30	7
Xiao 2006	China	Asian	Case-control	RT-PCR	30	30	6
Lan 2005	China	Asian	Case-control	RT-PCR	40	40	6
Lin 2011	China	Asian	Case-control	Western blot	6	6	6

N: number. NR: not report.

Table 3. Association between the clinicopathological characteristics and the DRs

Clinic Characteristics	DR4/TRAIL-R1		DR5/TRAIL-R2	
	He 2003	Xiao 2005	He 2003	Xiao 2005
Differentiation	46/6	64/19	48/7	56/18
TNM stage	31/21	50/36	32/23	12/34
AFP	37/15	60/22	41/16	58/20
vein thrombus	10/42	16/66	10/45	14/70
tumor size	18/34	52/19	19/36	14/20
metastasis	5/47	28/53	5/50	27/57

Clinicopathological characteristics of HCC included the tumor differentiation (well/moderately and poor), tumor TNM stage (I-II/III-IV), AFP (≥ 400 / < 400), portal vein thrombus (present/absent), tumor size (≤ 5 cm/ > 5 cm) and tumor metastasis (present/absent).

Infrastructure (CNKI) up to October 2015. After Scanning the titles and abstracts, 405 studies were excluded (reviews, experimental research, report, duplicate articles and studies irrelevant to the research aims). Finally seven articles [9-15] were included into this meta-analysis (Table 1). Totally, HCC specimens from 423 patients were used to explore the expression of TRAIL DR4/TRAIL-R1 and DR5/TRAIL-R2 in liver tissues (para-HCC tissues or HCC tissues). All studies used IHC, RT-PCR or Western blotting to detect the expression of DR4 and DR5 for estimating the expression of DRs and the relationship between TRAIL Death Receptors and the parameters of HCC patients.

The expression of TRAIL death receptors and the connection with the clinicopathological parameters in HCC

Among the seven studies, we used different ways to detect the expression of TRAIL Death Receptors, there was no significant association between the expression of DR4 or DR5 in HCC and Para-HCC tissues (Figures 1 and 2).

Figures 3 and 4 indicated that expression of DRs (DR4/TRAIL-R1 and DR5/TRAIL-R2) did not tend to be associated with AFP, tumor size and tumor metastasis. In addition, it also had no significant difference between the expression of DR4 and the portal vein thrombus with HCC.

However, two studies evaluated the connection of TRAIL Death Receptors (DR4 and DR5) with some clinicopathological characteristics in HCC.

The results implied that higher expression of DRs (DR4 and DR5) was lower in patients with

poor differentiation than that in those with moderate and well differentiation (pooled OR=0.15, 95% CI=0.06-0.38, $P < 0.0001$; pooled OR=0.3, 95% CI=0.13-0.70, $P = 0.005$, respectively), with an acceptable heterogeneity (chi-squared=0.05, $I^2 = 0\%$, $P = 0.83$; chi-squared=1.49, $I^2 = 33\%$, $P = 0.22$, respectively).

And the data still showed a relationship between DRs and tumor TNM. The results indicated higher expression of DRs was higher in patients with stage I/II than that in those with stage III/IV (pooled OR=4.81, 95% CI=1.68-13.80, $Z = 2.93$, $P = 0.003$; pooled OR=10.49, 95% CI=2.65-41.47, $Z = 3.35$, $P = 0.0008$, respectively), with no heterogeneity (chi-squared=0.75, $I^2 = 0\%$, $P = 0.39$; chi-squared=0.09, $I^2 = 0\%$, $P = 0.76$, respectively).

In addition, the expression of DR5 seemed to be correlated with portal vein thrombus of HCC (pooled OR=0.33, 95% CI=0.12-0.90, $Z = 2.17$, $P = 0.03$), without heterogeneity (chi-squared=0, $I^2 = 0\%$, $P = 1.00$). The data fingered that higher DR5 expression was lower in patients with por-

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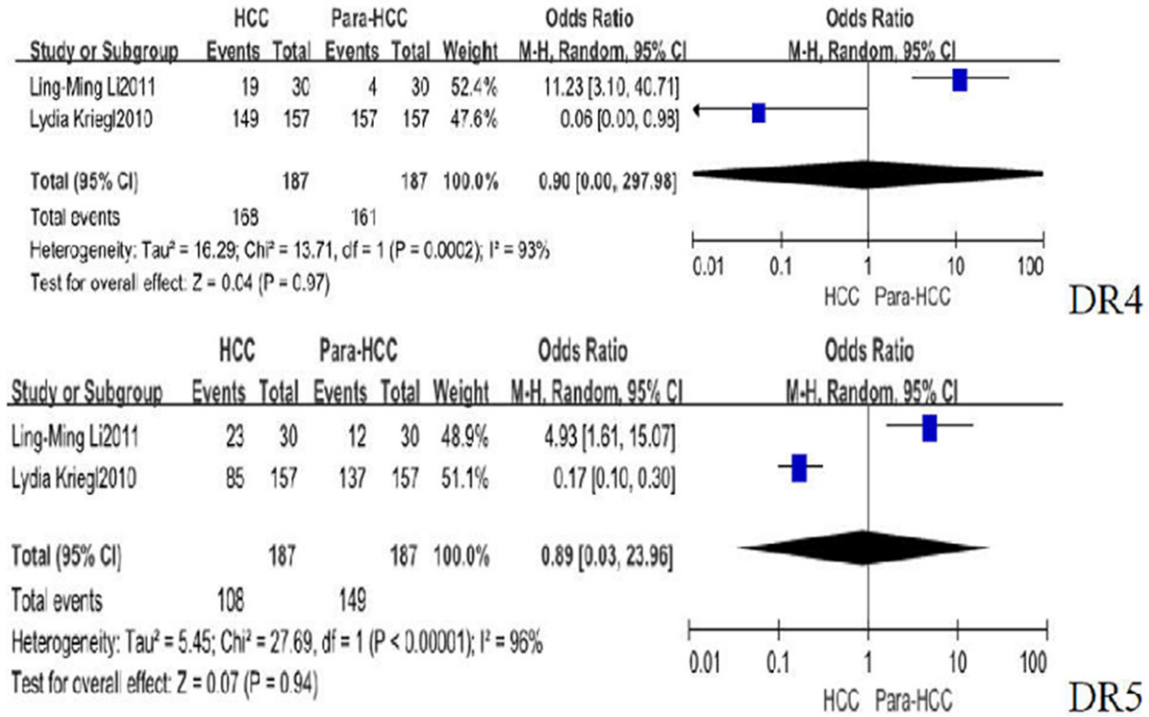


Figure 1. The expression of TRAIL-R1/DR4 and TRAIL-R2/DR5 in HCC tissues and para-HCC tissues by IHC, respectively.

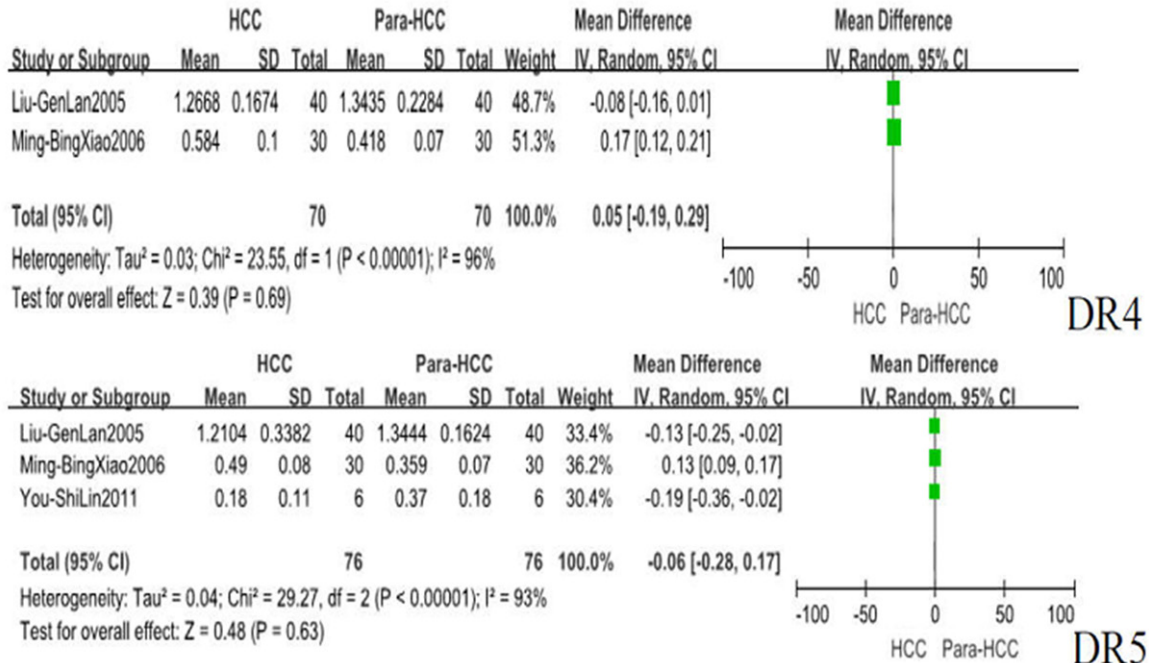


Figure 2. DRs expression in HCC and para-HCC (TRAIL-R1/DR4 was detected by RT-PCR; TRAIL-R2/DR5 was detected by RT-PCR and Western blot).

tal vein thrombus than in those without it (Figures 3 and 4). Analysis of clinicopathologi-

cal parameters revealed obvious symmetry in the funnel plots for publication bias (Figure 5),

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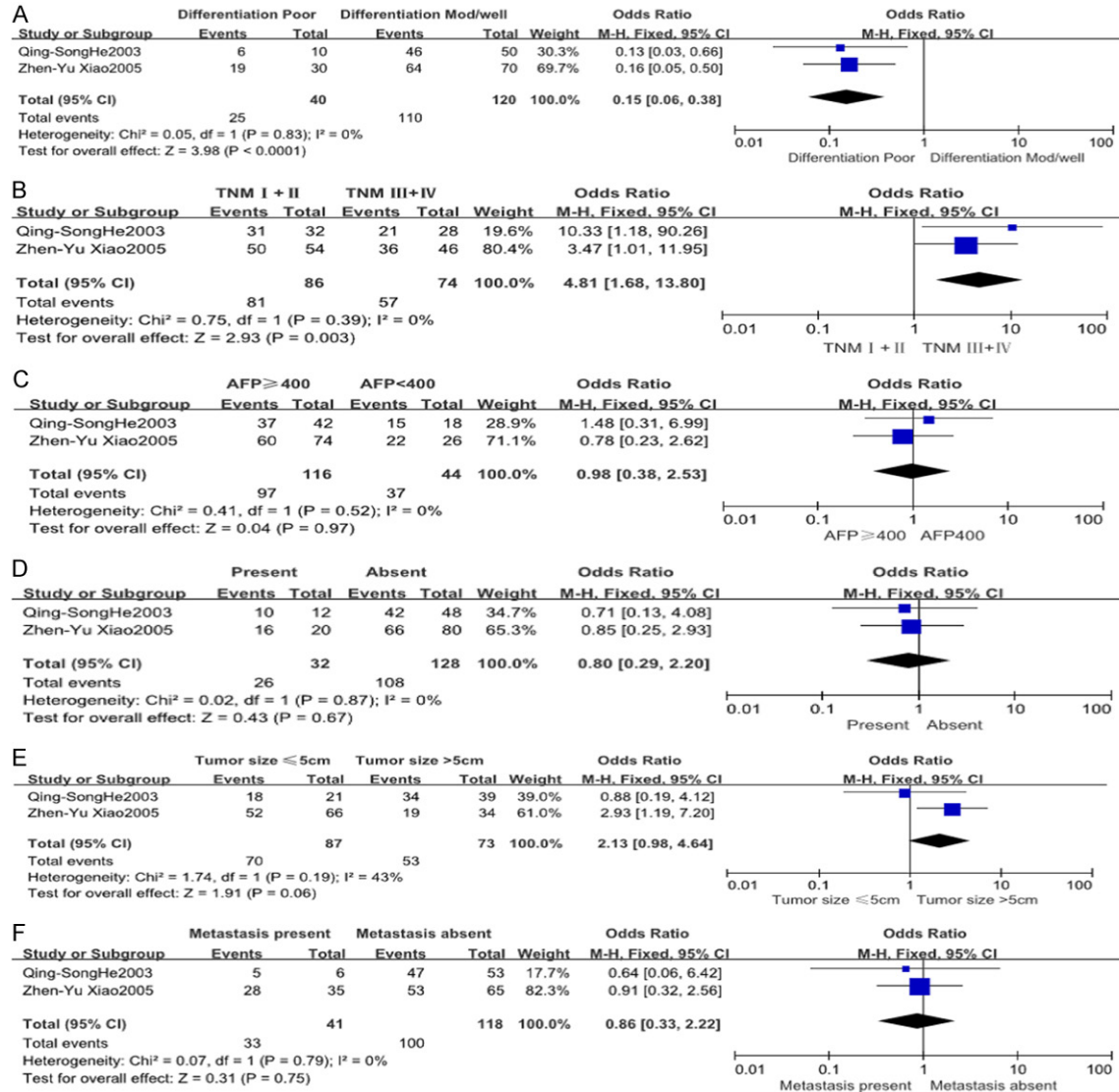


Figure 3. The clinicopathological parameters associated with DR4 in HCC. A. Tumor Differentiation. B. TNM stage. C. AFP. D. The portal vein thrombus. E. Tumor size. F. Tumor metastasis.

which was also supported by the Egger's test (P>0.05).

Discussion

TRAIL plays a major role in cancer therapy as a tumor suppressor protein [16]. Previous study indicated recombinant TRAIL protein was significantly effective in obstructing tumor cells in vivo [17]. And TRAIL deficiency in mice was involved in increased carcinogen-induced tumorigenesis and metastasis [18]. In general terms, the essence of TRAIL inducing apoptosis is initiated by TRAIL binding to its death receptors [19]. TRAIL binds to DR4/TRAIL-R1 or

DR5/TRAIL-R2 resulting in receptor trimerization and finally inducing apoptosis in a caspase-dependent manner. Down-regulation of TRAIL Death receptors has been associated with TRAIL resistance in human tumors [20]. And DR4/TRAIL-R1 and DR5/TRAIL-R2 have been studied as therapeutics in cancer cells [21]. But the expression of DRs and the clinicopathological characteristics in HCC patients are not clear. So it is severe need to refer these including studies to perform a meta-analysis to finally get a prone conclusion. We enrolled 7 studies to derive a more precise estimation about the TRAIL DR4/TRAIL-R1 and DR5/TRAIL-R2 expression in liver tissues and investigated the

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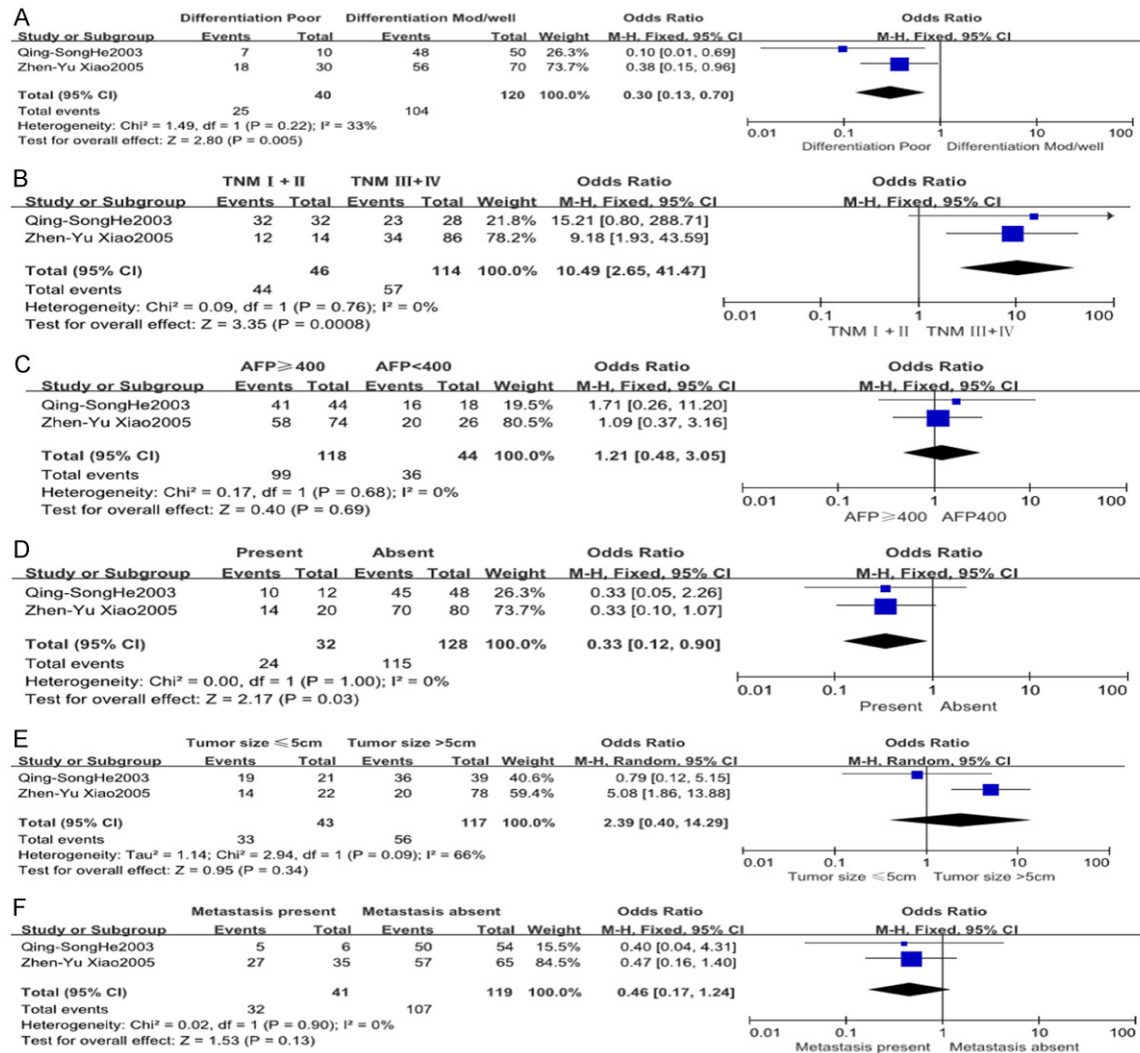


Figure 4. The clinicopathological parameters associated with DR5 in HCC. A. Tumor differentiation. B. TNM stage. C. AFP. D. The portal vein thrombosis. E. Tumor size. F. Tumor metastasis.

correlation between the clinicopathological parameters of HCC and the TRAIL Death Receptors. We respectively used IHC, Western blot analysis and PCR to detect the expression of DRs; the results implied there was no significant difference between the expression of DRs in HCC and Para-HCC tissues, which was one step along the oncogenetic pathway of tumor development (Figures 1 and 2).

To further study the relationship between Death Receptors (DR4, DR5) and clinicopathological parameters in HCC, the results implied higher expression of TRAIL DR4/TRAIL-R1 and DR5/TRAIL-R2 were both lower in patients with poor differentiation, stage III/IV than that in those with moderate and well differentiation, stage I/

II. In addition, DR5 expression was lower in liver cancer with portal vein thrombosis than those without it. All figures confirmed that down-regulated expression of TRAIL Death Receptors may relate with malignant progression of liver with poor prognosis or the possibility of a recurrence of liver cancer, because of HCC existing immune escape mechanisms in TRAIL-induced apoptosis HCC [22]. The study suggested that the majority of human HCC cell lines were resistant to TRAIL-induced apoptosis because of the expression of TRAIL Death Receptors were reduced. And it was important to note that our analysis exist some problems: the studies we internalized were just 7 and the small number of patients may influence our study.

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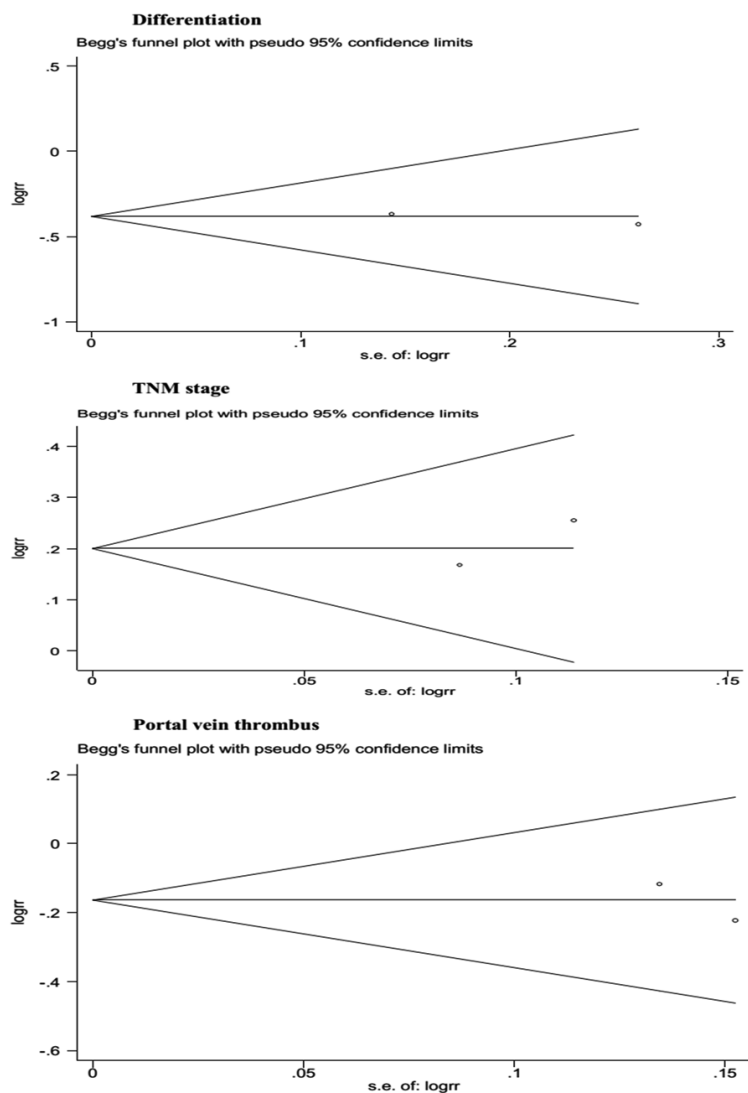


Figure 5. The Funnel plot of publication biases on the relationships between DRs and the clinicopathological parameters of patients with HCC.

In the current TNF family, tumor necrosis factor related apoptosis inducing ligand (TRAIL) is a new type of apoptosis member. Up regulation the expression of DR4/TRAIL-R1 and/or DR5/TRAIL-R2 by using of drugs, has been proven to be an important strategy, but there are still extra problems [23].

In conclusion, our study implied the TRAIL Death Receptors closely connected with the malignant progression of liver cancer. So it is urged for us to explore the mechanism of TRAIL Death Receptors to support a potential therapeutic in the treatment of human HCC in the future.

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Disclosure of conflict of interest

None.

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