**Research Article**

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# Immunohistochemical Determination of ATAD2 Protein Overexpression for Predicting Prognostic Value in Patients with Different Types of Cancer: A Meta-Analysis

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## Abstract

**Background**: Increasing number of studies has reported that Atpase family AAA domain-containing 2 (ATAD2) is nuclear coactivator, which is associated with tumor cell proliferation and invasion. Previous studies have demonstrated conflicting results on the relationship between high ATAD2 expression and clinicopathological characteristics of the tumors or patients' survival outcomes. Considering the discordant results of published studies, we performed a meta-analysis to evaluate the ATAD2 expression in predicting prognosis, and to assess the relationship between high ATAD2 expression and clinicopathological parameters.

**Methods:** We systematically searched electronic database of PubMed, Web of Science and Embase, and selected all immunohistochemical studies of hepatocellular cancer specimens for ATAD2, to analyze the relationship between high ATAD2 expression and prognosis of hepatocellular cancer patients. Pooled data of eligible studies together from individual studies and analyzed data using STATA software to perform this meta-analysis.

**Results:** A total of 5 studies with 719 liver cancer patients were included. ATAD2 protein overexpression was significantly correlated with poorer overall survival (HR 3.53, 95% CI: 1.87-6.63, P = 0.000). In addition, high ATAD2 expression was also negatively related with tumor stage [RR, relative risk: 1.46 (95% CI: 1.30-1.64); P < 0.001], as well as tumor size [RR: 1.23(95% CI: 1.06-1.43); P < 0.001], and tumor recurrence [RR: 1.34 (95% CI: 1.05-1.72); P < 0.001].

**Conclusions:** These data suggested that immunohistochemical determination of ATAD2 Protein might be a prognostic biomarker for the patients of hepatocellular cancer.

**Keywords:** ATAD2 protein, Hepatocellular cancer, Iimmunohistochemistry, Prognosis

## Introduction

ATPase family AAA domain-containing protein 2 (ATAD2), also listed as pro2000, ANCCA (AAA+ nuclear coregulator cancer-associated), shares the conserved region of ~ 220 amino acids where contains an ATP-binding site with other members of the ATPases family. ATAD2, as a critical oncogene [1], is associated with tumor cell proliferation and invasion. It contains two AAA+ ATPase domains (AAA-D1 and AAA-D2) and a bromodomain, and also plays a role of an androgen receptor (AR) coactivator [2,3]. ATAD2 has been identified as a candidate driver gene which maps to chromosome 8q24, where is the most frequently amplified region in various types of cancer [2,4]. The especial structure of ATAD2 indicates its critical roles in regulating cell growth, mobility, cell cycle and apoptosis [5-8]. Taghavi A et al.

[9] have revealed ATAD2 implicated in the pathogenesis of human breast cancer. Moreover, ATAD2 is a direct target of proto-oncogene ACTR/AIB1/SRC-3 [3] and it can functionally control several crucial regulators in survival pathways, including E2F1/3, EZH2, B-Myb and MYC in breast cancer, prostate cancer, lung adenocarcinoma and endometrial cancer [4, 10-13]. Many studies have researched the expression of ATAD2 in tumor cells and explored the relationship between ATAD2 expression and clinicopathological features. The results showed that ATAD2 protein expressed in the cytoplasm or cell nucleus in cancer specimens by immunohistochemistry (IHC). They found that ATAD2 usually overexpressed in tumor cells and correlated with poor survival, lymph node metastasis and disease recurrence [14,15]. Some studies identified that high expression of ATAD2 was significantly correlated with shorter DSS (P < 0.001) [10,16], tumor size (P < 0.001) [17,18], tumor stage (P < 0.001) [16,18,19], and tumor differentiation (P < 0.001) [16,20]. However, some researches indicated that ATAD2 overexpression was not associated with shorter disease specific survival (P = 0.109) [17] and clinicopathological features mentioned above [6,21]. Considering the discordant results of published studies, we performed a meta-analysis to evaluate the ATAD2 expression in predicting prognosis, and to assess the relationship between high ATAD2 expression and clinicopathological parameters.

## Materials and Methods

### Publication search strategy

We systematically performed an electronic searching of PubMed, Web of Science, Embase of all articles published up to November 5, 2019. Language was limited to English. The search strategy used a combination of key words: ("ATPase family AAA domain-containing 2/ATAD2" or "ANCCA" or "pro2000") AND ("cancer" or "carcinoma" or "neoplasm") AND ("hepatocellular" or "liver"). Titles and abstracts were screened to identify reports, which examined the relationship between ATAD2 expression and clinical outcomes, such as Overall Survival (OS), Disease Special Survival (DSS), recurrence free survival (RFS) and clinicopathological features. Reference lists of identified publications were also reviewed for additional relevant articles to ensure statistical integrity.

### Selection criteria

All of the included studies must fulfill the inclusion criteria: (1) observational studies concerning the association between "ATPase family AAA domain-containing 2/ATAD2/ANCCA/pro2000" and "hepatocellular or liver cancer/carcinoma/neoplasm"; (2) the expression of ATAD2 was detected on primary human tumor tissue specimens; (3)the detection method of ATAD2 was restricted to IHC; (4) clear definition of the criteria of high ATAD2 expression; (5) statistical analysis using univariate or multivariate hazards modeling reported HR for survival time with 95% confidence interval (CI).

### Data extraction

For relevant articles, variables were extracted. These included the following: first name of authors, country, year of publication, sample size, number of high expressed cases, number of low expressed cases, outcome, HRs with 95% CI, and *P*-values. Two reviewers independently extracted these data from the eligible publications, and discordant results were resolved by discussing.

### Quality assessment

Quality assessment of the eligible studies in the meta-analysis was evaluated by two reviewers independently based on the Newcastle-Ottawa quality assessment scale (NOS), a tool that developed to assess the quality of non-randomized studies in the interpretation of meta-analysis [22]. Assessed items included selection (four stars maximum), comparability (two stars maximum), and the ascertainment of outcomes of interest (three stars maximum), with a score range from 0 to 9 stars. Studies with an NOS score ≥6 were regarded as high-quality studies in this quality assessment system. While studies with scores 0-3, and 4-5 were assigned as low, medium quality, respectively. Disagreements about quality were resolved by discussing.

### Statistical analysis

The main dichotomous survival outcomes were expressed as relative HR. By convention, pooled HRs >1 implied poorer survival for high ATAD2 expression group compared with low expression group in various groups. We extracted HRs with the corresponding 95% CI according to the original articles. Statistical analyses of HRs for OS or DSS, RFS, and the RR for tumor stage, tumor differentiation, tumor size, tumor recurrence were all calculated by STATA SE12.0 (STATA Corporation, College Station, TX). We evaluated 95% CIs respectively for each estimate and presented them in forest plots. All 95% CIs were two-sided. The extent of variability contributing to statistical heterogeneity across trials was evaluated by I-square ($I^2$) statistic. P > 0.1 for the Chi-square test and $I^2$≤50% were interpreted as significant low-level heterogeneity among studies, the potential source of

which was assessed by a random effect model based on the DerSimonian and Laird method [23]. Otherwise, a fixed-effect model using the Mantel-Haenszel method [24] was adopted when $I^2>50\%$ or $P \leq 0.1$. Publication bias was assessed by Begg's funnel plots and Egger's tests. To confirm the convincing of outcomes in this meta-analysis, sensitivity analyses were conducted to explore the effects by sequential eliminate individual studies using the "metaninf" STATA command. Statistical significance was defined as a P value less than 0.05.

## Results

### Study selection

As shown in figure 1, through the electronic searching, a total of 84 potentially relevant articles were identified. The titles and abstracts were reviewed, and 53 duplicates and irrelevant articles were excluded. Nineteen full-text articles were assessed for eligibility after removing the studies of cell experiments, not in English or not for survival. Fourteen ineligible papers were eliminated due to unclear definition, insufficient survival data, non IHC, and reviews. In the final, a total of 5 studies with 719 cases were included in the current meta-analysis [6,17,21,25,26].

### Characteristics of included studies

In this meta-analysis, all necessary data were extracted from 5 studies. OS, as a primary outcome of survival analysis, was applied in 4 studies. The secondary outcomes were RFS and DSS. All included studies were detected by IHC and most primary antibodies of ATAD2 were bought from Abcam and Sigma companies. The main characteristics of included studies were presented in table 1 and details about NOS score of eligible studies were displayed in table 2.

## Meta-analysis of high ATAD2 expression and cancer prognosis

As shown in the figure 2, the pooled results indicated that highATAD2 expression was correlated with poorer OS (HR: 3.53, 95% CI: 1.87-6.63, P = 0.000) in hepatocellular cancer. The meta-analysis suggested that ATAD2 over-expression was positively associated with worse prognosis for cancer patients, especially for hepatocellular cancer.

## High ATAD2 expression and clinicopathological features

We explored the relationship between high ATAD2 expression and clinicopathological parameters in hepatocellular cancer. As the results suggested high ATAD2 expression was negatively related with tumor stage [RR, relative risk: 1.46 (95% CI: 1.30-1.64); P < 0.001] by the fixed-effects model ($I^2$=0.00%, P = 0.445, Figure 3A). The elevated expression of ATAD2 was also found to be significantly associated with tumor size [RR: 1.23(95% CI: 1.06-1.43); P < 0.001; Figure 3B]. The fixed-effects model analysis indicated that there was no evidence of statistically significant heterogeneity within tumor size ($I^2$=15.6%, P = 0.315). A pooled RR of 1.34 with 95%CI 1.05-1.72 (P < 0.001) by random-effects model ($I^2$=86.0%, P = 0.001) showed that high ATAD2 expression was associated with tumor recurrence (Figure 3C). But there was no relationship between ATAD2 protein expression and tumor differentiation (RR 1.23, 95%CI 0.94-1.61, P < 0.001, Figure 3D). In summary, compared with low ATAD2 expression group, high ATAD2 expression was associated with high tumor stage, large tumor size, and easy tumor recurrence. The pooled results revealed that high ATAD2 expression in tumor tissues were more susceptibility to develop poorer prognostic rate.
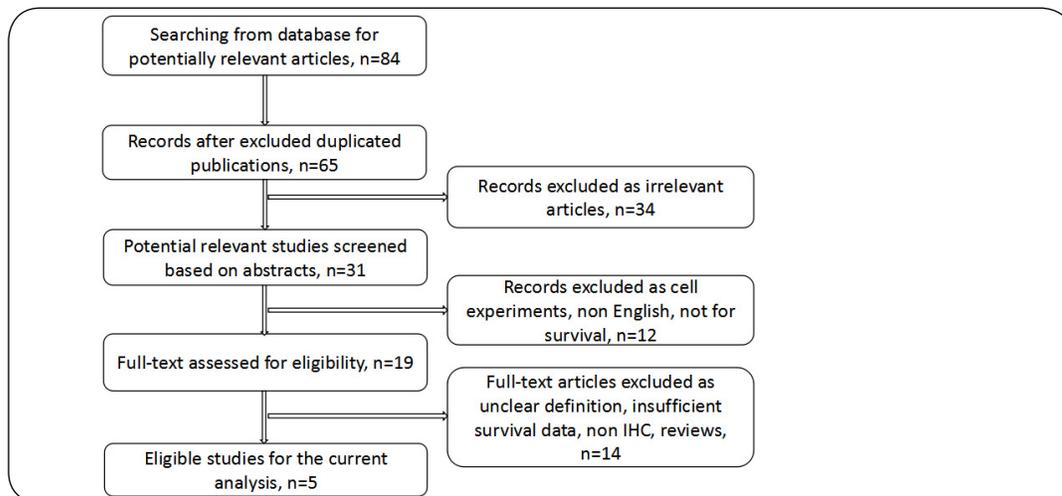


**Figure 1:** Flow diagram of the study selection process.

## Sensitivity analysis and publication bias

Sensitivity analysis was carried out to assess the effect on the pooled result by removing individual study at one time from the meta-analysis. The result indicated the combined HRs of high ATAD2 expression were reliable (Figure 4). The exclusion of any individual study did not change the significance of the pooled results, confirming that this study were robust. Subsequently, Begg funnel plot and Egger test were used to assess potential publication bias. The results showed that no publication bias was found for the survival analysis (Begg P value = 0.452, Egger P value = 0.220) (Figure 5).

**Table 1:** Characteristics of included studies.

| First Author | Cancer types | Country | No.of patients | ATAD2 Expression | | Outcome | Data extract | Method | Design |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Low | High | | | | |
| | | | | (%) | (%) | | | | |
| Wu et al. 2014 | HCC | China | 129 | 46 (35.7) | 83 (64.3) | OS | Reported | IHC | R |
| Wu et al. 2014 | HCC | China | 80 | 26 (32.5) | 54 (67.5) | OS | Reported | IHC | R |
| Hwang et al. 2015 | HCC | Korea | 182 | 63 (34.6) | 119 (65.4) | RFS, DSS | Reported | IHC | R |
| Huang et al. 2016 | HCC | China | 221 | 79 (35.7) | 142 (64.3) | OS | Reported | IHC | R |
| Yang et al. 2014 | HCC | China | 107 | 41 (38.3) | 66 (61.7) | OS | Reported | IHC | R |

Abbreviations: HCC: hepatocellular cancer; OS: overall survival; DSS: disease specific survival; RFS: recurrence free survival; IHC: immunohistochemistry; R: retrospective.
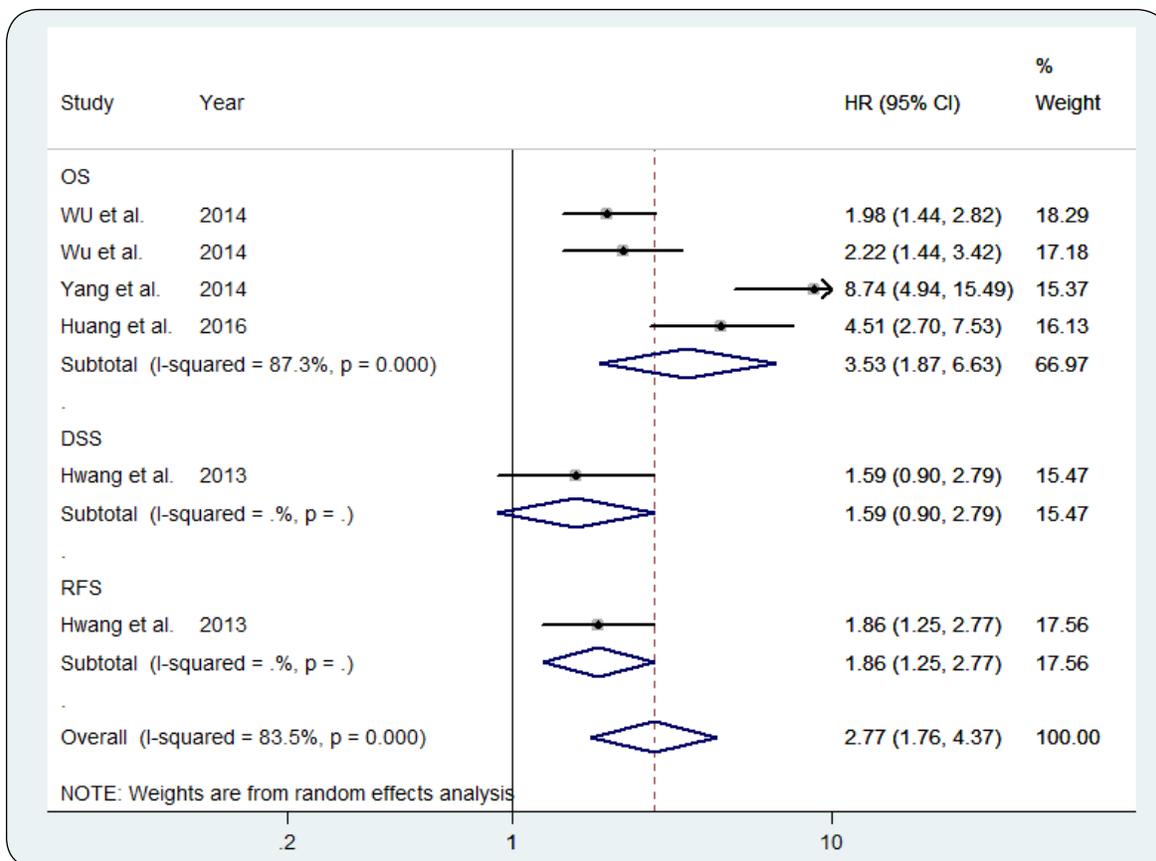


**Figure 2:** Forest plot of studies evaluating the association between ATAD2 expression and OS, RFS, and DSS in hepatocellular cancer.
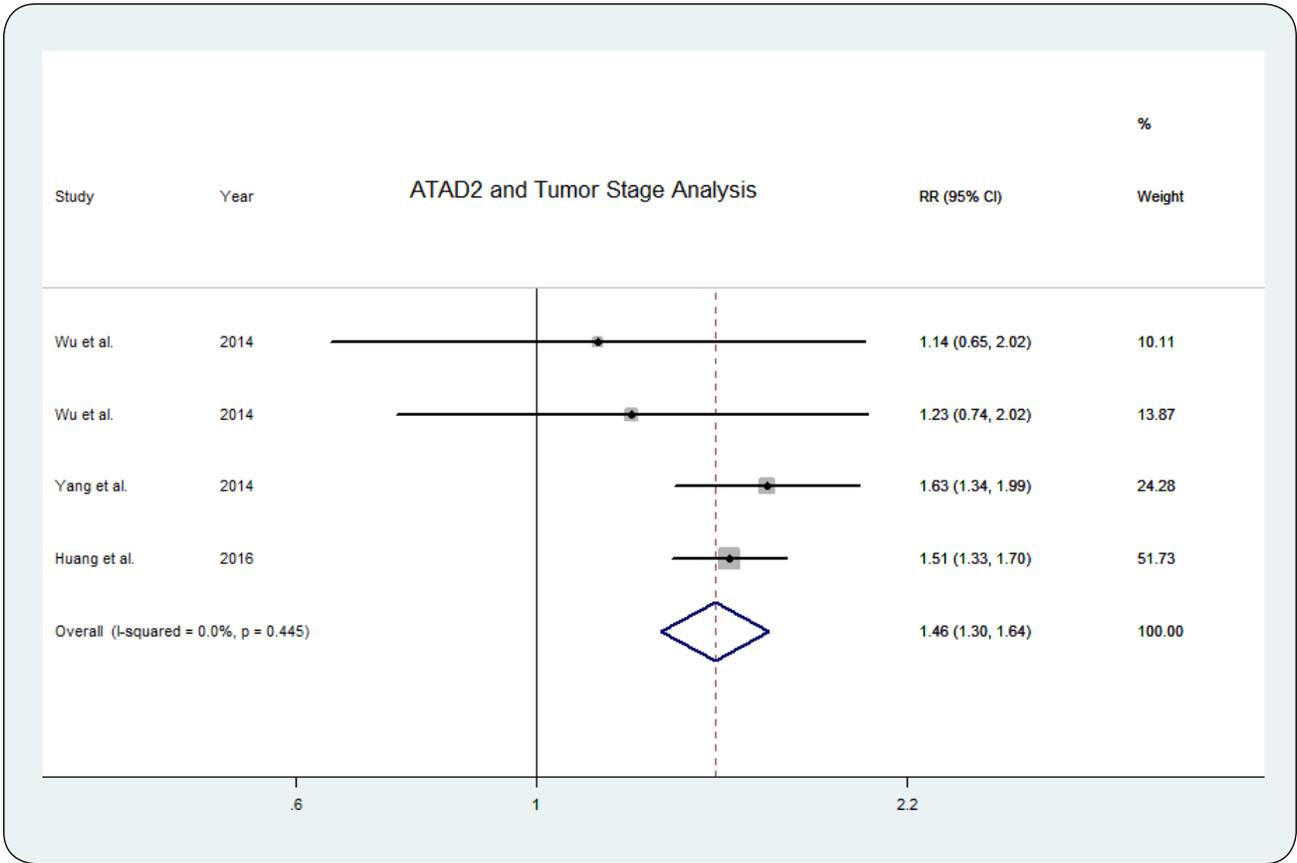
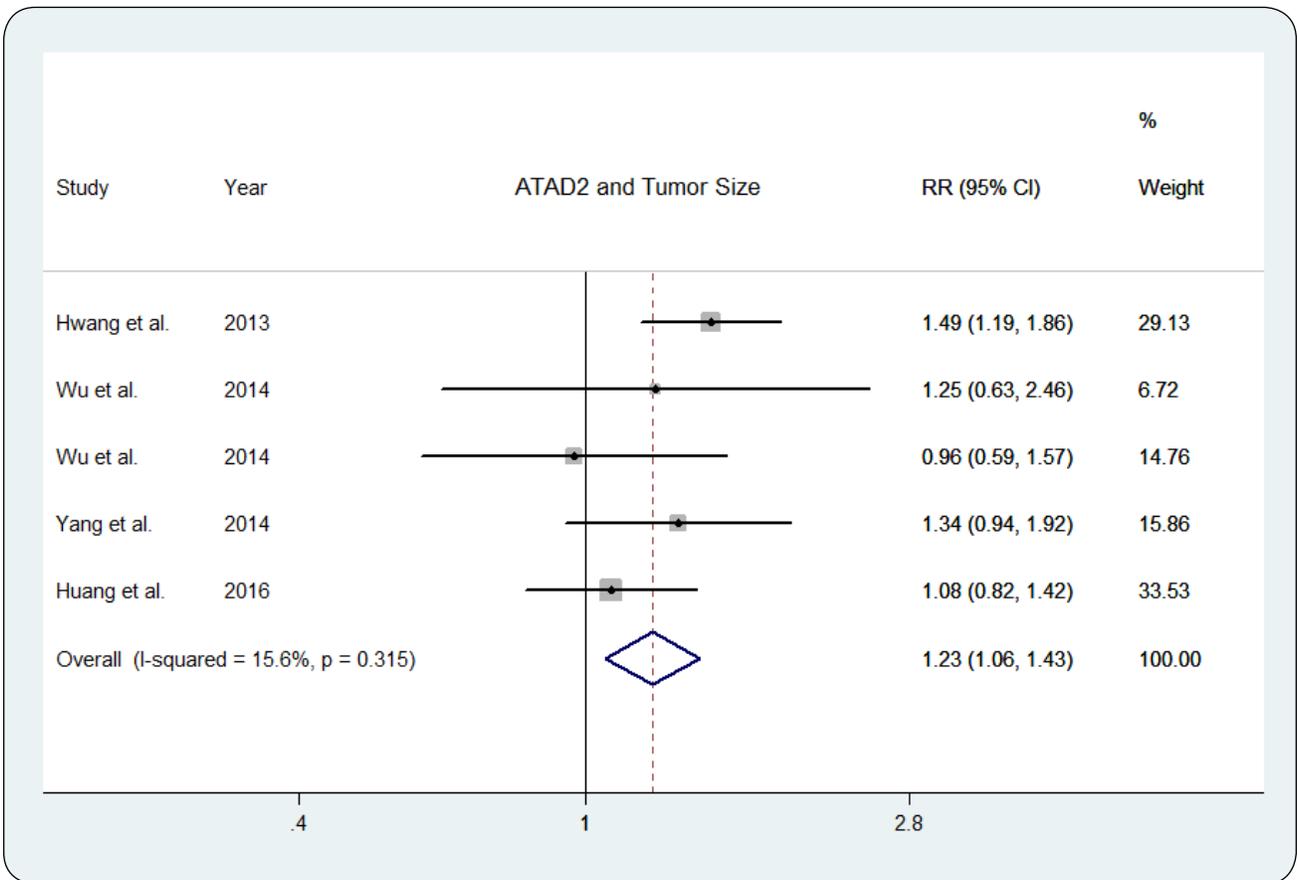**Figure 3A:** Meta-analysis the relationship between high ATAD2 expression and tumor stage.



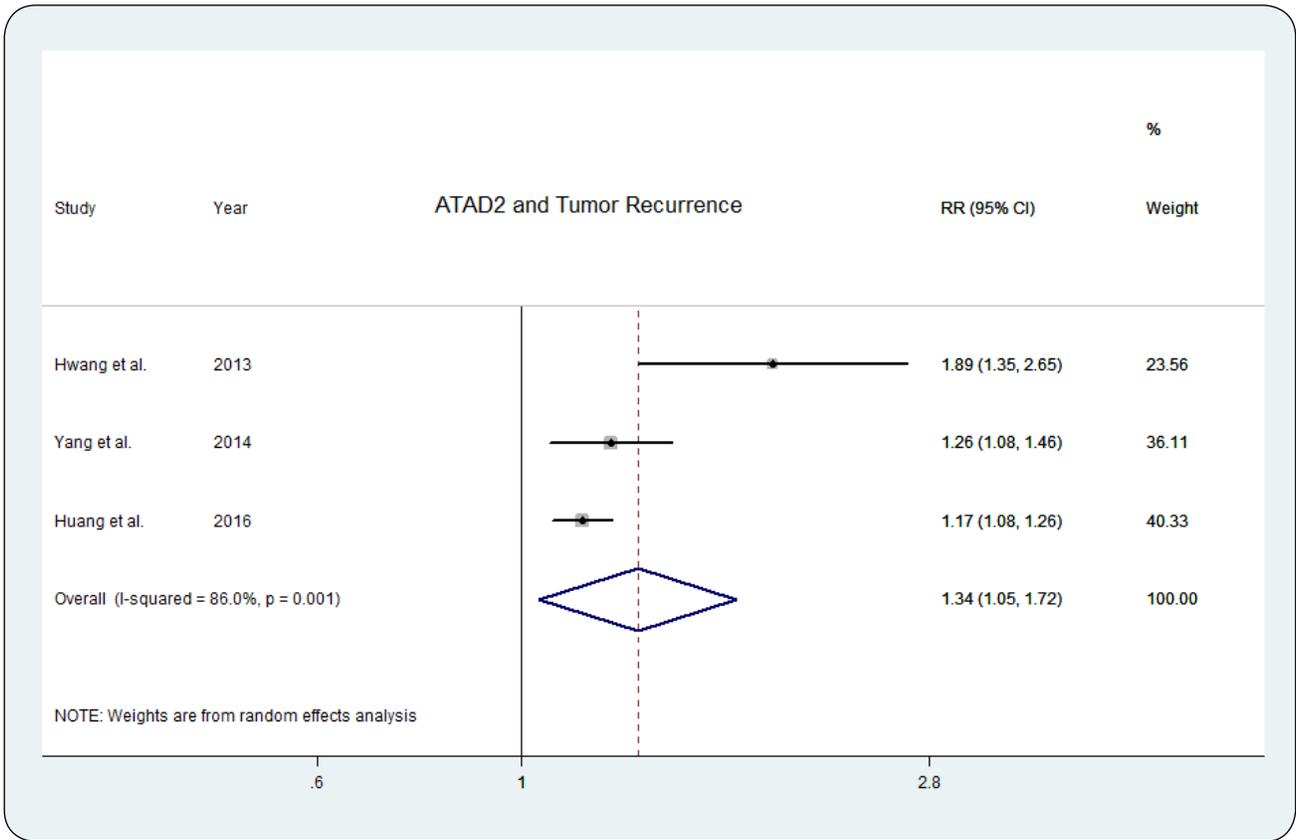**Figure 3B:** Meta-analysis the relationship between high ATAD2 expression and tumor size.

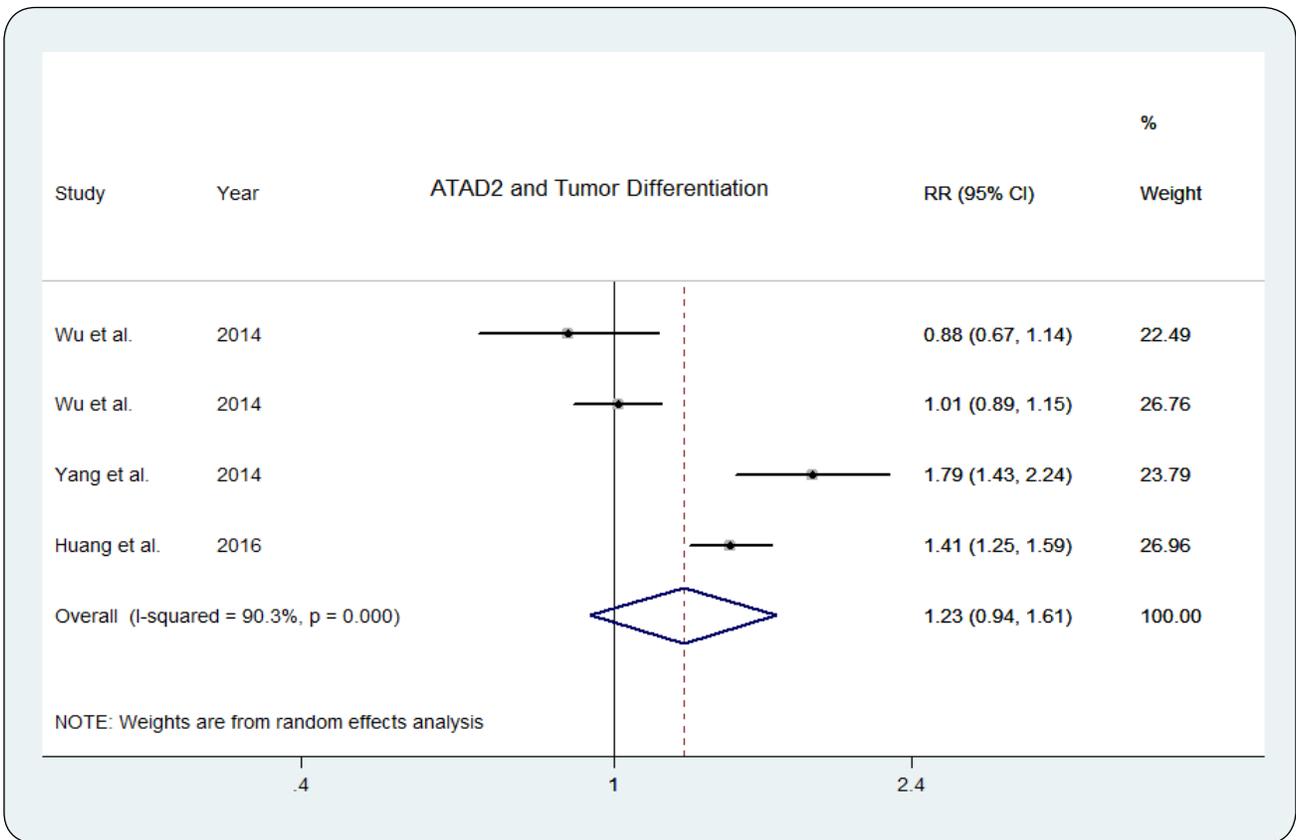**Figure 3C:** Meta-analysis the relationship between high ATAD2 expression and tumor recurrence.



**Figure 3D:** Meta-analysis the relationship between high ATAD2 expression and tumor differentiation.
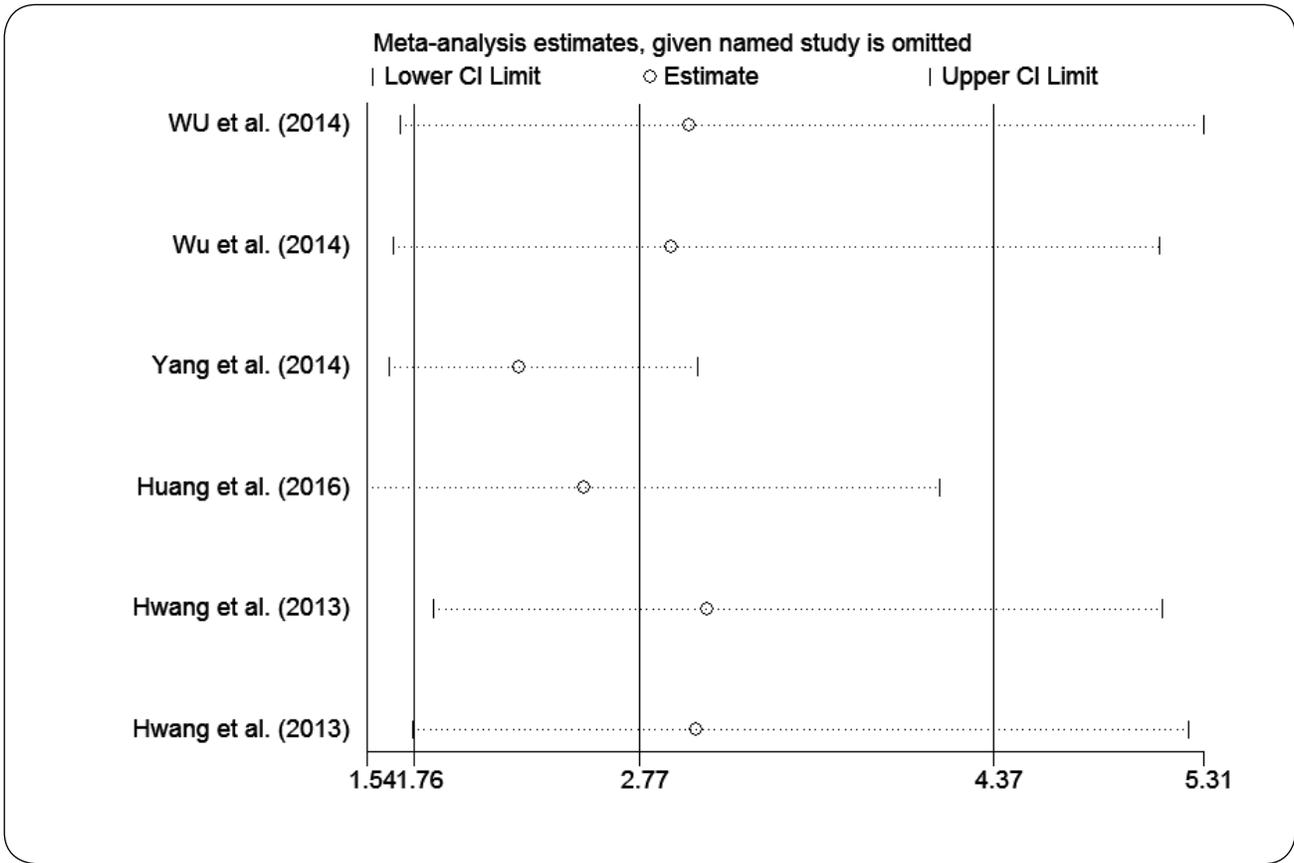
**Figure 4:** Sensitivity analyses for the survival of high ATAD2 expression.
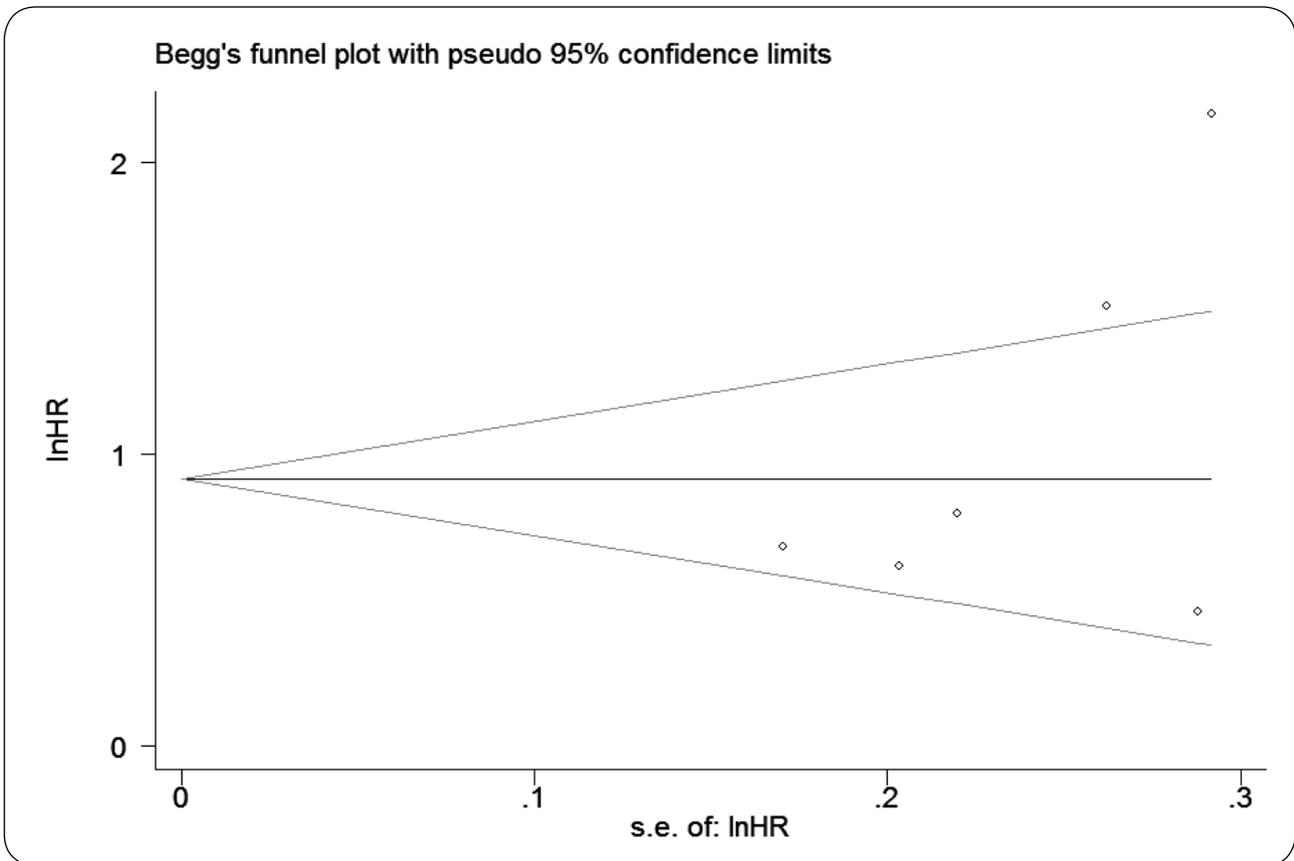


**Figure 5:** Begg's funnel plot showing publication bias for the pooled HRs of survival.

## Discussion

Molecular markers are promising for accurately predicting the prognosis of cancer patients. To date, many available studies have involved small sample sizes and given conflicting results, therefore unable to determine the value of ATAD2 expression in predicting prognosis and clinicopathological parameters of cancer patients. To the best of our knowledge, no meta-analysis has been performed to evaluate ATAD2 expression level as a prognostic indicator in published studies. Thus, we conducted the first meta-analysis to systematically explore the prevalence of high ATAD2 expression, the effect ofATAD2 expression on cancer patients' prognosis, and the relationship between high ATAD2 expression and clinicopathological features.

In the overall pooled analysis, the aggregation of data indicated that high ATAD2 protein expression was associated with poorer OS (HR 3.53, 95% CI: 1.87-6.63, P = 0.000) in hepatocellular cancer. In addition, high ATAD2 expression was also negatively related with tumor stage (RR:1.46, 95% CI: 1.30-1.64; P < 0.001), as well as tumor size (RR: 1.23, 95% CI: 1.06-1.43; P < 0.001) and tumor recurrence (RR:1.34, 95% CI: 1.05-1.72; P < 0.0010). Many studies have found that ATAD2 was a direct target of the oncogene, which can drive tumor cells proliferation and survival [3,4,27]. High mRNA level of ATAD2 was also an independent predictor for poor outcome and might be used for individual risk-adapted therapy in the future [27]. These could partly illustrate that ATAD2 over-expression was significantly associated with poor survival for cancer patients. As to the clinicopathological parameters, we analyzed the data as comprehensively as possible in this meta-analysis. The aggregation of data indicated that high ATAD2 expression was correlated with worse tumor stage, poor tumor differentiation, lymph node metastasis, distant metastasis, but not large tumor size. These would be another part of reason that high ATAD2 expression was associated with poorer survival in cancer patients.

There was strong evidence that ATAD2 was a driver of tumor cells proliferation. It was highly remarkable in mediating E2 induction of cyclin D1, c-myc and E2F1 in tumor cells [3]. ATAD2-E2F was also required for the activation of ACTR [28]. It had been found that ATAD2 worked as an important cofactor for MYC-dependent transcription. Through MYC and E2F transcription factors, ATAD2 increased the expression of proliferation-related and anti-apoptotic genes in many different types of cancer, including breast cancer, non-small cell lung cancer and prostate cancer [3,4,29,30]. ATAD2

could also indirectly act on histone post-translational modifications either by activating the expression of the H3K36 methyltransferase, NSD2/WHSC1 [31]. The two domains ofATAD2, the bromodomain and the AAA ATPase domain, could be targeted of small molecule inhibitors. Bromodomain inhibitors specifically inhibiting the BET family of bromodomain-containing factors were presenting significantly promising anti-cancer activities [32]. The AAA ATPase domain of ATAD2 also appeared as a good druggable target. Indeed, several small molecule inhibitors of AAAATPases had already been reported, inhibiting AAA ATPase members ranging from dynein [33] and p97/VCP [34] to pontin [35]. In summary, ATAD2 played an important role in human cancers. Blocking ATAD2 expression or activity might be an attractive therapeutic target for anticancer treatment.

When interpreted the results, a few limitations of this meta-analysis presented in several aspects. Firstly, the sample size of this meta-analysis was limited. Secondly, the potential publication bias might exist in this area of research. The current review was restricted to articles published in English. This might be a part of bias. Thirdly, there was statistical heterogeneity across studies. The heterogeneity possibly was caused by baseline characteristics of the patients, IHC technique, cutoff values and primary antibody selected. As the limited studies we could not perform meta-regression to further find out the source of heterogeneity. Finally, observational trials of all included studies with more potential confounding factors presented lower level of evidence than randomized controlled studies. Therefore, the results should be explained cautiously, and more studies especially randomized controlled studies should be performed.

## Conclusions

To conclude, the systematic review suggested high ATAD2 protein expression was correlated with worse OS in hepatocellular cancer. Immunohistochemical determination of ATAD2 Protein might be a promising prognostic biomarker and potential target in cancer treatment. Thus, further larger clinical studies are needed to make a comprehensive conclusion on the human cancers.

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