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# Prognostic Value of Tumor-Infiltrating FoxP3$^+$ T Cells in Gastrointestinal Cancers: A Meta Analysis

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## Abstract

***Purpose:*** Tumor-infiltrating FoxP3$^+$ T cells have been reported in various human tumors, which impaired cell-mediated immunity and promoted disease progression. However, its prognostic value for survival in patients with different gastrointestinal cancers [hepatocellular carcinoma (HCC), colorectal cancer (CRC), gastric cancer (GC)] remains controversial.

***Methods:*** Relevant literature was searched using PubMed, Embase, Cochrane, Ovid Medline and Chinese wanfang databases. A meta-analysis was conducted to estimate pooled survival and recurrence ratios. The odds ratio (OR) and 95% confidence intervals (CI) were calculated employing fixed- or random-effects models depending on the heterogeneity of the included trials.

***Results:*** For HCC and GC, the overall survival at 1, 3 and 5-year of high FoxP3$^+$ T cells infiltration patients were lower than low FoxP3$^+$ T cells infiltration patients ($P<0.05$). The recurrences at 1, 3 and 5-year of high FoxP3$^+$ T cells infiltration patients were higher than low FoxP3$^+$ T cells infiltration patients ($P<0.001$). But for CRC, the overall survival at 1, 3 and 5-year of high FoxP3$^+$ T cells infiltration patients were higher than low FoxP3$^+$ T cells infiltration patients ($P<0.001$). There were no differences in 1, 3 and 5-year recurrences between high and low FoxP3$^+$ T cells infiltration patients ($P>0.05$).

***Conclusions:*** Our findings suggested that tumor-infiltrating FoxP3$^+$ T cells were a factor for a poor prognosis for HCC and GC, but a good prognosis for CRC.

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## Introduction

Immune cells that infiltrate tumors engage in an extensive and dynamic crosstalk with cancer cells and some of the molecular events that mediate this dialog have been revealed [1]. In the past decade, much effort has been devoted to finding the function of regulatory T cells (Tregs) in tumor. Tregs are a subgroup of CD4$^+$ T helper cells with the function of suppressing T-cell immunity in both physiologic and disease statuses. Forkhead box protein P3 (FoxP3) is a transcription factor necessary and sufficient for induction of the immunosuppressive functions of Tregs, and it is now considered as the most specific marker for Tregs in tumors [2].

Abundance tumor-infiltrating FoxP3$^+$ T cells are expected to be associated with an unfavorable prognosis, as expected from their capacity to inhibit antitumor immunity. However, this idea has been challenged by recent studies showing that, high tumor infiltration by FoxP3$^+$ T cells is not always associated with a poor prognosis. On the contrary, it can improve survival in some tumors [3–5]. It was inconsistent with the initial hypothesis that FoxP3$^+$ T cells inhibit antitumor immunity. Even in the same kind of tumor, this conclusion was not entirely consistent [4,5]. The discrepancy was very obvious, especially in the gastrointestinal cancers such as hepatocellular carcinoma (HCC), colorectal cancer (CRC) and gastric cancer (GC) which all were considered as inflammation-associated cancers since with rich exogenous antigens.

To investigate this apparent discrepancy, we sought to conduct a meta-analysis to estimate the prognostic importance of tumor-infiltrating FoxP3$^+$ T cells level for overall survival (OS) and disease-free survival (DFS) among patients with HCC, CRC and GC, aiming to gain insights into whether FoxP3$^+$ T cells could provide useful guidance in the biological understanding and treatment of solid tumors.

## Materials and Methods

### Literature search

Relevant articles were identified by two reviewers via an electronic search of PubMed, EMBASE, Cochrane, Ovid Medline

**Table 1.** Main characteristics of studies about HCC included in the meta-analysis.

| Author | Year | Journal | Quality score | Number of cases | | Marker | Antibody | Cutoff | Survival |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M/F | High/low | | | | |
| Gao[9] | 2007 | J Clin Oncol | 6 | 260/42 | 147/155 | FoxP3 | Biolegend | Median | OS,DFS |
| Kobayashi[10] | 2007 | Clin Cancer Res | 6 | 113/34 | 73/74 | FoxP3/CD4 | Novocastra | Median | OS,DFS |
| Sasaki[11] | 2007 | Eur J Surg Oncol | 6 | 126/38 | 84/80 | FoxP3 | Abcan | Median | OS,DFS |
| Li[12] | 2008 | Zhonghua Zhong Liu | 7 | 54/9 | 20/43 | FoxP3 | Abcan | Other | OS |
| Shen[13] | 2009 | Can J Surg | 6 | 70/6 | 35/41 | FoxP3 | Abcan | Median | OS,DFS |
| Zhou[14] | 2009 | Int J Cancer | 7 | - | 36/49 | FoxP3 | Abcan | Median | OS,DFS |
| Lin[15] | 2010 | Chin J Cancer | 6 | 85/17 | 49/53 | FoxP3 | Abcan | Median | OS |
| Chen[16] | 2011 | PLoS One | 7 | - | 57/86 | FoxP3 | Abcan | Median | OS,DFS |
| Chen[17] | 2011 | Med Oncol | 7 | - | 70/71 | FoxP3 | Abcan | Median | OS,DFS |
| Huang[18] | 2012 | Digestion | 7 | 45/9 | 27/27 | FoxP3 | Abcan | Median | OS,DFS |
| Wu[19] | 2012 | J Gastroenterol Hepatol | 7 | 341/45 | 207/179 | FoxP3 | Abcan | Other | OS,DFS |
| Huang[20] | 2013 | J Gastroenterol Hepatol | 7 | 50/6 | 28/28 | FoxP3 | Abcan | Median | OS,DFS |
| Lin[21] | 2013 | Cancer Prev Res | 7 | - | 162/83 | FoxP3/CD4 | Abcan | Other | OS,DFS |

F, female; M, male; Quality score was assessed using the validated Jadad scale; High, high $FoxP3^+$ T cells infiltration; Low, low $FoxP3^+$ T cells infiltration.
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and Chinese wanfang databases using the following keywords: (FoxP3 or regulatory T cells), (hepatocellular carcinoma, colorectal cancer or gastric cancer) and "prognosis". And the search time period of the electronic database was from inception to Feb 8th, 2014. Additionally, possible missing papers were searched in reference lists of selected papers and systematic review. A search for unpublished literature was not performed. Disagreement on article inclusion between the two reviewers was resolved via a third reviewer.

### Inclusion and exclusion criteria

Inclusion criteria for this study were as follows: (1) patients were diagnosed clearly; (2) report of $FoxP3^+$ T cells in tumor surgical specimens; (3) $FoxP3^+$ T cells evaluation using immunohistochemical method; (4) association of high and low $FoxP3^+$ T cells infiltration patients with overall survival (OS), and/or disease-free survival (DFS) and contained survival curves. (5) when the same author or group reported results obtained from the same patient population in more than one article, the most recent report or the most informative report was included.

Exclusion criteria for this study were as follows: (1) letters, reviews, case reports, conference abstracts, editorials, and expert opinion were excluded; (2) articles in which have no information on survival rates or survival curve; (3) Non-surgical treatment study; (4) non-primary cancer, such as metastatic cancer or recurrent cancer; (5) peripheral blood or peritumoral specimens.

Name of authors or journals of the articles did not influence our decision in excluding or including the articles.

### Statistical analysis

Hazard ratio (HR) and its 95% confidence interval (CI) were used to estimate the association between $FoxP3^+$ T cells and patients' prognosis. If a direct report of survival and recurrence ratios were not available, then the survival data read from Kaplan-Meier curves were read by Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/) as described previously [6–8]. This work was performed by two independent persons to reduce inaccuracy in the extracted survival rates.

All analyses were performed with Review Manager version 5 (RevMan, Cochrane Collaboration, Oxford, England). Statistical heterogeneity between trials was evaluated by $\chi^2$ test and was considered significant when $P<0.05$. In the absence of statistically significant heterogeneity, the Mantel-Haenszel method in the fixed-effect model was used for the Meta analysis. Otherwise, the DerSimonian and Laird method in the random-effect model was selected. The odds ratio (OR) with 95%CI was used to assess treatment efficacy. The combined result was an average OR and 95%CI weighted according to the standard error of the OR of the trial. $P<0.05$ was considered statistically significant. We used funnel plots to assess the publication bias, and tested for funnel plot asymmetry using Egger's test and Begg's test.

## Results

### Study selection and characteristics

For HCC, 13 eligible trials involving 1964 patients were ultimately identified in Table 1 [9–21]. For CRC, 10 eligible trials involving 2756 patients were ultimately identified in Table 2 [22–31]. For GC, 16 eligible trials involving 1873 patients were ultimately identified Table 3 [32–47]. FigureS1A (for HCC), FigureS1B (for CRC) and FigureS1C (for GC) illustrate the search process and the final selection of relevant studies.

**Table 2.** Main characteristics of studies about CRC included in the meta-analysis.

| Author | Year | Journal | Quality score | Number of cases | | Marker | Antibody | Cutoff | Survival |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M/F | High/low | | | | |
| Sinicrope[22] | 2009 | Gastroenterology | 7 | 84/76 | 101/59 | FoxP3 | Abcan | Other | DFS |
| Lee[23] | 2010 | Cancer | 7 | 29/34 | 39/24 | FoxP3 | eBioscience | Other | OS |
| Suzuki[24] | 2010 | Cancer Immunol Immunother | 6 | 53/41 | 30/64 | FoxP3 | Abcan | Mean | OS,DFS |
| Frey[25] | 2010 | Int J Cancer | 6 | - | 614/616 | FoxP3 | Abcan | Other | OS,DFS |
| Nosho[26] | 2010 | J Pathol | 6 | - | 384/384 | FoxP3 | BioLegend | Other | OS |
| Tosolini[27] | 2011 | Cancer Research | 5 | - | 18/38 | FoxP3 | Abcan | Other | DFS |
| Yoon[28] | 2012 | PLoS One | 7 | - | 78/78 | FoxP3 | Abcan | Median | OS |
| Suzuki[29] | 2013 | Clinical Immunology | 6 | 49/39 | 34/54 | FoxP3 | Abcan | Mean | OS,DFS |
| Zeestraten[30] | 2013 | Cancer Microenvironment | 6 | 44/36 | 38/38 | FoxP3 | Abcan | Median | OS,DFS |
| Kim[31] | 2013 | PLoS One | 7 | 37/28 | 27/38 | FoxP3 | Abcan | Mean | OS |

F, female; M, male; Quality score was assessed using the validated Jadad scale; high $FoxP3^+$ T cells infiltration; Low, low $FoxP3^+$ T cells infiltration.

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**Table 3.** Main characteristics of studies about GC included in the meta-analysis.

| Author | Year | Journal | Quality score | Number of cases | | Marker | Antibody | Cutoff | Survival |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M/F | High/low | | | | |
| Mizukami[32] | 2008 | Br J Cancer | 7 | 56/24 | 40/40 | FoxP3 | eBioscience | Median | OS |
| Perrone[33] | 2008 | Eur J Cancer | 7 | 53/57 | 58/52 | FoxP3 | eBioscience | Median | OS,DFS |
| Haas[34] | 2009 | BMC Gastroenterol | 6 | 40/12 | 26/26 | FoxP3 | Abcan | Median | OS |
| Shen[35] | 2010 | J Cancer Res Clin Oncol | 7 | 89/44 | 66/67 | FoxP3 | Biolegend | Median | OS |
| Du[36] | 2011 | Cancer Sci | 6 | 131/48 | 87/92 | FoxP3 | Abcam | Median | OS,DFS |
| Kim[37] | 2011 | J Surg Oncol | 7 | 126/54 | 90/90 | FoxP3/CD4 | Abcan | Median | OS,DFS |
| Lu[38] | 2011 | J Surg Oncol | 7 | - | 30/30 | FoxP3 | Abcan | Median | OS |
| Shu[39] | 2011 | Zhonghua weichangwaike | 7 | - | 45/43 | FoxP3 | eBioscience | Median | OS |
| Wang[40] | 2011 | Ann Surg Oncol | 7 | 69/38 | 53/54 | FoxP3 | Abcan | Median | OS |
| Ishigami[41] | 2012 | Cancer Immunol Immunother | 7 | 99/42 | 76/65 | FoxP3 | Dako | Mean | OS |
| Kashimura[42] | 2012 | Gastric Cancer | 6 | 89/34 | 62/61 | FoxP3 | Abcan | Median | OS,DFS |
| Yoshii[43] | 2012 | Br J Cancer | 7 | 44/48 | 49/43 | FoxP3 | Abcan | Median | OS |
| Deng[44] | 2013 | PLoS One | 6 | 70/29 | 48/51 | FoxP3 | Abcan | Median | OS |
| Kim[45] | 2013 | Hum Pathol | 6 | 55/44 | 49/50 | FoxP3 | Abcan | Median | OS |
| Zhou[46] | 2013 | PLoS One | 7 | 89/44 | 87/46 | FoxP3 | Biolegend | Mean | OS |
| Ma[47] | 2014 | Br J Cancer | 7 | 132/65 | 24/173 | FoxP3 | Abcam | Other | OS |

F, female; M, male; Quality score was assessed using the validated Jadad scale; high $FoxP3^+$ T cells infiltration; Low, low $FoxP3^+$ T cells infiltration.

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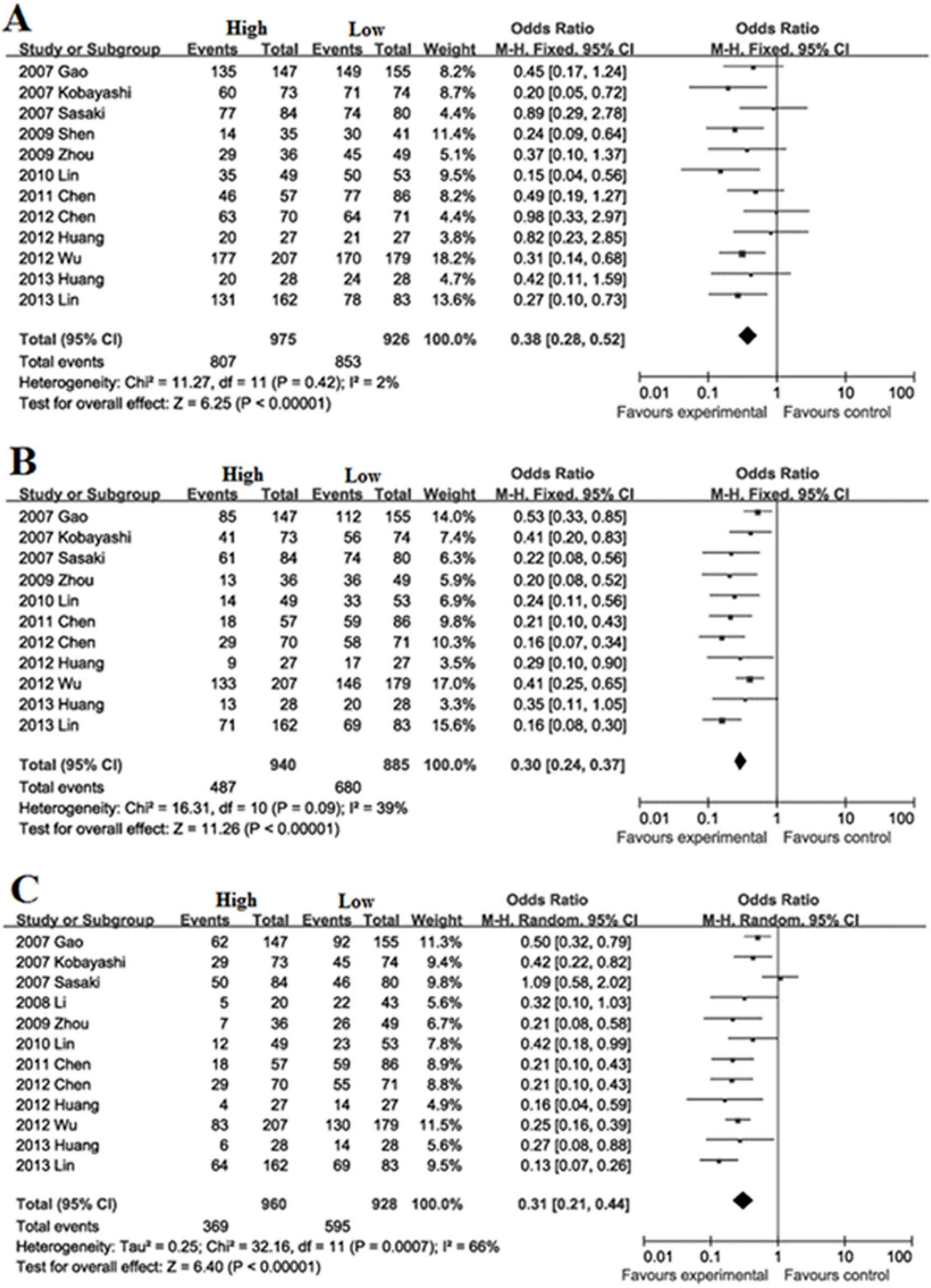


**Figure 1. Forest plot of Hazard ratio (HR) for survival of HCC patients.** Fixed effect model of odds ratio for survival of follow-up 1(A), 3-year (B) and random effect model of odds ratio for survival of follow-up 5-year (C) of HCC patients after surgery: high FoxP3$^+$ T cells infiltration patients *vs* low FoxP3$^+$ T cells infiltration patients.
doi:10.1371/journal.pone.0094376.g001

## Meta-analysis for HCC

Survival during follow-up 1, 3, 5-year after surgical resection: The overall survival rate during follow-up 1-year was significantly lower in high FoxP3$^+$ T cells infiltration patients (82.8%) than low FoxP3$^+$ T cells infiltration patients (92.1%) with a combined OR of 0.38 (95%CI = 0.28–0.52, $P<0.001$. Figure 1A). The overall survival rate during follow-up 3-year was significantly lower in high FoxP3$^+$ T cells infiltration patients (51.8%) than low FoxP3$^+$ T cells infiltration patients (76.8%) with a combined OR of 0.30 (95%CI = 0.24–0.37, $P<0.001$. Figure 1B). The overall survival

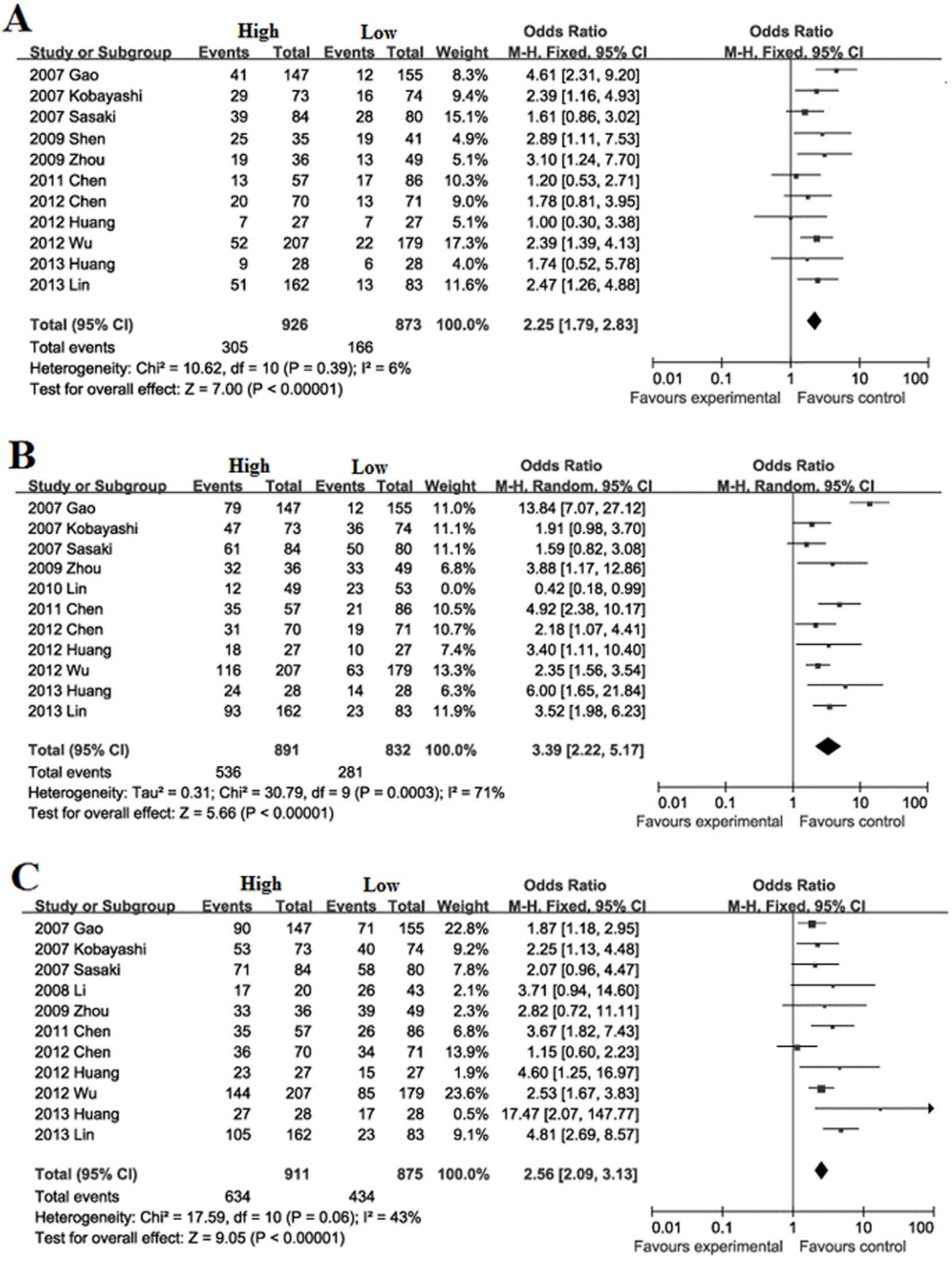


**Figure 2. Forest plot of HR for recurrence of HCC patients.** Fixed effect model of odds ratio for recurrence of follow-up 1(A), 5-year (C) and random effect model of odds ratio for recurrence of follow-up 3-year (B) of HCC patients after surgery: high FoxP3$^+$ T cells infiltration patients *vs* low FoxP3$^+$ T cells infiltration patients.
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rate during follow-up 5-year was significantly lower in high FoxP3$^+$ T cells infiltration patients (38.4%) than low FoxP3$^+$ T cells infiltration patients (64.1%) with a combined OR of 0.31 (95%CI = 0.21–0.44, $P<0.001$. Figure 1C).

Recurrence during follow-up 1, 3, 5-year after surgical resection: The recurrence rate during follow-up 1-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (32.9%) than low FoxP3$^+$ T cells infiltration patients (19.0%) with a combined OR of 2.25 (95%CI = 1.79–2.83, $P<0.001$. Figure 2A). The recurrence rate during follow-up 3-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (60.2%) than low FoxP3$^+$ T cells infiltration patients (33.8%) with a combined OR of 3.39 (95%CI = 2.22–5.17, $P<0.001$. Figure 2B). The recurrence rate during follow-up 5-year was

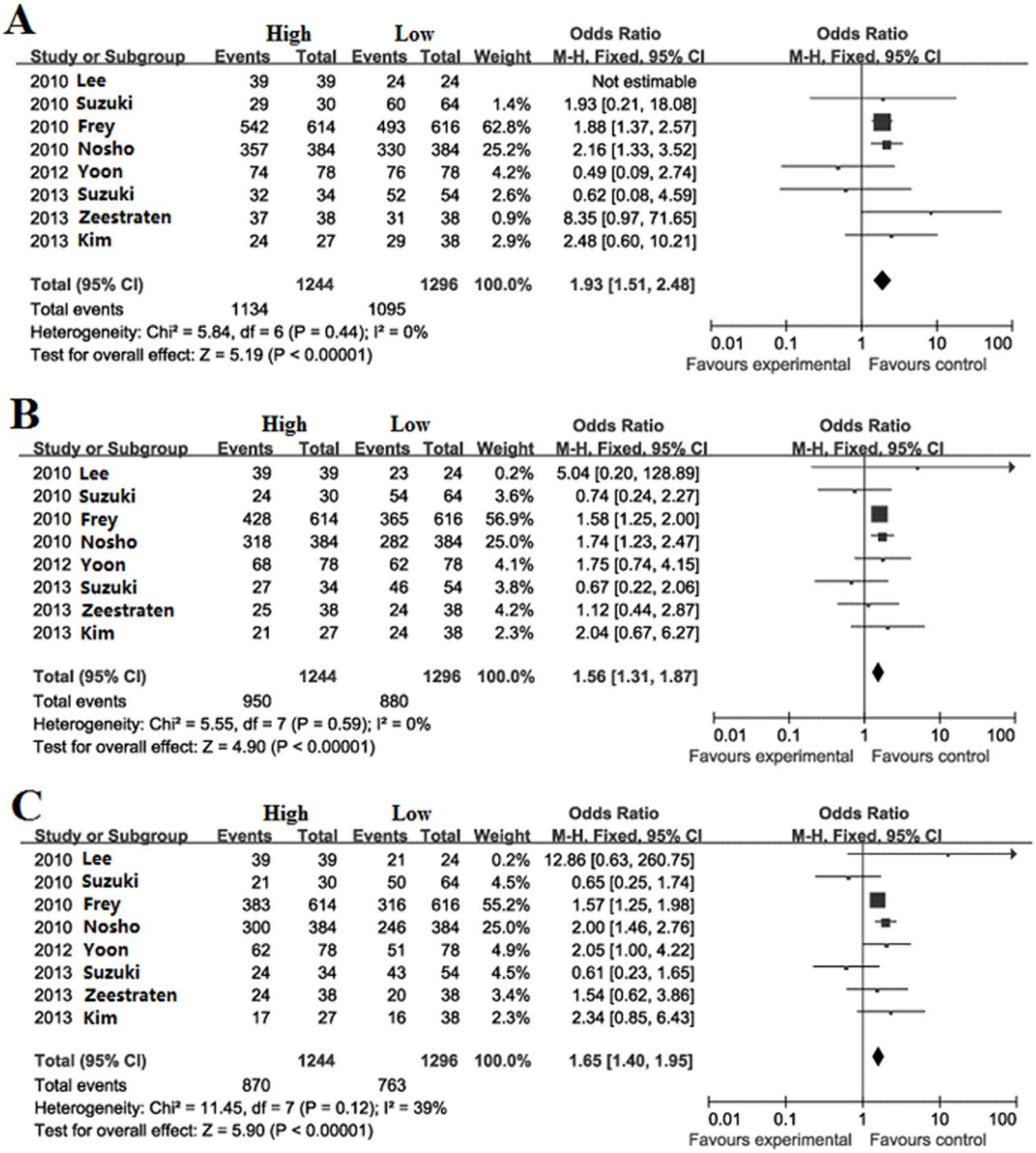


**Figure 3. Forest plot of HR for survival of CRC patients.** Fixed effect model of odds ratio for survival of follow-up 1 (A), 3 (B), 5-year (C) of CRC patients after surgery: high FoxP3$^+$ T cells infiltration patients *vs* low FoxP3$^+$ T cells infiltration patients.
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significantly higher in high FoxP3$^+$ T cells infiltration patients (69.6%) than low FoxP3$^+$ T cells infiltration patients (49.6%) with a combined OR of 2.56 (95%CI = 2.09–3.13, $P$<0.001. Figure 2C).

## Meta-analysis for CRC

Survival during follow-up 1, 3, 5-year after surgical resection: The overall survival rate during follow-up 1-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (91.2%) than low FoxP3$^+$ T cells infiltration patients (84.5%) with a combined OR of 1.93 (95%CI = 1.51–2.48, $P$<0.001. Figure 3A). The overall survival rate during follow-up 3-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (76.4%) than low FoxP3$^+$ T cells infiltration patients (67.9%) with a combined OR of 1.56 (95%CI = 1.31–1.87, $P$<0.001. Figure 3B). The overall survival rate during follow-up 5-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (69.9%) than low FoxP3$^+$ T cells infiltration patients (58.9%) with a combined OR of 1.65 (95%CI = 1.40–1.95, $P$<0.001. Figure 3C).

Recurrence during follow-up 1, 3, 5-year after surgical resection: There were no differences in 1(OR = 0.69, 95%CI = 0.23–2.01, $P$ = 0.49. Figure 4A), 3 (OR = 0.80, 95%CI = 0.37–1.72, $P$ = 0.57. Figure 4B) and 5-year (OR = 0.86, 95%CI = 0.34–2.18, $P$ = 0.75. Figure 4C) recurrences between high and low FoxP3$^+$ T cells infiltration patients.

## Meta-analysis for GC

Survival during follow-up 1, 3, 5-year after surgical resection: The overall survival rate during follow-up 1-year was significantly lower in high FoxP3$^+$ T cells infiltration patients (87.2%) than low FoxP3$^+$ T cells infiltration patients (92.8%) with a combined OR of 0.50 (95%CI = 0.28–0.88, $P$ = 0.02. Figure 5A). The overall

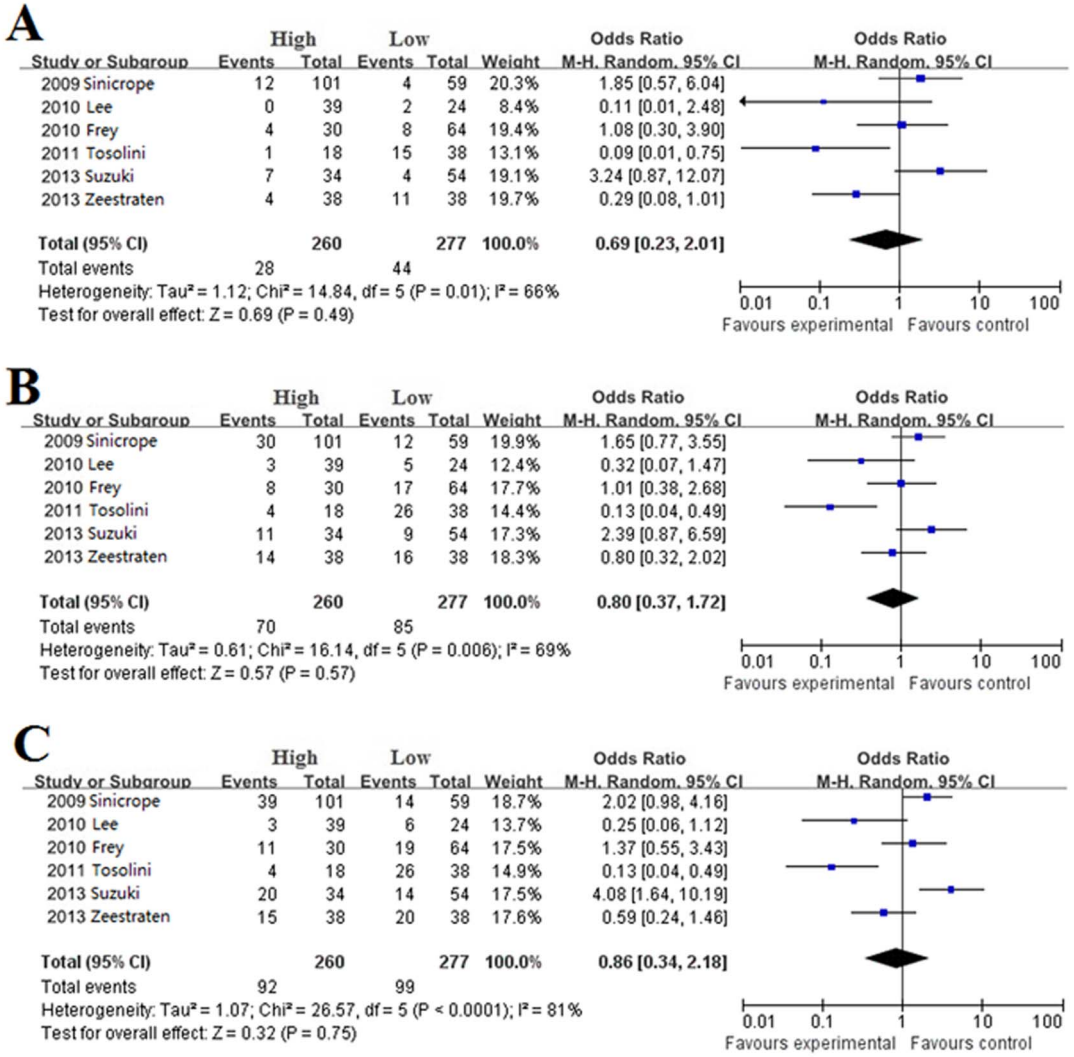


**Figure 4. Forest plot of HR for recurrence of CRC patients.** Random effect model of odds ratio for recurrence of follow-up 1 (A), 3 (B), 5-year (C) of CRC patients after surgery: high FoxP3$^+$ T cells infiltration patients *vs* low FoxP3$^+$ T cells infiltration patients.
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survival rate during follow-up 3-year was significantly lower in high FoxP3$^+$ T cells infiltration patients (65.4%) than low FoxP3$^+$ T cells infiltration patients (78.2%) with a combined OR of 0.51 (95%CI = 0.32–0.83, $P = 0.007$. Figure 5B). The overall survival rate during follow-up 5-year was significantly lower in high FoxP3$^+$ T cells infiltration patients (55.6%) than low FoxP3$^+$ T cells infiltration patients (69.0%) with a combined OR of 0.56 (95%CI = 0.38–0.84, $P = 0.005$. Figure 5C).

Recurrence during follow-up 1, 3, 5-year after surgical resection: The recurrence rate during follow-up 1-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (26.9%) than low FoxP3$^+$ T cells infiltration patients (10.8%) with a combined OR of 3.06 (95%CI = 1.95–4.80, $P<0.001$. Figure 6A). The recurrence rate during follow-up 3-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (43.4%) than low FoxP3$^+$ T cells infiltration patients (22.4%) with a combined OR of 2.77 (95%CI = 1.92–3.98, $P<0.001$. Figure 6B). The recurrence rate during follow-up 5-year was significantly higher in high FoxP3$^+$ T cells infiltration patients (52.5%) than low FoxP3$^+$ T cells infiltration patients (33.6%) with a combined OR of 2.52 (95%CI = 1.76–3.62, $P<0.001$. Figure 6C).

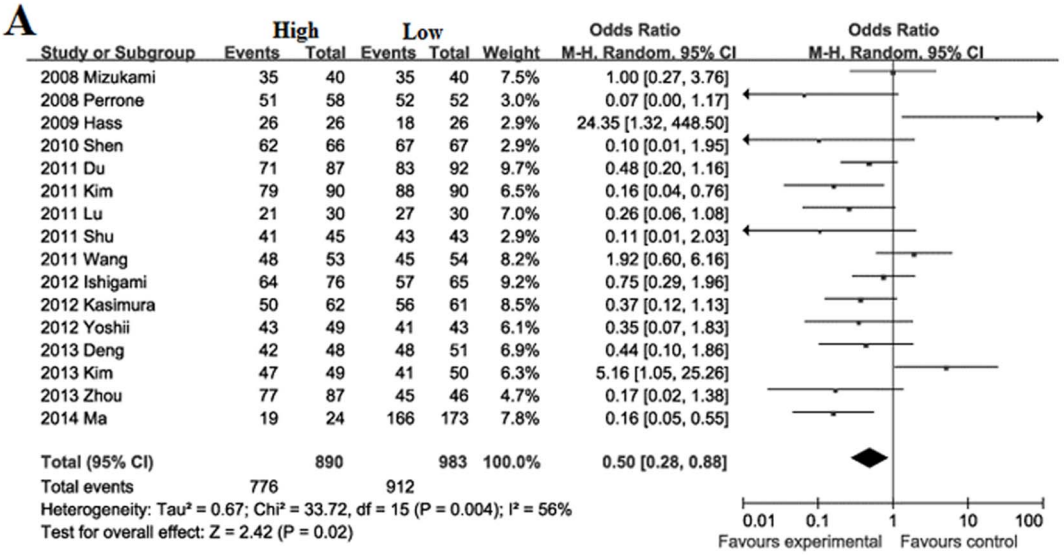
## Publication bias

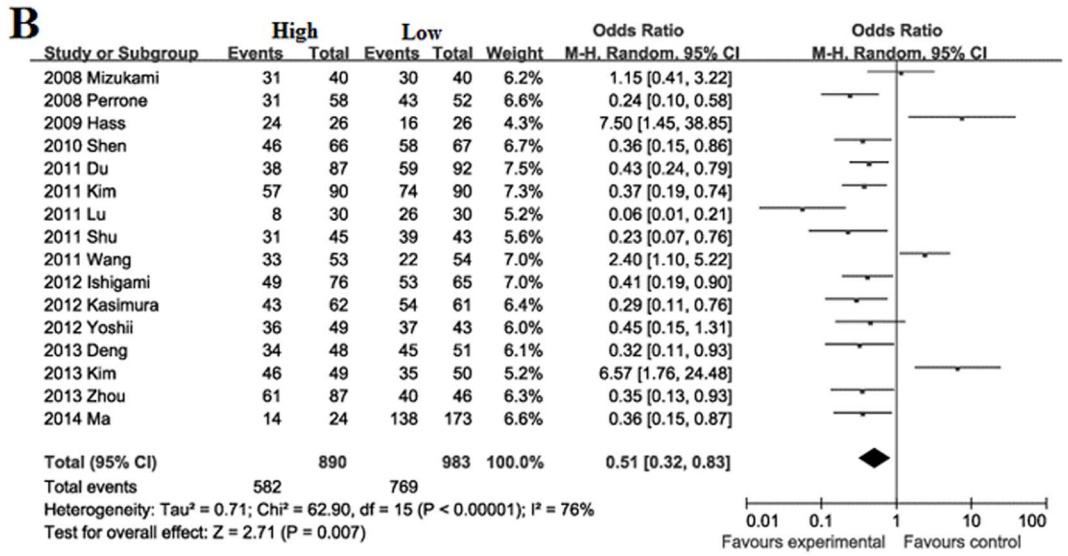
Publication bias may exist when no significant findings remain unpublished, thus artificially inflating the apparent magnitude of an effect. Survival and recurrences following high and low FoxP3$^+$ T cells infiltration patients with HCC, CRC and GC were calculated by the fixed-effect model and random-effect model, respectively. The results were similar and the combined results were highly reliable.

The funnel plots on survival and recurrence following high and low FoxP3$^+$ T cells infiltration patients with HCC (Figure S2), CRC (Figure S3) and GC (Figure S4) showed basic symmetry, which suggested no publication bias.

## Discussion

Tregs are functionally immunosuppressive subsets of CD4$^+$ T, which were found by Sakaguchi et al [2] in 1995. They control the balance between tolerance and rejection of self and altered self by



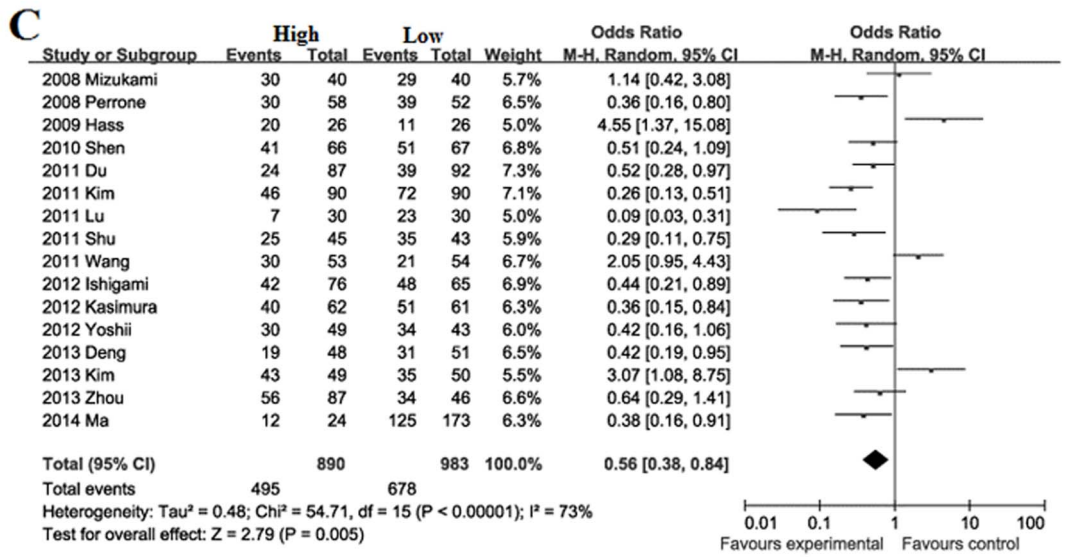




**Figure 5. Forest plot of HR for survival of GC patients.** Random effect model of odds ratio for survival of follow-up 1 (A), 3 (B), 5-year (C) of GC patients after surgery: high FoxP3$^+$ T cells infiltration patients *vs* low FoxP3$^+$ T cells infiltration patients.
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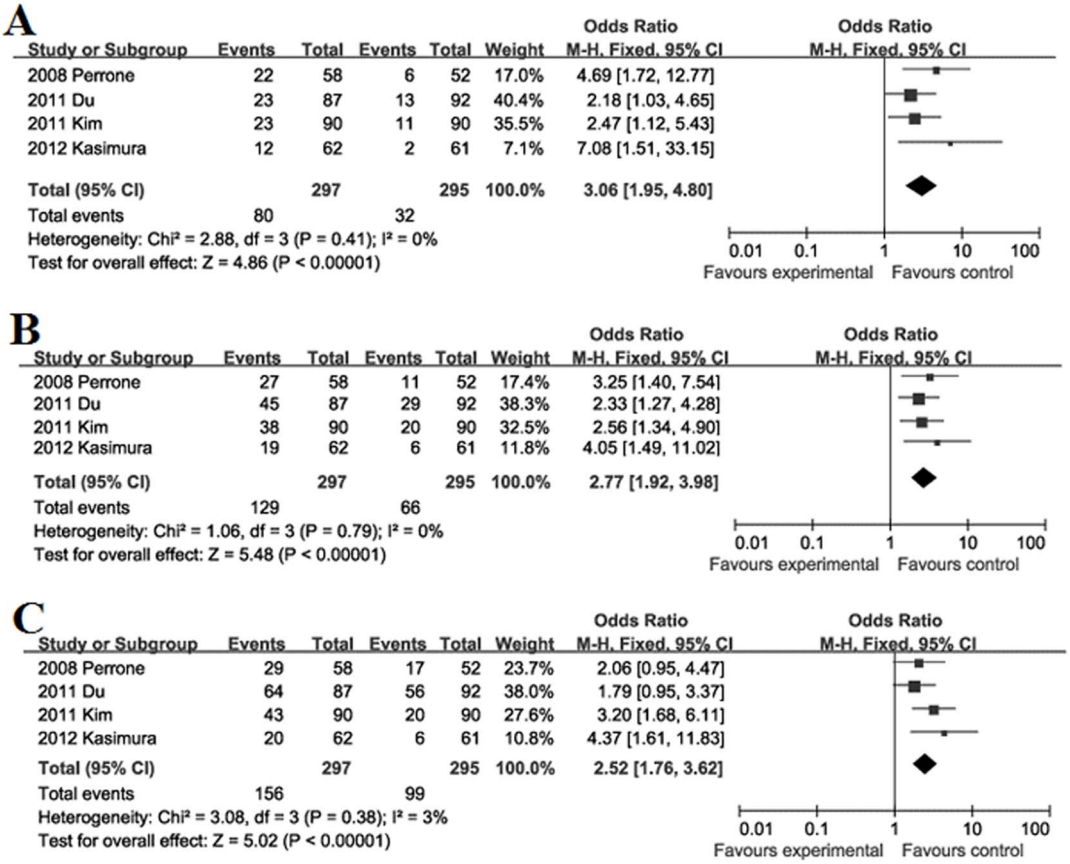


**Figure 6. Forest plot of HR for recurrence of GC patients.** Fixed effect model of odds ratio for recurrence of follow-up 1 (A), 3 (B), 5-year (C) of GC patients after surgery: high FoxP3$^+$ T cells infiltration patients *vs* low FoxP3$^+$ T cells infiltration patients.
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secreting IL-4, IL-10 and TGF-β and other cytokines [48]. For the identification of Tregs, many markers such as CTLA-4, GITR, OX-4, CD127 and transcription factor FoxP3 can be used [49]. FoxP3 is now considered as the most specific marker for Tregs [50], because it is critical for the development and function of Tregs. And FoxP3 became a popular single marker for Tregs studies in tumor. However, the conclusions from published research regarding its prognostic value for different tumors were controversial in different gastrointestinal cancers. Even in the same kind of tumor, this conclusion was not entirely consistent such as CRC and GC [3–5].

Meta-analysis is useful to integrate results from independent studies for a specified outcome. Pooled results from the combining relevant studies are statistical powerful, and make it possible to detecting effects that may be missed by individual studies. To date, no meta-analysis has been undertaken for any studies that evaluate tumor-infiltrating FoxP3$^+$ T cells as a prognostic marker in HCC, CRC or GC. In this meta-analysis, 13 studies involving 1964 patients were analyzed. All the studies consistently shown high density of tumor-infiltrating FoxP3$^+$ T cells have been associated with poor survival and high recurrences in HCC, consistent with the initial hypothesis that FoxP3$^+$ T cells inhibit antitumor immunity. These conclusions were confirmed by our previous reports [18,20,51]. In all our data clarified the results of individual studies and to identify patients at high risk for whom specific- or adjuvant-therapy might be necessary since high density of FoxP3$^+$ T cells is a prognostic factor for HCC.

For CRC, 10 studies involving 2756 patients were analyzed. Be different from HCC, studies of the prognostic value of FoxP3$^+$ T cells in CRC have lead to highly discrepant findings. Some studies investigating colorectal cancer concluded that FoxP3$^+$ T cells correlated with a good prognosis, whereas other studies found no prognostic association or even a bad prognostic claim [3–5,22–31]. The data were organized according to overall survival and recurrence; then combined results strongly demonstrated that high density of tumor-infiltrating FoxP3$^+$ T cells was a good prognosis for CRC. The result has challenged the conventionally theory that FoxP3$^+$ T cells can suppress tumor immunity. It is regrettable that very few studies in the literature have examined the exact functional properties of FoxP3$^+$ T cells isolated from human CRC. In considering CRC grows in a septic microenvironment, researchers recently hypothesized that the favorable prognostic effect of FoxP3$^+$ T cells may reflect their ability to preferentially suppress tumor-promoting inflammatory responses to gut microbes and Th17-cell-dependent proinflammatory [4].

For GC, the prognostic significance of tumor-infiltrating FoxP3$^+$ T cells for the survival of patients with gastric cancer remains controversial. There are 16 studies involving 1873 patients that compared the survival of HCC according to FoxP3$^+$ T cells expression level of the primary tumor met the enrollment criteria. In the 16 studies, studies looking at gastric cancers show a split among poor ($n = 11$), neutral ($n = 2$), and good ($n = 3$) prognostic claims. Base on those studies, the Meta-Analysis results consistent with HCC, high density of FoxP3$^+$ T cells was associated with poor survival and high recurrences.

However, one should be cautious when interrupting these results due to the limitations of our studies. Further high-quality studies are still needed to confirm these results. There are several important limitations also need to be considered. First of all, patients had received different treatments and postoperative treatment; preoperative TNM category and histologic types were various. Whereas, we were unable to assess these potential confounders present in individual studies. Second, although we tried to identify all relevant data, potential publication bias was unavoidable and some data could still be missing. Third, the antibody, cell-scoring strategy and the cutoff value were defined differently in some studies. Finally, this study was constrained to studies published in English and Chinese language; it was difficult to completely rule out publication bias.

HCC, CRC and GC are gastrointestinal tumors, and come from immune tolerance organs which exposed to high levels exogenous antigens. However, the role and function of $FoxP3^+$ T cells were different completely. Thus, the original view that $FoxP3^+$ T cells invariably suppress tumor immunity is oversimplified for CRC. The discrepancy in different tumors could arise from differences in study methodologies or in the biologic properties of specific tumor types. We require better understanding of the functional subtypes of $FoxP3^+$ T cells and their biologic properties in different tumor microenvironments if we wish to rationally modulate their behavior to enhance tumor immunity. We believe that the interaction between the different components of the tumor microenvironment and the diversity of signals provided by the tumor cells can explain these discrepancies in the prognostic studies relying on the presence of Tregs in tumor infiltrates. Recent findings have shown that a subset of $FoxP3^+$ Tregs could acquire the capacity to produce IL-17 instead of IL-10 and TGF-β [52]. The double-positive T cells exhibit functions of both Th17 and Tregs, or act as a transient population that may eventually generate either Th17 or Tregs, presenting a potential mechanism for the Tregs/Th17 regulation in the progression of tumor.

In summary, some studies fit with the general notion that $FoxP3^+$ T cells suppress adaptive immune responses and led many groups to pursue strategies to deplete $FoxP3^+$ T cells from patients or mouse with cancer as a means to enhance tumor immunity [53–55]. However, our findings suggest that the treatment of depletion or attenuation of $FoxP3^+$ T cells can be used for the treatment of HCC and GC but detrimental for CRC.

## Supporting Information

**Figure S1 Flow diagram of study selection.** A: Flow diagram of study selection for HCC; B: Flow diagram of study selection for CRC; C: Flow diagram of study selection for GC.
(DOC)

**Figure S2 Funnel plots for HCC.** A: 12 articles in the meta-analysis of survival during follow-up 1-year after treatment; B: 11 articles in the meta-analysis of survival during follow-up 3-year after treatment; C: 12 articles in the meta-analysis of survival during follow-up 5-year after treatment; D: 11 articles in the meta-analysis of recurrence during follow-up 1-year after treatment; E: 10 articles in the meta-analysis of recurrence during follow-up 3-year after treatment; F: 11 articles in the meta-analysis of recurrence during follow-up 5-year after treatment.
(TIF)

**Figure S3 Funnel plots for CRC.** A: 8 articles in the meta-analysis of survival during follow-up 1-year after treatment; B: 8 articles in the meta-analysis of survival during follow-up 3-year after treatment; C: 8 articles in the meta-analysis of survival during follow-up 5-year after treatment; D: 6 articles in the meta-analysis of recurrence during follow-up 1-year after treatment; E: 6 articles in the meta-analysis of recurrence during follow-up 3-year after treatment; F: 6 articles in the meta-analysis of recurrence during follow-up 5-year after treatment.
(TIF)

**Figure S4 Funnel plots for GC.** A: 16 articles in the meta-analysis of survival during follow-up 1-year after treatment; B: 16 articles in the meta-analysis of survival during follow-up 3-year after treatment; C: 16 articles in the meta-analysis of survival during follow-up 5-year after treatment; D: 4 articles in the meta-analysis of recurrence during follow-up 1-year after treatment; E: 4 articles in the meta-analysis of recurrence during follow-up 3-year after treatment; F: 4 articles in the meta-analysis of recurrence during follow-up 5-year after treatment.
(TIF)

**Checklist S1 PRISMA Checklist.**
(ZIP)

## Author Contributions

Conceived and designed the experiments: ZD FW. Performed the experiments: YH FW. Analyzed the data: YH HL RY FW. Contributed reagents/materials/analysis tools: YH HL FW PW YG. Wrote the paper: YH HL YZ EW PW YG.

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