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 were higher than low $FoxP3^+$ T cells infiltration patients 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 tumorinfiltrating 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 charact	teristics of stu	dies about HCC included in	the meta-analysis.						
Author	Year	Journal	Quality score	Number of ci	ses	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	9	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	SO
Shen[13]	2009	Can J Surg	6	70/6	35/41	FoxP3	Abcan	Median	OS,DFS
Zhou[14]	2009	Int J Cancer	7	I	36/49	FoxP3	Abcan	Median	OS,DFS
Lin[15]	2010	Chin J Cancer	6	85/17	49/53	FoxP3	Abcan	Median	SO
Chen[16]	2011	PLoS One	7	I	57/86	FoxP3	Abcan	Median	OS,DFS
Chen[17]	2011	Med Oncol	7	ı	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, Iow FoxP3⁺ T cells infiltration. I doi:10.1371/journal.pone.0094376.t001

FoxP3+ T Cells in Gastrointestinal Cancers

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 χ^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.

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	ı	384/384	FoxP3	BioLegend	Other	OS
Tosolini[27]	2011	Cancer Research	5	1	18/38	FoxP3	Abcan	Other	DFS
Yoon[28]	2012	PLoS One	7	ı	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 scor doi:10.1371/journal.pone.00943:	re was asse 76.t002	essed using the validated Jadad scale; high FoxF	P3 ⁺ T cells infiltration;	Low, low FoxP3 ⁺ T (cells infiltration.				

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

Table 3. Main characteristi	cs of studies about GC included in t	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	06/06	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
lshigami[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 w	is assessed using the validated Jadad scale; hi	igh FoxP3 ⁺ T cells infiltr	ation; Low, low	FoxP3 ⁺ T cells infiltra	ition.			

doi:10.1371/journal.pone.0094376.t003

\mathbf{A}	Hi	gh	L	w		Odds Ratio		Odds	Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% Cl		M-H. Fixe	d. 95% CI	
2007 Gao	135	147	149	155	8.2%	0.45 [0.17, 1.24]			t i	
2007 Kobayashi	60	73	71	74	8.7%	0.20 [0.05, 0.72]				
2007 Sasaki	77	84	74	80	4.4%	0.89 [0.29, 2.78]		_	_	
2009 Shen	14	35	30	41	11.4%	0.24 [0.09, 0.64]				
2009 Zhou	29	36	45	49	5.1%	0.37 [0.10, 1.37]			-	
2010 Lin	35	49	50	53	9.5%	0.15 [0.04, 0.56]				
2011 Chen	46	57	77	86	8.2%	0.49 [0.19, 1.27]			-	
2012 Chen	63	70	64	71	4.4%	0.98 [0.33, 2.97]			_	
2012 Huang	20	27	21	27	3.8%	0.82 [0.23, 2.85]				
2012 Wu	177	207	170	179	18.2%	0.31 [0.14, 0.68]				
2013 Huang	20	28	24	28	4.7%	0.42 [0.11, 1.59]			-	
2013 Lin	131	162	78	83	13.6%	0.27 [0.10, 0.73]				
Total (95% CI)		975		926	100.0%	0.38 [0.28, 0.52]		•		
Total events	807		853							
Heterogeneity: Chi2 =	11.27, df =	11 (P =	0.42); 12 :	= 2%						
Test for overall effect:	Z = 6.25 (P	< 0.000	001)				0.01	0.1	1 10	100
						Fa	vours (experimental	Favours con	trol

B						Odda Patia	0	dde Datio	
_	nig	in .	Lo	w		Odds Ratio		dus katio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	М-Н.	Fixed, 95% Cl	
2007 Gao	85	147	112	155	14.0%	0.53 [0.33, 0.85]			
2007 Kobayashi	41	73	56	74	7.4%	0.41 [0.20, 0.83]		-	
2007 Sasaki	61	84	74	80	6.3%	0.22 [0.08, 0.56]		-	
2009 Zhou	13	36	36	49	5.9%	0.20 [0.08, 0.52]		-	
2010 Lin	14	49	33	53	6.9%	0.24 [0.11, 0.56]		-	
2011 Chen	18	57	59	86	9.8%	0.21 [0.10, 0.43]		-	
2012 Chen	29	70	58	71	10.3%	0.16 [0.07, 0.34]			
2012 Huang	9	27	17	27	3.5%	0.29 [0.10, 0.90]			
2012 Wu	133	207	146	179	17.0%	0.41 [0.25, 0.65]	-		
2013 Huang	13	28	20	28	3.3%	0.35 [0.11, 1.05]			
2013 Lin	71	162	69	83	15.6%	0.16 [0.08, 0.30]			
Total (95% CI)		940		885	100.0%	0.30 [0.24, 0.37]	•		
Total events	487		680						
Heterogeneity: Chi2 =	16.31, df =	10 (P =	0.09); 12 =	: 39%					
Test for overall effect:	Z = 11.26 (P<0.00	0001)			-	0.01 0.1	1 10	100
						Fa	avours experiment	tal Favours cont	rol

C									
C	Hig	gh	Lo	W		Odds Ratio	Odds	Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Random. 95% C	M-H. Rand	om. 95% CI	
2007 Gao	62	147	92	155	11.3%	0.50 [0.32, 0.79]			
2007 Kobayashi	29	73	45	74	9.4%	0.42 [0.22, 0.82]			
2007 Sasaki	50	84	46	80	9.8%	1.09 [0.58, 2.02]	-	-	
2008 Li	5	20	22	43	5.6%	0.32 [0.10, 1.03]			
2009 Zhou	7	36	26	49	6.7%	0.21 [0.08, 0.58]			
2010 Lin	12	49	23	53	7.8%	0.42 [0.18, 0.99]			
2011 Chen	18	57	59	86	8.9%	0.21 [0.10, 0.43]			
2012 Chen	29	70	55	71	8.8%	0.21 [0.10, 0.43]			
2012 Huang	4	27	14	27	4.9%	0.16 [0.04, 0.59]			
2012 Wu	83	207	130	179	11.5%	0.25 [0.16, 0.39]	-		
2013 Huang	6	28	14	28	5.6%	0.27 [0.08, 0.88]			
2013 Lin	64	162	69	83	9.5%	0.13 [0.07, 0.26]			
Total (95% CI)		960		928	100.0%	0.31 [0.21, 0.44]	•		
Total events	369		595						
Heterogeneity: Tau ² =	0.25; Chi2 :	32.16,	df = 11 (i	P = 0.0	007); l² = 6	6%			
Test for overall effect:	Z = 6.40 (P	< 0.000	001)				0.01 0.1	1 10	100
			,			F	avours experimental	Favours cont	rol

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

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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

A	Hi	gh	Lo	W		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% Cl	M-H, Fixed, 95% Cl
2007 Gao	41	147	12	155	8.3%	4.61 [2.31, 9.20]	·
2007 Kobayashi	29	73	16	74	9.4%	2.39 [1.16, 4.93]	
2007 Sasaki	39	84	28	80	15.1%	1.61 [0.86, 3.02]	
2009 Shen	25	35	19	41	4.9%	2.89 [1.11, 7.53]	
2009 Zhou	19	36	13	49	5.1%	3.10 [1.24, 7.70]	
2011 Chen	13	57	17	86	10.3%	1.20 [0.53, 2.71]	
2012 Chen	20	70	13	71	9.0%	1.78 [0.81, 3.95]	
2012 Huang	7	27	7	27	5.1%	1.00 [0.30, 3.38]	
2012 Wu	52	207	22	179	17.3%	2.39 [1.39, 4.13]	
2013 Huang	9	28	6	28	4.0%	1.74 [0.52, 5.78]	
2013 Lin	51	162	13	83	11.6%	2.47 [1.26, 4.88]	
Total (95% CI)		926		873	100.0%	2.25 [1.79, 2.83]	•
Total events	305		166				
Heterogeneity: Chi ² = 1	0.62, df =	10 (P =	0.39); l ² =	- 6%			
Test for overall effect: 2	z = 7.00 (P	< 0.000	001)			Fa	avours experimental Favours control

Favours experimental Favours control

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Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% C	M-H, Random, 95% Cl
2007 Gao	79	147	12	155	11.0%	13.84 [7.07, 27.12]	
2007 Kobayashi	47	73	36	74	11.1%	1.91 [0.98, 3.70]	
2007 Sasaki	61	84	50	80	11.1%	1.59 [0.82, 3.08]	+
2009 Zhou	32	36	33	49	6.8%	3.88 [1.17, 12.86]	
2010 Lin	12	49	23	53	0.0%	0.42 [0.18, 0.99]	
2011 Chen	35	57	21	86	10.5%	4.92 [2.38, 10.17]	
2012 Chen	31	70	19	71	10.7%	2.18 [1.07, 4.41]	
2012 Huang	18	27	10	27	7.4%	3.40 [1.11, 10.40]	_ .
2012 Wu	116	207	63	179	13.3%	2.35 [1.56, 3.54]	-
2013 Huang	24	28	14	28	6.3%	6.00 [1.65, 21.84]	
2013 Lin	93	162	23	83	11.9%	3.52 [1.98, 6.23]	
Total (95% CI)		891		832	100.0%	3.39 [2.22, 5.17]	•
Total events	536		281				
Heterogeneity: Tau ² =	0.31; Chi2	= 30.79,	df = 9 (P	= 0.00	03); l ² = 7	1%	
Test for overall effect:	Z = 5.66 (P	< 0.000	001)			-	
						- Fi	avours experimental Favours control

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C	Hi	gh	L	w		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C	M-H, Fixed, 95% Cl
2007 Gao	90	147	71	155	22.8%	1.87 [1.18, 2.95]	
2007 Kobayashi	53	73	40	74	9.2%	2.25 [1.13, 4.48]	
2007 Sasaki	71	84	58	80	7.8%	2.07 [0.96, 4.47]	
2008 Li	17	20	26	43	2.1%	3.71 [0.94, 14.60]	
2009 Zhou	33	36	39	49	2.3%	2.82 [0.72, 11.11]	
2011 Chen	35	57	26	86	6.8%	3.67 [1.82, 7.43]	
2012 Chen	36	70	34	71	13.9%	1.15 [0.60, 2.23]	
2012 Huang	23	27	15	27	1.9%	4.60 [1.25, 16.97]	· · · · ·
2012 Wu	144	207	85	179	23.6%	2.53 [1.67, 3.83]	-=-
2013 Huang	27	28	17	28	0.5%	17.47 [2.07, 147.77]	
2013 Lin	105	162	23	83	9.1%	4.81 [2.69, 8.57]	
Total (95% CI)		911		875	100.0%	2.56 [2.09, 3.13]	•
Total events	634		434				
Heterogeneity: Chi ² = 1	7.59, df =	10 (P =	0.06); l ² =	= 43%			
Test for overall effect: 2	Z = 9.05 (P	< 0.000	001)			ı	Favours experimental Favours control

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. doi:10.1371/journal.pone.0094376.g002

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

A	н	igh	L	w		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	M-H, Fixed, 95% CI
2010 Lee	39	39	24	24		Not estimable	
2010 Suzuki	29	30	60	64	1.4%	1.93 [0.21, 18.08]	
2010 Frey	542	614	493	616	62.8%	1.88 [1.37, 2.57]	1
2010 Nosho	357	384	330	384	25.2%	2.16 [1.33, 3.52]	
2012 Yoon	74	78	76	78	4.2%	0.49 [0.09, 2.74]	
2013 Suzuki	32	34	52	54	2.6%	0.62 [0.08, 4.59]	
2013 Zeestraten	37	38	31	38	0.9%	8.35 [0.97, 71.65]	· · · · · · · · · · · · · · · · · · ·
2013 Kim	24	27	29	38	2.9%	2.48 [0.60, 10.21]	
Total (95% CI)		1244		1296	100.0%	1.93 [1.51, 2.48]	•
Total events	1134		1095				
Heterogeneity: Chi ² = 5	.84, df = 6	(P = 0.4	44); l ² = 0	%			
Test for overall effect: 2	z = 5.19 (P	< 0.000	001)			F	avours experimental Eavours control
							inours experimental in avours control
B	Hi	igh	Lo	W		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed. 95% C	M-H. Fixed, 95% Cl
2010 Lee	39	39	23	24	0.2%	5.04 [0.20, 128.89]	
2010 Suzuki	24	30	54	64	3.6%	0.74 [0.24, 2.27]	
2010 Frey	428	614	365	616	56.9%	1.58 [1.25, 2.00]	
2010 Nosho	318	384	282	384	25.0%	1.74 [1.23, 2.47]	-=-
2012 Yoon	68	78	62	78	4.1%	1.75 [0.74, 4.15]	+
2013 Suzuki	27	34	46	54	3.8%	0.67 [0.22, 2.06]	
2013 Zeestraten	25	38	24	38	4.2%	1.12 [0.44, 2.87]	_ _ _
2013 Kim	21	27	24	38	2.3%	2.04 [0.67, 6.27]	
Total (95% CI)		1244		1296	100.0%	1.56 [1.31, 1.87]	•
Total events	950		880				
Heterogeneity: Chi ² = 5	5.55, df = 7	7 (P = 0.	59); l ² = 0	%			
Test for overall effect:	Z = 4.90 (F	< 0.00	001)			F	avours experimental Favours control
C	U	iah	L			Odds Ratio	Odds Ratio
Study or Subgroup	Evente	Total	Events	Total	Weight	M-H Fixed 95% Cl	M-H Fixed 95% Cl
	L'YCHLS	Total	Evenus	Total	Telgill	10-11. FIXED. 35% CI	m-n, n Acu, 33/6 cr

		-									
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C		M-H, Fix	ed. 95%	CI	
2010 Lee	39	39	21	24	0.2%	12.86 [0.63, 260.75]		-			\rightarrow
2010 Suzuki	21	30	50	64	4.5%	0.65 [0.25, 1.74]			+-		
2010 Frey	383	614	316	616	55.2%	1.57 [1.25, 1.98]					
2010 Nosho	300	384	246	384	25.0%	2.00 [1.46, 2.76]			-		
2012 Yoon	62	78	51	78	4.9%	2.05 [1.00, 4.22]			<u> </u>		
2013 Suzuki	24	34	43	54	4.5%	0.61 [0.23, 1.65]			+		
2013 Zeestraten	24	38	20	38	3.4%	1.54 [0.62, 3.86]		-	+		
2013 Kim	17	27	16	38	2.3%	2.34 [0.85, 6.43]			+		
Total (95% CI)		1244		1296	100.0%	1.65 [1.40, 1.95]			•		
Total events	870		763								
Heterogeneity: Chi2 =	11.45, df =	7 (P = 0).12); l ² =	39%						+	400
Test for overall effect:	Z = 5.90 (P	< 0.00	001)				0.01	0.1	1	10	100
						F	avours	experimental	Favours	s contr	ol

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. doi:10.1371/journal.pone.0094376.q003

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. doi:10.1371/journal.pone.0094376.g004

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

\mathbf{A}	1	High	L	w		Odds Ratio	Odds	Ratio
Study or Sub	group Events	Total	Events	Total	Weight	M-H. Random. 95% C	M-H, Rand	om, 95% CI
2008 Mizukan	i 35	40	35	40	7.5%	1.00 [0.27, 3.76]		
2008 Perrone	51	58	52	52	3.0%	0.07 [0.00, 1.17]	←	t
2009 Hass	26	26	18	26	2.9%	24.35 [1.32, 448.50]		
2010 Shen	62	66	67	67	2.9%	0.10 [0.01, 1.95]	· ·	-
2011 Du	71	87	83	92	9.7%	0.48 [0.20, 1.16]		t
2011 Kim	79	90	88	90	6.5%	0.16 [0.04, 0.76]		
2011 Lu	21	30	27	30	7.0%	0.26 [0.06, 1.08]		t
2011 Shu	41	45	43	43	2.9%	0.11 [0.01, 2.03]	← .	-
2011 Wang	48	53	45	54	8.2%	1.92 [0.60, 6.16]	-	
2012 Ishigami	64	76	57	65	9.2%	0.75 [0.29, 1.96]		-
2012 Kasimur	a 50	62	56	61	8.5%	0.37 [0.12, 1.13]		t
2012 Yoshii	43	49	41	43	6.1%	0.35 [0.07, 1.83]	······	-
2013 Deng	42	48	48	51	6.9%	0.44 [0.10, 1.86]		-
2013 Kim	47	49	41	50	6.3%	5.16 [1.05, 25.26]		
2013 Zhou	77	87	45	46	4.7%	0.17 [0.02, 1.38]		-
2014 Ma	19	24	166	173	7.8%	0.16 [0.05, 0.55]	<u> </u>	
Total (95% CI)	890		983	100.0%	0.50 [0.28, 0.88]	+	
Total events	776		912					
Heterogeneity	Tau ² = 0.67; Chi	² = 33.72,	df = 15 (P = 0.0	04); l ² = 5	6%		
Test for overa	l effect: Z = 2.42	(P = 0.02))			-	0.01 0.1	1 10 100
		,,				Fi	avours experimental	Favours control

B	Hi	gh	Lo	W		Odds Ratio	Odds	Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Random, 95% C	M-H, Rand	om. 95% Cl
2008 Mizukami	31	40	30	40	6.2%	1.15 [0.41, 3.22]		
2008 Perrone	31	58	43	52	6.6%	0.24 [0.10, 0.58]		
2009 Hass	24	26	16	26	4.3%	7.50 [1.45, 38.85]		
2010 Shen	46	66	58	67	6.7%	0.36 [0.15, 0.86]		
2011 Du	38	87	59	92	7.5%	0.43 [0.24, 0.79]		
2011 Kim	57	90	74	90	7.3%	0.37 [0.19, 0.74]		
2011 Lu	8	30	26	30	5.2%	0.06 [0.01, 0.21]		
2011 Shu	31	45	39	43	5.6%	0.23 [0.07, 0.76]		
2011 Wang	33	53	22	54	7.0%	2.40 [1.10, 5.22]		
2012 Ishigami	49	76	53	65	7.0%	0.41 [0.19, 0.90]		
2012 Kasimura	43	62	54	61	6.4%	0.29 [0.11, 0.76]		
2012 Yoshii	36	49	37	43	6.0%	0.45 [0.15, 1.31]		-
2013 Deng	34	48	45	51	6.1%	0.32 [0.11, 0.93]		
2013 Kim	46	49	35	50	5.2%	6.57 [1.76, 24.48]		
2013 Zhou	61	87	40	46	6.3%	0.35 [0.13, 0.93]		
2014 Ma	14	24	138	173	6.6%	0.36 [0.15, 0.87]		
Total (95% CI)		890		983	100.0%	0.51 [0.32, 0.83]	+	
Total events	582		769					
Heterogeneity: Tau ² =	0.71; Chi ² =	= 62.90,	df = 15 (F	P < 0.0	0001); l ² =	76%		
Test for overall effect:	Z = 2.71 (P	= 0.007	0		,.	E.	0.01 0.1	10 100 100
			,			Fa	avours experimental	ravours control

	H	igh	L	ow		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% C	M-H, Random, 95% Cl
2008 Mizukami	30	40	29	40	5.7%	1.14 [0.42, 3.08]	_ _ _
2008 Perrone	30	58	39	52	6.5%	0.36 [0.16, 0.80]	
2009 Hass	20	26	11	26	5.0%	4.55 [1.37, 15.08]	
2010 Shen	41	66	51	67	6.8%	0.51 [0.24, 1.09]	
2011 Du	24	87	39	92	7.3%	0.52 [0.28, 0.97]	
2011 Kim	46	90	72	90	7.1%	0.26 [0.13, 0.51]	
2011 Lu	7	30	23	30	5.0%	0.09 [0.03, 0.31]	
2011 Shu	25	45	35	43	5.9%	0.29 [0.11, 0.75]	
2011 Wang	30	53	21	54	6.7%	2.05 [0.95, 4.43]	
2012 Ishigami	42	76	48	65	6.9%	0.44 [0.21, 0.89]	
2012 Kasimura	40	62	51	61	6.3%	0.36 [0.15, 0.84]	
2012 Yoshii	30	49	34	43	6.0%	0.42 [0.16, 1.06]	
2013 Deng	19	48	31	51	6.5%	0.42 [0.19, 0.95]	
2013 Kim	43	49	35	50	5.5%	3.07 [1.08, 8.75]	
2013 Zhou	56	87	34	46	6.6%	0.64 [0.29, 1.41]	
2014 Ma	12	24	125	173	6.3%	0.38 [0.16, 0.91]	
Total (95% CI)		890		983	100.0%	0.56 [0.38, 0.84]	•
Total events	495		678				
Heterogeneity: Tau ² =	0.48; Chi2 :	= 54.71,	df = 15 (P < 0.0	0001); l ² =	73%	
Test for overall effect:	Z = 2.79 (P	= 0.005	5)			E	avours experimental Eavours control
						F	avours experimental Pavours control

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. doi:10.1371/journal.pone.0094376.g005

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					Odds Ratio	Odds Ratio
Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% CI
22	58	6	52	17.0%	4.69 [1.72, 12.77]	
23	87	13	92	40.4%	2.18 [1.03, 4.65]	
23	90	11	90	35.5%	2.47 [1.12, 5.43]	
12	62	2	61	7.1%	7.08 [1.51, 33.15]	
	297		295	100.0%	3.06 [1.95, 4.80]	•
80		32				
2.88, df = 3	(P = 0.4)	1); l ² = 09	6			
Test for overall effect: Z = 4.86 (P < 0.00001)						ours experimental Eavours control
					Fav	ours experimental Pavours control
					Odds Ratio	Odds Ratio
Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
27	58	11	52	17.4%	3.25 [1.40, 7.54]	
45	87	29	92	38.3%	2.33 [1.27, 4.28]	
38	90	20	90	32.5%	2.56 [1.34, 4.90]	
19	62	6	61	11.8%	4.05 [1.49, 11.02]	
	297		295	100.0%	2.77 [1.92, 3.98]	•
129		66				
1.06, df = 3	B (P = 0.7	79); l ² = 0	%			
Z = 5.48 (F	< 0.000	001)			Fa	vours experimental Favours control
-	-	-	-		Odds Ratio	Odds Ratio
Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	M-H, Fixed, 95% Cl
29	58	17	52	23.7%	2.06 [0.95, 4.47]	
64	87	56	92	38.0%	1.79 [0.95, 3.37]	
43	90	20	90	27.6%	3.20 [1.68, 6.11]	
20	62	6	61	10.8%	4.37 [1.61, 11.83]	
	297		295	100.0%	2.52 [1.76, 3.62]	•
156	297	99	295	100.0%	2.52 [1.76, 3.62]	•
156 3.08, df = 3	297 3 (P = 0.	99 38); I² = 3	295 3%	100.0%	2.52 [1.76, 3.62]	♦
	Events 22 23 12 80 2.88, df = 3 Z = 4.86 (P Events 27 45 38 19 1.06, df = 3 Z = 5.48 (F Events 29 64 43 20	Events Total 22 58 23 87 23 90 12 62 297 80 2.88, df = 3 (P = 0.4 2 2 = 4.86 (P < 0.000)	Events Total Events 22 58 6 23 87 13 23 90 11 12 62 2 297 80 32 286, df = 3 (P = 0.41); I ² = 05 2 2 4.86 (P < 0.00001)	Events Total Events Total 22 58 6 52 23 87 13 92 23 90 11 90 12 62 2 61 297 295 80 32 2.86, df = 3 (P = 0.41); l ² = 0% 2 4.86 Z = 4.86 (P < 0.00001)	Events Total Events Total Weight 22 58 6 52 17.0% 23 87 13 92 40.4% 23 90 11 90 35.5% 12 62 2 61 7.1% 297 295 100.0% 80 32 2.86, df = 3 (P = 0.41); l ² = 0% Z 4.86 (P < 0.00001)	Odds Ratio Events Total Events Total Weight M-H. Fixed. 95% CI 22 58 6 52 17.0% 4.69 [1.72, 12.77] 23 87 13 92 40.4% 2.18 [1.03, 4.65] 23 90 11 90 35.5% 2.47 [1.12, 5.43] 12 62 2 61 7.1% 7.08 [1.51, 33.15] 297 295 100.0% 3.06 [1.95, 4.80] 80 80 32 28.86, df = 3 (P = 0.41); I ² = 0% Z 4.86 (P < 0.00001)

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. doi:10.1371/journal.pone.0094376.g006

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.

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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 metaanalysis 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 metaanalysis of recurrence during follow-up 1-year after treatment; E: 10 articles in the meta-analysis of recurrence during follow-up 3year 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 metaanalysis 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.



Figure S4 Funnel plots for GC. A: 16 articles in the metaanalysis 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 metaanalysis 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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