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Review Article

Ki67/MIB-1 predicts better prognoses in colorectal cancer patients received both surgery and adjuvant radio-chemotherapy: a meta-analysis of 30 studies

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Abstract: Ki67/MIB is a common cell proliferation marker and has been used to detect the proliferative activity of colorectal cancer (CRC). Previous studies have reported incongruous prognostic values of Ki67/MIB expression in CRC patients. Consequently, we performed the present meta-analysis to clarify the relationship between Ki67/MIB expression and prognoses in patients with CRC. We performed the literature retrieval through the following databases of PubMed, Wiley Online Library, Cochrane Central Register of Controlled Trials, Science Direct, Web of Science, CNKI, Chong Qing VIP, Wan Fang, China Biology Medicine disc (up to July 30, 2016) based on the defined search terms. Hazard ratios (HRs) combined with 95% confidence intervals (95% CIs) were calculated to evaluate the relationship of Ki67 expression with prognoses. A total of 30 publications with 8293 patients were identified eventually in our meta-analysis. Pooled results showed that Ki67/MIB-1 positive expression indicated better overall survival (OS, HR: 0.72; 95% CI: 0.62-0.85; P=0.001), cancer-specific survival (CSS, HR: 0.72; 95% CI: 0.55-0.94; P<0.001), and relapse-free survival (RFS, HR: 0.701; 95% CI: 0.551-0.892; P=0.001) in patients who received surgery combined with adjuvant radio-chemotherapy, but on the contrary, in patients who underwent surgery alone, Ki67/MIB-1 positive expression suggested worse OS (HR: 1.61; 95% CI: 1.10-2.37; P<0.001) and CSS (HR: 2.11; 95% CI: 1.58-2.80; P<0.001). In conclusion, high proliferative activity may increase the adjuvant radio-chemotherapy sensitivity of cancer cells, thus Ki67/MIB-1 can be used as a biomarker of favorable prognoses in CRC patients who received both surgical operation and adjuvant radio-chemotherapy.

Keywords: Colorectal cancer, Ki67/MIB-1, prognoses, meta-analysis

Introduction

Colorectal carcinoma (CRC) has become one of the most frequent malignancies and major causes of cancer-related death globally. As reported by The Global Burden of Cancer 2013, CRC ranks the third for cancer morbidity and the fourth for cancer mortality worldwide. In 2013, there existed 1.6 million incident cases and 771,000 deaths. Furthermore, its incidence rate has been showing an upward trend in recent years for various reasons [1]. Surgery combined with adjuvant radio-chemotherapy is the main treatment of CRC. Although the 5-year survival rate for early stage patients can reach 90%, the prognoses are still poor for advanced cases. Therefore, it is essential to uncover prognostic markers to improve the prognoses prediction in patients with CRC, especially in patients with advanced CRC. Several studies have reported the prognostic value of Ki67/MIB-1 expression in CRC patients.

Ki67 is a nonhistone protein that is located in the nucleus of proliferating cells and presents throughout the cell cycle of growing cells but absents in G0 phase [2]. MIB-1 is a monoclonal antibody of the Ki67 antigen. K67/MIB-1 has been used as a cell proliferation marker. And the expression level of Ki67/MIB-1 in tumor tissues is closely related to the cancer cell proliferation. Some reports have evaluated the prog-

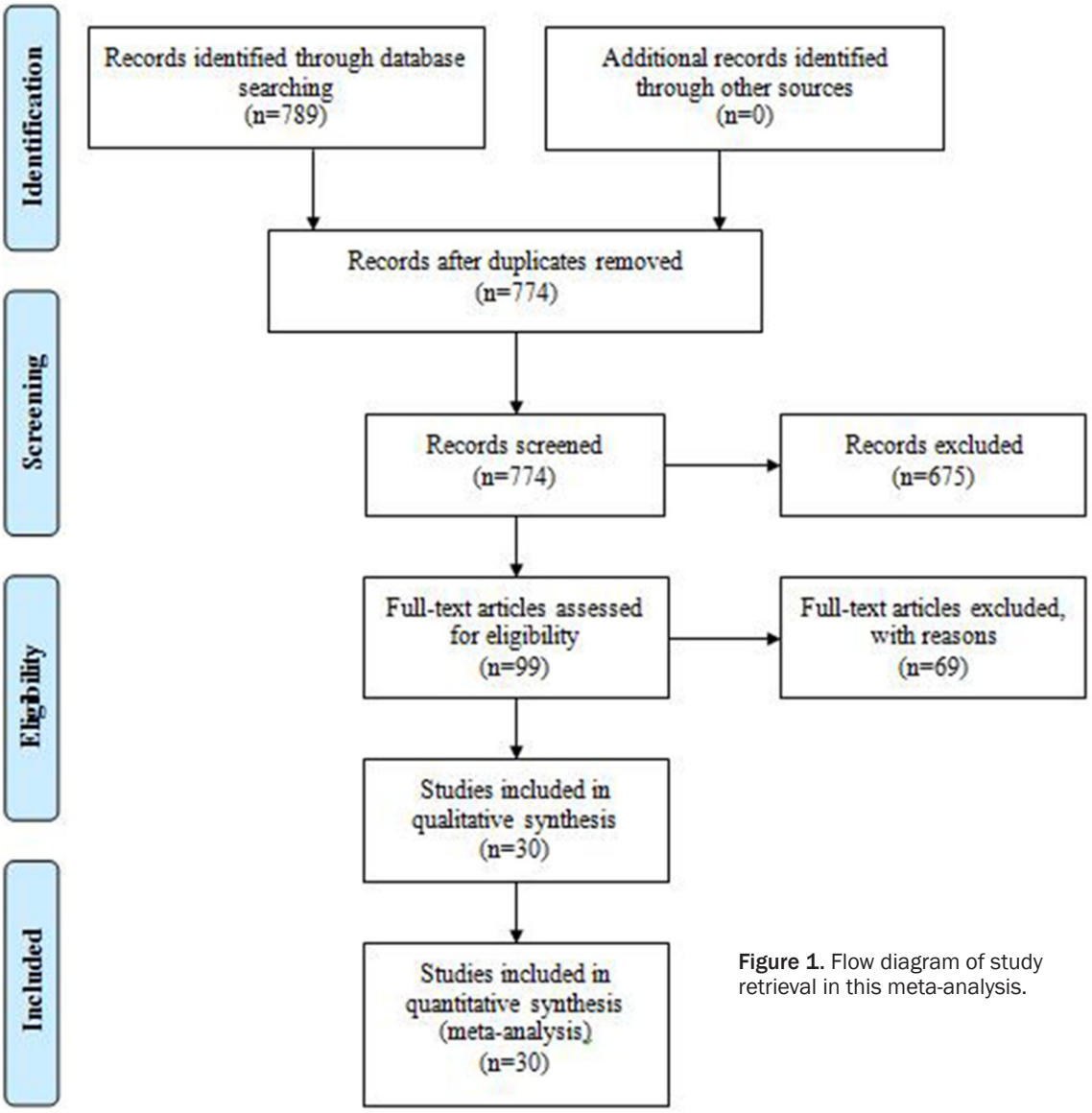


Figure 1. Flow diagram of study retrieval in this meta-analysis.

nostic significance of Ki67/MIB-1 immunostaining in several types of cancer. Rossi demonstrates that high expression of Ki67 indicates poorer survival in breast cancer [3]. Liang shows that Ki67 is an independent factor for the prognosis of cervical cancer patients [4]. We have also investigated the prognostic value of Ki67/MIB-1 expression in glioma, cervical cancer and hepatocellular carcinoma by conducting respective meta-analyses. Finally, we found that high Ki67/MIB-1 staining predicted unfavorable prognoses in all three cancers [5-7]. However, the prognostic roles of Ki67/MIB-1 in colorectal cancer are still controversial. Petrowsky reports that high Ki67 scores suggest significant decreased survival for CRC patients [8]. In contrast, zlobec shows an improved survival in CRC patients with high Ki67/MIB-1 expression [9].

Because of the inconsistent prognostic roles of Ki67/MIB-1 expression in CRC patients, we performed the present meta-analysis of available studies to evaluate the predictive value of Ki67/MIB-1 in CRC prognoses.

Materials and methods

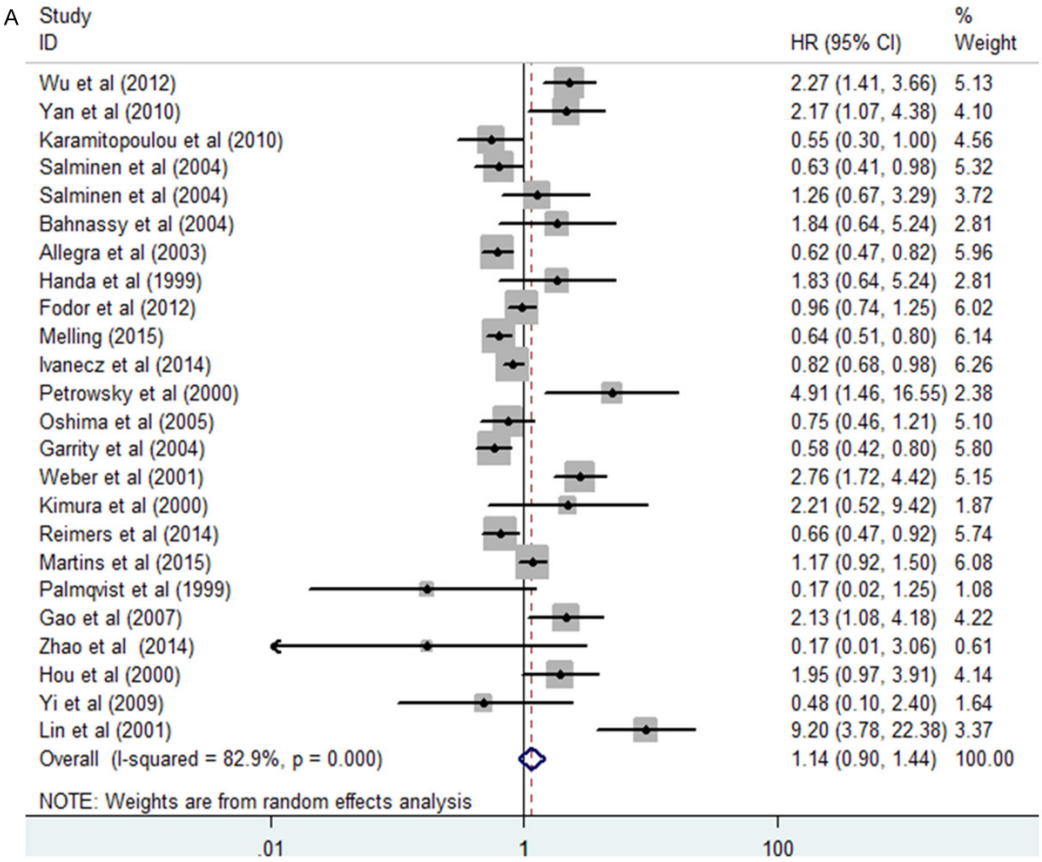
Search strategy

We conducted a literature search via nine online databases of PubMed, Wiley Online Library, Cochrane Central Register of Controlled Trials,

Table 1. Main characteristics of included 30 publications

Study (year)	Region	Language	Sample size	Cancer type	Follow up	Test method	Cut-off value	Analysis method	Adjuvant chemo-radiotherapy	HR (95% CI)	Prognostic value
Shin et al (2014)	Korea	English	266	CRC	NA	IHC	50%	Univariate analysis Multivariate analysis	No	U: DFS: HR 0.92 (0.48-1.78) CSS: HR 1.20 (0.25-5.76) M: DFS: HR 0.74 (0.38-1.43) CSS: HR 0.80 (0.16-4.04)	No significance
Wu et al (2012)	America	English	192	CRC	59 m (median)	IHC	NA	Multivariate analysis	No	OS: HR 2.267 (1.406-3.657)	Poor
Roxburgh et al (2013)	UK	English	230	CRC	113 m (median)	IHC	NA	Univariate analysis Multivariate analysis	No	U: CSS: HR 2.32 (1.48-3.64) M: CSS: HR 1.89 (1.15-3.11)	Poor
Yan et al (2010)	China	English	203	CC	61 m (median)	IHC	10%	Univariate analysis Multivariate analysis	No	U: OS: HR 2.17 (1.07-4.38) DFS: HR 2.06 (1.09-3.89) M: DFS: HR 1.88 (0.96-3.69)	Poor
Karamitopoulou et al (2010)	Greece	English	82	CRC	NA	IHC	NA	Univariate analysis	Yes	OS: HR 0.55 (0.3-1.0)	No significance
Fluge et al (2009)	Norway	English	412	CC	5-7 y	IHC	40%	Multivariate analysis	Yes	RFS: HR 0.55 (0.34-0.89)	Good
Zlobec et al (2008)	Switzerland	English	392	CRC	51 m (median)	IHC	15%	univariate analysis	Yes	CSS: HR 0.72 (0.53-0.97)	Good
Salminen et al (2004)	Finland	English	146	RC	99 m (median)	IHC	40%	Univariate analysis Multivariate analysis	Yes	U: OS: HR 0.63 (0.41-0.98) M: OS: HR 1.26 (0.67-3.29)	Good
Bahnassy et al (2004)	Egypt	English	60	CRC	30 m (mean)	IHC	11.5%	Univariate analysis	No	OS: HR 1.826 (0.636-5.243)	No significance
Allegra et al (2003)	America	English	907	CRC	5 y	IHC	40%	Multivariate analysis	Yes	RFS: RR 0.76 (0.58-1.01) OS: RR 0.62 (0.47-0.82)	Good
Handa et al (1999)	Japan	English	73	CRC	40 m (mean)	IHC	19.5%	Univariate analysis	No	OS: HR 1.826 (0.636-5.243)	No significance
Petrowsky et al (2000)	Germany	English	41	CRC	39 m (median)	IHC	50%	Survival curve	No	OS: HR 4.91 (1.46-16.55)	Poor
Nash et al (2010)	America	English	251	CRC	2.3 y (median)	IHC	50%	Multivariate analysis	No	CSS: HR 2.6 (1.4-4.8)	Poor
Fodor et al (2012)	UK	English	867	CRC	5 y	IHC	60%	Multivariate analysis	Yes	OS: HR 0.96 (0.74-1.25)	No significance
Melling (2015)	Germany	English	1653	CRC	46 m (median)	IHC	25%	Survival curve	Yes	OS: HR 0.64 (0.51-0.8)	Good
Peng et al (2013)	China	English	161	CRC	3 y	IHC	40%	Multivariate analysis	No	DFS: OR 3.298 (0.799-13.610)	No significance
Oshima et al (2005)	Brazil	English	89	CRC	33 m (average)	IHC	40%	Survival curve	No	OS: HR 0.75 (0.46-1.21)	No significance
Yang et al (2014)	China	English	36	CRC	46 m (mean)	IHC	50%	Survival curve	No	DFS: HR 0.63 (0.09-4.34)	No significance
Ivanecz et al (2014)	Slovenia	English	98	CRC	103 m (median)	IHC	50%	Multivariate analysis	Yes	OS: HR 0.82 (0.68-0.98) DFS: HR 0.88 (0.73-1.05)	Good
Garrity et al (2004)	Rochester	English	366	CRC	8.7 y (median)	IHC	27%	Multivariate analysis	Yes	OS: HR 0.58 (0.42-0.80) DFS: HR 0.61 (0.43-0.87)	Good
Weber et al (2001)	France	English	221	CRC	21.1 m (median)	IHC	50%	Multivariate analysis	No	OS: RR 2.76 (1.72-4.42) DFS: RR 1.84 (1.51-2.47)	Poor
Kimura et al (2000)	Japan	English	110	CRC	68.5 m (mean)	IHC	51%	Survival curve	Yes	OS: HR 2.21 (0.52-9.42)	No significance
Reimers et al (2014)	Leiden	English	285	CRC	NA	IHC	27%	Survival curve e	No	OS: HR 0.66 (0.47-0.92) DFS: HR 0.68 (0.49-0.96)	Good
Palmqvist et al (1999)	Sweden	English	65	CRC	67 m (median)	IHC	27%	Survival curve	No	OS: HR 0.17 (0.02-1.25)	No significance
Martins et al (2015)	Portugal	English	672	CRC	NA	IHC	NA	Survival curve	No	OS: HR 1.17 (0.92-1.50	No significance
Gao et al (2007)	China	Chinese	120	CRC	NA	IHC	10%	Survival curve	No	OS: HR 2.13 (1.08-4.18)	Poor
Zhao et al (2014)	China	Chinese	64	CRC	5 y	IHC	10%	Survival curve	No	OS: HR 0.17 (0.01-3.06)	No significance
Hou et al (2000)	China	Chinese	107	CRC	NA	IHC	40%	Survival curve	No	OS: HR 1.95 (0.97-3.91)	No significance
Yi et al (2009)	China	Chinese	64	CRC	59.6 m (mean)	IHC	5%	Survival curve	No	OS: HR 0.48 (0.1-2.4)	No significance
Lin et al (2001)	China	Chinese	60	CRC	36-81 m (range)	IHC	50%	Survival curve	No	OS: HR 9.2 (3.78-22.38)	Poor

CRCA: colorectal adenocarcinoma; CRC: colorectal cancer; CC: colon cancer; RC: rectal cancer; NA: not available; m, month; y: year; IHC: immunohistochemistry; HR: hazard ratio; CI: confidence interval; U: univariate analysis; M: multivariate analysis; OS: overall survival; DFS: disease-free survival; CSS: cancer-specific survival; RFS: relapse-free survival.


A
Study ID
HR (95% CI)
% Weight
Wu et al (2012) 2.27 (1.41, 3.66) 5.13
Yan et al (2010) 2.17 (1.07, 4.38) 4.10
Karamitopoulou et al (2010) 0.55 (0.30, 1.00) 4.56
Salminen et al (2004) 0.63 (0.41, 0.98) 5.32
Salminen et al (2004) 1.26 (0.67, 3.29) 3.72
Bahnassy et al (2004) 1.84 (0.64, 5.24) 2.81
Allegra et al (2003) 0.62 (0.47, 0.82) 5.96
Handa et al (1999) 1.83 (0.64, 5.24) 2.81
Fodor et al (2012) 0.96 (0.74, 1.25) 6.02
Melling (2015) 0.64 (0.51, 0.80) 6.14
Ivanecz et al (2014) 0.82 (0.68, 0.98) 6.26
Petrowsky et al (2000) 4.91 (1.46, 16.55) 2.38
Oshima et al (2005) 0.75 (0.46, 1.21) 5.10
Garrity et al (2004) 0.58 (0.42, 0.80) 5.80
Weber et al (2001) 2.76 (1.72, 4.42) 5.15
Kimura et al (2000) 2.21 (0.52, 9.42) 1.87
Reimers et al (2014) 0.66 (0.47, 0.92) 5.74
Martins et al (2015) 1.17 (0.92, 1.50) 6.08
Palmqvist et al (1999) 0.17 (0.02, 1.25) 1.08
Gao et al (2007) 2.13 (1.08, 4.18) 4.22
Zhao et al (2014) 0.17 (0.01, 3.06) 0.61
Hou et al (2000) 1.95 (0.97, 3.91) 4.14
Yi et al (2009) 0.48 (0.10, 2.40) 1.64
Lin et al (2001) 9.20 (3.78, 22.38) 3.37
Overall (I-squared = 82.9%, p = 0.000) 1.14 (0.90, 1.44) 100.00
NOTE: Weights are from random effects analysis
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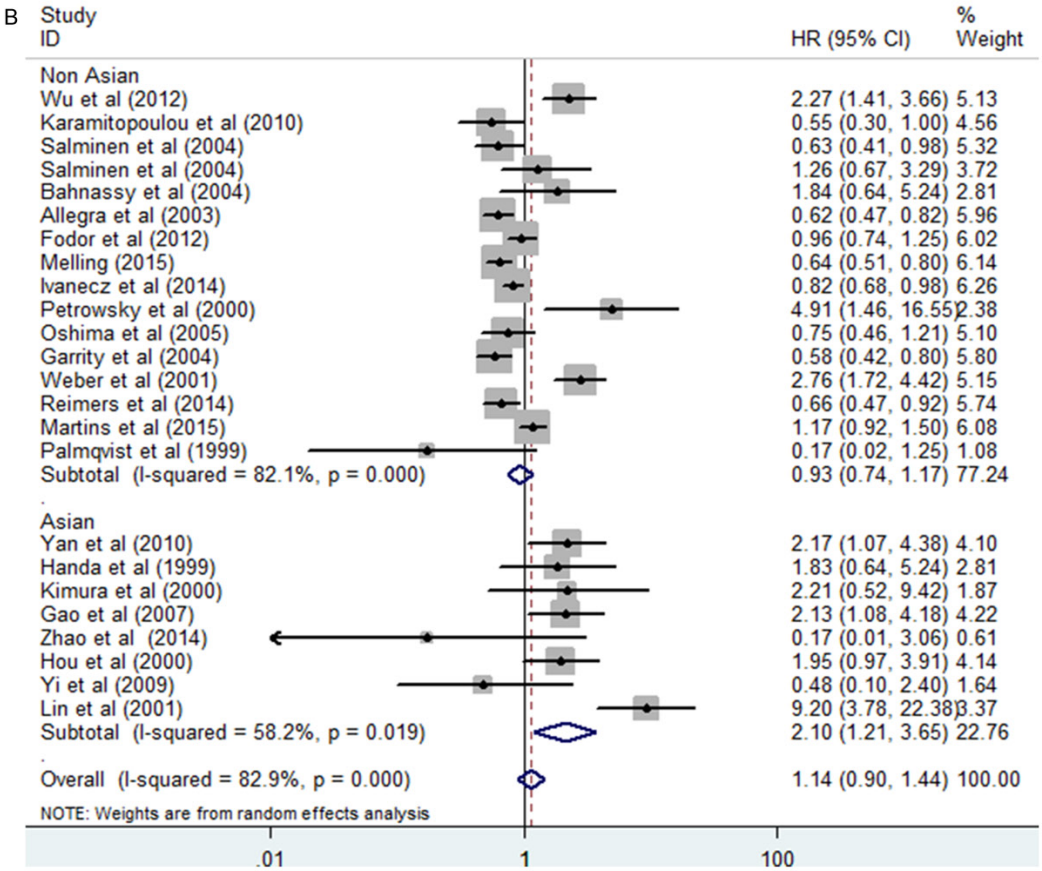
B
Study ID
HR (95% CI)
% Weight
Non Asian
Wu et al (2012) 2.27 (1.41, 3.66) 5.13
Karamitopoulou et al (2010) 0.55 (0.30, 1.00) 4.56
Salminen et al (2004) 0.63 (0.41, 0.98) 5.32
Salminen et al (2004) 1.26 (0.67, 3.29) 3.72
Bahnassy et al (2004) 1.84 (0.64, 5.24) 2.81
Allegra et al (2003) 0.62 (0.47, 0.82) 5.96
Fodor et al (2012) 0.96 (0.74, 1.25) 6.02
Melling (2015) 0.64 (0.51, 0.80) 6.14
Ivanecz et al (2014) 0.82 (0.68, 0.98) 6.26
Petrowsky et al (2000) 4.91 (1.46, 16.55) 2.38
Oshima et al (2005) 0.75 (0.46, 1.21) 5.10
Garrity et al (2004) 0.58 (0.42, 0.80) 5.80
Weber et al (2001) 2.76 (1.72, 4.42) 5.15
Reimers et al (2014) 0.66 (0.47, 0.92) 5.74
Martins et al (2015) 1.17 (0.92, 1.50) 6.08
Palmqvist et al (1999) 0.17 (0.02, 1.25) 1.08
Subtotal (I-squared = 82.1%, p = 0.000) 0.93 (0.74, 1.17) 77.24
Asian
Yan et al (2010) 2.17 (1.07, 4.38) 4.10
Handa et al (1999) 1.83 (0.64, 5.24) 2.81
Kimura et al (2000) 2.21 (0.52, 9.42) 1.87
Gao et al (2007) 2.13 (1.08, 4.18) 4.22
Zhao et al (2014) 0.17 (0.01, 3.06) 0.61
Hou et al (2000) 1.95 (0.97, 3.91) 4.14
Yi et al (2009) 0.48 (0.10, 2.40) 1.64
Lin et al (2001) 9.20 (3.78, 22.38) 3.37
Subtotal (I-squared = 58.2%, p = 0.019) 2.10 (1.21, 3.65) 22.76
Overall (I-squared = 82.9%, p = 0.000) 1.14 (0.90, 1.44) 100.00
NOTE: Weights are from random effects analysis
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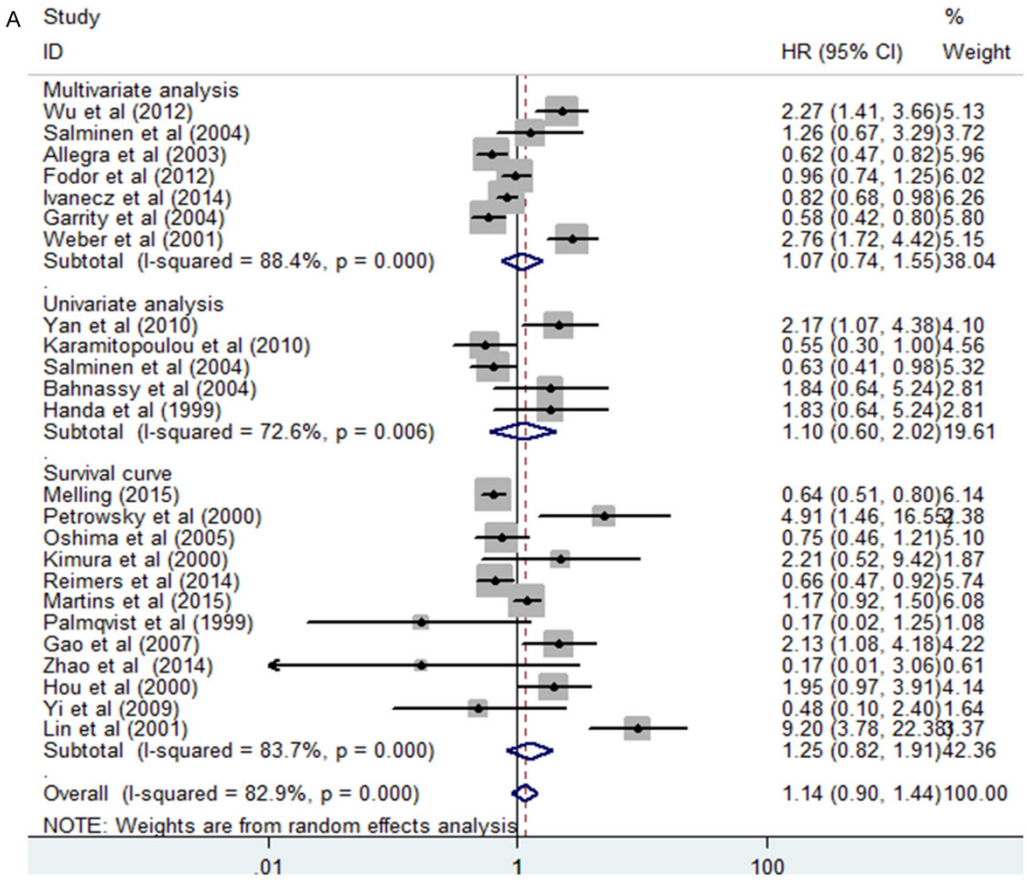
Figure 2. Meta-analysis of Ki67 expression with overall survival (OS). A: Pooled result with all available publications for OS. B: Subgroup analysis by ethnicity.

Table 2. Meta-analysis of Ki67/MIB-1 expression with OS, DFS, CSS, RFS in CRC patients

Groups	Number of studies	HR (95% CI)	*P* value	Heterogeneity	
				I^2 (%)	*P* value
OS	23	1.14 (0.90-1.44)	0.276	82.9	<0.001
Ethnicity					
Asians	8	2.10 (1.21-3.65)	0.008	58.2	0.019
Non-Asians	15	0.93 (0.74-1.17)	0.544	82.1	<0.001
Analysis method					
Survival curve	12	1.25 (0.82-1.91)	0.291	83.7	<0.001
Multivariate analysis	7	1.07 (0.74-1.55)	0.707	88.4	<0.001
Univariate analysis	5	1.10 (0.60-2.02)	0.754	72.6	0.006
Treatment					
Surgery alone	15	1.61 (1.10-2.37)	0.015	80.7	<0.001
Surgery combined with adjuvant radio-chemotherapy	8	0.72 (0.62-0.85)	<0.001	49	0.047
DFS	8	1.09 (0.78-1.52)	0.628	82	<0.001
Ethnicity					
Asians	3	1.96 (1.27-3.00)	0.002	0	0.599
Non-Asians	5	0.89 (0.61-1.30)	0.577	87.1	<0.001
Analysis method					
Survival curve	2	0.68 (0.49-0.94)	0.022	0	0.939
Multivariate analysis	6	1.14 (0.73-1.79)	0.555	87.2	<0.001
Univariate analysis	2	1.38 (0.63-3.05)	0.422	66.6	0.084
Treatment					
Surgery alone	6	1.26 (0.82-1.95)	0.293	77.5	<0.001
Surgery combined with adjuvant radio-chemotherapy	2	0.76 (0.53-1.08)	0.122	69.5	0.07
CSS	4	1.50 (0.82-2.75)	0.189	84.4	<0.001
Analysis method					
Multivariate analysis	3	2.03 (1.39-2.96)	<0.001	0	0.372
Univariate analysis	3	1.26 (0.48-3.29)	0.642	89.7	<0.001
Treatment					
Surgery alone	3	2.11 (1.58-2.80)	<0.001	0	0.612
Surgery combined with adjuvant radio-chemotherapy	1	0.72 (0.55-0.94)	0.015	None	None
RFS	2	0.70 (0.55-089)	0.004	23.2	0.254

Science Direct, Web of Science, CNKI, Chong Qing VIP, Wan Fang, China Biology Medicine disc (up to July 30, 2016). The following terms were utilized: (1) "colorectal cancer" or "colon cancer" or "rectal cancer" or "intestinal cancer" or "bowel cancer" or "colorectal carcinoma" or "colon carcinoma" or "rectal carcinoma" or "intestinal carcinoma" or "bowel carcinoma" or "colorectal neoplasm" or "colon neoplasm" or "rectal neoplasm" or "intestinal neoplasm" or "bowel neoplasm" or "colorectal tumo*" or "colon tumo*" or "rectal tumo*" or "intestinal tumo*" or "bowel tumo*" or "intestinal adenocarcinoma" or "colorectal adenocarcinoma" or "colon adenocarcinoma" or "rectal adenocarcinoma" or "signet-ring cell carcinoma" or "CRC" or "CRAC", and (2) "Ki67" or "Ki-67" or "MIB-1" or "proliferative index" or "proliferative marker" or "proliferative activity" or "mitotic index" or "labeling index" or "mitotic count", and (3) "prognosis" or "prognoses" or "prognostic" or "survive" or "survival" or "follow-up" or "mortality" or "incidence" or "predict" or "course" or "outcome" [5]. We have also further screened the references of the included articles carefully to search additional eligible publications.


A
Study
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HR (95% CI)
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Weight
Multivariate analysis
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Fodor et al (2012) 0.96 (0.74, 1.25) 6.02
Ivanecz et al (2014) 0.82 (0.68, 0.98) 6.26
Garrity et al (2004) 0.58 (0.42, 0.80) 5.80
Weber et al (2001) 2.76 (1.72, 4.42) 5.15
Subtotal (I-squared = 88.4%, p = 0.000) 1.07 (0.74, 1.55) 38.04
Univariate analysis
Yan et al (2010) 2.17 (1.07, 4.38) 4.10
Karamitopoulou et al (2010) 0.55 (0.30, 1.00) 4.56
Salminen et al (2004) 0.63 (0.41, 0.98) 5.32
Bahnassy et al (2004) 1.84 (0.64, 5.24) 2.81
Handa et al (1999) 1.83 (0.64, 5.24) 2.81
Subtotal (I-squared = 72.6%, p = 0.006) 1.10 (0.60, 2.02) 19.61
Survival curve
Melling (2015) 0.64 (0.51, 0.80) 6.14
Petrowsky et al (2000) 4.91 (1.46, 16.55) 2.38
Oshima et al (2005) 0.75 (0.46, 1.21) 5.10
Kimura et al (2000) 2.21 (0.52, 9.42) 1.87
Reimers et al (2014) 0.66 (0.47, 0.92) 5.74
Martins et al (2015) 1.17 (0.92, 1.50) 6.08
Palmqvist et al (1999) 0.17 (0.02, 1.25) 1.08
Gao et al (2007) 2.13 (1.08, 4.18) 4.22
Zhao et al (2014) 0.17 (0.01, 3.06) 0.61
Hou et al (2000) 1.95 (0.97, 3.91) 4.14
Yi et al (2009) 0.48 (0.10, 2.40) 1.64
Lin et al (2001) 9.20 (3.78, 22.38) 3.37
Subtotal (I-squared = 83.7%, p = 0.000) 1.25 (0.82, 1.91) 42.36
Overall (I-squared = 82.9%, p = 0.000) 1.14 (0.90, 1.44) 100.00
NOTE: Weights are from random effects analysis
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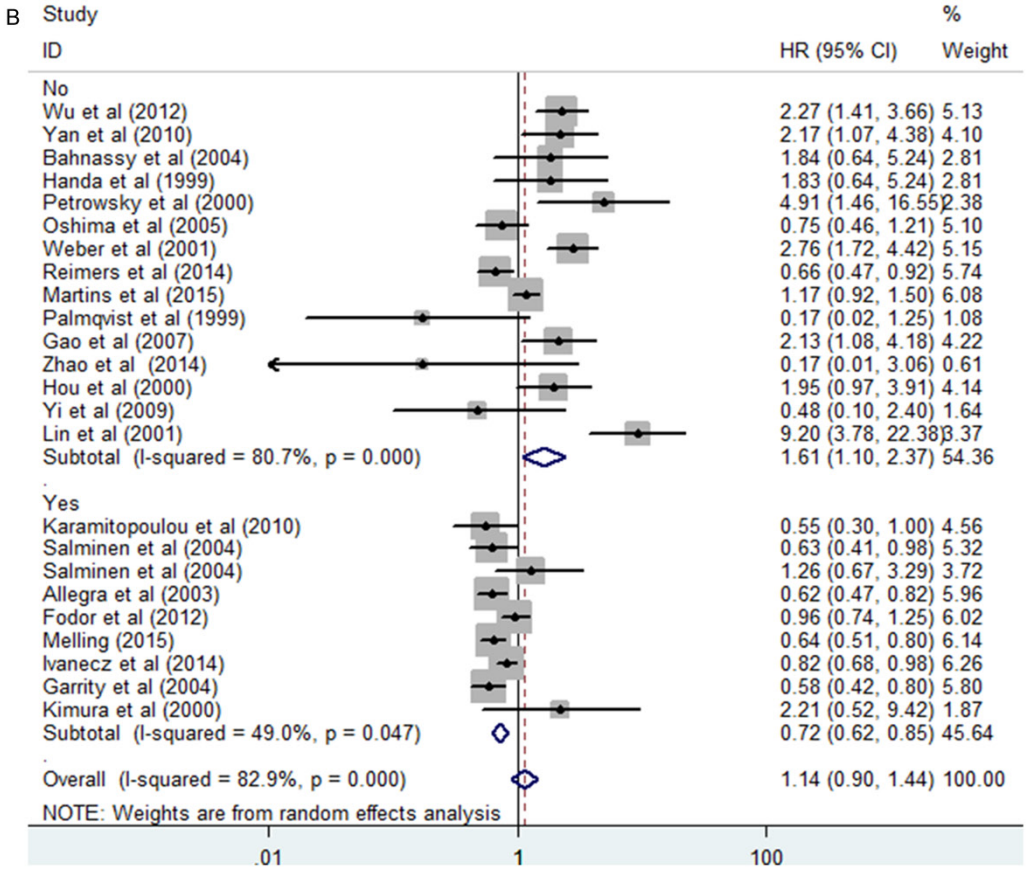
B
Study
ID
HR (95% CI)
%
Weight
No
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Yan et al (2010) 2.17 (1.07, 4.38) 4.10
Bahnassy et al (2004) 1.84 (0.64, 5.24) 2.81
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Gao et al (2007) 2.13 (1.08, 4.18) 4.22
Zhao et al (2014) 0.17 (0.01, 3.06) 0.61
Hou et al (2000) 1.95 (0.97, 3.91) 4.14
Yi et al (2009) 0.48 (0.10, 2.40) 1.64
Lin et al (2001) 9.20 (3.78, 22.38) 3.37
Subtotal (I-squared = 80.7%, p = 0.000) 1.61 (1.10, 2.37) 54.36
Yes
Karamitopoulou et al (2010) 0.55 (0.30, 1.00) 4.56
Salminen et al (2004) 0.63 (0.41, 0.98) 5.32
Salminen et al (2004) 1.26 (0.67, 3.29) 3.72
Allegra et al (2003) 0.62 (0.47, 0.82) 5.96
Fodor et al (2012) 0.96 (0.74, 1.25) 6.02
Melling (2015) 0.64 (0.51, 0.80) 6.14
Ivanecz et al (2014) 0.82 (0.68, 0.98) 6.26
Garrity et al (2004) 0.58 (0.42, 0.80) 5.80
Kimura et al (2000) 2.21 (0.52, 9.42) 1.87
Subtotal (I-squared = 49.0%, p = 0.047) 0.72 (0.62, 0.85) 45.64
Overall (I-squared = 82.9%, p = 0.000) 1.14 (0.90, 1.44) 100.00
NOTE: Weights are from random effects analysis
.01
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Figure 3. Meta-analysis of Ki67 expression with overall survival (OS). A: Subgroup analysis by analysis method. B: Subgroup analysis by the condition of adjuvant therapy.

Selection criteria

Study selection was conducted by two investigators independently and all publications included in our meta-analysis must fulfill these criteria: (1) the research objects must be human beings rather than animals; (2) patients were confirmed pathologically; (3) the studies must evaluate the prognostic value of Ki67/MIB-1 in colorectal cancer; (4) the trials should report clear methods to detect Ki67/MIB-1 expression in colorectal cancer patients; (5) Hazard ratios (HRs) and 95% confidence intervals (CIs) for overall survival (OS), cancer-specific survival (CSS), disease-free survival (DFS), and relapse-free survival (RFS) were provided directly or could be calculated through the sufficient survival data. While animal-based investigations, letters, comments, reviews, and articles lack of sufficient data to estimate HRs with 95% CIs were ineligible. Furthermore, if multiple publications contained the same patients, the most complete and latest studies were selected.

Data extraction

Data was retrieved from the included articles by two independent reviewers, with any inconsistence was resolved by conversation with a third and fourth investigators. The main information was collected from each eligible study: first author, publication year, study region, sample size, cancer type, follow-up time, testing method of Ki67/MIB-1, cut-off value, condition of adjuvant therapy, HRs with 95% CIs. Additionally, we marked several data as "not available (NA)" for they were not reported in original articles. We have contacted authors for missing information, however, partial data was still unavailable. All databases were screened and discussed by two investigators to ensure the accuracy.

The cut-off values of Ki67 expression are varied among the different trials, and we defined the positive or negative group according to the cut-off points shown in original articles.

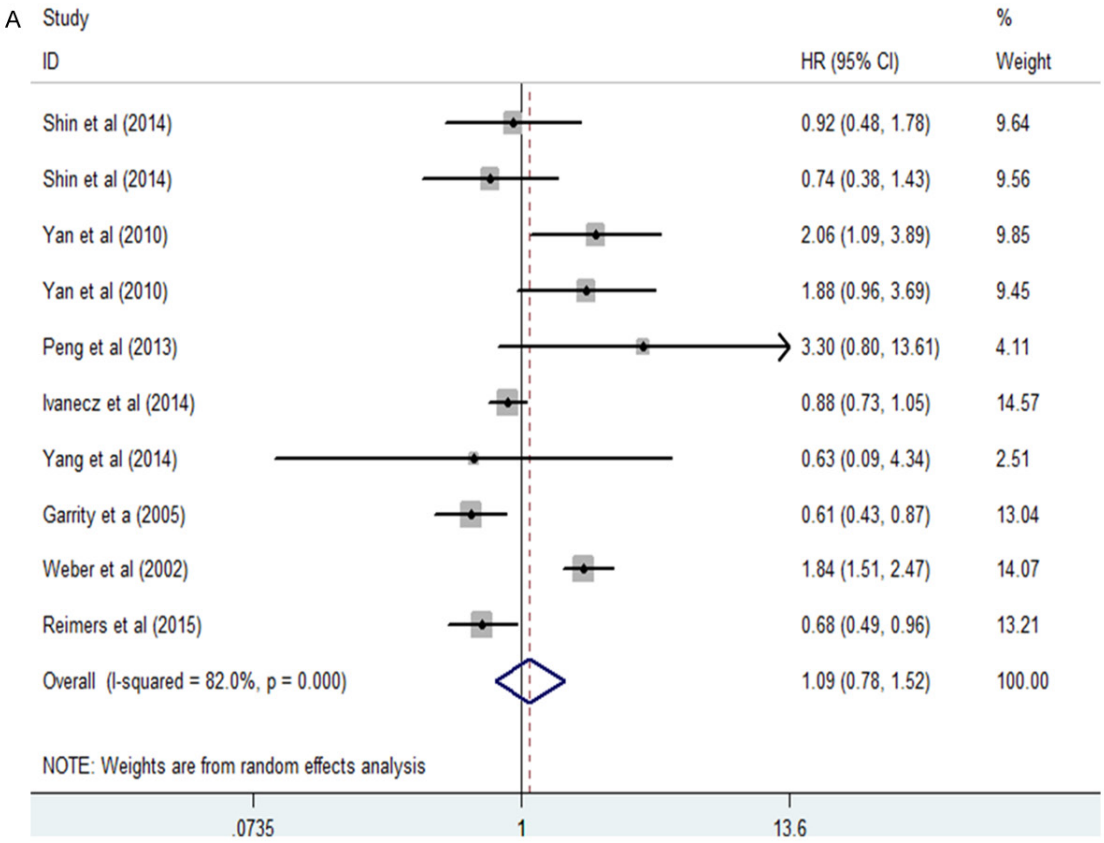
Statistical analysis

HR combined with its 95% CI was applied to evaluate the association between Ki67/MIB-1 expression and prognostic outcomes. If HRs and 95% CIs were described in the articles, we extracted them directly. Otherwise, we estimated them on the basis of the Kaplan-Meier survival curves utilizing Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/) [10]. Then the overall HR and 95% CI were calculated with STATA, version 12.0 (StataCorp, College Station, TX, USA). An observed HR>1 suggested an unfavorable survival for patients with Ki67/MIB-1 positive expression, and the effect of Ki67/MIB-1 expression on survival would be considered statistical significance when the 95% CI corresponding to the HR did not overlap 1. The inter-study heterogeneity was assessed by Q test and I-squared statistic (I^2 test) [11]. A random effect model was selected if the heterogeneity among those studies existed (Q test $P<0.05$, $I^2>50\%$), otherwise, a fixed effect model was chosen. In addition, subgroup analyses according to ethnicity (Asians and non-Asians), analysis method (univariate analysis, multivariate analysis, and survival curve) and the condition of adjuvant radio-chemotherapy were also carried out to investigate the possible source of the heterogeneity among eligible studies. Publication bias was estimated quantitatively by Egger's test and graphically by Begg's funnel plot [12]. All *P* values <0.05 (tested by two-sided) were considered statistically significant.

Results

Search results and description of included studies

As shown in **Figure 1**, a total of 789 studies (403 in English and 386 in Chinese) were retrieved through an initial literature search. After scrutinizing the titles and abstracts, we excluded 675 publications because they were irrelevant or duplicate records, case reports, reviews and animal-based studies. Then we further reviewed the full-text of the remaining articles and excluded 69 studies due to they did not provide sufficient survival data. Eventually 30 articles (25 in English and 5 in Chinese) with 8293 patients met our inclusion criteria and were included in this meta-analysis [8, 9, 13-40]. The main characteristics of all 30 included reports were summarized in **Table 1**. All


A
Study
ID
HR (95% CI)
%
Weight
Shin et al (2014)
0.92 (0.48, 1.78)
9.64
Shin et al (2014)
0.74 (0.38, 1.43)
9.56
Yan et al (2010)
2.06 (1.09, 3.89)
9.85
Yan et al (2010)
1.88 (0.96, 3.69)
9.45
Peng et al (2013)
3.30 (0.80, 13.61)
4.11
Ivanecz et al (2014)
0.88 (0.73, 1.05)
14.57
Yang et al (2014)
0.63 (0.09, 4.34)
2.51
Garrity et a (2005)
0.61 (0.43, 0.87)
13.04
Weber et al (2002)
1.84 (1.51, 2.47)
14.07
Reimers et al (2015)
0.68 (0.49, 0.96)
13.21
Overall (I-squared = 82.0%, p = 0.000)
1.09 (0.78, 1.52)
100.00
NOTE: Weights are from random effects analysis
.0735
1
13.6

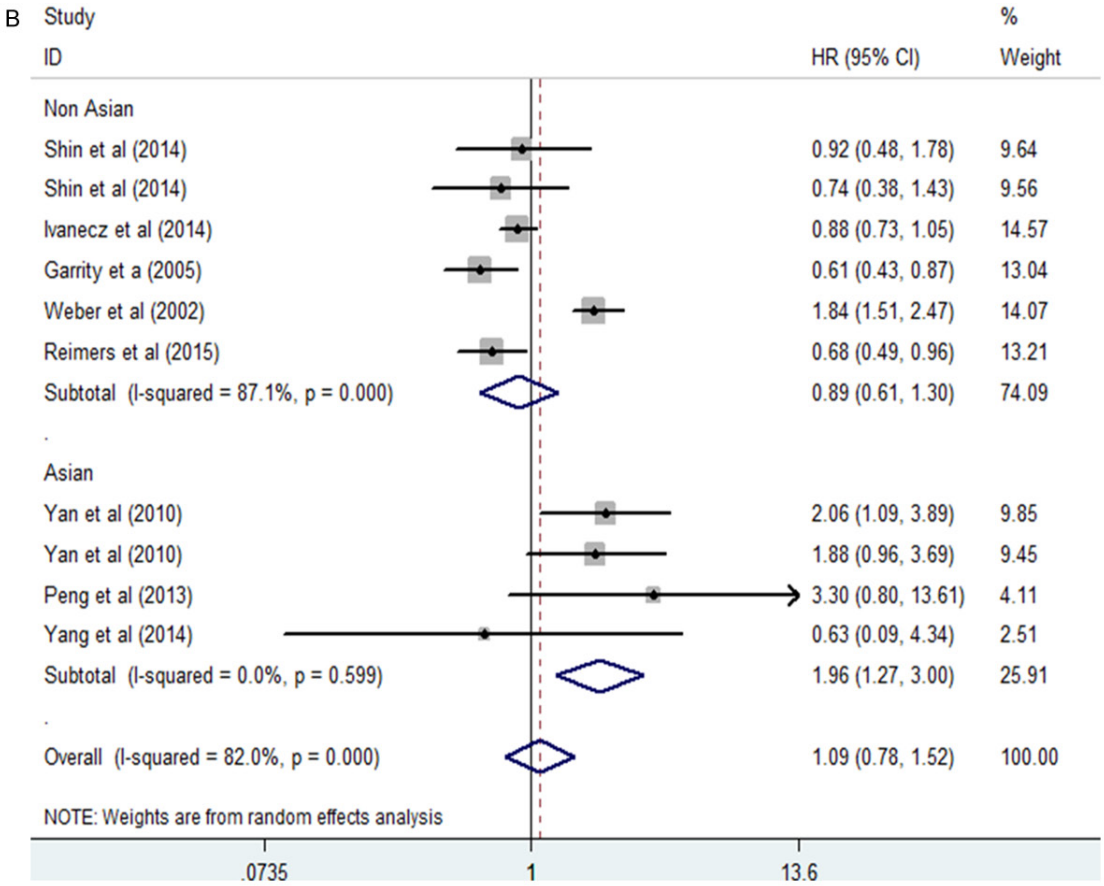

B
Study
ID
HR (95% CI)
%
Weight
Non Asian
Shin et al (2014)
0.92 (0.48, 1.78)
9.64
Shin et al (2014)
0.74 (0.38, 1.43)
9.56
Ivanecz et al (2014)
0.88 (0.73, 1.05)
14.57
Garrity et a (2005)
0.61 (0.43, 0.87)
13.04
Weber et al (2002)
1.84 (1.51, 2.47)
14.07
Reimers et al (2015)
0.68 (0.49, 0.96)
13.21
Subtotal (I-squared = 87.1%, p = 0.000)
0.89 (0.61, 1.30)
74.09
Asian
Yan et al (2010)
2.06 (1.09, 3.89)
9.85
Yan et al (2010)
1.88 (0.96, 3.69)
9.45
Peng et al (2013)
3.30 (0.80, 13.61)
4.11
Yang et al (2014)
0.63 (0.09, 4.34)
2.51
Subtotal (I-squared = 0.0%, p = 0.599)
1.96 (1.27, 3.00)
25.91
Overall (I-squared = 82.0%, p = 0.000)
1.09 (0.78, 1.52)
100.00
NOTE: Weights are from random effects analysis
.0735
1
13.6

Figure 4. Meta-analysis of Ki67 expression with disease-free survival (DFS). A: Pooled result with all available publications for DFS. B: Subgroup analysis by ethnicity.

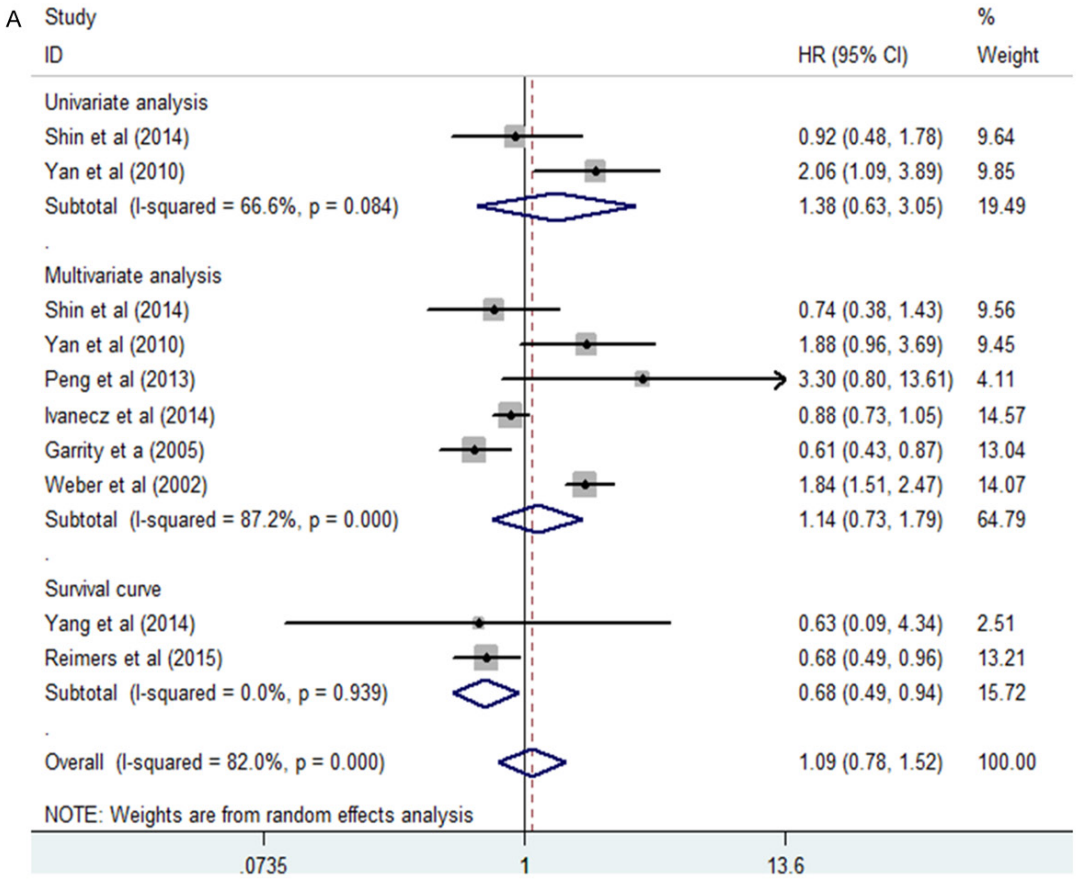
of the eligible studies were published from 1999 to 2015. Among the 30 studies, 11 were conducted in Asia (China, Korea and Japan), 19 were conducted in Europe, America and Egypt. The sample size in each study ranged from 36 to 1653 and the follow-up time varied from 21.1 months to 104.4 months (median). All trials used immunohistochemistry (IHC) method to detect Ki67 expression: MIB-1 was applied in 11 researches, and other anti-Ki67 antibodies were utilized in another 19 articles. All of the patients received surgical treatment, while partial patients from 10 of the trials were also treated with adjuvant radio-chemotherapy.

The prognostic value of Ki67/MIB-1 in colorectal cancer

The relationship between Ki67/MIB-1 expression and OS: A total of 23 publications with 6545 cases assessed the association of Ki67/MIB-1 expression with OS. A random effect model was chosen since noticeable heterogeneity was observed among the 23 reports (I^2=82.9%, P<0.001). The pooled result suggested that no significant correlation between Ki67/MIB-1 expression and OS existed (HR: 1.14; 95% CI: 0.90-1.40; P=0.276; **Figure 2A**; **Table 2**). Furthermore, we carried out subgroup analyses by ethnicity, analysis method and the condition of adjuvant therapy (**Figures 2B**, **3A** and **3B**; **Table 2**). By ethnicity subgroup analysis, we found that Ki67/MIB-1 positive expression was related to poor OS in Asians (HR: 2.10; 95% CI: 1.21-3.65; P=0.008) but not in non-Asians (HR: 0.93; 95% CI: 0.74-1.17; P= 0.544). By analysis method subgroup analysis, we observed no statistical significance in multivariate analysis group (HR: 1.27; 95% CI: 0.74-1.55; P=0.707), univariate analysis group (HR: 1.10; 95% CI: 0.60-2.02; P=0.754), or survival curve group (HR: 1.25; 95% CI: 0.82-1.91; P=0.291). In treatment subgroup, Ki67/MIB-1 positive expression was correlated with worse OS for patients who received surgical treatment alone (HR: 1.61; 95% CI: 1.10-2.37; P< 0.001). While on the contrary, for CRC patients who underwent surgery combined with adjuvant radio-chemotherapy, Ki67/MIB-1 positive expression predicted favorable OS (HR: 0.72; 95% CI: 0.62-0.85; P<0.001).

The relationship between Ki67/MIB-1 expression and DFS: Eight studies with 1636 participants analyzed the relationship between Ki67/MIB-1 expression and DFS. The inter-study heterogeneity existed (I^2=82.0%, P<0.001), so a random effect model was selected. The pooled result showed that no significant relationship of Ki67 expression with DFS was discovered (HR: 1.09; 95% CI: 0.78-1.52; P=0.623; **Figure 4A**; **Table 2**). Moreover, subgroup analyses were also conducted (**Figures 4B**, **5A** and **5B**; **Table 2**). Subgroup analysis on ethnicity implied that an elevated Ki67/MIB-1 expression predicted an unfavorable DFS in Asians (HR: 1.96; 95% CI: 1.27-3.00; P=0.002) but not in non-Asians (HR: 0.89; 95% CI: 0.61-1.30; P=0.557). Additionally, subgroup analysis on analysis method showed that Ki67/MIB-1 positive expression indicated a better DFS in survival curve group (HR: 0.68; 95% CI: 0.49-0.94; P=0.022) but not in univariate analysis group (HR: 1.38; 95% CI: 0.63-3.05; P=0.422) or multivariate analysis group (HR: 1.14; 95% CI: 0.73-1.79; P=0.555). By treatment subgroup analysis, we discovered no statistical difference in surgical group (HR: 1.26; 95% CI: 0.82-1.95; P=0.293) or in surgery combined with adjuvant radio-chemotherapy group (HR: 0.76; 95% CI: 0.53-1.08; P=0.122).

The relationship between Ki67/MIB-1 expression and CSS: Four trials with 1139 cases evaluated the relationship between Ki67/MIB-1 expression and CSS in CRC patients. A random effect model was applied since inter-study heterogeneity was detected (I^2=84.4%, P<0.001). As shown in **Figure 6A** and **Table 2**, no association between Ki67/MIB-1 expression and CSS was detected (HR: 1.50; 95% CI: 0.82-2.75; P=0.189). In addition, we performed subgroup analyses of analysis method and treatment method (**Figure 6B** and **6C**; **Table 2**). In analysis method group, the pooled result in multivariate analysis subgroup suggested an unfavorable CSS with an increased Ki67 expression (HR: 2.03; 95% CI: 1.39-2.96; P<0.001), while in univariate analysis group the combined result failed to identify a significant relationship between Ki67/MIB-1 expression and CSS (HR: 1.26; 95% CI: 0.48-3.29; P=0.189). In treatment method group, a poor CSS was observed


A
Study
ID
HR (95% CI)
%
Weight
Univariate analysis
Shin et al (2014) 0.92 (0.48, 1.78) 9.64
Yan et al (2010) 2.06 (1.09, 3.89) 9.85
Subtotal (I-squared = 66.6%, p = 0.084) 1.38 (0.63, 3.05) 19.49
Multivariate analysis
Shin et al (2014) 0.74 (0.38, 1.43) 9.56
Yan et al (2010) 1.88 (0.96, 3.69) 9.45
Peng et al (2013) 3.30 (0.80, 13.61) 4.11
Ivanecz et al (2014) 0.88 (0.73, 1.05) 14.57
Garrity et a (2005) 0.61 (0.43, 0.87) 13.04
Weber et al (2002) 1.84 (1.51, 2.47) 14.07
Subtotal (I-squared = 87.2%, p = 0.000) 1.14 (0.73, 1.79) 64.79
Survival curve
Yang et al (2014) 0.63 (0.09, 4.34) 2.51
Reimers et al (2015) 0.68 (0.49, 0.96) 13.21
Subtotal (I-squared = 0.0%, p = 0.939) 0.68 (0.49, 0.94) 15.72
Overall (I-squared = 82.0%, p = 0.000) 1.09 (0.78, 1.52) 100.00
NOTE: Weights are from random effects analysis
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13.6

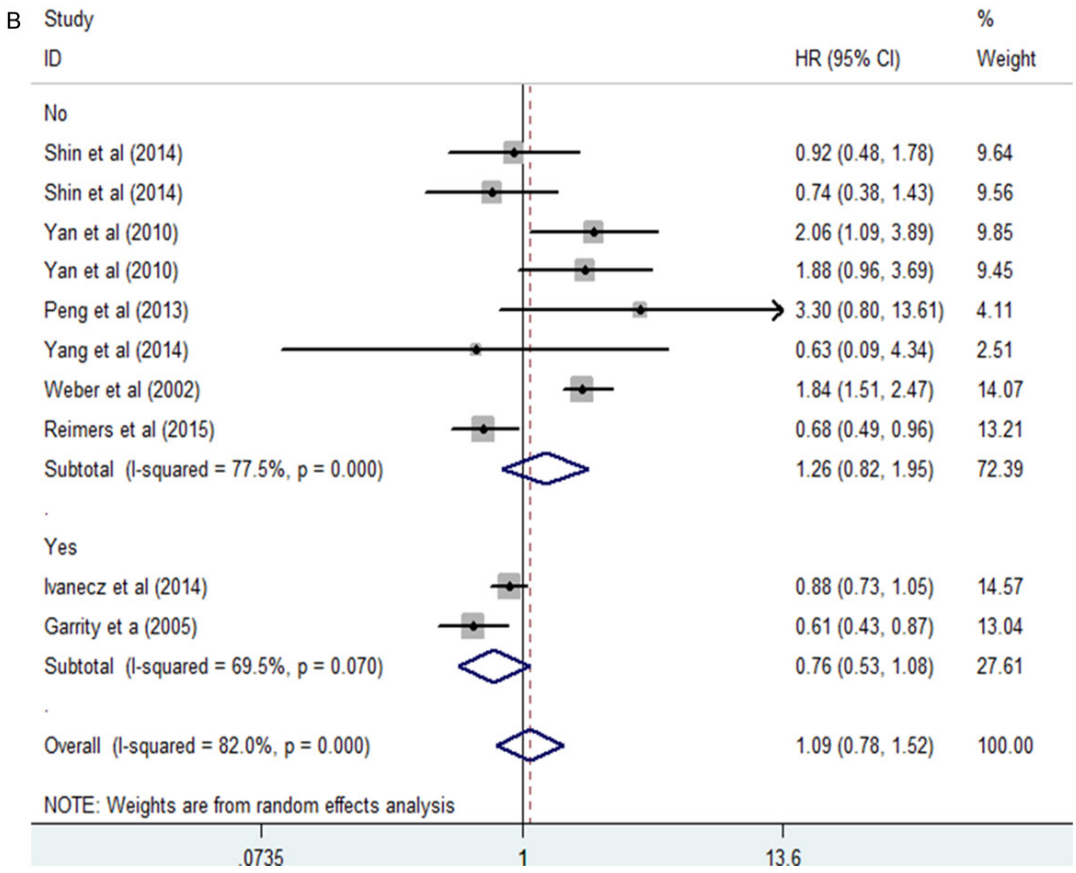
B
Study
ID
HR (95% CI)
%
Weight
No
Shin et al (2014) 0.92 (0.48, 1.78) 9.64
Shin et al (2014) 0.74 (0.38, 1.43) 9.56
Yan et al (2010) 2.06 (1.09, 3.89) 9.85
Yan et al (2010) 1.88 (0.96, 3.69) 9.45
Peng et al (2013) 3.30 (0.80, 13.61) 4.11
Yang et al (2014) 0.63 (0.09, 4.34) 2.51
Weber et al (2002) 1.84 (1.51, 2.47) 14.07
Reimers et al (2015) 0.68 (0.49, 0.96) 13.21
Subtotal (I-squared = 77.5%, p = 0.000) 1.26 (0.82, 1.95) 72.39
Yes
Ivanecz et al (2014) 0.88 (0.73, 1.05) 14.57
Garrity et a (2005) 0.61 (0.43, 0.87) 13.04
Subtotal (I-squared = 69.5%, p = 0.070) 0.76 (0.53, 1.08) 27.61
Overall (I-squared = 82.0%, p = 0.000) 1.09 (0.78, 1.52) 100.00
NOTE: Weights are from random effects analysis
.0735
1
13.6

Figure 5. Meta-analysis of Ki67 expression with disease-free survival (DFS). A: Subgroup analysis by ethnicity. B: Subgroup analysis by analysis method.

in patients who received surgery alone (HR: 2.11; 95% CI: 1.58-2.80; P<0.001). While, only one study with 392 patients was included in group which patients received both surgery and adjuvant radio-chemotherapy and the result showed a favorable prognosis (HR: 0.72; 95% CI: 0.55-0.94; P=0.015).

The relationship between Ki67/MIB-1 expression and RFS: Only two reports with 1319 patients analyzed the relationship between Ki67/MIB-1 expression and RFS. Because no heterogeneity was observed (I^2=23.2%, P=0.254), a fixed effect model was utilized. **Figure 7** and **Table 2** illustrated the pooled HR with its 95% CI, and the result suggested that Ki67/MIB-1 positive expression was associated with a better RFS in CRC patients (HR: 0.701; 95% CI: 0.551-0.892; P=0.001). Patients in the two researches received both surgery and adjuvant radio-chemotherapy.

Publication bias

The potential publication bias of the 30 accepted studies was evaluated by Begg's and Egger's tests. And no obvious publication bias was observed among the studies included in our meta-analysis (**Figure 8A-C**).

Discussion

Ki67 is one of the most common cell proliferation markers, and it has been considered as an effective target to detect tumor cell proliferation. In clinical practices, Ki67 has been extensively used as a diagnostic indicator in breast cancer, lung cancer, and so on. In addition, the prognostic value of Ki67 expression in various malignant tumors has also received considerable attention.

Preceding publications have demonstrated the prognostic role of Ki67/MIB-1 expression in several malignancies such as breast cancer [41], cervical cancer [42], glioma [43], hepatocellular carcinoma [44], and non-small cell lung carcinoma (NSCLC) [45]. Meta-analyses of Ki67 expression in certain cancers were also conducted. Martin performed a meta-analysis with 37 studies, and he found that an increased Ki67 expression predicted a decreased survival in patients with NSCLC [35]. Azambuja demonstrated a poor prognostic role of Ki67 expression in early breast cancer by carrying out a meta-analysis with 46 trials including 12155 patients [46]. Our previous meta-analyses also uncovered that a high Ki67/MIB-1 immunostaining indicated worse prognoses in glioma, cervical cancer, and hepatocellular carcinoma [5-7]. Evidences from those original studies and system reviews show that Ki67/MIB-1 is a significant predictive factor in partial carcinomas (breast cancer, cervical cancer, gliomas, hepatocellular carcinoma and non-small cell lung carcinoma) and can be used as a prognostic biomarker in clinical practices.

However, the prognostic values of Ki67 expression in CRC were incongruous. Investigation by Weber shows that positive Ki67 expression indicates decreasing survival rates in CRC patients with liver metastases, hence, ki67 labeling index can be a reliable predictor of worse OS and DFS [31]. Handa also analyzed the prognostic significance of Ki67 expression; however, he found it was an insignificant prognostic biomarker in CRC cases [22]. Furthermore, several reports demonstrate that a high Ki67 expression is related to improved prognoses in CRC patients, especially in patients who received both surgery and adjuvant radio-chemotherapy [9, 18, 19, 21, 25, 29, 30]. Fluge studied Ki67 expression in 412 CRC patients and discovered that a high Ki67 staining was significantly linked to a favorable outcome in patients who received surgery combined with adjuvant chemotherapy, but not in patients who received surgery alone. The author explained that more rapidly proliferating cancer cells may be more vulnerable to chemotherapy-induced cancer cell death [18]. Also, Itamochi put forward that lower proliferation of tumor cells may contribute to chemotherapy-resistant in renal clear cell carcinoma [47]. Suzuki presented that a high MIB-1 index predicted a better OS in cervical cancer because high proliferative indices may be indicative of increased response to adjuvant radiation therapy [48].

According to our meta-analysis, no significant correlation between Ki67/MIB-1 expression and OS, DFS, or CSS was observed in overall

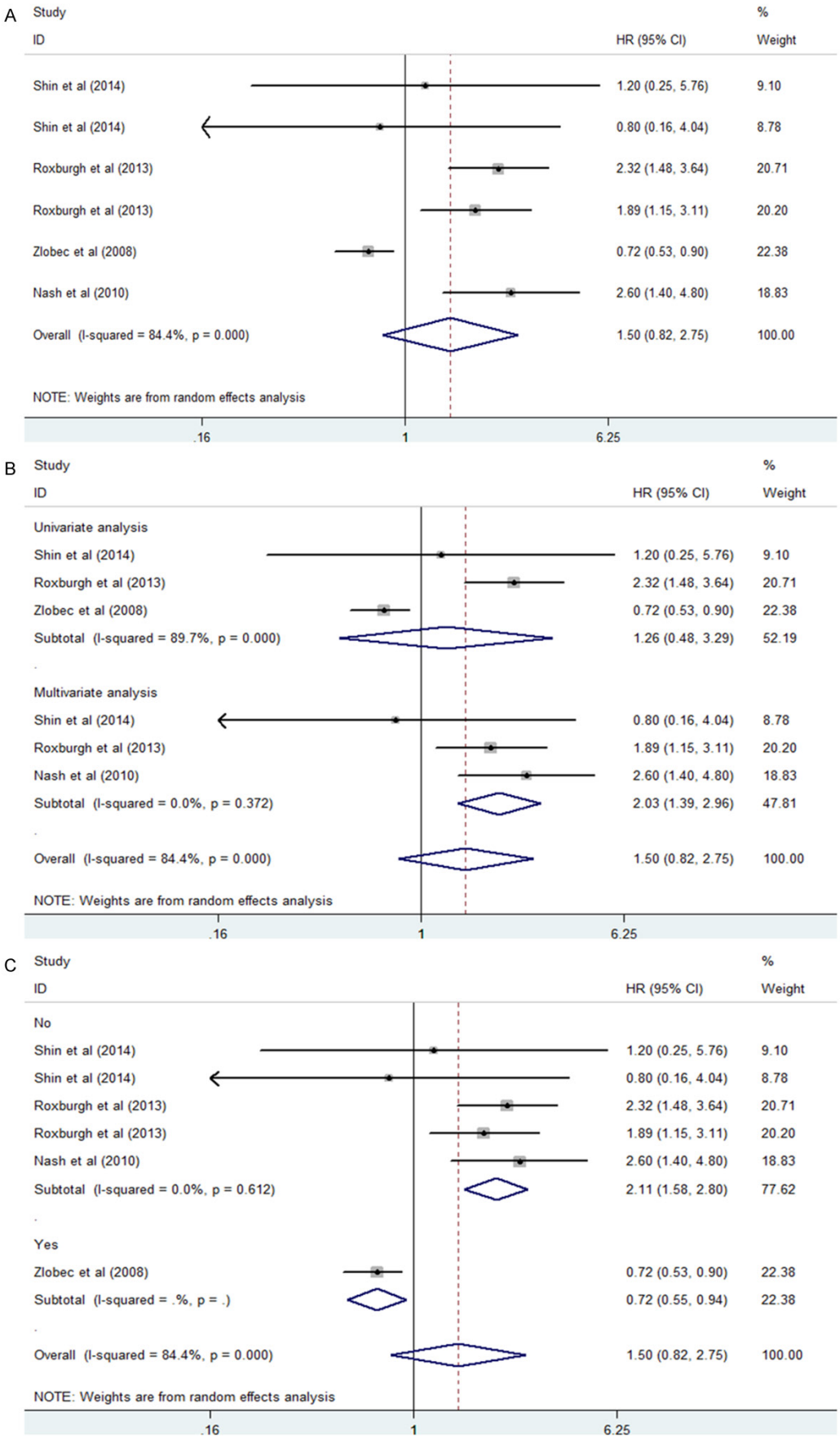
A
Study
ID
HR (95% CI)
%
Weight
Shin et al (2014)
1.20 (0.25, 5.76)
9.10
Shin et al (2014)
0.80 (0.16, 4.04)
8.78
Roxburgh et al (2013)
2.32 (1.48, 3.64)
20.71
Roxburgh et al (2013)
1.89 (1.15, 3.11)
20.20
Zlobec et al (2008)
0.72 (0.53, 0.90)
22.38
Nash et al (2010)
2.60 (1.40, 4.80)
18.83
Overall (I-squared = 84.4%, p = 0.000)
1.50 (0.82, 2.75)
100.00
NOTE: Weights are from random effects analysis
.16
1
6.25
B
Study
ID
HR (95% CI)
%
Weight
Univariate analysis
Shin et al (2014)
1.20 (0.25, 5.76)
9.10
Roxburgh et al (2013)
2.32 (1.48, 3.64)
20.71
Zlobec et al (2008)
0.72 (0.53, 0.90)
22.38
Subtotal (I-squared = 89.7%, p = 0.000)
1.26 (0.48, 3.29)
52.19
Multivariate analysis
Shin et al (2014)
0.80 (0.16, 4.04)
8.78
Roxburgh et al (2013)
1.89 (1.15, 3.11)
20.20
Nash et al (2010)
2.60 (1.40, 4.80)
18.83
Subtotal (I-squared = 0.0%, p = 0.372)
2.03 (1.39, 2.96)
47.81
Overall (I-squared = 84.4%, p = 0.000)
1.50 (0.82, 2.75)
100.00
NOTE: Weights are from random effects analysis
.16
1
6.25
C
Study
ID
HR (95% CI)
%
Weight
No
Shin et al (2014)
1.20 (0.25, 5.76)
9.10
Shin et al (2014)
0.80 (0.16, 4.04)
8.78
Roxburgh et al (2013)
2.32 (1.48, 3.64)
20.71
Roxburgh et al (2013)
1.89 (1.15, 3.11)
20.20
Nash et al (2010)
2.60 (1.40, 4.80)
18.83
Subtotal (I-squared = 0.0%, p = 0.612)
2.11 (1.58, 2.80)
77.62
Yes
Zlobec et al (2008)
0.72 (0.53, 0.90)
22.38
Subtotal (I-squared = .%, p = .)
0.72 (0.55, 0.94)
22.38
Overall (I-squared = 84.4%, p = 0.000)
1.50 (0.82, 2.75)
100.00
NOTE: Weights are from random effects analysis
.16
1
6.25

Figure 6. Meta-analysis of Ki67 expression with cancer-specific survival (CSS). A: Pooled result with all available publications for CSS. B: Subgroup analysis by analysis method. C: Subgroup analysis by the condition of adjuvant therapy.

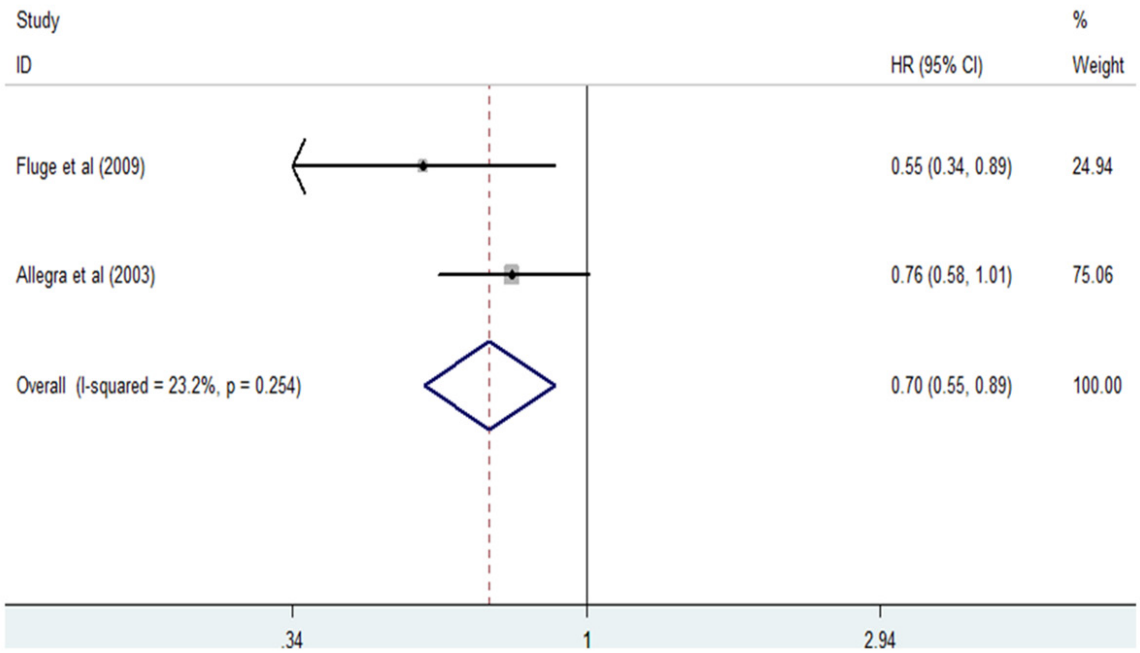

Figure 7. Meta-analysis of Ki67 expression with relapse-free survival (RFS).

populations. However, when subgroup analyses were conducted, we discovered that Ki67/MIB-1 positive expression probably related to favorable prognoses in patients who received both surgery and adjuvant radio-chemotherapy but not in patients who received surgery alone. A possible explanation of the contradictory impacts of Ki67/MIB-1 expression on prognoses is that colorectal cancer cells with a higher proliferative activity are more responsive to radio-chemotherapy. The mechanism of radio-chemotherapy for cancer therapy is to kill some specific cells, especially those rapidly proliferating cells. And the evaluated Ki67 expression in carcinoma tissues often suggests that the cells are in high proliferation states and are more likely to be killed. A study carried out by Willett demonstrates that adjuvant radiotherapy can preferentially kill rapidly proliferating and dividing cells in rectal cancer [49]. Garrity also pointed out that carcinomas with faster proliferation showed greater sensitivity to chemotherapy [30]. Consequently, a high Ki67 immunostaining may hint an increased radio-chemotherapy response and thus indicate better prognoses in CRC patients who received both operation and adjuvant therapy.

There still existed several limitations which should be noted in the present meta-analysis. First, the amount of included literatures was only 30 and in subgroup analyses the number of available reports was even smaller. In addition, the sample size in several studies was so small that the reliabilities of the results decreased. Second, the cut-off points of Ki67 expression were varied in different publications. It was difficult to determine a unified threshold in clinical trials because investigators often chose different cut-off values according to their clinical objectives. Consequently, choosing different Ki67 cut-off values may affect the classification of high and low proliferative carcinomas. Third, in several included studies, HRs and 95% CIs were not provided directly; otherwise, they were calculated from the Kaplan-Meier survival curves. And the accuracy of those calculated HRs is less than HRs obtained from articles directly. Fourth, the antibodies used to detect Ki67 expression were not unified in different publications. Some studies used MIB-1, and others used anti-Ki67 antibodies. Lindboe used four equivalent antibodies to record labeling indices and found that different antibodies revealed different staining

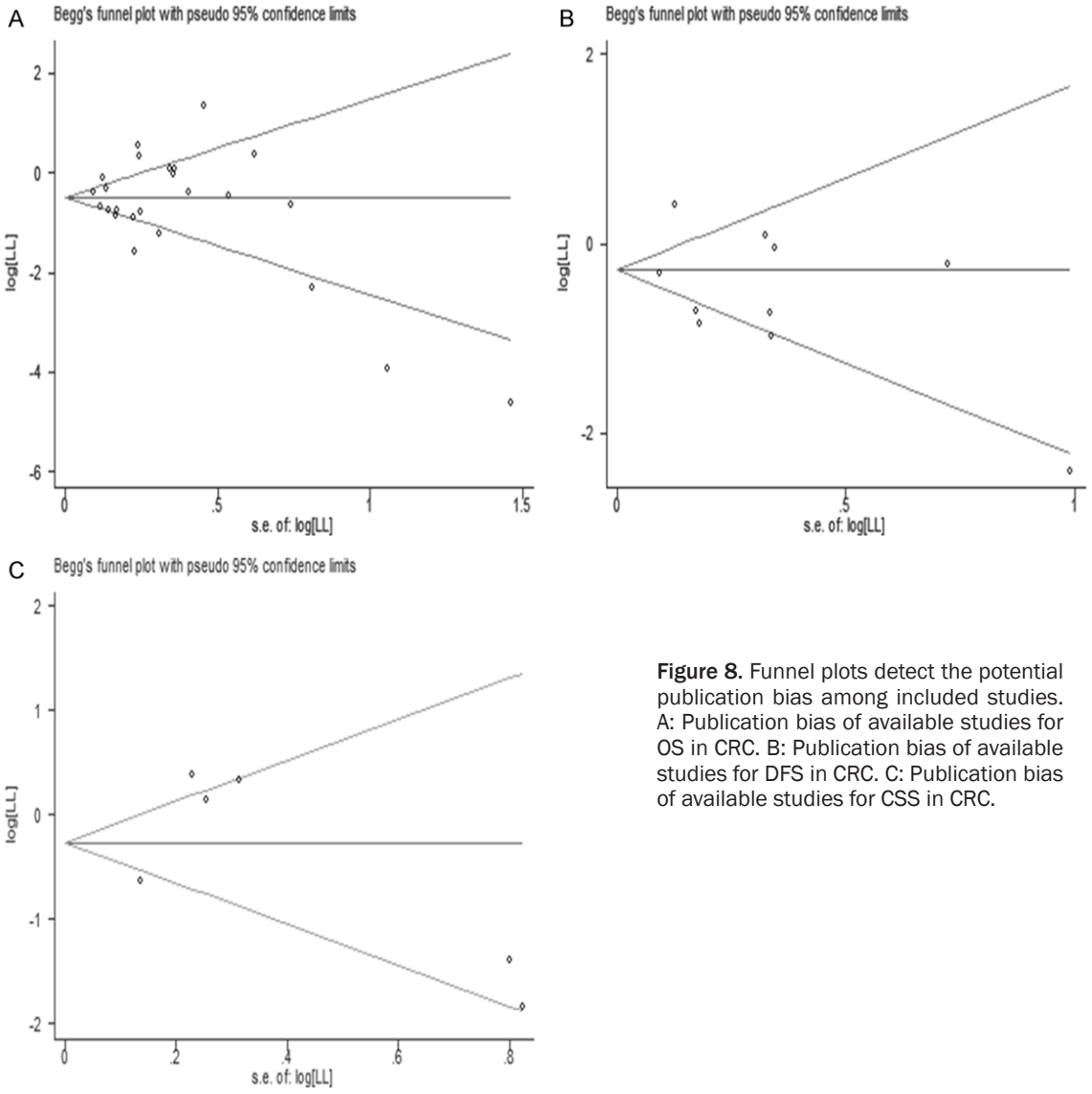

Figure 8. Funnel plots detect the potential publication bias among included studies. A: Publication bias of available studies for OS in CRC. B: Publication bias of available studies for DFS in CRC. C: Publication bias of available studies for CSS in CRC.

characteristics [50]. Among the four antibodies, MIB-1 seems to have the highest sensitivity for detecting Ki67 expression. Therefore, selecting different antibodies may result in alterations among the 30 studies. Fifth, we only accepted English and Chinese papers; consequently, some publications met our inclusion criteria were excluded due to the limitations of language. Finally, the full-text of some publications was not available although we have contacted the original authors, which may result in selection bias.

Despite these limitations, the results of this meta-analysis uncovered that a positive Ki67/MIB-1 expression could predict unfavorable OS and DFS in Asians, and more importantly, Ki67 positive staining suggested better prognoses in CRC patients who received both surgery and adjuvant radio-chemotherapy. Furthermore, better and stricter trials with more cases are necessary to provide stronger evidences on the prognostic significance of Ki67/MIB-1 expression in CRC patients. In addition, further research is needed to explore the mechanism of regulating Ki67 gene expression in carcinoma cells.

In conclusion, according to our meta-analysis, a high Ki67/MIB-1 immunostaining predicts favorable prognoses in patients who received combined surgery and adjuvant chemo-radiotherapy but not in patients who received surgery alone. The result indicates that Ki67/MIB-

1 can be a biomarker to reflect the response of radio-chemotherapy in patients with CRC. Additionally, clinical trials involving larger numbers of participants should be conducted to strengthen our conclusions.

Disclosure of conflict of interest

None.

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