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ORIGINAL ARTICLE

Loss of PTEN expression as a predictor of resistance to anti-EGFR monoclonal therapy in metastatic colorectal cancer: evidence from retrospective studies

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Abstract
Purpose To investigate the predictive value of loss of PTEN expression in patients with metastatic colorectal cancer (mCRC) treated with anti-EGFR monoclonal therapy.
Methods Studies were systematically identified to investigate the relationship between PTEN expression and clinical outcome in mCRC patients treated with anti-EGFR MoAbs. Clinical outcomes included objective response rate (ORR), progression-free survival (PFS) and overall survival (OS). The pooled relative risk (RR) or hazard ratio (HR) was estimated using a fixed-effects model or a random-effects model according to the heterogeneity between the studies.
Results A total of 852 patients were included in the final meta-analysis. The rate of loss of PTEN expression was 28.4 % (242/852). The overall pooled RR for ORR was 0.413 (95 % confidence intervals (CI), 0.177–0.965) when patients with loss of PTEN expression were compared with those with normal PTEN expression. Anti-EGFR monoclonal therapy resulted in improved PFS (HR, 0.466; 95 % CI, 0.292–0.640) and OS (HR, 0.689 [95 % CI, 0.482–0.896]) in patients unselected by KRAS mutation with normal PTEN expression over loss of PTEN expression. A better prognosis, as reflected by PFS (HR, 0.344; 95 % CI, 0.154–0.533) and OS (HR, 0.544; 95 % CI, 0.285–0.803), was observed in wild-type KRAS patients with normal PTEN expression versus loss of expression.
Conclusions Loss of expression of PTEN is a potential biomarker for resistance to anti-EGFR monoclonal therapy, particularly in mCRC patients with KRAS wild type.

Keywords PTEN · Metastatic colorectal cancer · Monoclonal antibodies · Meta-analysis

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Background

Metastatic colorectal cancer (mCRC) is one of the leading causes of cancer-related deaths in the world. Recently, the median survival of patients with mCRC has improved to current reports of between 24 and 30 months [1, 2], as two monoclonal antibodies (MoAbs), which are targeted at epidermal growth factor receptor (EGFR), the chimeric immunoglobulin G1 MoAb cetuximab and the fully humanized immunoglobulin G2 panitumumab, have been shown to be effective in chemotherapy-resistant mCRC [3, 4]. However, less than 12 % of patients with mCRC are responsive to anti-EGFR therapy as a single agent [5, 6]. Therefore, predictive biomarkers are needed to enable decisions on cost-effectiveness. Oncogenic activation of intracellular signaling pathways downstream of EGFR, including the RASRAF-MAPK and PI3K-PTEN-AKT signaling pathways, has been reported as a key mechanism for generating resistance to anti-EGFR MoAbs. Increasing evidence has shown that KRAS mutations in the RAS-RAF-

MAPK pathway are predictive markers of resistance to anti-EGFR MoAbs [7–9]. Although 40 % of CRCs are KRAS mutated [10–12], the response rate to anti-EGFR monoclonal therapy is approximately 10 % [5, 6], which suggests that alternative predictive factors of resistance to anti-EGFR MoAb exist. Another EGFR pathway also exists, the PTEN/PI3K/AKT pathway. PTEN (phosphatase and tensin homolog gene) is an important tumor-suppressor gene that negatively regulates Akt activities [13]. Loss of PTEN function results in Akt pathway overactivation and predicts anti-EGFR monoclonal therapy resistance in patients with mCRC. Recently, an increasing number of studies have evaluated the role of loss of PTEN expression in predicting resistance to anti-EGFR monoclonal therapy. However, the results remain inconclusive due to the relatively small sample sizes in the studies. Therefore, we conducted a meta-analysis to pool available trial data in order to derive a more precise estimation of predictive and prognostic values of loss of PTEN expression in patients with mCRC treated with anti-EGFR MoAbs.

Materials and methods

Systematic computerized searches of Pubmed (1966 to January 2012), Embase (1980 to January 2012), the Science Citation Index (1945 to January 2012) and the Chinese Biomedical Database (1981 to January 2012) were performed using the following search terms: metastatic colorectal cancer (for example, 'metastatic colon cancer,' 'metastatic rectal cancer,' 'mCRC'), PTEN (for example, 'phosphatase and tensin homolog gene'), anti-EGFR (for example, 'cetuximab and panitumumab') and clinical outcomes (for example, 'objective response,' 'progression-free survival,' 'overall survival'). All eligible studies were retrieved, and the bibliographies were scrutinized for further relevant publications.

Inclusion criteria

1. Studies exploring the relationship between loss of PTEN expression and clinical outcome in patients with mCRC treated with anti-EGFR MoAbs.
2. Studies using one or more of the following as outcome measures to assess tumor response and prognosis: objective response rate (ORR), progression-free survival (PFS) and overall survival (OS).

Data extraction

Data, including the first author's name, year of publication, study design, total number of patients in the study, number of patients with loss of PTEN expression, line of treatment (first- or subsequent-line), study treatment protocols and response criteria, were collected. At the same time, key outcome data, such as ORR, PFS and OS, were abstracted from all included studies. Two reviewers (Wang and Gao) independently reviewed the papers for eligibility and quality. Disagreements were resolved by a third reviewer (Fang).

Statistical analysis

The primary endpoint was ORR. The ORR was defined as the sum of complete response (CR) and partial response (PR). The association between PTEN expression and ORR was expressed as a relative risk (RR) for ORR in patients with loss of PTEN expression over those with normal PTEN expression. Thus, a RR of 1 indicated a lack of association between PTEN expression and tumor response to anti-EGFR MoAbs therapy. A RR greater than 1 corresponded to a direct correlation between a higher ORR and loss of PTEN expression. Finally, a tendency toward a correlation between loss of PTEN expression and non-responsiveness was indicated by a RR less than 1.

The secondary endpoints were PFS and OS. The PFS was defined as the interval from random assignment of treatment to radiological evidence of disease progression or death. Time-to-event PFS data and median OS for each trial were summarized by the log hazard ratio (HR) and its variance for the normal PTEN expression group compared with the loss of PTEN expression group following anti-EGFR monoclonal therapy. If a study provided separate HR estimates for KRAS wild type and mutant groups, we treated them as two different studies. If the trials reported log HR and variance directly, the reported values were used. For those trials that did not provide this information, data were extracted from published survival curves where available, to estimate the values of the log HR and variance according to previously described methods [14]. When survival curves for treatment arms were not available, other data, such as log-rank test p values and the number of events in each treatment arm, were extracted to allow estimation of the log HR and variance [14]. A HR <1 indicates an improvement in PFS and OS for normal PTEN expression compared with loss of PTEN expression.

The Stata version 11.0 software (Stata Corporation, College Station, TX, USA) was used for all data analyses. Heterogeneity of effect sizes across the studies was examined using the Q statistic [15]. Statistical significance was set at 0.10 for heterogeneity [16]. The ORR, PFS and OS were analyzed based on a fixed-effects model using the Mantel–Haenszel method. If significant heterogeneity was found to be less than 0.10, the random-effects model instead of the fixed-effects model was used for further

analysis. Funnel plots with the Begg's test [17] were visually evaluated for any asymmetry, in order to detect a possible publication bias, in which a p value <0.10 was considered representative of a statistically significant bias [18].

Subgroup analysis was performed to evaluate the results for wild-type KRAS groups compared with unselected groups. Sensitivity analyses were carried out to check whether modification of the inclusion criteria of the meta-analysis affected the final results.

Results

Characteristics of the included studies

Twelve studies [12, 19–29] were identified according to the inclusion criteria in the meta-analysis. Tables 1 and 2 show the main characteristics and effects in the twelve studies of patients treated with anti-EGFR MoAbs, all of which were retrospective cohort studies. Of these studies, sample sizes ranged from 27 to 162. Anti-EGFR MoAb was given either as first-line or as subsequent-line treatment in seven studies [12, 20–22, 24, 26, 28] and as second-line or subsequent-line treatment in five studies [19, 23, 25, 27, 29].

PTEN expression and ORR in patients with mCRC unselected by KRAS mutation

A PTEN expression analysis was successfully performed in 852 mCRC samples in the twelve studies. Loss of PTEN expression was detected in 242 (28.4 %) primary tumors. Nine studies [12, 19–26] provided data on ORR in patients unselected by KRAS mutation. The ORR of patients with loss of PTEN expression was 14.5 % (22/152), whereas the ORR of those with normal PTEN expression was 37.4 %

Table 1 Main characteristics of studies included in the meta-analysis

First author, year, region	Study design	Patients assessed	Loss of PTEN expression (%)	Line of treatment	Study treatment protocols	Response criteria
Frattini [12], Italy	Retrospective	27	11/27 (40.7)	≧1st	C + I based, or C + CAPOX (C administered at 400 mg/m^2 over 2 h, followed by weekly 250 mg/m^2 over 1 h)	RECIST
Razis [19], Greece	Retrospective	72	10/72 (13.9)	≧2nd	C combined with various regimens in all patients except one treated with single agent C only	Not reported
Loupakis [20], Italy	Retrospective	85	36/85 (42.4)	≧1st	C + I for patients with I-refractory mCRC (whose disease had progressed during or within 3 months after treatment with an I-based regimen)	RECIST
Molinari [21], Switzerland	Retrospective	38	8/38 (21.1)	≧1st	C or P combined with I in all patients who failed at least one prior chemotherapy regimen based on I with the exception of one patient who received C as a frontline therapy	RECIST
Sartore-Bianchi [22], Italy	Retrospective	81	32/81 (39.5)	≧1st	C alone; P alone; or C + I based (with the exception of 13 patients who received C as frontline therapy; the others had failed at least one prior chemotherapy. For those progressed on I-based chemotherapy, C was administered in combination with I)	RECIST
Perrone [23], Italy	Retrospective	32	4/32 (12.5)	≧2nd	I at a dose of 300 mg/m^2 every 3 weeks and C at 400 mg/m^2 followed by a weekly dose of 250 mg/m^2	RECIST
Laurent-Puig [24], France	Retrospective	162	31/162 (19.0)	≧1st	C alone; C + I or C + folfiri (all but one patient received C-based therapy as a second-line or greater treatment for mCRC)	RECIST
Li [25], China	Retrospective	61	24/61 (39.3)	≧2nd	C alone; C + OX based or C + I based	RECIST
Negri [26], Italy	Retrospective	43	5/43 (16.7)	≧1st	C + OX or C + I (80 % patients received ≥ 3 lines of chemotherapy)	Not reported
Saridaki [27], Greece	Retrospective	106	21/106 (19.8)	≧2nd	C + OX based or C + I based	Not reported
Park [28], Korea	Retrospective	69	38/69 (55.1)	≧1st	C alone; or C + I; or C + OX; or C + 5FU (cetuximab at 400 mg/m^2, followed by 250 mg/m^2 every week)	RECIST
Sood [29], US	Retrospective	76	22/76 (28.6.)	≧2nd	C alone; or P alone; or C + I or P + I	RECIST

C cetuximab, *P* panitumumab, *Chem* chemotherapy, *I* irinotecan, *OX* oxaliplatin, *CAPOX* capecitabine with oxaliplatin, *5-FU* 5-fluorouracil, *FOLFIRI* 5-fluorouracil, calcium folinate with irinotecan

Table 2 Effect of PTEN expression on the efficacy of anti-EGFR monoclonal therapy in patients with mCRC

First author	ORR		PFS			OS		
	Loss of PTEN expression	Normal of PTEN expression	HR and variance			HR and variance		
			Unselected by KRAS mutation	KRAS wt	KRAS mt	Unselected by KRAS mutation	KRAS wt	KRAS mt
Frattini [12]	0/11	10/16	NR	NR	NR	NR	NR	NR
Razis [19]	5/10	19/62	NC	NC	NC	NR	NR	NR
Loupakis [20]	4/36	11/49	NR	0.45 (0.12 –0.87)	0.54 (0.13 –1.35)	NR	0.50 (0.15–1.26)	1.30 (0.48–3.60)
Molinari [21]	0/8	2/30	NR	NR	NR	NR	NR	NR
Sartore-Bianchi [22]	1/32	17/49	0.527 (0.321–1.124)[a]	NR	NR	HR: 0.883 (0.271–1.496)[a]	NR	NR
Perrone [23]	0/4	10/28	NR	NR	NR	NR	NR	NR
Laurent-Puig [24]	10/22 KRAS wt	41/89 KRAS wt	NR	NC	NR	0.556 (0.323–0.909)	0.556 (0.323–0.909)	NR
Li [25]	1/24	18/37	0.605 (0.382–1.682)[a]	NR	NR	1.006 (0.321–1.691)[a]	NR	NR
Negri [26]	1/5	21/38	NC	NR	NR	NC	NR	NR
Saridaki [27]	NR	NR	0.588 (0.357–1.031)	0.371 (0.196–0.714)	NR	0.909 (0.556–1.667)	0.909 (0.5–1.429)	NR
Park [28]	NR	NR	0.141 (0.023–0.857)	0.141 (0.023–0.857)	NR	NR	NR	NR
Sood [29]	NR	NR	NR	NC	NR	NR	NC	NR

NR not reported, *NC* not converted, *KRAS wt* KRAS wild type, *KRAS mt* KRAS mutation type, *ORR* objective response rate, *PFS* progression-free survival, *OS* overall survival

[a] The values of the log HR and variance were estimated according to previously described methods [14]

(149/398). The RR of ORR in patients with loss of PTEN expression over those with normal expression was 0.413 (95 % CI, 0.177–0.965), based on a random-effects model (p for heterogeneity = 0.001) using the Mantel–Haenszel method (Fig. 1) without significant publication bias (p for Begg's test = 0.602; Fig. 2).

Due to the presence of significant heterogeneity, a sensitivity analysis was performed by excluding trials that used anti-EGFR MoAb as second-line or as subsequent-line treatment. The RR of ORR in patients with loss of PTEN expression over those with normal expression was 0.286 (95 % CI, 0.095–0.857), with significant persistent heterogeneity (p = 0.056).

PTEN expression and PFS in patients with mCRC

Ten studies [19, 20, 22, 24–29] provided data on PFS in patients unselected by KRAS mutation. Four studies [20, 27, 28] reported HR and variance directly. HR and variance were estimated from original data provided by two studies [22, 25], and another four studies [19, 24, 26, 29] reported original data without survival curves, which could not be converted to HR and variance. Therefore, HR and variance from six studies [20, 22, 25, 27, 28] on PFS in patients unselected by KRAS mutation were pooled in the meta-analysis. The HR of PFS in patients with normal PTEN expression over those with loss of expression was 0.466 (95 % CI, 0.292–0.640), based on a fixed-effects model (p for heterogeneity = 0.675) using the Mantel–Haenszel method (Fig. 3) without significant publication bias (p for Begg's test = 0.133; Fig. 4; Table 3). In subgroup analysis, the HR of PFS in patients with normal PTEN expression over those with loss of expression in the wild-type KRAS group was 0.344 (95 % CI, 0.154–0.533), based on a fixed-effects model (p for heterogeneity = 0.533) using the Mantel–Haenszel method without significant publication bias from three studies [20, 27, 28] (HR and variance provided directly; p for Begg's test = 1.000; Table 3).

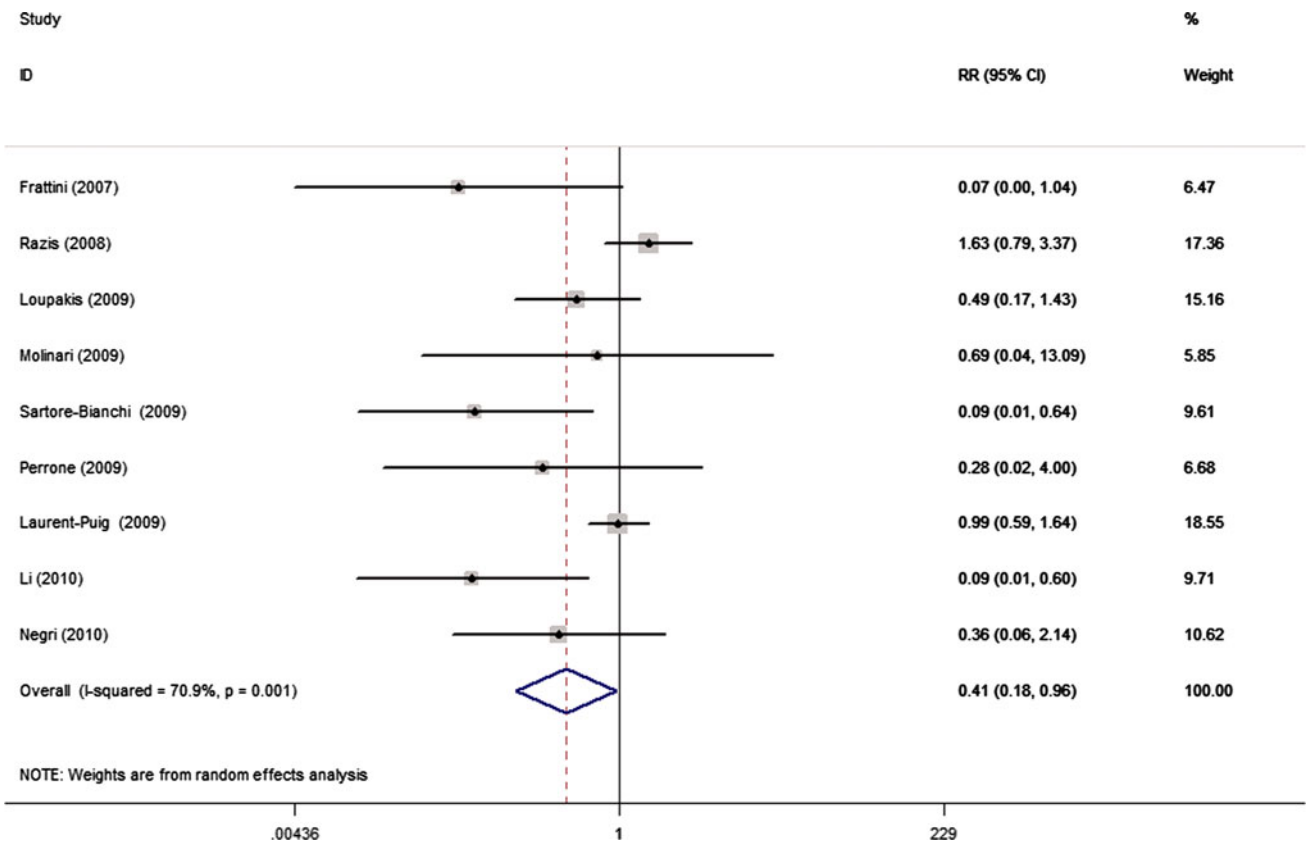

Fig. 1 Forest plot of studies of objective response rate in patients with loss of PTEN expression over those with normal PTEN expression

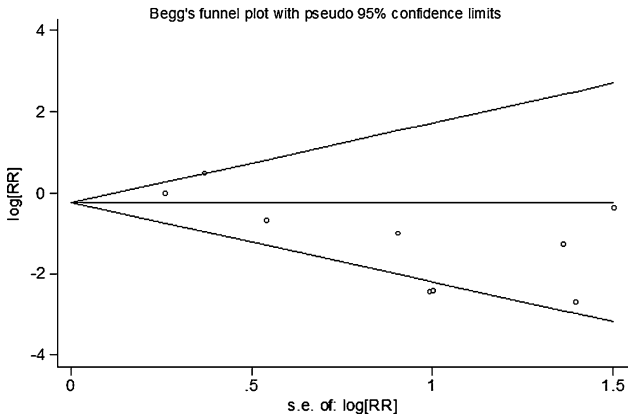

Fig. 2 Begg's funnel plot of studies on objective response rate in patients with loss of PTEN expression over those with normal PTEN expression

PTEN expression and OS in patients with mCRC

Eight studies [20, 22, 24–27, 29] provided data on OS in patients unselected by KRAS mutation. Four studies [20, 24, 27] reported HR and variance directly. HR and variance were estimated from original data provided by two studies [22, 25], and another two studies [26, 29] reported original data without survival curves, which could not be converted to HR and variance. Therefore, HR and variance from six studies [20, 22, 24, 25, 27] on PFS in patients unselected by KRAS mutation were pooled in the meta-analysis. The HR of OS in patients with normal PTEN expression over those with loss of expression was 0.689 (95 % CI, 0.482–0.896), based on a fixed-effects model (p for heterogeneity = 0.603) using the Mantel–Haenszel method (Fig. 5) without significant publication bias (p for Begg's test = 1.000; Fig. 6; Table 3). In subgroup analysis, the HR of OS in patients with normal PTEN expression over those with loss of expression in the wild-type KRAS group was 0.544 (95 % CI, 0.285–0.803), based on a fixed-effects model (p for heterogeneity = 0.985) using the Mantel–Haenszel method without significant publication bias from three studies [20, 24, 27] (HR and variance provided directly; p for Begg's test = 1.000; Table 3).

Discussion

A previous meta-analysis [30], which included four studies and a total of 231 cases of mCRC, suggested that loss of PTEN expression is associated with clinical resistance to anti-EGFR monoclonal therapy in patients with mCRC. However, the number of studies involved and the pooled

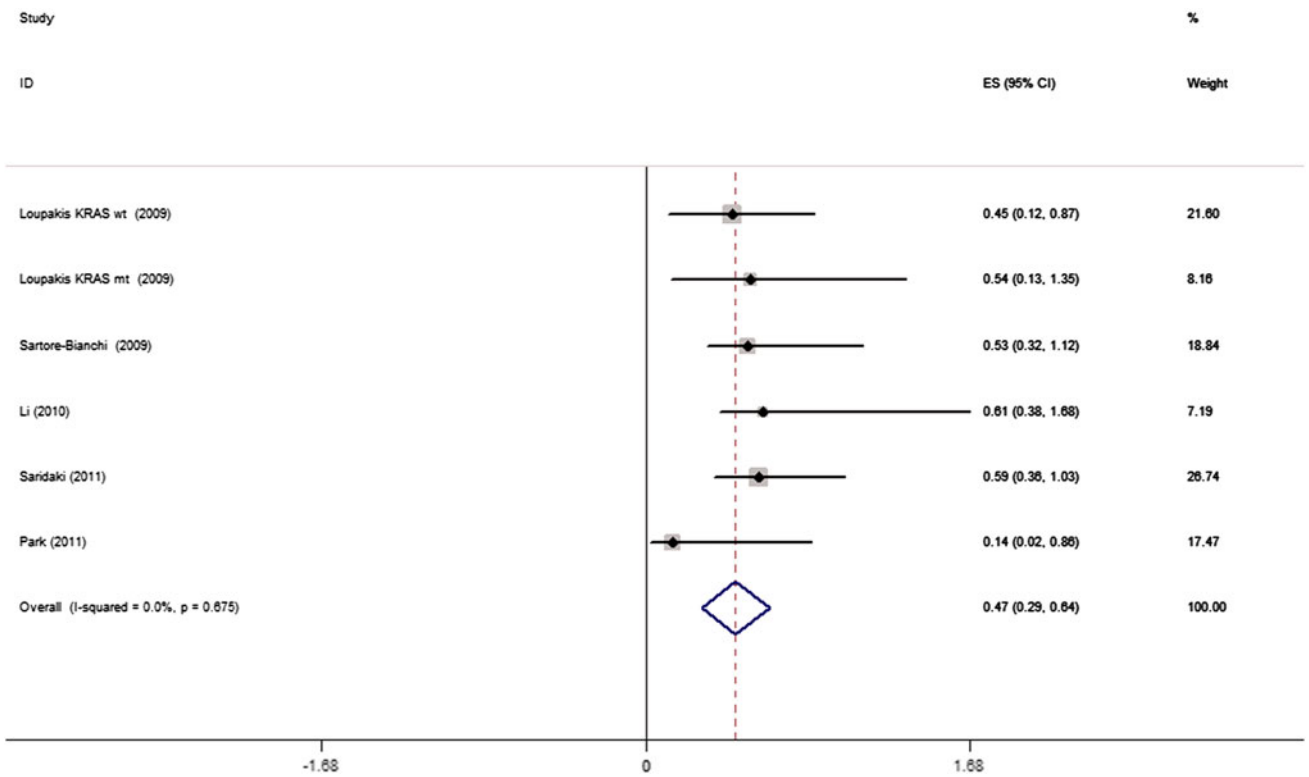

Fig. 3 Forest plot of studies of progression-free survival in patients with loss of PTEN expression over those with normal PTEN expression

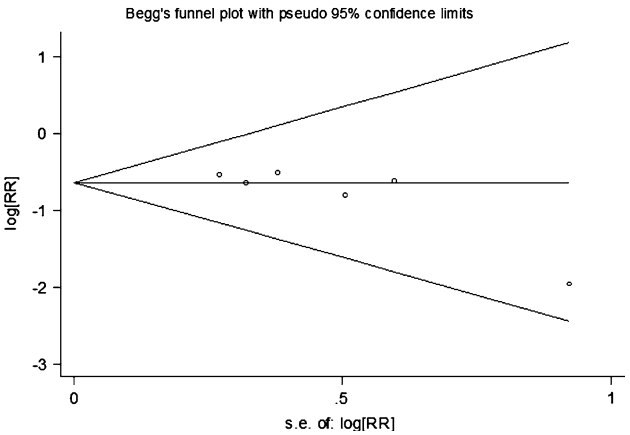

Fig. 4 Begg's funnel plot of studies on progression-free survival in patients with loss of PTEN expression over those with normal PTEN expression

sample sizes were relatively small. Data concerning PFS and OS were also not available in this meta-analysis. However, several additional larger studies have been published since this review. Therefore, we performed the current meta-analysis to more precisely investigate the predictive and prognostic values of loss of PTEN expression in patients with mCRC treated with anti-EGFR monoclonal therapy.

The overall RR of ORR indicated that patients with mCRC and loss of PTEN expression were less sensitive to anti-EGFR monoclonal therapy than those with normal expression. Significant heterogeneity for RRs of ORR existed in overall comparisons. This heterogeneity continued after excluding the trials that used anti-EGFR MoAb as second-line or as subsequent-line treatment. Therefore, heterogeneity was a potential problem in this

Table 3 Hazard ratio of PTEN expression with PFS and OS by KRAS mutation status

Characteristics	PFS				OS			
	No. of studies	*p* for heterogeneity	HR (95 % CI)	*p* for Begg's test	No. of studies	*p* for heterogeneity	HR (95 % CI)	*p* for Begg's test
Unselected patients	6	0.675	0.466 (0.292–0.640)	0.133	6	0.603	0.689 (0.482–0.896)	1.000
Patients with KRAS wild type	3	0.533	0.344 (0.154–0.533)	1.000	3	0.985	0.544 (0.285–0.803)	1.000

PFS progression-free survival, *OS* overall survival

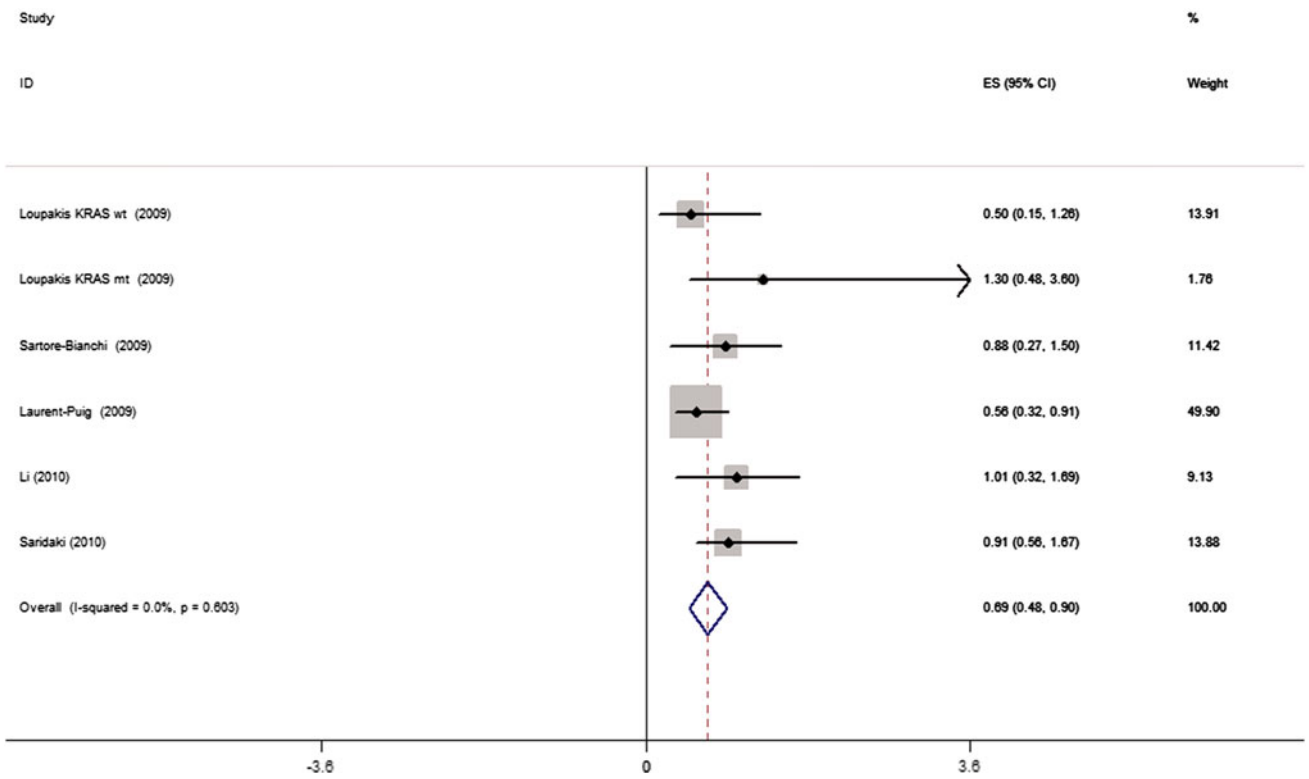

Fig. 5 Forest plot of studies of overall survival in patients with normal PTEN expression patients over those with loss of PTEN expression

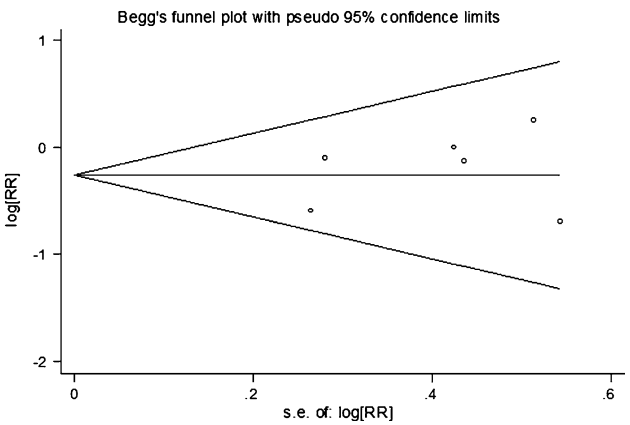

Fig. 6 Begg's funnel plot of studies on overall survival in patients with normal PTEN expression over those with loss of PTEN expression

meta-analysis, which may affect interpretation of the ORR results.

As KRAS mutation has been suggested as a clear predictor of resistance to anti-EGFR MoAbs therapy, the value of PTEN expression in predicting non-responders should primarily affect patients with KRAS wild type. Therefore, we performed a subgroup analysis to evaluate the results of PFS and OS for the wild-type KRAS groups compared with the unselected groups. Although the pooled HR of PFS and OS in normal PTEN expression over loss of PTEN expression in patients with unselected KRAS mutation status was significantly associated with a worse prognosis and a shorter survival, this tendency was more apparent in wild-type KRAS patients. The results of subgroup analysis suggest that a comprehensive analysis of KRAS mutation and PTEN expression is a better predictor of clinical outcome in patients with mCRC treated with anti-EGFR MoAbs, which requires further confirmation in prospective trials.

Several limitations of our meta-analysis need to be considered. Firstly, the inclusion of retrospective studies may introduce selection bias. Secondly, inadequate reporting of survival curves precluded conversion to HR and variance from original data. Therefore, we did not perform a pooled analysis for HR. Thirdly, our findings were based on unadjusted estimates, while a more precise analysis should be carried out if more detailed individual data allowed for an adjusted estimate by other factors such as age, sex, ethnicity, treatment protocols and other biomarkers.

Despite these limitations, the major finding in our study is the confirmation that loss of expression of PTEN is a potential biomarker for resistance to anti-EGFR monoclonal therapy, particularly in patients with mCRC and wild-type KRAS. These findings need to be explored in large prospective randomized studies.

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Conflict of interest No company had any input into or influence on the design, analysis, interpretation, or content of this manuscript. There are no conflicts of interest with any of the authors.

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