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Review Article

Clinicopathological and prognostic significance of MUC4 expression in cancers: evidence from meta-analysis

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Abstract: Mucin4 (MUC4) is a secreted glycoprotein. Numerous studies had indicated that MUC4 was an attractive prognostic tumor biomarker. However, the results of different studies have been inconsistent. So we conducted this meta-analysis to explore the association between MUC4 expression and cancer prognosis. A systematically comprehensive search was performed through PubMed, EMBASE and CNKI (Chinese National Knowledge Infrastructure). Prognostic value of MUC4 expression in malignancy patients was evaluated by pooled hazard ratios (HRs) and their 95% confidence intervals (CIs). Meanwhile, pooled odds ratio (OR) with 95% CI was appropriate for the association between MUC4 expression and clinicopathological parameters. Eighteen studies including 1,933 patients were enrolled in this meta-analysis. Significant association was found between elevated MUC4 expression and poorer overall survival (OS) with pooled hazard ratio (HR) of 1.87 [95% confidence interval (CI): 1.58-2.23, P<0.001]. Significant associations were also detected in biliary tract carcinoma (HR: 2.41, 95% CI: 1.69-3.42, P<0.001), pancreatic cancer (HR: 2.01, 95% CI: 1.42-2.86, P<0.001) and colorectal cancer (HR: 1.73, 95% CI: 1.17-2.54, P=0.006). Moreover, combined odds ratio (OR) of MUC4 indicated that MUC4 overexpression was associated with tumor stage, tumor invasion and lymph node metastasis. Our results demonstrated that MUC4 may be exploited as a novel prognostic biomarker for cancer patients.

Keywords: MUC4, mucin, cancer, prognosis, meta-analysis

Introduction

Mucins are heavily glycosylated proteins that synthesized by epithelial cells and participate in the protection, repair and survival of the epithelia. To date, about 20 human mucins have been identified and categorized into two classes (secreted/gel forming mucins and transmembrane mucins) based on their structural characteristics and physiological functions.

As a critical member of transmembrane mucins, MUC4 was first identified in 1991 from a tracheobronchial cDNA library [1], and could be found expressed in various normal tissues [2-5]. Under normal conditions, MUC4 is localized at the apical surface of the epithelial cells. During cancer progression, MUC4 could act as an intramembrane ligand for receptor tyrosine kinase ErbB2 and thus participated in cancer cell signaling [6, 7]. Furthermore, MUC4 was involved the regulation of p27 [8], which is a cyclin-dependent kinase inhibitor that regulates the G1 and S phases of the cell cycle [9].The association between MUC4 expression and malignancies had hitherto been indicated in amount of reports, and most of them suggested that overexpression of MUC4 was a potential predictor of poor outcome in cancer patients [10-24]. However, some researchers arrived at the opposite conclusions [25, 26]. Thus, the prognostic value of hyper-expression of MUC4 remains inconclusive. Given these discrepancies of the results and the relatively small sample sizes of studies, we conducted this meta-analysis of all available studies to investigate the relationship between MUC4 expression and their prognosis effect in cancer patients.

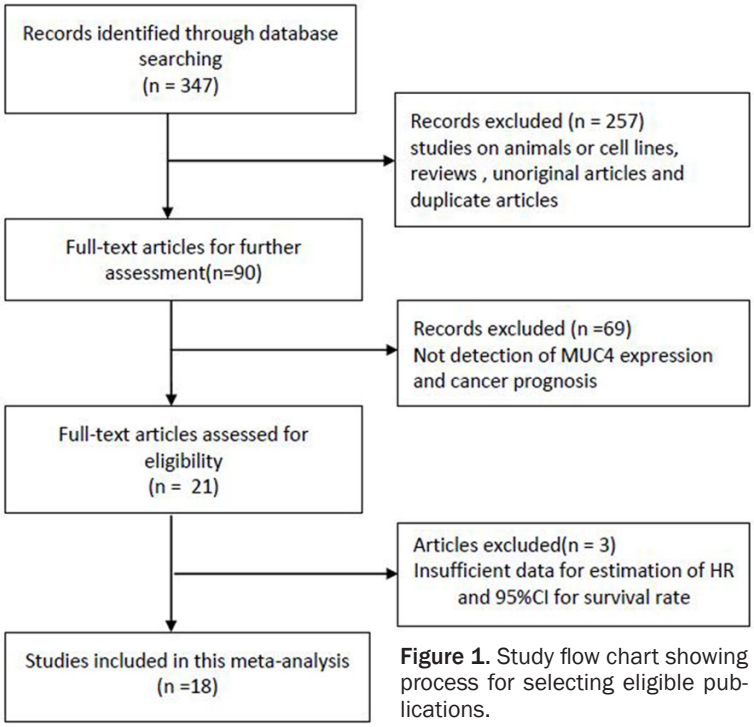

Figure 1. Study flow chart showing process for selecting eligible publications.

Materials and methods

Search and selection process

A systematic literature search was conducted via the databases PubMed, EMBASE and CNKI (Chinese National Knowledge Infrastructure), covering all relevant studies published up to Apr 27, 2015, with a combination of the following keywords: "mucin 4" OR "MUC4" AND "prognosis" OR "survival" OR "outcome" AND "cancer" OR "carcinoma" OR "neoplasm". Cited references in these papers had been surveyed as well to find additional eligible studies. Two investigators (Huang and Wang) performed the search independently.

Inclusion criteria

To be eligible for inclusion, studies have to meet the following criteria: (a) trials have to be published as a full paper in English or Chinese literature; (b) investigating the association between MUC4 and cancer prognosis; (c) sufficient data for estimating hazard ratio (HR) with 95% confidence interval (CI). The major reasons for exclusion of studies were:(a) overlapping data; (b) abstract, comment, and review; (c) studies without detailed data. The flow diagram was shown in **Figure 1**.

Data extraction and quality assessment

Two reviewers (Huang and Wang) did the search and identification independently using the standard approach [27]. The following items were collected from each eligible publication: first author's name, publication year, nationality, geography (Asian or Western), cancer type, quantitative method (IHC or PCR or Others), cut-off value, follow-up months, hazard ratios (HR) with corresponding 95% confidence intervals (CI) for overall survival (OS), disease-free survival (DFS) and progression-free survival (PFS) and the total number of participants, respectively. In case of discrepancies, another investigator (Ren) was invited to discuss and check the original data until a consensus was reached. Quality assessment for each study included in final analysis was carried out by the same two reviewers according to the Newcastle-Ottawa quality assessment scale (NOS) [28]. NOS scores ranged from 0 to 9, and a score ≥7 indicates good quality in our present study.

Statistical analysis

Hazard ratio (HR) with a 95% confidence interval was calculated for the association between MUC4 expression and cancer prognosis (OS and DFS/PFS/DFS, respectively). Meanwhile, pooled odds ratio (OR) with 95% CI was appropriate for the association between MUC4 expression and clinicopathological parameters. When the statistical variables were described in text or tables, we obtained them directly. Otherwise, the methods reported by Tierney [29] was used to calculate data from Kaplan-Meier survival curves. The heterogeneity among these studies was checked using Chi-square based Q test and considered statistically significant when I^2>50% or P<0.1. The fixed effects model (Mantel-Haenszel method) was picked if there was no significant heterogeneity; otherwise, the random effects model (the Der Simonian and Laird method) was utilized

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

First author	Publication year	Case nationality	Dominant geography	Sample size	Mean age	Malignant disease	Survival analysis	Source of HR	Follow-up months	NOS score
Higashi1	2015	Japan	Asian	114	67.4	Pancreatic cancer	OS	Reported	40	7
Majhi	2013	USA	Western	29	NA	NSCLC	OS	SC	144	5
Khanh	2013	Japan	Asian	206	NA	Colorectal cancer	OS/RFS	Reported	144	8
Lee	2012	Korea	Asian	63	66.9	Gallbladder cancer	OS	Reported	122	7
Higashi2	2012	Japan	Asian	63	67.4	Cholangio cancer	OS	Reported	NA	8
Hamada	2012	Japan	Asian	150	64.5	OSCC	OS/DFS	Reported/SC	206	8
Yi Zhu	2011	China	Asian	57	61.7	Pancreatic cancer	OS	Reported	40	7
Shanmugam	2010	England	Western	132	65	Colorectal cancer	OS	Reported	300	8
Aloysius	2010	England	Western	104	NA	Periampullary cancer	OS	Reported	36	6
Yeh CN	2009	China	Asian	51	60	Cholangio cancer	OS	Reported	70.1	8
Westgaard	2009	Norway	Western	65	68	Pancreatic cancer	OS	SC	60	7
Tsutsumida	2007	Japan	Asian	185	67	Lung cancer	OS/RFS	SC	100	7
Morrison	2007	USA	Western	295	NA	Endometrial cancer	OS/PFS	Reported	NA	6
Tamada	2006	Japan	Asian	70	69.2	Cholangio cancer	OS	Reported	100	8
Chauhan	2006	USA	Western	38	NA	Ovarian cancer	OS	SC	108	7
Saitou	2005	Japan	Asian	135	65.8	Pancreatic cancer	OS	Reported	155	7
Weed	2004	USA	Western	149	NA	Upper Aerodigestive Tract cancer	OS/RFS	Reported	108	6
Shibahara	2004	Japan	Asian	27	65.3	Cholangio cancer	OS	Reported	60	7

OS, overall survival; DFS, disease-free survival; RFS, recurrence-free survival; PFS,progression-free survival; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; NA, not available; SC, survival curve.

Table 2. HRs and 95% CIs for patient survival (OS) in association with MUC4 expression in enrolled studies

First author	Publication year	Detecting method	Cut-off value	Case Number		HR (95% CI)	
				High expression	Low expression	OS	DFS/RFS/PFS
Higashi1	2015	IHC-8G7	10%	106	8	1.00 (0.31-4.12) M	NA
Majhi	2013	NA	Score >12	16	13	0.32 (0.05-1.92) U*	NA
Khanh	2013	IHC-1G8	5%	68	138	1.51 (0.91-2.53)M	2.30 (1.21-4.36) M
Lee	2012	IHC-1G8	5%	35	28	2.89 (0.884-9.451) M	NA
Higashi2	2012	IHC-8G7	5%	19	44	1.73 (0.83-3.60) M	NA
Hamada	2012	IHC-8G7	5%	61	89	1.619 (1.115-2.409) M	1.00 (0.97-1.03) U*
Yi Zhu	2011	qRT-PCR	50%	29	28	2.571 (1.277-5.177) M	NA
Shanmugam	2010	IHC-8G7	75%	33	99	2.07 (1.14-3.75) M	NA
Aloysius	2010	IHC-1G8	5%	53	51	1.79 (0.88-3.7) M	NA
Yeh CN	2009	IHC-1G8	1%	13	38	3.40 (1.56-7.41) M	NA
Westgaard	2009	IHC-1G8	10%	44	21	2.02 (1.02-3.98) U*	NA
Tsutsumida	2007	IHC-8G7	25%	25	160	3.21 (1.39-7.42) U*	1.00 (0.96-1.04) U*
Morrison	2007	IHC-1G8	5%	69	226	2.15 (0.85-5.48) M	1.44 (0.51-4.04) M
Tamada	2006	IHC-8G7	5%	19	51	2.655 (1.125-6.625) M	NA
Chauhan	2006	IHC-8G7	25%	23	15	1.61 (0.5-5.23) U*	NA
Saitou	2005	IHC-8G7	5%	21	114	1.956 (1.13-3.384) M	NA
Weed	2004	IHC-1G8	10%	19	130	0.27 (0.08-0.88) M	0.27 (0.10-0.77) M
Shibahara	2004	IHC-8G7	5%	10	17	4.560 (1.190-17.478) M	NA

The source of HR and 95% CI is described as derived from univariate analysis (U) or multivariateanalysis (M). *HR and 95% CI calculated from survival curves. OS, overall survival; DFS,disease-free survival; RFS, recurrence-free survival; PFS, progression-free survival; HR, HR (high vslow); qRT-PCR, quantitative real-time PCR; NA, not available; IHC-1G8: Immunohistochemistryusing 1G8 antibody; IHC-8G7: Immunohistochemistry using 8G7 antibody.

[16]. Sub-group analyses and logistic meta-regression analyses were conducted to explore the source of heterogeneity among variables, such as cancer types, geography, quantitative method, cut-off level and study quality. Sensitivity analysis was carried out to identify the effect of data from each study on pooled HRs. Publication bias was determined by Egger's test and Begg's funnel plots [17]. All statistical tests were conducted with STATA software version 12.0 (STATA Corporation, College Station, TX, USA) and $P<0.05$ was considered significant.

Results

Study characteristics

A total of 347 potentially relevant studies were identified after the initial database searches. After a rough review of the titles and abstracts of all studies, 257 studies were excluded; then, with a systematical review of the full texts by the same two reviewers, another 69 studies were excluded (**Figure 1**). Three studies were excluded because of insufficient data [30-32]. Eventually, 18 eligible studies containing 1,933 patients were included in this meta-analysis [10-26, 33].

The main characteristics of the included studies are summarized in **Tables 1** and **2**. Of the 18 studies, 11 (1121 patients: 61.1%) were performed in Asian area [10-14, 16, 18, 21, 23, 24, 33], and the rest 7 studies (812 patients: 38.9%) were conducted in European or American areas [15, 17, 19, 20, 22, 25, 26]. All of these studies were retrospective in design. The malignant neoplasms assessed in these studies included biliary tract carcinoma [11, 12, 16, 19, 23, 24], pancreatic cancer [14, 18, 20, 33], colorectal cancer [10, 15], lung cancer [21, 25], oral squamous cell carcinoma (OSCC) [13], endometrial cancer [22], ovarian cancer [17] and Upper Aerodigestive Tract cancer [26]. Immunohistochemistry was used to detect the expression of MUC4 in all studies except one, which performed quantitative real-time PCR (qRT-PCR) [14].

Meta-analysis

MUC4 expression and OS: There were 18 studies with a total of 1,933 patients providing sur-

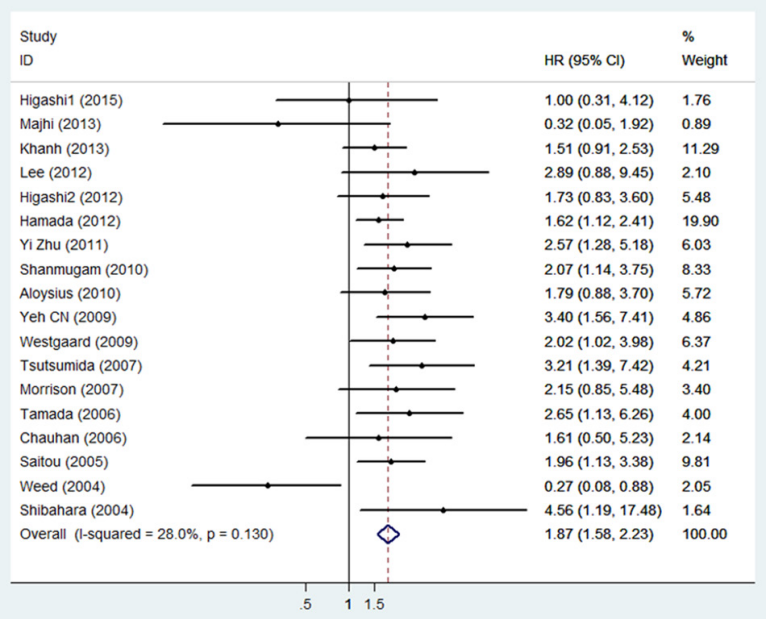

Figure 2. Forest plot of hazard ratio (HR) for the association between high MUC4 expression and overall survival in patients with malignant tumors.

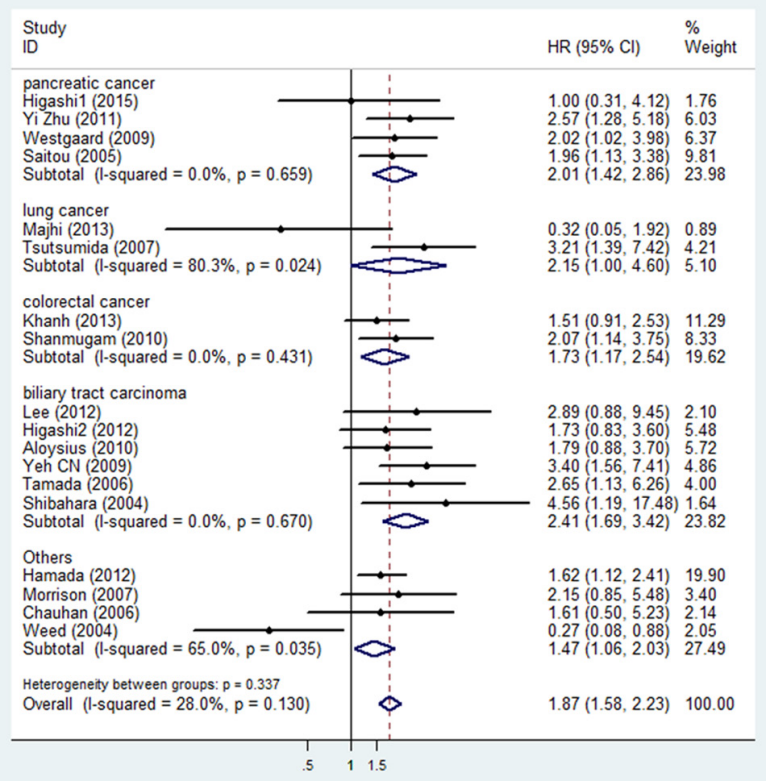

Figure 3. Meta-analysis (Forest plot) of the evaluable studies assessing MUC4 expression and overall survival stratified by cancer type.

vival results in the form of OS. Since the heterogeneity was not statistic significant (I^2=28.0%, P=0.130), the fixed model was used to pool HRs. Our result showed that MUC4 overexpression was significantly associated with poor OS in various carcinomas, with the pooled HR of 1.87 (95% CI: 1.58-2.23, P<0.001) (**Figure 2**).

To determine the prognostic role of MUC4 in different cancers, studies were divided into subgroups by cancer types. The results indicated that high MUC4 expression was an unfavorable prognostic indicator in biliary tract carcinoma (HR: 2.41, 95% CI: 1.69-3.42, P<0.001), pancreatic cancer (HR: 2.01, 95% CI: 1.42-2.86, P<0.001) and colorectal cancer (HR: 1.73, 95% CI: 1.17-2.54, P=0.006), but not in lung cancer (HR: 1.18, 95% CI: 0.13-11.05, P=0.888) (**Figure 3**).

We also performed subgroup analysis by geography, detecting methods, cut-off level and study quality. And the results indicated that a significant relationship between MUC4 overexpression and poor OS was also exhibited in studies with an Asian country (HR: 1.99, 95% CI: 1.63-2.44, P<0.001), IHC-1G8 (HR: 1.74, 95% CI: 1.11-2.75, P=0.017), IHC-8G7 (HR: 1.93, 95% CI: 1.54-2.42, P<0.001), the cut-off level >5% (HR: 1.83, 95% CI: 1.48-2.27, P<0.001) and the high quality study (HR: 1.99, 95% CI: 1.66-2.39, P<0.001) (**Table 3**).

MUC4 expression and DFS / RFS/PFS: A total of five studies [10, 13, 21, 22, 26] were used for DFS/PFS/RFS analysis with a random-effects model due to significant heterogeneity (I^2=69.9%, P=0.010). Our results failed to dem-

Table 3. Main results of meta-analysis

Categories	Studies	Patients	MUC4+	HRs	95% CI	Heterogeneity		P
						I-Square	P_h	
Overall	18	1933	663	1.87	1.58-2.23	28.00%	0.13	<0.001
Cancer type								
Lung cancer	2	214	41	1.18	0.13-11.05	80.30%	0.024	0.888
Colorectal cancer	2	338	101	1.73	1.17-2.54	0.00%	0.431	0.006
Biliary tract carcinoma	6	378	149	2.41	1.69-3.42	0.00%	0.67	<0.001
Pancreatic cancer	4	371	200	2.01	1.42-2.86	0.00%	0.659	<0.001
Others	4	632	172	1.22	0.59-2.55	65.00%	0.035	0.589
Geography								
Western	7	812	257	1.4	0.84-2.33	54.20%	0.041	0.191
Asian	11	1121	406	1.99	1.63-2.44	0.00%	0.503	<0.001
Methods								
IHC-1G8	7	933	301	1.74	1.11-2.75	55.50%	0.036	0.017
IHC-8G7	9	914	317	1.93	1.54-2.42	0.00%	0.698	<0.001
Others	2	86	45	1.08	0.14-8.10	77.10%	0.037	0.938
Cut-off Value								
>5%	9	1113	355	1.83	1.48-2.27	0.00%	0.839	<0.001
Study quality								
High	14	1333	506	1.99	1.66-2.39	0.00%	0.738	<0.001
Low	4	600	157	0.89	0.32-2.47	71.90%	0.014	0.825

IHC-1G8: Immunohistochemistry using 1G8 clone antibody, IHC-8G7: Immunohistochemistry using 8G7 clone antibody, MUC4+: MUC4 positive patients number, Ph: $P_{Heterogeneity}$; HR: hazard ratio, CI: confidence interval.

Table 4. Meta-analysis of MUC4 expression and clinicopathological parameters intumor patients

Categories	Studies	Patients	OR (95% CI)	I^2%	P_h	P
Age (≥65 vs <65)	9	928	1.38 (1.00, 1.91)	0.00%	0.483	0.05
Gender (Male vs Female)	11	1304	1.08 (0.82, 1.43)	0.00%	0.996	0.562
Tumor stage (III/IV vs I/II)	6	941	1.82 (1.30, 2.56)	36.90%	0.161	0.001
Tumor invasion (T3/T4 vs T1/T2)	5	603	2.01 (1.27, 3.15)	39.90%	0.155	0.003
Histological grade (Moderate/Poor vs Well)	9	1048	1.22 (0.88, 1.68)	0.00%	0.731	0.236
Lymph node metastasis (Positive vs Negative)	9	991	1.92 (1.36, 2.69)	3.50%	0.405	<0.001

OR, odds ratio; 95% CI, 95% confidence interval; P_h: $P_{Heterogeneity}$.

onstrate any significant association between MUC4 expression and DFS/PFS/RFS (HR: 1.01, 95% CI: 0.93-1.09, P=0.869). Subgroup analysis, meta regression and sensitivity analysis were not applicable in analysis of the relationship between MUC4 expression and DFS/RFS/PFS because of the limited number of studies.

MUC4 expression and clinicopathological parameters: As shown in **Table 4**, overexpression of MUC4 was significantly associated with tumor stage (III/IV vs. I/II: OR 1.82, 95% CI 1.30-2.56) [10, 13, 15, 21, 26, 33], tumor invasion (T3/T4 vs. T1/T2: OR 2.01, 95% CI 1.27-3.15) [10, 11, 13, 23, 33] and lymph node metastasis (positive vs. negative: OR 1.92, 95% CI 1.36-2.69) [10-13, 18, 21, 23, 24, 33].

Sensitivity analysis

We adopted the "leave-one-out" scheme (i.e., analysis is conducted using all studies but one) to explore individual study's influence on the pooled HRs. The results showed that pooled HRs was not materially altered which suggested that no individual study significantly affected the pooled results (**Figure 4**).

Publication bias

Begg's funnel plot and the Egger's linear regression test were conducted to evaluate the publi-

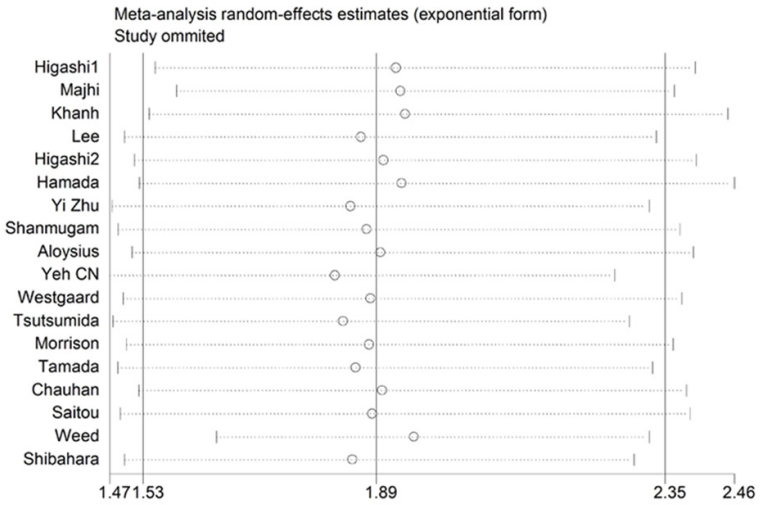

Figure 4. Sensitivity analysis for overall survival: effect of individual studies on pooled hazard ratios (HR) for cancer patients.

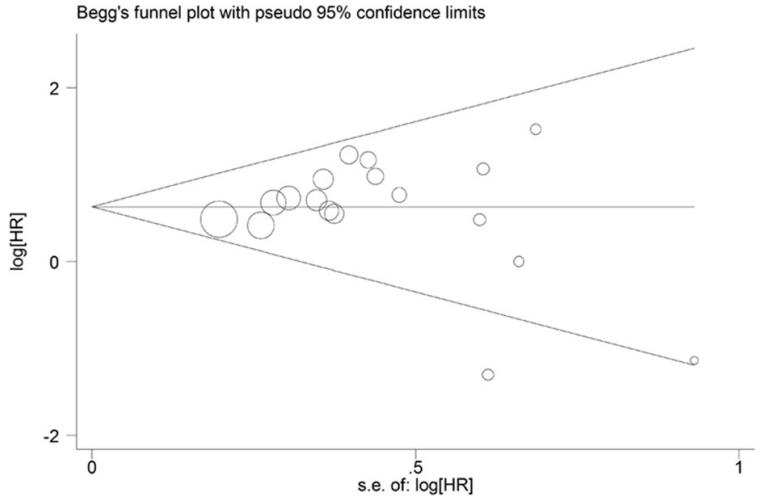

Figure 5. Begg's funnel plot of MUC4 expression and OS in tumor patients.

cation bias of the literature. In the pooled analyses of OS and DFS/RFS/PFS, the Egger's test *p* values were 0.695 and 0.865, respectively, as shown by symmetric funnel plots (**Figure 5**). Therefore, no evidence of publication bias was noted.

Discussion

Cancer remains the major public health burden which counts for one in four deaths in the United States [34]. It is of great interest in identifying reliable and informative prognostic biomarkers for cancer patients to provide valuable information for clinical decision-making. Recently, many studies have suggested that mucins are potential biomarkers of cancer prognosis given their unique expression profiles in cancer patients compared with normal individuals [35, 36]. Among them, MUC4 is considered to be a promising one. As a transmembrane glycoprotein, MUC4 has been considered a pivotal factor to regulate the cell proliferation and survival through interaction with ErbB2 family [6, 7]. Moreover, MUC4 promotes tumor progression by repression apoptosis by both ErbB2 dependent and independent mechanisms [37]. Recently , several researches have reported that elevated expression of MUC4 might be a predictive factor for tumor prognosis, including bile duct carcinoma, colorectal cancer, oral squamous cell carcinoma, invasive ductal carcinoma of the pancreas, and small sized lung adenocarcinoma [10, 11, 13-15, 18, 23]. However, other researches arrived at the opposite conclusions [25, 26]. Thus, the prognostic value of high MUC4 expression remained inconclusive. To address the prognostic value of MUC4 expression, we conducted this meta-analysis.

To the best of our knowledge, this is the first meta-analysis focused on the association between elevated MUC4 expression and the prognosis and clinicopathological characteristics of patients with various cancers. A total of 18 eligible studies [10-26, 33], including 1,933 cases, were identified and analyzed in the present meta-analysis. The results revealed that elevated MUC4 expression was significantly associated with poor OS (HR 1.87, 95% CI 1.58-2.23, P<0.001) of tumor patients. Moreover, there seems to be a correlation between MUC4 overexpression and tumor stages, tumor invasion and lymph node metastasis. These results might be important for the understanding of

cancer biology and help us to distinguish high-risk groups of patients and improve the clinical outcomes.

To determine the prognostic role of MUC4 in different cancers, we conducted subgroup analysis by cancer types. The results showed that elevated MUC4 expression was significantly associated with worse OS in patients with biliary tract carcinoma (HR 2.41, 95% CI 1.69-3.42, P<0.001), pancreatic cancer (HR 2.01, 95% CI 1.42-2.86, P<0.001), and colorectal cancer (HR 1.73, 95% CI 1.17-2.54, P=0.006). Thus, MUC4 could serve as a novel prognostic marker for carcinomas aforementioned. But in lung cancer, the prognostic role evidence of MUC4 is not powerful. We also conducted subgroup analysis by geography, detecting methods and study quality. In geography subgroup analysis, significant association was only found in Asian patients (HR 1.99, 95% CI 1.63-2.44, P<0.001), suggesting MUC4 had more prognostic value in Asian patients. When in terms of detecting methods, we found that IHC-1G8 (HR 1.74, 95% CI 1.112.75, P=0.017) and IHC-8G7 (HR 1.93, 95% CI 1.54-2.42, P<0.001) were both effective methods for detecting the expression of MUC4 in cancer patients. Besides, tumor patients had shorter OS only in high-quality studies (HR 1.99, 95% CI 1.66-2.39, P<0.001).

Several studies had indicated that the presence of MUC4 on the tumor cell can mask the surface epitopes to the cytotoxic immune cells such as cytotoxic-T lymphocytes or NK cells and, hence, escape from immune response [38, 39]. But in this meta-analysis we failed to reveal any significant association between MUC4 expression and DFS/RFS/PFS (HR 1.01, 95% CI 0.93-1.09, P=0.869) with significant heterogeneity (I^2=69.90%, P=0.010). Consider the small sample size (only five studies), it may be too early to reach a conclusion and more large size studies are needed to strengthen our conclusions.

Although the present study is the first meta-analysis on the association between MUC4 expression and patient survivals, some limitation should be noted. Firstly, our meta-analysis only encompassed a total of 18 studies, thus the results might be a fluke because sample error of eligible studies could lead to insufficient statistical power. Secondly, although most of the method for detecting MUC4 expression of all enrolled studies was IHC, the dyeing operation, antibody concentration and cutoff value of different tissues varied in different studies. Thirdly, not all of the HRs with 95% CIs was directly extracted from the studies, so we had to evaluate the HRs via Kaplan-Meier curves and these calculated HRs and 95% CIs might be less reliable than the directly given data. Finally, although no significant difference was detected according to the results of sensitivity analysis and publication bias assay, publication bias cannot be totally ruled out.

In conclusion, the present meta-analysis indicated that MUC4 overexpression may be positively correlated with poor prognosis in cancer patients. Therefore, MUC4 may be used as a prognostic marker and a novel potential therapeutic target for cancer patients. To strengthen our conclusion, standardized prospective studies with high quality are recommended to scoop the relationship between high MUC4 expression and prognosis for cancer patients.

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Disclosure of conflict of interest

None.

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