Prognostic Value of SMAD4 in Pancreatic Cancer: A Meta-Analysis[1,2]

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Abstract

PURPOSE: The prognostic value of SMAD4 in pancreatic cancer has been evaluated in several studies. However, the conclusions remain controversial. Therefore, we aimed to evaluate the association between SMAD4 expression and the outcome of pancreatic cancer patients by performing a meta-analysis. *METHODS:* We systematically searched for relevant studies evaluating the relationship between SMAD4 expression and the outcome of pancreatic cancer patients until May 2015. A meta-analysis was performed using STATA 12.0, and pooled hazard ratios (HRs) with 95% confidence intervals (CIs) were used to estimate the strength of the association between SMAD4 expression and the prognosis of pancreatic cancer patients. *RESULTS:* The analysis included 1762 patients from 14 studies, with 1401 patients from 11 studies and 927 patients from 8 studies included in the univariate and multivariate analyses, respectively. Loss of SMAD4 expression was found to be significantly correlated with poor overall survival, with the combined HR (95% CI) of 1.20 (1.03-1.40). After adjusting for potential confounders using the Cox regression model, the pooled HR (95% CI) was 1.88 (1.31-2.70). In subgroup analysis, study region, number of patients, follow-up duration, and cutoff value were found to affect the significance of the association between loss of SMAD4 expression and poor prognosis. In addition, there was no evidence of publication bias, as suggested by Begg's and Egger's test. *CONCLUSIONS:* Loss of SMAD4 was associated with poor survival and was a negative prognostic indicator in patients with pancreatic cancer.

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Introduction

Pancreatic cancer is the eighth leading cause of cancer-related deaths worldwide [1]. Surgery remains the primary treatment modality and the only chance of cure. However, more than 80% of patients have advanced disease at presentation (metastasis or invasion of the superior mesenteric artery or celiac trunk in case of locally advanced tumors), which makes the tumor unsuitable for surgical resection [2]. Even if resection can be performed, the median survival is still only 18 months [3]. Despite the improvements in diagnostic and therapeutic techniques for pancreatic cancer over the past few decades, the prognosis is still poor, with an estimated survival of only 5.8% at 5 years [4,5]. Clinicopathological characteristics, for example, tumor size and stage, cannot reliably predict individual clinical outcomes. Thus, the identification of accurate molecular prognostic factors may contribute to a better understanding of cancer development and clinical outcome and eventually facilitate the rational selection of therapeutic strategies.

Pancreatic cancer is a genetic disease characterized by somatic mutations. An important genetic change in pancreatic cancer is the *SMAD4* mutation, which leads to the loss of SMAD4 protein expression. *SMAD4* is a tumor suppressor gene that is inactivated in

more than 50% of pancreatic cancer cases [6] either by the intragenic mutation of one allele in combination with the loss of the other allele or by homozygous deletion of both alleles [7]. In the cytoplasm, SMAD4 mediates signals from a family of transforming growth factor-β ligands and their transmembrane receptors through the phosphorylation of SMAD proteins, which heterodimerize with SMAD4. This SMAD4/SMAD complex transmits upstream signals by translocating to the nucleus, binding to specific DNA sequences, and activating gene transcription.

Recently, many studies demonstrated that the loss of SMAD4 protein expression was associated with lymph node metastasis and TNM stage in pancreatic cancer patients [8,9]. Moreover, its prognostic role in pancreatic cancer has been widely investigated [10–12].

However, the results are conflicting. Many studies have shown that the loss of SMAD4 expression is positively associated with poor prognosis [12,13]. However, other studies have found no correlation between the loss of SMAD4 expression and a poor clinical outcome [7,8]. Whether the discrepancies in these data are due to limited sample sizes or genuine heterogeneity is still unknown. Thus, current clinical data are insufficient to determine the prognostic value of SMAD4 in pancreatic cancer. Accordingly, to clarify the exact prognostic value of SMAD4 in pancreatic cancer, we conducted a meta-analysis of eligible studies to investigate the association between the loss of SMAD4 expression and the prognosis of pancreatic cancer patients.

Materials and Methods

Search Strategy

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematics Reviews and Meta-Analyses guidelines [14]. A comprehensive literature search of the electronic databases PubMed, Embase, and Web of Science was performed up to May 20, 2015. Studies were selected using the following search terms: *SMAD4 or DPC4* and *cancer or tumor or carcinoma or neoplasm* and *prognosis or prognostic or outcome* and *pancreatic*. The references of articles and reviews identified through the search were also manually searched for the possible inclusion of additional studies.

Study Selection

To be eligible for inclusion in this meta-analysis and data extraction, studies had to 1) be on patients with pancreatic cancer, 2) provide overall survival (OS) data to evaluate the role of SMAD4 expression in the prognosis of pancreatic cancer patients, 3) provide hazard ratios (HRs) with 95% confidence intervals (CIs) or enable calculation of these statistics from the data presented, 4) classify SMAD4 expression as "high" and "low" or "positive" and "negative", and 5) be published as a full paper in English. The exclusion criteria were 1) data from review, animal, or cell line studies; 2) no information on survival; and 3) publication in any language other than English. In cases of multiple publications on the same population, only the most recent or the most complete study was included in the analysis.

Data Extraction and Quality Assessment

Two investigators reviewed each eligible article independently and extracted information from all publications meeting the inclusion criteria. The following information was culled from each study: first author name, publication year, country where the research was performed, number of patients, mean or median age of patients,

histology, disease stage, follow-up duration, detection method, antibody used and its dilution, cutoff value for positivity, and OS data. OS was defined as the interval between initial diagnosis and death. Inconsistencies in the research process were resolved through debate and consultations.

The quality of each eligible study was assessed using the Newcastle-Ottawa quality assessment scale [15]. The scale includes eight items on methodology under three main dimensions: selection (four items, one point for each item), comparability (one item, up to two points), and outcome (three items, one point for each item). Item scores were added up and used to quantitatively compare study quality. A higher score indicated higher quality. Inconsistencies in the scoring were resolved through discussion and consultations.

Statistical Methods

The HR and 95% CI were used to estimate the effect of the loss of SMAD4 expression on the prognosis of patients with pancreatic cancer. For multivariate analysis, the HRs (95% CIs) were computed using the Cox regression model. If the HRs (95% CIs) were not provided directly in the articles, we contacted the authors to gain more information or calculated these using the methods provided by Tierney et al. [16]. We used the Cochran's Q test ($P < .10$, significant heterogeneity) and the I^2 statistic ($I^2 \leq 50\%$, no or moderate heterogeneity; $I^2 > 50\%$, strong heterogeneity) to estimate the heterogeneity among studies. The random-effects model was used to pool the HRs (95% CI) in case of significant heterogeneity ($P < .10$ or $I^2 > 50\%$); otherwise, the fixed-effects model was used. A sensitivity analysis was performed by removing one study at a time to estimate the stability of the results. Publication bias was assessed using the funnel plot and Egger's test. All the analyses were performed using STATA 12.0 software (Stata Corporation, College Station, TX).
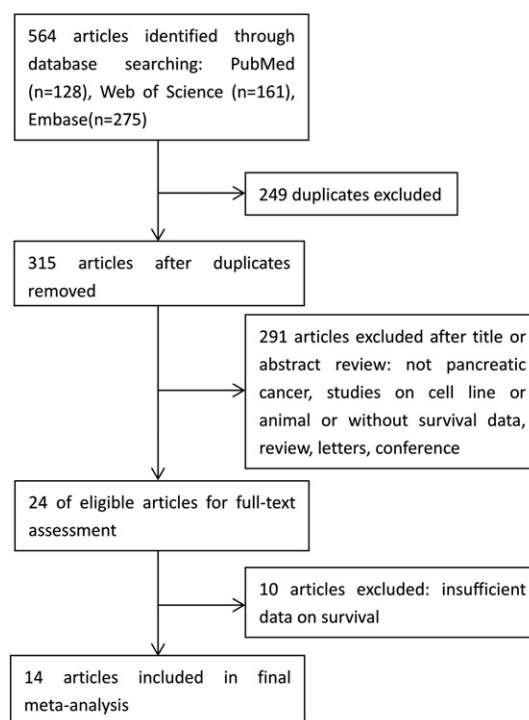

Figure 1. Flow diagram of the selection procedure of studies.

Results

Study Characteristics

We searched articles from PubMed, Web of Science, and Embase systematically, and 564 potentially relevant articles were identified. After reading the titles, abstracts, tables, figures, and key data carefully, 14 articles [7–13,17–23] with 1762 patients were found to meet the eligibility criteria and were included in the meta-analysis (Figure 1). The characteristics of these studies are listed in Table 1. SMAD4 expression in all studies was evaluated using immunohistochemistry. The cutoff value for negative versus positive SMAD4 staining varied among the studies. Positivity was determined by visible staining in nine studies, whereas different scores were used as the cutoff value in the other studies. The included studies were of high quality.

Loss of SMAD4 Expression and Prognosis in Pancreatic Cancer

Among the 14 studies eligible for estimating the correlation between the loss of SMAD4 expression and OS, 11 were suitable for univariate analysis and 8 for multivariate analysis (Figure 2); the rest could not be included because of insufficient data. For univariate analysis, because of the significant heterogeneity between studies (I^2 = 58.8%, P = .007), the random-effects model was used to combine the effect of the loss of SMAD4 expression and the pooled HR (95% CI) was 1.20 (1.03-1.40), indicating that the loss of SMAD4 expression predicted poor OS in pancreatic cancer patients. Considering the potential confounding factors and significant heterogeneity between studies (I^2 = 71.0%, P = .001), the pooled HR (95% CI) of the loss of SMAD4 expression for OS was 1.88 (1.31-2.70) after adjusting for age, tumor size, differentiation, stage, lymph node status, and grade.

We performed a subgroup analysis of the correlation between the loss of SMAD4 and OS by study region, patient age, sample size, follow-up duration, tumor stage, and cutoff value. The results are listed in Tables 2 and 3.

In univariate analysis, the loss of SMAD4 expression was significantly associated with poor OS in the studies with Asian patients (HR: 1.19, 95% CI: 1.03-1.38), those with sample sizes of ≤ 100 (HR: 1.36, 95% CI: 1.18-1.56), and those with a cutoff value of 0 (HR: 1.21, 95% CI: 1.08-1.34). In all other subgroups, loss of SMAD4 was not significantly associated with poor OS. In multivariate analysis, the pooled HRs indicated that SMAD4 expression was not significantly associated with poor OS only in the subgroup with a cutoff value >0 (HR: 1.24, 95% CI: 0.84-1.84). In the multivariate analysis, we noted that studies in which more than 80% of patients had stage I to II disease may have had a poorer OS (HR: 2.91, 95% CI: 1.51-5.61) than did those with fewer stage I to II patients (HR: 1.53, 95% CI: 1.11-2.10). This result suggests that the loss of SMAD4 might be more relevant in patients with less advanced disease.

Publication Bias Assessment

We used Begg's funnel plot and Egger's test to evaluate publication bias (Figure 3). For both univariate and multivariate analyses, no funnel plot asymmetry was found. Moreover, Egger's test did not indicate significant funnel plot asymmetry (P = .441 for univariate analysis; P = .173 for multivariate analysis). Therefore, publication bias was not detected in the meta-analysis.

Sensitivity Analysis

We used the leave-one-out method for sensitivity analysis by removing one study at a time to determine whether any single study

Table 1. Characteristics of the Eligible Studies

First Author	Year	Country	Number of Patients (Enrolled/Positive/Negative)	Age (Years)	Histology	Stage (I-II/III-IV)	Follow-up (Median/Months)	Method	Antibody (Dilution)	Cutoff (%)	Univariate Analysis HR (95% CI)	Multivariate Analysis HR (95% CI)	Study Quality Score
Javel M	2014	USA	81/47/34	Mean 60.6	PDAC	8/83	NA	IHC	Proteintech Group 1:450	>0	NA	1.190 (0.730-1.961)	6
Bachet JB	2012	France	453/166/287	Median 63.0	PAC	453/0	54	IHC	Santa 1:50	>0	1.087 (0.855-1.389)	NA	9
Biankin AV	2002	Australia	119/56/63	Mean 64.0	PDAC	29/100	3.5	IHC	Santa	>5	0.60 (0.41-0.89)	1.14 (0.71-1.81)	7
Oshima M	2013	Japan	106/42/64	Mean 68.0	PDAC	106/0	17.3	IHC	Santa 1:100	>0	NA	2.045 (1.154-3.624)	8
Kadera BE	2013	USA	32/16/16	Median 60.0	PDAC	0/32	48.9	IHC	Santa 1:100	>0	4.9 (1.4-16.6)	9.3 (2.0-42.5)	7
Ottenhof NA	2012	Netherlands	78/44/34	Mean 63.0	PDAC	20/58	NA	IHC	Santa 1:300	>0	1.354 (1.082-1.693)	2.34 (1.30-4.21)	8
Voorneveld PW	2013	Netherlands	41/19/22	NA	PDAC	NA	NA	IHC	Santa 1:1600	Score > 1	1.379 (1.015-1.876)	NA	6
Jiang H	2012	China	70	Mean 59.0	PDAC	160/2	NA	IHC	Abcam 1:15	Score > 3	1.94 (0.98-3.84)	NA	7
Yamada S	2015	Japan	174/70/104	Mean 63.7	PDAC	150/24	16.7	IHC	Santa 1:100	>0	NA	4.004 (2.488-6.666)	7
Toga T	2004	Japan	88/13/75	Mean 65.9	IDC	17/71	NA	IHC	Santa 1:100	>10	1.362 (1.015-1.832)	1.536 (0.744-3.175)	6
Tascilar M	2001	USA	249/111/138	Mean 65.4	PAC	59/190	17	IHC	Santa 1:100	>0	1.36 (1.03-1.81)	1.36 (1.01-1.83)	9
Kborana AA	2005	USA	124/59/65	Mean 66.5	PDAC	67/57	16	IHC	Santa 1:400	>5	1.136 (0.769-1.695)	NA	9
Yamazaki K	2014	Japan	113/46/67	NA	PDAC	NA	NA	IHC	Santa	>0	1.125 (0.932-1.357)	NA	7
Hua Z	2003	China	34/26/8	Mean 55.2	PAC	18/16	NA	IHC	Santa 1:100	>0	1.031 (0.694-1.533)	NA	6

IHC: immunohistochemistry, *HR (95% CI)*: hazard ratio (95% confidence interval), *NA*: not available, *PDAC*: pancreatic ductal adenocarcinoma, *PAC*: pancreatic adenocarcinoma, *IDC*: invasive ductal adenocarcinoma.
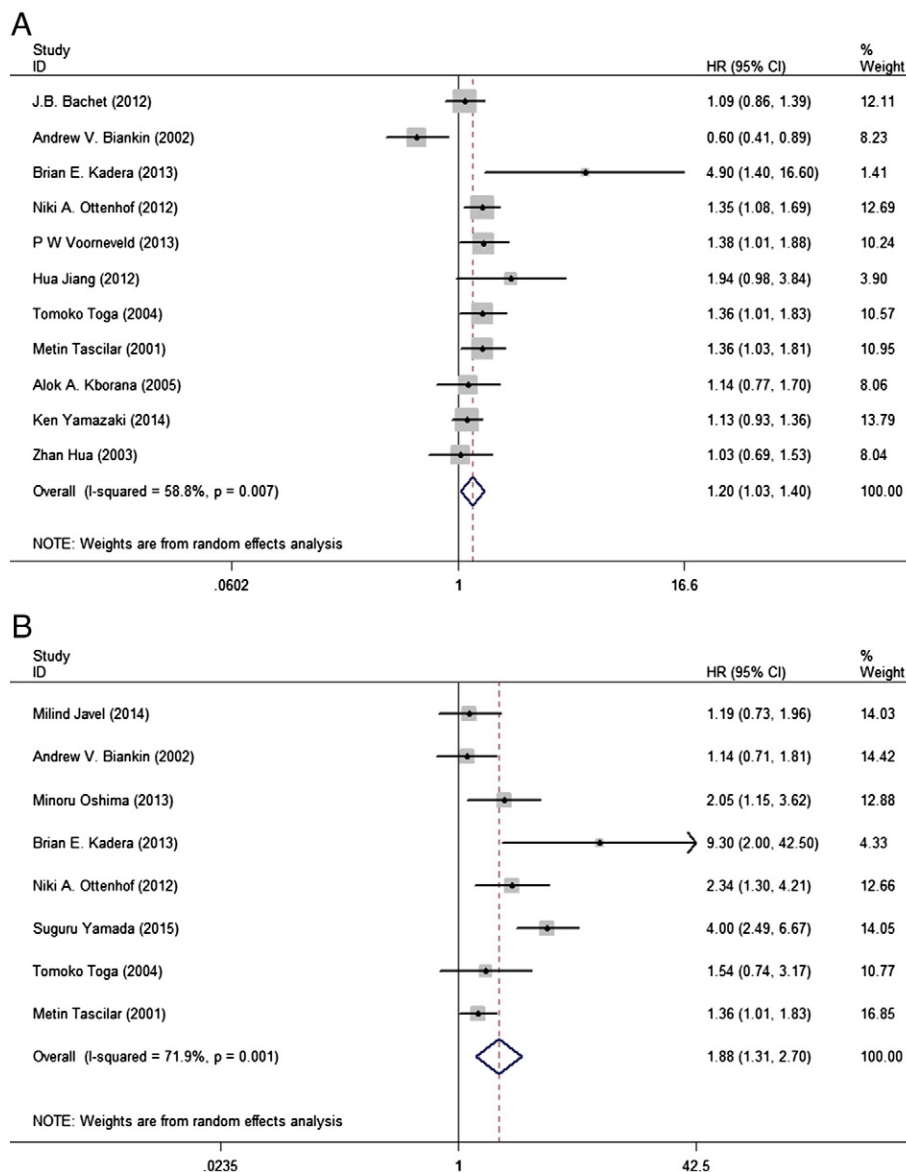
Figure 2. Forest plot showing the association between the loss of SMAD4 expression and OS in pancreatic cancer using univariate and multivariate analyses. (A) Univariate analysis. (B) Multivariate analysis.

influenced the results (Figure 4). The sensitivity analysis indicated that the studies by Ottenhof et al. [13] and Tascilar et al. [11] significantly influenced the pooled HR in univariate analysis. However, the result was stable and not obviously influenced by any single study in multivariate analysis.

Discussion

Pancreatic cancer is associated with the highest mortality rates among malignant conditions worldwide. Its prognosis is poor, although various treatment options have been applied. Currently, surgical resection is still the only curative treatment for pancreatic cancer and the only method to improve survival. In addition, adjuvant systemic treatment also plays a role in the treatment of pancreatic cancer. Despite recent improvements in survival, most patients who undergo surgical resection and adjuvant treatment ultimately die of the disease. This necessitates the identification of more sensitive and specific prognostic indicators to accurately predict a response to treatment. SMAD4, which is a mediator between extracellular growth factors

from the transforming growth factor-β family and genes inside the cell nucleus, has been reported to be related with the prognosis of cervical cancer [24,25], colorectal cancer [26], hepatocellular carcinoma [27], and gastric cancer [28]. Studies have shown that the loss of SMAD4 expression is closely associated with tumor prognosis in pancreatic cancer as well.

In recent years, SMAD4 expression has been shown to be associated with disease progression in pancreatic ductal adenocarcinoma [29,30], where the loss of SMAD4 expression was associated with distant metastasis. However, reports on the association of SMAD4 expression with patient survival are inconsistent. For example, Bachet et al. [17] reported that the loss of SMAD4 expression was not significantly associated with survival in pancreatic cancer patients and could not be viewed as a prognostic marker. Furthermore, Biankin et al. [19] even found that the loss of SMAD4 was associated with improved outcomes in pancreatic cancer patients (HR: 0.60, 95% CI: 0.41-0.89). Therefore, to verify the true relationship between the loss of SMAD4 expression and the prognosis

Table 2. Stratified Analysis of Pooled HRs for Pancreatic Cancer Patients Using Univariate Analysis

Variable	No. of Studies	No. of Patients	HR(95% CI)	Heterogeneity			Model Used
				χ^2	I^2	P Value	
Region							
Asian	4	305	1.19 (1.03-1.38)	3.62	17.1%	.306	Fixed
Non-Asian	7	1096	1.19 (0.94-1.50)	20.63	70.9%	.002	Random
Age (mean)							
>60	5	658	1.14 (0.88-1.49)	14.93	73.2%	.005	Random
≤60	2	104	1.33 (0.72-2.43)	2.46	59.4%	.117	Random
Stage							
>80%	2	523	1.33 (0.77-2.27)	2.45	59.3%	.117	Random
≤80%	7	724	1.18 (0.92-1.52)	20.47	70.7%	.002	Random
Follow-up							
>36	2	485	2.03 (0.47-8.70)	5.49	81.8%	.019	Random
≤36	3	492	0.99 (0.61-1.60)	11.40	82.5%	.003	Random
No. of patients							
>100	5	1058	1.05 (0.85-1.31)	11.64	65.6%	.02	Random
≤100	6	343	1.36 (1.18-1.56)	7.05	29.1%	.217	Fixed
Cutoff							
0	6	959	1.21 (1.08-1.34)	8.50	41.1%	.131	Fixed
>0	5	442	1.17 (0.83-1.64)	15.65	74.4%	.004	Random

of patients with pancreatic cancer, we conducted a meta-analysis, including recent related studies, using a comprehensive search strategy. We found that the loss of SMAD4 expression was a significant predictor for OS in patients with pancreatic cancer. The pooled HRs (95% CIs) were 1.20 (1.03-1.40) for univariate analysis and 1.88 (1.31-2.70) for multivariate analysis. In addition, for the multivariate analysis, the sensitivity analysis indicated that the significant association between the loss of SMAD4 expression and poor OS was not altered, regardless of whether one of these studies was omitted, suggesting the robustness of this result. However, for the univariate analysis, the result was significantly influenced by two studies, Ottenhof et al. and Tascialar et al., and after being excluded, the pooled HRs (95% CIs) were 1.1827 (0.9952-1.4055) and 1.1843 (0.9998-1.4029), respectively. To some degree, potential confounding factors might increase the heterogeneity among studies and influence the results of univariate analysis. All studies included in this meta-analysis were found to be of high quality.

For both univariate and multivariate analyses, the heterogeneity among studies was found to be strong. To decrease this heterogeneity,

Table 3. Stratified Analysis of Pooled HRs for Pancreatic Cancer Patients Using Multivariate Analysis

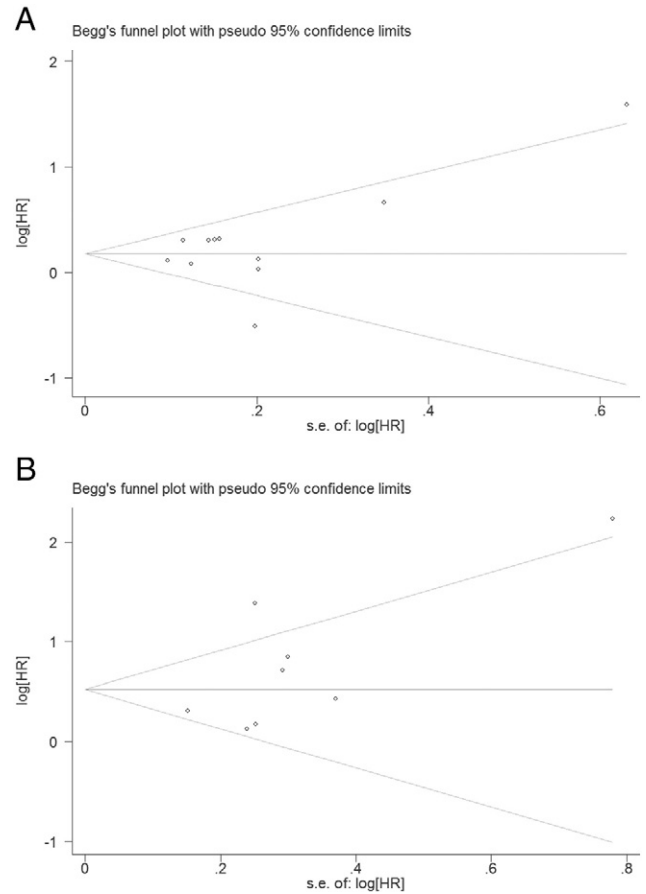| Variable	No. of Studies	No. of Patients	HR (95% CI)	Heterogeneity			Model Used
				χ^2	I^2	P Value	
Region							
Asian	3	368	2.43 (1.37-4.30)	5.65	64.6%	.059	Random
Non-Asian	5	559	1.55 (1.07-2.26)	10.00	60.0%	.04	Random
Age (mean)							
>60	7	895	1.75 (1.24-2.46)	20.07	70.1%	.003	Random
≤60	NA						
Stage							
>80%	2	280	2.91 (1.51-5.61)	3.04	67.1%	.081	Random
≤80%	6	647	1.53 (1.11-2.10)	10.04	50.2%	.074	Random
Follow-up							
>36	1	32	9.3 (2.0-42.5)				
≤36	4	648	1.86 (1.09-3.15)	16.97	82.3%	.001	Random
No. of patients							
>100	4	648	1.86 (1.09-3.15)	16.97	82.3%	.001	Random
≤100	4	279	1.95 (1.09-3.51)	7.96	62.3%	.047	Random
Cutoff							
0	6	720	2.17 (1.37-3.42)	21.61	76.9%	.001	Random
>0	2	207	1.24 (0.84-1.84)	0.46	0%	.498	Fixed

Figure 3. Begg's funnel plot for all studies included in this meta-analysis. (A) Univariate analysis. (B) Multivariate analysis.

much attention should be paid to the baseline characteristics of patients, which might have affected the conclusion of each study that was included in the meta-analysis. These baseline characteristics included patient age, study region, sample size, follow-up duration, tumor clinical stage, and cutoff value for the definition of positive staining. Therefore, further subgroup analysis was performed according to these baseline characteristics. The results of subgroup analysis should be interpreted because of the existence of heterogeneity among studies and the small sample size. In the subgroups where no significant association was found between the SMAD4 expression and the prognosis, strong heterogeneity existed among these studies, and random-effect model was chosen to pool the HRs. Therefore, we also performed multivariate analysis. In the multivariate analysis, the HR was adjusted by potential confounding factors, and the pooled HRs indicated that only in the subgroup where the cutoff value was more than 0 was the loss of SMAD4 expression not significantly associated with the poor OS (HR: 1.24, 95% CI: 0.84-1.84). This may because of the lack of a uniform standard for defining positive SMAD4 expression. The cutoff value greatly varied among studies, and this may have affected the study results.

Several limitations of this meta-analysis must be acknowledged, as we could not completely eliminate potential bias. First, the studies included in our meta-analysis were restricted to those published in English, and the number of studies is small. Therefore, we could not analyze the association between SMAD4 expression and disease-free survival as only one study [12] reported this association (HR: 1.888,
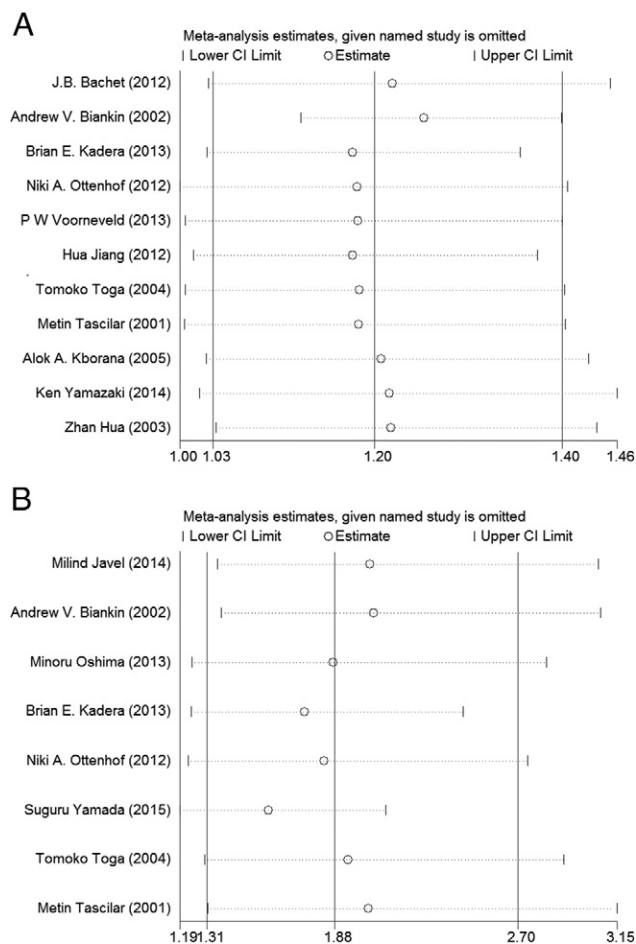
Figure 4. Effect of individual studies on the pooled HR for OS. (A) Univariate analysis. (B) Multivariate analysis.

95% CI: 1.133-3.146). Second, the studies had significant heterogeneity. Different patient selection criteria, treatment protocols, and detection methods for SMAD4 expression are possible explanations for the heterogeneity. For example, the expression of SMAD4 was determined by immunohistochemistry in all the included studies. However, differences in the primary antibody type and concentration could affect the results of immunohisto-chemistry, resulting in heterogeneity among studies. Therefore, a random-effects model was adopted, and subgroup analysis was performed to minimize the effect of this limitation. Third, although we extracted HRs and 95% CIs using the strategies reported by Tierney et al. [16], the data calculated from the Kaplan-Meier curve or log-rank test may not be as precise as obtaining data directly from the original article. In addition, although Begg's funnel plot and Egger's test indicated no publication bias, this could still influence the results and lead to a false-positive association. An asymmetrical funnel plot may indicate publication bias or be due to an inflated estimate in small studies of low quality. Thus, although the funnel plot is often used to assess publication bias, it should be noted that the asymmetry may also be due to other sources of bias [31].

Conclusions

In conclusion, pancreatic cancer patients with loss of SMAD4 expression were found to have shorter OS in multivariate analysis as well as subgroup analysis. Therefore, it can be considered a poor predictor of survival in patients with pancreatic cancer. SMAD4 expression assessment could provide more detailed information for patients with pancreatic cancer and could be used to optimize therapeutic schemes. Further studies, especially large well-matched prospective studies, are needed to clarify the prognostic value of SMAD4 for survival in patients with pancreatic cancer.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tranon.2015.11.007.

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