Original Article

Clinicopathological Significance of Mucin 2 Immunohistochemical Expression in Colorectal Cancer: A Meta-Analysis

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ABSTRACT

Objective: To evaluate the association between mucin 2 (MUC2) expression and clinicopathological characters of colorectal cancer.

Methods: A literature search was performed on December 31, 2010 according to defined selection criteria. We evaluated the correlation between MUC2 (detected by immunohistochemistry) and clinicopathological characters of colorectal cancer. According to the tumor histological type, differentiation, location and TNM staging of colorectal carcinoma, we divided the clinicopathological characteristics into different subgroups. Fixed and random effects models were applied for estimation of the summarized risk ratios (RRs) and 95% confidence intervals (CIs) in different subgroups. Finally, forest plots and funnel plots were created to allow for visual comparison of the results or the effect of publication bias.

Results: According with the inclusive criteria, fourteen studies (*n*=1,558) were eligible for the meta-analysis. We observed a trend towards a correlation of MUC2 higher positivity in mucinous than non-mucinous carcinoma (RR, 2.10; 95% CI, 1.30–3.40; *P*=0.002) and less positivity in distal than proximal colon (RR, 0.74; 95% CI, 0.64–0.85; *P*=0.000). There was no statistically significance for the association between MUC2 expression and differentiation or TNM staging of colorectal cancer, but MUC2 overexpression tended to be associated with the presence of T stage tumor (RR, 1.17; *P*=0.052).

Conclusion: MUC2 overexpression was associated with the mucinous and proximal colorectal cancer.

Key words: Colorectal cancer; MUC2; Immunohistochemistry; Meta-analysis

INTRODUCTION

Mucins, whose protein backbones are encoded by *MUC* genes, are the major secreted glycoproteins of the gastrointestinal tract and play a role in normal physiological processes and in the neoplastic progression of colorectal cancer[1]. Mucin 2 (MUC2), which represents the prominent gel-forming mucin in the colon, has been found to undergo significant changes in malignant transformation of colorectal tumor. MUC2–/– mice displayed aberrant intestinal crypt morphology and altered cell maturation and migration. Most notably, the mice frequently developed adenomas in the small intestine that progressed to invasive adenocarcinoma, as well as rectal tumor[2]. In a number of immunohistochemical studies of MUC2 expression in colorectal cancer, it has been found that mucinous carcinomas are positive for MUC2 expression, in contrast to MUC2 down-regulation in non-mucinous adenocarcinomas. It is still not clear if patients with mucinous carcinomas have poorer prognoses than those with non-mucinous adenocarcinomas, or if the excessive mucin production worsens the prognosis, and if so, by which mechanisms[3]. Moreover, the expression profiles of mucins might determine subtle differences in tumor phenotypes. These data suggest that MUC expression profiling can be used diagnostically to distinguish individual histologic subclassifications and may guide

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the selection of target therapeutics. Elucidation of their pathophysiological mechanisms might lead to new concepts for the diagnosis and treatment of these alterations. Thus, the identification of these molecular changes that hold prognostic significance is vital.

Immunohistochemistry is a widely accepted and well documented method for characterizing patterns of protein expression while preserving tissue and cellular architecture[4]. Several studies have evaluated the relations between MUC2 protein immunohistochemical expression and clinicopathological characters in patients with colorectal carcinoma. However, the results of various studies are conflicting or inconclusive. It is unknown whether differences in these investigations have been mostly due to their limited sample size or genuine heterogeneity. Thus, we conducted a meta-analysis of all available studies relating MUC2 expression with the clinicopathological characters in colorectal cancer patients.

MATERIALS AND METHODS

Identification and Eligibility of Relevant Studies

A literature search was performed on December 31, 2010 utilizing the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed), ISI Web of SCIENCE (http://apps.isiknowledge.com/) for articles in English, and China National Knowledge Infrastructure (http://dlib.cnki.net/kns50/) for articles in Chinese. The search strategy was based on combinations of "MUC2", "colorectal cancer", "immunohistochemistry", "colon cancer" and "rectal cancer". References of retrieved articles also were screened for relevance. We accepted for the meta-analysis studies measuring MUC2 by immunohistochemistry in patients with colorectal cancer, provided that measurements had been done in the primary tumor. Whenever reports pertained to overlapping patients, we retained only the largest study to avoid duplication of information. We used prespecified rules to standardize as much as possible the definition of MUC2 positivity. We defined MUC2 positivity as positive cell stain in at least 25% of the tumor cells in continuous scales or at least moderate staining in qualitative scales. The above cutoff was used by the majority of the enrolled studies. When data with this cutoff were not possible to retrieve, we accepted the cutoff that was closest to this 25% cutoff level.

Data Extraction

Two investigators extracted data from eligible studies independently and reached consensus to all items. We extracted data on major clinicopathological characteristics of patients, measurements, and results. In particular, in each report we recorded the first author, year of publication, country of origin, antibodies used for immunohistochemistry, number of patients analyzed, tumor histological type, differentiation, location, and TNM staging of colorectal carcinoma. We divided the clinicopathological characteristics into different subgroup: mucinous vs. non-mucinous carcinoma; poorly vs. well & moderately differentiated carcinoma; distal (descending colon & sigmoid colon & rectum) vs. proximal (cecum & ascending colon & transverse colon) carcinoma; T3 & T4 vs. T1 & T2; N1 & N2 & N3 vs. N0; M1 vs. M0. In every subgroup, non-mucinous, well & moderately differentiated, proximal, T1 & T2, N0 or M0 carcinoma was used as a control.

Statistical Analyses

Data on the predictive ability of MUC2 positivity for different clinicopathological characteristics were combined across studies using fixed and random effects models for the synthesis of risk ratios (RRs). By convention, an observed RR>1 implied a higher MUC2 positivity than control in each subgroup. The homogeneity of separate study in each subgroup was assessed using *I*-squared statistic and the Chi-square test. With a $P>0.05$, the included studies were considered homogeneous and the fixed effect model should be selected, otherwise, the random effect model should be used. The overall pooled RR estimates, along with 95% confidence intervals (95% CIs), were calculated for each outcome. Forest plots were created to allow a visual comparison of the results and an estimation of the heterogeneity. The effect of publication bias was assessed graphically using funnel plots and evaluated with the Begg's test and Egger's regression asymmetry test. All statistical analyses were performed using Stata for Windows statistical software version 8.0 (Stata Corp LP, College Station, Texas, USA). $P<0.05$ was considered statistically significant.

RESULTS

Eligible Studies

The initial search algorithm retrieved a total of 225 references (including 12 articles in Chinese) and we evaluated these reports in full text or abstract. Overall, we identified 33 reports associating with MUC2 expression in colorectal carcinoma patients. Of the published studies, 19 reports were excluded: 2 studies were written in Russian[5, 6]; 1 was overlapped with another study because of duplicate reports from the same study population[7]; 5 studies were written by the same first author and also overlapped with their another study; and 11 studies evaluated MUC2 positive expression with other methods or did not accord with the inclusive criteria. Finally, fourteen studies (n=1,558) were eligible for the meta-analysis. Characteristics of the 14 eligible studies are listed in Table 1. Nine reports

originated from Asia, 3 from Europe, 1 from Australia and 1 from the United States. The most commonly used MUC2 antibody was Ccp58 (Novocastra, UK).

Data Synthesis: MUC2 Association with Tumor Type, Differentiation and Location

There were 9 studies eligible for the analysis of the relation between MUC2 positivity and tumor types (mucinous vs. non-mucinous carcinoma, *n*=1,142), 6 studies eligible for the analysis of the relation between MUC2 positivity and differentiation (poorly vs. well & moderately differentiated, *n*=518) and 6 studies eligible for the analysis of the relation between MUC2 positivity and tumor location (distal vs. proximal, *n*= 610).

We observed a trend towards a correlation of higher MUC2 positivity in mucinous than non-mucinous carcinoma (RR, 2.10; 95% CI, 1.30−3.40; *P*=0.002) and less positivity in distal than proximal colon (RR, 0.74; 95% CI, 0.64−0.85; *P*=0.000). Figure 1A and B illustrated forest plots of studies, including the common RRs and the heterogeneity test for both subgroups. The breadth and length of the CIs showed the value of the published studies and the calculated common RRs. Publication bias was evident in mucinous carcinoma vs. non-mucinous subgroup (Egger's test, *P*=0.006), but not evident in distal vs. proximal subgroup (Egger's test, *P*=0.895). This findings was supported by the Begg's funnel plots based on studies, which is displayed in Figure 1C and D. Poorly histologic differentiation tumors were not more likely to show higher MUC2 positivity than well and moderate differentiation (RR, 0.93; 95% CI, 0.77−1.14; *P*=0.496).

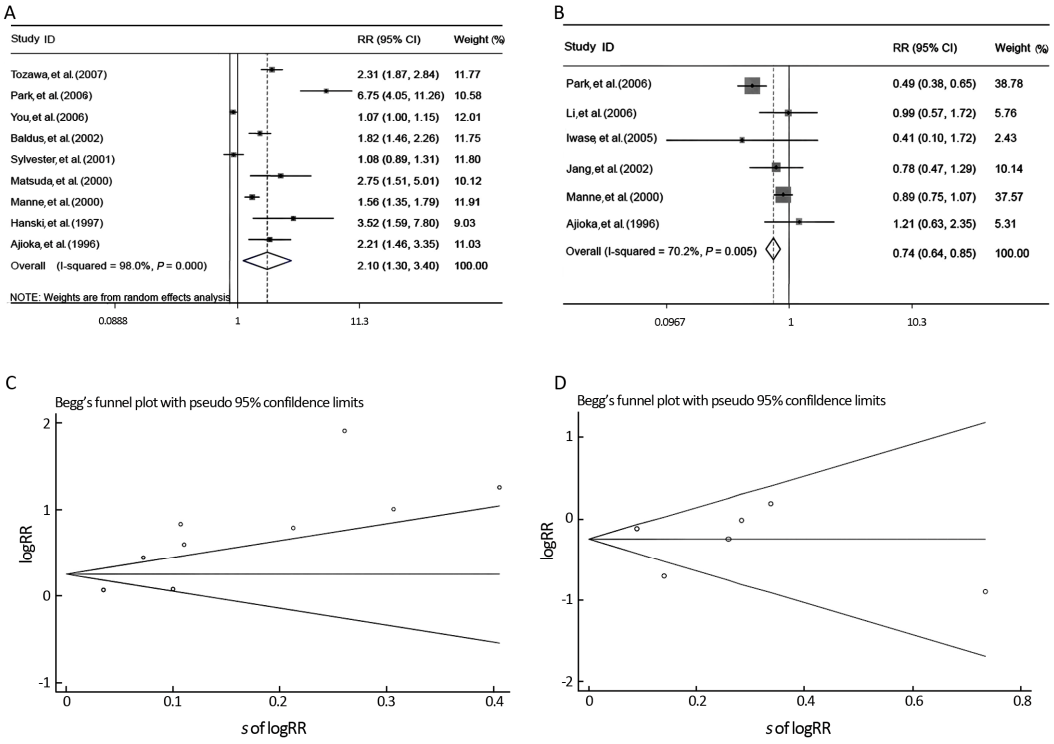

Figure 1. Study-specific RRs with 95% CIs of MUC2 positivity and funnel plots. **A, C:** Mucinous vs. non-mucinous carcinoma; **B, D:** Distal vs. proximal subgroup. *s* indicates standard error.

Data Synthesis: MUC2 Association with TNM Stage of Colorectal Carcinoma

Due to a lack of original data, the literature research identified several publications that examined the relation between MUC2 expression and TNM staging, including 4 publications for T stage, 7 for N stage and only 2 for M stage. For the current meta-analysis, the positive rate of MUC2 was compared in T3 & T4 vs. T1 & T2 patients, N1 & N2 & N3 vs. N0 patients, M1 vs. M0 patients. As shown in Figure 2A, MUC2 overexpression tended to be associated with the presence of T3 & T4 stage, but the effect was modest and not formally statistically significant (RR, 1.17; *P*=0.052). Publication bias was not evident based on the Begg's test and Egger's regression analysis (*P*=0.374, Figure 2B). In other subgroups about N or M stage of colorectal cancer, there was no statistical significance in MUC2 positivity (N1 & N2 & N3 vs. N0, RR, 1.04; 95% CI, 0.77−1.40; *P*=0.798 and M1 vs. M0, RR, 0.97; 95% CI, 0.79−1.18; *P*=0.252). Other forest plots and funnel plots of meta-analyses were not shown.

Table 1. Characteristics of the eligible studies

Author (year-country)	*N*	No. of MUC2 positive cases												Antibody (dilution)
		NMC	MC	W & M	P	C & A & A	D & A & T	T1–2	T3–4	N0	N1–3	M0	M1	
Tozawa, et al.[8] (2007-Japan)	152	52/122	30/30	–	–	–	–	–	–	–	–	–	–	Ccp58, Novocastra (–)
Park, et al.[9] (2006-Korea)	194	13/98	86/96	–	–	56/76	43/118	–	–	–	–	–	–	Ccp58, Novocastra (1:100)
You, et al.[10] (2006-China)	167	127/138	29/29	–	–	–	–	–	–	–	–	–	–	Ccp58, Novocastra (1:100)
Yang, et al.[11] (2006-China)	90	–	–	–	–	–	–	–	–	9/40	23/50	–	–	Ccp58, Neomarker (1:100)
Li, et al.[12] (2006-China)	64	38/64	–	25/43	13/21	6/10	32/54	–	–	29/37	9/27	–	–	Ccp58, Santa cruz (1:100)
Iwase, et al.[13] (2005-Japan)	38	6/38	–	6/38	–	3/11	3/27	–	–	–	–	–	–	Ccp58Novocastra (1:100)
Baldus, et al.[14] (2002-Germany)	243	105/221	19/22	–	–	–	–	56/124	68/119	56/126	68/117	11/18	113/225	Ccp58, Novocastra (1:200)
Jang, et al.[15] (2002-Korea)	97	–	–	–	–	12/25	27/72	12/33	27/64	20/53	19/44	–	–	Ccp58, Novocastra (1:100)
Huang, et al.[16] (2002-China)	126	–	–	25/73	21/53	–	–			26/56	20/70	–	–	Ccp58, Santa cruz (1:50)
Sylvester, et al.[17] (2001-UK)	37	25/28	9/9	16/17	9/11	–	–	–	–	–	–	–	–	LUM2-3, – (1:3,000)
Matsuda, et al.[18] (2000-Japan)	86	25/83	2/2	25/82	0/1	–	–	8/33	20/53	–	–	–	–	Ccp58, Novocastra (1:100)
Manne, et al.[19] (2000-USA)	166	72/113	53/53	109/142	16/24	66/83	59/83	32/42	92/123	64/86	59/77	106/142	19/24	Ccp58, Novocastra (1:200)
Hanski, et al.[20] (1997-Germany)	47	5/22	20/25	–	–	–	–	–	–	–	16/27	–	–	4F1, – (1:300)
Ajioka, et al.[21] (1996-Australia)	51	19/46	5/5	21/40	3/11	7/17	17/34	–	–	8/24		–	–	Ccp58, – (1:1,000)

NMC: non-mucinous carcinoma; MC: mucinous carcinoma; W & M: well and moderately differentiated; P: poorly differentiated; C & A & T: cecum, ascending colon and transverse colon; D & S & R: descending colon, sigmoid colon and rectum; –: no data were shown in reference.

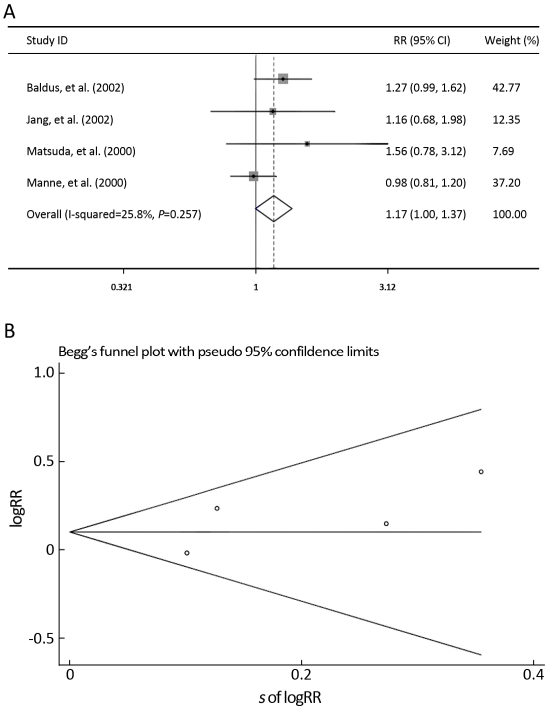

Figure 2. Study-specific RRs with 95% CIs of MUC2 positivity **(A)** and funnel plots **(B)** in T3 & T4 vs. T1 & T2 subgroup. *s* indicates standard error.

DISCUSSION

The objective of the current meta-analysis was to investigate whether there is a correlation between MUC2 positivity and clinicopathological characters in patients with colorectal cancer by evaluating the MUC2 expression profile using immunohistochemistry in the primary tumor. Due to lack of standard evaluation systems for declaring a case as positive or negative for MUC2 expression, we used prespecified rules to standardize as much as possible the definition of MUC2 positivity. According our prespecified rules as a cutoff level, only 14 published investigations were included in the current meta-analysis and other articles were excluded because they did not accord with the inclusive criteria or lacked the original data. Despite the fact that we tried to optimize standardization, some remaining variability in definitions was unavoidable. Although the final estimates of the eligible studies using the standardized cutoff did some positive results in the present meta-analysis, conclusions need to be drawn cautiously. One problem is related to the selection bias of positive results, which may be facilitated by the statistical method of the meta-analysis. Positive correlations may be represented excessively due to the following facts: there is a tendency to publish only positive results; conversely, correlations that could not be proven remain unpublished. If the published results are now bundled statistically, and if new objectives are deduced from these data, then an overly positive bias can be assumed. For example, MUC2 overexpression tended to be associated with the presence of T3 & T4 stage and no publication bias was observed in this subgroup, but only 4 eligible publications (n=591) were analyzed in our present study and the statistical P value was critical (P=0.052). Further investigations based on larger clinical samples are needed to confirm this finding.

All studies that were included in the current meta-analysis investigated MUC2 expression in colorectal tumors with different clinicopathological status. However, some studies showed a total lack of information. Because the validity of eligible studies largely depends on clinicopathological confirmation, it appears difficult to draw clear conclusions from these studies. This is true for the patient who had higher MUC2 positivity in the mucinous carcinoma and proximal colon location of the primary tumor, which also was reported in some of the studies that were included in the current meta-analysis. Further, we found trends for modest correlations of MUC2 positivity with higher T stage tumors. In the current meta-analysis, the estimates that we obtained were not adjusted for other variables such as sex, age, tumor size and prognosis. Prognostic biomarkers may be useful for identifying high-risk patients, leading to an improvement in their clinical or therapeutic management. There have not been sufficient studies to assess the association of MUC2 with prognosis in colorectal carcinomas. Previous studies had shown that MUC2 was not significantly associated with prognosis[14, 19], however, Perez, et al.[22] reported that MUC2 overexpression was correlated with worse overall survival. Contrarily, some researchers reported loss of MUC2 expression was a poor prognostic factor in mismatch repair proficient colorectal carcinomas and stage II and III colorectal carcinomas[23, 24]. Thus, it is difficult to determine whether, in fact, MUC2 plays a key role in the very complex cascade of the colorectal carcinogenesis.

In conclusion, our meta-analysis represented a quantified synthesis of all eligible studies and found a statistically significant relationship between higher MUC2 positivity and colorectal mucinous carcinoma or proximal location. Interestingly, MUC2 overexpression tended to be associated with the T3 & T4 patients, but the relationship between MUC2 expression and invasion of colorectal cancer needs further investigation. The following suggestions should be made to future authors: include a large series of patients, stratify by tumor stage, fully describe the clinical characteristics of the study population, present the results both as comparison of survival curves and as multivariate regression analysis and provide a full description of survival events to allow calculations.

Surely, the standardization of immunohistochemical staining procedures and evaluation protocols will be required to achieve comparable results for further evaluation on MUC2 expression significance in colorectal carcinoma progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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