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ORIGINAL ARTICLE

Clinicopathological Significance of RhoA Expression in Digestive Tract Cancer: A Systematic Review and Meta-Analysis

Ping Wang [1], Wanyu Li [1], Jifeng Peng [1], Shengnan Qi [1], Lingxie Song [1], Chunxia Liu [1, 2], Feng Li [1, 2]

[1] *Department of Pathology, Shihezi University School of Medicine and The Key Laboratories for Xinjiang Endemic and Ethnic Diseases, Chinese Ministry of Education, Shihezi, Xinjiang 832002, China*
[2] *Department of Pathology, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, Xinjiang 832002, China*

SUMMARY

Background: **RhoA protein expression has been reported in different types of cancer. We performed an up-to-date meta-analysis to evaluate the clinicopathological characteristics of RhoA protein expression in patients with gastrointestinal cancer.**
Methods: **We searched in several databases, including MEDLINE (PubMed) and China National Knowledge Infrastructure, to identify studies examining the association between RhoA protein and cancer. The quality of the included studies was assessed. Cochrane Collaboration's Software Review Manager 5.3 was utilized to test the heterogeneity, overall effect, and publication bias of the combined studies. The reported odds ratio and 95% confidence interval (CI) were calculated by using fixed and random effects models depending on the heterogeneity of the included studies.**
Results: **A total of 15 studies met the inclusion criteria of the meta-analysis. RhoA expression was significantly higher in gastrointestinal cancer than in normal tissues. RhoA protein expression in digestive system neoplasms was significantly associated with tumor clinical staging, metastatic status and differentiated degree. However, no association with gender was found. RhoA mRNA expression was no associated with clinicopathological significance.**
Conclusions: **Current evidence supports the conclusion that RhoA expression is associated with clinical staging, metastatic status, and differentiated degree in digestive system neoplasms. RhoA expression may play an important role in the carcinogenesis and metastasis of gastrointestinal cancer.**
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Correspondence:
Chunxia Liu, MD, PhD
Department of Pathology
Shihezi University School of Medicine
2nd North Road
Shihezi, 832002, Xinjiang
China
Phone/Fax: +86-993-205-7136
Email: liuliu2239@sina.com

Feng Li, MD, PhD
Department of Pathology
Shihezi University School of Medicine
2nd North Road
Shihezi, 832002, Xinjiang
China
Phone/Fax: +86-993-205-7136
Email: lifeng7855@126.com

KEY WORDS

RhoA protein, clinicopathological significance, meta-analysis, digestive tract cancer

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INTRODUCTION

The global incidence rate of cancers has increased in recent years. The mortality rate of malignant tumors, especially gastrointestinal tumors, including cancers of the stomach and intestine, are higher than other tumors worldwide. Morbidities and mortalities from gastric cancer, esophageal cancer, and colorectal cancer are the highest among malignant tumors in China. Thus, digestive tract malignant tumors have to be investigated [1]. The Rho small GTP binding protein (Rho) family, consisting of Rho, Rac, and Cdc42 subfamilies, regulates cell migration through the reorganization of actin cytoskeleton. Through its combination of guanosine triphosphate (GTP), this protein is widely recognized to play a significant role in cancer progression. Further research has found that Rho GTPases previously played prominent roles in cancer development and progression, including processes of cell morphology, proliferation, invasion, metastasis, and apoptosis in a variety of biological processes [2-5]. The Rho subfamily, consisting of RhoA, RhoB, and RhoC isoforms, regulates the formation of stress fibers and focal adhesions in cells [6,7]. Reports have revealed that RhoA is up-regulated in gastric, testicular, pancreatic, ovarian, and breast cancers [8-12]. The Rho-mediated process is involved in the migration and invasion of cancer; as observed, the activation of RhoA increases the invasiveness of cultured rat hepatoma cells [13,14]. However, several available clinical studies are not unified when it comes to the significance of the expression of Rho GTPases in human cancer specimens. We investigated whether or not RhoA is associated with the progression and metastasis in gastrointestinal cancer patients. Hence, we performed a meta-analysis to reveal the clinicopathological significance of RhoA protein expression in patients. To improve the accuracy and reliability of the investigation, we enlarged the sample size by data-pooling. Studies were summarized in this meta-analysis to systematically qualify the RhoA and tumor clinicopathological characteristics in gastrointestinal cancer.

MATERIALS AND METHODS

Literature search strategy and selection criteria

Two investigators performed the search independently. Comprehensive literature searches were performed in electronic databases, including PubMed, EMBASE, and CNKI, until 1 June 2015. Without the limitation of language or source, various search terms and combinations were used, including (1) "RhoA," (2) "cancer or neoplasm or carcinoma," and (3) "clinicopathological characteristics". We also performed a manual search for articles in the reference. In our meta-analysis, the included studies should meet a number of criteria, as follows: (1) all patients had complete clinicopathological data; (2) RhoA protein was detected in all patients; and (3) article must have an English abstract. For inclusion into the analysis, the minimum number of patients was limited to more than 10 in every study. If the author reported results that were obtained from the same patient population in several studies, we will use the most recently published study or the one with the larger sample size. Studies were excluded based on the following criteria: (1) reviews, case-only studies, or familial studies; (2) lacking sufficient data for calculation of incidence; and (3) previous publications were duplicated or samples were replicated. Abstracts and unpublished studies were excluded.

Data extraction

Information was carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria listed above. When a study can be included in the meta-analysis, a consensus must be reached by both investigators. Data extracted for each study included name of the first author, year of publication, sample source, number of cases, and clinicopathological parameters.

Quality assessment

Study quality was assessed independently by the two reviewers using various factors, including (1) clear definition of the study population and the type of carcinoma; (2) researchers revealed the relationship between RhoA expression and cancer clinicopathological parameters; (3) sample size is larger than 10; and (4) studies provided sufficient information to estimate OR and 95% confidence interval (CI). Studies lacking any of these factors were excluded from the final analysis.

Statistical analysis

Statistical analysis was performed using Review Manager 5.3 (provided by The Cochrane Collaboration, Oxford, England). Heterogeneity among the studies was tested using I^2 statistic. If the $I^2 < 50\%$ and $p > 0.05$, the fixed effect model was used, otherwise, the random effect model was used. $P < 0.05$ was considered statistically significant. We then evaluated the potential publication bias with funnel plots.

RESULTS

Selection and characteristics of studies

Fifteen studies were included in the final meta-analysis, as shown in Figure 1. The basic characteristics of these patients are summarized in Table 1 [15-26] and Table 2 [27-29]. Positive RhoA expression was defined by immunohistochemistry (IHC), Western Blot (WB) or quantitative real-time polymerase chain reaction (qRT-PCR). All studies provided data on the correlations between RhoA and cancer clinicopathological characteristics in gastrointestinal cancer patients.

Table 1. Basic information and quality evaluation of the included literature at protein level.

References	Country	Cases of number (M/F)	Cancer	Methods	Antibody Source (dilution)	Clinicopathological factors
Tang (2013)	China	189 (145/44)	GC	IHC (NR)	Abcam (1:150)	Gender, Differentiation, Stage, LNM
Jiang (2011)	China	107 (62/45)	GC	IHC (SP)	Bioworld (1:50)	Gender, Differentiation, Stage, LNM
Gu (2012)	China	179 (137/42)	GC	IHC (Modified two step)	Santa (1:50)	Gender, Differentiation, Stage, LNM
Wang (2014)	China	46 (NR)	GC	IHC (SABC)	Bioss (1:100)	Differentiation, LNM
Huang (2015)	Chinese Taiwan	206 (150/56)	GC	IHC (NR)	Santa (1:600)	Gender, Differentiation, Stage, LNM
Yang (2010)	China	92 (54/38)	CC	IHC (SP)	Santa (NR)	Differentiation, Stage, LNM
Yoji (2008)	Japan	27 (19/8)	CC	WB	Santa	Gender, Differentiation, LNM
Faried (2005)	Japan	122 (105/17)	ESCC	IHC (SABC)	Santa (1:100)	Gender, Differentiation, Stage
Zhang (2012)	China	140 (95/45)	ESCC	IHC (SABC)	Santa (NR)	Gender, Differentiation, Stage, LNM
Zhang (2014)	China	160 (104/56)	ESCC	IHC (SABC)	NR	Gender, Differentiation, Stage, LNM
Zhang (2013)	China	95 (65/30)	ESCC	IHC (SABC)	Santa (NR)	Gender, Differentiation, Stage, LNM
Koji (2006)	Japan	26 (13/13)	HCC	IHC	Santa (1:400)	Gender, Stage

GC - gastric cancer, CC - colorectal cancer, ESCC - esophageal squamous cell carcinoma, HCC - hepatocellular carcinoma, LNM - lymph node metastasis, IHC - immunohistochemistry, NR - not reported.

Table 2. Basic information and quality evaluation of the included literature at mRNA level.

References	Country	Cases of number (M/F)	Cancer	Methods	Clinicopathological factors
Faried (2007)	Japan	50 (45/5)	ESCC	qRT-PCR	Gender, Differentiation, Stage, LNM
Hu (2013)	China	80 (56/24)	HCC	qRT-PCR	Gender, Differentiation, Stage
Wang (2010)	China	42 (24/18)	CC	qRT-PCR	Gender, Differentiation, LNM

ESCC - esophageal squamous cell carcinoma, HCC - hepatocellular carcinoma, CC - colorectal cancer, LNM - lymph node metastasis, qRT-PCR - quantitative real-time polymerase chain reaction.

RhoA expression and clinicopathological parameters in gastrointestinal and gastric cancer

In our present study, a total of 12 articles were included to evaluate the RhoA expression in gastrointestinal cancer tissue. In Figure 2A, RhoA expression is significantly higher in gastrointestinal cancer (III and IV) than in early stage cancer (I and II) (OR = 0.30, 95% CI 0.22 - 0.41; $p < 0.00001$). In Figure 2B, a significant difference exists between the presence and the absence of lymph node (OR = 3.45, 95% CI 2.16 - 5.51; $p < 0.00001$). In Figure 2C, the RhoA expression of the low differentiation was significantly higher than that of

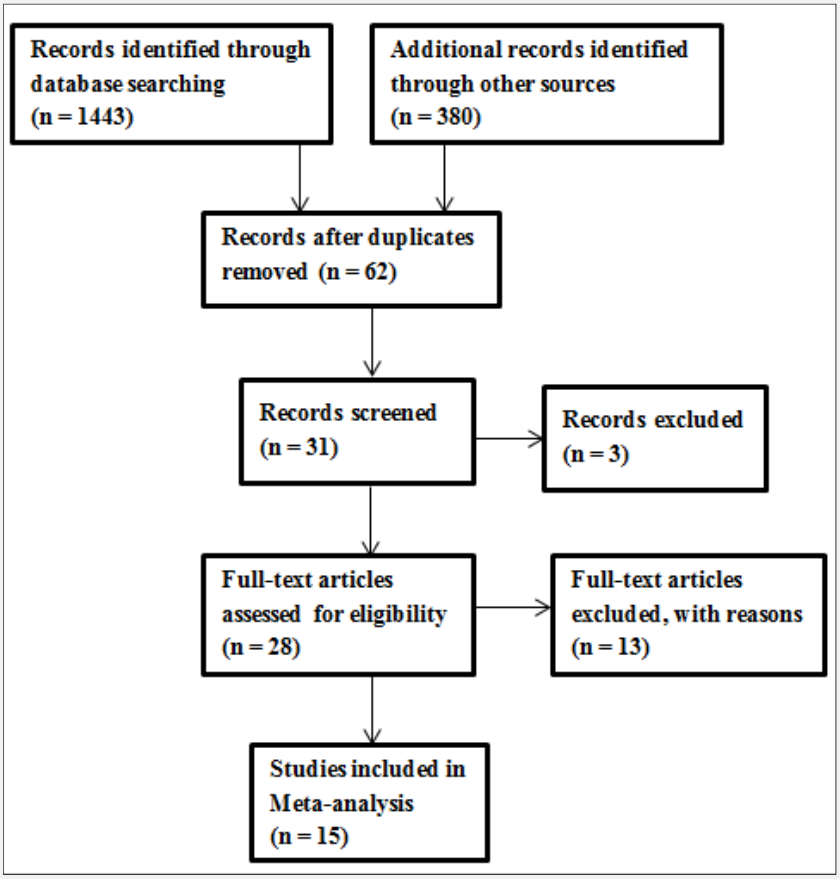


Figure 1. Flow chart of study selection.

high and moderate differentiation (OR = 1.94, 95% CI 1.17 - 3.20; p = 0.01).

We analyzed gastric cancer separately. Figure 3A reveals that RhoA expression is significantly higher in gastric cancer than in normal tissues (OR = 5.76, 95% CI 2.81 - 11.84; p < 0.00001). Additionally, gastric cancer tissue further strengthens the evidence comparing RhoA with stage and lymph node metastasis (in Figure 3B and 3C). It must be noted in Figure 3D (OR = 2.08, 95% CI 0.81 - 5.33; p = 0.13), however, that RhoA expression is no significant between low differentiation and high and moderate differentiation in gastric cancer patients. No significant difference was reported for patients between male and female (not provided).

Relationship between RhoA expression and clinicopathological parameters on protein-based level

In our present study, Figure 4 reveals that relevant studies in colorectal cancer (CC), esophageal squamous cell carcinoma (ESCC), and hepatocellular carcinoma (HCC) were analyzed. A total of 7 articles were included to evaluate the RhoA expression in cancer tissue. In Figure 4A, RhoA expression is significantly higher in gastric cancer (III and IV) than in early stage cancer (I and II) (OR = 0.28, 95% CI 0.18 - 0.43; p < 0.00001). In Figure 4B, a significant difference exists between the presence and the absence of lymph node metastasis (OR = 4.45, 95% CI 2.93 - 6.75; p < 0.00001). In figure 4C, the RhoA expression of the low differentiation was significantly higher than that of high and moderate differentiation (OR = 2.49, 95% CI 1.64 - 3.78; p < 0.00001). No significant difference was reported for patients between male and female (not provided).

Relationship between RhoA expression and clinicopathological parameters on mRNA-based level

Supplementary Figure 1 shows the association between RhoA expression and clinicopathological features on mRNA-based level of CC and HCC. Three studies were included in the study. No significant difference was found in the clinicopathological parameters.

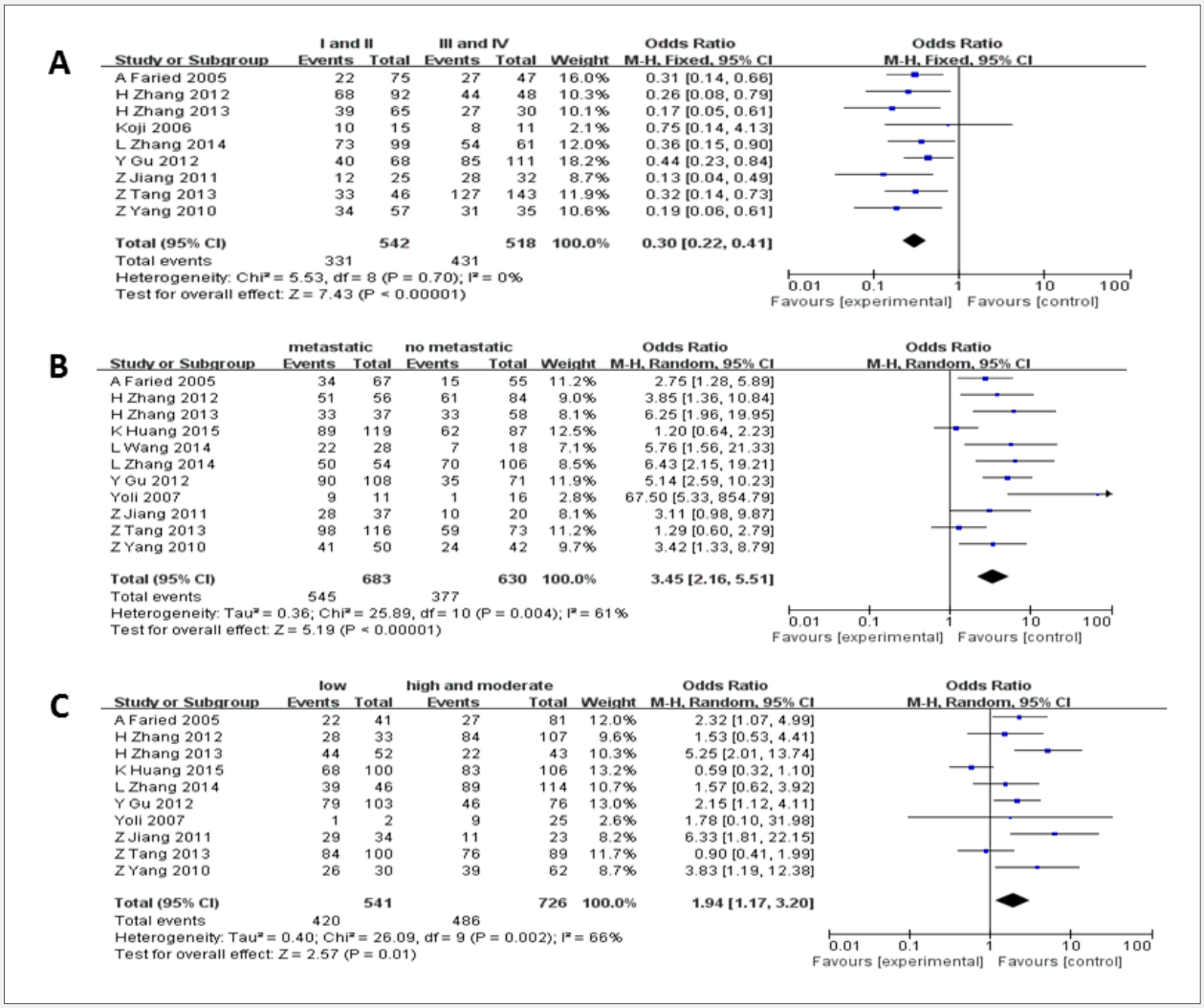


Figure 2. (A) The pooled OR from nine studies about clinical stages. (B) The pooled OR from eleven studies about lymph node metastatic status. (C) The pooled OR from ten studies including high, moderate, and low differentiation.

Sensitivity analysis and Publication bias

Our results were analyzed and removed one by one to illuminate the sensitivity. For lymph node metastasis and clinical stage, the pooled OR were not significantly altered in sensitivity analyses, showing our results were reliable. As shown in Figure 2D, we found that the result of Huang et al. [26] was different from the others, there was no significant difference of clinicopathological characteristics. However, we excluded Huang et al. [26] from the analyses and found that RhoA expression was related between low differentiation and high and moderate differentiation in gastric cancer patients. The differences may be caused by different types of tumor and different groups of people.

The funnel plots were used to assess publication bias (Figures 5), suggesting that the evidence of publication biases was not obvious in the meta-analysis of RhoA expression and clinicopathological features. However, the funnel plots were used to assess publication bias (Supplementary Figures 2), suggesting that some publication biases exist in the meta-analysis. The problems were largely due to the low numbers of the included literature, which was less than 10.

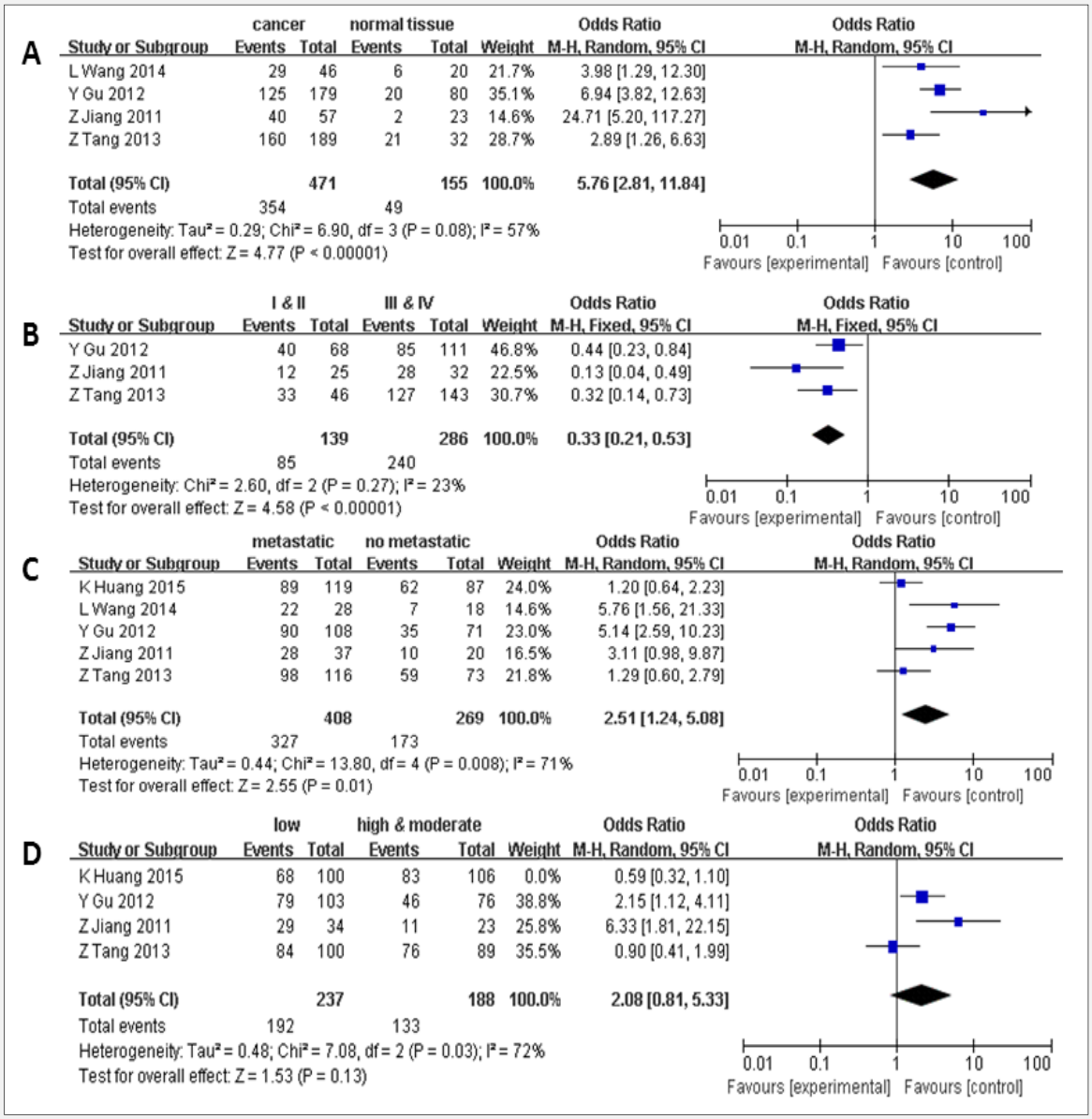


Figure 3. (A) The pooled OR from four studies including gastric cancer and normal tissue samples. (B) The pooled OR from three studies about clinical stages. (C) The pooled OR from five studies about lymph node metastatic status. (D) The pooled OR from four studies including high, moderate, and low differentiation.

DISCUSSION

The Rho family is involved in nearly every process in the occurrence and development of tumors, including the regulation of cytoskeleton, cell cycle, changes in cell adhesion and movement, and cell transformation in tumor cells, whereas RhoA protein may be an important factor and molecular basis in tumor cell invasion and metastasis[30,31]. RhoA protein may be involved in the proliferation and survival of the tumor. For example, constitutively active RhoA protein can stimulate metastasis *in vitro*. In normal epithelial cells, RhoA protein contributes to the generation of epithelial polarity and assembly connection and executive ability. In the progression of tumors, RhoA can also affect epithelial cell damage [32]. The activity of Rho protein can inhibit downstream cadherin, thereby leading to a more motile phenotype [33]. Rho GTPases can also regulate the gen-

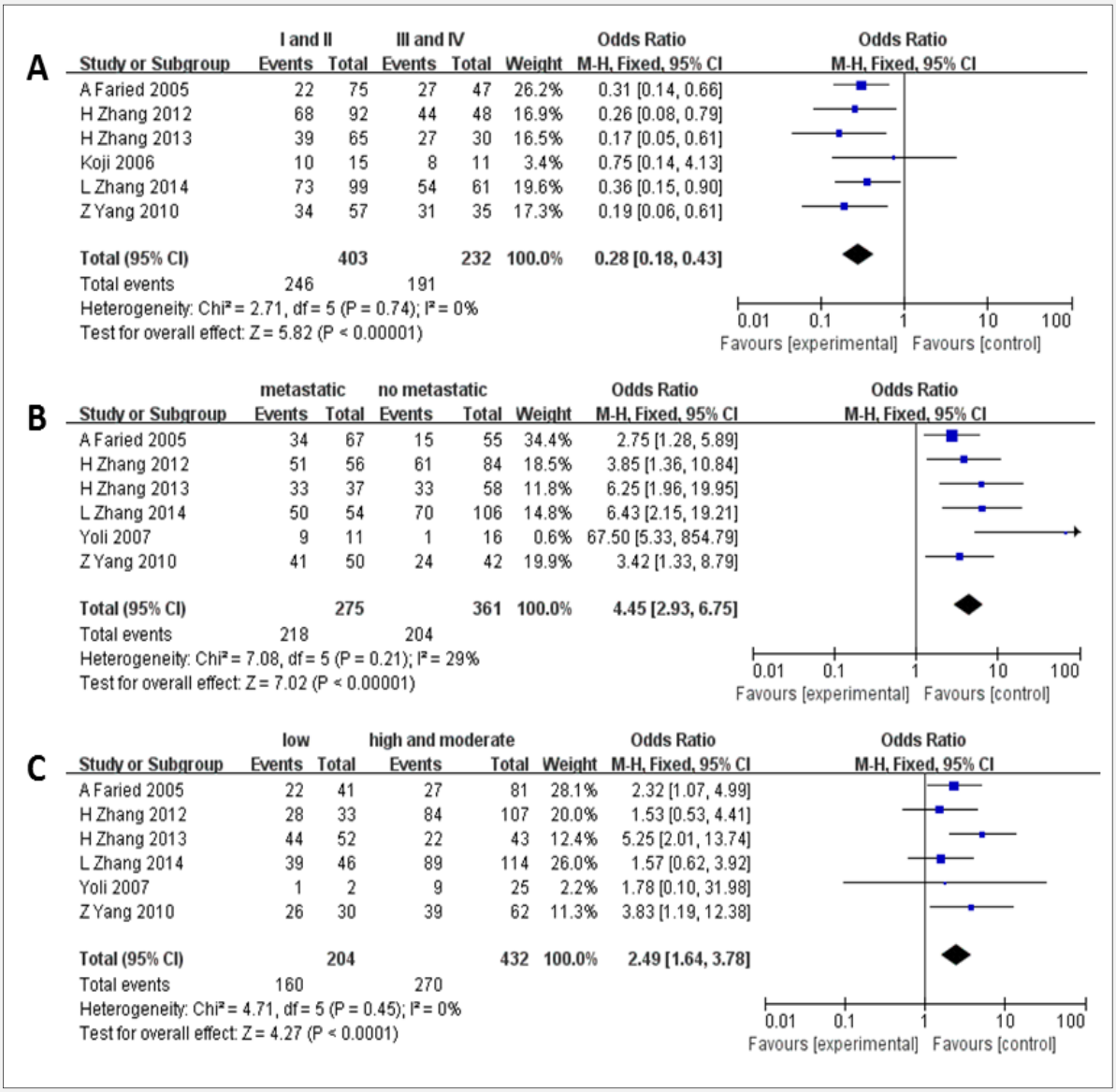
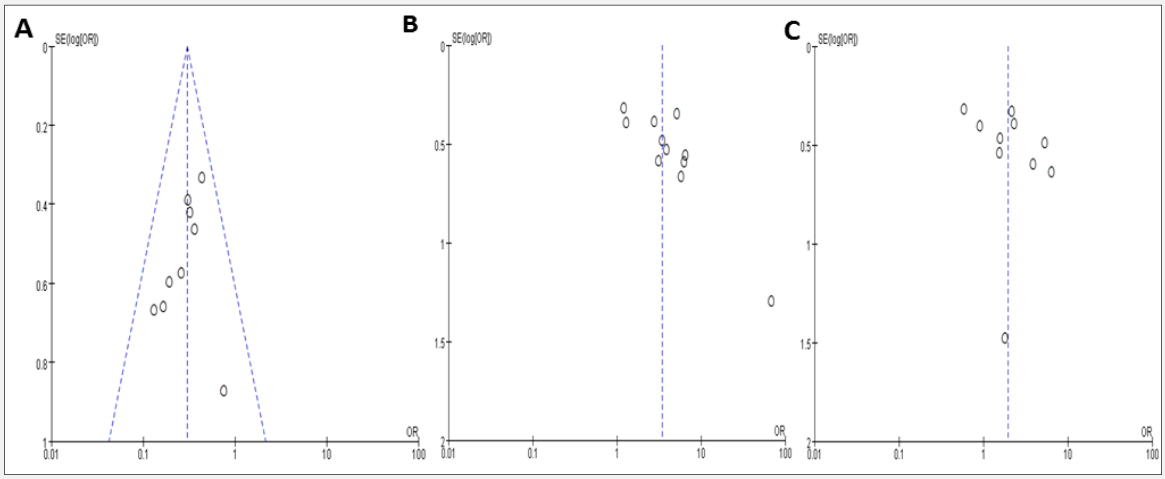


Figure 4. Forest plots of studies conducted on RhoA expression and features in colorectal cancer (CC), esophageal squamous cell carcinoma (ESCC) and hepatocellular carcinoma (HCC) on protein-based level. (A) The pooled OR from six studies about clinical stages of gastrointestinal cancer. (B) The pooled OR from six studies about lymph node metastatic status of gastrointestinal cancer. (C) The pooled OR from six studies including high, moderate, and low differentiation of gastrointestinal cancer.

eration of matrixmetalloproteinase (MMPs), thereby influencing matrix remodeling and tumor cell invasion. However, results obtained by using RhoA cells in RNA interference studies suggest that tumor cell migration results showed variability, which may be due to migration and invasion ability, and have very strong dependence on the cells [34-36]. In addition, the small GTPase RhoA is widely recognized as an important regulator of contractility and shortening in smooth muscle tissues in response to stimulation with contractile agonists. RhoA can regulate phosphorylation by inhibiting the catalytic activity of myosin light chain (MLC) phosphatase, which leads to an increase in MLC phosphorylation. RhoA activates Rho kinase, which inhibits MLC phosphatase activity by phosphorylating its regulatory subunit myosin phosphatase target subunit 1(MYPT1) or by phosphorylating the inhibitory peptide of MLC phosphatase CPI-17 [37, 38].



Figures 5. Funnel plots of studies conducted on RhoA expression and features in gastrointestinal cancer on protein-based level. (A) Clinical stages in gastrointestinal cancer. (B) Metastatic status in gastrointestinal cancer. (C) Differentiation in gastrointestinal cancer.

At the same time, researchers observed that RhoA protein gene has a cell transformation function. Usually, RhoA can participate in various cancer cells by genetically causing the malignant transformation. RhoA protein can also induce angiogenesis through vascular endothelial cell growth; finally, tumor cells pass through the vascular endothelium for distant metastasis [39]. Previous reports have confirmed that abnormal expression and activation of RhoA are correlated with the development and metastasis of malignant carcinoma [40]. However, the role that RhoA expression plays in gastrointestinal cancer and its clinical significance has not been thoroughly investigated. These data revealed the following: (1) gastrointestinal cancer has higher expression than normal tissue; (2) RhoA expression is not strongly associated with gender in gastrointestinal cancer patients; (3) RhoA expression is significantly higher in gastrointestinal cancer patients with lower differentiation than in those with high and moderate differentiation; (4) RhoA expression is associated with the clinical staging of gastrointestinal cancer patients; and (5) RhoA expression is higher in the presence rather than the absence of metastatic gastrointestinal cancer. To the best of our knowledge, the present meta-analysis study is the first to systematically evaluate the association among RhoA expression and clinicopathological features in gastrointestinal cancer.

The clinicopathological significance of RhoA expression for patients with gastrointestinal cancer remains controversial. The activity of RhoA is correlated with lymph node metastasis in human colorectal cancer [20]. However, Ahmad Faried revealed that RhoA is correlated with tumor differentiation, not with lymph node metastasis [27]. For gastric cancer tissues, the expression of RhoA is related to lymph node metastasis [16, 17], but Tang et al. revealed that the expression of RhoA is not associated with lymph node metastasis or differentiation degree [15]. Studies that were included had varying results. Therefore, several potential limitations of our study need to be considered. First, our meta-analysis is unlikely to obtain enough necessary data to analyze RhoA expression with specific parameters. Second, the reliability of this study may be limited by the information provided by the included studies. Third, the studies included were from Asia. Thus, these results may not represent the results obtained in other countries. Fourth, we used different methods to explore the relationship of RhoA expression and cancer. Fifth, there were different types of tumors itself to produce differences, for example, there is no significant difference between the clinicopathological characteristics and RhoA expression in intestinal-type gastric cancer, but for diffuse-type gastric cancer, high RhoA expression was involved with more advanced pathological N category compared to low RhoA expression [26]. Furthermore, the definition of RhoA positivity varied between the involved studies, and variability may lead to potential bias. Clinical outcome data for analyzing the prognostic significance of RhoA expression in patients with gastrointestinal cancer are lacking. Despite the aforementioned limitations, our study is the first meta-analysis on the association of RhoA expression with clinicopathological characteristics of gastrointestinal cancer.

CONCLUSION

In summary, we demonstrated that RhoA overexpression is associated with clinical staging, poor differentiation, and metastasis in gastrointestinal cancer. Our findings strongly suggest that RhoA is a promising biomarker for gastrointestinal cancer and possibly a therapeutic target for cancer treatment. However, more clinical studies with refined design and large samples are required to push our conclusion further, as well as reach our derived prospective.

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Contributions:
Wang P wrote the manuscript. Liu C and Li F provided the content and checked the manuscript for grammar and format. Li W, Peng J, Qi S, and Song L helped in collection and analysis the data.

Declaration of Interest:
The authors have declared that no competing interest exists.

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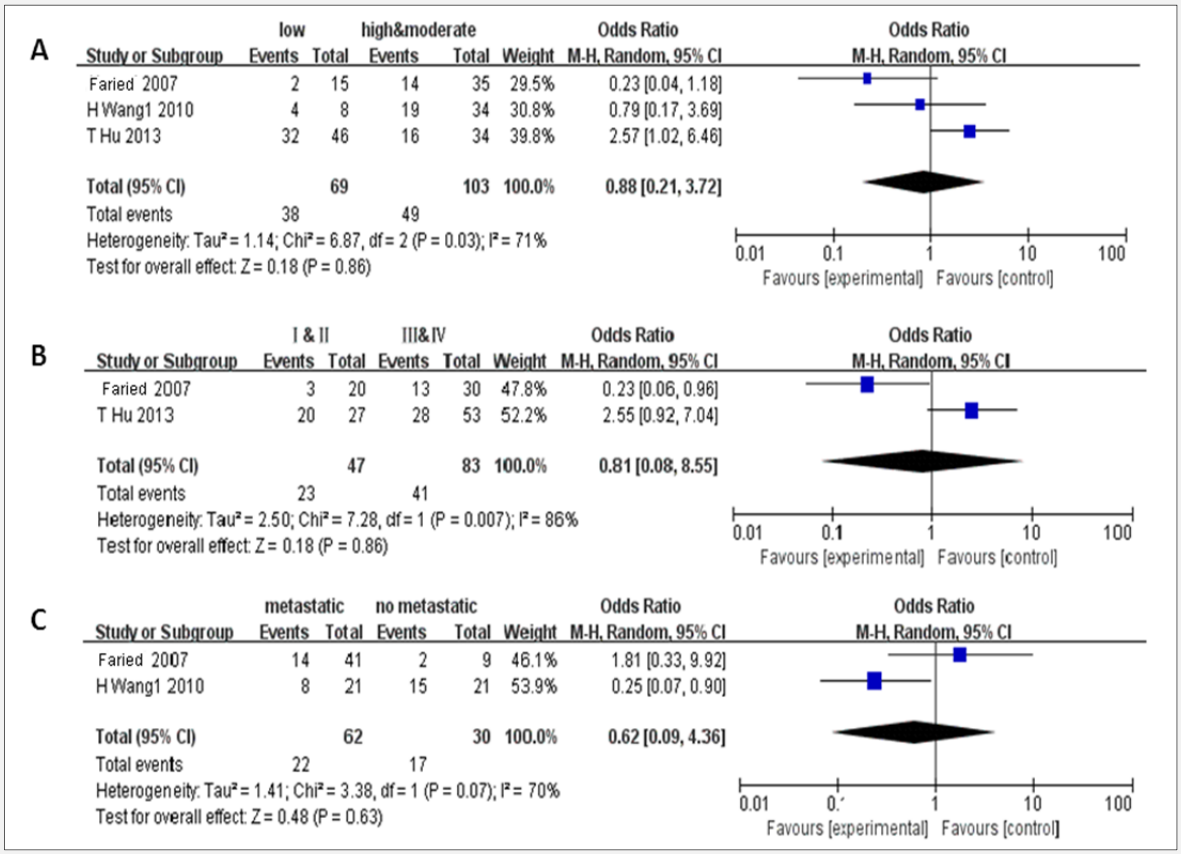
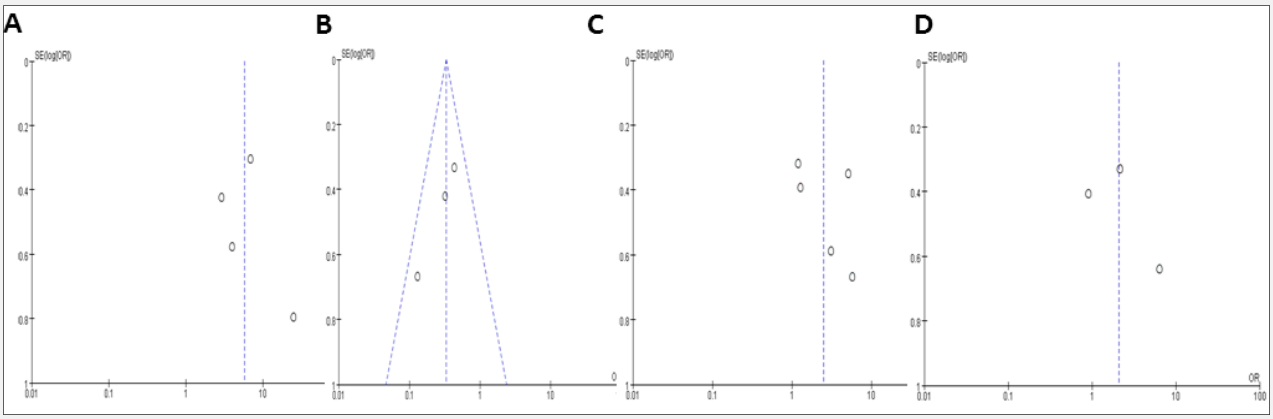


Supplementary Figure 1. RhoA expression on mRNA-based level. (A) The pooled OR from three studies including high, moderate, and low differentiation of gastrointestinal cancer. (B) The pooled OR from two studies about clinical stages of gastrointestinal cancer. (C) The pooled OR from two studies about lymph node metastatic status of gastrointestinal cancer.



Supplementary Figure 2. Funnel plots of studies conducted on RhoA expression and features in gastric cancer. (A) Gastric cancer and normal tissue samples. (B) Clinical stages in gastric cancer. (C) Metastatic status in gastric cancer. (D) Differentiation in gastric cancer.