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Original Article

Meta-analysis of RhoE expression in human cancers and the effects of its deficiency on animal models

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Abstract: RhoE protein expression is reportedly related to various cancers. We performed an up-to-date meta-analysis of RhoE protein expression in some digestive tract carcinoma and other solid tumors. In addition, RhoE deficiency in mice was analyzed using several databases, STATA version12.0, and Review Manager Version 5.3 software. The RhoE protein expression levels in neoplasms present in the digestive system and breast cancer were remarkably lower than those in normal or adjacent tissues. RhoE protein in gastrointestinal cancer was associated with early clinical stage (P = 0.020) and early differentiation grade (P = 0.001), whereas the RhoE protein in breast cancer was associated with lymph node metastasis. However, the RhoE protein expression was unrelated to lung cancer. The absence of RhoE expression resulted in growth retardation in mice (P < 0.00001). Thus, RhoE may serve as a tumor-suppressor gene in the carcinogenesis and metastasis of gastrointestinal and breast cancers.

Keywords: Cancer, RhoE, meta-analysis

Introduction

In recent years, the overall incidence of malignant tumors has increased with gastrointestinal cancer causing the highest mortalities. Simultaneously, breast cancer is one of the most commonly occurring cancers with increasing mortality rates in women worldwide. The burden of the disease is still growing, and stronger prevention and treatment systems are needed [1]. Lung cancer also affects the health of Chinese residents. Many cancer studies have been conducted, but the results are often contradictory.

The relationship between tumors and Rho family members has gained considerable research interest. RhoE, also known as Rnd3, is an atypical member of the Rho protein family without detectable GTPase activity and belongs to the Ras superfamily members. The Rho family has 22 members present in humans, including Rac (1, 2, and 3), Rho (A, B, and C), and Cdc42. Particularly, the Rnd subfamily including Rnd1, Rnd2, and RhoE/Rnd3 exist in mammals, fish, and birds but not in invertebrates [2]. The Rho family has extensive biological effects, such as adjusting and restructuring the cytoskeleton to regulate the shape of cells, and influencing cell growth, proliferation, and transfer. Compared with other Rho family members, RhoE exists only in combination with GTP in cells [3, 4]. Under the regulation of RhoA, RhoE can improve the disappearance of stress fibers in epithelial cells and thus lead to changes in cell morphology [5]. Wennerberg et al. [6] found that Rnd proteins can resist the effect of classical RhoA by stimulating a GTPase-activating protein. Li et al. [7] showed that RhoE protein can influence the multidrug-resistant phenotype of gastric cancer cells by suppressing Bax expression at the posttranscriptional level to inhibit vincristine-induced apoptosis.

The mechanism of RhoE protein in cancer cells has not yet been clearly assessed. However,

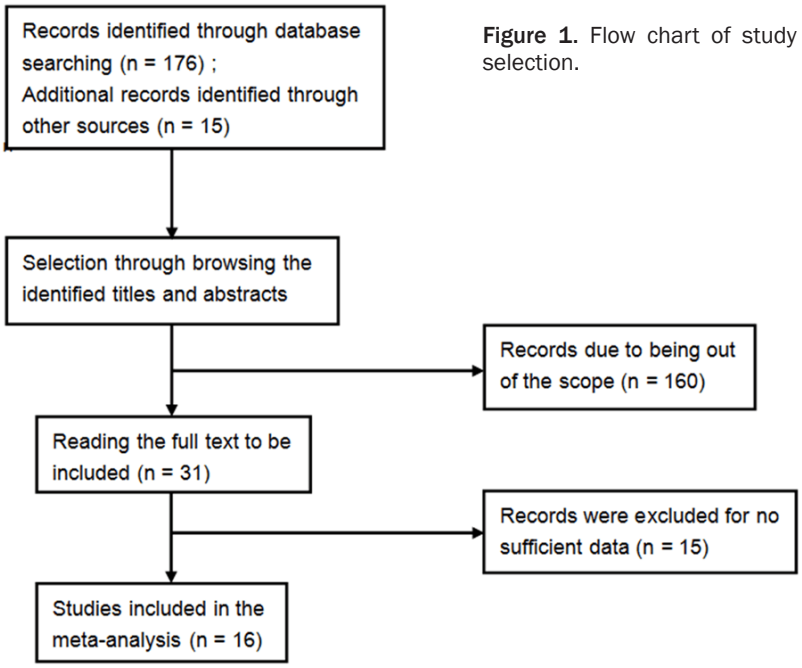

Figure 1. Flow chart of study selection.

current research data show that RhoE is associated with tumor occurrence and strongly influences the prognosis of patients. Some researchers have confirmed that RhoE could induce the invasion of gastric cancer [8], and that its overexpression is a poor prognostic factor in non-small-cell lung cancer [9]. RhoE protein can play a positive role in regulating the invasiveness and metastasis of mesenchymal tumor cells [10]. However, RhoE acts as a suppressor protein of tumor metastasis in hepatocellular carcinoma [11]. Liu et al. [12] revealed that RhoE downregulation in glioblastoma patients promotes tumorigenesis through the augmentation of Notch transcriptional complex activity. To date, information on the relationship between RhoE and cancer is limited. Therefore, we conducted this meta-analysis to investigate RhoE expression and its relationship with clinicopathological parameters to reveal the role of RhoE in cancer.

Materials and methods

Literature search strategy

Two investigators independently performed the search in the electronic databases of PubMed, EMBASE, Wanfang, and CNKI until October 5, 2015. The following search terms and their combinations were used: (cancer OR neoplasm OR carcinoma) AND (RhoE OR Rnd3) AND (clinicopathological features OR animals). The titles and abstracts of all studies identified in the search were meticulously browsed to exclude the clearly irrelevant publications. The remaining articles were verified to determine whether or not they contained useful information on the topic. We also performed manual viewing of the articles through references.

Selection criteria and data extraction

Studies included in the meta-analysis had to meet the following criteria: (1) all patients had clinicopathological data, (2) RhoE/Rnd3 proteins were detected in all patients, and (3) the scope of literature research was the same as that of the research method. We used the complete study if the author found some data with patient population that was the same as those in several studies. Studies were excluded if they (1) were reviews or case-only studies, (2) lacked sufficient data for the calculation of incidence and/or risk ratio (RR) with 95% confidence interval (CIs), and (3) were duplications of previous publications or replicated samples.

The investigators strived to reach an agreement to determine if a study could be included in the meta-analysis. Each study recorded the following information: first author, year of publication, region, number of cases, methods, antibody source, clinicopathological parameters, and animal weight. Investigation heterogeneity was assessed to determine whether or not these data could be analyzed for meta-analysis.

Quality assessment

Two reviewers used the following factors to independently assess the quality of the study: (1) the study population and type of carcinoma were clearly defined; (2) the relationships among RhoE/Rnd3 expression, cancer clinicopathological parameters, and prognosis were revealed; (3) sample size was larger than 10, but no limitation on animal models was im-

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

Author	Year	Region	Cancer	Patient (M/F)	Antibody source	Methods	RhoE expression	Clinicopathological factors
Wang [13]	2014	Heilongjiang	PLC	68 (54/14)	Cell Signing	IHC	Down	Sex, differentiation, stage
Zhang [14]	2009	Xian	HCC	106 (62/44)	Sigma	IHC	Down	Sex, differentiation, stage
Luo [15]	2012	Guangdong	HCC	99 (84/15)	Abcam	IHC	Down	Sex, differentiation, stage
Sun [16]	2009	Chongqing	CC	48 (NR)	NR	IHC	Down	Differentiation, stage, LNM
Zhou [17]	2012	Xian	CC	202 (104/98)	Santa Cruz	IHC	Up	Sex, differentiation, LNM
Zhu [18]	2008	Sichuan	CC	32 (23/9)	NR	IHC	Down	Differentiation
			GC	74 (54/20)				
Zhao [19]	2012	Henan	ESCC	128 (81/47)	Santa Cruz	IHC	Down	Sex, differentiation, Stage, LNM
Feng [20]	2013	Shanghai	GC	90 (67/23)	Upstate	IHC	Up	Sex, differentiation, LNM
Feng [21]	2010	Xian	GC	34 (NR)	Upstate	IHC	Down	NR
Liu [22]	2014	Zhejiang	BC	71 (NR)	Santa Cruz	IHC	Down	Stage, LNM
Li [23]	2012	Liaoning	BC	60 (0/60)	Millipore	IHC	Down	Stage, LNM
Zeng [24]	2011	Guangdong	BC	106 (0/106)	Abgent	IHC	Down	Stage
Zhu [25]	2008	Sichuan	LC	62 (50/12)	NR	IHC	UP	Stage
			BC	34 (NR)			Down	
Liu [26]	2009	Chongqing	LC	60 (NR)	NR	IHC	Down	Stage, LNM

PLC: primary liver carcinoma; HCC: hepatocellular carcinoma; CC: colorectal cancer; ESCC: esophageal squamous cell carcinoma; GC: gastric cancer; BC: breast cancer; LC: lung cancer; LNM: lymph-node metastasis; IHC: immunohistochemistry; NR: not reported.

Table 2. Characteristics of animal studies included in the meta-analysis

Author (year)	N	Cell	Type of animal	Time	Data analysis
Mocholi et al. 2011	19 (11RhoEgt/gt 8RhoE+/+)	Embryonic stem (ES) cells	Mice	Postnatal day 15	Variance analysis
Olga et al. 2014	8 (3RhoEgt/gt 5RhoE+/+)	Embryonic stem (ES) cells	Mice	Postnatal day 15	Unpaired Student's t-test

posed; and (4) sufficient information for estimating the RR of overall survival (OS) and 95% confidence interval CI were provided. Normal tissues also included adjacent non-tumor tissues.

Statistical analysis

The random effect model was applicable to the pooling method if substantial heterogeneity was explored, whereas the fixed-effect model was suitable in the case of heterogeneity among studies. I^2 index and *P* value were consistent in terms of heterogeneity. The fixed effect model was used at I^2 index < 50% and P > 0.05; otherwise, the random-effect model was adopted. RR and 95% CI were used to analyze the categorical variables. All *P* values were two sided, and P < 0.05 was considered statistically significant. Continuous variables were estimated as weighted mean differences with 95% CI between the RhoEgt/gt animals and the control animals. All analyses were performed using STATA version 12.0 (Stata, College Station, TX, USA) and Review Manager Version 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2012).

Results

Selection and characteristics of studies

Sixteen studies were included in the final meta-analysis as shown in **Figure 1**. Initially, 191 articles appeared using the keywords. By reading the article titles and abstracts, 160 articles were subsequently excluded from the meta-analysis because the studies were unrelated to RhoE or Rnd3. Three articles were not defined by immunohistochemistry (IHC), eight duplicated studies and reviews had no detailed information, and four articles discussed non-digestive tract tumor, thus resulting in their exclusion from the meta-analysis. Finally, 1168 cancer patients and 2 studies about animals were included. Their basic characteristics are summarized in **Table 1** [13-26] and **Table 2** [27, 28]. Positive RhoE expression was defined by IHC.

Table 3. Meta-analysis results of RhoE expression in gastrointestinal, breast, and lung cancers

Tumor type	Outcome of interest	No. of studies	Number of tissue samples	RR/ WMD	95% CI	Heterogeneity (%)	P	Z
Gastrointestinal cancer	Cancer and normal tissue	7	E. RhoE = 218	0.52	0.37-0.73	I^2 = 89.7, P = 0.000	0.000*	3.79
			C. RhoE = 269					
	OS	3	E. RhoE = 112	1.44	0.33-6.26	I^2 = 94.1, P = 0.000	0.628	2.87
			C. RhoE = 44					
	Differentiation grade	5	E. RhoE = 67	0.55	0.29-1.05	I^2 = 79.4, P = 0.001	0.001*	3.42
			C. RhoE = 206					
	Stage	5	E. RhoE = 67	0.44	0.22-0.88	I^2 = 89.8, P = 0.000	0.020*	2.32
			C. RhoE = 139					
	Lymph node involvement	3	E. RhoE = 77	0.44	0.06-2.99	I^2 = 91.8, P = 0.000	0.400	0.84
			C. RhoE = 79					
	Sex	5	E. RhoE = 187	1.07	0.92-1.25	I^2 = 0.0, P = 0.783	0.364	0.91
			C. RhoE = 111					
Breast Cancer	Cancer and normal tissue	3	E. RhoE = 77	0.56	0.47-0.67	I^2 = 0.0, P= 0. 904	0.000*	6.44
			C. RhoE = 116					
	Lymph node involvement	3	E. RhoE = 27	0.34	0.12-0.96	I^2 =81.7, P = 0.004	0.041*	2.04
			C. RhoE = 46					
	Stage	3	E. RhoE = 22	0.75	0.39-1.46	I^2 = 61.0, P = 0.077	0.401	0.84
			C. RhoE = 57					
Lung Cancer	Cancer and normal tissue	2	E. RhoE = 49	0.30	0.03-2.70	I^2 = 95.9, P = 0.000	0.285	1.07
			C. RhoE = 75					
	Stage	2	E. RhoE = 33	0.55	0.08-3.89	I^2 = 71.8, P = 0.060	0.547	0.60
			C. RhoE = 16					

*P < 0.05. C: control group; E: experiment group; RR: risk ratios; WMD: weighted mean difference; CI: confidence interval; OS: overall survival; I^2: heterogeneity detection; Z: Z-test statistics.

These studies provided sufficient data on the correlations among RhoE expression, clinicopathological characteristics, and mouse weight. The results of RhoE expression in different cancers are shown in **Table 3**.

RhoE expression in gastrointestinal cancer

The pooled RRs from seven studies on gastrointestinal cancer, including 461 cancer patients and 329 normal gastrointestinal tissues, are illustrated in **Figure 2A** (RR = 0.52, 95% CI = 0.37-0.73; P < 0.0001). The results indicated that RhoE expression was significantly lower in gastrointestinal cancer than in normal tissues. The pooled RRs from five studies, including 170 low differentiation and 382 high and moderate differentiations, are shown in **Figure 2B** (RR = 0.55, 95% CI = 0.29-1.05; P = 0.001). This finding indicated that RhoE expression was significantly higher in gastrointestinal cancer patients with high and moderate differentiation than in those with low differentiation. Similarly, the pooled estimates for RR in **Figure 2C** revealed that RhoE expression was higher in gastrointestinal cancer (I and II) than in early-stage cancer (III and IV), and the difference was significant (RR = 0.44, 95% CI = 0.22-0.88; P = 0.020). The pooled RR from five studies including 384 males and 219 females (RR = 1.07, 95% CI = 0.92-1.25; P = 0.364) showed that RhoE expression was not statistically significant with sex in cancer patients and with lymph node metastasis (RR = 0.44, 95% CI = 0.06-2.99; P = 0.400). Moreover, RhoE expression was not significant with OS in gastrointestinal cancer patients. We then used the random effect model for analysis as I^2 = 86.6%.

RhoE expression in breast and lung cancers

Similarly, the pooled RR from three studies in breast cancer showed that RhoE expression was significantly lower in breast cancer than in normal tissues. Thus, the fixed effect model was applied to examine as I^2 = 0% (**Figure 3A**). RhoE protein was also associated with lymph node metastasis in breast cancer. Thus, the random effect model was applied to examine as I^2 = 81.7% (**Figure 3B**). However, no correlation was observed between RhoE expression and lung cancer, and we usedthe random effect model for analysis as I^2 = 58.8% (**Figure 4**).

RhoE expression in animal models

Mice without RhoE expression (RhoEgt/gt) showed a slower daily increase in bodyweight

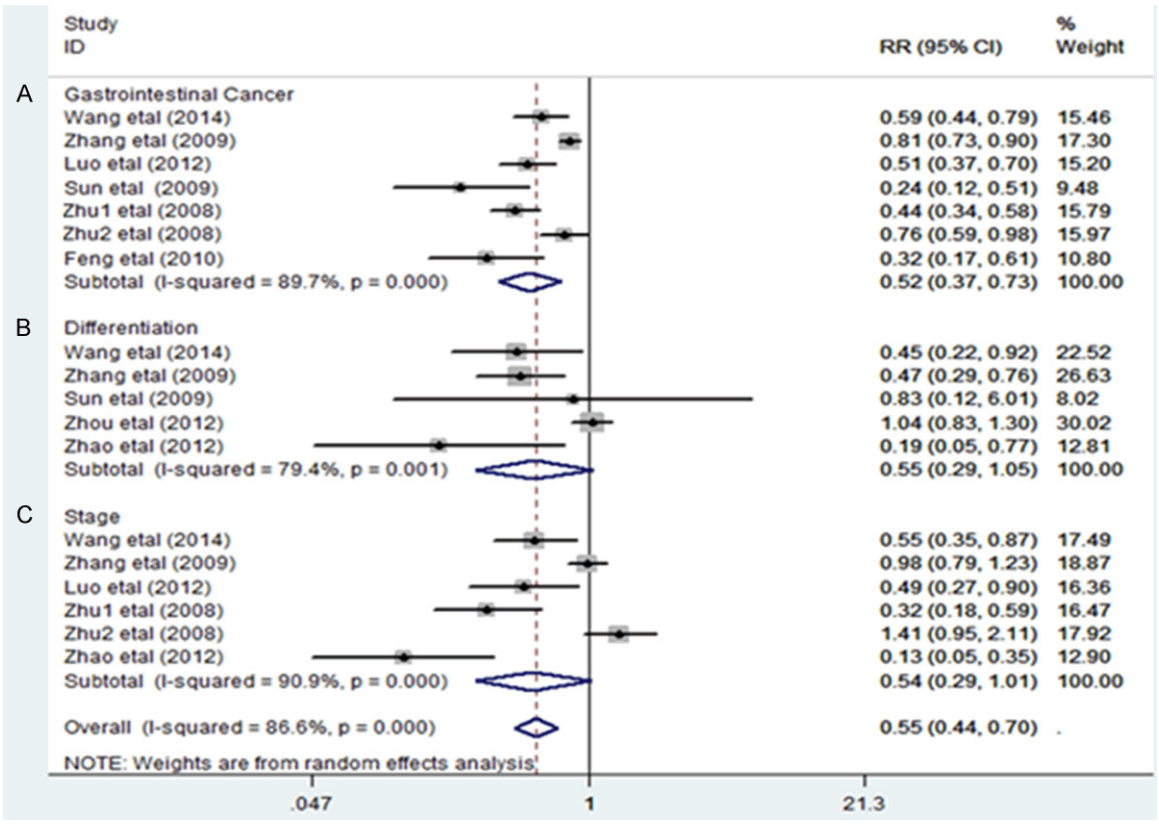

Figure 2. A. Pooled RRs from seven studies, including gastrointestinal cancer and normal tissues; B. Pooled RRs from five studies on differentiation in gastrointestinal cancer; C. Pooled RRs from six studies on the clinical stage in gastrointestinal cancer.

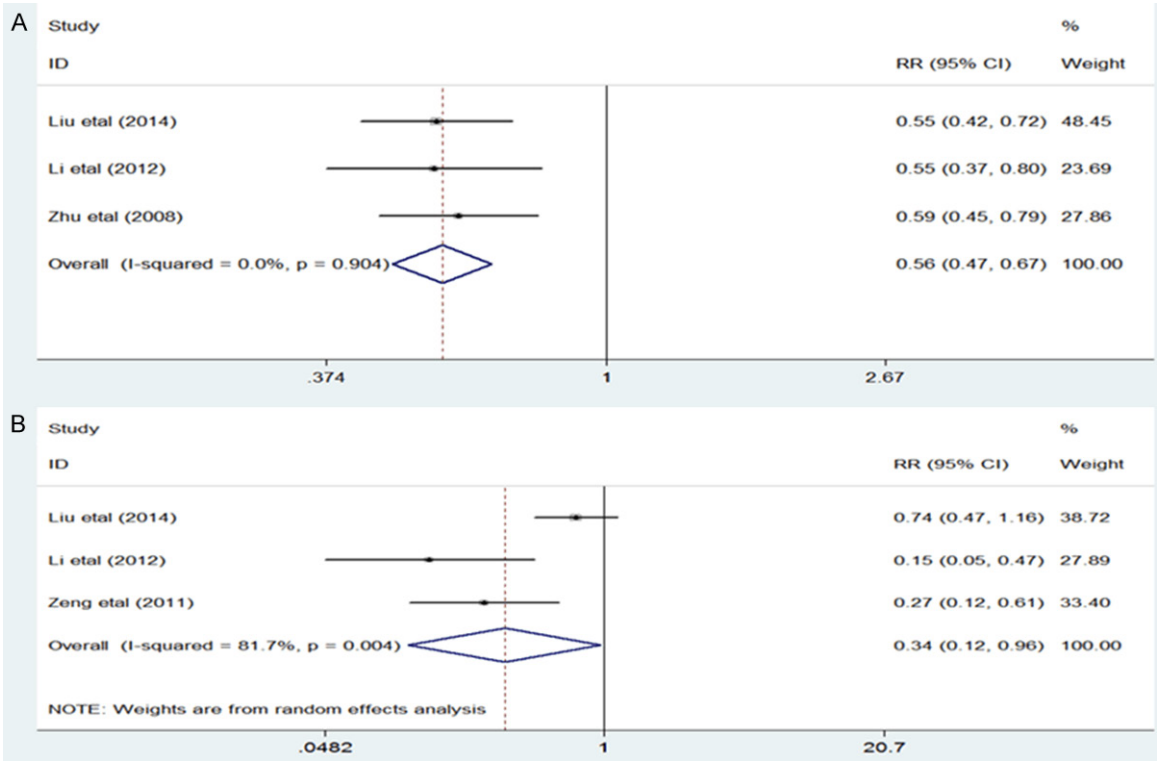

Figure 3. A. Pooled RRs from three studies including breast cancer and normal tissues; B. Pooled RRs from three studies on lymph node metastasis in breast cancer.

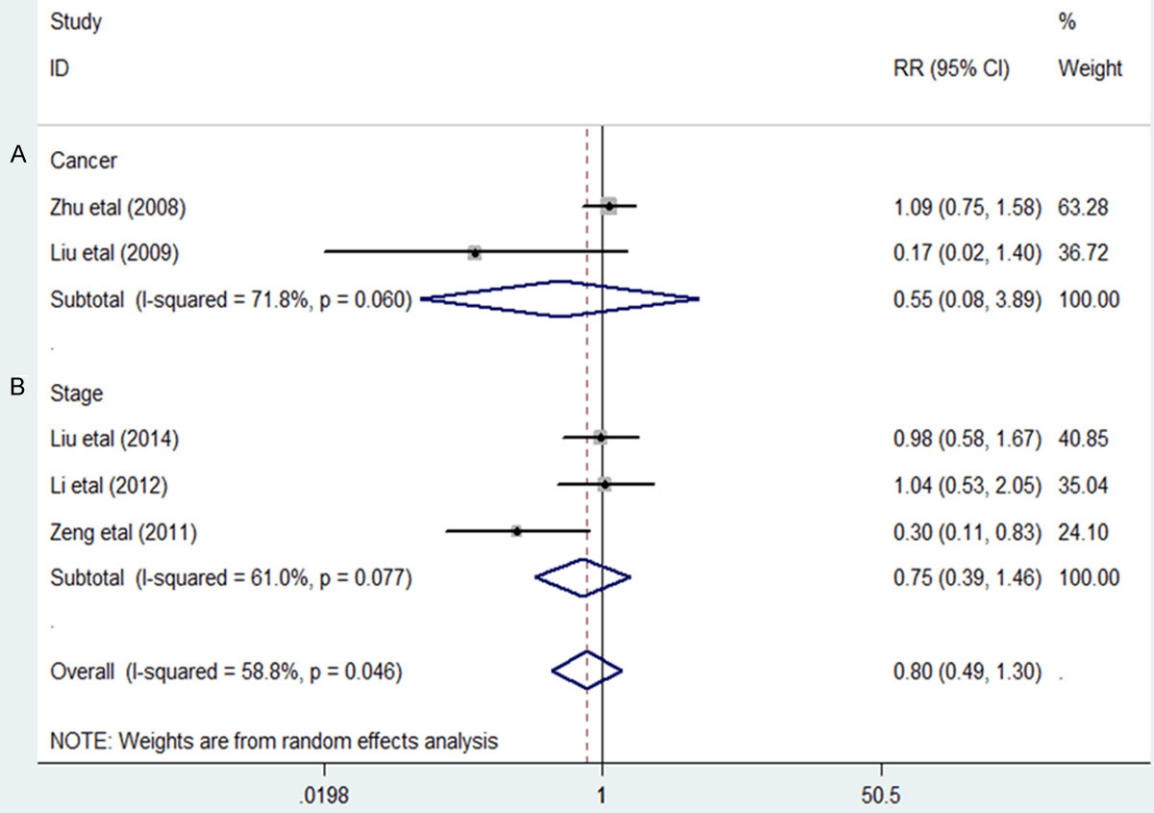

Figure 4. A. Pooled RRs from two studies including lung cancer and normal tissues; B. Pooled RRs from three studies on the clinical stage in lung cancer.

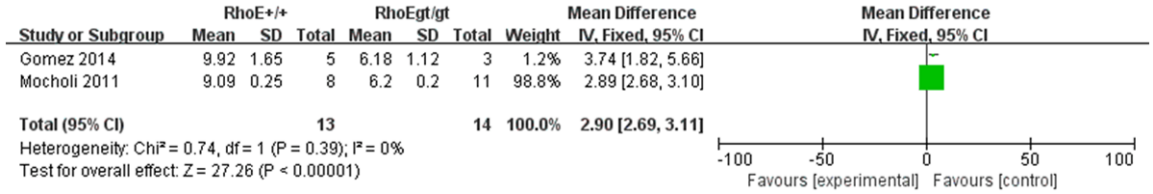

Figure 5. Forest plot of RhoEgt/gt and wild-type postnatal 15-day-old mice compared in terms of body weight.

(P < 0.00001) than mice with the wildtype (RhoE+/+) at postnatal day 15. Significant differences were found between the RhoE+/+ and RhoEgt/gt groups (**Figure 5**). Continuous variables were assessed by the weighted mean difference with 95% CI between the RhoE-treated animals and the control animals.

Publication bias

Begg's funnel plots were used to detect publication bias in gastrointestinal cancer. No significant evidence of publication bias was found in the meta-analysis of RhoE expression (**Figure 6**). The deviation was due to the sample size, area, type of tumor, and diagnostic criteria. The expression levels of RhoE protein in tumor tissues were different. Given their rare inclusion in the literature, other aspects failed the publication bias tests.

Discussion

RhoE/Rnd3 was first described by Pierre Chardin and colleagues in 1996. It was isolated and combined with p190RhoGAP to create the

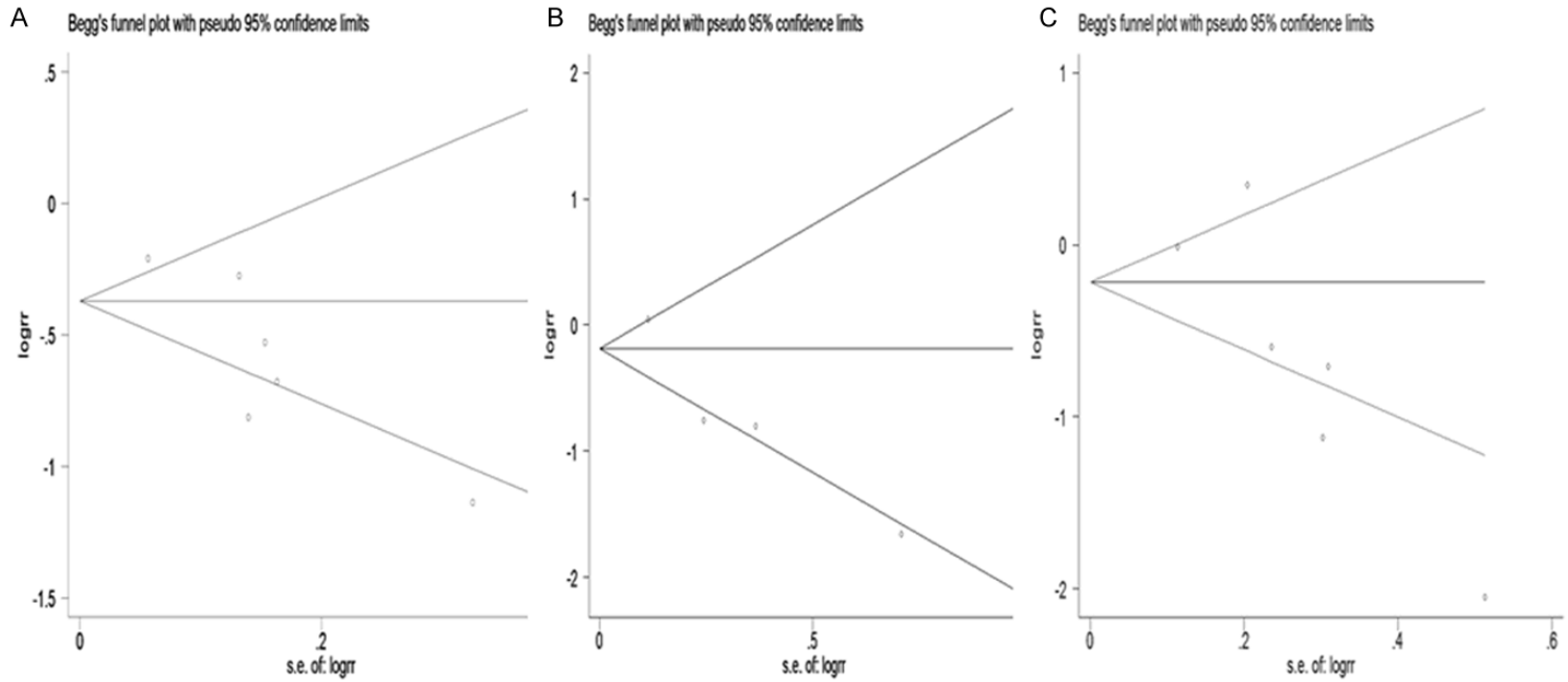

Figure 6. A. Begg's funnel plots on gastrointestinal cancer and normal tissues; B. Begg's funnel plots on differentiation in gastrointestinal cancer; C. Begg's funnel plots on the clinical stage in gastrointestinal cancer.

GTPase-activating protein for RhoA. Independently, the actions and localization of RhoE are regulated by its phosphorylation state [4, 29]. RhoE is involved in many physiological functions, including tumor development, invasion, and metastasis, and it is closely related to tumor prognosis. On one hand, RhoE overexpression can inhibit the proliferation of cancer cells by preventing cell cycle progression and inducing transformation. On the other hand, RhoE is also a transcriptional p53 target gene that can perform expected functions by regulating ROCK I signals to influence the cell states under genotoxic stress conditions, and it can be used as a survival factor to promote tumor progression [30, 31]. Interestingly, RhoE inhibits Notch signaling, and the downregulation of RhoE in cancers promotes tumorigenesis by augmenting the Notch transcriptional complex activity [32-34]. All existing studies used tumor patients or animal models. Simply put, RhoE expression in cancer is currently controversial. Some studies have suggested a possible tumor-suppressive role for RhoE expression in human cancer [10, 11, 15, 34-39]. However, previous evidence suggests a positive function with some tumor types [8, 9, 17, 20, 40-42]. These results indicate that RhoE expression plays specific roles in different cell lines. Moreover, RhoE could be related to nervous system diseases similar to other Rho family members, and it reveals itself as a promoter in neurite formation [27, 43, 44]. RhoE-deficient mice showed that the knockdown of RhoE weakens the contact inhibition of growth and affects the metastatic capability of cancer cells, and RhoE shows inhibitory action at the initiation and in the progression of tumors [45]. Therefore, the difference in RhoE expression in tumorigenesis may be due to the different cell or tumor types and to the changes in mediators or experimental details in vivo and in vitro.

Our meta-analysis aimed to observe the relationship between RhoE expression in digestive tract cancer, breast cancer, lung cancer, and mice to provide a foundation for further studies on the RhoE protein. The results showed a significantly lower RhoE protein expression level in gastrointestinal cancer than in normal tissues. At the same time, RhoE protein was correlated with early differentiation degree and clinical staging but not with sex, lymph node metastasis, and OS. These results indicated that RhoE could play a major role in gastrointestinal cancer. In breast cancer, we found that RhoE protein had a significantly lower expression than normal tissues and that it was associated with lymph node metastasis. However, the literature on lung cancer was insufficient, and the results showed no significance. Some researchers have shown that the downregulation of RhoE in human glioblastoma could enhance Notch activity and promote genesis and that the low expression of RhoE could be associated with poor prognosis [12, 46]. However, reports on the clinical parameters of glioma tissue are insufficient, and our meta-analysis cannot be used for comprehensive analysis. Animal models by gene trapping in ES cells were used to observe the significance of RhoE expression. Mocholi et al. [27] revealed that RhoE is essential for postnatal development as null mice died shortly after birth. Gomez et al. [28] observed Leydig cells within the testes, and the lack of RhoE expression resulted in testicular anomalies. However, the existing data on RhoE expression in animal models do not reveal comprehensive results. We only found that RhoE deficiency displayed growth retardation. This finding may shed new light on the effects of RhoE expression deficiency in mice. Moreover, it indicates that RhoE may serve as an important protein in the normal development of vertebrates.

The results of our meta-analysis seemed to differ from those of other studies. For example, Zhou et al. [17] found that RhoE expression was high in normal colorectal tissues and was also related to lymph node metastasis and distant metastasis. Zhao et al. [19] discovered that RhoE expression was downregulated in esophageal squamous cell carcinoma (ESCC) and was associated with lymph node metastasis. In Luo et al.'s [15] study, RhoE was also downregulated in hepatocellular carcinoma (HCC) and was negatively associated with tumor grade and poor survival with low expression.

On the basis of these observations, we collected and summarized data to reach a final conclusion and confirm the lower RhoE protein expression levels in cancerous and normal gastrointestinal tissue, and found a negative regulation in gastrointestinal cancer. Research shows that RhoE overexpression may be a poor prognostic value in patients with non-small-cell lung cancer, whereas the down-regulation of

RhoE expression is correlated with stages and lymph node metastases [9, 26]. This meta-analysis showed that RhoE expression was not significant in lung cancer. Available studies on the prognosis of OS are scarce, and thus we need to continue to expand the search for prognostic data in the future.

This meta-analysis has some limitations. First, the total number of data involved in this study was too small. Given the minimal literature sample size, we need to validate the results by using large samples and conducting more studies in the future. In addition, this method of meta-analysis was tested by IHC, and thus the evaluation of staining was not the same. We also observed only Chinese patients, and fewer cases could exist in foreign countries. Heterogeneity could not be avoided, and it forced us to use the highly conservative random effect model. Despite these limitations, the collected data showed the relationship among RhoE protein, clinicopathological features, and prognosis in patients with gastrointestinal and breast cancers. As some animal studies were conducted to test the role of RhoE protein, this meta-analysis was based on evidence from a wide number of both human and animal studies.

In conclusion, this study revealed that RhoE expression was downregulated in gastrointestinal and breast cancers. We also confirmed the relationship of RhoE protein with the clinicopathological features of gastrointestinal, breast, and lung cancers. In vivo RhoE protein played an important role in normal development. RhoE protein may be a suppressor of tumor progression, but better resources should be utilized to identify the effect and prognostic significance of RhoE protein in different cancers in the future. Moreover, future research should examine the role of RhoE protein in drug treatment.

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Disclosure of conflict of interest

None.

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