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Review Article

Status of integrin subunit alpha 4 promoter DNA methylation in colorectal cancer and other malignant tumors: a systematic review and meta-analysis

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Abstract

Background and purpose: Although many recent studies have analyzed the validation of integrin subunit alpha 4 (ITGA4) biomarker for cancer detection in patients with various malignancies, the diagnostic value of *ITGA4* methylation for malignant tumors remains uncertain. We performed a systematic review and meta-analysis to unravel the relationship between *ITGA4* promoter methylation status and malignant tumors.
Experimental approach: A meta-analysis was performed using the metaphor package in R 3.5 and Meta-Disc 1.4 software. Data were derived from a search of main electronic databases up to January 2022. SROC analysis was used to evaluate the status of *ITGA4* promoter methylation in colorectal cancer (CRC) and other cancers. A total of 1232 tumor samples and 649 non-tumor samples from 13 studies were analyzed.
Findings/Results: The pooled results including all types of cancer provided evidence that *ITGA4* hypermethylation was more frequent in tumor samples than non-tumor samples (OR 13.32, 95% CI 7.96-22.29). Methylation of *ITGA4* has a pooled sensitivity of 0.95 (95% CI: 0.94-0.97), a pooled specificity of 0.57 (95% CI: 0.54-0.60), and an area under the curve (AUC) of 0.94. When the analysis was performed independently for CRC, it revealed a higher association (OR = 20.77, 95% CI: 9.15-47.15). The assessment of *ITGA4* methylation of tissue samples resulted in a pooled sensitivity of 0.99 (95% CI: 0.97-1.00) and a pooled specificity of 0.90 (95% CI: 0.86-0.93), and AUC of 0.94 for the diagnosis of CRC.
Conclusion and implications: *ITGA4* methylation analysis is a reliable method for CRC screening in tissue samples.

Keywords: Colorectal cancer; *ITGA4* gene; Meta-analysis; Promoter methylation.

INTRODUCTION

Cancer is the second most common cause of mortality globally, which led to 9.6 million deaths in 2018 (1,2). As the mean age of the global population is increasing, cancer incidence rates have also increased in most countries (3,4). It is widely accepted that earlier detection of malignancy reduces overall mortality rates. Therefore, early monitoring of diagnostic biomarkers can help to identify individuals at risk early allowing the institution of necessary precautions (5).

Gene promoter methylation is known to be an important epigenetic factor that results in the deregulation of gene expression (6). Gene products that are crucial for the cell cycle and their related regulation are under spatiotemporal control.

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One of the main phenomena contributing to the dysregulation of these important genes is hyper- or hypo-methylation, which triggers cell cycle aberration (7,8). The gradual accumulation of methylation errors and/or mutations contributes to the initiation and progression of sporadic malignant neoplasms. Newly formed tumors usually remained undetected up to their advanced stages which dramatically reduces the possibility and chances of effective treatments. There is an inverse relation between the stage of cancer diagnosis, treatment outcome, and survival rate. Therefore early-stage cancer screening modalities have been gaining vital importance (9).

To date, there are a vast variety of biomarkers available (10-13) and these have been subject to intensive research in order to develop early screening tools for cancer, however, due to the conflicting data, it would be difficult to find out the suitability of any marker for early-stage cancer screening. In a review conducted by Laugsand *et al*. several single markers for colorectal cancer (CRC) with a sensitivity of 75% and specificity of ≥ 90% were identified. Amongst the markers listed, the *ITGA4* gene has been reported to be one of the superior single markers with a specificity of 90% or more (14). *ITGA4* gene methylation status in chronic lymphocytic leukemia (CLL) was examined by Attia *et al*. who concluded that hypermethylation at three different CpG islands of the *ITGA4* gene is a common event in CLL when compared with healthy controls (15). Methylation of the *ITGA4* gene emerged as having a significant negative association with the overall survival rate in pancreatic adenocarcinoma patients and was positively associated with tumor recurrence (16).

The ITGA4 protein is a component of integrins, α4β1 and α4β7, and has been subjected to detailed studies to date. Integrins are heterodimeric transmembrane receptors that mediate adhesion-dependent cellular functions. Various combinations of two subunits (α and β) make 24 different integrin molecules in mammalian cells (17). These molecules link with a variety of biological molecules and promote multiple processes such as signaling pathways, growth, inflammation, and cell migration (Fig. 1) (18,19).

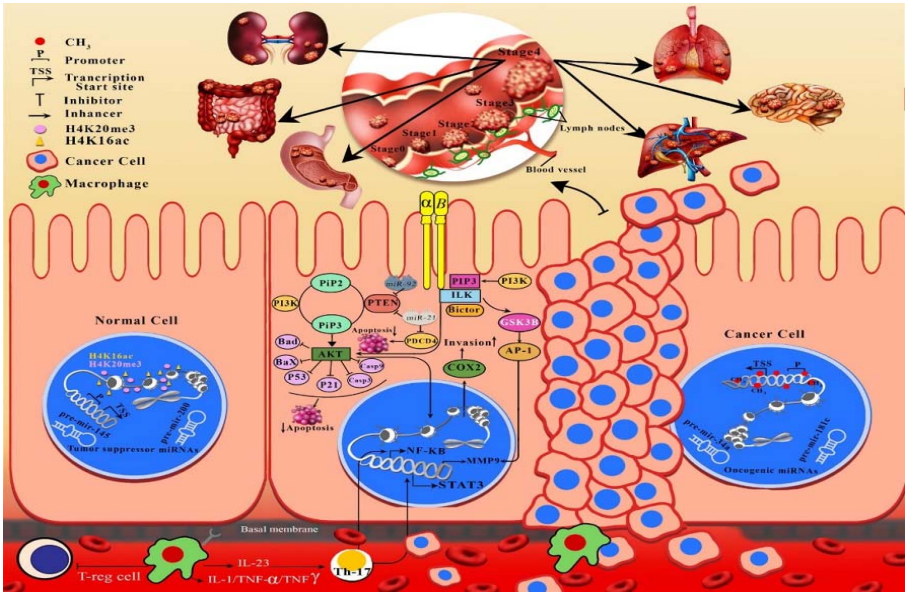

Fig. 1. Schematic representation of integrin signaling pathway and its deregulation in colorectal cancer. Cooperative signaling between integrins and NF-κB or AKT-mediated pathway led to tumor cell migration to the blood vessels and invasion to the distant organs. Various epigenetic modifiers like DNA methylation, histone methylation and acetylation, miRNAs down- or up-regulation are also depicted. NF-κB, Nuclear factor-κB.

Cell-to-cell adhesion is due to the family of ITGA4 integrins which are particularly important for immune function. The α4 peptide (CD49d) binds to the β1 chain (CD29) or the β7 chain producing integrins of α4β1 (VLA-4 or very late antigen-4) and α4β7 (molecule of lymphocyte Peyer's patch adhesion). Alpha-4 integrins play a role in cases such as myogenesis, hematopoiesis, heart, and placental development. The alpha 4 integrins also play a role in cardiovascular disease surveillance, inflammation, and pathogenesis. α4β1 binds to vascular cell adhesion molecule-1 (VCAM-1) on the surface of endothelial and stromal cells and to fibronectin that is in the extracellular matrix. α4β7, on the other hand, binds to mucosal vascular addressing cell adhesion molecule-1 (MAd-CAM-1). The US Food and Drug Administration (FDA) has approved natalizumab as a humanized monoclonal antibody targeting *ITGA4* that treats multiple sclerosis and Crohn's disease (20). However, the role played by ITGA4 in tumorigenesis still remains controversial. Some studies reported that ITGA4 is a tumor suppressor, whereas a correlation of its expression with the extent of malignant cell transformation and metastasis has been proposed by others (21-23). For example, a study that examined abnormally expressed integrin subunits as biomarkers in skin cutaneous melanoma revealed that *ITGA4* expression levels were closely related to metastasis and pathological stage (24). Also, low expression of *ITGA4* in CRC cells was associated with poor prognosis and results from analysis of TCGA high-throughput RNA sequencing data revealed that *ITGA4* might participate in apoptosis and survival *via* the PI3K/Akt pathway (25). In spite of several recent studies, the power and suitability of *ITGA4* promoter methylation for malignant tumor screening still remain inconclusive (26-30). Therefore, we have conducted a systematic review and meta-analysis of the available literature to assess the significance of *ITGA4* promoter methylation evaluation in CRC and other cancers screening.

METHODS

Methods and search strategy

Studies were identified by the PRISMA statement (31). In this meta-analysis, we performed a comprehensive search related to *ITGA4* gene methylation. Two researchers (S. Jafarpour and N. Vatandoost) separately searched the electronic databases: PubMed, Embase, Web of Science, Cochrane Library, and Scopus. Eligible literature was retrieved until Jan 2022 using the following terms: ("Cancer" OR "Tumor" OR "Neoplasm" OR "Carcinoma ") AND ("ITGA4" OR "integrin alpha 4" OR "antigen CD49D" OR "alpha 4 subunits of VLA-4 receptor ") AND ("methyl*" OR "epigene*"). The abstract and the title of the publications were retrieved and the full text of relevant articles was reviewed to make sure that the data of interest were included. Any conflicts were solved through discussion with a third researcher (R. Salehi).

Inclusion and exclusion criteria

The search strategy was according to PICO characteristics based on the following criteria: 1) English as the publication language; 2) original studies evaluated the association between *ITGA4* methylation and any type of cancer or tumor; 3) studies using a case-control design; 4) comparison with healthy persons, or adjacent non-cancer tissues must be considered as control samples in the studies; 5) studies with sufficient data to be able to calculate the odds ratio or the number of true positives, false positives, true negatives, and false negatives. All, non-human experiments, cell line studies, non-serum/tissue/urine samples in the study, and studies with incomplete data were excluded. Other types of studies such as reviews, letters, meeting abstracts, and redundant studies were also excluded.

Data extraction and quality assessment

Two investigators (S. Jafarpour and R. Salehi) independently assessed the quality of eligible studies according to the quality assessment of diagnostic accuracy studies (QUADAS) tool in which a study with a score ≥ 9 was considered high quality. Information extracted from the studies included the first author's name, year, ethnicity, detection methods, sample size, tumor type, and experimental results. Also, additional data such as information about subgroups, primer sequences, product size, and annealing temperature were collected. Any conflicts were solved through consensus and a third researcher.

Statistical analysis

For the diagnostic meta-analysis, we extracted true positive, false positive, true negative, and false negative from each eligible study. The ORs and 95% CIs were extracted or calculated to evaluate the strength of the association between *ITGA4* methylation and cancer risk. The pooled odds ratio was computed using the method of Peto because of the sparseness of contingency tables (32,33). Synthesizing odds ratios were done using random effects meta-analysis. Heterogeneity across the enrolled studies was evaluated by Cochran's Q-statistic and I^2 statistic was used to estimate the heterogeneity of the studies in the meta-analysis (34). Subgroup analyses and sensitivity analyses were also performed to explore the source of heterogeneity. To further assess the diagnostic performance of *ITGA4* methylation, we calculated pooled sensitivity and specificity and we also drew the summary receiver operator characteristic (SROC) curve (35). Deeks' funnel plot asymmetry test was performed to assess the potential publication bias. A significant probability level was considered $P < 0.05$. Meta-analysis was performed using the metaphor package in R 3.5 (37) and Meta-Disc 1.4.

RESULTS

Search results

In the primary search based on the terms, 127 pieces of literature were identified from electronic databases (PubMed, Embase, Web of Science, Cochrane Library, and Scopus) and manual search. All studies were exported into EndNote software and two investigators (S. Jafarpour and N. Vatandoost) independently selected the studies. Subsequently, 58 studies remained after the removal of duplicates and irrelevant titles and/or abstracts. By reviewing the full text of the articles, a total of 13 studies met the inclusion criteria for the current meta-analysis, and others were removed for the following reasons: studies with insufficient data, reviews, non-human studies, and studies unrelated to this meta-analysis. Any conflicts were solved through discussion with a third researcher (R. Salehi). Fig 2 shows the flow of the chart of the study selection process.

Study characteristics and quality assessment

In the current meta-analysis, a total of 1232 tumor samples and 649 non-tumor samples were analyzed. The included studies reported about six types of cancers, including CRC (n = 6), gastric cancer (n = 2), breast cancer (n = 1), cholangiocarcinoma (n = 1), prostate cancer (n = 1), oropharyngeal squamous cell carcinoma (n = 1), and bladder cancer (n = 1). Methylation-specific polymerase chain reaction (MSP) and quantitative methylation-specific polymerase chain reaction (QMSP) were used to measure the *ITGA4* methylation status in all the included studies. Samples in the studies included stool, plasma, and tissue.

About the ethnicity of the patients; 8 Asian studies, 2 Caucasians studies, and 3 mixed populations studies. More details of the studies are shown in Table 1. Based on the QUADAS assessment, all included studies met the quality requirements based on the guideline (score ≥ 9).

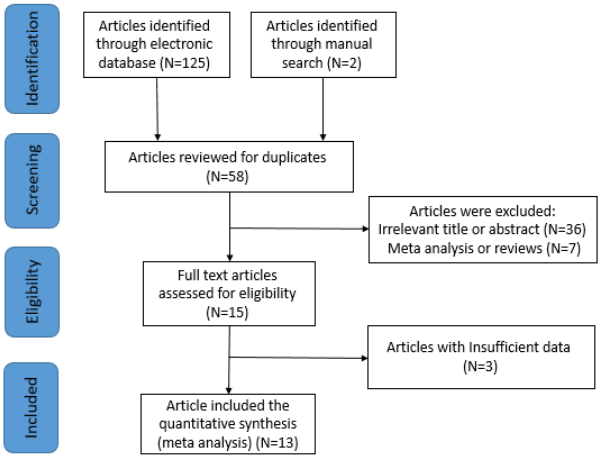

Fig. 2. The flow chart of study selection.

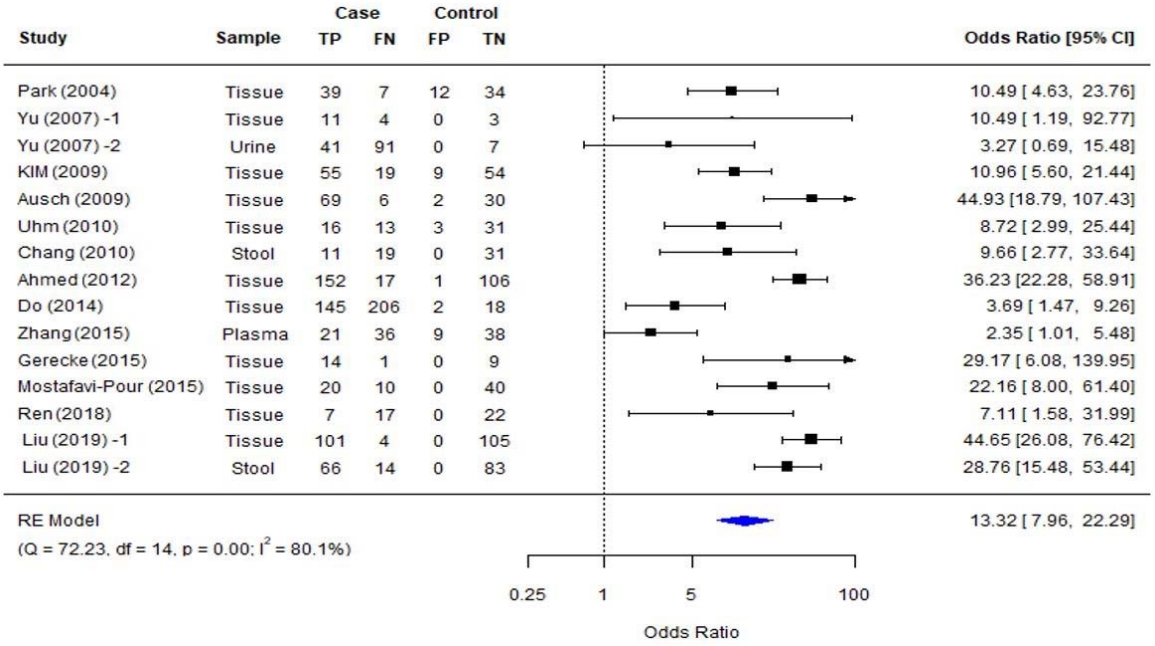

Fig. 3. Forest plot of *ITGA4* methylation in tumor cases and non-tumor controls. ITGA4, Integrin subunit alpha 4.

Meta-analysis of ITGA4 methylation in all types of tumors

ITGA4 promoter methylation was assessed among a total of 1232 cases and 649 control samples from 13 studies; two of these studies (29,38) analyzed both tissue and stool samples of the patients which are the reasons for considering the analysis of tissue and stool samples separately. Further analysis indicated high heterogeneity in the current meta-analysis (I^2 = 80.1%). Therefore, a random-effect model was applied for the current meta-analysis. Our results showed a significant association of *ITGA4* methylation with cancer (OR = 13.32, 95%, CI = 7.96-22.29, I^2=80.1; Fig. 3). The SROC curve indicated that *ITGA4* methylation might be a promising biomarker for tumor diagnosis (AUC = 0.94, Fig. 4A; pooled sensitivity = 0.95, 95%, CI = 0.94-0.97, pooled specificity = 0.57, 95% CI = 0.54-0.60, Fig. 4B).

To investigate some sources of heterogeneity, we performed a subgroup analysis according to ethnicity, tumor type, detection method, and sample type (Table 2). Comprising 4 studies, QMSP (OR = 34.26, 95%, CI = 26.25-46.49, I^2 = 0.0%) resulted in a higher diagnostic value and higher sensitivity and specificity compared with the MSP method (OR = 9.77, 95% CI = 5.51-17.32, I^2 = 69.7%). The diagnostic value of *ITGA4* methylation as a biomarker for cancer was not substantially different between Caucasian and Asian ethnicity. Overall, the assessment of studies used tissue samples (OR = 16.66, 95%, CI = 9.77-28.41, I^2 = 74.9%) for *ITGA4* methylation showed a higher diagnostic value compared to other kinds of samples (OR = 7.22, 95%, CI = 2.16-24.16, I^2 = 83.3%) along with a higher sensitivity (0.96 *vs* 0.94) and specificity (0.60 *vs* 0.50).

Diagnostic value of ITGA4 for colorectal tumors

Our meta-analysis showed that *ITGA4* methylation could be used as a diagnostic biomarker for CRC (OR = 20.77, 95% CI = 9.15-47.15, I^2 = 87.8%; Fig. 4). Subsequently, we estimated the diagnostic values of *ITGA4* methylation in CRC tissues and other types of samples (stool, urine, and plasma), respectively. There was a higher pooled odds ratio using *ITGA4* methylation as a diagnostic biomarker for CRC in tissue (OR = 39.49, 95% CI = 28.83-55.33, I^2 = 0.0%) compared to other types of samples (OR = 8.83, 95% CI = 2.00-39.08, I^2 = 88.5%; Fig. 5).

Table 1. Study characteristics included in the meta-analysis.

First author (year)	Country	Ethnicity	Sample	Method	Tumor type	Case			Control		
						Age of cases	TP	Total case	Age of controls	FP	Total control
Park (2004)	Korea	Asian	Tissue	MSP	Gastric	NA	39	46	NA	12	46
Yu (2007)	China	Asian	Tissue	MSP	Bladder	63.4 (34-88)	11	15	55.7 (16-83)	0	3
Yu (2007)	China	Asian	Stool	MSP	Bladder	NA	41	132	NA	0	7
KIM (2009)	Korea	Asian	Tissue	MSP	Gastric	57.7	55	74	NA	9	63
Ausch (2009)	USA &	Mix	Tissue	MSP	Colorectal	62 (35-80)	69	75	NA	2	32
Chang (2010)	Korea	Asian	Stool	MSP	Colorectal	61.7 ± 7.5	11	30	58.8 ± 10.2	0	31
Uhm (2010)	Korea	Asian	Tissue	MSP	CC	57 ± 10.42	16	29	55 ± 11.53	3	34
Ahmed (2012)	Norway	Mix	Tissue	QMSP	Colorectal	71 (33-92)	152	169	67(63-72)	1	107
Do (2014)	Korea	Asian	Tissue	MSP	Breast	25-83	145	351	NA	2	20
Zhang (2015)	China	Asian	plasma DNA	MSP	Colorectal	56.64 ± 8.27	21	57	61.40 ± 12.41	9	47
Gerecke (2015)	Germany	Caucasian	Tissue	MSP	Colorectal	71.7	14	15	NA	0	9
Mostafavi-Pour	Iran	Caucasian	Tissue	MSP	Prostate	NA	20	30	NA	0	40
Ren (2018)	USA	Mix	Tissue	QMSP	OPSCC	55.2 (9.0)	7	24	29.4 (9.6)	0	22
Liu (2019)	China	Asian	Tissue	QMSP	Colorectal	59 (26-82)	101	105	58 (45–77)	0	105
Liu (2019)	China	Asian	Stool	QMSP	Colorectal	60 (46-78)	66	80	59 (48–74)	0	83

ITGA4, Integrin subunit alpha 4; MSP, methylation-specific polymerase chain reaction; QMSP, quantitative methylation-specific PCR; OPSCC, oropharyngeal squamous cell carcinoma NA not available; CC, cholangiocarcinoma; TP, true positive; FP, false positive.

Table 2. Subgroup analysis of *ITGA4* methylation as a diagnostic biomarker for cancer.

Subgroup	Number of studies	OR (95%CI)	I^2%	Q	Q_p	AUC (SE)	Sensitivity (95% CI)	Specificity (95% CI)
Overall	15	13.32 (7.96,22.29)	80.1	72.23	< 0.001	0.94 (0.039)	0.95 (0.94, 0.97)	0.57 (0.54, 0.60)
Tumor type								
Colorectal	7	20.77 (9.15,47.15)	87.8	40.55	< 0.001	0.59 (0.297)	0.97 (0.95, 0.99)	0.81 (0.77, 0.84)
Others	8	8.75 (5.66,13.52)	25.9	8.82	0.2655	0.94 (0.113)	0.99 (0.97, 1.00)	0.90 (0.86, 0.93)
Race								
Caucasian	5	8.75 (5.65,13.52)	25.9	8.82	0.2655	0.88 (0.176)	0.99 (0.98, 1.00)	0.80 (0.75, 0.85)
Asian	10	9.67 (5.12,18.25)	80.4	53.61	< 0.001	0.89 (0.063)	0.93 (0.90-0.95)	0.45 (0.41-0.48)
Detection method								
MSP	11	9.77 (5.51,17.32)	69.7	33.36	< 0.001	0.89 (0.044)	0.92 (0.90, 0.95)	0.42 (0.38, 0.45)
QMSP	4	34.26 (26.25,46.49)	0.0	5.49	0.1393	0.99 (0.085)	1.00 (0.98,1.00)	0.86 (0.81, 0.90)
Sample								
Tissue	11	16.66 (9.77, 28.41)	74.9	40.56	< 0.001	0.95 (0.036)	0.96 (0.94, 0.97)	0.60 (0.56, 0.63)
Others	4	7.22 (2.16, 24.16)	83.3	24.31	< 0.001	0.88 (0.208)	0.94 (0.89, 0.97)	0.50 (0.44, 0.55)

ITGA4, integrin subunit alpha 4; Q, Cochran's statistic; Q_p, corresponded *P*-value of Cochran's statistics; SE, standard error; OR, odds ratio; AUC, area under the curve; CI, confidence interval.

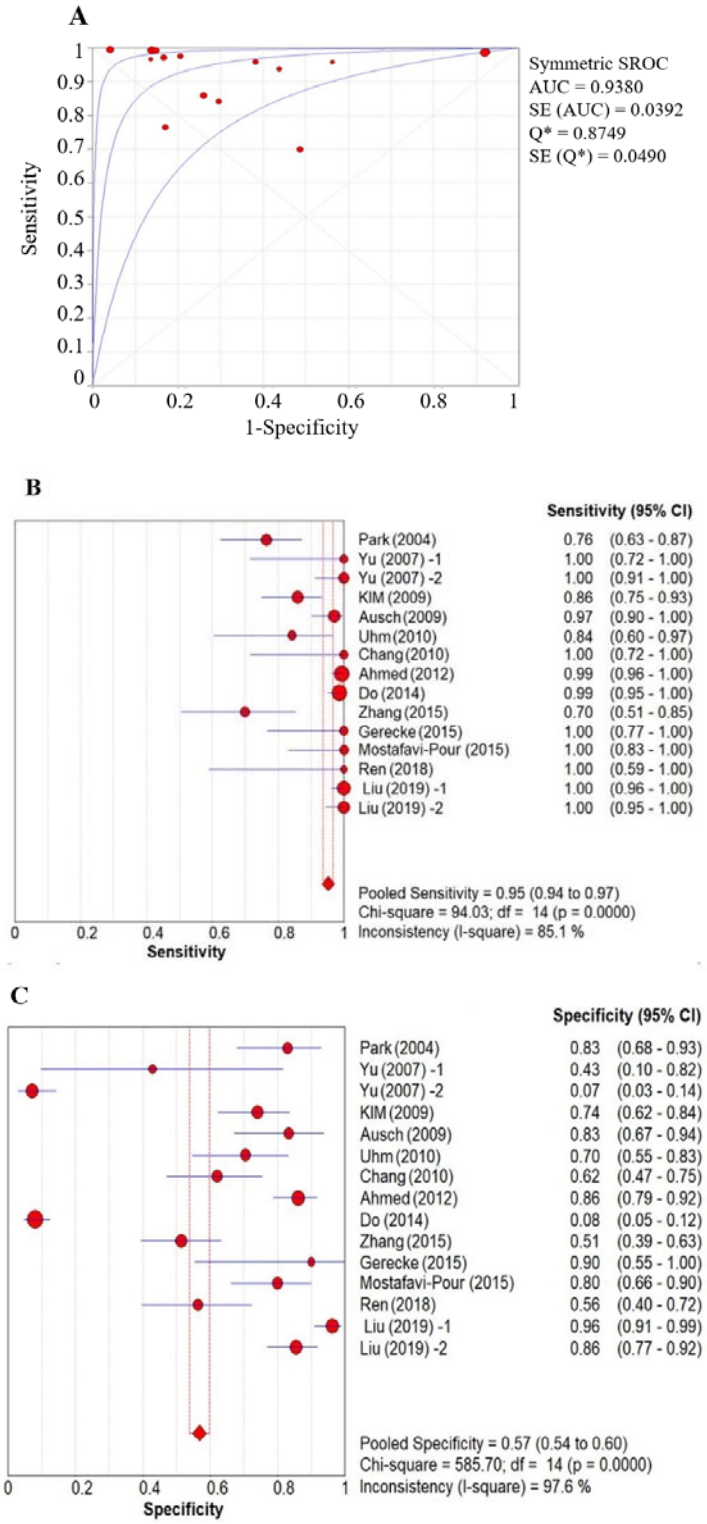

Fig. 4. (A) Summary receiver operating characteristic curves, (B) sensitivity, and (C) specificity of *ITGA4* methylation as a diagnostic biomarker for cancer. ITGA4, Integrin subunit alpha 4; AUC, the area under curve; SE, standard error.

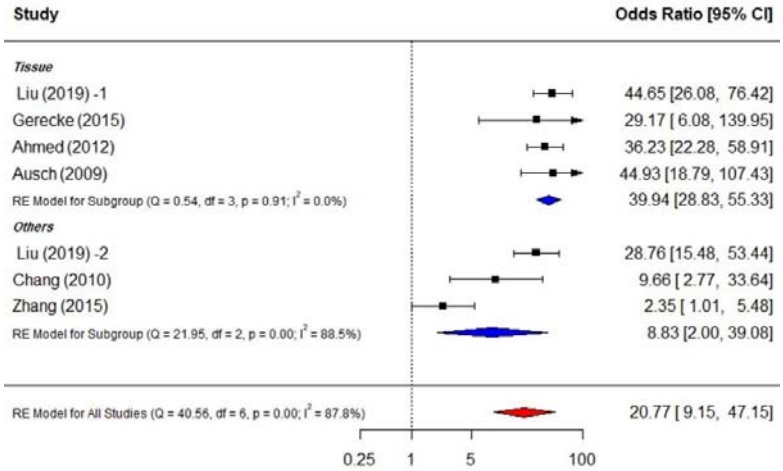

Fig. 5. Forest plot of *ITGA4* methylation for diagnosis of colorectal cancer based on tissue and other type of samples. ITGA4, Integrin subunit alpha 4.

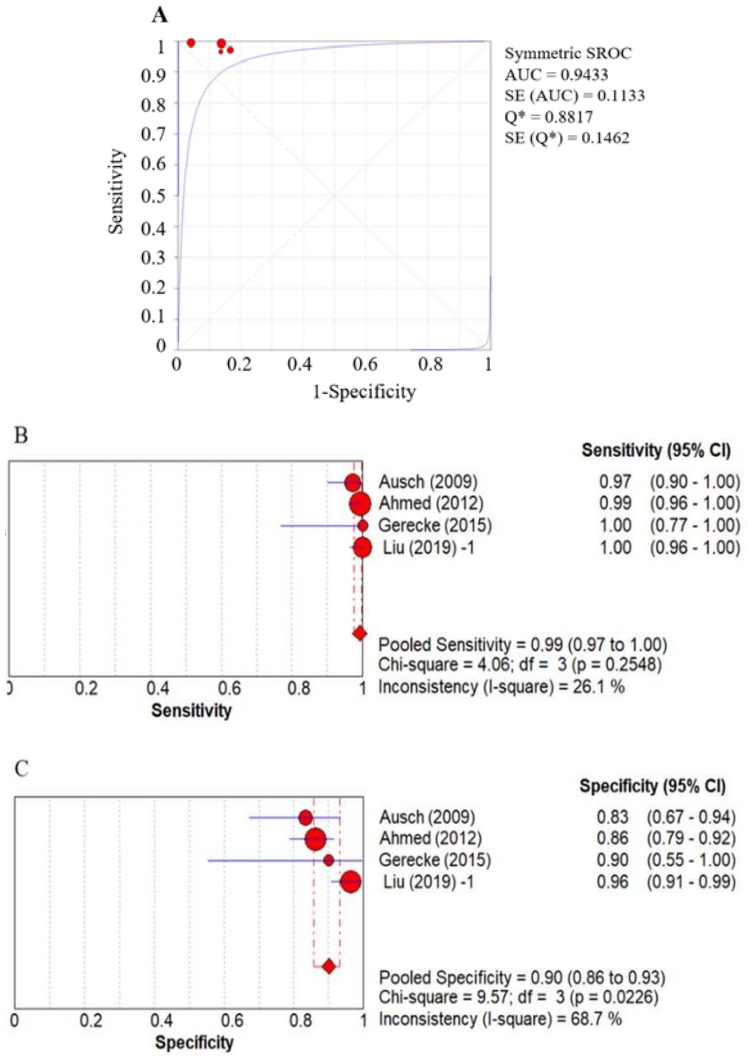

Fig. 6. (A) SROC curves, (B) sensitivity, and (C) specificity of *ITGA4* methylation in tissue as a diagnostic biomarker for CRC. SROC, Summary receiver operating characteristic; ITGA4, integrin subunit alpha 4; AUC, area under the curve; SE, standard error.

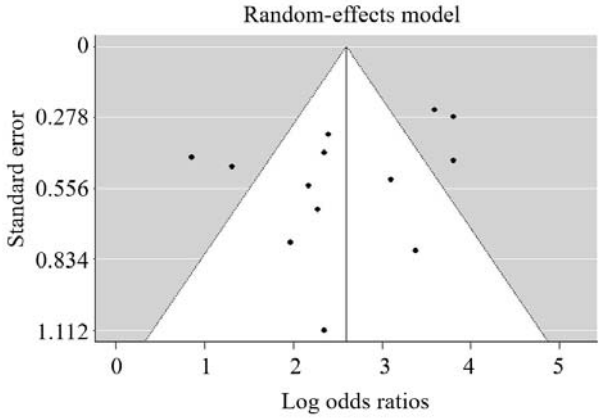

Fig. 7. Funnel plot of assessment of publication bias in the association of *ITGA4* methylation with cancer risk. ITGA4, integrin subunit alpha 4.

Figure 6 shows a further assessment of the diagnostic value of *ITGA4* methylation in tissue samples. There was a pooled sensitivity of 0.99 (95% CI = 0.97-1.00; Fig. 5B), pooled specificity of 0.90 (95% CI = 0.86-0.93; Fig. 6C), and an AUC of 0.94 (Fig. 6A) when CRC tissue sample was used for *ITGA4* methylation assessment.

Publication bias and sensitivity analysis

The funnel plot indicated that there was no publication bias in our meta-analysis (Fig. 7). The rank correlation test (Kendall's tau = -0.11, $P = 0.68$) and regression test for funnel plot asymmetry (z = -1.41, $P = 0.157$) did not infer to publication bias, too. We perform a sensitivity analysis by deleting each study one time and recomputing pooled odds ratio. Results did not differ significantly in pooled effect and heterogeneity.

DISCUSSION

The present meta-analysis was conducted to clarify the relationship between *ITGA4* promoter methylation with CRC and other cancers. Thirteen studies consisting of data from a total of 1232 tumor samples and 649 non-tumor control samples were systematically analyzed. The main outcome of the study is indicative of *ITGA4* methylation which is more frequently detected in tumor samples than that of normal controls. Also, the AUC for the SROC curve was 0.94 indicating that *ITGA4* methylation could be considered a single biomarker for tumor diagnosis.

In order to reduce heterogeneity, sub-group and meta-regression analyses were conducted according to the tumor types, detection methods, ethnicity, and type of samples. We performed a stratified analysis based on the method used for methylation detection (Table 2). AUC and sensitivity were well comparable but specificity emerged differently between MSP and QMSP methods (Table 2). Methylation assessment methods are typically chosen based on the study design as well as the technology available in the research facility (39). However, in the present study based on our stratified analyses results, we decided to include papers that adopted the MSP method as well as the QMSP method.

Subsequently, subgroup analysis according to ethnicity revealed that the diagnostic value of *ITGA4* methylation was not substantially different between Caucasian and Asian ethnicity suggesting that the methylation status of *ITGA4* in cancer could be ethnically independent. We also analyzed *ITGA4* methylation status in all types of samples (stool, urine and plasma, and tissues) and concluded that the predictive value of *ITGA4* methylation in tissue samples was higher than in other kinds of samples.

Besides DNA mutations and genetic changes, epigenetic alterations are involved in cancer initiation and tumorigenesis (40). Chemical modifications of DNA, chromatin remodeling, and histone modifications are common epigenetic events involved in the pathogenesis of human cancers (41,42). Among these epigenetic modifications, DNA methylation has a prominent role and is considered an early signature of malignant tumors (43).

Given the multiple functions of integrins in malignant transformation and metastasis, it is clear that different results have been reported regarding the *ITGA4* methylation status in malignancies (19,44). High levels of *ITGA4* play a dual role in cancer, preventing cancer cells from separating and invading, and facilitating metastasis of cancer cells by binding to surface ligands in endothelial cells (22). *ITGA4* expression is found in a subset of breast

cancer. Hypermethylation of *ITGA4* is strongly associated with HER2-positive tumors. The presence of α4 in *ITGA4* appears to be not beneficial for HER2-positive tumors, although its signaling pathways and molecular mechanism are not fully investigated (45). *ITGA4* gene methylation status and gene expression pattern in CLL was examined by Attia *et al.* who stated that the *ITGA4* expression pattern appears to be regulated at the mRNA level by local methylation CpG sites. Methylation of *ITGA4* in CpG sites-2 and 3 was detected in CLL patients with del13q14+ and hypermethylation of *ITGA4* in CpG sites-1 can be a potential prognostic biomarker for CLL patients (15). Aberrant promoter methylation of the *ITGA4* gene was reported in different malignancies including CRC (46,47), cholangiocarcinoma (48), breast (23), and gastric tumors (49). To the best of our knowledge, this is the first diagnostic meta-analysis to evaluate the diagnostic value of *ITGA4* methylation for CRC and other malignancies and our result here showed that *ITGA4* could be effective for the diagnosis of cancer.

A considerable number of included articles in our meta-analysis were related to colorectal cancer. It seems that the methylation of *ITGA4* is more widespread in CRC than in other cancers. Therefore, we performed a similar analysis to evaluate the diagnostic values of *ITGA4* methylation in CRC patients. The outcomes indicated that *ITGA4* methylation may be a more reliable diagnostic biomarker for CRC than other cancers. Pulkka *et al.* showed that *ITGA4* is the main integrin in the pathogenesis of gastrointestinal stromal tumors and is associated with poor overall survival (20). Our results may support a model that DNA methylation mediates downregulation and are consistent with the study carried out by Mo *et al*. (25), which revealed that downregulation of *ITGA4* was associated with poor prognosis in CRC patients and *ITGA4* could be an early predictor of CRC.

The gene methylation frequency in the various stages of the adenoma-carcinoma sequence changes in a gene-specific manner (50). Consequently, sensitive and precise diagnostic markers are needed that can be used to control early adenoma patterns for the sequence of carcinomas in the colon epithelium. Ausch *et al*. observed that in 75% (27 out of 36) of adenomas, *ITGA4* was hypermethylated and it is a suitable biomarker for the early detection of colonic neoplasms (51). The *ITGA4* promoter methylation is an early and recurrent phenomenon of precancerous and cancerous lesions. In addition, methylation appears in colon tissue in individuals with severe inflammation and has not been reported in any sample with a normal colon (27). It was obvious that during colorectal carcinogenesis, *ITGA4* as putative early markers tended to be methylated slightly earlier and our meta-analysis also suggested that *ITGA4* promoter methylation is a useful marker for the early diagnosis of neoplastic lesions in colitis-associated cancer.

However, the following points may be accounted as limitations of our meta-analysis. First, we observed notable heterogeneity in our results. This heterogeneity may be influenced by the difference in tumors types, ethnicity, experimental methods, and sample differences. Second, limiting the language of study to English might also induce publication bias. Third, due to the insufficient information about clinicopathological features in the included studies, the association of *ITGA4* methylation with clinicopathological characteristics has not been done. Fourth, due to the limited number of studies, we pooled all tumor types in our analysis, therefore the conclusion we presented is based on the pooled data analysis and should be confirmed in the case of each cancer independetly. However, we have minimized biases by heterogeneity analysis.

CONCLUSION

Methylation of the *ITGA4* gene is associated with a variety of malignancies. *ITGA4* methylation has a good potential to be a possible broad-spectrum epigenetic screening marker for the diagnosis of cancers at early stages and could be effective in the future for CRC screening.

Acknowledgments

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

R. Salehi contributed to the conceptualization; M. Yazdi contributed to data curation; S. Jafarpour and R. Nedaeinia contributed to the investigation; R. Salehi contributed to the methodology; S. Jafarpour and N. Vatandoost wrote the original draft of the article; G.A. Ferns revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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