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Association between *GPX3* promoter methylation and malignant tumors: A meta-analysis

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ABSTRACT

Glutathione peroxidase 3 (GPX3) has an important function of scavenging hydrogen peroxide and preventing cancer. The purpose of this meta-analysis was to analyze the relationship between *GPX3* gene methylation and cancer and to further evaluate its diagnostic value for cancer. We screened eligible literatures from the PubMed, Embase, CNKI and Wanfang databases. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to measure the association of *GPX3* methylation with cancer. Summary receiver operating characteristics (SROC) analysis was used to assess the diagnostic value of *GPX3* methylation for cancer. A total of 17 eligible articles were included in the meta-analysis involving a total of 960 tumor samples and 445 non-tumor samples. The results showed that *GPX3* hypermethylation was significantly associated with cancer (OR = 17.32, 95% CI = 8.22–36.51, $P < 0.00001$). Compared with cancer patients without lymph node metastasis, cancer patients with lymph node metastasis were more associated with *GPX3* hypermethylation (OR = 2.97, 95% CI = 1.53–5.76, $P = 0.001$). SROC analysis showed for *GPX3* methylation was a promising biomarker for cancer risk (AUC = 0.89, pooled sensitivity = 0.93, pooled specificity = 0.54, NLR = 0.15, PLR = 2.05, DOR = 17.32). TCGA database bioinformatics analysis of 696 pairs of tumor and non-tumor tissues further validate the association of *GPX3* methylation with the risk of cancer [cg21504918: 0.10 (0.08, 0.15) vs. 0.09 (0.08, 0.11), $P = 5.8E-28$; cg26638444: 0.05 (0.04, 011) vs. 0.04 (0.03, 0.06), $P = 8.7E-29$]. In summary, our study indicates that *GPX3* methylation is associated with cancer and has the potential to become a broad-spectrum tumor screening marker and has a value in predicting tumor lymph node metastasis.

1. Introduction

In 2018, there were 18.1 million new cases of cancer patients and 9.6 million cancer deaths, so cancer has become the world's second leading cause of death [1]. The prevention and treatment of cancer depends on early detection, early diagnosis and early treatment. Therefore, high-efficiency tumor markers, sensitive diagnostic methods and targeted therapeutic techniques are the main entry points for researchers to seek breakthroughs [2]. The mechanism of tumor occurrence is complex, mainly including genetic and environmental factors [3]. Recent evidence suggests that abnormal DNA methylation is one of the important molecular mechanisms of tumorigenesis and development [4], and is expected to become a new means of tumor screening, diagnosis and treatment [5].

Glutathione peroxidase 3 (GPX3) is the most widely studied member of the glutathione peroxidase family [6], whose main function is to act as a by-product of phosphonic peroxides in the elimination of cellular oxidative metabolism by electron donors. And fatty acid hydroperoxide, reducing the accumulation of hydrogen peroxide in the body [7–9]. Numerous studies have shown that glutathione peroxidase is involved in the development and progression of tumors. Abnormal inactivation or low expression of *GPX3* may induce tumorigenesis due to excessive reactive oxygen species (ROS) including hydrogen peroxide, which may lead to tumorigenesis [10–12]. In addition, the main biochemical role of hydrogen peroxide is to regulate the characteristics of cancer cells, including proliferation, invasion, migration, angiogenesis and apoptosis, so it is currently believed that *GPX3* can regulate cancer progression by regulating the level of intracellular hydroperoxide. An RNA transcriptome sequencing report containing 27 different tissues suggests that *GPX3* expression in normal humans is tissue specific [13], while a study by Chen B et al based on six tumor types and 63 human tumor cell lines confirmed that *GPX3* was highly expressed in normal non-tumor cell lines, and the expression rate of *GPX3* was significantly reduced or even could not be detected in the malignant tumor cell lines

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of different cancer types [14].

DNA methylation is one of the important mechanisms leading to silencing of gene expression [15]. At present, there is no systematic understanding of the relationship between *GPX3* methylation and various tumors. The experimental subjects have obvious ethnic and regional differences, and the experimental methods are also different. The experimental materials include tissues, plasma or cell lines. Therefore, the purpose of this meta-analysis is to summarize the existing literature reports, to explore the possible effects of *GPX3* gene methylation on malignant tumors, and to further analyze the diagnostic value of *GPX3* gene methylation for tumors.

2. Materials and methods

2.1. Literature screening

We used the keyword "(Glutathione Peroxidase 3 or GPX3) and (cancer or tumor or carcinoma or neoplasm) and (methylation or epigene*)" to conduct a comprehensive search of PubMed, Embase, CNKI and Wanfang databases. The search deadline was October 5, 2018. The inclusion criteria for valid literature were as follows: (1) This document contains case control groups; (2) This document is the original literature that clearly states the association between *GPX3* methylation and tumors; (3) Control samples must be from normal subjects or paratumor tissues or non-cancerous samples of patients; (4) The study has sufficient data to calculate true positives, false positives, true negatives, and false negatives; (5) When the same group of patients participated in the study multiple times, only the most complete data sets was used to avoid duplication. The literature exclusion criteria are as follows: (1) cell line-based studies; (2) incomplete data of case and control groups; (3) reviews. The flow chart of literature screening was shown in Fig. 1A.

2.2. Data collection and literature quality evaluation

Each study was extracted by two independent authors, including the author's last name, year of publication, country, ethnicity, study tumor type, sample type, and experimental results. In addition, we collected detailed information on assay methods, primer sequences, fragment size, annealing temperature, clinical features of the tumor, clinical phenotype of the patient, and etc. The details were summarized in Tables 1 and 2. Two authors assessed the quality of the literature according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) (Supplemental Table 1).

2.3. Meta-analysis

Review Manager 5 and Stata SE12.0 software were used to perform meta-analysis. Odds ratios (ORs) and corresponding 95% confidence intervals were used to assess the association between *GPX3* methylation and tumor risk or individual tumor subgroups, and based on independent OR values between studies and subgroups, final evaluation of pooled OR value. Select the appropriate effect model based on the heterogeneity statistic I^2. If $I^2 > 50\%$, we selected the random effects model. Otherwise, we selected the fixed effect model. Heterogeneity across the enrolled studies was evaluated by Cochran's Q-statistic ($P < 0.05$ was regarded as statistically significant, $P < 0.1$ was considered as a tendency for heterogeneity) [16]. $P < 0.05$ was considered as significant. All the P values were calculated under two-sided tests.

2.4. SROC analysis and DOR calculation

We used the Meta-Disc 1.4 software to calculate the Summary receiver operator characteristic (SROC) curve and output the pooled sensitivity and pooled specificity, along with the corresponding 95% confidence interval (95% CI). At the same time, since the diagnostic odds ratio (DOR) is an evaluation index that combines the sensitivity and specificity and is not affected by the disease incidence of the tested population [17], we further calculated the positive-likelihood ratio. (PLR), negative-likelihood ratio (NLR) and DOR.

2.5. TCGA database bioinformatics analysis

In order to further verify the association of *GPX3* methylation with cancer risk, we extracted the methylation data of *GPX3* in the TCGA pan-cancer (PANCAN) database (http://xena.ucsc.edu/). The methylation levels of paired tumor and non-tumor samples were compared by a nonparametric Wilcoxon paired test.

3. Results

Our meta-analysis included a total of 17 eligible articles involving 960 tumor samples and 445 non-tumor samples (Fig. 1A). As shown in Table 1, the meta-analysis involved 9 gastrointestinal tumor-related studies and 8 studies on other tumors. There were 15 tissue studies, 2 plasma studies. There were 14 semiquantitative studies (MSP), and 3 quantitative methylation studies. There were 10 Asian studies and 7 Caucasian studies. Of the 17 studies, 4 studies did not mention primer sequences in detail, and the remaining 13 studies indicated that the amplified *GPX3* fragments were located in the promoter (Table 1).

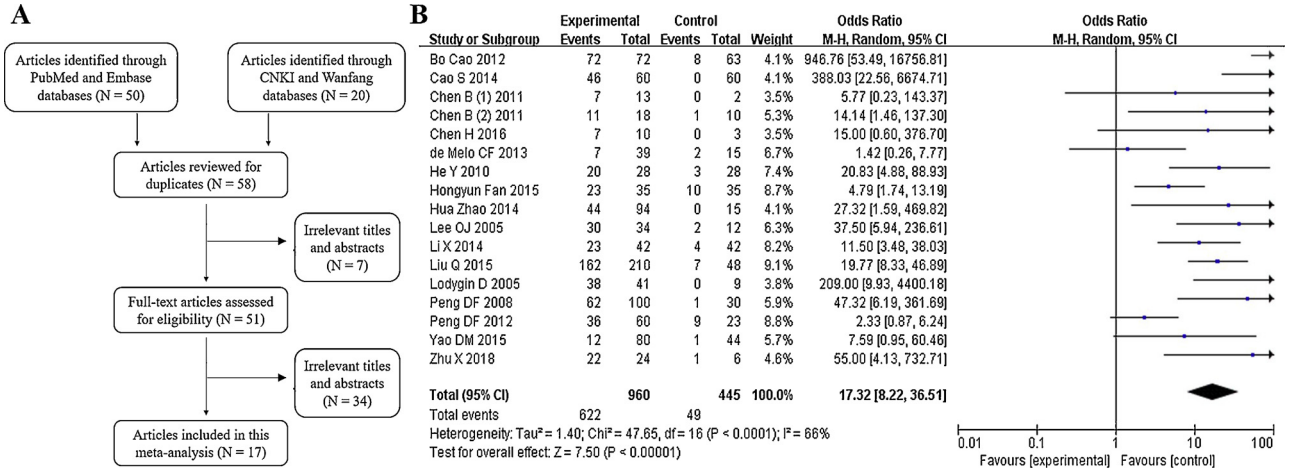

Fig. 1. Flow diagram and forest plot of eligible articles.
(A) Flow of study identification, inclusion, and exclusion. (B) Forest plot of *GPX3* different methylation rate between tumor cases and non-tumor controls.

Table 1
Parameters of all available studies.

First author	Country	Year	Ethnicity	Sample	Method	Tumor type	Case		Control	
							M+	total	M+	total
Bo Cao	China	2012	Asian	Tissue	MSP	Cervical cancer	72	72	8	63
Cao S	China	2014	Asian	tissue	MSP	Hepatocellular carcinoma	46	60	0	60
Chen B	USA	2011	Caucasian	Tissue	MSP	Bladder cancer	7	13	0	2
Chen B	USA	2011	Caucasian	Tissue	MSP	Head and neck cancer	11	18	1	10
Chen H	China	2016	Asian	Tissue	MSP	Melanoma	7	10	0	3
de Melo CF	Brazil	2013	Mix	Tissue	MSP	Gastric cancer	7	39	2	15
He Y	China	2010	Asian	Tissue	MSP	Esophageal squamous cell carcinoma	20	28	3	28
Hongyun Fan	China	2015	Asian	Plasma	MSP	Gastric cancer	23	35	10	35
Hua Zhao	China	2014	Asian	Tissue	MSP	Thyroid cancer	44	94	0	15
Lee OJ	USA	2005	Caucasian	Tissue	MSP	Barrett's adenocarcinoma	30	34	2	12
Li X	China	2014	Asian	Plasma	MSP	Esophageal squamous cell carcinoma	23	42	4	42
Liu Q	China	2015	Asian	Tissue	MSP	Renal cell carcinoma	162	210	7	48
Lodygin D	Germany	2005	Caucasian	Tissue	MSP	Prostate cancer	38	41	0	9
Peng DF	USA	2012	Caucasian	Tissue	Pyrosequencing	Gastric cancer	36	60	9	23
Peng DF	USA	2008	Caucasian	Tissue	Pyrosequencing	Barrett's adenocarcinoma	62	100	1	30
Yao DM	China	2015	Asian	tissue	BSP	Chronic myeloid leukemia	12	80	1	44
Zhu X	China	2018	Asian	Tissue	MSP	Esophageal squamous cell carcinoma	22	24	1	6

M+: the number of methylation; total: the number of case or control. MSP: Methylation-specific PCR. BSP: Bisulfite sequencing PCR.

In this study, we used the QUADAS assessment tool to evaluate the quality of the literature. As shown in Supplementary Table 1, the five articles in our meta-analysis did not clearly indicate the time interval between the trial to be evaluated and the gold standard experiment (item 4). All 17 articles did not mention whether the results of the trial to be evaluated were under the results of the gold standard test (item 10). In addition, the Deeks' funnel plot indicated that there was a publication bias in our meta-analysis ($P = 0.03$, Fig. 1).

As shown in Fig. 1B, tumor tissue had a higher *GPX3* methylation rate than normal tissue, and *GPX3* methylation was a risk factor for tumors (OR = 17.32, 95% CI = 8.22–36.51, $P < 0.00001$). It also showed that there was moderate heterogeneity in our meta-analysis ($I^2 = 66\%$). To analyze the source of heterogeneity, we divided 17 studies into two subgroups of gastrointestinal tumors and other tumors by tumor type (Fig. 2). Our subgroup analysis showed that gastrointestinal tumors were significantly associated with high *GPX3* methylation (OR = 11.09, 95% CI = 7.19–17.12, $P < 0.00001$), and other tumor groups were also associated with high *GPX3* methylation (OR = 29.22, 95% CI = 15.50–55.08, $P < 0.00001$). There was a high heterogeneity ($I^2 = 73\%$, $P = 0.0002$) in the meta-analysis of gastrointestinal tumor group, and there was no heterogeneity in the meta-analysis of other tumor group ($I^2 = 37\%$, $P = 0.13$). This suggests that

Table 2
Primers used to amplify bisulfite converted DNA at *GPX3* promoter regions.

Study	Year	Method	Forward	Reverse	Amplicon size (bp)	Annealing temperature (℃)
Bo Cao et al	2012	MSP	m5'-CGATTGGTTGTAAGGGTTTCGGTT-3'; u5'-TGATTGGTTGTAAGGGTTTTGGTT-3'	m5'-CTCAAAATCGCCTAAACCGCTAC-3'; u5'-CTCAAAATCACCTAAACCACTAC-3'	130	62
Cao S et al	2014	MSP	m5'-TTACGAGGGGCGGTCGTACGAGGG-3'; u5'-TTATGAGGGGTGGTTGCATGTGGG-3'	m5'-AAAACGACCGACGCGAACGCCTGC-3'; u5'-AAAACAATCAACACAAACACCTCC-3'	NA	m:65; u:60
Chen B et al	2011	MSP	NA	NA	NA	m:60; u:58
Chen B et al	2011	MSP	NA	NA	NA	m:60; u:58
Chen H et al	2016	MSP	m5'-TATGTTATTGTCGTTTCGGGAC-3'; u5'-TTTATGTTATTGTTTTGGGATG-3'	m5'-GTCCGTCTAAAATATCCGACG-3'; u5'-ATCCATCTAAAATATCCAACACTCC-3'	170	59
de Melo CF et al	2013	MSP	5′-CTGGAGARACTAAGYCCTCC-3′	5′-GAGGAATACTCATTGCGAAGGCGA-3′	150	59
He Y et al	2010	MSP	m5'-TATGTTATTGTCGTTTCGGGAC-3'; u5'-TTTATGTTATTGTTGTTTTGGGATG-3'	m5'-GTCCGTCTAAAATATCCGACG-3'; u5'-ATCCATCTAAAATATCCAACACTCC-3'	170	59
Hongyun Fan et al	2015	MSP	m5'-TATGTTATTGTCGTTTCGGGAC-3'; u5'-TTTATGTTATTGTTGTTTTGGGATG-3'	m5'-GTCCGTCTAAAATATCCGACG-3'; u5'-ATCCATCTAAAATATCCAACACTCC-3'	170	62
Hua Zhao et al	2014	MSP	m5'-TATGTTATTGTCGTTTCGGGAC-3'; u5'-TTTATGTTATTGTTGTTTTGGGATG-3'	m5'-GTCCGTCTAAAATATCCGACG-3'; u5'-ATCCATCTAAAATATCCAACACTCC-3'	170	55
Lee OJ et al	2005	MSP	m5'-GGTGGGGAGTTGAGGGTAAGTC-3'; u5'-GGTGGGGAGTTGAGGGTAAGTT-3'	m5'-CCTACAACAACCGAACCATAACGAAA-3'; u5'-CCTACAACAACCAAACCATAACAAAA-3'	219	m64; u60
Li X et al	2014	MSP	m5'-CGTTCGTTTTTGAAATTTTAGTC-3'; u5'-TGTTTGTTTTTGAAATTTTAGTTGT-3'	m5'-CTACCTAATCCCTAACCACCGT-3'; u5'-CTACCTAATCCCTAACCACCATC-3'	140	62
Liu Q et al	2015	MSP	m5'-TATGTTATTGTCGTTTCGGGAC-3'; u5'-TTTATGTTATTGTTGTTTTGGGATG -3'	m5'-GTCCGTCTAAAATATCCGACG-3'; u5'-ATCCATCTAAAATATCCAACACTCC-3'	170	59
Lodygin D et al	2005	MSP	NA	NA	NA	NA
Peng DF et al	2012	Pyrosequencing	5'-AGGTGGGGAGTTGAGGGTAA3'	Biotin-5'-TCCCAACCACCTTTCAAAC-3'	122	NA
Peng DF et al	2008	Pyrosequencing	NA	NA	NA	NA
Yao DM et al	2015	BSP	5'-ATTTTGGAGTTAAAAGAGGAAG-3'	5'-CTACCTAATCCCTAACCACC-3'	268	56
Zhu X et al	2018	MSP	m5'-CGTTCGTTTTTGAAATTTTAGTC-3'; u5'-TGTTTGTTTTTGAAATTTTAGTTGT-3'	m5'-CTACCTAATCCCTAACCACCGT-3'; u5'-CTACCTAATCCCTAACCACCATC-3'	240	NA

MSP, methylation-specific PCR; NA, not applicable.

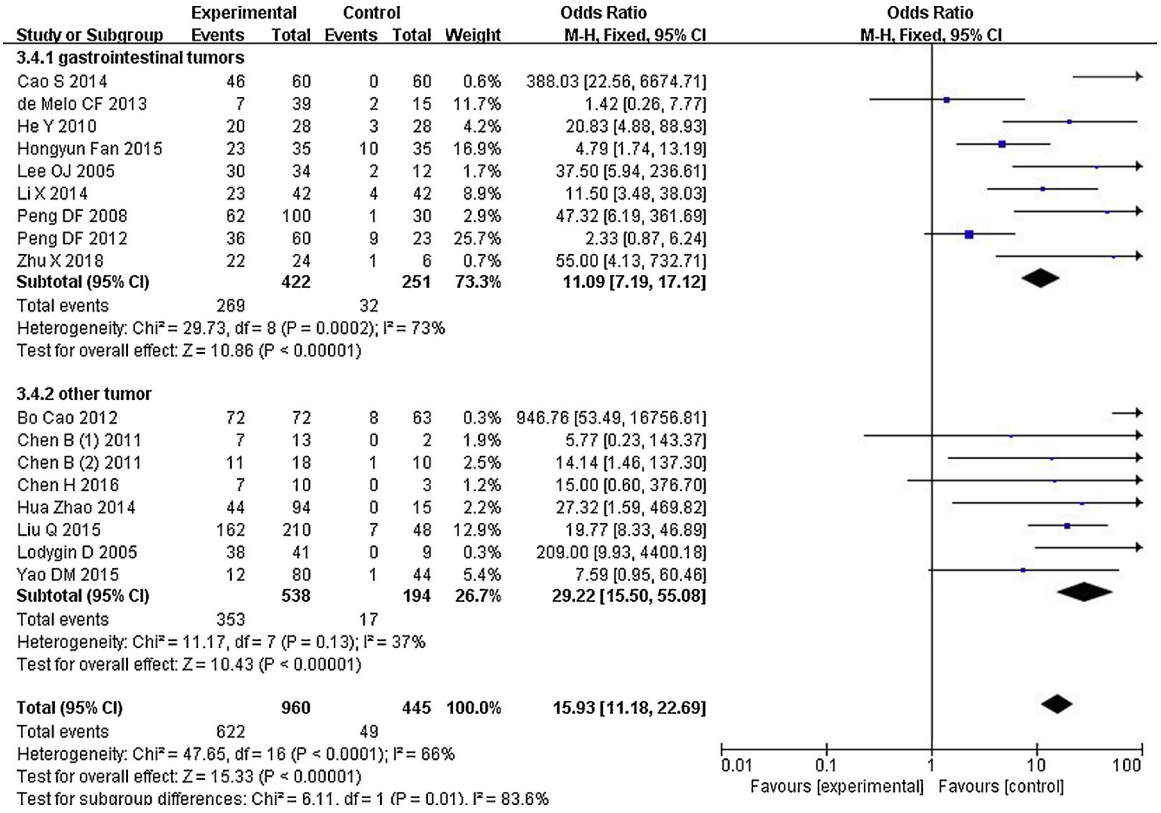

Fig. 2. Subgroup meta-analysis by different tumor types.
Effective literatures were stratified into groups of gastrointestinal tumors and other tumors.

heterogeneity is mainly derived from studies of gastrointestinal tumors.

In addition, we performed subgroup analyses for different races, experimental methods, and sample types (Table 3). Our results supported *GPX3* hypermethylation was associated with tumor risk. In addition, we stratified the subjects of this study according to different clinical phenotypes. The results showed that patients with lymph node metastasis had more significant *GPX3* methylation than patients without lymph node metastasis (OR = 2.97, 95% CI = 1.53–5.76, P = 0.001, Fig. 3A), while there were no differences of *GPX3* methylation between male and female cancer patients (P = 0.68, I^2 = 0%, Fig. 3B), and there was no difference in tumor patients with age greater than or equal to 60 and age less than 60 (P = 0.80, I^2 = 0%, Fig. 3C). The SROC curve indicated that *GPX3* methylation had an important diagnostic value for tumors (AUC = 0.89, Fig. 4A; pooled sensitivity = 0.93, pooled specificity = 0.54, Fig. 4B). In addition, the DOR results showed that the diagnostic test had a better discriminant effect (NLR = 0.15, PLR = 2.05, DOR = 17.32, Fig. 4C).

We used the TCGA database to further validate the association of *GPX3* methylation with the risk of cancer. As shown in Fig. 5, the DNA Methylation 450 K microarray in the TCGA pan-cancer (PANCAN) database contained 12 CpG sites on *GPX3* locus. At the same time, the amplified region in the methylation studies from our meta-analysis covered two CpG sites (cg21504918 and cg26638444). By comparing *GPX3* methylation data from 696 tumor tissues and 696 paired non-tumor tissues, we found that the two CpG sites (cg21504918 and cg26638444) were significantly hypermethylated in tumor tissues [cg21504918: 0.10 (0.08, 0.15) vs. 0.09 (0.08, 0.11), P = 5.8E-28; cg26638444: 0.05 (0.04, 011) vs. 0.04 (0.03, 0.06), P = 8.7E-29]. In addition, most other CpG sites were significantly hypermethylated in tumor tissues (Fig. 5).

Table 3
The association between *GPX3* methylation and ethnicity, detect method and sample type.

Variables	N	Test of association		Heterogeneity	
		OR (95% CI)	*P* Value	I^2 (%)	*P* Value
Overall	17	17.32 (8.22, 36.51)	< 0.00001	66%	< 0.0001
Ethnicity					
Asian	10	22.50 (14.42, 35.13)	< 0.00001	59%	0.008
Caucasian	7	8.41 (4.67, 15.14)	< 0.00001	71%	0.002
Method					
MSP	14	20.53 (13.61, 30.95)	< 0.00001	61%	0.002
Non-MSP	3	6.99 (3.37, 14.52)	< 0.00001	76%	0.02
Sample type					
Tissue	15	19.00 (12.62, 28.62)	< 0.00001	68%	< 0.0001
Plasma	2	7.11 (3.32, 15.22)	< 0.00001	17%	0.27

N: number of articles; OR: Odds Ratio; 95% CI: 95% Confidence Interval. MSP: Methylation-Specific PCR.

4. Discussion

This meta-analysis mainly explored the correlation between *GPX3* methylation and various tumors. The main result was that *GPX3* gene methylation was more likely to occur in tumor samples than non-tumor samples. Subgroup analysis suggested that patients with lymph node metastasis had more significant *GPX3* methylation than patients without lymph node metastasis. In addition, the SROC curve indicated that *GPX3* methylation had a high diagnostic value for tumors.

Numerous studies have confirmed that abnormal inactivation of *GPX3* may result in excessive hydrogen peroxide production and accumulation in tissue cells, causing DNA damage in tissue cells, and is closely related to tumor development [10–12,18]. The current studies showed that numerous genetic changes of *GPX3* gene in cancer, including mainly increased *GPX3* gene copy number in renal clear cell carcinoma [19], *GPX3* gene deletion in liver cancer [20], *GPX3* single nucleotide polymorphism in gastric cancer [21], rectal cancer [22],

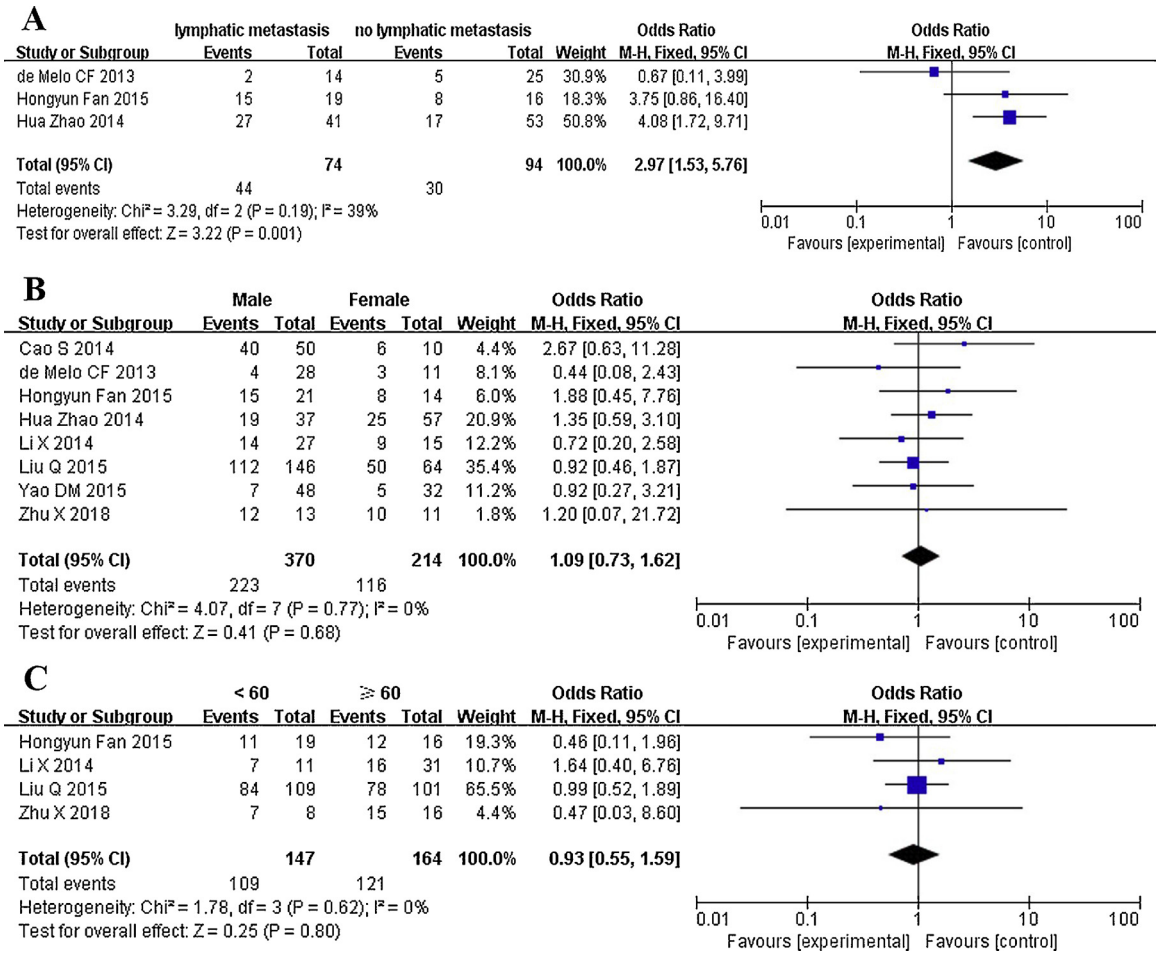

Fig. 3. Subgroup association tests in tumor group by gender, age and lymphatic metastasis.
(A) *GPX3* methylation in patients with or without lymphatic metastasis. (B) *GPX3* methylation in male and female tumor patients. (C) *GPX3* methylation in patients aged less than 60 and aged over 60.

thyroid cancer [23] and prostate cancer [24]. Besides, a number of studies were devoted to reporting aberrant methylation of *GPX3*. Our meta-analysis showed that tumor samples were more prone to higher *GPX3* methylation than non-tumor samples. Since gene methylation often leads to low expression of corresponding mRNA and protein [15], *GPX3* methylation may lead to the loss of expression of *GPX3* protein, which is likely to be an important mechanism leading to tumorigenesis.

Our study found that patients with lymph node metastasis had a higher rate of *GPX3* methylation compared with tumor patients without lymph node metastasis, and the tumor types included were gastric cancer and thyroid cancer. Studies have shown that low expression of *GPX3* by promoter hypermethylation can be used as a marker for lymphatic metastasis of cervical cancer [25] and gallbladder carcinoma [26]. Lymph node metastasis often indicates tumor progression and deterioration. Previously, Zhou JD et al. pointed out that *GPX3* hypermethylation was associated with poor prognosis in patients with myelodysplastic syndrome [27], and *GPX3* hypermethylation may serve as an independent prognostic marker for non-M3 acute myeloid leukemia [28]; Chen B et al confirmed that *GPX3* hypermethylation could be used as a prognostic indicator for head and neck cancer chemotherapy [14], indicating that *GPX3* aberrant methylation has a certain prognostic value. In addition, low expression of *GPX3* was also associated with tumor prognosis. For example, Qi X et al found that *GPX3* down-regulation indicates a lower overall survival rate for the liver cancer patients after hepatectomy [29]; Yang ZL et al found that low expression of *GPX3* was a poor prognosis in patients with gallbladder cancer [26]. These findings provide evidence for the prognostic value of *GPX3* methylation, and our meta-analysis also suggested that *GPX3* methylation may serve as a prognostic marker for tumor progression and progression.

The SROC curve in this study indicated that *GPX3* had a certain tumor diagnostic value, and its sensitivity and medium specificity indicated that *GPX3* methylation had the potential to become a broad-spectrum tumor screening marker. CEA is currently the most commonly used broad-spectrum tumor marker and can be elevated in a variety of malignancies, especially rectal cancer, pancreatic cancer, and breast cancer. However, the sensitivity of CEA for early diagnosis of tumor is not high [30]. For example, in the diagnosis of colorectal cancer, CEA had a sensitivity of 0.36, a specificity of 0.87 [31]; and CEA had a sensitivity of 0.40, and a specificity of 0.81 when diagnosing pancreatic cancer [32]; and the sensitivity and the specificity of CEA in the diagnosis of breast cancer was 0.58 and 0.87, respectively [33]. Therefore, clinically more sensitive markers than CEA are needed for early screening of tumors. In the present work, we calculated the DOR to eliminate the potential effects of differences in morbidity. Our results showed that the DOR of the *GPX3* methylation screening tumor was 17.32. In contrast, the DOR of CEA screening for breast cancer was 7.07 [33], the DOR of screening pancreatic cancer was 3.35 [34], and the DOR of screening for esophageal cancer was 9.26 [35]. Therefore, *GPX3* methylation may be a broad-spectrum tumor marker with higher diagnostic efficacy and has the potential to be an ideal indicator for early tumor screening.

TCGA PANCAN database involves 33 of the most common cancers. We extracted *GPX3* methylation data from 696 pairs of tumor and non-

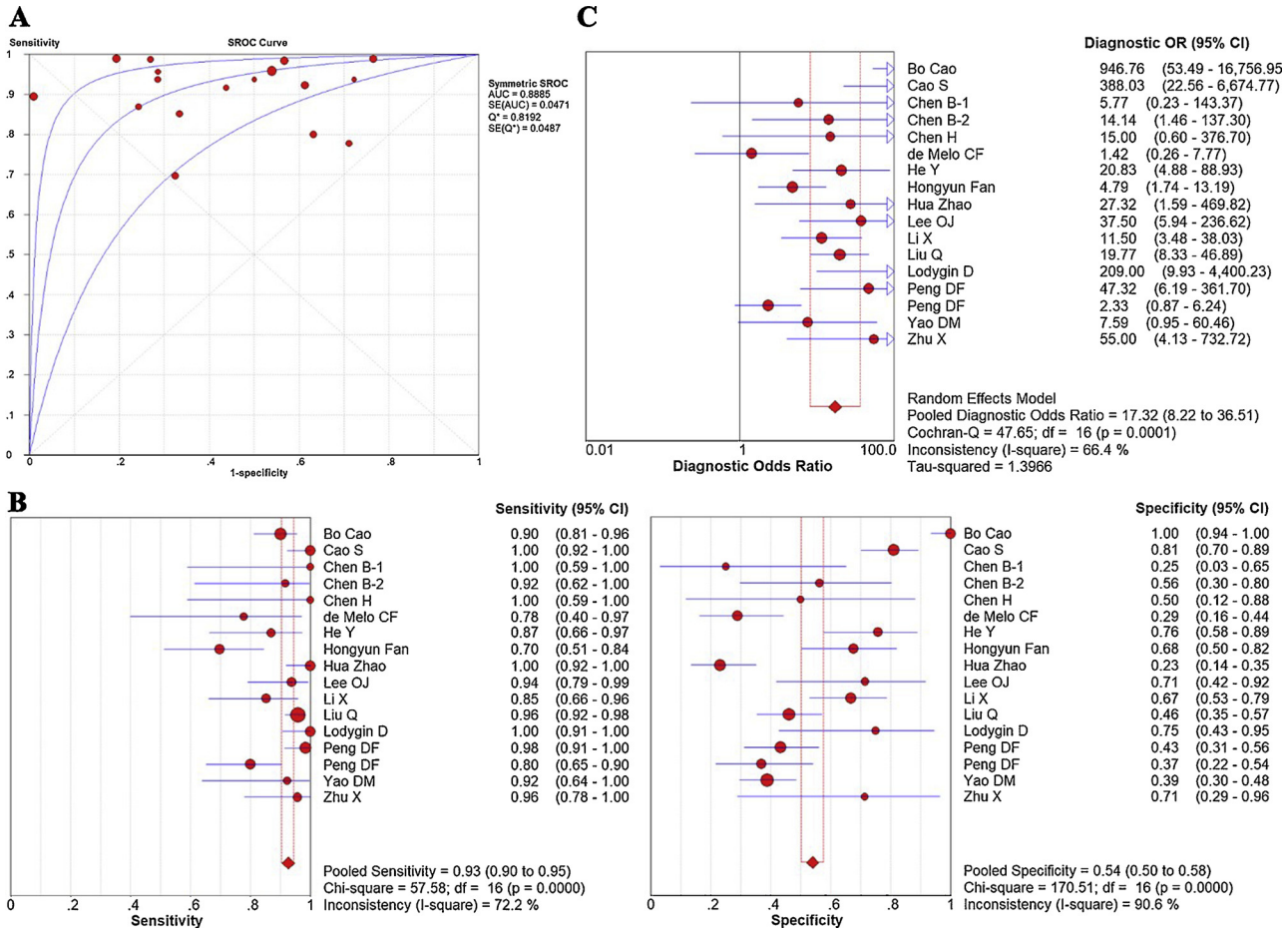

Fig. 4. Diagnostic value of *GPX3* methylation for tumor.
(A) Meta-analysis with the SROC curve, AUC = 0.89. (B) The pooled sensitivity (SENS) and specificity (SPEC) of *GPX3* methylation in diagnosing tumor, SENS = 0.93, SPEC = 0.54. (C) The forest plot of diagnostic odds ratio (DOR), pooled NLR = 0.15, pooled PLR = 2.05, pooled DOR = 17.32.

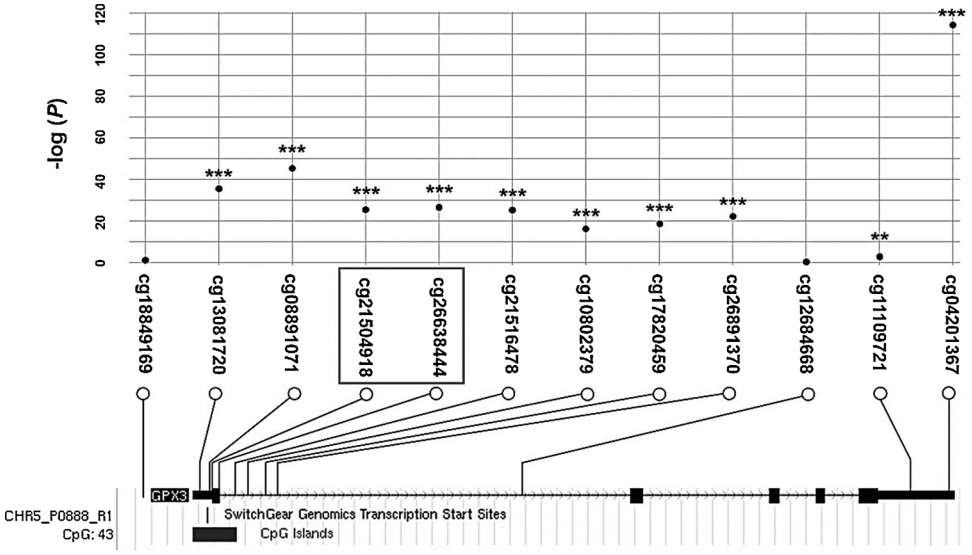

Fig. 5. Comparison of *GPX3* methylation between tumor and tumor-free tissues from TCGA Pan-Cancer Atlas database.
The DNA Methylation 450 K microarray in the TCGA PANCAN database contained 12 CpG sites on *GPX3* locus. The two CpG sites (cg21504918 and cg26638444) in the box were covered in the amplified region of the methylation studies from our meta-analysis. ***: $P < 0.0001$; **: $P < 0.001$.

tumor samples. Our analysis found that *GPX3* was significantly hypermethylated in cancerous tissues. This finding not only validates the results of our meta-analysis, but also provides a reference for the clinical detection sites of this broad-spectrum tumor marker.

However, there were some shortcomings in this study. First, the results of our meta-analysis are heterogeneous. The heterogeneity may be derived from different tumor types, different populations, different methylation detection methods, and different tissue sources. Second, due to the limited number of studies, the *GPX3* methylation ROC model incorporates all tumor types, and a more accurate assessment of the diagnostic value of a particular type of tumor should be made in the future. In addition, this meta-analysis has a mild publication bias due to

the inclusion of multiple tumor types, although we have minimized this by rigorous literature quality assessment and heterogeneity analysis, but this inevitable publication bias can still cause certain errors.

In summary, our study shows that *GPX3* methylation rate is associated with a variety of tumor risks. *GPX3* methylation has high sensitivity and DOR in tumor diagnosis, and has the potential to become a broad-spectrum tumor screening marker, and have certain value in the prediction of tumors with lymph node metastasis and tumor progression.

Conflicts of interest statement

The authors declare they have no conflict of interest.

Author contributions

SD, CZ and HH contribute to the conception, design and final approval of the submitted version. CZ, ZZ, CC and YL contribute to interpretation of data. HH and BL contribute to the completion of figures and tables. CZ and HH contribute to performing the data analyses. CZ and SD contribute to writing the paper. All the authors have read and approved the final manuscript.

Research involving human participants and/or animals

Our study followed all applicable international, national and/or institutional guidelines for animal care and use.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152443.

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