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Review Article

Epigenetic alterations in CHFR promoter hypermethylation as a cancer biomarker of digestive system carcinomas: a meta-analysis

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Abstract: Recently, it was reported that methylation of the checkpoint protein with forkhead-associated and ring finger domains (CHFR) gene could be related with some gastrointestinal cancers including gastric cancer and esophageal cancer. However, a couple of other studies had different results. The aim of this article was to perform a meta-analysis to have a better understanding of the possible association between the CHFR methylation and gastrointestinal cancers. Three frequently used databases, Pubmed, Medline and Web of Science, were searched and the 54 relevant articles were studied. Based on the specific criteria, 10 qualified articles were finally enrolled into this meta-analysis. The results revealed that the methylation of CHFR was significantly related with the risk of digestive system cancer (OR=4.21, 95% CI: 3.20-5.53). The results of subgroup analysis on disease type showed the methylation of CHFR was significantly associated with the risk of gastric cancer (OR=4.95, 95% CI: 3.66-6.71). Subgroup analysis on detection methods showed that the methylation of CHFR greatly increased the risks of gastrointestinal cancers detected by MSP (OR=8.02, 95% CI: 5.29-12.16). The results of Egger's test suggested that there was no evidence of publication bias (P=0.522). Therefore, this meta-analysis showed a significant association between the CHFR methylation and digestive system cancers, indicating that CHFR may be regarded as a diagnostic and therapeutic indicator.

Keywords: CHFR methylation, gastrointestinal cancers, CHFR methylation and digestive system carcinomas

Introduction

Gastrointestinal cancers have become a global problem recently [1]. About 3.4 million new diagnosed digestive cancer cases occurred in 2012 worldwide based on GLOBOCAN estimates [2]. It was once considered that tumor angiogenesis were only associated with the genetic mutation and environmental factors [3]. However, epigenetic abnormalities were proved to have a significant correlation with the initiation and development of cancers [4-6]. DNA methylation is currently recognized to be one of the essential epigenetic mechanisms and DNA hyper-methylation is involved in the formation and progress of digestive carcinomas [7].

The CHFR gene is located on chromosome 12q24.33, which is regarded as the cell-cycle checkpoint gene and the tumor suppressor gene as well [8]. The CHFR has the function to delay chromosome condensation and reduce the mitotic stress [9]. In view of biochemical function, CHFR is an ubiquitin ligase that involves cell cycle regulatory proteins for degradation. CHFR also contains an FHA domain in its N terminus, which is involved in phosphopeptide binding [8]. CHFR is widely expressed in normal tissues and the loss or reduced expression of CHFR due to hyper-methylation of a CpG island in the promoter region, which has been observed in tumor cell lines and digestive system carcinomas, such as gastric, esophagus and colon [10, 11].

The relationship between CHFR gene methylation and digestive carcinomas has been studied, however, the research results are inconsistent. Thus, it is necessary to conduct an up-to-date meta-analysis on the association between CHFR gene methylation and digestive carcinomas.

Table 1. Quality evaluation parameters and criterion [12, 13]

Parameter	Score		
	2	1	0
Sample size	> 100	50-100	< 50
Control source	Carcinomatous and adjacent tissues	Carcinomatous and normal tissues	Unclear
Detection for CHFR methylation	q-MSP	MSP	Others
Basic information	Adequate	Sectional	None
Diagnostic method	Pathology	Clinic diagnosis	Other methods

Abbreviation: MSP, methylation-specific PC; qMSP, quantitative methylation-specific PCR.

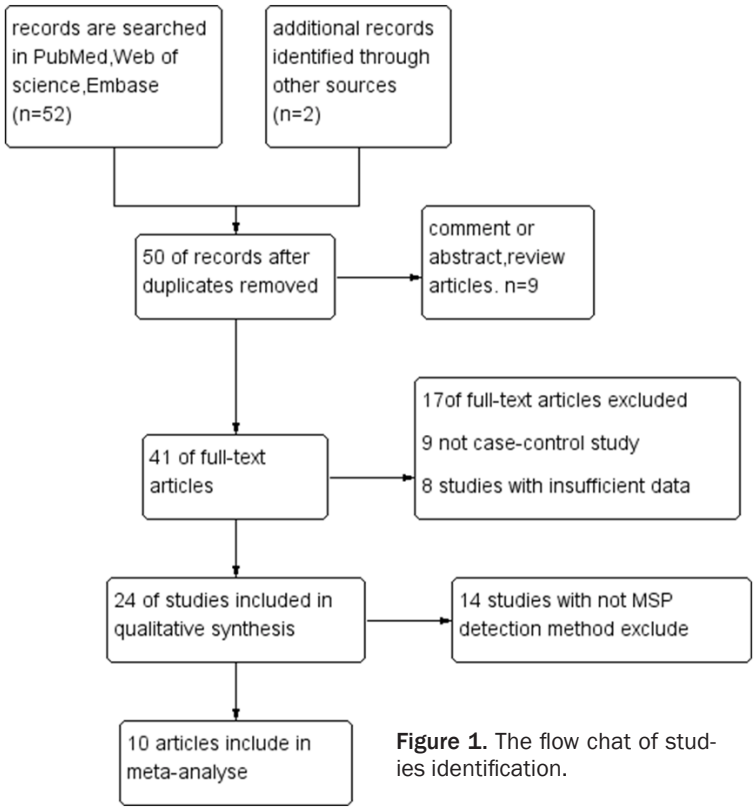

Figure 1. The flow chat of studies identification.

Materials and methods

Search strategy and data extraction

A detailed literature search strategy was performed using the keywords "CHFR methylation", "DNA methylation", "CHFR methylation and digestive carcinomas" to search all the related articles in Pubmed, Web of Science and Embase databases (updated on December 30, 2016). Two independent investigators screened the relevant articles using standardized screening guide. The eligible articles were enrolled into meta-analysis according to the inclusion and exclusion criteria. The general information of the eligible articles including the first author, publication year, original country, disease category, numbers of cases and controls, specimen source and diagnostic method were collected by two independent investigators.

Inclusion criteria

a. The included studies must be concentrated on the relationship between CHFR methylation and the digestive carcinomas. b. The methodology of case and control group must be the consistent in selected articles. c. Studies with enough data to calculate odds ratios and corresponding 95% confidence intervals (ORs, 95% CIs) were included. d. Cases are diagnosed by pathological diagnosis. e. Articles must be published in English or Chinese. All selected articles must be from Asian and the study materials must be tissues specimens. The detection method for CHFR methylation in each study must be MSP or q-MSP.

Exclusion criteria

a. Abstracts, letters, comments, editorials, reviews, single-case reports and family-based studies are excluded. b. The articles with insufficient data or overlapped data are excluded. c. The articles in which patients received chemotherapy or radiotherapy are excluded.

Quality assessment

We evaluated the quality of eligible studies in accordance with an improved 10-point scale, which is a suitable quality assessment method

Table 2. Characteristics of eligible articles

First Author	Publication year	Country	Continent	Case (n)	Control (n)	Cancer type	Sample source	Detection method	Score
HirakiM	2010	Japan	Asian	49	49	GC	T_1	q-MSP	8
Oki E	2009	Japan	Asian	59	59	GC	T_1	MSP	8
Homma N	2005	Japan	Asian	52	52	GC	T_1	MSP	8
Cheng ZD	2010	China	Asian	64	64	GC	T_2	MSP	9
Hu SL	2011	China	Asian	123	123	GC	T_2	MSP	8
Hu SL	2010	China	Asian	70	70	GC	T_1	MSP	9
Honda T	2004	Japan	Asian	71	71	GC	T_1	MSP	8
Hu SL	2009	China	Asian	42	42	GC	T_2	MSP	8
Xie K	2007	China	Asian	50	50	Liver cancer	T_1	MSP	8
Mei XY	2015	China	Asian	28	28	EC	T_2	MSP	8

Abbreviation: GC, gastric cancer; EC, esophageal cancer; T_1, Carcinomatous and normal tissues; T_2, Carcinomatous and adjacent tissues.

Table 3. The distribution of methylation and non-methylation

First Author	Case group			Control group		
	n1	M1	U1	n0	M0	U0
HirakiM [18]	49	31	18	49	15	34
Oki E [19]	59	20	39	59	6	53
Homma N [20]	52	18	34	52	4	48
Cheng ZD [21]	64	33	31	64	12	52
Hu SL [22]	123	51	72	123	14	109
Hu SL [23]	70	34	36	70	15	56
Honda T [24]	71	25	46	71	4	67
Hu SL [25]	42	23	19	42	3	9
Xie K [26]	50	21	29	50	11	39
Mei XY [27]	28	13	15	28	12	16

M, methylation; U, non-methylation.

for case-control study [12, 13]. A modified 0-10 points scoring system, shown in **Table 1**, was used to evaluate the quality of eligible studies. The higher the articles score, the better quality the articles have. The average score of the eligible studies was 8.2 points.

Statistical analysis

This meta-analysis was performed using STATA software (version12.0, STATA Corp, College Station, Tex). Crude Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to assess the strength of association between CHFR gene methylation and the digestive system carcinomas. Pooled ORs were calculated using the data of eligible articles in random-effect model (M-H heterogeneity method) or fixed-effect model (Mantel and Haenszel method). I^2 index and *p* value of the chi-squared test were used to inspect the heterogeneity among enrolled literature [14]. If there existed a notable heterogeneity ($P < 0.05$ and/or $I^2 > 50\%$), the random-effect model was used to estimate Ors [15]. Conversely, the fixed-effects model was performed [16]. Subgroup analysis was performed by disease type and detection methods respectively. The Z test and *p* value of 0.05 were used to judge whether the differences of OR values had statistical significance. Sensitivity analysis was conducted to assess the influence of individual studies. Egger's test was applied to evaluate the publication bias [17].

Results

Search strategy and characteristics of eligible articles

The complete searching procedure is shown in **Figure 1**. 10 eligible studies [18-27] including eight gastric cancer studies, one liver cancer study and one esophageal cancer were enrolled in this meta-analysis on the basis of the inclusion and exclusion criteria. The characteristics of included studies are shown in **Table 2**. The distribution of CHFR methylation and non-methylation is shown in **Table 3**. 608 cases and 608 controls from these articles were employed for the analysis of CHFR methylation. In addition, the total case numbers and control numbers of included studies were collected to calculate the pooled odds ratio (OR).

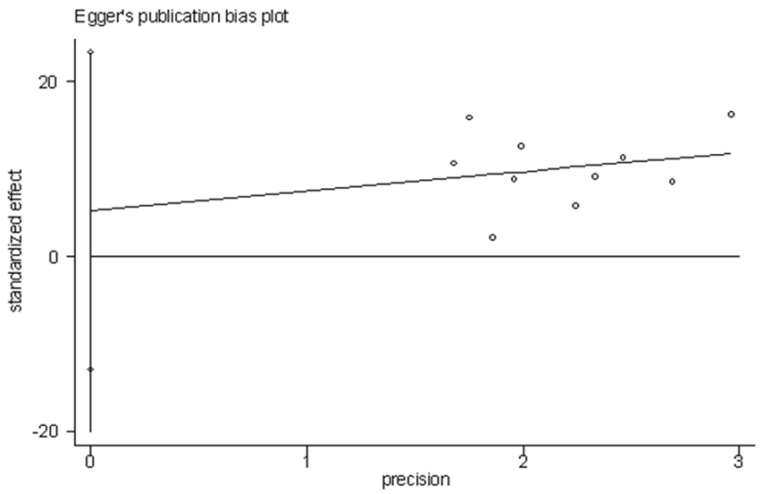

Figure 2. Egger's test for publication bias.

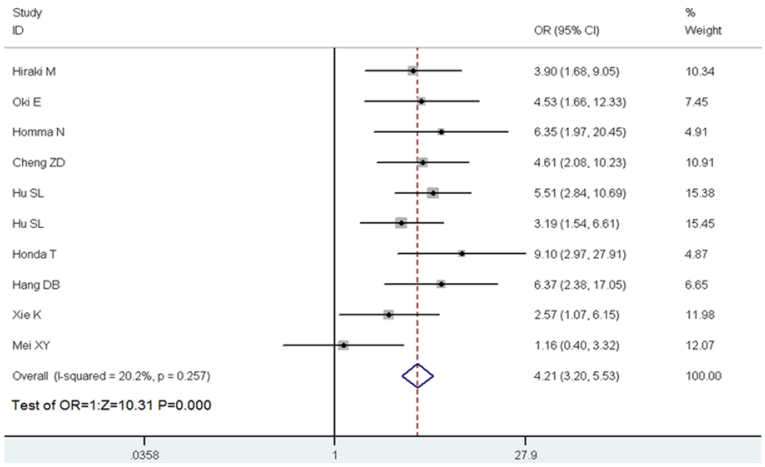

Figure 3. Forest plots of the association between CHFR methylation and digestive system cancer risk.

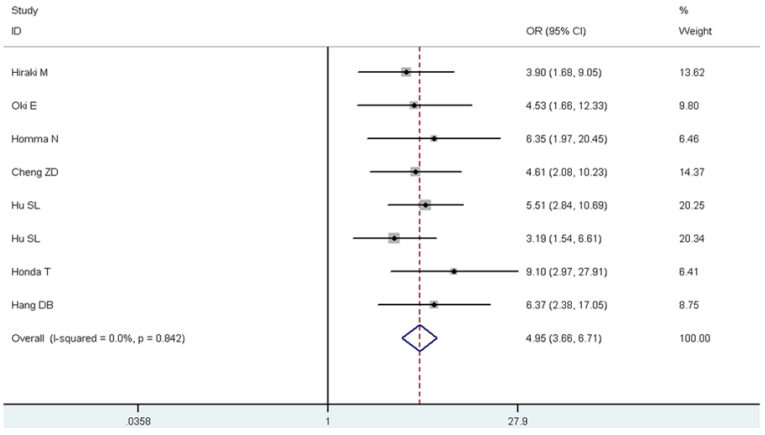

Figure 4. Forest plots of the association between CHFR methylation and gastric cancer risk.

Results of meta-analysis

The between-study heterogeneity of all the 10 eligible studies was firstly analyzed and no significant heterogeneity was found (P=0.257, I^2=20.2%, **Figure 3**). Therefore, the strength of the association between methylation of CHFR and risk of digestive cancers were determined by the fixed-effects model. Overall, compared with control groups, the pooled OR of CHFR methylation in digestive cancer specimens was 4.21 (95% CI: 3.20-5.53, P < 0.001, **Figure 3**), indicating that CHFR methylation was associated with an increased risk of digestive carcinomas.

Subgroup analysis

Subgroup analysis was conducted based on cancer type and methylation testing methods respectively. The pooled OR of CHFR methylation in gastric cancer tissues was 4.95 (95% CI: 3.66-6.71, P < 0.001, **Figure 4**), suggesting that CHFR methylation was a risk factor of gastric cancer. After stratified analysis by testing methods, significantly increased risks were found in MSP (OR=8.02, 95% CI: 5.29-12.16, P < 0.001, **Figure 5**).

Publication bias

In this study, publication bias was recognized by Egger's test. The results of Egger's linear regression showed that there was no obvious evidence of publication bias (P= 0.522). The shape of Egger's plot was shown in **Figure 2**. There was no publication bias in this meta-analysis.

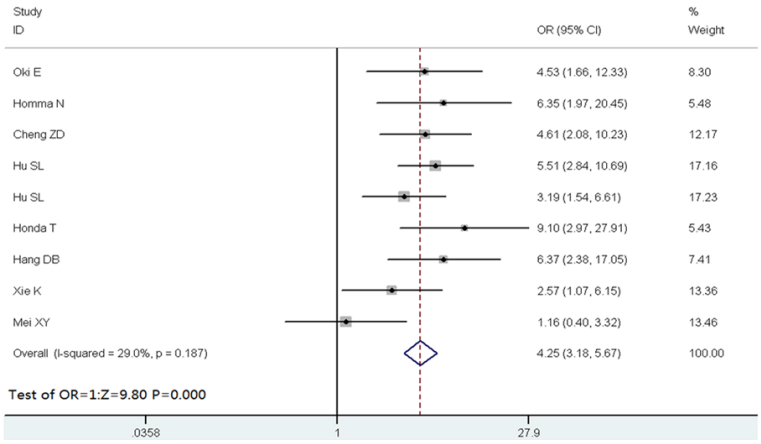

Figure 5. Forest plots of the studies using the MSP testing method.

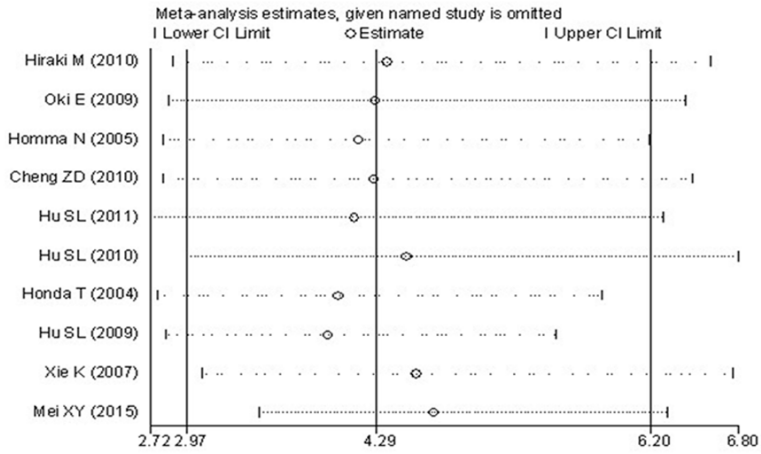

Figure 6. Sensitivity analysis.

Sensitivity analysis

Sensitivity analysis was performed to evaluate the influence of each study on the pooled OR and STATA command "metaninf" was used. The new combined ORs were compared with the original pooled ORs after expurgation of one study from all eligible articles each time. The results remained unchanged. After removing several studies successively, which obviously varied from other eligible articles, the results were still consistent with the original results. The results of Sensitivity analysis was shown in **Figure 6**.

Discussion

To date, a growing number of cancer genes have been recognized to involve the methylation in normally unmethylated promoter CpG islands [28, 29]. No expression of the tumor suppressor gene could be caused by this epigenetic change. It plays a key role in an epigenetically-mediated loss-of-gene function, which is as critical for tumorigenesis as mutations in the coding regions [30].

In recent years, the association of aberrant methylation of the CHFR gene and tumor suppressor gene silencing has been identified in several gastrointestinal cancers [11, 31] and has become a rising concern. We conducted the first meta-analysis to assess the association between CHFR methylation and digestive system cancer risk in the present study. The results revealed that the methylation of CHFR increased the risk of gastrointestinal cancers.

In subgroup analysis of cancer type, the overall OR in gastric cancer vs non-cancer samples was 4.95 (95% CI: 3.66-6.71, $P < 0.001$), indicating that the CHFR methylation had a significant association with the risk of gastric cancer. On the other hand, the CHFR methylation had no association with the risk of the esophageal cancer and liver cancer (95% CI: 3.66-6.71, $P < 0.001$). However, a small amount of eligible studies in subgroup analysis may induce the credibility of this association. In the present study, the association of CHFR methylation with digestive system cancer risk was also stratified by methylation testing methods. And significant ORs in subgroups with MSP methods was found.

In conclusion, this meta-analysis showed that the CHFR methylation could increase the susceptibility of gastrointestinal cancers, particularly the gastric cancer. The CHFR methylation could be considered as a candidate of biomarker for cancer screening, diagnosis and therapy

in the future. Further well-designed studies with larger sample size in gastrointestinal cancers are needed to confirm our findings.

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Disclosure of conflict of interest

None.

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