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Review Article

P15 gene methylation in hepatocellular carcinomas: a systematic review and meta-analysis

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Abstract: Objective: This study was performed to investigate the correlation between P15 methylation and hepatocellular carcinoma (HCC) and hepatocirrhosis using a meta-analysis of available case control studies. Methods: Previous studies have primarily evaluated the incidence of P15 methylation in HCC and corresponding control groups, and compared the incidence of P15 methylation in liver cirrhosis and control groups. Data regarding publication information, study characteristics, and incidence of P15 methylation in both groups were collected from these studies and summarized. Results: Ten studies that assessed P15 gene methylation in 824 HCC tumour tissues and five studies analyzing P15 methylation in 155 liver cirrhosis tissues met our inclusion criteria. Our meta-analysis revealed that the rate of P15 methylation was significantly higher in HCCs than in adjacent non-tumour tissues (OR 9.04, 95% CI 5.80-14.09, $P < 0.00001$). Moreover, P15 methylation was significantly higher in liver cirrhosis tissues than in control tissues (OR 7.82, 95% CI 3.58-17.07, $P < 0.00001$). Conclusions: we found that P15 methylation was associated with an increased risk of HCC and liver cirrhosis. P15 hypermethylation induced the inactivation of the P15 gene, which played an important role in hepatocarcinogenesis.

Keywords: P15 methylation, hepatocellular carcinoma, liver cirrhosis, meta-analysis

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and leading cause of death among patients with cirrhosis and the third leading cause of cancer-related deaths worldwide [1, 2]. Several risk factors have been suggested to be involved in the development of HCC, including aflatoxin exposure, alcohol consumption, chronic inflammation associated with viral hepatitis and familial tendency [3]. In China, HCC is now second only to lung cancer in terms of total deaths [4]. The high mortality rate of HCC is due to the fact that no systemic therapy is available for advanced cases of the disease [5]. Previous studies have suggested that epigenetics modification may play a role in the development of HCC [6, 7]. The molecular genetics of HCC have recently been extensively characterized. Among these molecular genetics, aberrant DNA cytosine methylation is one of the most consistent epigenetic changes in HCC [8].

P15 is a common tumour suppressor genes [9, 10]. In recent years, the loss of P15 expression and their aberrant gene methylation have been reported in the tumour tissues of various human neoplasias, such as laryngeal squamous cell cancer [11], leukemia [12], colorectal carcinoma [13] and HCC [14, 15]. Therefore, these genes methylation may be useful biomarkers for the diagnosis of HCC [16]. HBV infection and HBV-DNA replication are associated with P14 (ARF), P15 (INK4B), P16 (INK4A), and RB gene methylation, high rate of P14 (ARF), P15 (INK4B), and P16 (INK4A) in HCC with HBV infection suggests that HBV-induced hypermethylation may be one of the mecha-

nisms of HBV involved in hepatocellular carcinogenesis [9]. In consistent with previous finding, disease progression from chronic HCV to cirrhosis and hepatocellular carcinoma is associated with increasing P14 and P15 promoter methylation [17].

The P15 is well-known cell cycle progression inhibitors. They may bind to cyclin-dependent kinases CDK4/6, abrogates their ability to form kinase active complex with D-type cyclins, and therefore, maintains Rb-family proteins in a hypophosphorylated state, which promote E2F binding and G1 cell-cycle arrest [18]. Although several recent studies have assessed the association of P15 methylation with the risk of HCC, the data are highly diverse due to differences among studies, the range of P15 methylation in HCC tissues is 12%-82% [17, 19-24]. Generally, the overall DNA methylation level is higher in cancer cells than in normal cells. In a cohort of high-risk subjects, P15 methylation is associated with hepatitis B virus infection, implying an environment epigenetic interaction in the development of HCC [22]. Although previous reports indicates that inactivation of the P15 gene is mainly induced by the methylation, and it is one of the important epigenetic alterations in HCC. Interestingly, the reported rates of P15 methylation in HCC is remarkably diverse. Moreover, whether it is associated with the incidence of hepatocirrhosis is still unclear. The various results of these studies underpin the need for assessing the evidence of the relationship between P15 inactivation and HCC. Hence, we conducted a systematic review and meta-analysis to quantitatively evaluate the effects of P15 methylation on the incidence of HCC.

Materials and methods

Date source and search

We screened a comprehensive search of the related studies using PubMed (1976 onward), EMBASE (1966 onward), Cochrane Library (no date restriction), the Chinese Biomedical Database (no date restriction) and the China National Knowledge Infrastructure (no date restriction). Medical Subject Headings were used in the searching in both Chinese and English languages. The databases up to December 2014 regarding the correlation between methylation of the P15 gene and the risk of HCC were included in this study. The following keywords used were human hepatocellular carcinoma (HCC), liver cirrhosis, P15, P15 (INK4B) and methylation. Relevant reviews and meta-analysis of the role of P15 methylation in the incidence of HCC and hepatocirrhosis were examined for potential inclusive studies.

Inclusion and exclusion criteria

The following studies included in this meta-analysis could meet the following criteria: (1) They were case-control studies regarding the correlation between methylation of P15 gene and the risk of HCC and liver cirrhosis; (2) They were performed in the HCC and liver cirrhosis patients; (3) P15 gene methylation status were detected by using methylation-specific PCR (MSP), real-time quantitative MSP (QMSP) or pyrosequencing; (4) Specimens of HCC must be surgically removed tumor tissues and the control groups were composed of adjacent or other non-tumor tissues; (5) The number of samples were more than 10; (6) These studies must include sufficient data about rate of P15 methylation.

Data extraction

Data extraction was systematically performed by three reviewers using standardized data extraction forms. For each study, the following information was extracted: first author, year of publication, country, sample size, detection method and the rate of gene methylation. The discrepancy was resolved by discussion.

Statistical analysis

Odds ratios (ORs) were used as a measure of the relationship between P15 methylation and the risk of HCC for case-control studies and the corresponding 95% confidence interval (CI). The pooled ORs were combined by the Mantel-Haenszel methods [25]. $P < 0.05$ was taken to indicate significant differences. All significance tests were two-sided. An OR > 1 indicated a higher incidence of P15 methylation in HCC tissues than in corresponding controls. The percentage of variability across studies attributable to heterogeneity beyond chance was assessed by χ^2 test ($P < 0.1$) and I^2 statistics. When there was no statistically significant heterogeneity, a pooled effect was calculated with a fixed-effects model; otherwise, a random-effects model was employed. We also assessed

Table 1. Main characteristics and methodological quality of all eligible studies included in meta-analysis

	Year	Gender (M/F)	Age (Year)	Country or Area	HCC tissue/Control	Method
Fukai [10]	2005	25/14	53.7 ± 8.6	Japan	Tumor/non-tumor tissues	MSP
Iyer [21]	2009	20/8	56.3 ± 9.1	Egyptian	Tumor/non-tumor tissues	MSP
Ko [14]	2008	No date	53 ± 9	Korea	Tumor/non-tumor tissues	MSP
Liu [23]	2006	46/4	48.5 ± 9.2	China	Tumor/non-tumor tissues	MSP
Oh [29]	2007	16/9	53.8	Korea	Tumor/non-tumor tissues	MSP
Qin [24]	2004	No date	No date	China	Tumor/non-tumor tissues	MSP
Yang [28]	2003	30/21	No date	USA	Tumor/non-tumor tissues	MSP
Zekri [17]	2013	166/44	55.6 ± 7.5	USA	Tumor/non-tumor tissues	MSP
Zhang [22]	2007	39/11	54.5 ± 7.1	Taiwan	Tumor/non-tumor tissues	MSP
Zhang [9]	2014	70/18	No date	China	Tumor/non-tumor tissues	MSP

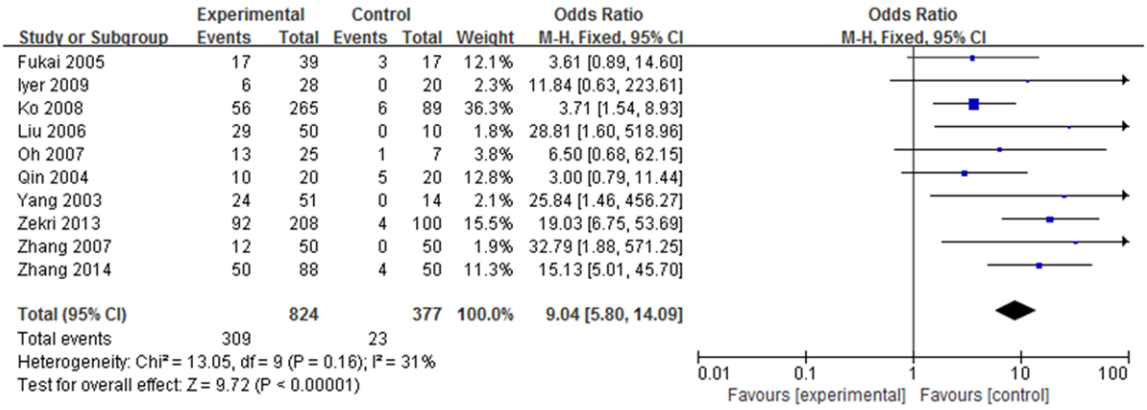

Figure 1. Forest plot for the association between P15 methylation and the risk of human hepatocellular carcinoma.

the probability of publication bias with funnel plots and Egger's test [26, 27]. $P < 0.1$ indicated statistically significant publication bias. In addition, we conducted a sensitivity analysis to evaluate whether the results were statistically affected. Dates were analyzed using RevMan version 5 from the Cochrane Collaboration.

Results

Study characteristics

In our study, 10 studies assessing P15 gene methylation in 824 HCC tumour tissues and 5 studies analyzing P15 gene methylation in 155 liver cirrhosis tissues met our inclusion criteria. Methylation-specific polymerase chain reaction (MSP) was conducted in all included studies. The study characteristics and methodological quality regarding P15 methylation was summarized in **Table 1**.

P15 gene methylation in HCC tumor tissues and non-tumor tissues

Ten studies included 824 HCC tumor tissues and 377 control tissues concentrated on the association between P15 methylation and the risk of HCC. As shown in (**Figure 1**), there was no significant heterogeneity among these studies ($P = 0.16$, $I^2 = 31\%$), which analyzed using the random-effects model. The meta-analysis indicated that the rate of P15 methylation was significantly higher in HCC than in adjacent non-tissues tissues (OR 9.04, 95% CI 5.80-14.09, $P < 0.00001$).

P15 gene methylation in liver cirrhosis tissues and control tissues

Ten studies included 155 liver cirrhosis tissues and 137 control tissues concentrated on the association between P15 methylation and the risk of liver cirrhosis. As shown in (**Figure 2**),

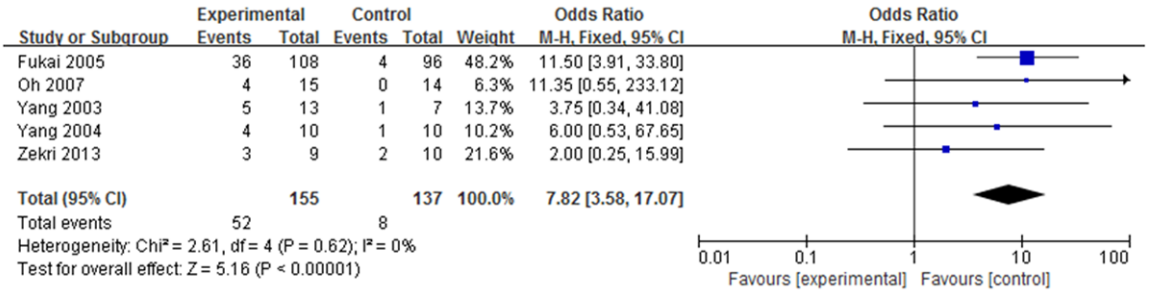

Figure 2. Forest plot for the association between P15 methylation and the risk of liver cirrhosis.

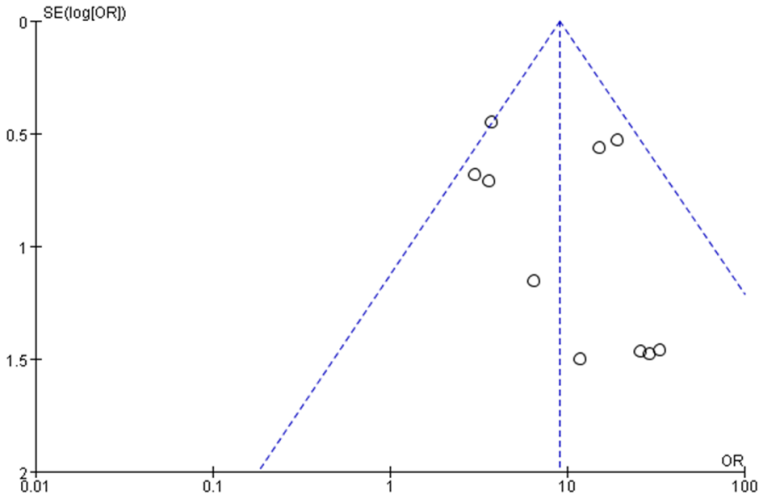

Figure 3. Begg's funnel plot of publication biases on the relationship between P15 methylation and the risk of human hepatocellular carcinoma.

there was no significant heterogeneity among these studies (P = 0.62, I^2 = 0%), which analyzed using the random-effects model. The meta-analysis indicated that the rate of P15 methylation was significantly higher in liver cirrhosis than in adjacent control tissues (OR 7.82, 95% CI 3.58-17.07, P < 0.00001).

Publication bias and sensitivity analysis

We conducted Begg's funnel plot and Egger's test to detect the publication bias of included studies. As shown in (**Figures 3** and **4**), although the Begg's funnel plots revealed a little asymmetry, Egger's tests which offered specific data showed no obvious Publication bias.

Discussion

Gene-specific promoters epigenetic modification are common found in human hepatocellular carcinoma, however, the epigenetic alterations of P15 gene methylation specific to the underlying disease etiology remains elusive. Based on 10 studies and a total of 824 cases of HCC tumor tissues and 377 cases of non-tumor tissues, this pooled analysis comprehensively assessed the relationship between P15 gene methylation and the incidence of HCC or liver cirrhosis. Using the pooled crude ORs from the included studies, we found that P15 gene methylation was associated with 9.04 fold increased risks of HCC compared with non-tumorous tissues of HCC patients. Moreover, a 7.82 fold increased risk of liver cirrhosis was also found when compared with non-cirrhotic tissues.

The relationship between P15 gene methylation and the incidence of HCC has been reported by some studies. Roncalli *et al* [20], showing that P15 is frequently altered in HCC (42%, 14/33) and liver cirrhosis (33%, 11/33). Zekri et al reports that the methylation frequency of P15 gene is detected in 92/208 (44.2%) and 36/108 (33.3%) in HCC and liver cirrhosis respectively [17]. Yang et al reports that P15 is frequently altered in HCC (47%, 24/51) and no methylation in liver cirrhosis [28]. In our study, consistent with those of other reports, we suggested that P15 gene methylation might highly relate to hepatocarcinogenesis, and it might be the major mechanism of P15 gene inactivation.

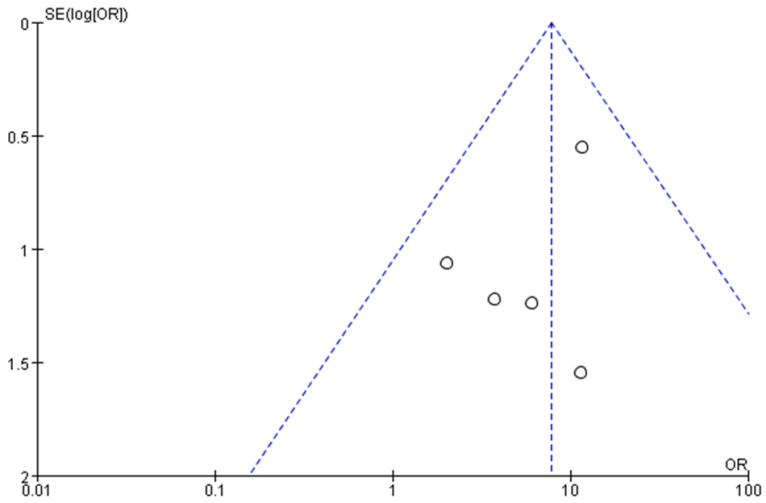

Figure 4. Begg's funnel plot of publication biases on the relationship between P15 methylation and the risk of liver cirrhosis.

It is known that most HCC is associated with chronic liver disease resulting from HBV, HCV or liver cirrhosis [10, 21, 22]. Zekri et al have recently reported that the prevalence of P15 methylation was higher in HCV-related HCCs than in the others [17]. In addition, P15 methylation has a statistically significant difference between patients with chronic hepatitis C and control group, but there is no significant difference between patients with P15 positive and negative methylation regarding age and gender [25]. Among the 50 serum samples from HCC patients, the association of p15 promoter hypermethylation with hepatitis B virus infection is also statistically significant. (HBsAg-positive cases, 36.0% (9/25); HBsAg-negative cases, 12.5% (3/24); $P = 0.04$) [22]. In contrast, P15 methylation in either HCC or non-tumorous liver tissue is more likely to occur in patients over the age of 65, suggesting that P15 methylation may be age related but not cancer specific [10]. Moreover, P15 methylation is not demonstrated in chronic hepatitis or cirrhosis, in contrast to HCCs with expression of 12% [29]. However, a significant association is observed in the P15 gene between gender and promoter methylation. The proportion of males with methylated p15 promoters is greater than the proportion of females with P15 promoter methylation [21]. Regarding P15 methylation in different tissues from HCC patients, there are conflicting reports [30]. Aberrant P15 promoter methylation is demonstrated in 64% (16 of 25), a substantial proportion, in tumor tissues from HCC patients, but only 16% (4/25) in plasma or serum from HCC patients [30].

Our meta-analysis revealed that the overall P15 methylation rate was 37.5% (309/824) in HCC tumour tissues compared to 6% (23/377) in adjacent tissues. The pooled OR was 9.04 (95% CI 5.80-14.09), which suggested that P15 gene methylation was highly correlated to the incidence of HCC. Moreover, P15 methylation has also been reported in other cancers [31, 32]. For example, Bodoor et al [12] reports that the P15 methylation rate is 45% in peripheral blood of acute myeloid leukemia patients, 19% in chronic lymphoid leukemia patients and 14% in acute leukemia patients. Similarly, Majchrzak-Celińska et al [33] shows that the P15 methylation rate is 12% in the serum of central nervous system cancer patients. These data suggest that P15 gene methylation may play an important role in cancer development.

In conclusion, we found that P15 methylation was associated with an increased risk of HCC and liver cirrhosis. P15 hypermethylation induced the inactivation of the P15 gene, which played an important role in hepatocarcinogenesis.

Disclosure of conflict of interest

None.

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