Original Article

Association between *APC* promoter methylation and clinicopathological features of patients with hepatocellular carcinoma: a meta-analysis with PRISMA guideline

Yaqiong Zhang[1], Bo Li[2], Qianqian Sun[3], Xiaoyu Wu[1], Qi Chen[1], Zhaoyun Li[1], Jinggang Mo[3]

[1]Department of Clinical Laboratory, Taizhou Central Hospital, Taizhou 318000, China; [2]Ultrasound Department of Taizhou Hospital, Taizhou 318000, China; [3]School of Life Science, Wenzhou Medical University, Wenzhou 325000, China

Contributions: (I) Conception and design: Z Li, J Mo, Y Zhang; (II) Administrative support: Y Zhang; (III) Provision of study materials or patients: Y Zhang, B Li, Q Sun; (IV) Collection and assembly of data: Y Zhang, X Wu, Q Chen; (V) Data analysis and interpretation: Y Zhang, X Wu, B Li, Q Sun; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Zhaoyun Li. Department of Clinical Laboratory, Taizhou Central Hospital, Taizhou 318000, China. Email: kyyx111111@163.com; Jinggang Mo, The First Affiliated Hospital, Wenzhou Medical University, Wenzhou 325000, China. Email: ayaya0305@163.com.

Background: The adenomatous polyposis coli (*APC*) has been reported as a key tumor suppressor gene in hepatocellular carcinoma (HCC). However, the clinical significance of *APC* promoter methylation in HCC remains unclear. This meta-analysis was conducted to assess the relationship between *APC* promoter methylation and clinicopathological characteristics of patients with HCC.

Methods: Eligible publications were identified by online electronic databases (PubMed, Embase, EBSCO and the Cochrane Library). The combined odds ratios (ORs) with their corresponding 95% confidence intervals (95% CIs) were calculated and summarized under the random-effects model.

Results: Final 21 available studies were included in the current study. *APC* promoter methylation was significantly higher in HCC than in normal live tissues, liver cirrhosis, and healthy blood samples (OR =11.46, P<0.001; OR =6.04, P<0.001; OR =93.83, P<0.001; respectively). No significant correlation was found between HCC and chronic hepatitis, and dysplastic nodules (P>0.1). *APC* promoter methylation was shown to be associated with hepatitis B virus (HBV), and hepatitis C virus (HCV) infection status of patients with HCC (OR =2.86, P=0.005; OR =3.50, P=0.001; respectively), but not correlated with clinical stage, gender, and vascular invasion status (P>0.1).

Conclusions: *APC* promoter methylation may be correlated with HCC, especially for patients with HBV and HCV infection status. Promoter methylation of the *APC* may be a potential noninvasive biomarker using blood samples in the detection of HCC. More studies with large samples sizes are needed to validate our findings in the future.

Keywords: Adenomatous polyposis coli (*APC*); promoter methylation, hepatocellular carcinoma (HCC); blood; hepatitis B virus (HBV); hepatitis C virus (HCV) infection

Submitted Dec 31, 2016. Accepted for publication Mar 02, 2017.
doi: 10.21037/tcr.2017.03.71
View this article at: http://dx.doi.org/10.21037/tcr.2017.03.71

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor and the second most common cause of cancer-related deaths among the world (1). On the basis of global cancer statistics, an estimated 782,500 new cases were diagnosed as HCC in 2012, leading to approximately 745,500 deaths of this disease (1). Some risk factors have been showed to be involved in the development of HCC, including hepatitis B virus (HBV), hepatitis C virus (HCV) infection, liver cirrhosis, aflatoxin exposure, and alcohol

consumption etc. (2-5).

Studies suggest that epigenetic alterations (DNA methylation, histone modifications, nucleosome positioning and non-coding RNAs) are an early biotic event in human cancers (6,7). As a common epigenetic change, DNA methylation plays an important role in the tumorigenesis, progression and prognosis of cancer (8-10). Mapped to human chromosome 5q21-5q22, the *adenomatous polyposis coli* (*APC*) gene, a key tumor suppressor gene (TSG), encodes a large multidomain protein and involves in the Wnt signaling pathway (11,12). Moreover, the *APC* gene has many biological functions, such as the regulation of cell cycle, cell migration and adhesion, transcriptional activation, apoptosis, and chromosomal instability (13-15). *APC* promoter methylation has been found in many human cancers, including prostate cancer (16), colorectal cancer (17), breast cancer (18), and HCC (19).

The results with respect to promoter methylation of the *APC* in HCC *vs.* benign lesions remains controversial. For example, Harder *et al.* reported that *APC* promoter had a same methylation frequency in HCC and liver cirrhosis (20). *APC* promoter methylation frequency was significantly higher in HCC than in liver cirrhosis by Lee *et al.* (21). Thus, the current meta-analysis was performed to evaluate the relationship between *APC* promoter methylation and HCC in cancer *vs.* liver cirrhosis, chronic hepatitis, and dysplastic nodules. Additionally, we also assessed the correlation between *APC* promoter methylation and clinicopathological features of patients with HCC, including gender, tumor stage, vascular invasion, HBV, and HCV infection status.

Methods

Search strategy

Eligible publications were identified through searching the relevant databases before December 10th, 2016, including the PubMed, Embase, EBSCO and the Cochrane Library. The search strategy was conducted based on the following search terms and key words: (APC OR adenomatous polyposis coli) AND (liver OR hepatocellular OR hepatic) AND (cancer OR tumor OR neoplasm OR carcinoma) AND (methylation OR epigene*). The reference lists from the eligible studies were carefully checked to identify other potential articles.

Inclusion criteria

Eligible articles must fulfil the following selection criteria in this meta-analysis: (I) patients were diagnosed with HCC by histopathological confirmation; (II) studies provided sufficient information to estimate the relationship between *APC* promoter methylation and HCC in the comparison of cancer and control groups; (III) studies provide sufficient dada to assess the correlation of *APC* promoter methylation with clinicopathological characteristics in HCC. When authors published several articles using the same sample population, only the most complete publication with more information were selected in our meta-analysis.

Data extraction and quality assessment

Two authors independently extracted the relevant information from the included studies: first author's surname, year of publication, country, ethnicity, detection method of methylation, sample type, number of patients in case and control groups, methylation level, and clinicopathological parameters such as gender, tumor stage, vascular invasion, HBV, and HCV infection status. Control tissue samples included chronic hepatitis, liver cirrhosis, dysplastic nodules, and normal liver tissues. Additionally, the quality evaluation of the eligible articles was performed using the Newcastle–Ottawa Scale (NOS), ranging from 0 to 9. An individual study with a NOS score of ≥6 was considered as high quality, and a NOS score of ≤3 was considered as low quality (22,23) (*Table S1*).

Data analysis

The data were analyzed using Stata software (version 12.0, Stata Corporation, College Station, TX, USA). The pooled odds ratios (ORs) with their corresponding 95% confidence intervals (95% CIs) were calculated to determine whether *APC* promoter methylation was correlated with the risk of HCC in cancer *vs.* control groups. In addition, the relationship between *APC* promoter methylation and clinicopathological features of patients with HCC was also analyzed by the combined ORs with the corresponding 95% CIs. The chi-square test and Q statistics were applied to assess the possible heterogeneity among studies (24). The random-effects model was used in the current meta-analysis. If there was obvious evidence of heterogeneity (I^2 >50% or P<0.1), a sensitivity analysis was carried out to determine the influence of the pooled OR and the change of heterogeneity by omitting an individual study (25,26). The potential publication bias was carried out using Egger's

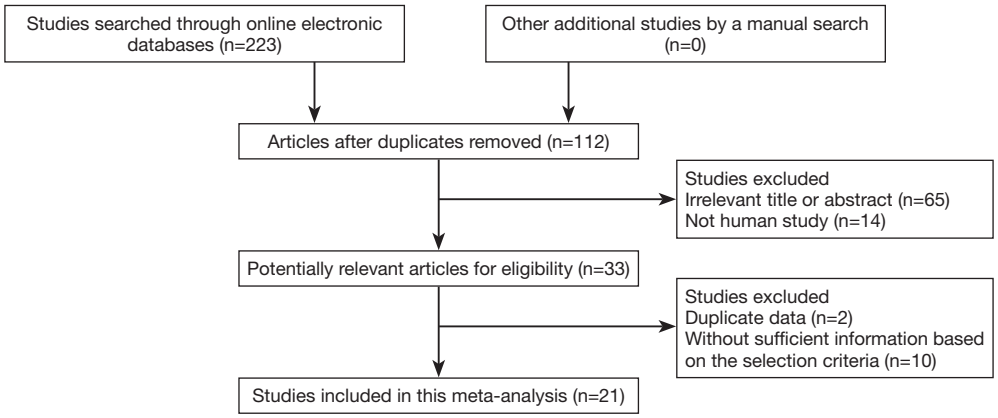

Figure 1 Flow chart of the study selection in the meta-analysis.

test in cancer *vs.* normal liver tissue samples (27).

Results

Study characteristics

A total of 223 relevant publications were searched from a range of online electronic databases as above described (*Figure 1*). Based on the above selection criteria, 21 available studies (19-21,28-39,40-45) were identified in the current meta-analysis. 16 studies evaluated the relationship between *APC* promoter methylation and HCC in tissue samples of HCC patients *vs.* normal live tissues (19-21,28,30, 32-35,37,38,40,41,43-45). Three studies evaluated the correlation between *APC* promoter methylation and HCC in blood samples of HCC patients *vs.* healthy blood samples (29-31). Six publications analyzed the relationship between *APC* promoter methylation and HCC in cancer *vs.* liver cirrhosis (20,21,33,37,39,44). Three publications analyzed the association between *APC* promoter methylation and HCC in cancer *vs.* dysplastic nodules (21,33,43), three publications estimated the association between *APC* promoter methylation and HCC in cancer *vs.* chronic hepatitis (21,28,37). Nine studies analyzed the correlation between *APC* promoter methylation and clinicopathological features of patients with HCC (31,33,35-37,39,40,42,44). All eligible articles met a score of more than or equal to 5. The basic characteristics of the included studies are presented in *Table S1*.

Association between APC promoter methylation and HCC in cancer vs. normal controls

The data included the comparison of 1004 HCC patients and 212 normal live tissues, and 203 HCC patients and 109 healthy blood samples. The results demonstrated that promoter methylation of the *APC* gene was significantly higher in HCC than in normal live tissues and healthy blood samples (OR =11.46, 95% CI: 5.01–26.21, P<0.001; OR =93.83, 95% CI: 20.33–432.97, P<0.001; respectively) (*Figure 2*).

Association between APC promoter methylation and HCC in cancer vs. liver cirrhosis, chronic hepatitis, and dysplastic nodules

The result involving 296 patients with HCC and 173 patients with liver cirrhosis showed that *APC* promoter methylation was significantly higher in HCC than in liver cirrhosis (OR =6.04, 95% CI: 2.59–14.09, P<0.001) (*Figure 3*).

No significant correlation was observed between *APC* promoter methylation and HCC in cancer *vs.* chronic hepatitis and dysplastic nodules (OR =9.51, 95% CI: 0.04–2,387.75, P=0.424; OR =1.36, 95% CI: 0.24–7.66, P=0.728; respectively) (*Figure 3*), including 151 HCC patients *vs.* 77 patients with chronic hepatitis and 147 HCC patients *vs.* 74 patients with dysplastic nodules.

Subgroup analysis of APC promoter methylation in cancer vs. normal live tissues

Table 1 summarizes the pooled OR based on ethnicity (Asians, Caucasians and mixed population) and detection method (MSP and non-MSP). Subgroup analysis by ethnicity showed that *APC* promoter methylation was associated with an increased risk of HCC in Asian, Caucasian and mixed populations (OR =18.91, 95% CI: 5.26–67.96, P<0.001; OR

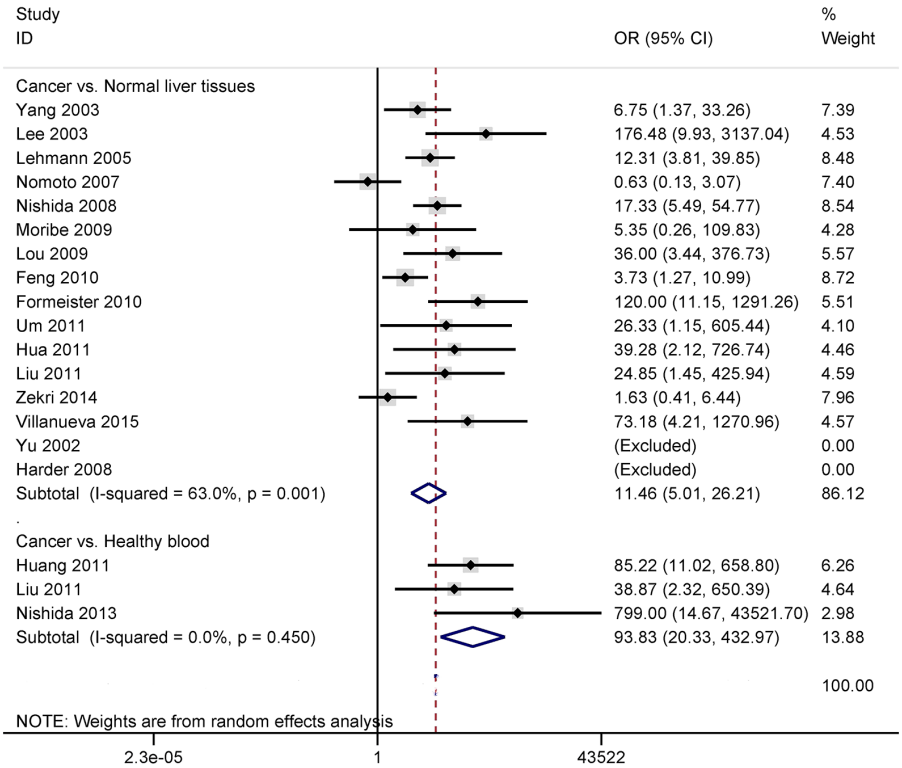

Figure 2 Forest plots of the association between *APC* promoter methylation and HCC in cancer *vs*. normal live tissues and healthy blood.

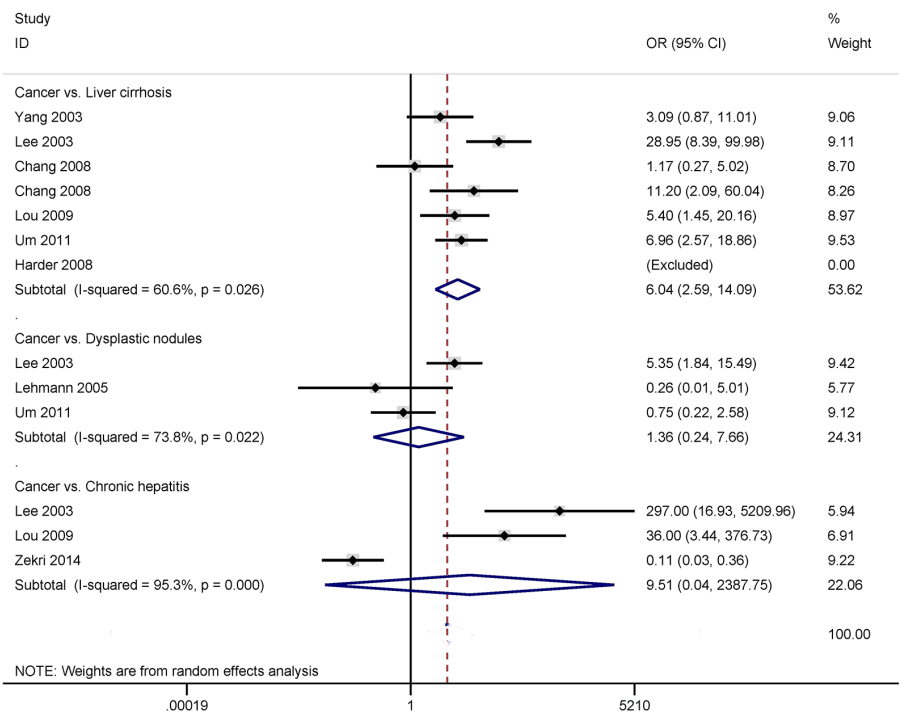

Figure 3 Forest plots of the association between *APC* promoter methylation and HCC in cancer *vs*. liver cirrhosis, chronic hepatitis, and dysplastic nodules.

Table 1 Subgroup analyses of *APC* promoter methylation in HCC *vs.* normal liver tissues

Subgroup	Studies	Overall OR (95% CI)	I^2; P	P value	Cases	Controls
Ethnicity						
Asians	10	18.91 (5.26–67.96)	64.7%; 0.004	<0.001	586	106
Caucasians	5	7.56 (2.05–27.91)	62.2%; 0.048	0.002	378	81
Mix	1	3.73 (1.27–10.99)	NA; NA	0.017	40	25
Detection method						
MSP	8	11.48 (2.35–56.16)	77.3%; <0.001	0.003	456	98
Non-MSP	8	11.38 (5.52–23.46)	20.3%; 0.275	<0.001	548	114

Mix, mixed population; MSP, methylation-specific polymerase chain reaction; NA, not applicable; OR, odds ratio; 95% CI, 95% confidence interval; HCC, hepatocellular carcinoma; *APC*, adenomatous polyposis coli.

=7.56, 95% CI: 2.05–27.91, P=0.002; OR =3.73, 95% CI: 1.27–10.99, P=0.017; respectively).

Subgroup analysis based on detection method showed that the pooled OR of *APC* promoter methylation for the MSP subgroup was 11.48 (95% CI: 2.35–56.16, P=0.003), and 11.38 (95% CI: 5.52–23.46, P<0.001) for the non-MSP subgroup.

Sensitivity analysis in cancer vs. normal live tissues and liver cirrhosis

We conducted a sensitivity analysis to assess the stability of the pooled result based on the omission of one study. When cancer was compared to normal live tissues, we deleted two studies (28,41), and re-calculated the combined OR from the remaining 14 studies (OR =16.60, 95% CI: 8.36–32.96, P<0.001), with a significantly decreased heterogeneity (I^2=32.5%, P=0.131).

In the comparison of cancer and liver cirrhosis, one study (21) was removed, the pooled OR from the remaining five studies was 4.40 (95% CI: 2.21–8.78, P<0.001). Meanwhile, there was no substantial evidence of heterogeneity (I^2=28.1%, P=0.234).

Association between APC promoter methylation and gender, tumor stage, and vascular invasion status

The results showed that *APC* promoter methylation was not correlated with gender, tumor stage, and vascular invasion status of patients with HCC (OR =1.33, 95% CI: 0.57–3.08, P=0.51; OR =1.10, 95% CI: 0.36-3.37, P=0.87; OR =0.77, 95% CI: 0.05–12.35, P=0.851; respectively), including 188, 238 and 120 HCC patients, respectively (*Figure 4*).

Association between APC promoter methylation and HBV and HCV infection status

The result from seven studies with 209 HCC patients demonstrated that *APC* promoter methylation was significantly correlated with HBV infection status of HCC patients (OR =2.86, 95% CI: 1.38–5.92, P=0.005) (*Figure 5*).

The result from five studies with 223 HCC patients showed that a significant correlation was found between *APC* promoter methylation and HCV infection status of HCC patients (OR =3.50, 95% CI: 1.63–7.52, P=0.001) (*Figure 5*).

Publication bias

Egger's test was used to detect the potential publication bias in the comparison of cancer and normal live tissues. No obvious evidence of publication bias was found in this meta-analysis (P=0.076>0.05).

Discussion

Promoter methylation of tumor suppressor genes (TSGs) leads to the downregulation or loss of gene expression (46,47), and may markedly influence the initiation and progression of cancer (48). The reduced expression of the *APC* gene through promoter methylation has been found in various types of human cancers (49-51). *APC* promoter methylation may play a crucial role in cancer carcinogenesis and progression (52-55). The promoter of the *APC* gene has been shown to be frequently methylated in HCC (19,28,30). However, the clinical significance of *APC* promoter methylation remains unclear. We

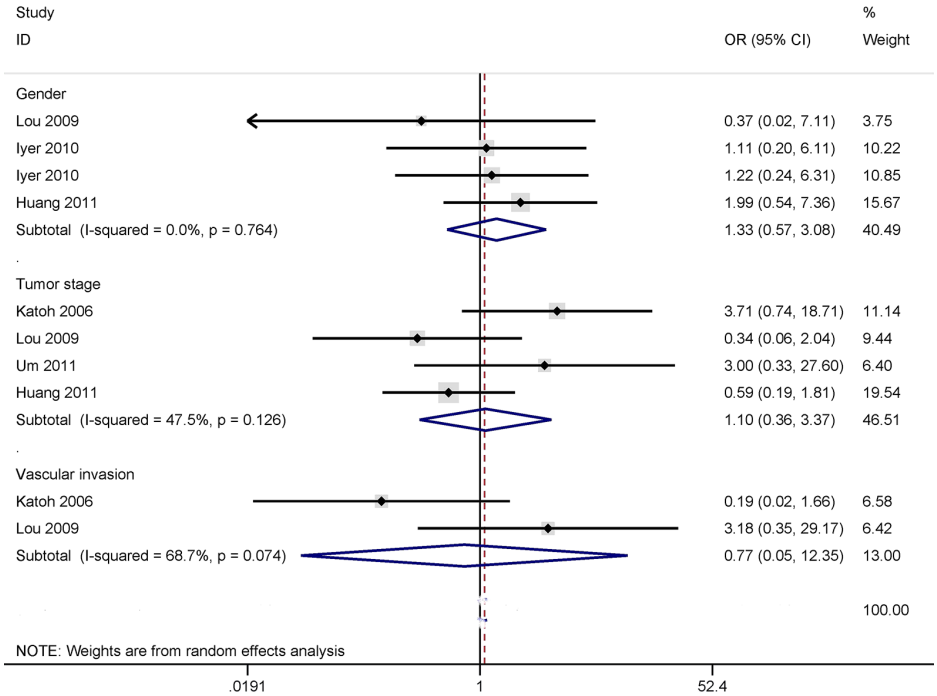

Figure 4 Forest plots of the correlation between *APC* promoter methylation and gender, tumor stage, and vascular invasion status of patients with HCC.

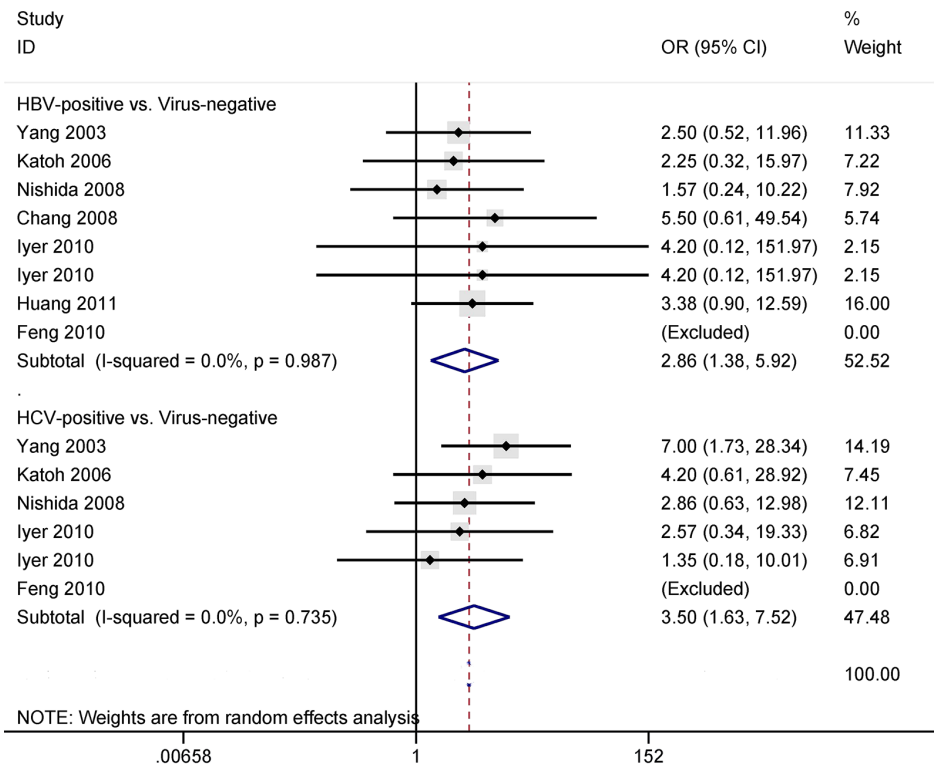

Figure 5 Forest plots of the correlation between *APC* promoter methylation and HBV and HCV infection status of patients with HCC.

performed this meta-analysis to evaluate the association between *APC* promoter methylation and HCC in cancer *vs*. different benign lesions (liver cirrhosis, chronic hepatitis and dysplastic nodules), and the relationship of *APC* promoter methylation with clinicopathological characteristics of HCC patients.

Our result showed that *APC* promoter methylation was significantly higher in tissue samples of patients with HCC than in normal live tissues, suggesting that *APC* promoter methylation may play a key role in the initiation of HCC. Furthermore, subgroup analyses of ethnicity (Asian, Caucasian and mixed populations) and detection method (MSP and non-MSP) were conducted to find the difference among the different subgroups. A subgroup analysis by ethnicity revealed that *APC* promoter methylation was correlated with Asian, Caucasian and mixed populations with HCC, indicating that the Asian, Caucasian and mixed populations were susceptible to *APC* promoter methylation. Moreover, *APC* promoter methylation was found to be correlated with HCC risk in the MSP and non-MSP, suggesting that the MSP and non-MSP methods were sensitive to the *APC* gene. Interestingly, in the analysis of blood samples, the result from three studies showed that *APC* promoter methylation was notably higher in blood samples of HCC patients than in healthy subjects (OR =93.83, P<0.001), which indicated that *APC* promoter methylation may be a useful noninvasive biomarker based on blood detection. However, the results of subgroup analyses and blood samples should be carefully considered because of small sample sizes.

When HCC was compared to liver cirrhosis, chronic hepatitis, and dysplastic nodules. A significant correlation in the promoter methylation of the *APC* gene was observed between HCC and liver cirrhosis (OR =6.04, *P*<0.001), but not between HCC and chronic hepatitis, and dysplastic nodules (*P*>0.1). Our findings suggested that *APC* promoter methylation may only contribute to the progression from liver cirrhosis into HCC. Because the sample size in this study is small, more studies with large sample sizes are necessary to further validate the relationship between HCC and chronic hepatitis, and dysplastic nodules for *APC* promoter methylation.

Finally, the correlation of *APC* promoter methylation with clinicopathological features of HCC patients was also evaluated. The results revealed that *APC* promoter methylation was lined to HBV and HCV infection status of patients with HCC (OR =2.86, P=0.005; OR =3.50, P=0.001; respectively), but not associated with tumor stage, gender and vascular invasion status.

Substantial heterogeneity was found in cancer *vs*. normal live tissues and liver cirrhosis, therefore, the sensitivity analysis was performed in the current meta-analysis. Two studies (28,41) were removed in the comparison of cancer and normal live tissues, and one study (21) was removed in the comparison of cancer and liver cirrhosis. The pooled results remained significant, suggesting the stability of our analyses.

Our results compare favorably with the previous meta-analysis by Zhang *et al*. (56), which also found that *APC* promoter methylation was correlated with an increased risk of HCC in cancer *vs*. normal live tissues. The number of sample sizes included in the current meta-analysis (n=1,216 tissues) was larger than in the previous meta-analysis (n=944 tissues). In addition, the previous meta-analysis did not analyze whether *APC* promoter methylation was associated with HCC in the MSP and non-MSP subgroups.

Several limitations should be illustrated in this meta-analysis. First, our study mainly included Asian and Caucasian populations, and other ethnic groups, such as African population, were lacking. Second, based on small sample sizes, the relationship of *APC* promoter methylation with clinicopathological features of patients with HCC should be further studied in the future. Third, the association comparing HCC and liver cirrhosis, chronic hepatitis, or dysplastic nodules is needed in the future. Finally, based on blood samples, additional studies are essential to confirm whether *APC* promoter methylation may become a potential biomarker using blood samples for HCC diagnosis.

In conclusion, our findings suggest that HCC has a notably higher *APC* promoter methylation than normal live tissues and liver cirrhosis, but not higher than chronic hepatitis and dysplastic nodules. *APC* promoter methylation is correlated with HBV and HCV infection status of HCC patients, but not linked to tumor stage, gender and vascular invasion status. *APC* promoter methylation may be potential noninvasive biomarker in the blood. More well-matched studies with large sample sizes are essential to confirm our findings.

Acknowledgments

Funding: The research was supported by grants from the Health & Medical Science and Technology Program of Zhejiang Province (2017171691).

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2017.03.71). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Zhang Y, Li B, Sun Q, Wu X, Chen Q, Li Z, Mo J. Association between *APC* promoter methylation and clinicopathological features of patients with hepatocellular carcinoma: a meta-analysis with PRISMA guideline. Transl Cancer Res 2017;6(2):275-284. doi: 10.21037/tcr.2017.03.71

Supplementary

Table S1 General characteristics of the eligible studies

First author	Country	Ethnicity	Method	Sample	Control sample	Case	Control	Male	Female	Stage 1–2	Stage 3–4	Only HBV+	Negative	Only HCV+	Negative	Vascular invasion +	Vascular invasion –	NOS
						M+/N	M+/N	M+/N	M+/N	M+/N	M+/N	M+/N	M+/N	M+/N	M+/N	N	N	
Yu 2002 (45)	China	Asians	MSP	Tissue	NLT	0/29	0/4											5
Yang 2003 (44)	USA	Caucasians	MSP	Tissue	NLT	27/51	2/14					5/9	8/24	14/18	8/24			7
Lee 2003 (21)	Korea	Asians	MSP	Tissue	NLT	49/60	0/20											7
Katoh 2006 (42)	Japan	Asians	MSP	Tissue		53/60				39/42	14/18	15/17	10/13	28/30	10/13	28/34	25/26	6
Lehmann 2005 (43)	Germany	Caucasians	RTPCR	Tissue	NLT	35/41	9/28											7
Nomoto 2007 (41)	Japan	Asians	MSP	Tissue	NLT	61/74	15/17											7
Nishida 2008 (40)	Japan	Asians	COBRA	Tissue	NLT	65/75	6/22					11/13	14/18	40/44	14/18			8
Harder 2008 (20)	Germany	Caucasians	QMSP	Tissue	NLT	34/34	16/16											6
Moribe 2009 (38)	Japan	Asians	QMSP	Tissue	NLT	19/44	0/3											7
Lou 2009 (37)	China	Asians	MSP	Tissue	NLT	54/60	1/5	45/51	9/9	22/26	32/34					21/22	33/38	6
Feng 2010 (35)	USA	Mix	MethyLight	Tissue	NLT	31/40	12/25					8/12	0/0	23/28	0/0			6
Formeister 2010 (34)	USA	Asians	MSP	Tissue	NLT	40/43	1/10											5
Iyer 2010 (36)	USA	Caucasians	MSP	Tissue		18/28		13/20	5/8			1/1	2/5	12/19	2/5			6
Iyer 2010 (36)	USA	Caucasians	MSP	Blood		15/28		11/20	4/8			1/1	2/5	9/19	2/5			6
Um 2011 (33)	Korea	Asians	MethyLight	Tissue	NLT	39/46	0/2			13/14	26/32							8
Hua 2011 (32)	China	Asians	*	Tissue	NLT	33/47	0/8											6
Liu 2011 (30)	China	Asians	MSP	Tissue	NLT	48/108	0/15											7
Zekri 2014 (28)	Egypt	Caucasians	MSP	Tissue	NLT	13/31	4/13											6
Villanueva 2015 (19)	USA	Caucasians	PSQ	Tissue	NLT	172/221	0/10											7
Yang 2003 (44)	USA	Caucasians	MSP	Tissue	LC	27/51	4/15											7
Lee 2003 (21)	Korea	Asians	MSP	Tissue	LC	49/60	4/30											7
Harder 2008 (20)	Germany	Caucasians	QMSP	Tissue	LC	34/34	34/34											6
Chang 2008 (39)	China	Asians	MSP	Tissue	LC	14/19	12/17					11/13	3/6					6
Chang 2008 (39)	China	Asians	MSP	Blood	LC	16/26	2/16											6
Lou 2009 (37)	China	Asians	MSP	Tissue	LC	54/60	10/16											6
Um 2011 (33)	Korea	Asians	MethyLight	Tissue	LC	39/46	20/45											8
Lee 2003 (21)	Korea	Asians	MSP	Tissue	DN	49/60	10/22											7
Lehmann 2005 (43)	Germany	Caucasians	RTPCR	Tissue	DN	35/41	10/10											7
Um 2011 (33)	Korea	Asians	MethyLight	Tissue	DN	39/46	37/42											8
Lee 2003 (21)	Korea	Asians	MSP	Tissue	CH	49/60	0/34											7
Lou 2009 (37)	China	Asians	MSP	Tissue	CH	54/60	1/5											6
Zekri 2014 (28)	Egypt	Caucasians	MSP	Tissue	CH	13/31	33/38											6
Huang 2011 (31)	China	Asians	*	Blood	HB	49/72	1/41	43/61	6/11	10/17	39/55	45/61	5/11					6
Liu 2011 (30)	China	Asians	MSP	Blood	HB	26/108	0/60											7
Nishida 2013 (40)	Japan	Asians	COBRA	Blood	HB	23/23	0/8											5

Mix, mixed population; MSP, methylation-specific polymerase chain reaction; RTPCR, real-time PCR-based quantification; COBRA, combined bisulfite restriction analysis; QMSP, quantitative methylation-specific PCR; PSQ, pyrosequencing; *, methylation-sensitive restriction enzyme-based quantitative PCR; HBV, hepatitis B virus; HCV, hepatitis C virus; NLT, normal liver tissues; LC, liver cirrhosis; DN, dysplastic nodules; CH, chronic hepatitis; HB, healthy blood; M+, number of methylation positive status; N, total sample size; NOS, newcastle-ottawa scale.