Meta-analysis of DNA methylation biomarkers in hepatocellular carcinoma

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ABSTRACT

DNA methylation is an epigenetic mechanism in the pathogenesis of hepatocellular carcinoma (HCC). Here, we conducted a systematic meta-analysis to evaluate the contribution of DNA methylation to the risk of HCC. A total of 2109 publications were initially retrieved from PubMed, Web of Science, Cochrane Library, Embase, CNKI and Wanfang literature database. After a four-step filtration, we harvested 144 case-control articles in the meta-analysis. Our results revealed that 24 genes (carcinoma tissues *vs* adjacent tissues), 17 genes (carcinoma tissues *vs* normal tissues) and six genes (carcinoma serums *vs* normal serums) were significantly hypermethylated in HCC. Subgroup meta-analysis by geographical populations showed that six genes (carcinoma tissues *vs* adjacent tissues) and four genes (carcinoma tissues *vs* normal tissues) were significantly hypermethylated in HCC. Our meta-analysis identified the correlations between a number of aberrant methylated genes (*p16*, *RASSF1A*, *GSTP1*, *p14*, *CDH1*, *APC*, *RUNX3*, *SOCS1*, *p15*, *MGMT*, *SFRP1*, *WIF1*, *PRDM2*, *DAPK1*, *RARβ*, *hMLH1*, *p73*, *DLC1*, *p53*, *SPINT2*, *OPCML* and *WT1*) and HCC. Aberrant DNA methylation might become useful biomarkers for the prediction and diagnosis of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent malignancies and the sixth leading cause of cancer-related deaths in the United States [1]. The development of HCC is caused by the interaction of environmental, genetic and epigenetic factors [2, 3], such as aflatoxin exposure, alcohol consumption, hepatitis virus infection and familial tendency [4–6]. In addition, epigenetic modification is involved in HCC pathogenesis [3], and aberrant DNA methylation is the primary mediator of epigenetic changes in HCC [7].

Methylation of CpG islands in the gene promoters is recognized as a common epigenetic mechanism of transcriptional regulations [8, 9], and it has been shown to have a relation to the occurrence and development of several types of carcinomas [10–14]. Aberrant DNA methylation

of the gene promoters may become promising biomarkers for the early diagnosis of diseases [15, 16]. Several studies have suggested that aberrant methylation of multiple tumor suppressor genes may contribute to the pathogenesis of HCC and epigenetic inactivation provides a prognostic value for determining the risk for the development of HCC [17, 18].

Aberrant patterns of DNA methylation in HCC can be useful for the prediction of cancer risk. We conduct a comprehensive meta-analysis based on the accumulation of the HCC association studies on DNA methylation to provide molecular clues of the potential diagnostic biomarkers with aberrant DNA methylation in HCC.

RESULTS

A total of 2109 studies were identified by our initial research using the keywords "hepatocellular

carcinoma or hepatocarcinoma or primary liver cancer or HCC or hepatic carcinoma or liver tumor" and "DNA methylation" from PubMed, Web of Science, Cochrane Library, Embase, CNKI and Wanfang literature database. After a series of selection procedure shown in Figure 1, we excluded 983 irrelevant studies, 611 non-case control studies, 218 studies without methylation frequency data and 153 studies less than 3 articles. Thus, a total of 144 eligible studies were included in the current meta-analysis (Supplementary Document S1). The 144 case-control studies published from 2000 to 2016 included 6523 HCC tumor tissues, 5498 adjacent tissues, 689 normal tissues, 2044 HCC serums and 1371 normal serums within 24 genes. Among these 24 genes, the meta-analysis of *p16*, *RASSF1A*, *CDH1*, *RUNX3* and *GSTP1* genes methylation was performed between HCC tumor tissues *vs* adjacent tissues, HCC tumor tissues *vs* normal tissues and HCC serums *vs* normal serums. The meta-analysis of *p14*, *p15*, *p73*, *APC*, *SOCS1*, *MGMT*, *SFRP1*, *PRDM2*, *DAPK1*, *RARβ*, *IGF2* and *hMLH1* genes methylation was performed between HCC tumor tissues *vs* adjacent tissues and HCC tumor tissues *vs* normal tissues. The meta-analysis of *WIF1* gene methylation was performed between HCC tumor tissues vs adjacent tissues and HCC serums vs normal serums, and the meta-analysis of *DLC1*, *p53*, *SPINT2*, *RB1*, *OPCML* and *WT1* genes methylation was performed between HCC tumor tissues *vs* adjacent tissues.

For 24 genes reported in at least three studies between HCC tumor tissues and adjacent tissues (Table 1), no evidence of statistical heterogeneity was observed for nine genes, including *GSTP1* (I^2 = 35%), *PRDM2* (I^2 = 40%), *DAPK1* (I^2 = 19%), *p73* (I^2 = 48%), *hMLH1* (I^2 = 0%), *DLC1* (I^2 = 0%), *p53* (I^2 = 16%), *OPCML* (I^2 = 0%) and *WT1* (I^2 = 0%). No visual bias was shown in the meta-analysis of the above nine genes (Figure 2). Our data also demonstrated a significant heterogeneity of the remaining 15 genes that included *p16* (I^2 = 68%), *RASSF1A* (I^2 = 53%), *p14* (I^2 = 77%), *APC* (I^2 = 71%), *RUNX3* (I^2 = 68%), *SOCS1* (I^2 = 82%), *CDH1* (I^2 = 82%), *p15* (I^2 = 51%), *MGMT* (I^2 = 79%), *WIF1* (I^2 = 71%), *SFRP1* (I^2 = 67%), *RARβ* (I^2 = 62%), *IGF2* (I^2 = 86%), *SPINT2* (I^2 = 71%) and *RB1* (I^2 = 64%). Therefore, random effect tests were applied for the meta-analysis of the above 15 genes. Their funnel plots were shown in Figure 2.

For 17 genes reported in at least three studies between HCC tumor tissues and normal tissues (Table 2), no evidence of statistical heterogeneity was observed for 11 genes, including *p16* (I^2 = 13%), *RASSF1A* (I^2 = 37%), *CDH1* (I^2 = 17%), *p15* (I^2 = 0%), *RUNX3* (I^2 = 0%), *MGMT* (I^2 = 36%), *SFRP1* (I^2 = 0%), *PRDM2* (I^2 = 0%), *DAPK1* (I^2 = 0%), *RARβ* (I^2 = 14%) and *p73* (I^2 = 0%). No visual bias was shown in the meta-analysis of the above 11 genes (Figure 2). Our data also demonstrated a significant heterogeneity of 5 genes that included *APC* (I^2 = 73%), *GSTP1* (I^2 = 57%), *SOCS1* (I^2 = 66%), *p14* (I^2 = 56%) and *IGF2* (I^2 = 89%). Therefore, random effect tests were applied for the meta-analysis of the above 5 genes. The heterogeneity of *hMLH1* was not applicable, because two of three case-control studies were not estimable. Their funnel plots were shown in Figure 2.

For six genes reported in at least three studies between HCC tumor serums and normal serums (Table 3), no evidence of statistical heterogeneity was observed for four genes, including *RASSF1A* (I^2 = 0%), *p16* (I^2 = 0%), *CDH1* (I^2 = 0%), *RUNX3* (I^2 = 0%), *GSTP1* (I^2 = 0%) and *WIF1* (I^2 = 0%). No visual bias was shown in the meta-analysis of the above six genes and their funnel plots were shown in Figure 2.

As shown in Table 1, the meta-analysis of *p16* gene was involved with 43 studies between 2185 HCC tumor tissues and 2081 adjacent tissues. Our results revealed that the frequency of *p16* gene methylation in carcinoma tissues was significantly higher than adjacent tissues (the overall OR = 5.10, 95% CI = 3.81–6.84, *p* < 0.00001). The meta-analysis of *RASSF1A* methylation between 1414 HCC tumor tissues and 1265 adjacent tissues indicated a statistical difference (the overall OR = 6.70, 95% CI = 4.83–9.30, *p* < 0.00001). The same consequence was also found in the other 18 genes including *APC* (the overall OR = 5.14, 95% CI = 3.18–8.30, *p* < 0.00001), *RUNX3* (the overall OR = 19.99, 95% CI = 10.06–39.72, *p* < 0.00001), *SOCS1* (the overall OR = 3.47, 95% CI = 1.80–6.71, *p* = 0.0002), *CDH1* (the overall OR = 2.31, 95% CI = 1.13–4.74, *p* = 0.02), *p15* (the overall OR = 2.03, 95% CI = 1.23–3.36, p = 0.006), *WIF1* (the overall OR = 6.53, 95% CI = 3.33–12.80, *p* < 0.00001), *PRDM2* (the overall OR = 12.33, 95% CI = 8.54–17.81, *p* < 0.00001), *SFRP1* (the overall OR = 3.95, 95% CI = 1.91–8.14, *p* = 0.0002), *RARβ* (the overall OR = 5.27, 95% CI = 1.53–18.10, *p* = 0.008), *p73* (the overall OR = 6.15, 95% CI = 3.09–12.24, *p* < 0.00001), *hMLH1* (the overall OR = 5.10, 95% CI = 2.20–11.85, *p* = 0.0001), *DLC1* (the overall OR = 17.30, 95% CI = 6.71–44.58, *p* < 0.00001), *p53* (the overall OR = 5.12, 95% CI = 1.27–20.59, *p* = 0.02), *SPINT2* (the overall OR = 18.38, 95% CI = 3.81–88.61, *p* = 0.0003), *OPCML* (the overall OR = 1.93, 95% CI = 1.20–3.11, *p* = 0.006) and *WT1* (the overall OR = 5.08, 95% CI = 2.41–10.69, *p* < 0.0001). Our meta-analysis was unable to find any statistical significance between HCC tumor tissues and adjacent tissues for the methylation of the remaining four genes, including *MGMT*, *DAPK1*, *IGF2* and *RB1*.

As shown in Table 2, the meta-analysis of *p16* gene was involved with 31 studies between 1415 HCC tumor tissues and 399 normal tissues. Our results revealed that the frequency of *p16* gene methylation in carcinoma tissues was significantly higher than normal tissues (the overall OR = 13.41, 95% CI = 9.18–19.59, *p* < 0.00001). The meta-analysis of *RASSF1A* methylation between 900 HCC tumor tissues and 341 normal tissues indicated a statistical difference (the overall OR = 43.70, 95% CI = 26.92–70.96, *p* < 0.00001). The same consequence was also found in the other 13 genes including *APC* (the overall OR = 17.20, 95% CI = 5.75–51.41, *p* < 0.00001), *GSTP1* (the overall OR = 13.56, 95% CI = 5.52–33.29, *p* < 0.00001), *CDH1* (the overall OR = 4.93, 95% CI = 2.70–8.99, *p* < 0.00001), *p15* (the overall OR = 5.48, 95%

Table 1: Characteristics of 24 aberrant methylated genes between carcinoma tissues and adjacent tissues in HCC

Gene	Studies (n)	Overall OR (95% CI)	I²	*P* value	Carcinoma tissues/adjacent tissues
p16	43	5.10 [3.81, 6.84]	68%	< 0.00001	2185/2081
RASSF1A	28	6.70 [4.83, 9.30]	53%	< 0.00001	1414/1265
GSTP1	20	6.81 [5.39, 8.62]	35%	< 0.00001	1011/883
p14	17	2.67 [1.26, 5.64]	77%	0.01	911/717
APC	15	5.14 [3.18, 8.30]	71%	< 0.00001	888/762
RUNX3	13	19.99 [10.06, 39.72]	68%	< 0.00001	1025/998
SOCS1	13	3.47 [1.80, 6.71]	82%	0.0002	767/674
CDH1	12	2.31 [1.13, 4.74]	82%	0.02	575/533
p15	12	2.03 [1.23, 3.36]	51%	0.006	535/446
MGMT	10	1.66 [0.64, 4.28]	79%	0.3	560/470
PRDM2	9	12.33 [8.54, 17.81]	40%	< 0.00001	470/452
WIF1	8	6.53 [3.33, 12.80]	71%	< 0.00001	654/506
DAPK1	6	1.03 [0.64, 1.66]	19%	0.91	271/231
SFRP1	5	3.95 [1.91, 8.14]	67%	0.0002	304/275
RARβ	5	5.27 [1.53, 18.10]	62%	0.008	276/170
IGF2	5	0.18 [0.02, 1.55]	86%	0.12	160/200
p73	5	6.15 [3.09, 12.24]	48%	< 0.00001	275/169
hMLH1	4	5.10 [2.20, 11.85]	0%	0.0001	251/182
DLC1	3	17.30 [6.71, 44.58]	0%	< 0.00001	206/200
p53	3	5.12 [1.27, 20.59]	16%	0.02	107/107
SPINT2	3	18.38 [3.81, 88.61]	71%	0.0003	130/130
RB1	3	7.33 [0.58, 92.08]	64%	0.12	123/123
OPCML	3	1.93 [1.20, 3.11]	0%	0.006	213/144
WT1	3	5.08 [2.41, 10.69]	0%	< 0.0001	113/113

CI = 2.54–11.79, $p < 0.0001$), *RUNX3* (the overall OR = 31.16, 95% CI = 12.24–79.31, $p < 0.00001$), *SOCS1* (the overall OR = 9.73, 95% CI = 2.85–33.27, $p = 0.0003$), *MGMT* (the overall OR = 10.84, 95% CI = 3.46–33.91, $p < 0.0001$), *SFRP1* (the overall OR = 13.97, 95% CI = 5.28–36.92, $p < 0.00001$), *PRDM2* (the overall OR = 24.86, 95% CI = 10.44–59.17, $p < 0.00001$), *DAPK1* (the overall OR = 5.32, 95% CI = 2.38–11.91, $p < 0.0001$), *p14* (the overall OR = 6.42, 95% CI = 1.54–26.69, $p = 0.01$), *RARβ* (the overall OR = 7.37, 95% CI = 1.79–30.39, $p = 0.006$) and *p73* (the overall OR = 27.59, 95% CI = 4.87–156.35, $p = 0.0002$). Our meta-analysis was unable to find any statistical significance between HCC tumor tissues and adjacent tissues for the methylation of the remaining two genes, including *IGF2* and *hMLH1*.

As shown in Table 3, Our meta-analysis showed statistical significance between HCC tumor serums and normal serums for the methylation of all six genes, including *RASSF1A* (the overall OR = 83.81, 95% CI = 47.35–148.36, $p < 0.00001$), *p16* (the overall OR = 123.15, 95% CI = 49.12–308.74, $p < 0.00001$), *CDH1* (the overall OR = 23.70, 95% CI = 5.39–104.28, $p < 0.0001$), *RUNX3* (the overall OR = 103.92, 95% CI = 16.33–661.45, $p < 0.00001$), *GSTP1* (the overall OR = 21.09, 95% CI = 4.02–110.65, $p = 0.0003$) and *WIF1* (the overall OR = 53.65, 95% CI = 10.62–271.09, $p < 0.00001$).

In this meta-analysis, we selected geographical populations to analyze sources of heterogeneity. We found that there was significant difference in *RASSF1A* and *GSTP1* between HCC tumor tissues and normal tissues in Japan ($p = 0.041$) and America ($p = 0.021$) respectively (Supplementary Table S2). However, there was no significance in other genes between HCC tumor tissues and normal tissues in geographical populations (Supplementary Table S2). In addition, we didn't find any significance in 24 methylated genes between HCC tumor tissues and adjacent tissues (Supplementary Table S1) and six methylated genes between HCC tumor serums and normal serums (Supplementary Table S3) in geographical populations. Our meta-analysis indicated

Table 2: Characteristics of 17 aberrant methylated genes between carcinoma tissues and normal tissues in HCC

Gene	Studies (n)	Overall OR (95% CI)	I²	*P* value	Carcinoma tissues/normal tissues
p16	31	13.41 [9.18, 19.59]	13%	< 0.00001	1415/399
RASSF1A	21	43.70 [26.92, 70.96]	37%	< 0.00001	900/341
APC	14	17.20 [5.75, 51.41]	73%	< 0.00001	727/217
GSTP1	13	13.56 [5.52, 33.29]	57%	< 0.00001	646/199
CDH1	10	4.93 [2.70, 8.99]	17%	< 0.00001	469/111
p15	9	5.48 [2.54, 11.79]	0%	< 0.0001	382/70
RUNX3	8	31.16 [12.24, 79.31]	0%	< 0.00001	549/152
SOCS1	8	9.73 [2.85, 33.27]	66%	0.0003	474/118
MGMT	8	10.84 [3.46, 33.91]	36%	< 0.0001	458/93
PRDM2	8	24.86 [10.44, 59.17]	0%	< 0.00001	422/116
SFRP1	7	13.97 [5.28, 36.92]	0%	< 0.00001	372/91
DAPK1	7	5.32 [2.38, 11.91]	0%	< 0.0001	328/77
p14	6	6.42 [1.54, 26.69]	56%	0.01	317/57
RARβ	5	7.37 [1.79, 30.39]	14%	0.006	230/54
IGF2	5	0.48 [0.02, 9.59]	89%	0.63	207/36
hMLH1	3	3.24 [0.18, 58.10]	—	0.42	204/34
p73	3	27.59 [4.87, 156.35]	0%	0.0002	174/27

The heterogeneity of *hMLH1* gene methylation was not applicable, because two of three case-control studies were not estimable.

Table 3: Characteristics of six aberrant methylated genes between carcinoma serums and normal serums in HCC

Gene	Studies (n)	Overall OR (95% CI)	I²	*P* value	Carcinoma serums/normal serums
RASSF1A	16	83.81 [47.35, 148.36]	0%	< 0.00001	1110/783
p16	9	123.15 [49.12, 308.74]	0%	< 0.00001	510/352
CDH1	3	23.70 [5.39, 104.28]	0%	< 0.0001	216/90
RUNX3	3	103.92 [16.33, 661.45]	0%	< 0.00001	172/110
GSTP1	3	21.09 [4.02, 110.65]	0%	0.0003	155/100
WIF1	3	53.65 [10.62, 271.09]	0%	< 0.00001	252/180

that the heterogeneity was not contributed by geographical populations, except *RASSF1A* and *GSTP1* between HCC tumor tissues and normal tissues in Japan and America respectively.

Subgroup meta-analysis by geographical populations was performed for *p16*, *RASSF1A*, *GSTP1*, *APC*, *RUNX3*, *SOCS1* and *PRDM2* between HCC tumor tissues and adjacent tissues. As shown in Figure 3, we found a statistical difference between HCC tumor tissues and adjacent tissues for *p16* methylation in China from 28 studies (OR = 4.88, 95% CI = 3.50–6.81, I² = 66%, *p* < 0.00001) and in Japan from 6 studies (OR = 8.43, 95% CI = 3.71–19.19, I² = 68%, *p* < 0.00001), but not in

Germany from 3 studies (*p* = 0.12, I² = 86%). In addition, there was a significant geographical difference in the meta-analysis of *RASSF1A* gene in China (OR = 6.07, 95% CI = 4.18–8.80, I² = 57%, *p* < 0.00001) and Japan (OR = 18.52, 95% CI = 2.30–149.14, I² = 72%, *p* = 0.006, Figure 3). The same consequence was also found in the other four genes including *GSTP1* (China: OR = 5.12, 95% CI = 3.89–6.73, I² = 7%, *p* < 0.00001; Japan: OR = 15.06, 95% CI = 8.59–26.40, I² = 18%, *p* < 0.00001), *APC* (China: OR = 4.88, 95% CI = 3.63–6.56, I² = 34%, *p* < 0.00001; Japan: OR = 11.32, 95% CI = 1.97–64.94, I² = 89%, *p* = 0.006), *RUNX3* (China: OR = 24.10, 95% CI = 9.00–64.52, I² = 75%, *p* < 0.00001; Japan: OR = 15.11,

Records identified through database
searching for Pubmed, Web of Science,
Cochrane Library and Embase
(n = 1732)

Additional records identified through
CNKI and Wanfang
(n = 426)

Records after duplicates removed
(n = 2109)

Records excluded with
irrelevant titles and abstracts
(n = 983)

Records screened
(n = 1126)

Full-text articles excluded with
non case-control
(n = 611)

Full-text articles assessed
for eligibility
(n = 515)

Studies excluded with no
methylation frequency data
(n = 218)

Potential studies included in
qualitative synthesis
(n = 297)

Records excluded: the number
of articles is less than 3
(n = 153)

Studies included in quantitative
synthesis (meta-analysis)
(n = 144)

Meta-analysis of hepatocellular carcinoma:
p16 (n = 62), *RASSF1A* (n = 47), *GSTP1* (n = 25), *p14* (n = 19),
CDH1 (n = 18), *APC* (n = 18), *RUNX3* (n = 17), *SOCS1* (n = 15),
p15 (n = 13), *MGMT* (n = 11), *WIF1* (n = 9), *PRDM2* (n = 9),
SFRP1 (n = 8), *DAPK1* (n = 8), *RARβ* (n = 7), *IGF2* (n = 6),
hMLH1 (n = 5), *p73* (n = 5), *DLC1* (n = 3), *p53* (n = 3),
SPINT2 (n = 3), *RB1* (n = 3), *OPCML* (n = 3), *WT1* (n = 3)

Figure 1: Flow diagram of the stepwise selection from the relevant studies.

95% CI = 4.46–51.24, I² = 62%, *p* < 0.0001) and *PRDM2* (China: OR = 9.77, 95% CI = 6.01–15.88, I² = 38%, *p* < 0.00001; Japan: OR = 20.24, 95% CI = 10.80–37.95, I² = 47%, *p* < 0.00001)(Figure 4). The subgroup meta-analysis of *SOCS1* was unable to observe any significant result in each geographical population (Supplementary Figure S1).

The subgroup meta-analysis by geographical populations was also performed for *p16*, *APC*, *RUNX3* and *PRDM2* between HCC tumor tissues and normal tissues. As shown in Figure 5, we found a statistical difference between HCC tumor tissues and normal tissues for *p16* methylation in China from 15 studies (OR = 21.31, 95% CI = 11.10–40.94, I² = 0%, *p* < 0.00001), in Japan from 6 studies (OR = 9.20, 95% CI = 4.58–18.51, I² = 0%, *p* < 0.00001) and in Germany from 3 studies (OR = 30.43, 95% CI = 7.32–126.41, I² = 41%, *p* < 0.00001). The same consequence was also found in the other two genes including *RUNX3* (China: OR = 22.22, 95% CI =
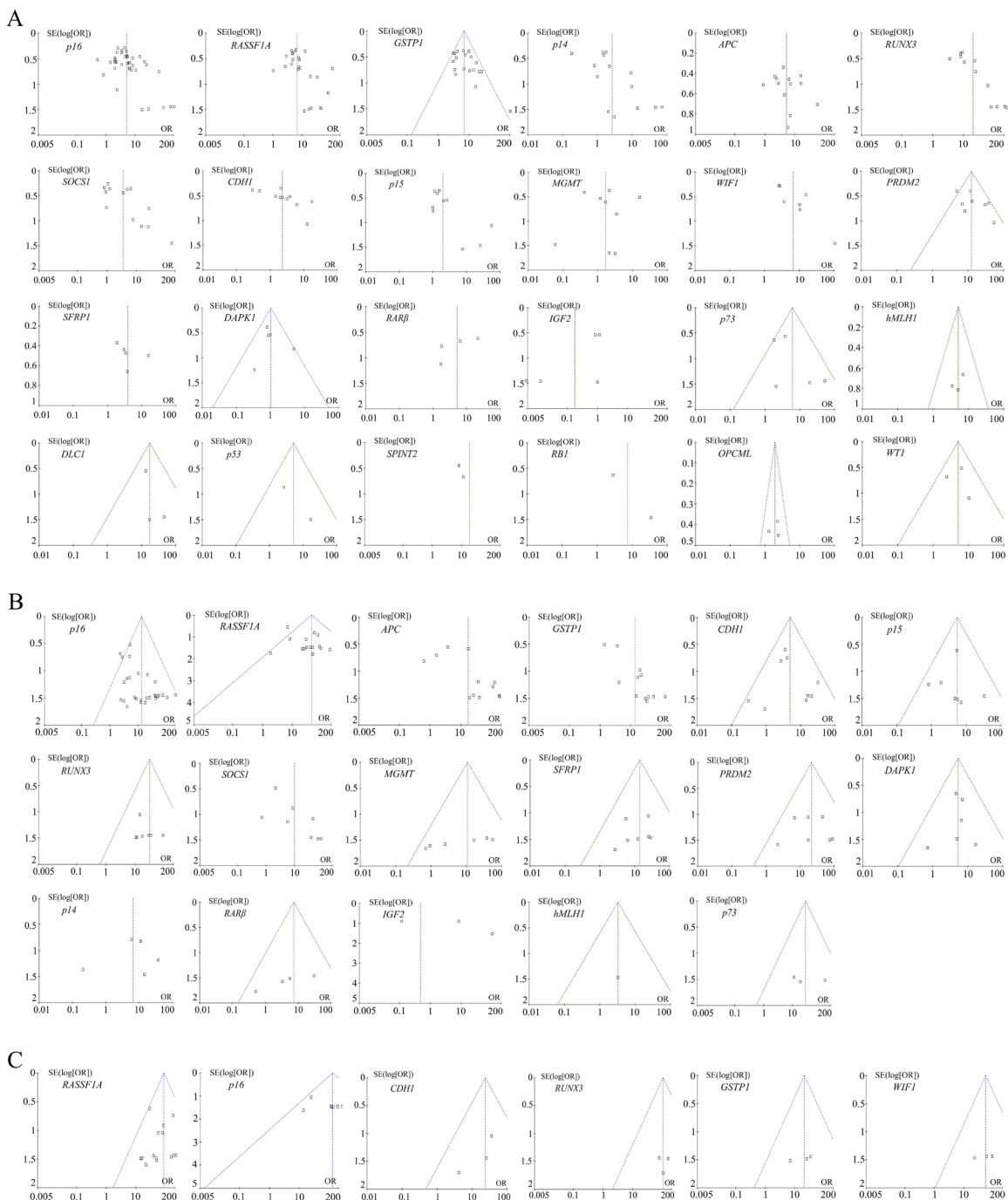
Figure 2: (**A**) Funnel plots of DNA methylation of 24 genes between HCC tumor tissues and adjacent tissues in the meta-analysis. (**B**) Funnel plots of DNA methylation of 17 genes between HCC tumor tissues and normal tissues in the meta-analysis. (**C**) Funnel plots of DNA methylation of four genes between HCC tumor serums and normal serums in the meta-analysis.

p16

Carcinoma tissues / Adjacent tissues / Odds Ratio / Odds Ratio

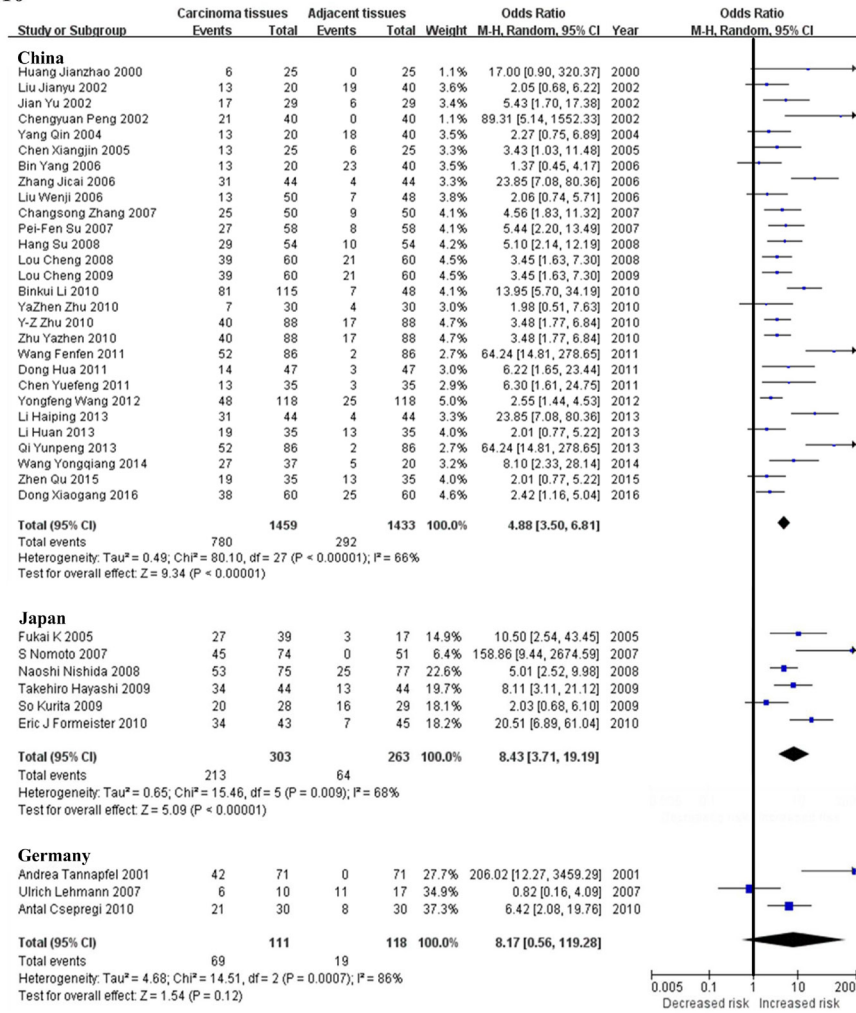| Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	Year
China							
Huang Jianzhao 2000	6	25	0	25	1.1%	17.00 [0.90, 320.37]	2000
Liu Jianyu 2002	13	20	19	40	3.6%	2.05 [0.68, 6.22]	2002
Jian Yu 2002	17	29	6	29	3.4%	5.43 [1.70, 17.38]	2002
Chengyuan Peng 2002	21	40	0	40	1.1%	89.31 [5.14, 1552.33]	2002
Yang Qin 2004	13	20	18	40	3.5%	2.27 [0.75, 6.89]	2004
Chen Xiangjin 2005	13	25	6	25	3.3%	3.43 [1.03, 11.48]	2005
Bin Yang 2006	13	20	23	40	3.5%	1.37 [0.45, 4.17]	2006
Zhang Jicai 2006	31	44	4	44	3.3%	23.85 [7.08, 80.36]	2006
Liu Wenji 2006	13	50	7	48	3.8%	2.06 [0.74, 5.71]	2006
Changsong Zhang 2007	25	50	9	50	4.1%	4.56 [1.83, 11.32]	2007
Pei-Fen Su 2007	27	58	8	58	4.1%	5.44 [2.20, 13.49]	2007
Hang Su 2008	29	54	10	54	4.2%	5.10 [2.14, 12.19]	2008
Lou Cheng 2008	39	60	21	60	4.5%	3.45 [1.63, 7.30]	2008
Lou Cheng 2009	39	60	21	60	4.5%	3.45 [1.63, 7.30]	2009
Binkui Li 2010	81	115	7	48	4.1%	13.95 [5.70, 34.19]	2010
YaZhen Zhu 2010	7	30	4	30	3.0%	1.98 [0.51, 7.63]	2010
Y-Z Zhu 2010	40	88	17	88	4.7%	3.48 [1.77, 6.84]	2010
Zhu Yazhen 2010	40	88	17	88	4.7%	3.48 [1.77, 6.84]	2010
Wang Fenfen 2011	52	86	2	86	2.7%	64.24 [14.81, 278.65]	2011
Dong Hua 2011	14	47	3	47	3.0%	6.22 [1.65, 23.44]	2011
Chen Yuefeng 2011	13	35	3	35	2.9%	6.30 [1.61, 24.75]	2011
Yongfeng Wang 2012	48	118	25	118	5.0%	2.55 [1.44, 4.53]	2012
Li Haiping 2013	31	44	4	44	3.3%	23.85 [7.08, 80.36]	2013
Li Huan 2013	19	35	13	35	4.0%	2.01 [0.77, 5.22]	2013
Qi Yunpeng 2013	52	86	2	86	2.7%	64.24 [14.81, 278.65]	2013
Wang Yongqiang 2014	27	37	5	20	3.2%	8.10 [2.33, 28.14]	2014
Zhen Qu 2015	19	35	13	35	4.0%	2.01 [0.77, 5.22]	2015
Dong Xiaogang 2016	38	60	25	60	4.6%	2.42 [1.16, 5.04]	2016
Total (95% CI)		1459		1433	100.0%	4.88 [3.50, 6.81]	
Total events	780		292				

Heterogeneity: Tau² = 0.49; Chi² = 80.10, df = 27 (P < 0.00001); I² = 66%
Test for overall effect: Z = 9.34 (P < 0.00001)

Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	Year
Japan							
Fukai K 2005	27	39	3	17	14.9%	10.50 [2.54, 43.45]	2005
S Nomoto 2007	45	74	0	51	6.4%	158.86 [9.44, 2674.59]	2007
Naoshi Nishida 2008	53	75	25	77	22.6%	5.01 [2.52, 9.98]	2008
Takehiro Hayashi 2009	34	44	13	44	19.7%	8.11 [3.11, 21.12]	2009
So Kurita 2009	20	28	16	29	18.1%	2.03 [0.68, 6.10]	2009
Eric J Formeister 2010	34	43	7	45	18.2%	20.51 [6.89, 61.04]	2010
Total (95% CI)		303		263	100.0%	8.43 [3.71, 19.19]	
Total events	213		64				

Heterogeneity: Tau² = 0.65; Chi² = 15.46, df = 5 (P = 0.009); I² = 68%
Test for overall effect: Z = 5.09 (P < 0.00001)

Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	Year
Germany							
Andrea Tannapfel 2001	42	71	0	71	27.7%	206.02 [12.27, 3459.29]	2001
Ulrich Lehmann 2007	6	20	11	17	34.9%	0.82 [0.16, 4.09]	2007
Antal Csepregi 2010	21	30	8	30	37.3%	6.42 [2.08, 19.76]	2010
Total (95% CI)		111		118	100.0%	8.17 [0.56, 119.28]	
Total events	69		19				

Heterogeneity: Tau² = 4.68; Chi² = 14.51, df = 2 (P = 0.0007); I² = 86%
Test for overall effect: Z = 1.54 (P = 0.12)

0.005 0.1 1 10 200
Decreased risk Increased risk

RASSF1A

Carcinoma tissues / Adjacent tissues / Odds Ratio / Odds Ratio

Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	Year
China							
Jian Yu 2002	29	29	23	29	1.4%	16.32 [0.87, 304.72]	2002
Changsong Zhang 2007	44	50	24	50	5.6%	7.94 [2.87, 21.98]	2007
Zhou Xiaojun 2007	20	24	9	24	4.3%	8.33 [2.15, 32.30]	2007
Hang Su 2008	50	54	50	54	4.0%	1.00 [0.24, 4.22]	2008
Lou Cheng 2009	57	60	43	60	4.5%	7.51 [2.07, 27.28]	2009
Chen Yuefeng 2011	23	35	10	35	5.7%	4.79 [1.74, 13.19]	2011
Zhang Huijin 2011	65	97	11	26	6.3%	2.77 [1.14, 6.72]	2011
Chen Wusong 2011	51	60	3	60	4.2%	107.67 [27.63, 419.56]	2011
Dong Hua 2011	30	47	9	47	6.0%	7.45 [2.91, 19.06]	2011
Fei Bojian 2011	14	20	5	20	4.1%	7.00 [1.74, 28.17]	2011
Ying Feng 2012	82	103	40	103	7.7%	6.15 [3.30, 11.45]	2012
Xue Wanjiang 2012	60	80	33	80	7.4%	4.27 [2.18, 8.38]	2012
Chen Tangen 2013	69	100	15	100	7.3%	12.61 [6.30, 25.23]	2013
Xiaoying Zhang 2013	48	48	40	48	1.4%	20.36 [1.14, 363.58]	2013
Fan Haiyan 2013	65	97	11	26	6.3%	2.77 [1.14, 6.72]	2013
Zhang Yuanying 2013	14	32	5	32	4.9%	4.20 [1.29, 13.70]	2013
Li Huan 2013	31	35	26	35	4.5%	2.68 [0.74, 9.73]	2013
Wang Yongqiang 2014	35	37	7	20	3.2%	32.50 [5.96, 177.11]	2014
Zhen Qu 2015	31	35	26	35	4.5%	2.68 [0.74, 9.73]	2015
Dong Xiaogang 2016	44	60	22	60	6.8%	4.75 [2.18, 10.33]	2016
Total (95% CI)		1103		944	100.0%	6.07 [4.18, 8.80]	
Total events	862		412				

Heterogeneity: Tau² = 0.37; Chi² = 44.42, df = 19 (P = 0.0008); I² = 57%
Test for overall effect: Z = 9.50 (P < 0.00001)

Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	Year
Japan							
Naoshi Nishida 2008	64	75	43	77	44.6%	4.60 [2.10, 10.06]	2008
Toyoki Moribe 2009	19	20	4	20	30.2%	76.00 [7.70, 750.49]	2009
Eric J Formeister 2010	43	43	31	45	25.2%	40.05 [2.30, 696.67]	2010
Total (95% CI)		138		142	100.0%	18.52 [2.30, 149.14]	
Total events	126		78				

Heterogeneity: Tau² = 2.38; Chi² = 7.03, df = 2 (P = 0.03); I² = 72%
Test for overall effect: Z = 2.74 (P = 0.006)

0.005 0.1 1 10 200
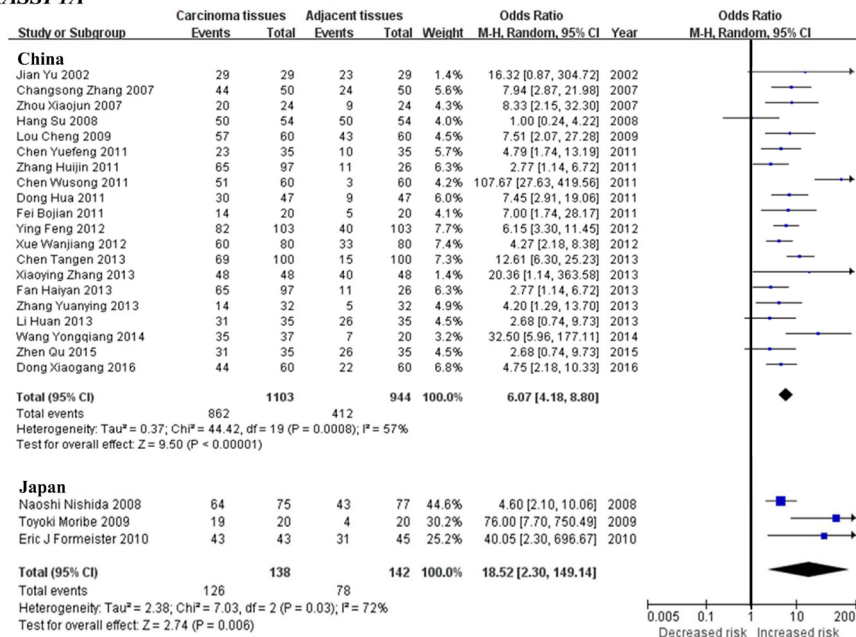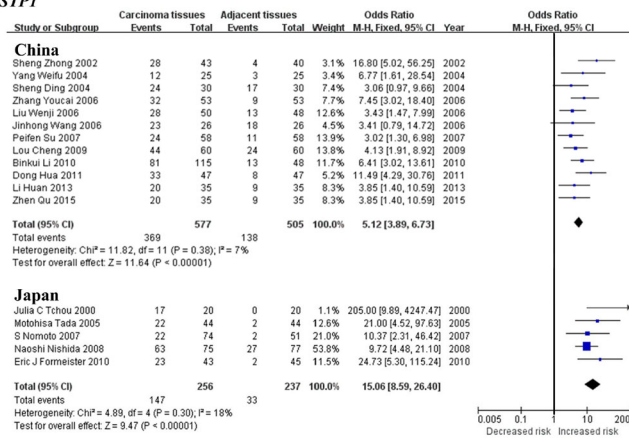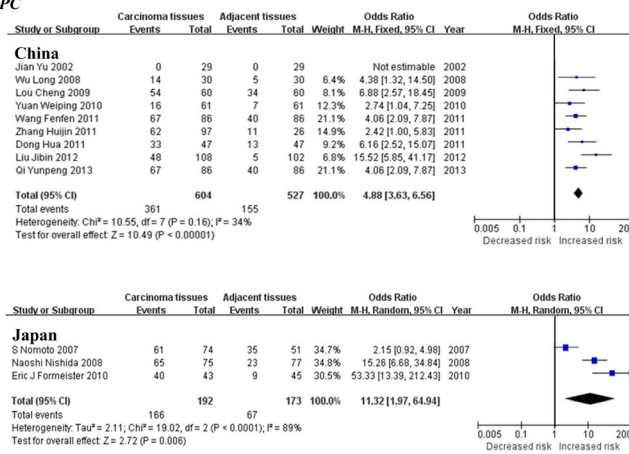Decreased risk Increased risk

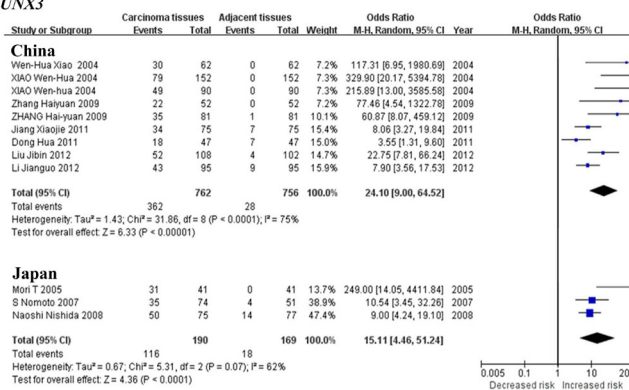**Figure 3: Forest plots of *p16* and *RASSF1A* methylation between HCC tumor tissues and adjacent tissues in the meta-analysis.**
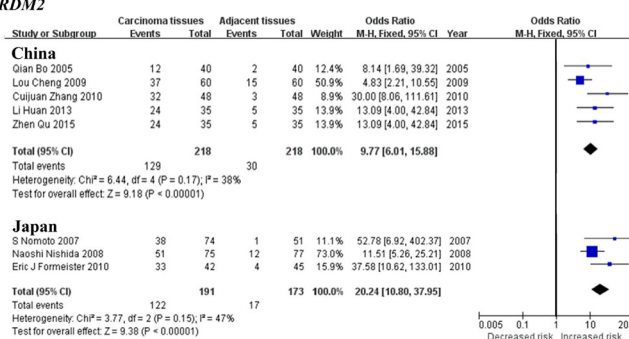
Figure 4: Forest plots of *GSTP1*, *APC*, *RUNX3* and *PRDM2* methylation between HCC tumor tissues and adjacent tissues in the meta-analysis.

5.36–92.12, $I^2 = 0\%$, $p < 0.0001$; Japan: OR = 53.40, 95% CI = 13.03–218.85, $I^2 = 36\%$, $p < 0.00001$) and *PRDM2* (China: OR = 30.54, 95% CI = 7.33–127.34, $I^2 = 23\%$, $p < 0.00001$; Japan: OR = 33.16, 95% CI = 8.97–122.59, $I^2 = 0\%$, $p < 0.00001$)(Figure 5). In addition, there was a significant geographical difference in the meta-analysis of *APC* gene in China (OR = 43.92, 95% CI = 12.13–159.06, $I^2 = 0\%$, $p < 0.00001$) but not in Japan (OR = 9.96, 95% CI = 0.64–155.15, $I^2 = 88\%$, $p = 0.10$, Figure 5).

DISCUSSION

Our meta-analysis has included a large amount of studies that evaluated the contribution of aberrant DNA methylation to the risk of HCC. The meta-analysis mainly focused on 24 tumor suppressor genes between HCC tumor tissues and adjacent tissues, 17 tumor suppressor genes between HCC tumor tissues and normal tissues and six tumor suppressor genes between HCC serums and normal serums (≥3 studies per gene). 20 HCC-associated genes (*p16*, *RASSF1A*, *GSTP1*, *p14*, *APC*, *RUNX3*, *SOCS1*, *CDH1*, *p15*, *WIF1*, *PRDM2*, *SFRP1*, *RARβ*, *p73*, *hMLH1*, *DLC1*, *p53*, *SPINT2*, *OPCML* and *WT1*) hypermethylation showed significant evidences between HCC tumor tissues and adjacent tissues to the risk of HCC. 15 HCC-associated genes (*p16*, *RASSF1A*, *APC*, *GSTP1*, *CDH1*, *p15*, *RUNX3*, *SOCS1*, *MGMT*, *SFRP1*, *PRDM2*, *DAPK1*, *p14*, *RARβ* and *p73*) hypermethylation showed significant evidences between HCC tumor tissues and normal tissues to the risk of HCC. Six HCC-associated genes (*RASSF1A*, *p16*, *CDH1*, *RUNX3*, *GSTP1* and *WIF1*) hypermethylation showed significant evidences between HCC serums and normal serums to the risk of HCC.

This meta-analysis showed that methylation of *p16*, *RASSF1A*, *APC*, *GSTP1*, *CDH1*, *p15*, *RUNX3*, *SOCS1*, *SFRP1*, *PRDM2*, *p14*, *RARβ* and *p73* genes in HCC tumor tissues was significantly higher than both adjacent tissues and normal tissues, revealing that the number of hepatic cells with these methylated genes may increase significantly in hepatocarcinogenesis process from normal liver to adjacent liver and HCC. In addition, methylation of *MGMT* and *DAPK1* genes in HCC tumor tissues was significantly higher than normal tissues but not adjacent tissues, revealing that methylation of two genes may play a significant role in the early stage of hepatocarcinogenesis process. However, methylation of *IGF2* gene in HCC tumor tissues was not significantly higher than neither adjacent tissues nor normal tissues, revealing that methylation of *IGF2* gene didn't play a significant role during the hepatocarcinogenesis process.

Subgroup meta-analysis by geographical populations found that *RASSF1A*, *GSTP1*, *APC*, *RUNX3* and *PRDM2* hypermethylation was a common risk factor between HCC tumor tissues and adjacent tissues in HCC patients, however, the statistical significance of *p16* hypermethylation was only found in China and Japan,
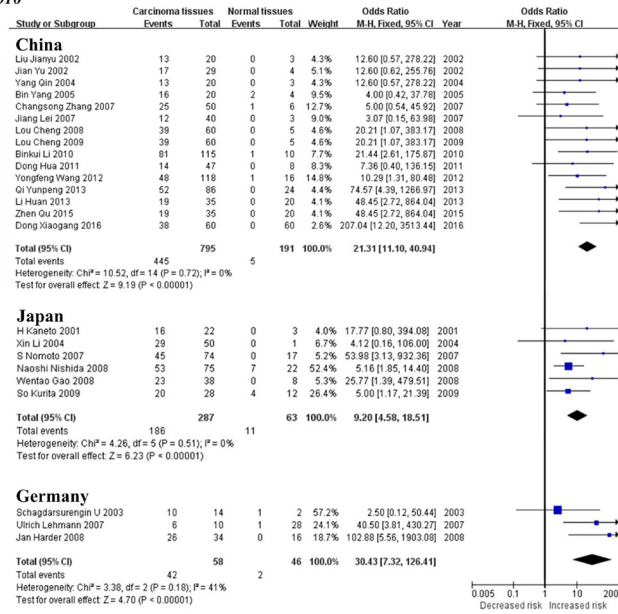
but not in Germany. In addition, we found that subgroup meta-analysis by geographical populations showed *p16*, *RUNX3* and *PRDM2* hypermethylation was a common risk factor between HCC tumor tissues and normal tissues in HCC patients. However, the statistical significance of *APC* hypermethylation was only found in China but not in Japan. The effects of *p16* hypermethylation on China, Japan and Germany, and *APC* hypermethylation on China and Japan are different, due to the diversities of hereditary, alcohol consumption and hepatitis virus infection in the different geographic regions [4, 19, 20]. Our results suggested that aberrant DNA methylation might become useful biomarkers in the early diagnosis of HCC.

On the other hand, some meta-analyses showed that global DNA hypomethylation in peripheral blood leukocytes may be a promising biomarker for cancer risk [21]. The association between global DNA methylation and cancer risk may depend on different factors, such as cancer and sample types [22]. For example, Barchitta found that LINE-1 methylation, a representative biomarker for global DNA methylation, increased significantly in colorectal carcinoma and gastric carcinoma, but not hepatocellular carcinoma. In addition, the significant difference in methylation levels was confirmed in tissue samples, but not in blood samples [22]. However, our meta-analysis showed that methylation of four genes (*RASSF1A*, *p16*, *CDH1* and *RUNX3*) in both tissue and serum samples were significantly different for the risk of HCC.
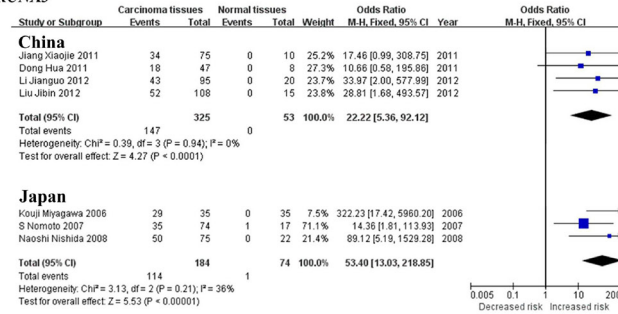
Aberrant gene methylation is recognized as one of the main mechanisms of triggering HCC [23], and it may serve as a useful biomarker for the prediction of HCC risk. The silencing of tumor suppressor genes by hypermethylation in the promoter regions also contributed to HCC progression [24]. For example, *RASSF1A* promoter methylation may conduce to the loss of RASSF1A expression, thereby leading to the silencing of *RASSF1A* gene and decrease of its function, which is one of the most common early events in HCC [25]. The same phenomenon occurred in many other genes, such as *p16*, *GSTP1*, *APC*, *RUNX3*, *SOCS1*, *WIF1*, *p73*, *hMLH1*, *DLC1*, *OPCML* and *WT1* [18, 26–33]. The frequencies of methylation of these gene promoters were significant higher in HCC than in nonneoplastic liver tissues and normal liver tissues. In addition, Feng revealed that *HOXA9*, *RASSF1* and *SFRP1* were more frequently methylated in HBV-positive HCC cases, while *CDKN2A* were significantly more frequently methylated in HCV-positive HCC cases, suggesting that methylation of these gene promoters may be involved in virus-induced hepatocarcinogenesis [34].

The present meta-analysis has several limitations that need to be taken with the following cautions. First, the selection bias is inevitable due to the search strategy restricted to the articles published in English or Chinese. Secondly, the heterogeneity existed in 15 genes between HCC tumor tissues and adjacent tissues and five genes
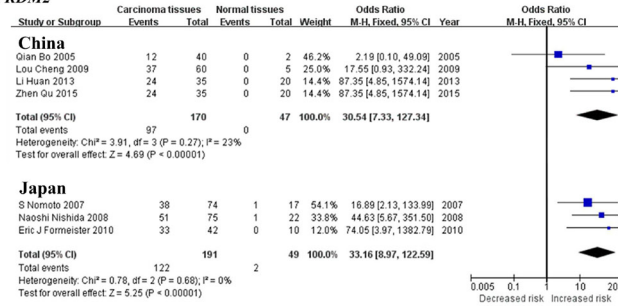
p16

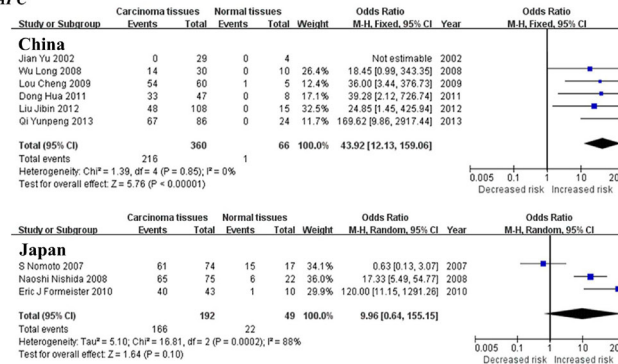| Study or Subgroup	Carcinoma tissues Events	Total	Normal tissues Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
China							
Liu Jianyu 2002	13	20	0	3	4.3%	12.60 [0.57, 278.22]	2002
Jian Yu 2002	17	29	0	4	5.1%	12.60 [0.62, 255.76]	2002
Yang Qin 2004	13	20	0	3	4.3%	12.60 [0.57, 278.22]	2004
Bin Yang 2005	16	20	2	4	9.5%	4.00 [0.42, 37.78]	2005
Changsong Zhang 2007	25	50	1	6	12.7%	5.00 [0.54, 45.92]	2007
Jiang Lei 2007	12	40	0	3	9.0%	3.07 [0.15, 63.98]	2007
Lou Cheng 2008	39	60	0	5	4.6%	20.21 [1.07, 383.17]	2008
Lou Cheng 2009	39	60	0	5	4.6%	20.21 [1.07, 383.17]	2009
Binkui Li 2010	81	115	1	10	7.7%	21.44 [2.61, 175.87]	2010
Dong Hua 2011	14	47	0	8	8.3%	7.36 [0.40, 136.15]	2011
Yongfeng Wang 2012	48	118	1	16	14.8%	10.29 [1.31, 80.48]	2012
Qi Yunpeng 2013	52	86	0	24	4.4%	74.57 [4.39, 1266.97]	2013
Li Huan 2013	19	35	0	20	4.1%	48.45 [2.72, 864.04]	2013
Zhen Qu 2015	19	35	0	20	4.1%	48.45 [2.72, 864.04]	2015
Dong Xiaogang 2016	38	60	0	60	2.6%	207.04 [12.20, 3513.44]	2016
Total (95% CI)		795		191	100.0%	21.31 [11.10, 40.94]	
Total events	445		5				

Heterogeneity: Chi² = 10.52, df = 14 (P = 0.72); I² = 0%
Test for overall effect: Z = 9.19 (P < 0.00001)

Japan							
H Kaneto 2001	16	22	0	3	4.0%	17.77 [0.80, 394.08]	2001
Xin Li 2004	29	50	0	1	6.7%	4.12 [0.16, 106.00]	2004
S Nomoto 2007	45	74	0	17	5.2%	53.98 [3.13, 932.36]	2007
Naoshi Nishida 2008	53	75	7	22	52.4%	5.16 [1.85, 14.40]	2008
Wentao Gao 2008	23	38	0	8	5.3%	25.77 [1.39, 479.51]	2008
So Kurita 2009	20	28	4	12	26.4%	5.00 [1.17, 21.39]	2009
Total (95% CI)		287		63	100.0%	9.20 [4.58, 18.51]	
Total events	186		11				

Heterogeneity: Chi² = 4.26, df = 5 (P = 0.51); I² = 0%
Test for overall effect: Z = 6.23 (P < 0.00001)

Germany							
Schagdarsurengin U 2003	10	14	1	2	57.2%	2.50 [0.12, 50.44]	2003
Ulrich Lehmann 2007	6	10	1	28	24.1%	40.50 [3.81, 430.27]	2007
Jan Harder 2008	26	34	0	16	18.7%	102.88 [5.56, 1903.08]	2008
Total (95% CI)		58		46	100.0%	30.43 [7.32, 126.41]	
Total events	42		2				

Heterogeneity: Chi² = 3.38, df = 2 (P = 0.18); I² = 41%
Test for overall effect: Z = 4.70 (P < 0.00001)

0.005 0.1 1 10 200
Decreased risk   Increased risk

RUNX3

Study or Subgroup	Carcinoma tissues Events	Total	Normal tissues Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
China							
Jiang Xiaojie 2011	34	75	0	10	25.2%	17.46 [0.99, 308.75]	2011
Dong Hua 2011	18	47	0	8	27.3%	10.66 [0.58, 195.86]	2011
Li Jianguo 2012	43	95	0	20	23.7%	33.97 [2.00, 577.99]	2012
Liu Jibin 2012	52	108	0	15	23.8%	28.81 [1.68, 493.57]	2012
Total (95% CI)		325		53	100.0%	22.22 [5.36, 92.12]	
Total events	147		0				

Heterogeneity: Chi² = 0.39, df = 3 (P = 0.94); I² = 0%
Test for overall effect: Z = 4.27 (P < 0.0001)

Japan							
Kouji Miyagawa 2006	29	35	0	35	7.5%	322.23 [17.42, 5960.20]	2006
S Nomoto 2007	35	74	1	17	71.1%	14.36 [1.81, 113.93]	2007
Naoshi Nishida 2008	50	75	0	22	21.4%	89.12 [5.19, 1529.28]	2008
Total (95% CI)		184		74	100.0%	53.40 [13.03, 218.85]	
Total events	114		1				

Heterogeneity: Chi² = 3.13, df = 2 (P = 0.21); I² = 36%
Test for overall effect: Z = 5.53 (P < 0.00001)

0.005 0.1 1 10 200
Decreased risk   Increased risk

PRDM2

Study or Subgroup	Carcinoma tissues Events	Total	Normal tissues Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
China							
Qian Bo 2005	12	40	0	2	46.2%	2.19 [0.10, 49.09]	2005
Lou Cheng 2009	37	60	0	5	25.0%	17.55 [0.93, 332.24]	2009
Li Huan 2013	24	35	0	20	14.4%	87.35 [4.85, 1574.14]	2013
Zhen Qu 2015	24	35	0	20	14.4%	87.35 [4.85, 1574.14]	2015
Total (95% CI)		170		47	100.0%	30.54 [7.33, 127.34]	
Total events	97		0				

Heterogeneity: Chi² = 3.91, df = 3 (P = 0.27); I² = 23%
Test for overall effect: Z = 4.69 (P < 0.00001)

Japan							
S Nomoto 2007	38	74	1	17	54.1%	16.89 [2.13, 133.99]	2007
Naoshi Nishida 2008	51	75	1	22	33.8%	44.63 [5.67, 351.50]	2008
Eric J Formeister 2010	33	42	0	10	12.0%	74.05 [3.97, 1382.79]	2010
Total (95% CI)		191		49	100.0%	33.16 [8.97, 122.59]	
Total events	122		2				

Heterogeneity: Chi² = 0.78, df = 2 (P = 0.68); I² = 0%
Test for overall effect: Z = 5.25 (P < 0.00001)

0.005 0.1 1 10 200
Decreased risk   Increased risk

APC

Study or Subgroup	Carcinoma tissues Events	Total	Normal tissues Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
China							
Jian Yu 2002	0	29	0	4		Not estimable	2002
Wu Long 2008	14	30	0	10	26.4%	18.45 [0.99, 343.35]	2008
Lou Cheng 2009	54	60	1	5	12.4%	36.00 [3.44, 376.73]	2009
Dong Hua 2011	33	47	0	8	17.1%	39.28 [2.12, 726.74]	2011
Liu Jibin 2012	48	108	0	15	32.5%	24.85 [1.45, 425.94]	2012
Qi Yunpeng 2013	67	86	0	24	11.7%	169.62 [9.86, 2917.44]	2013
Total (95% CI)		360		66	100.0%	43.92 [12.13, 159.06]	
Total events	216		1				

Heterogeneity: Chi² = 1.39, df = 4 (P = 0.85); I² = 0%
Test for overall effect: Z = 5.76 (P < 0.00001)

0.005 0.1 1 10 200
Decreased risk   Increased risk

Study or Subgroup	Carcinoma tissues Events	Total	Normal tissues Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Japan							
S Nomoto 2007	61	74	15	17	34.1%	0.63 [0.13, 3.07]	2007
Naoshi Nishida 2008	65	75	6	22	36.0%	17.33 [5.49, 54.77]	2008
Eric J Formeister 2010	40	43	1	10	29.9%	120.00 [11.15, 1291.26]	2010
Total (95% CI)		192		49	100.0%	9.96 [0.64, 155.15]	
Total events	166		22				

Heterogeneity: Tau² = 5.10; Chi² = 16.81, df = 2 (P = 0.0002); I² = 88%
Test for overall effect: Z = 1.64 (P = 0.10)

0.005 0.1 1 10 200
Decreased risk   Increased risk

Figure 5: Forest plots of *p16*, *RUNX3*, *PRDM2* and *APC* methylation between HCC tumor tissues and normal tissues in the meta-analysis.

between HCC tumor tissues and normal tissues. This phenomenon may be caused by the moderate number of samples in the involved studies and the inconsistent criteria in the selection of controls. We expect a larger size of samples to be tested in the future for a more reliable conclusion. Thirdly, our meta-analysis focused on the genes with at least three independent studies, and this might prevent those genes reported in two large studies from being included in the current meta-analysis. Finally, the status of DNA methylation was qualitative (M+ or M−) in all the selected studies that were performed with methylation specific PCR (MSP). The tested CpG sites might not stand for the whole promoter regions.

In summary, our meta-analysis indicated that aberrant DNA methylation was associated with the risk of HCC. Aberrant DNA methylation might become useful biomarkers for the prediction and prognostication of HCC.

MATERIALS AND METHODS

Study identification

We conducted a comprehensive systematic literature via PubMed, Web of Science, Cochrane Library, Embase, CNKI and Wanfang using the "hepatocellular carcinoma or hepatocarcinoma or primary liver cancer or HCC or hepatic carcinoma or liver tumor" and "DNA methylation" as keywords in titles and abstracts. The search was updated until September 1, 2016. The search was limited to the articles published in English and Chinese. A preliminary review of abstracts was conducted to determine the relevance on methylation study. Studies were selected if they met the following criteria: (1) they were the case-control associations of gene methylation with the risk of HCC in humans; (2) they had the sufficient methylation informations to calculate the odds ratios (ORs) and 95 % confidence intervals (CIs) for the meta-analysis. We excluded the studies such as letters, reviews, abstracts and conference articles. Studies with non case-control or no methylation frequency data were also removed from the meta-analysis. Furthermore, we excluded the genes with the number of articles less than 3. The selection process of the included studies was shown in the flow chart of Figure 1.

Data extraction and quality assessment

All the literatures and data included in the meta-analysis were retrieved and extracted independently by five authors (CZ, JL, TH, DD and DJ) to improve subjective bias, and any discrepancy was checked again and resolved through discussion. For each eligible article, we extracted the following information: first author's name, publication year, geographical populations, the numbers of cases and controls.

Quality assessment was performed by three authors (CZ, XS and DL) independently and any disagreement was discussed with the fourth author (KW). Due to the observational study design of the included studies, the Newcastle-Ottawa Scale was used to assess the methodological quality of the included studies. It assessed the selection, comparability and exposure of a case-control study.

Meta-analysis

The Review Manager software (version 5.0, Cochrane Collaboration, Oxford, UK) and STATA (version 10.0 Stata Corporation College Station, TX) were used for the current meta-analysis. The combined ORs and the corresponding 95% CIs were calculated and computed in the forest plots for each gene to evaluate the contribution of its DNA methylation to the risk of HCC. A fixed-effect model was applied for the meta-analysis with the moderate heterogeneity ($I^2 < 50\%$), otherwise a random-effect model was used. Funnel plots were used to check whether there was an obvious publication bias among the involved studies. *P* values less than 0.05 were considered to be significant.

Abbreviations

HCC: Hepatocellular carcinoma; CNKI: China National Knowledge Infrastructure; OR: Odds ratio; CI: Confidence interval; *RASSF1A*: Ras association domain family member1A; *GSTP1*: Glutathione S-transferase pi 1; *APC*: Adenomatous polyposis coli; *RUNX3*: Runt-related transcription factor 3; *SOCS1*: Suppressor of the cytokine signalling 1; *MGMT*: O6-methylguanine-DNA methyltransferase; *WIF1*: WNT inhibitory factor 1; *PRDM2*: PR domain containing 2; *SFRP1*: Secreted frizzled-related protein 1; *RARβ*: Retinoic acid receptor beta; *hMLH1*: Human MutL homolog 1; *DLC1*: Deleted in liver cancer 1; *SPINT2*: Serine peptidase inhibitor, Kunitz type, 2; *OPCML*: Opioid binding protein/cell adhesion molecule-like; *WT1*: Wilms tumor 1.

CONFLICTS OF INTEREST

The authors have declared that no competing interests exist.

Authors contributions

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