The prognostic value of CDKN2A hypermethylation in colorectal cancer: a meta-analysis

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Background: The prognostic value of CDKN2A promoter hypermethylation in colorectal cancer remains controversial. We systematically reviewed the evidence for assessment of CDKN2A methylation in colorectal cancer to elucidate this issue.

Methods: Pubmed, Embase and ISI web of knowledge were searched to identify eligible studies to evaluate the association of CDKN2A hypermethylation and overall survival and clinicopathological features of colorectal cancer patients. Combined hazard ratios (HRs) or odds ratios (ORs) with 95% confidence interval (95% CI) were pooled using a random-effects model.

Results: A total of 11 studies encompassing 3440 patients were included in the meta-analysis. CDKN2A hypermethylation had an unfavourable impact on OS of patients with colorectal cancer (HR 1.65, 95% CI 1.29–2.11). Subgroup analysis indicated that CDKN2A hypermethylation was significantly correlated with OS in Europe (HR 1.49; 95% CI 1.28–1.74) and Asia (HR 3.30; 95% CI 1.68–6.46). Furthermore, there was a significant association between CDKN2A hypermethylation and lymphovascular invasion (OR 1.68, 95% CI 1.15–2.47), lymph node metastasis (OR 1.68, 95% CI 1.09–2.59) and proximal tumour location (OR 2.09, 95% CI 1.34–3.26) of colorectal cancer.

Conclusion: This meta-analysis indicated that CDKN2A hypermethylation might be a predictive factor for unfavourable prognosis of colorectal cancer patients.

Colorectal cancer is the third most frequent malignancy worldwide, and represents the fourth most common cause of cancer-related deaths, leading to an estimated 608 000 deaths by 2008 (Ferlay et al, 2010). Despite recent advances in the management of colorectal cancer and new developments of cancer surveillance, the majority of colorectal cancer patients are still diagnosed at an advanced stage when the therapeutic options are limited, and the 5-year survival rate of colorectal cancer patients remains much lower than expected (Ferlay et al, 2010; Jemal et al, 2010).

Many efforts have been made to identify the molecular prognostic biomarkers including epigenetic markers for patients with colorectal cancer, in order to make a better selection of therapeutic approaches after surgery and improve patients’ survival (Draht et al, 2012). CDKN2A gene functions as an important tumour suppressor in various human malignancies including colorectal cancer, and its activation prevents carcinogenesis via induction of cell growth arrest and senescence (Collado et al, 2007; Rayess et al, 2012). CDKN2A promoter methylation is a frequent epigenetic event and an important mechanism leading to silencing...
and dysfunction of CDKN2A gene, which further results in uncontrolled cell proliferation and tumour development and progression (Samowitz et al., 2005). Ever since Liang et al. (1999) reported that the presence of CDKN2A promoter hypermethylation predicted shorter survival in colorectal cancer patients, the prognostic value of CDKN2A hypermethylation in colorectal cancer have been widely investigated, owing to the fact that these results exhibited an increasing relevance with clinical practice. Although multiple studies were conducted on colorectal cancer patients, whether CDKN2A hypermethylation is a predictive factor for poor prognosis remains controversial, and several studies in this field possessed a small sample size. Therefore, we conducted the present meta-analysis to appraise the prognostic value of CDKN2A hypermethylation in colorectal cancer.

MATERIALS AND METHODS

Search strategy and selection criteria. We conducted and reported this systematic review and meta-analysis following the PRISMA statement (Moher et al., 2009). The following were the criteria for the eligibility of included studies: (1) the study assessed the association between CDKN2A methylation and overall survival (OS) of patients with colorectal cancer; (2) the study evaluated the methylation status of CDKN2A promoter in primary tumour tissues after surgical resection; (3) the study provided a hazard ratio (HR) and 95% confidence interval (CI) directly or gave the data, which allow for the estimation of the HR and 95% CI; (4) the sample size of the study is not <40 patients.

Pubmed, Embase and ISI web of knowledge were searched by using the combinations of following terms: ‘CDKN2A’, ‘p16’, ‘methylation’, ‘colon’, ‘rectum’, ‘colorectal’, ‘cancer’, ‘carcinoma’, ‘prognosis’, ‘prognostic’ and ‘survival’. The search concluded in February 2013, and no lower date limit was used. We only included the studies that were published in the English language, given the fact that other languages were often not available for both authors and readers.

The bibliographies in selected articles were also examined to identify other relevant studies. Conference abstracts were not in the scope of this analysis owing to the insufficient data provided by the authors. All studies were carefully examined to avoid the inclusion of the duplicate data. Two reviewers (XX and CW) assessed the eligibility of the screened studies independently. Agreement was reached for the discrepancies by discussion.

Data extraction and management. Data were extracted independently by two authors (XX and CW) from each eligible study. The predefined form used for data extraction documented the most relevant items including author’s name, year of publication, study location, number of patients, methylation detection method, methylation rate and follow-up.

Methodological assessment. Methodological assessment for each of the included studies was performed by three investigators (XX, CW and CM) and scored them using REMARK guidelines and ELCWP quality scale (Steele et al., 2001; McShane et al., 2005). The three investigators reported the quality scores of reviewed studies independently, and reach a consensus value for each item.

Statistical analysis. For the quantitative aggregation of the survival data, HRs and their 95% CIs were used to assess the impact of CDKN2A hypermethylation on survival of colorectal cancer patients. Studies reporting results of multivariate or univariate analysis for survival were used for the aggregation of the survival data. If HRs and their corresponding SEs were not directly reported in the included studies, they were estimated according to the available survival data by using a method reported by Parmar et al. (1998). The individual HR estimates were pooled into a summary HR using the approach provided in a previous published study (Yusuf et al., 1985). For the analysis of the association of CDKN2A hypermethylation and clinicopathological features, odds ratios (ORs) and their 95% CIs were applied to estimate the effect. An observed HR or OR >1 implied a worse survival for the group with CDKN2A hypermethylation or the significant association between CDKN2A hypermethylation and clinicopathological characteristics, respectively. We pooled HRs and ORs of the studies by using Review Manager Version 5.1 (The Nordic Cochrane Centre, Copenhagen, Denmark). Publication bias was assessed by using a method reported by Egger et al. (1997a). We also explored reasons for inter-study heterogeneity using meta-regression analysis and subgroup analysis by study location, publication year, number of patients, methylation rate and quality score. The analysis of publication bias and meta-regression was performed by using STATA version 10.0 (StataCorp, College Station, TX, USA). Sensitivity analysis was conducted by omission of each single study to investigate the stability of the results.

RESULTS

Study selection and characteristics. Through the database search, a total of 135 articles were identified for initial evaluation (Figure 1). After excluding the studies out of the scope of our meta-analysis, 46 studies evaluating the prognostic effect of CDKN2A hypermethylation in patients with colorectal cancer were considered for further assessment in detail. By further review, 28 were excluded because the estimation of HRs in these studies was not allowed because of the insufficient data provided by the authors, three were excluded because the authors determined the methylation status of CDKN2A promoter by using DNA from other than tumour tissues (e.g., serum), one was excluded because it had overlapped data with another study, two were excluded because the studies were not publications in English, and one was excluded because of the small sample size in this study. Therefore,

![Figure 1. Flow diagram of study inclusion.](image-url)
three studies (27.3%; Table 1). Five of 11 studies (45.5%) revealed that CDKN2A hypermethylation was a poor prognostic factor for survival of patients, and 6 (54.5%) reported that CDKN2A hypermethylation did not have an unfavourable impact on survival.

The individual HRs of the 11 included studies were estimated using the methods reported by Parmar et al (1998). Five of these 11 studies directly provided their HRs. Two studies reported the total number of events and the log-rank statistic or its P-value according to which HRs can be approximated. In the four remaining studies, HRs had to be extrapolated from the graphical representations of the survival distributions.

Figure 2 demonstrates a forest plot of the individual HRs and results from the meta-analysis. Overall, CDKN2A hypermethylation in the primary tumour had significant association with enhanced mortality risk of colorectal cancer patients in the random-effects model (combined HR 1.65, 95% CI 1.29–2.11), despite the exhibition of heterogeneity among studies (I² 69%, P = 0.0004). For the exploration of the source of heterogeneity, the characteristics of the 11 eligible studies are extracted and summarised in Table 1. One study assessed the patients from China, one from Korea, two from Japan, two from Spain, one from France, one from Switzerland, two from USA and one from Australia. The total study sample size was 3440 with a mean of 313 (range, 84–902 patients). Four of these studies included <100 patients and 5 studies enrolled >200 patients (Table 1). These eligible studies were published from 1999 to 2012.

Study results report and meta-analysis. CDKN2A gene was found to be methylated in 23% of total colorectal cancer patients. The survival data by multivariate analysis can be obtained from three studies (27.3%; Table 1). Five of 11 studies (45.5%) revealed that CDKN2A hypermethylation was a poor prognostic factor for survival of patients, and 6 (54.5%) reported that CDKN2A hypermethylation did not have an unfavourable impact on survival.

Table 1. Major features of the included studies

| Study          | Study location | Number of patients | Methylation rate (%) | Methylation detection method | Stage | Median | Range | Survival analysis |
|----------------|----------------|--------------------|----------------------|-----------------------------|-------|--------|-------|-------------------|
| Barault et al, 2008 | France         | 582                | 26.3                 | MSP                         | I–IV  | —      | 60    | Multivariate      |
| Bihl et al, 2012     | Switzerland    | 422                | 20.6                 | Pyrosequencing and MSP      | I–IV  | 60     | 46–74 | Multivariate      |
| Esteller et al, 2001 | Spain          | 86                 | 34.9                 | MSP                         | Dukes’ A–C | 68     | 16–89 | Univariate        |
| Ishiguro et al, 2006 | Japan          | 88                 | 22.7                 | MSP                         | I–IV  | 53.2   | —     | Univariate        |
| Kim et al, 2010      | Korea          | 131                | 10.7                 | MSP                         | I–IV  | 49     | 1–116 | Univariate        |
| Liang et al, 1999    | China          | 84                 | 28.6                 | MSP                         | Dukes’ B2 | 60–86 | —     | Univariate        |
| Maeda et al, 2003    | Japan          | 90                 | 13.3                 | qMSP                        | II–IV | 54.5   | —     | Univariate        |
| Shen et al, 2007     | USA            | 182                | 17.0                 | MSP                         | IV    | 14     | —     | Univariate        |
| Shima et al, 2011a   | USA            | 902                | 29.8                 | qMSP                        | I–IV  | 146    | —     | Multivariate      |
| Veganzones-de-Castro et al, 2012 | Spain      | 318                | 24.8                 | qMSP                        | Dukes’ A–D | 92     | 75–111| Univariate        |
| Ward et al, 2003     | Australia      | 555                | 23.1                 | MSP                         | I–IV  | 32     | 1–60  | Univariate        |

Follow-up (months)

| Hazard ratio | Hazard ratio |
|--------------|--------------|
| IV, random, 95% CI | IV, random, 95% CI |
| 1.65 (1.29, 2.11) | Test for overall effect: Z = 4.03 (P < 0.0001) |

Figure 2. Meta-analysis of impact of CDKN2A hypermethylation on overall survival of patients with colorectal cancer. Results are presented as individual and pooled HR, and 95% CI.

11 studies meeting the inclusion criteria were finally enrolled in this systematic review and meta-analysis (Liang et al, 1999; Esteller et al, 2001; Maeda et al, 2003; Ward et al, 2003; Ishiguro et al, 2006; Shen et al, 2007; Barault et al, 2008; Kim et al, 2010; Shima et al, 2011a; Bihl et al, 2012; Veganzones-de-Castro et al, 2012; Figure 1).

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The meta-analysis of the correlation between CDKN2A tumour location (proximal metastasis (positive vs negative: OR 1.68, 95% CI 1.15–2.47), lymph node metastasis (positive vs negative: OR 1.68, 95% CI 1.09–2.59) and tumour location (proximal vs distal: OR 2.09, 95% CI 1.34–3.26). The meta-analysis of the correlation between CDKN2A hypermethylation and lymphovascular invasion, lymph node metastasis, Duke’s stage and tumour size did not exhibit inter-study heterogeneity ($I^2=0\%$), whereas the analysis of other clinico-pathological parameters exhibited heterogeneity ($I^2=51–92\%$).

Sensitivity analysis revealed that omitting any single study did not affect the pooled HR significantly. The evaluation of publication bias indicated that the Egger test reached the significance ($P=0.063<0.1$) for studies involved in the analysis of OS. The funnel plots for publication bias also demonstrated a certain degree of asymmetry (Figure 3).

### DISCUSSION

Although promoter methylation of a number of tumour suppressors (e.g., RASSF1A, MGMT) have been studied for their prognostic value in colorectal cancer patients recently, the identification of an epigenetic marker with established predictive value for survival of colorectal cancer patients remains a issue that needs to be addressed (Shima et al, 2011b; Chen et al, 2012). CDKN2A promoter hypermethylation been found to lead to silencing of the tumour-suppressor CDKN2A gene, which might further result in the development, progression and invasion of colorectal cancer, and could be associated with poor prognosis of patients (Tada et al, 2003; Rayess et al, 2012). Despite an increasing number of studies performed on promoter status of CDKN2A gene, there is still controversy over the prognostic value of CDKN2A hypermethylation in colorectal cancer. We thus conducted this systematic review and meta-analysis to assess whether CDKN2A hypermethylation could predict the prognosis of colorectal cancer patients.

This study aggregated the outcomes of 3440 colorectal cancer patients from 11 individual trials, revealing that CDKN2A hypermethylation is a significant predictor for poor OS of colorectal cancer patients (HR 1.65, 95% CI 1.29–2.11).

### Table 2. Meta-regression and subgroup analysis of the studies reporting the association of CDKN2A hypermethylation and overall survival of cancer patients

| Stratified analysis | No. of studies | No. of patients | Fixed | Random | Meta-regression P-value | $I^2(\%)$ | P-value |
|--------------------|----------------|----------------|-------|--------|-------------------------|---------|---------|
| Study location     |                |                |       |        |                         |         |         |
| Europe             | 4              | 1408           | 1.49 (1.28, 1.74) | 1.49 (1.27, 1.76) | 0.008 | 4 | 0.37 |
| Asia               | 4              | 393            | 3.32 (2.14, 5.15) | 3.30 (1.68, 6.46) | 56 | 0.08 |
| USA                | 2              | 1084           | 1.13 (0.92, 1.39) | 1.20 (0.83, 1.74) | 59 | 0.12 |
| Oceania            | 1              | 555            | 1.40 (0.91, 2.16) | 1.40 (0.91, 2.16) | – | – |
| Publication year   |                |                |       |        |                         | 0.510   |         |
| ≥ 2007             | 6              | 1085           | 1.73 (1.35, 2.21) | 1.85 (1.29, 2.65) | 44 | 0.11 |
| > 2007             | 5              | 2355           | 1.37 (1.20, 1.56) | 1.52 (1.09, 2.13) | 80 | 0.0004 |
| No. of patients    |                |                |       |        |                         | 0.226   |         |
| < 200              | 6              | 661            | 2.14 (1.61, 2.85) | 2.45 (1.46, 4.10) | 63 | 0.02 |
| > 200              | 5              | 2779           | 1.33 (1.18, 1.51) | 1.34 (1.08, 1.65) | 58 | 0.05 |
| Methylation rate (%) |            |                |       |        |                         | 0.592   |         |
| < 24               | 6              | 1468           | 1.56 (1.33, 1.82) | 1.76 (1.27, 2.43) | 57 | 0.04 |
| > 24               | 5              | 1972           | 1.31 (1.11, 1.56) | 1.57 (1.03, 2.39) | 78 | 0.001 |
| REMARK score (%)   |                |                |       |        |                         | 0.770   |         |
| < 12               | 5              | 1001           | 1.54 (1.19, 2.00) | 1.54 (1.19, 2.00) | 0 | 0.65 |
| > 12               | 6              | 2439           | 1.42 (1.25, 1.61) | 1.75 (1.22, 2.51) | 83 | 0.0001 |
| ELCWP score (%)    |                |                |       |        |                         | 0.279   |         |
| < 66               | 6              | 1399           | 1.64 (1.40, 1.92) | 1.92 (1.36, 2.70) | 57 | 0.04 |
| > 66               | 5              | 2041           | 1.24 (1.05, 1.47) | 1.45 (1.01, 2.08) | 73 | 0.006 |

Abbreviations: CI = confidence interval; HR = hazard ratio.

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The significant association between CDKN2A hypermethylation and OS of patients was also present in the analysis of studies following REMARK guidelines more rigorously (HR 1.49; 95% CI 1.28–1.74) and Asia (HR 3.30, 95% CI 1.68–6.46) while not in other study locations. As the characteristics of colorectal cancer in different regions might differ because of diverse environmental factors and genetic and epigenetic background of human races, the prognostic value of biomarkers such as CDKN2A promoter hypermethylation in colorectal cancer might be different among different study locations.

A significant heterogeneity of the included studies was observed in this systematic review except for the analysis of studies on OS in Europe. In sensitivity analysis, omission of any individual study did not reduce the heterogeneity or help to elucidate the source of heterogeneity. Meta-regression and subgroup analysis indicated that study location might account for part of inter-study heterogeneity. Moreover, subgroup analysis indicated that CDKN2A hypermethylation was significantly correlated with poor prognosis of patients in Europe (HR 1.49; 95% CI 1.28–1.74) and Asia (HR 3.30, 95% CI 1.68–6.46) while not in other study locations. As the characteristics of colorectal cancer in different regions might differ because of diverse environmental factors and genetic and epigenetic background of human races, the prognostic value of biomarkers such as CDKN2A promoter hypermethylation in colorectal cancer might be different among different study locations. However, the pooled analysis of all included studies from different regions in this meta-analysis indicated an overall predictive role of CDKN2A hypermethylation for poor survival in colorectal cancer patients worldwide. More multicenter studies might further clarify whether CDKN2A serves as a prognostic factor for colorectal cancer patients worldwide.

As for the detection of methylation status of CDKN2A promoter in tumour tissues, the studies included in this meta-analysis used either MSP that is a semi-quantitative approach or qMSP that is a quantitative approach. Moreover, the studies did not apply the same PCR primers and the CDKN2A promoter regions examined for methylation status was also not always uniform, leading to a potential bias because the sensitivity and specificity of MSP or qMSP might depend on the primers used, regions detected and other PCR conditions. Subgroup analysis was not possible to be conducted to address this technical issue, because the small groups of studies used the same primers and other PCR conditions. Furthermore, owing to the fact that an optimal threshold has not been defined for qMSP or pyrosequencing, the cutoff defining a colorectal cancer with CDKN2A promoter hypermethylation is not identical, which might also potentially generate a certain degree of heterogeneity.

The approach of extrapolating the HRs from studies was also a potential factor that might lead to bias. If HRs were not directly provided by the studies, we calculated them according to the data reported in the publications or estimated them by extrapolating the information from the survival curves. Although two of the investigators (XX and CW) extracted the survival rates according to the graphical representation of the survival curves, this did not reduce the heterogeneity or help to elucidate the source of heterogeneity.

### Table 3. Meta-analysis of the association between CDKN2A hypermethylation and clinicopathological features of colorectal cancer

| Stratification of CRC   | No. of studies | No. of patients | Fixed HR (95%CI) | Random HR (95%CI) | P-value | I^2 (%) | P-value |
|-------------------------|---------------|-----------------|-----------------|-------------------|---------|---------|---------|
| Gender                  | 6             | 2369            | 1.31 (1.09, 1.58) | 1.27 (0.94, 1.71) | 0.12    | 51      | 0.07    |
| Stage of disease        | 3             | 1168            | 1.24 (0.95, 1.62) | 1.63 (0.84, 3.16) | 0.14    | 65      | 0.06    |
| Dukes’ stage            | 2             | 406             | 1.49 (0.94, 2.35) | 1.49 (0.94, 2.35) | 0.09    | 0       | 0.51    |
| Lymphovascular invasion | 3             | 784             | 1.68 (1.15, 2.47) | 1.68 (1.15, 2.47) | 0.008   | 0       | 0.89    |
| Lymph node metastasis   | 2             | 496             | 1.68 (1.09, 2.59) | 1.68 (1.09, 2.59) | 0.02    | 0       | 0.99    |
| Grade of differentiation| 7             | 2597            | 0.55 (0.42, 0.72) | 1.23 (0.39, 3.83) | 0.72    | 92      | <0.00001|
| Mucinous type           | 4             | 1379            | 1.36 (0.94, 1.94) | 0.98 (0.35, 2.71) | 0.97    | 82      | 0.001   |
| Tumour location         | 7             | 2616            | 2.68 (2.23, 3.23) | 2.09 (1.34, 3.26) | 0.001   | 77      | 0.0002  |
| Tumour size             | 2             | 369             | 1.08 (0.56, 2.09) | 1.07 (0.54, 2.10) | 0.81    | 0       | 0.32    |

Abbreviations: CRC = colorectal cancer; CI = confidence interval; OR = odds ratio.
The estimated HRs might thus be not as reliable as those retrieved directly from reported statistics. However, we compared our estimated HRs and 95% CIs with the results reported in papers and did not identify any major deviation of our results from the results available in the publications.

Egger tests and funnel plots exhibited a certain degree of publication bias for the analysis of association between CDKN2A methylation and OS. As known, studies that did not possess statistically significant results are less frequently published, and even if these results are published, they are more frequently reported in a brief way and not easily available for analysis. It should be also noted that the positive studies are more frequently published in English language, whereas unpublished studies and conference abstracts were not enrolled, because the data that can be used for methodological evaluation and meta-analysis were only available in full published studies. Moreover, studies that did not report sufficient data for estimation of HRs were ruled out from this systematic review, and this might potentially generate bias. However, we conducted a complete literature search through the above databases for the eligible studies to minimise the possible bias, and the large sample of colorectal cancer patients (n = 3440) enrolled in this analysis ensures the reliability and stability of the results.

In addition, CDKN2A promoter hypermethylation is a frequent epigenetic event, and is often included in the panel of genes to assess the CIMP in colorectal cancer. Several studies in this meta-analysis included the analysis on the prognostic value of CIMP, which included CDKN2A, hypermethylation and other gene methylations (Ward et al, 2003; Shen et al, 2007; Barault et al, 2008; Kim et al, 2010; Shima et al, 2011a). Whereas, these studies usually applied the CIMP’s comprising various gene patterns different among studies, which might lead to the difficulty of direct comparison and could generate more heterogeneity if the data were pooled. Therefore, more homogenous studies on the association between identical CIMP and cancers are needed in the future to allow for the meta-analysis on the prognostic value of CIMP in colorectal cancer.

To sum up, this meta-analysis indicated that CDKN2A hypermethylation was significantly associated with poor OS as well as lymphovascular invasion, lymph node metastasis, and proximal tumour location in colorectal cancer. CDKN2A hypermethylation might be a predictive factor of poor prognosis in patients with colorectal cancer particularly in Europe and Asia, and might predict invasion and metastasis. However, more prospective studies with homogeneity are needed to further confirm the results in this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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