Original Article
The prognostic value of CDX2 in colorectal cancer: a meta-analysis

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Abstract: Caudal-type homeodomain transcription factors 2 (CDX2) acts as an intestine-specific transcription factor is essential for intestinal differentiation and development, which shows a low expression in colorectal cancer tissues. However, the prognostic role of CDX2 in colorectal cancer remains controversial. Here, a meta-analysis of eight published studies containing 2547 patients was conducted to evaluate the prognostic value of CDX2 in colorectal cancer by calculating the hazard ratio (HR) and its 95% confidence interval (CI). The summary results revealed that the low expression of CDX2 was association with poor prognosis of OS (HR=1.99, 95% CI: 1.31-3.02) and DFS (HR=1.81, 95% CI: 1.08-3.05). Subgroup analysis showed decreased CDX2 was a significant prognostic marker in Asian patients (HR=2.66, 95% CI: 1.38-5.13), but not in Caucasian (HR=1.56, 95% CI: 0.94-2.59). This meta-analysis demonstrated that CDX2 could act as a significant biomarker in the prognosis of colorectal cancer.

Keywords: CDX2, colorectal cancer, prognosis, meta-analysis

Introduction
Colorectal cancer (CRC) is a most common malignant tumor worldwide, and its incidence has increased in recent years [1]. Cancer staging according to the guidelines of the American Joint Committee on Cancer helps to estimate prognosis and to select primary and adjuvant therapy in CRC, but the results of the treatment are variable within the same cancer stage because of the heterogeneity of the molecular changes [2]. It is useful to identify new biomarkers to assist in predicting the response to therapy and disease outcome.

Caudal-type homeodomain transcription factors 2 (CDX2) plays an essential role in the intestinal development [3-6]. The expression of CDX2 in adults is restricted to the intestine, from the duodenum to the rectum [7]. Knockdown of CDX2 expression increases susceptibility for tumors and accelerates G1-S cell cycle transition in heterozygous Cdx2+/− mice [8]. Overexpression of CDX2 inhibits growth and promotes differentiation of colorectal cancer cells [9, 10]. In addition, CDX2 has been shown to disrupt the β-catenin TCF protein complexes by binding to β-catenin, thereby resulting in the suppression the signaling of Wnt/β-catenin and cell proliferation [11]. Some clinical studies showed that low expression of CDX2 is associated with poor outcome in the patients of colorectal cancer [12, 13], but this association could be not validated by some other studies [14, 15].

Here, we present a meta-analysis to evaluate the influence of NRP-1 overexpression on the clinical outcomes in colorectal cancer to verify development of therapeautic strategies.

Materials and methods
Literature search

PubMed and the Web of Science databases were searched for studies which evaluated the level of NRP-1 expression and overall survival (OS) in patients with colorectal cancer between 1987 and 2015. Studies were selected using the following search terms: CDX2; CDX-2; colon cancer; colorectal cancer; and oncogene. We
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identified a total of 280 studies. Additionally, we also experimented to trace find the unpublished data though a search in Google, Baidu, and Wikipedia, but no additional studies were proper for inclusion.

Inclusion and exclusion criteria

The meta-analysis included studies that met the inclusion criteria as follows: (1) evaluation of CDX2 expression based on the human CRC tissues; (2) publications in English language; (3) studies reported the association between CDX2 expression level and CRC prognosis; and (4) studies with the hazard ratio (HR) and its 95% confidence intervals (CIs) or Kaplan-Meier curve to calculate these data. And the exclusion criteria were as follows: (1) letters, case reports, reviews, and conference abstracts without original data; (2) articles from which the relevant data could not be extracted; and (3) overlapping articles or ones with duplicate data.

Data extraction and assessment of study quality

Two of the colleagues (Hongbin Yu, and Heng Zhang) independently extracted data from eligible studies. The following data was extracted: name of first author; publication year; patients number; country; ethnicity; age; follow-up time; method of assessment; antibody source. The two reviewers checked the data again and discussed the data if the results differed to reach a consensus. A third author was invited to the discussion when the two primary authors could not reach an agreement. Study quality was evaluated independently by reviewers of Hongbin Yu and Heng Zhang according to the Newcastle-Ottawa quality assessment scale.

Statistical analysis

Survival data were extracted or calculated by Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/). The impact of CDX2 expression on OS and DFS was estimated by HR and 95% CI. Tests for heterogeneity were performed for each analysis, with significance set at P<0.05 [15]. In each analysis, heterogeneity was carried out with P<0.05 showed the significance, and I² was also calculated with ≥50% standing for substantial heterogeneity. The potential risk of publication bias was evaluated by Egger’s test, in which the P value<0.05 showed the significance. We also estimated the effect of individual studies on the summary HR by reestimating and plotting the summary HR in the absence of each study. The statistical analyses were carried out using STATA version 12.0 software (Stata Corporation, Collage Station, Texas, USA). P<0.05 showed significance in two-sided test.

Results

Characteristics of eligible studies

The combined search displays 280 references from Web of Science and PubMed databases. Among these references, 246 non-relevant articles, including review articles, articles only with abstract and duplicate and meta-analysis studies, were excluded through reviewing the titles and abstracts. The remaining 34 articles were reviewed and analyzed in detail, of which, four articles reported in CDX2 gene polymorphism, 19 did not describe patients overall survival and three cannot extract HR and 95% CI of OS. Finally, eight relevant articles with nine studies involving 2547 patients evaluated CDX2 expression level and overall survival (OS) in patients with colorectal cancer were eligible for this meta-analysis [12-19]. The study flowchart of references selection process is shown in
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**Table 1.** Summary sheet of patient characteristics in the selected studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Number (M/F)</th>
<th>Age</th>
<th>Rate of low CDX2 expression</th>
<th>Methods</th>
<th>Quality score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bae et al.</td>
<td>2015</td>
<td>South Korea</td>
<td>Asian</td>
<td>713 (434/279)</td>
<td>62</td>
<td>5.9%</td>
<td>IHC</td>
<td>7</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>2013</td>
<td>South Korea</td>
<td>Asian</td>
<td>109 (66/43)</td>
<td>56.9</td>
<td>13.8%</td>
<td>IHC</td>
<td>7</td>
</tr>
<tr>
<td>Hong et al.</td>
<td>2013</td>
<td>South Korea</td>
<td>Asian</td>
<td>207 (119/88)</td>
<td>63.2</td>
<td>5.3%</td>
<td>IHC</td>
<td>7</td>
</tr>
<tr>
<td>Dawson et al.</td>
<td>2013</td>
<td>Switzerland</td>
<td>Caucasian</td>
<td>201 (125/76)</td>
<td>72</td>
<td>34.5%</td>
<td>IHC</td>
<td>7</td>
</tr>
<tr>
<td>Knösel et al.</td>
<td>2012</td>
<td>Germany</td>
<td>Caucasian</td>
<td>402 (205/197)</td>
<td>65.4</td>
<td>42.0%</td>
<td>IHC</td>
<td>7</td>
</tr>
<tr>
<td>Bauer et al.</td>
<td>2012</td>
<td>USA</td>
<td>Caucasian</td>
<td>102</td>
<td>NR</td>
<td>15.7%</td>
<td>Genechip arrays</td>
<td>6</td>
</tr>
<tr>
<td>Bauer et al.</td>
<td>2012</td>
<td>USA</td>
<td>Caucasian</td>
<td>95</td>
<td>NR</td>
<td>10.5%</td>
<td>Genechip arrays</td>
<td>6</td>
</tr>
<tr>
<td>Matsuda et al.</td>
<td>2010</td>
<td>Japan</td>
<td>Asian</td>
<td>97</td>
<td>NR</td>
<td>9.3%</td>
<td>IHC</td>
<td>7</td>
</tr>
<tr>
<td>Baba et al.</td>
<td>2009</td>
<td>USA</td>
<td>Caucasian</td>
<td>621 (242/379)</td>
<td>NR</td>
<td>29.0%</td>
<td>IHC</td>
<td>7</td>
</tr>
</tbody>
</table>

M: male; F: female; NR: no reported; IHC: Immunohistochemistry.

**Figure 1.** and the patient characteristics and study quality score of the 9 studies are summarized in **Table 1**.

**Results of meta-analysis**

The meta-analysis was carried out on nine studies evaluating the relation of CDX2 expression in colorectal cancer with OS. The pooled HR was 1.99 (95% CI: 1.31-3.02; Z=3.25; P=0.001) (**Figure 2**) with heterogeneity ($I^2=77\%$, $p<0.001$). Three studies were used to evaluate the relation of CDX2 expression in colorectal cancer with DFS; the pooled HR was 1.81 (95% CI: 1.08-3.05; Z=2.24; P=0.025) (**Figure 3**) without heterogeneity ($I^2=64.2\%$, $P=0.061$). These results suggested that low expression of CDX2 was significantly correlated with a worse prognosis of colorectal cancer and that CDX2 low expression may serve as an independent prognostic factor in colorectal cancer.

Subgroup analysis was performed by the ethnicity, source of primary antibodies, rates of CDX2 low expression (**Table 2**). The results revealed that a significant relationship between...
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CDX2 expression in colorectal cancer and OS was shown in Asian countries (HR 2.66, 95% CI: 1.38-5.13, Z=2.91, P=0.004) with heterogeneity ($I^2$ 70.7%, P=0.017) (Table 2), but not in Caucasian patients (HR=1.56, 95% CI: 0.94-2.59) with heterogeneity ($I^2$ 74.4%, P=0.004). Heterogeneity was smaller when the rate of CDX2 low expression was a percentage >15.7% ($I^2$ 53.90%, P=0.114) than ≤15.7%. When OS analysis was limited to studies with primary antibodies derived from the same company (Biogenex) in difference concentration, heterogeneity was still significant ($I^2$ 82.3%, P=0.001), which was larger than the heterogeneity in all other companies except for Biogenex ($I^2$ 45.2%, P=0.121). It indicated that the evaluation standards of CDX2 low express and the antibody in difference concentration contributed to heterogeneity in the results.

Publication bias and sensitivity analysis

In this meta-analysis, publication bias was evaluated by Begg’s Test and Egger’s test. All analyses demonstrated a high probability of publication bias with Begg’s Test (P=0.029) and Egger’s test (P=0.001). We performed a sensitivity analysis in which one study was excluded.
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Discussion

Cdx2 is an intestine-specific transcription factor, which is essential for intestinal differentiation and development. CDX2 protein (but not gene polymorphism) is a prognostic factor in gastric cancer, which acts as a marker of good outcome in patients with gastric cancer [20-22]. However, its prognostic significance in colorectal cancer remains controversial. In this article, eight articles of nine studies were contained in this meta-analysis to evaluate the prognostic value of CDX2 expression in colorectal cancer. We found decreased CDX2 expression in colorectal tissue was significantly associated with worse overall survival and progression-free survival in patients. Subgroup analysis showed decreased CDX2 was a negative prognostic marker in Asian patients, but not in Caucasian. We found that the evaluation standards of CDX2 low express and the antibody in difference concentration may contribute to heterogeneity in the results.

There are four limitations to this meta-analysis. First, eight articles of nine studies published in English probably produced additional bias in the study included in this meta-analysis. Second, HRs calculated from data or extrapolated from survival curves might be less reliable than direct analysis of variance. Third, significant heterogeneity between studies resulting from some reasons, such as methods of detection, quality of antibody, and the difference standard for CDX2 negative expression was detected in this meta-analysis. Among all the studies, studies of Baba et al. and Matsuda et al. used the antibody from the same company with similar concentration of detection (1:50 and 1:20, respectively), but the ratio of CDX2 negative expression (29% vs 9.3%) and HR (1.16 vs 28.6) are difference.

In conclusion, our meta-analysis of 9 studies showed that low expression of CDX2 is relevant to a poor outcome in colorectal cancer, which demonstrated that CDX2 could act as a significant biomarker in the prognosis of colorectal cancer.

Disclosure of conflict of interest

None.

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