Int J Clin Exp Med 2016;9(8):15955-15960
www.ijcem.com /ISSN:1940-5901/IJCEM0028752

Original Article

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

Hongbin Yu[1*], Heng Zhang[2*], Qixin Cao[3], Wei Zhu[1]

[1]Department of General Surgery, First People's Hospital of Huzhou, Zhejiang Province, China; [2]Department of General Surgery, Central Hospital of Lishui, Zhejiang Province, China; [3]Department of Surgery, Huzhou Hospital of Traditional Chinese Medicine, Zhejiang Province, China. [*]Equal contributors.

Received March 20, 2015; Accepted June 12, 2016; Epub August 15, 2016; Published August 30, 2016

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 therapeutic 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

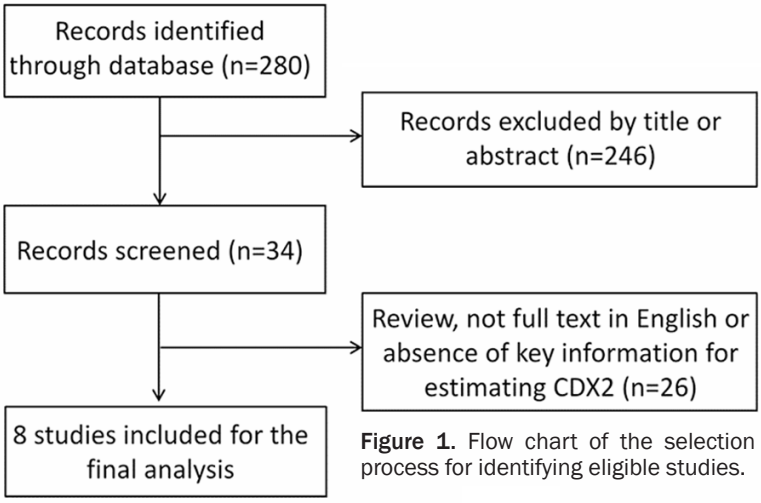

Figure 1. Flow chart of the selection process for identifying eligible studies.

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 KaplanMeier 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 I^2 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

Table 1. Summary sheet of patient characteristics in the selected studies

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

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

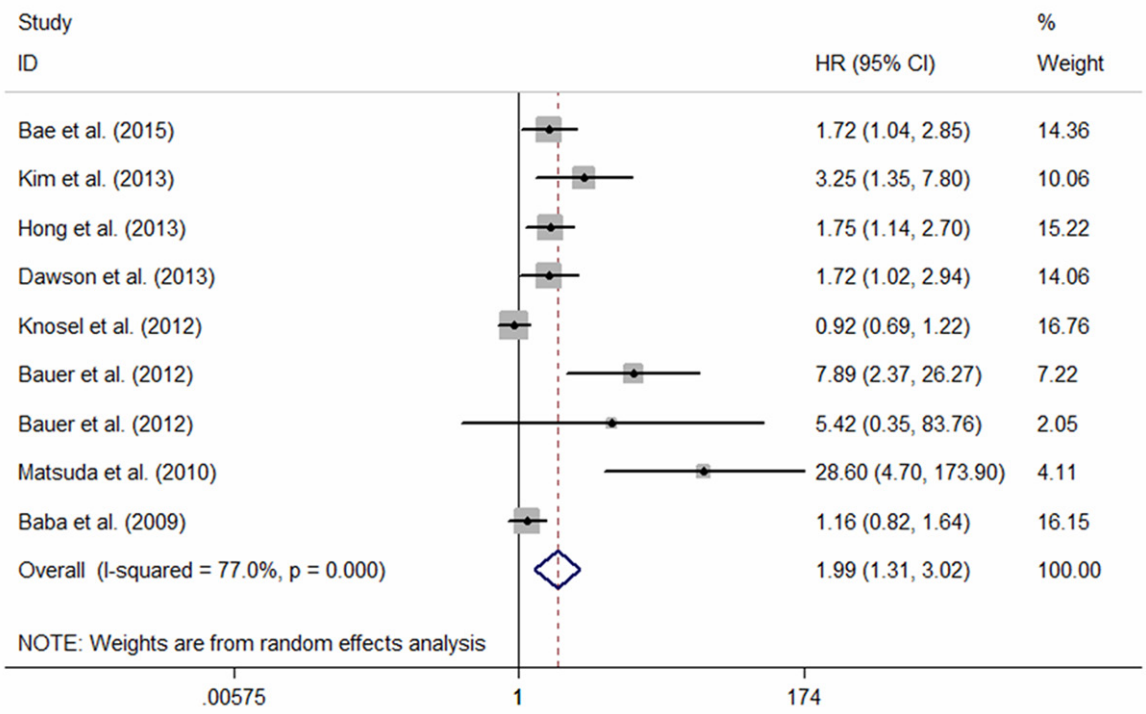

Figure 2. Forest plot showed the meta-analysis of the hazard ratio estimates for OS in the patients.

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

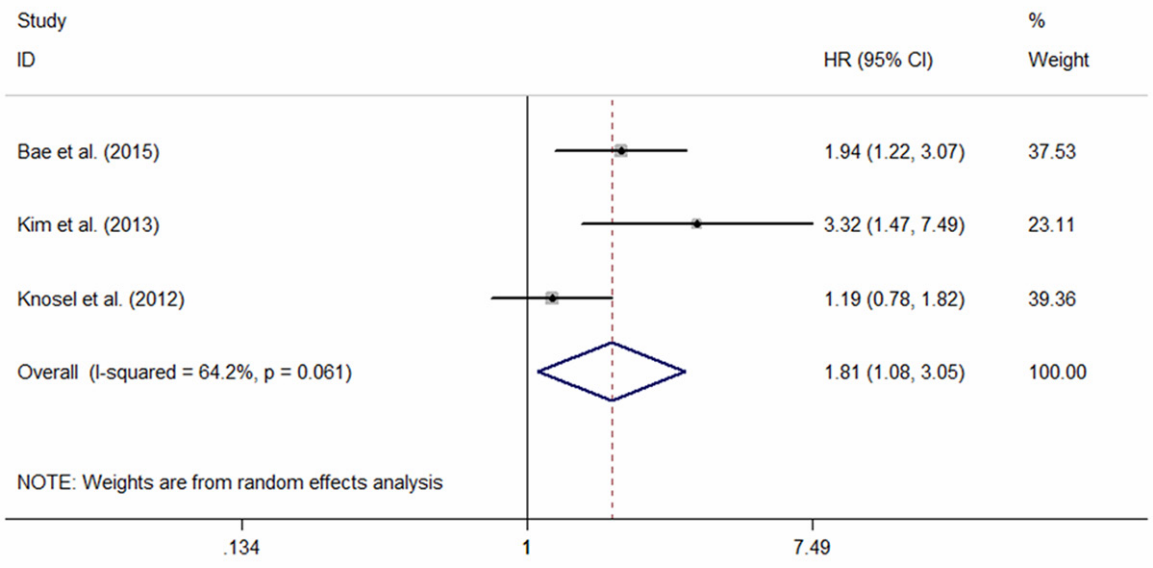

Figure 3. Forest plot showed the meta-analysis of the hazard ratio estimates for DFS in the patients.

Table 2. Summary sheet of the hazard ratio estimates for OS or DFS in the patients in subgroup analysis

Subgroup analysis	No. of studies (No. of patients)	Pooled OR (95% CI)	Z	P	Model	Heterogeneity		Publication bias	
						I^2 (%)	P_{het}	Begg's *P*	Egger's *P*
OS	9 (2547)	1.99 (1.31, 3.02)	3.25	*0.001*	R	77.00%	<0.001	0.029	0.001
DFS	2 (822)	1.81 (1.08, 3.05)	2.24	*0.025*	R	64.20%	0.061	0.296	0.1
Asian	4 (1126)	2.66 (1.38, 5.13)	2.91	0.004	R	70.70%	0.017	0.089	0.02
Caucasian	5 (1421)	1.56 (0.94, 2.59)	1.73	0.084	R	74.40%	0.004	0.2	0.063
Genechip	2 (197)	7.43 (2.47, 22.34)	3.57	<0.001	R	0.00%	0.806	1	-
IHC	7 (2350)	1.69 (1.14, 2.51)	2.63	0.008	R	76.50%	<0.001	0.035	<0.001
Biogenex	4 (1833)	1.54 (0.87, 2.72)	1.49	0.137	R	82.30%	0.001	0.089	0.008
Novocastra	2 (408)	1.74 (1.24, 2.43)	3.24	<0.001	R	0.00%	0.96	1	-
Ventana Medical Systems	1 (109)	3.25 (1.35, 7.81)	2.63	0.008	R	-	-	-	-
Companies (except for Biogenex)	5 (714)	2.08 (1.54, 3.81)	3.61	<0.001	F	45.20%	0.121	0.221	0.093
Rate of low expression ≤15.7%	6 (1323)	3.30 (1.75, 6.23)	3.68	<0.001	R	67.20%	0.009	0.133	0.026
Rate of low expression >15.7%	3 (1224)	1.15 (0.84, 1.58)	0.88	0.38	R	53.90%	0.114	0.296	0.104

P<0.05 was considered to indicate statistically significant differences; R: random effect model; F: fix effect model; OS: overall survival; DFS: disease-free survival.

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

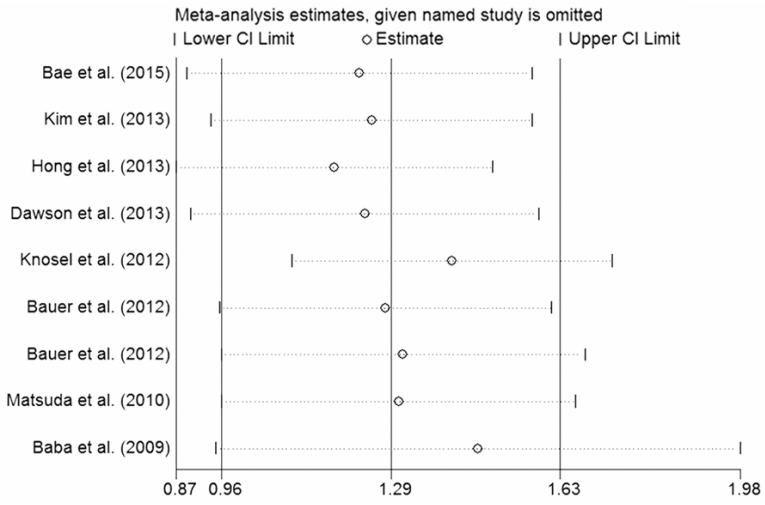

Figure 4. One-way sensitivity analysis of the hazard ratio estimates for OS in the patients.

at a time to determine the stability of our results (**Figure 4**). We found that the corresponding HRs was changed by Knösel et al. 2012 and Baba et al. 2015, which indicated that these two studies contributed mainly to publication bias in the results.

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 compa ny 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.

Address correspondence to: Wei Zhu, Department of General Surgery, First People's Hospital of Huzhou, Zhejiang Province, China. Tel: +86572-2023728; E-mail: weizhu188@163.com

References

[1] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008; 58: 71-96.

[2] Nozoe T, Kohno M, Iguchi T, Maeda T and Ezaki T. Five-point scoring system based on clinicopathological data: A convenient criterion to determine prognosis of patients with colorectal carcinoma. Oncol Lett 2013; 5: 978-982.

[3] Silberg DG, Swain GP, Suh ER and Traber PG. Cdx1 and cdx2 expression during intestinal development. Gastroenterology 2000; 119: 961-971.

[4] Guo RJ, Suh ER and Lynch JP. The role of Cdx proteins in intestinal development and cancer. Cancer Biol Ther 2004; 3: 593-601.

[5] Beck F, Erler T, Russell A and James R. Expression of Cdx-2 in the mouse embryo and

placenta: possible role in patterning of the extra-embryonic membranes. Dev Dyn 1995; 204: 219-227.
[6] James R, Erler T and Kazenwadel J. Structure of the murine homeobox gene cdx-2. Expression in embryonic and adult intestinal epithelium. J Biol Chem 1994; 269: 15229-15237.
[7] Suh E, Chen L, Taylor J and Traber PG. A homeodomain protein related to caudal regulates intestine-specific gene transcription. Mol Cell Biol 1994; 14: 7340-7351.
[8] Chawengsaksophak K, James R, Hammond VE, Kontgen F and Beck F. Homeosis and intestinal tumours in Cdx2 mutant mice. Nature 1997; 386: 84-87.
[9] Soubeyran P, Mallo GV, Moucadel V, Dagorn JC and Iovanna JL. Overexpression of Cdx1 and Cdx2 homeogenes enhances expression of the HLA-I in HT-29 cells. Mol Cell Biol Res Commun 2000; 3: 271-276.
[10] Mallo GV, Soubeyran P, Lissitzky JC, Andre F, Farnarier C, Marvaldi J, Dagorn JC and Iovanna JL. Expression of the Cdx1 and Cdx2 homeotic genes leads to reduced malignancy in colon cancer-derived cells. J Biol Chem 1998; 273: 14030-14036.
[11] Funakoshi S, Ezaki T, Kong J, Guo RJ and Lynch JP. Repression of the desmocollin 2 gene expression in human colon cancer cells is relieved by the homeodomain transcription factors Cdx1 and Cdx2. Mol Cancer Res 2008; 6: 1478-1490.
[12] Bae JM, Lee TH, Cho NY, Kim TY and Kang GH. Loss of CDX2 expression is associated with poor prognosis in colorectal cancer patients. World J Gastroenterol 2015; 21: 1457-1467.
[13] Bauer KM, Hummon AB and Buechler S. Right-side and left-side colon cancer follow different pathways to relapse. Mol Carcinog 2012; 51: 411-421.
[14] Baba Y, Nosho K, Shima K, Freed E, Irahara N, Philips J, Meyerhardt JA, Hornick JL, Shivdasani RA, Fuchs CS and Ogino S. Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. Clin Cancer Res 2009; 15: 4665-4673.
[15] Knosel T, Chen Y, Hotovy S, Settmacher U, Altendorf-Hofmann A and Petersen I. Loss of desmocollin 1-3 and homeobox genes PITX1 and CDX2 are associated with tumor progression and survival in colorectal carcinoma. Int J Colorectal Dis 2012; 27: 1391-1399.
[16] Kim JH, Rhee YY, Bae JM, Cho NY and Kang GH. Loss of CDX2/CK20 expression is associated with poorly differentiated carcinoma, the CpG island methylator phenotype, and adverse prognosis in microsatellite-unstable colorectal cancer. Am J Surg Pathol 2013; 37: 1532-1541.
[17] Hong KD, Lee D, Lee Y, Lee SI and Moon HY. Reduced CDX2 expression predicts poor overall survival in patients with colorectal cancer. Am Surg 2013; 79: 353-360.
[18] Dawson H, Koelzer VH, Lukesch AC, Mallaev M, Inderbitzin D, Lugli A and Zlobec I. Loss of Cdx2 Expression in Primary Tumors and Lymph Node Metastases is Specific for Mismatch Repair-Deficiency in Colorectal Cancer. Front Oncol 2013; 3: 265.
[19] Matsuda M, Sentani K, Noguchi T, Hinoi T, Okajima M, Matsusaki K, Sakamoto N, Anami K, Naito Y, Oue N and Yasui W. Immunohistochemical analysis of colorectal cancer with gastric phenotype: claudin-18 is associated with poor prognosis. Pathol Int 2010; 60: 673-680.
[20] Goto Y, Kato T, Ando T, Goto H and Hamajima N. No association between the CDX2 G543C polymorphism and risk of gastric atrophy and cancer. Asian Pac J Cancer Prev 2012; 13: 5691-5694.
[21] Xiao ZY, Ru Y, Sun JT, Gao SG, Wang YF, Wang LD and Feng XS. Expression of CDX2 and villin in gastric cardiac intestinal metaplasia and the relation with gastric cardiac carcinogenesis. Asian Pac J Cancer Prev 2012; 13: 247-250.
[22] Wang XT, Wei WY, Kong FB, Lian C, Luo W, Xiao Q and Xie YB. Prognostic significance of Cdx2 immunohistochemical expression in gastric cancer: a meta-analysis of published literatures. J Exp Clin Cancer Res 2012; 31: 98.