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*Original Article*

# A Clinicopathological Study on the Expression of Cadherin-17 and Caudal-related Homeobox Transcription Factor (CDX2) in Human Gastric Carcinoma

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**ABSTRACT:**
***Aims:*** **To analyse the clinicopathological characteristics of the expression of cadherin-17 (CDH17) and caudal-related homeobox transcription factor (CDX2) in human gastric carcinoma, and to evaluate the clinical significance of these two markers in the histological classification and prognosis of gastric carcinoma.**
***Materials and methods:*** **CDH17, CDX2 protein expression in paraffin-embedded specimens gathered from 166 patients with gastric carcinoma were detected by immunohistochemistry. The association of CDH17, CDX2 protein expression with the clinicopathological characteristics, and with the prognosis of gastric carcinoma were subsequently assessed.**
***Results:*** **CDH17, nucleus and cytoplasm CDX2 expression were positively expressed in 101/166 (60.8%), 59/166 (35.5%) and 57/166 (34.3%) gastric carcinoma patients, respectively. The expression of both CDH17 and CDX2 is associated with the intestinal-type gastric carcinoma ($P < 0.01$). Positive expression of CDH17 was significantly associated with the depth of gastric wall invasion ($P = 0.04$), lymph node metastasis ($P < 0.01$) and stages of gastric carcinoma ($P = 0.01$). Positive expression of CDX2 in the nucleus was mainly found in male patients ($P = 0.02$), in early stage ($P = 0.01$) and medullary-type gastric carcinoma ($P = 0.02$). There was a negative association between nuclear CDX2 expression and lymph node metastasis of gastric carcinoma ($P < 0.01$). The combined expression of CDH17 and CDX2 was significantly lower in diffuse-type carcinoma than intestinal- or mixed-type carcinoma ($P < 0.01$ and $P = 0.01$, respectively). The patients with CDH17 expression associated with poor prognosis of gastric carcinoma ($P < 0.01$), as opposed to patients with CDX2 expression ($P < 0.01$). The survival rate of patients with CDH17+/CDX2− expression was the lowest ($P < 0.01$), and conjoined expressions of CDH17+/CDX2− and CDH17+/CDX2+ were independent prognostic indicators of gastric carcinoma (both $P < 0.01$).**
***Conclusion:*** **The results suggest that the expression of CDH17 or CDX2 may be an important feature of gastric carcinoma. A combined detection of CDH17/CDX2 co-expression may benefit us in predicting the prognosis of gastric carcinoma. Ge, J. *et al*. (2008). *Clinical Oncology* 20, 275–283**


**Key words:** Cadherin-17, CDX2, clinical pathology, gastric carcinoma, prognosis

## Introduction

The incidence of gastric carcinoma has been decreasing in recent years. However, it remains a major medical challenge and one of the most common malignant diseases [1,2].

Recently, intense interests and extensive investigations have surrounded the cause of gastric carcinoma, but the prognosis of this malignant tumour has not significantly improved. There are a number of clinical reports showing that its biological behaviour and prognosis could be greatly different among patients at the same stages and with the same histological types or differentiation grades. A number of biomarkers have been found to be involved in the development and progression of gastric carcinoma [3,4].

Caudal-related homeobox transcription factor (CDX2), which is a member of the caudal-related homeobox gene family, plays an important role in mammalian early intestinal development and the maintenance of intestinal epithelia through its regulation of intestine-specific gene transcription [5]. Normally, CDX2 is expressed in small intestinal and colonic epithelia, but not in gastric epithelium [6]. However, CDX2 expression is observed in intestinal metaplasia of the stomach and in intestinal-type gastric adenocarcinomas. In gastric carcinogenesis, intestinal metaplasia is a well-known component of the stepwise series of mucosal changes, especially in intestinal-type adenocarcinomas. CDX2 has been documented to have an important role in intestinal metaplastic differentiation [7,8]. Interestingly, CDX2 has recently been implicated in

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the regulation of cadherin-17 (CDH17) in the intestinal tract, which is one of the effective factors for gastric carcinoma prognosis [3,9,10]. CDH17 is a structurally unique member of the cadherin superfamily and has only 20 amino acids in the cytoplasmic domain [11,12]. CDH17 is expressed in mice and humans almost exclusively in epithelial cells of both embryonic and adult small intestine and colon [13,14]. It was suggested that the expression of CDH17 was characteristic of the advanced gastric carcinoma that was associated with poor prognosis [3]. The mRNA and protein expression of both CDX2 and CDH17 have been shown to be highly expressed in stomach tumour compared with non-cancerous mucosa. Moreover, the over-expression of CDX2 was significantly associated with CDH17 in gastric adenocarcinoma. Furthermore, the expression of CDX2 and CDH17 proteins was strongly coupled in intestinal metaplasia [15]. The tissues with intestinal metaplasia and intestinal-type carcinomas may partly share similar expression of some markers, such as CDH17 and CDX2.

Both CDH17 and CDX2 have been found to be associated with the outcome of stomach cancer, and CDH17 is not supposed to be an independent prognosis factor of gastric carcinoma [3,16]. However, there are a few reports that simultaneously investigated the clinicopathological features of the co-expression of CDH17 and CDX2 in gastric carcinoma. In the present study, we analysed the association between CDH17 and CDX2, as separate factors or in combination, and Lauren's classification, lymph metastasis, invasion depth, distant metastasis, vascular invasion, tumour size, as well as TNM stages, to evaluate the clinical significance of these two markers in the histological classification and the prognosis assessment of gastric carcinoma.

## Materials and Methods

### *Patients and Tissue Samples*

One hundred and sixty-six fresh gastric carcinoma tissues and paired normal tissues located at least 10 cm away from the tumour were obtained immediately after gastrectomy (109 men, 57 women, aged 32–72 years, mean ± standard deviation = 52.2 ± 10.2 years, TNM staging from I to IV).

None of the patients recruited in this study had chemotherapy or radiotherapy before surgery. All received resection of primary gastric carcinomas between 8 February 1999 and 19 May 2005 in the Department of Gastrointestinal Surgery, Xiangya Hospital, Central South University, China. The pathological diagnosis was carried out preoperatively and confirmed by surgery. All patients were reviewed and all specimens were re-examined in April 2007. The depth of wall invasion, vascular invasion, lymph node metastasis and histological grade were examined by the same group of two senior pathologists experienced in stomach cancer diagnosis. All patients were given a follow-up ranging from 2 to 8 years. For the analysis of survival and follow-up, the date of gastrectomy was used to represent the beginning of the follow-up period. All patients who died of other diseases but not gastric carcinoma or unexpected events were excluded from the case collection. The cause of death of all 103 patients recruited in this study was aggravation of gastric carcinoma. The study was approved by the Research Ethics Committee of Central South University, China. Informed consent was obtained from all patients. All specimens were handled and made anonymous according to the ethical and legal standards.

Based on Lauren's classification system, all gastric carcinomas were categorised into three histological types: intestinal, diffuse, and mixed [17]. The patients were also categorised based on the differentiation status of the malignant cells into three histological grades: well, moderate and poor. Based on a combination of locoregional tumour involvement and the presence of metastases, all patients were staged according to the TNM stage grouping [18]. Classification of the quantity of stroma was made according to the criteria of the Japanese Classification of Gastric Cancer [19] as follows: scirrhous type, stroma is abundant and fibrous; medullary type, stroma is scanty; intermediate type, the quantity of stroma is between scirrhous type and medullary type.

### *Western Blotting*

The specificity of the anti-CDH17 antibody and anti-CDX2 antibody was examined using Western blotting. Human gastric carcinoma cell line SGC7901 was used in this study. The cells were collected by trypsinisation and spin. The cell pellets were lysed on ice for 10 min in 100 μl lysis buffer, which contained 50 mM Tris–HCl pH 7.4, 125 mM NaCl, 0.1% (v/v) NP-40, 5 mM ethylene diamine tetraacetic acid, 50 mM NaF, 50 μg/ml phenylmethylsulphonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor and 1 μg/ml aprotinin. Protein (50 μg) was subjected to

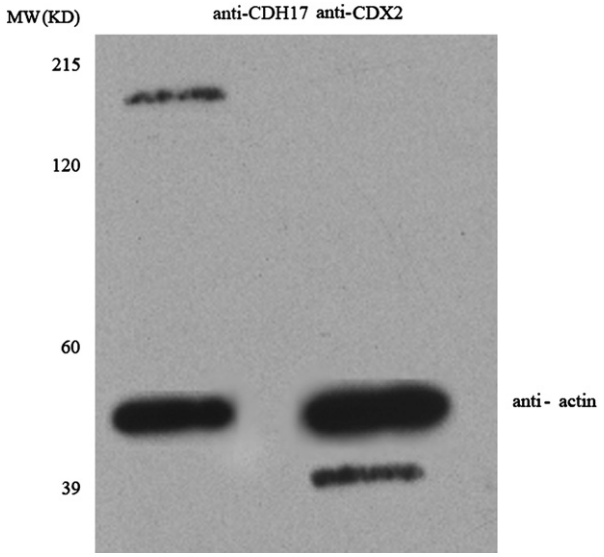


Fig. 1 — The specificity of CDH17 antibody and CDX2 antibody was analysed by Western blot testing. Two single bands of 120 and 36 kDa were detected in SGC7901 gastric carcinoma cell lines. β-actin antibody was used as the control with the single band of 42 kDa.