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# Overexpression of N-cadherin is correlated with metastasis and worse survival in colorectal cancer patients

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N-cadherin is related to the progression and metastases of several solid carcinomas. However, it was still unclear whether N-cadherin is overexpressed in colorectal malignant tumors that have stronger malignant tendency. In this study, we used immunohistochemistry to detect the expression patterns of N-cadherin in both the primary tumors and their normal mucosa tissues of 120 patients with colorectal cancer. We revealed that N-cadherin was expressed in 78.3% (94/120) of colorectal tumor tissues and in only 9.2% (11/120) of paired distant normal mucosa tissues with a significant difference ($P$=0.000). The low, moderate, and high expression of N-cadherin protein was 42.5%, 30.8%, and 26.7%, respectively. N-cadherin overexpression was associated with advanced TNM stage, lymph nodes metastasis and distant metastasis ($P$<0.05). Patients with N-cadherin overexpressed showed the obvious lower overall survival rate than those with moderate and low expression, and patients with low expression had a better survival rate than those with moderate and high expression ($P$<0.05). In conclusion, high N-cadherin expression may lead to tumor aggressiveness and metastatic potential in colorectal cancer, and may prove to be a possible prognostic factor.

**colorectal neoplasms, N-cadherin, immunohistochemistry, prognosis**

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Colorectal cancer is one of the most common malignancies, and causes cancer-related deaths ranked third in the word-worldwide [1]. China, as well as some other Asian countries, has experienced a 2–4-fold increase in colorectal cancer incidence during the past several decades [2]. The majority of patients are diagnosed with advanced stage cancer, which is generally resistant to treatment efforts, therefore restricting further treatment to improve the prognosis. Therefore, candidate biomarkers for early detection and prognosis of colorectal cancer are urgently needed to early treatment and improve survival rate. Thus, candidate biomarkers for early detection and prognostication of colorectal cancer are urgently required to guide early treatment and improve survival rates [3].

N-cadherin is a potential biomarker that may predict the metastasis and prognosis of colorectal cancer. N-cadherin was first identified in the brains of mouse embryos, then in murine nervous tissues, lens and cardiac muscle [4,5]. N-cadherin overexpression has been observed in several carcinomas. High expression of N-cadherin in breast cancer induces invasion and metastasis [6,7], while reduced N-cadherin inhibits tumor cell growth, migration and invasion, resulting in a better prognosis in the pancreatic ductal adenocarcinoma mouse model [8]. Expression of N-cadherin is also related to metastasis in prostate cancer and increases metastases in patients of castration-resistant tumors [9].

Increased N-cadherin expression, together with decreased expression of E-cadherin, is a prominent characteristic of epithelial-mesenchymal transition (EMT), which arises during tumor progression; this "cadherin switching" plays an important role in the most of tumor cells [10]. EMT process

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is very important for colorectal cancer. Epithelial mesenchymal proteins, including E-cadherin, LAMC2, beta-catenin and cathepsin L, play key roles in the occurrence and development of scolorectal cancer [11]. These findings suggest that N-cadherin, an epithelial mesenchymal protein, may also play a critical role in colorectal cancer; however, its expression in this cancer remains to be ascertained. In our study, we detected the expression of N-cadherin in colorectal tissues to clear and definite its relationship with clinicopathological characteristics and prognosis of colorectal cancer patients.

## 1 Materials and methods

### 1.1 Patients and follow-up

A total of 120 patients with colorectal cancer were enrolled, as described in our previous study [12]. All patients were confirmed by pathology and underwent radical surgery at Peking University People's Hospital from September 2002 to April 2004. There were 74 men and 46 women, with a mean age of 64.5±12.8 years (range, 26–89 years). Seventy-five (62.5%) patients had colon tumors and 45 (37.5%) patients had rectal tumors. No preoperative chemotherapy or radiotherapy was performed. The clinicopathological features are listed in Table 1.

The median survival time of patients was 53.3 months (range, 1–78 months). At the end of the study, 43 patients died. All the patients were followed up by phone interview or direct evaluation until death or December 2008. Our study was consented by the Ethics Committee of Peking University People's Hospital.

### 1.2 Tissue samples and tissue microarray

Circular specimens of 2 mm diameter were from formalin-fixed paraffin-embedded colorectal tumor tissues and paired distant normal mucosa tissues. The tissues were arranged in a new tissue microarray (Beecher Instruments, Microarray Technologies, Silver Spring, MD). For the 120 pairs of tumor and distant normal mucosa tissues, 11 tissue microarraies were fabricated, each containing 10–12 tumor and normal mucosa tissues. In order to control the quality, area of tumor should be accounted for 10% of the total at least. Each case contains at least two qualified specimens at the microarray.

### 1.3 Immunohistochemistry and scoring

Tissue array sample dissolved in xylene to deparaffinize, rehydrated in a graded ethanol, and then treated to antigen retrieval by 6.5 mmol/L sodium citrate buffer (pH 6.0). The tissues were incubated with sheep polyclonal anti-N-cadherin antibody (5 mg/L) (R&D Systems Inc., Minneapolis, MN, USA) at room temperature for 2 h. Primary antibodies were detected using the Powervision two-step histostaining reagent (Zhongshan Inc., Beijing, China), with PV-9003 as the secondary antibody, and detection was performed by diaminobenzidine chromogenic reaction. Immunostaining scores accorded to the previous method [13]. N-cadherin expression was graded according to the area and intensity of staining. The intensity was classified by the following principle: 3, intense staining; 2, moderate staining; 1, mild staining; 0, no staining. The area was measured as the following codex: 3, >60% positively stained; 2, 30%–60% positively stained; 1, <30% of cells positively stained; 0, no staining of cells in any microscopic fields. The summed score (intensity+area) of ⩽2 indicated low expression, the score between 3 and 4 indicated moderate expression, whereas the score between 5 and 6 indicated high expression.

### 1.4 Statistical analyses

All data were analyzed using SPSS 16.0 software (SPSS Inc, Chicago, IL, USA). The association of N-cadherin expression with clinicopathological characteristics was counted by $\chi^2$ tests. Cumulative survival rate was calculated by the Kaplan-Meier curve, and differences of survival rates were compared by log-rank test. $P$ values of <0.05 were as statistically significant.

## 2 Results

### 2.1 N-cadherin expression in tumor and normal mucosa tissues

The expression of N-cadherin was identified by immunohistochemistry to appear as the cellular origin and distribution. N-cadherin staining was negative or weak in paired distant normal mucosa tissues (Figure 1), but stronger in colorectal cancer tissues. Results of the microarray immunohistochemistry showed that the positive staining rate of N-cadherin was 78.3% (94/120) in colorectal cancer tissues and 9.2% (19/120) in normal mucosa, with a statistically significant difference ($P$=0.000).

### 2.2 Clinicopathological significance of N-cadherin expression

Elevated levels of N-cadherin expression in colorectal cancer tissues were significantly related to more malignant phenotypes, including advanced TNM stage ($P$=0.009), lymph node metastasis ($P$=0.002) and distant metastasis ($P$=0.004). In contrast, there were no significant differences in N-cadherin expression in colorectal cancer tissues for other clinicopathological features, including age, gender, site of tumor, tumor location, tissue differentiation, depth of wall invasion (T-stage) and recurrence ($P$>0.05).

### 2.3 Prognostic implications of N-cadherin expression

The five-year survival rates of low, moderate, and high

**Table 1** Relationship of clinicopathological features and N-cadherin expression in 120 colorectal cancer patients

| | *n* | N-cadherin expression[a)] | | | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| | | Low | Mod | High | | |
| Tissue | | | | | 67.108 | 0.000 |
| Non-neoplastic samples | 120 | 109 | 11 | 0 | | |
| Tumor samples | 120 | 51 | 37 | 32 | | |
| Age | | | | | 1.996 | 0.369 |
| >65 years | 63 | 23 | 18 | 19 | | |
| ⩽65 years | 57 | 28 | 19 | 13 | | |
| Gender | | | | | 2.544 | 0.280 |
| Male | 74 | 33 | 19 | 22 | | |
| Female | 46 | 18 | 18 | 10 | | |
| Size of tumor | | | | | 2.839 | 0.242 |
| >5 cm | 35 | 11 | 14 | 10 | | |
| ⩽5 cm | 85 | 40 | 23 | 22 | | |
| Location | | | | | 1.471 | 0.479 |
| Colon | 75 | 35 | 21 | 19 | | |
| Rectum | 45 | 16 | 16 | 13 | | |
| Differentiation grade | | | | | 4.646 | 0.098 |
| Poor | 21 | 5 | 7 | 9 | | |
| Moderate or well | 99 | 46 | 30 | 23 | | |
| TNM staging | | | | | | |
| I | 11 | 5 | 4 | 2 | 17.096 | 0.009 |
| II | 43 | 23 | 12 | 8 | | |
| III | 44 | 21 | 13 | 10 | | |
| IV | 22 | 2 | 8 | 12 | | |
| Depth of wall invasion | | | | | | |
| T1 | 1 | 0 | 1 | 0 | 11.605 | 0.071 |
| T2 | 18 | 12 | 5 | 1 | | |
| T3 | 95 | 38 | 28 | 29 | | |
| T4 | 6 | 1 | 3 | 2 | | |
| Lymph nodes metastasis | | | | | 12.247 | 0.002 |
| No | 56 | 33 | 14 | 9 | | |
| Yes | 64 | 18 | 23 | 23 | | |
| Distant metastasis | | | | | 10.897 | 0.004 |
| M0 | 96 | 47 | 29 | 20 | | |
| M1 | 24 | 4 | 8 | 12 | | |
| Recurrence | | | | | 2.883 | 0.237 |
| No | 98 | 44 | 31 | 23 | | |
| Yes | 22 | 7 | 6 | 9 | | |

a) N-cadherin expression: well, well differentiated; mod, moderately differentiated; poor, poorly differentiated.

expression of N-cadherin in colorectal cancer patients were 77.2%, 56.6% and 28.5%, respectively. The mean survival time was 66.4±3.4 months in patients with low expression of N-cadherin, with a comparison of 49.9±5.1 months for those individuals with moderate expression and 30.5±5.5 months for those with high expression. The survival curves proved that the overall survival rates decreased significantly with upregulated N-cadherin expression (Figure 2).

## 3 Discussion and conclusions

N-cadherin is located at adherens junctions, where it mediates a dynamic contact not only between cells but also between matrix and cells [14]. In addition, its cytoplasmic expression took part in multiple intracellular signaling pathways [15]. Several studies showed that N-cadherin enhanced tumor cell motility and promoted invasion and metastasis for lots of experimental models of tumor [9]. In this study, we showed that N-cadherin expression in human was related to metastasis and prognosis of colorectal cancer.

First, we found that N-cadherin protein was more highly expressed in colorectal tumor than paired distant normal mucosa tissues. The positive expression rate of N-cadherin in colorectal cancer tissues was 78.3%, which was similar to other tumor types. It has been found that N-cadherin is expressed in 58% of melanoma cell lines [16], 53.6% of breast invasive ductal carcinoma, 68.4% of invasive lobular

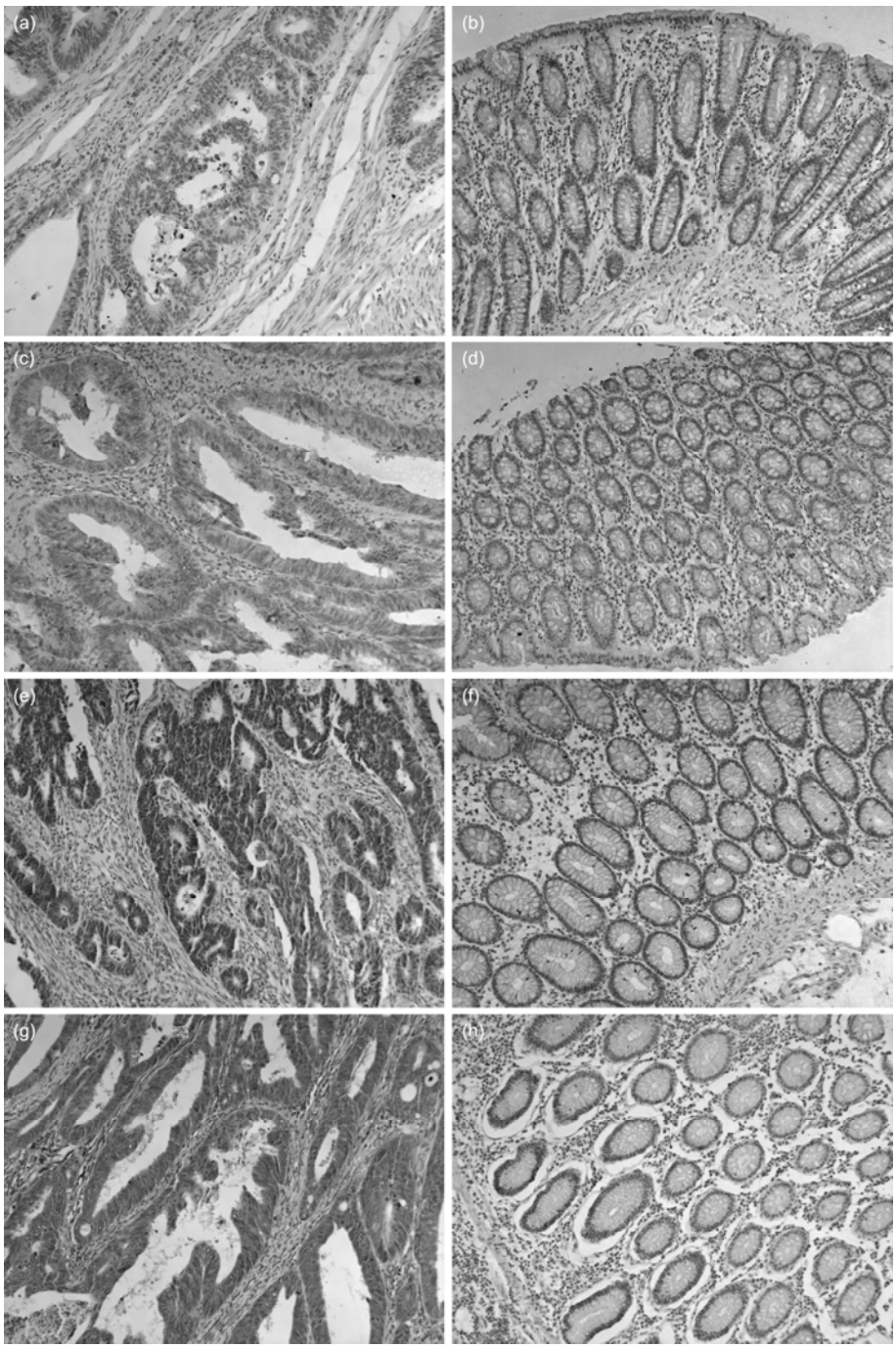


**Figure 1** Immunohistochemical staining of N-cadherin expression in colorectal tumors (a), (c), (e), (g) and paired normal mucosa (b), (d), (f), (h)**.** Positive staining in tumor: negative (a), weak (c), moderate (e), and strong staining (g); negative or weak staining (b), (d), (f), (h) in distant normal mucosa. Original magnification ×100.

carcinoma and 31.7% of carcinoma *in situ* [17]. These high expression rates in malignant tumors suggest that it may play an important role in tumor progression.

We noted that N-cadherin expression was positively associated with clinical TNM stage. However, no relationship was found between expression of N-cadherin and the depth of wall invasion (T-stage). We deduced that the relationship of N-cadherin expression and TNM stage was due to lymph node metastasis (N-stage) and/or distant metastasis (M-stage).

As expected, this presumption was proved by our further investigations, which demonstrated that N-cadherin expression was positively associated with lymph node metastasis and distant metastasis in a large number of colorectal cancer patients. In previous studies, N-cadherin expression was thought to have a link with pelvic lymph node invasion and little time to skeletal metastasis [18]. High N-cadherin

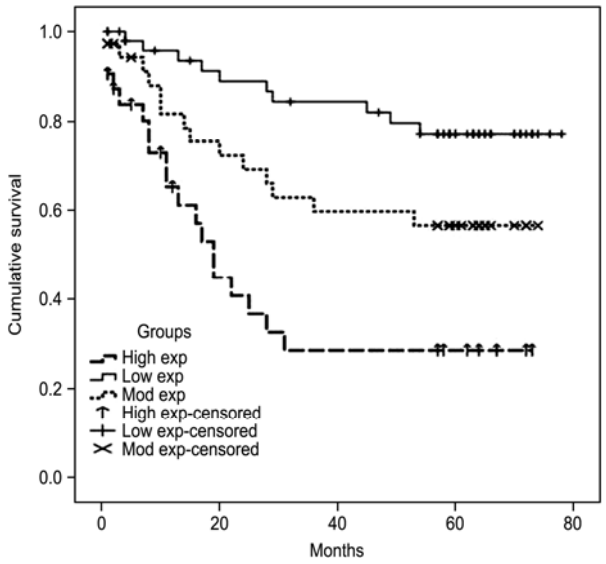


**Figure 2** Kaplan-Meier survival curves according to the expression of N-cadherin for colorectal cancer patients. High exp, high expression; mod exp, moderate expression; low exp, low expression. low exp vs mod exp ($P$=0.035); mod exp vs high exp ($P$=0.018); low exp vs high exp ($P$=0.000).

expression was shown in triple-negative breast cancer with lymphatic infiltration [19]. It plays a vital role in metastasis of head and neck spindle cell carcinoma [20], and is well related to the invasion and lymph node metastasis in squamous cell carcinoma of head and neck region [21]. Conversely, a recent study showed that there was no association of N-cadherin expression with either nodal or distant metastasis of extrahepatic cholangiocarcinoma [22]. Based on the previous findings and our study, we can conclude that N-cadherin may promote the metastatic process of colorectal cancer, as well as that of other solid malignant tumors.

The functional mechanism of N-cadherin in inducing metastasis of colorectal cancer is still unclear. Much effort in elucidating its biological behavior in various other tumors has been made. N-cadherin facilitates the dissociation of single cells from the primary tumor [23] and most probably renders the cells more motile by homophilic adhesion to some other cells [24]. Then, homophilic interactions between N-cadherin-expressing tissues and cancer cells, such as vasculature and stroma, promote transit through the tissue and the live of cancer cells in other organs [25]. Knockdown of N-cadherin expression leads to decreased cell aggregation and increased cell migration and invasion [26]. At the same time, many genes influence metastasis through regulation of N-cadherin and other EMT participators, such as MUC4 [27], WIF1 [28], EIF5A2 [29], and HIF-1alpha [30]. Our next study will focus on clarifying the undefined mechanism of N-cadherin in colorectal cancer metastasis.

Most important of all, N-cadherin protein was closely correlated with prognosis of colorectal cancer patients; the higher the N-cadherin expression, the worse the outcome will be for patient survival. It has been found that high expression of N-cadherin usually means a reduced survival time in several malignant tumors, including extrahepatic cholangiocarcinoma [22], osteosarcoma [31], urothelial carcinoma [32], prostate cancer, and breast cancer [33]. These results certify that N-cadherin is a valuable prognostic biomarker of colorectal cancer, as well as in other tumors. However, further research should be carried out in multiple centers, for our study was merely based on patients attending a single hospital and included just 120 patients.

In conclusion, N-cadherin expression was significantly associated with TNM stage, lymph node metastasis and distant metastasis. It may be a potential biomarker in the clinical setting for predicting metastasis and prognosis of colorectal cancer.

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