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# Immunohistochemical study of E-cadherin and β-catenin expression in colorectal carcinomas

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Parameters of histological type, differentiation, lymph node metastasis and stage have been observed to indicate the prognosis of colorectal carcinomas. Immunohistochemically E-cadherin and β-catenin expression of tumour cells have been evaluated to define life expectancy, response to the treatment, metastatic disease and recurrence of tumour in correlation with these prognostic parameters.

60 cases diagnosed as colorectal adenocarcinoma were selected to be studied retrospectively. Immunohistochemistry was performed using E-cadherin and β-catenin primary antibodies and avidin-biotin-peroxidase.

53 of 60 adenocarcinoma tissues were evaluated as classical type adenocarcinoma and 7 of them as mucinous carcinoma. 48 classical type adenocarcinoma tissues showed membranous staining for E-cadherin, 13 tissues showed cytoplasmic staining. All 53 adenocarcinoma tissues expressed nuclear or membranous type β-catenin in different intensities. Reduced E-cadherin expression significantly correlated with lymph nodes metastasis ($p = 0.01$). E-cadherin expression significantly correlated with increasing histological differentiation ($p = 0.04$).

When E-cadherin and β-catenin expressions were compared, there was a significant difference between the tumour stage, histological differentiation and the existence of lymph node metastasis. When both E-cadherin and β-catenin expressions were reduced, there was a significant unfavourable prognosis.

**Key words:** colorectal cancer, E-cadherin, β-catenin, immunohistochemistry, prognostic factor.

## Introduction

Colorectal carcinoma is the major cause of morbidity and mortality worldwide. The parameters such as histological type, differentiation, presence of lymph node metastasis and stage emphasize the prognosis of colorectal carcinomas. Tumour formation is a complex, multistep process involving the accumulation of genetic lesions in genes that regulate the pathways of cell proliferation, adhesion, differentiation, and death required for normal development [1]. Glycoproteins involved in the cell-cell and cell-extracellular matrix adhesion of tumour cells are believed to participate in the acquisition of an invasive and metastatic phenotype. In this sense, the E-cadherin/β-catenin complex has a critical role in cell-to-cell adhesion.

A potential role for β-catenin in colorectal carcinogenesis first seemed likely in view of the importance of disruption of cell-to-cell adhesion to cancer invasion and metastasis [1]. However, a more primary role in the pathogenesis of colorectal adenocarcinoma (CRC) soon became apparent with the discovery that adenomatous polyposis coli (APC) protein binds to cytosolic β-catenin, and that adenomatous polyposis coli (APC) mutation leads to nuclear accumulation of β-catenin, a feature associated with progression along the adenoma-carcinoma sequence [2]. The significance of this primary role now seems to be ever increasing as several of the reg-

ulators and effectors of the β-catenin signalling pathway are being found to represent molecular pathways that have well-characterized links with colorectal carcinogenesis. β-catenin may be regarded as existing in three different subcellular forms: membrane-bound (as part of the adherens complex), cytosolic, and nuclear [1].

E-cadherin, a cadherin family member of the cell surface glycoprotein, is a $Ca^{2+}$-dependent cell-to-cell adhesion molecule found mainly in epithelial tissue. It is thought to implicate embryogenesis, cellular migration in inflammatory tissue, and cellular differentiation or dedifferentiation [3].

The extracellular domain interacts homotypically with the E-cadherin molecules of neighbouring cells while maintaining intercellular adhesion. Its cytoplasmic tail constitutes a complex with a group of intracytoplasmic proteins, such as catenins [4].

E-cadherin and β-catenin unit binds to the cytoskeleton that is essential for the adhesive function of E-cadherin. Furthermore, E-cadherin and catenins appear to act as more of an intercellular glue. β-catenin seems to participate in the signal transduction from the cellular surface to the cytoplasm independently of the cell-to-cell adhesion. Many investigators have suggested the suppressor role of E-cadherin in tumour invasion [5].

Loss or diminished expression by mutation or epigenetic change have been demonstrated in many epithelial cancers. The down-regulation of E-cadherin is seen most prominently in carcinomas showing infiltrative growth associated with little intercellular cohesion, such as invasive lobular carcinoma of the breast and diffuse gastric adenocarcinoma including gastric signet-ring cell carcinoma [6, 7]. Therefore, the loss of the E-cadherin function could be associated with invasiveness, lymph node metastasis and distant metastasis resulting in poor prognosis. Few investigations have explained the mechanism of the distinctive phenotype and aggressive clinical behaviour in colorectal signet-ring cell carcinoma [8].

This study was carried out to observe the relationship between the parameters such as histological type, differentiation, lymph node metastasis and stage in correlation with E-cadherin and β-catenin expressions of tumour cells to evaluate life expectancy, response to treatment, metastatic illness and recurrence of colorectal carcinoma.

## Material and methods

Sixty cases diagnosed as colorectal adenocarcinoma between 2004 and 2005 were selected to be studied retrospectively. There were 38 male and 22 female patients. Their mean age was 62.3 (range 16-82), median 66. Four patients were younger than 40.

Haematoxylin-eosin stained specimens were examined and the differentiation and prognostic parameters of the tumours were evaluated. Along with the tumour, a block which has non-tumoral tissue was selected. Immunohistochemistry was performed using E-cadherin (36B5 clone, Neomarkers) and β-catenin (Cat-a.1. clone, Immunovision) primary antibodies and avidin-biotin-peroxidase. In this study, formalin-fixed paraffin-embedded sections of normal and tumoral colonic tissue were stained by an indirect avidin-biotin immunohistochemical technique using mouse monoclonal antibodies, and membranous, cytoplasmic and nuclear cellular localization were assessed by light microscopy. Staining distribution was scored as follows: 3, >90% of positive epithelial cells; 2, >50% of positive epithelial cells; 1, <50% of positive epithelial cells.

## Results

Fifty three of 60 adenocarcinoma tissues were evaluated as classical type adenocarcinoma and 7 of them as mucinous carcinoma. Forty eight classical type adenocarcinoma tissues showed membranous staining for E-cadherin, 13 tissues showed cytoplasmic staining and no staining was observed in 5 tissues. The staining results of the cases with E-cadherin and β-catenin can be found in Tables I-IV. The follow-up period of the cases ranges between 6 and 25 months; the results are listed in Table V. The comparison of the lymph node metastases of the cases with the tumour differentiation can be found in Table VI. Four of mucinous carcinoma tissues showed membranous type staining. Three of them showed no staining.

All 53 adenocarcinoma tissues expressed nuclear or membranous type β-catenin in different intensities. In all of 7 mucinous carcinoma tissues, nuclear or membranous staining was observed.

Evaluation for stages (TNM): 2 patients were stage I, 12 patients were stage II, 46 patients were stage III. Stage I and II patients were observed, 39 stage III patients received chemotherapy, 27 patients with rectosigmoid tumours received sequential radiotherapy (50 Gy) and chemotherapy: 5 Fluorouracil 500 mg/m$^2$, Ca-Leucoverin 50 mg/m$^2$, day 1 to 5, 28 days intervals for a total of 6 cycles. Twelve patients with colon cancer received only chemotherapy.

No patient with colorectal signet ring cell carcinoma had a familial history of malignant disease, including colorectal cancer and hereditary non-polyposis colorectal cancer with its associated malignancy. Six of 14 patients died due to recurrence who had no distant metastasis on primary surgery, during a median follow-up of 15.7 months. Four patients (15%) showed liver metastases, 2 patients had locoregional metastases. Two-year overall survival

rates were 72%. Reduced E-cadherin expression significantly correlated with lymph nodes metastasis ($p = 0.01$). E-cadherin expression significantly correlated with increasing histological differentiation ($p = 0.04$). E-cadherin staining did not correlate with the prognosis ($p = 0.06$). Reduced β-catenin staining was related to a poor prognosis but was not significantly important ($p = 0.72$). When both E-cadherin and β-catenin were reduced, there was a significant unfavourable prognosis compared with the reduced expression ($p = 0.05$). The analysis of E-cadherin and β-catenin expression can provide clinically important on treatment.

When E-cadherin and β-catenin expressions were compared, there was a significant difference between the tumour stage, histological differentiation and the existence of lymph node metastasis. When both E-cadherin and β-catenin were reduced, there was a significant unfavourable prognosis.

There were no significant correlations between β-catenin expression and histology, differentiation, vascular invasion, or lymphatic invasion.

**Table I.** The distribution of membranous staining characteristics with E-cadherin

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Well differentiated adenocarcinoma | 2 | 2 | 3 | 1 |
| Moderately differentiated adenocarcinoma | 1 | 8 | 19 | 6 |
| Poorly differentiated adenocarcinoma | 2 | 3 | 4 | 2 |
| Mucinous adenocarcinoma | 3 | 3 | 1 | 0 |

**Table II.** The distribution of cytoplasmic staining characteristics with E-cadherin

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Well differentiated adenocarcinoma | 6 | 0 | 1 | 0 |
| Moderately differentiated adenocarcinoma | 26 | 2 | 5 | 2 |
| Poorly differentiated adenocarcinoma | 8 | 3 | 0 | 0 |
| Mucinous adenocarcinoma | 7 | 0 | 0 | 0 |

**Table III.** The distribution of membranous staining characteristics with β-catenin

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Well differentiated adenocarcinoma | 0 | 2 | 2 | 4 |
| Moderately differentiated adenocarcinoma | 1 | 7 | 8 | 18 |
| Poorly differentiated adenocarcinoma | 0 | 4 | 5 | 2 |
| Mucinous adenocarcinoma | 0 | 4 | 2 | 1 |

**Table IV.** The distribution of nuclear staining characteristics with β-catenin

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Well differentiated adenocarcinoma | 4 | 2 | 1 | 1 |
| Moderately differentiated adenocarcinoma | 14 | 2 | 11 | 7 |
| Poorly differentiated adenocarcinoma | 6 | 1 | 2 | 2 |
| Mucinous adenocarcinoma | 5 | 1 | 0 | 1 |

**Table V.** The numerical distribution of follow-up results between the groups

| | Healthy | Recurrence | Exitus |
|---|---|---|---|
| Well differentiated adenocarcinoma | 6 | 0 | 0 |
| Moderately differentiated adenocarcinoma | 19 | 0 | 3 |
| Poorly differentiated adenocarcinoma | 6 | 0 | 1 |
| Mucinous adenocarcinoma | 2 | 0 | 0 |

**Table VI.** The distribution of lymph node metastases between the groups

| LYMPH NODE METASTASIS | POSITIVE | NEGATIVE |
|---|---|---|
| Well differentiated adenocarcinoma | 1 | 7 |
| Moderately differentiated adenocarcinoma | 16 | 19 |
| Poorly differentiated adenocarcinoma | 5 | 6 |
| Mucinous adenocarcinoma | 5 | 1 |

Results of E-cadherin and β-catenin expression were analyzed with regard to survival time. 5-year survival rates were 42.3% with the reduced expression of E-cadherin. Patients with the reduced expression tended to have a poorer prognosis. 5-year survival rate was 46.0% with the reduced expression of β-catenin.

### Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 11.0 was used for statistical analysis. Statistical analyses between groups were performed using Mann Whitney U test for numeric values and $\chi^2$ tests for nominal values. Spearman's rho test was used to detect the correlations. Probability values of less than 0.05 were considered significant.

Multivariate analysis on disease-related survival (Cox proportional hazard model).

## Discussion

The protein association with adenomatous polyposis coli (APC) protein, and presence of dysregulation of β-catenin protein expression at all stages of the adenoma-carcinoma sequence have indicated an important role for β-catenin pathways in colorectal carcinogenesis. However, recent studies have shown that more components of colorectal carcinogenesis are linked to β-catenin pathways. Dysregulation of apoptosis plays an important role in colorectal carcinogenesis. The protein β-catenin acts together with components of almost all stages and aspects of colorectal carcinogenesis via the adenoma-carcinoma sequence [1].

The reciprocal relation between tumour dedifferentiation, infiltrative growth and lymph node involvement and several human malignancies like thyroid, oesophagus, gastric and colon adenocarcinomas and reduced E-cadherin expression have been reported in many studies [9, 10].

E-cadherin loss has frequently been reported in gastric signet ring cell carcinoma and lobular breast carcinoma [8].

Clear consecutive steps defined by explicit genetic events cause colorectal cancer. Abnormalities in the expression and functional activity of cell adhesion molecules are implicated in the development and progression in the majority of colorectal cancers. Intercellular (e.g. E-cadherin/catenin complex) and cell-matrix (e.g. integrins) adhesion molecules are more than just cementing substances; they regulate cell polarity, differentiation, proliferation, migration and invasion. In colorectal cancer, cells lose actin cytoskeletal organization and normal cell adhesion when they become invasive [11]. Most colorectal carcinomas arise from adenomas through an archetypal pathway, the adenoma-carcinoma-metastasis sequence. Aberrant expression of β-catenin, p16, E-cadherin and c-myc appears to have played an important role in the development and/or progression of CRC, but their precise distribution pattern and associations in different pathologic loci along CRC's pathogenic pathway have not been thoroughly examined [12]. β-catenin is a multifunctional protein originally identified as a component of the cadherin cell-to-cell adhesion complex. It also binds the adenomatous polyposis coli (APC) tumour suppressor which controls β-catenin cellular levels through its degradation [13]. The E-cadherin/β-catenin system acts as an invasion suppressor of epithelial malignancies. This invasion suppressive activity seems to be mediated not only by the cell adhesive activity of E-cadherin but by other indefinite signalling pathways evoked by β-catenin. In fact, cancer cells that have infiltrated the stroma reduce the expression of E-cadherin and accumulate β-catenin [14].

Various authors have reported that reduced synthesis of epithelial junctional proteins during dedifferentiation, tumorigenesis and metastasis in a great variety of tumours. Therefore, it is a common view that loss of adhesive molecules and adhesion structures is associated with the development of an invasive phenotype and unfavourable prognosis. Colon carcinomas, on the other hand, were shown to behave differently as synthesis of main adhesive proteins continues despite the development of an invasive phenotype [15]. Alterations in adhesion molecules, angiogenesis, and matrix metalloproteinases have been associated with metastasis and intravasation [16]. The E-cadherin/β-catenin complex is a prime mediator of cell-to-cell adhesion. APC mutations can result in the loss of β-catenin downregulation and an accumulation of β-catenin in the

cell. β-catenin mutations can have a similar effect [17]. It is known that normal epithelial cells express the transmembrane glycoprotein E-cadherin that mediates the calcium-dependent adhesion between cells, and its intracellular partners – catenins at the cell membrane [18]. Reduced expression of both E-cadherin and β-catenin in carcinoma cells has been associated with dedifferentiation, increased invasiveness, and advanced stage in a number of tumours [19, 20], including tumours of the digestive system [21, 22].

β-catenin can activate target genes associated with proliferation and invasion, linking with the APC gene. The purpose of this study was to investigate whether nuclear expression of β-catenin in cells at the invasive front or in the vessels was associated with liver metastasis in human colon cancer. Nuclear accumulation of β-catenin in cellular cells at the invasive front and in the vessels was the most powerful predictor of liver metastasis in colorectal cancer. This may be an important marker in the selection of patients for adjuvant therapy or other treatment modalities [23].

β-catenin has a central role not only in linking the cadherin-mediated cell adhesion system but also in the intercellular signalling pathway. In a study, in order to investigate alterations of β-catenin in the development of colorectal carcinoma, a pattern of β-catenin expression was studied using immunohistochemistry in 74 sporadic colorectal adenomas, in histologically normal mucosa adjacent to 65 of these adenomas, and in 52 carcinomas arising in adenomas. All normal epithelia displayed cell boundary staining for β-catenin. Adenomas and carcinomas showed varying degrees of membranous staining. However, some tumours also showed nuclear staining of β-catenin protein. Decreased membranous and increased nuclear β-catenin staining was associated with increasing degrees of dysplasia in adenomas. Carcinomas manifested significantly reduced membranous, but enhanced nuclear β-catenin expression compared with their associated adenomas [2]. In this study, E-cadherin and β-catenin showed membranous, β-catenin nuclear, and E-cadherin cytoplasmic expressions. The expression of E-cadherin and β-catenin could be relevant in determining the prognosis of colorectal carcinomas and providing a more accurate mechanism for their classification.

The staining intensity of E-cadherin and β-catenin was compared separately with prognostic parameters (tumour type: mucinous or classical adenocarcinoma, degree of differentiation, vascular invasion, existence of lymph node metastasis, metastatic illness and recurrence of tumour). It has been found out that increasing nuclear β-catenin expression and loss of membranous E-cadherin are independent, adverse prognostic factors in colorectal carcinomas [24].

In this study, although we have found out the membranous expression of E-cadherin and β-catenin, and the nuclear expression of β-catenin, we have not found a significant difference with prognostic parameters statistically.

When E-cadherin and β-catenin expressions were compared, no significant difference was observed. On the other hand, there was a significant difference between the tumour stage and the existence of lymph node metastasis.

In this study, we evaluated immunohistochemically the expression of E-cadherin and β-catenin in formalin-fixed, paraffin-embedded tissue specimens of colorectal cancer tissue, and we analyzed clinicopathological findings, as related to survival time.

We considered E-cadherin and β-catenin expression levels to be reduced, tumour cells were stained, because the distribution of ratio of staining cells showed bipolarity.

In conclusion, the cadherin-catenin complex may play a major role in cell-to-cell adhesion systems, and down-regulation of E-cadherin and β-catenin indicates an unfavourable prognosis. This is a useful prognostic factor in colorectal cancer with β-catenin expression. For patients with a reduced expression of the cadherin-catenin complex, follow-up should be close because optional chemotherapy or radiation may be required.

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