

Abstracts of the 2005th Maruyama Memorial Lectures of the 74th Annual Meeting of the Medical Association of Nippon Medical School

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Abstracts of the 2005th Maruyama Memorial Research Fund Prize Memorial Lecture (1)

Clinical and Biological Significance of Lymph Node Micrometastasis in Colorectal Cancer

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Introduction

Lymph node (LN) metastasis and the depth of tumor invasion are important prognostic factors in colorectal cancer (CRC). LN status is the key criterion for selecting patients with CRC who require adjuvant chemotherapy. Adjuvant chemotherapy can reduce the mortality down to 22% in patients with LN-metastasis-positive CRC. However, some patients without LN metastasis relapse and die of cancer after curative surgery, and adjuvant chemotherapy is not always beneficial for the survival of patients with LN-metastasis-negative CRC. Therefore, predictors of recurrence of LN-metastasis-negative CRC remain controversial. The histopathological examination of resected LNs stained with hematoxylin and eosin (H&E) has been the standard method for the diagnosis of LN metastasis. Recent technological advances has enabled the detection of LN micrometastasis in patients with LN-metastasis-negative cancer with various methods, including immunohistochemistry (IHC) and polymerase chain reaction (PCR) analysis. Several researchers have reported that cytokeratin (CK) immunostaining can be used to identify LN micrometastasis not identified by routine H&E staining in patients with CRC. However, the clinical significance of LN micrometastasis is controversial. In addition, the mechanism of LN micrometastasis remains unknown. E-cadherin is an adhesion molecule that plays an important role in the formation and maintenance of the epithelial architecture. It has been reported that the reduced expression level of E-cadherin is closely associated with LN metastasis in patients with CRC. However, the changes in E-cadherin expression during the induction of LN metastasis have not been extensively studied. The purpose of this study was to clarify the clinical and biological significance of LN micrometastasis detectable

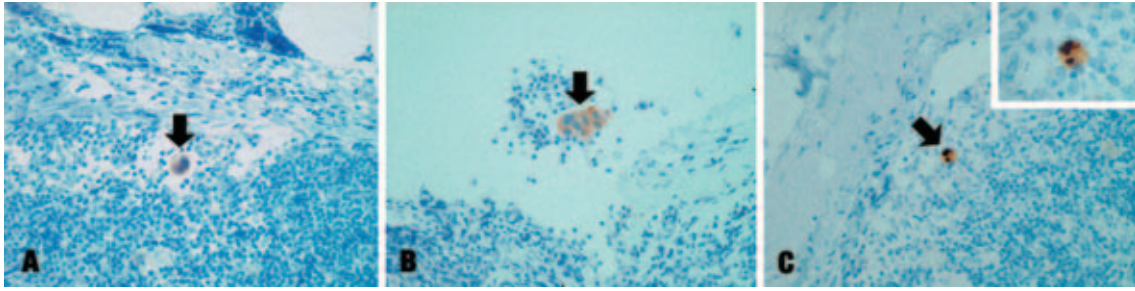


Fig. 1 Immunohistochemistry of CK20

Staining for CK20 was observed in the cytoplasm of single (A, **arrow**) and clustered cancer cells (B, **arrow**) in the subcapsular sinus of LNs. We excluded one case that showed an apoptotic body in a CK20-positive cell (C, **arrow**).

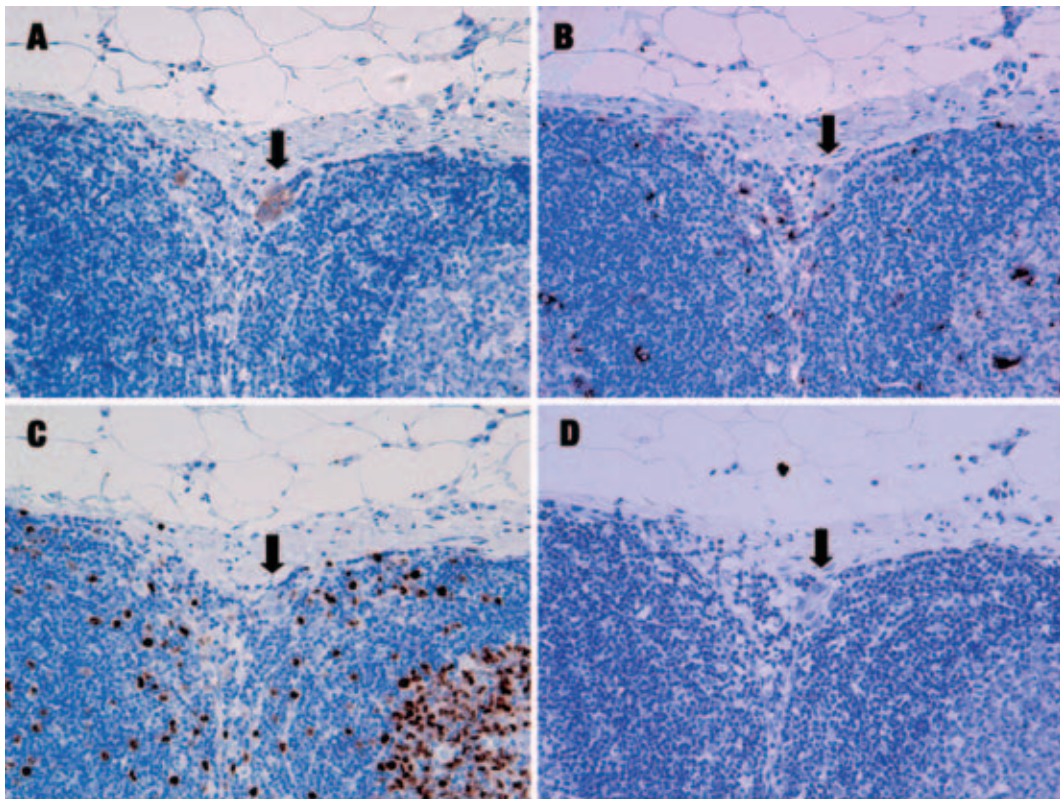


Fig. 2 Immunohistochemistry of CK20, CD68, Ki-67 and cleaved-caspase 3

CK20-positive cells are found in the subcapsular sinus of LN (A, **arrow**); however, these cells are negative for CD68 (B, **arrow**), Ki-67 (C, **arrow**) and cleaved-caspase 3 (D, **arrow**).

with IHC using the CK20 antibody.

Materials and Methods

Clinical Significance of LN Micrometastasis

Forty-two patients with stage I and II LN-metastasis-negative CRC who underwent surgery at Nippon Medical School Hospital (Bunkyo-ku, Tokyo) from January through December 1999 were enrolled in this study. We defined LN micrometastasis as either single tumor cells or a small cell cluster that could not be identified with routine pathological examination. A total of 525 LNs were examined with IHC using the CK20 antibody.

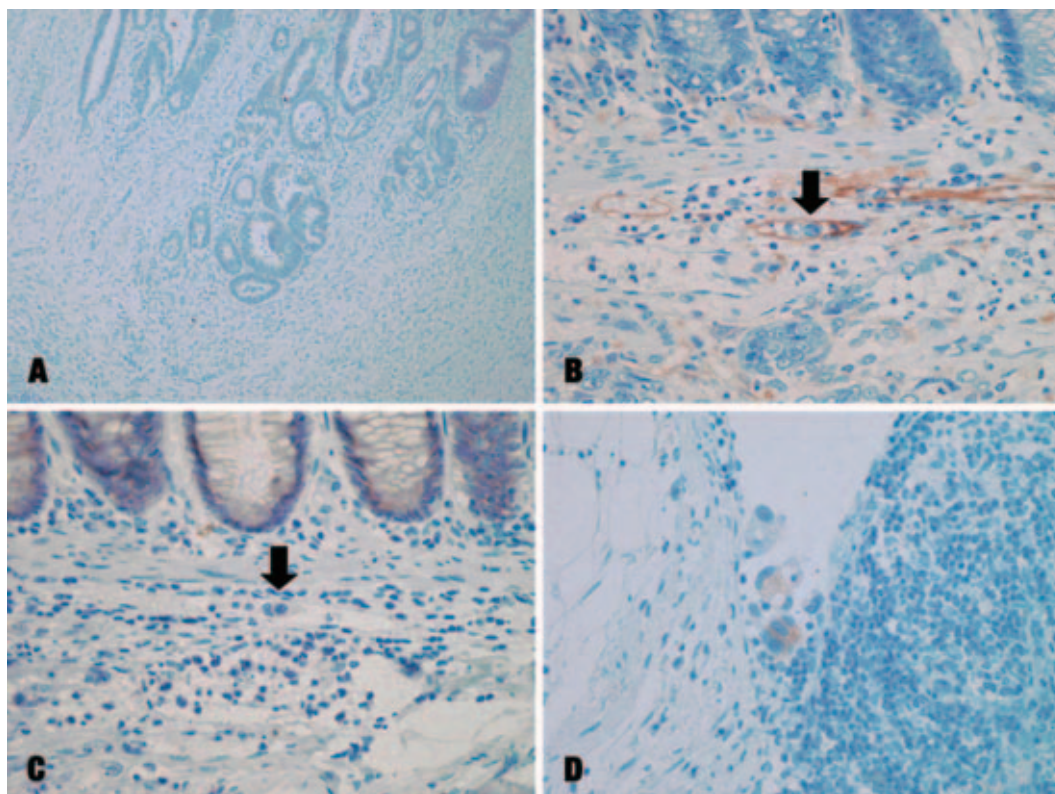


Fig. 3 Immunohistochemistry of E-cadherin

E-cadherin expression levels in the invasive front of the primary tumor decreased (A). Expression of E-cadherin in a single cancer cell in the D2-40-positive lymphatic vessel (B, **arrow**) was also decreased (C, **arrow**). However, various E-cadherin expression levels were observed in LN micrometastasis (D).

Paraffin-embedded specimens were cut into 6 slices: 1 slice 3 μm thick for H&E staining and 5 slices 6 μm thick for IHC. All slices were prepared as serial sections.

Biological Significance of LN Micrometastasis

We performed IHC using the serial sections to investigate the biological characteristics (Ki-67 as a cell proliferation marker, CD68 as a macrophage marker and cleaved-caspase 3 as an apoptotic marker). Moreover, we performed IHC staining for E-cadherin using a primary tumor, cancer cells in lymphatic vessels and LN micrometastasis to investigate changes in adhesive molecule expression during the induction of LN metastasis.

IHC

IHC was performed with the following primary antibodies: an anti-human CK20 antibody at 1 : 50 dilution, an anti-human CD68 antibody at 1 : 100 dilution, an anti-human Ki-67 antibody at 1 : 1,000 dilution, an anti-human cleaved-caspase 3 antibody at 1 : 200 dilution, an anti-human E-cadherin antibody at 1 : 200 dilution and an anti-human D2-40 antibody at 1 : 200 dilution.

Results

Clinical Significance of LN Micrometastasis

CK20 staining was observed in the cytoplasm of single (**Fig. 1A**, arrow) or clustered cancer cells (**Fig. 1B**, arrow) in the subcapsular sinus of the LN, and 12 nodes (8 of 42 patients) were positive. We excluded one case that showed an apoptotic body in a CK20-positive cell (**Fig. 1C**, arrow and inset). No micrometastasis was

detected in LNs from any of the patients with submucosal CRC. The depth of tumor invasion and pathological stage showed no correlation with the presence of LN micrometastasis: micrometastasis was present in 21.1% of stage I cases (4 of 19 patients) and 17.4% of stage II cases (4 of 23 patients). The patients with LN micrometastasis had a significantly lower disease-free survival rate than did patients without LN micrometastasis (log-rank test, $p=0.0093$). However, no significant difference between the two groups was found in the overall survival rate or any clinicopathological factor.

Biological Significance of LN Micrometastasis

CK20-positive cells were found in the subcapsular sinus of LNs (**Fig. 2A**, arrow), but these cells were negative for CD68 (**Fig. 2B**, arrow), Ki-67 (**Fig. 2C**, arrow) and cleaved-caspase 3 (**Fig. 2D**, arrow). The E-cadherin expression level in the invasive front of the primary tumor was decreased (**Fig. 3A**). Single cancer cell in D2-40-positive lymphatic vessel (**Fig. 3B**, arrow) also decreased (**Fig. 3C**, arrow). However, various levels of E-cadherin expression were observed in LN micrometastasis (**Fig. 3D**).

Discussion and Conclusions

Davidson *et al.* firstly reported the detection of micrometastasis with IHC in 1990. In the present study, we detected LN micrometastasis with IHC in CRC using an anti-CK20 antibody and suggested its usefulness as a predictor of the recurrence of cancer in patients with node-negative CRC. The detection of LN micrometastasis with IHC is advantageous in that we could discriminate cancer cells from macrophages on the basis of the shape of positive cells and nuclei and could exclude apoptotic cells. Our results concerning the biological significance of LN micrometastasis indicate that single or clustered cancer cells in LNs are associated with cells under cell-cycle arrest, but not cells approaching apoptosis. In addition, the change in E-cadherin expression may contribute to the induction of LN metastasis.
