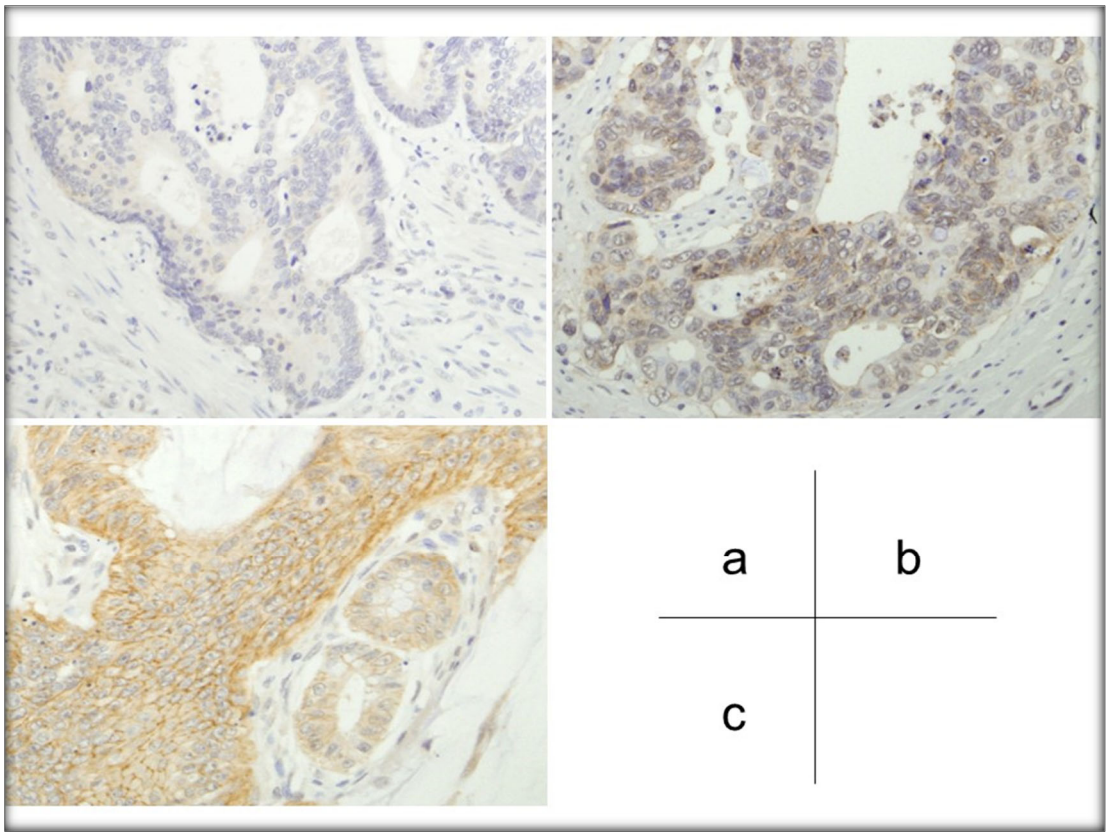

Figure 1. Immunohistochemical expression patterns of claudin-1 (CL-1). Expression of +1, <30% positive at cell membrane and weakly positive at cell cytoplasm (a: top, left); Expression of +2, >30% positive at cell membrane and weakly positive at cell cytoplasm or <30% positive at cell membrane and moderately positive at cell cytoplasm (b: top, right), and expression of +3, >30% positive at cell membrane and moderately positive at cell cytoplasm (c: bottom, left).

Digital quantitative evaluation of CL-1 expression demonstrated 117.3 ± 14.6 in colorectal cancer cases with recurrence/metastasis and 118.7 ± 17.7 in cases without recurrence/metastasis (mean ± standard deviation) (**Table 2 & Figure 2**). CL-1 expression levels at the invasive front (98.3 ± 14.1, $p<0.05$) were significantly decreased in colorectal cancer cases with recurrence/metastasis, compared with cases without recurrence/metastasis (107.0 ± 18.3).

Table 2. Digital quantitative evaluation of CL-1 expression.

	Total	Whole (mean±SD)	Invasive front (mean±SD)
Recurrence/metastasis group	46	117.3±14.6	98.3±14.1
No recurrence/metastasis group	43	118.7±17.7	107.0±18.3
		p=0.69	p=0.013*

Comparing the quantity of expression of non-neoplastic glands as 100

**p<0.05: statistical significance, Student's T test; SD: Standard deviation*

Recurrence/metastasis group: lymph node metastasis, liver metastasis, distant metastasis, peritoneal dissemination, and recurrence after operation

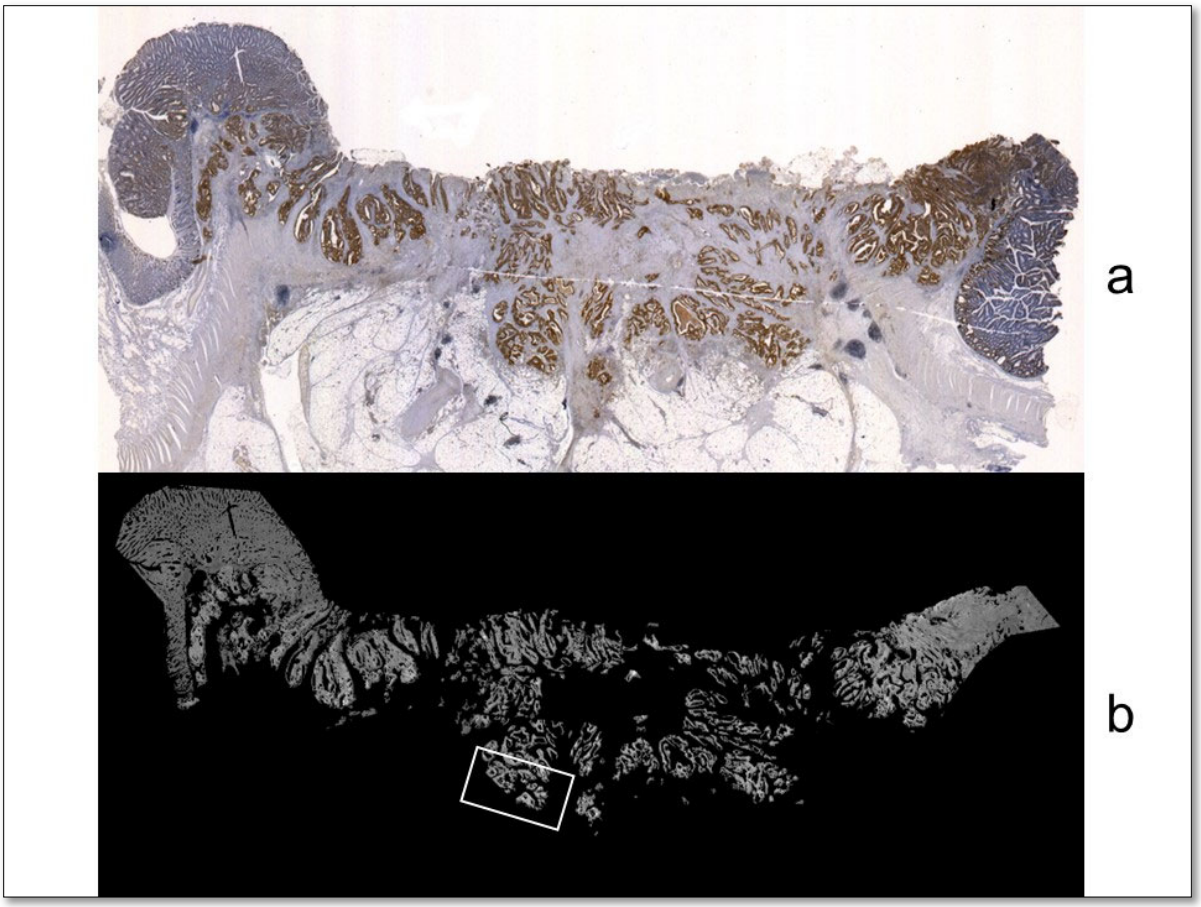

Figure 2. Digital quantitative evaluation of claudin-1 (CL-1). CL-1 immunostaining sections of colorectal cancer were scanned at 1200dpi (1200 pixels/inch) (a: top). Color components of the scanned preparation were separated into RGB (red-green-blue) (b: bottom). The intensity of immunohistochemical expression was represented as 100 x red / blue component. Mean expression levels were quantitative evaluated at the invasive front (200 x 50 pixels: square frame), as well as the entire tumor.

DISCUSSION

We examined the immune histochemical expression of CL1, CL4 and E-cad in human colorectal adenocarcinomas and analyzed their clinicopathological significance. This study examined the relationship between low-grade CL-1 expression at the invasive front and metastatic potentials (lymph node and liver metastases) in colorectal cancer patients. In addition, this is the first study demonstrating decreased CL-1 expression in colorectal cancers with metastasis/recurrence using digital quantitative evaluation. There were no apparent statistical correlations between clinicopathological features and the expressions of CL4 and E-cad.

Cell adhesion is crucial for the assembly of individual cells into three-dimensional tissues [1-3]. The functional units of cell adhesion are typically multi protein complexes consisting of three general classes, i.e., cell adhesion molecules/adhesion receptors, extracellular matrix proteins and cytoplasmic plaque/peripheral membrane proteins. Recent advances in molecular biology have clarified the structures and functional regulations of cell adhesion including the tight junction, adherens junction and desmosome. The claudin family and occludin have been identified as the major proteins of the tight junction, while the cadherin family has been discovered as adherence junction proteins [2].

On the other hand, oncological studies have focused on the relations between cancer invasion and the expression of cell adhesion molecules because decreased molecules may affect cancer invasion (cell migration) and metastasis, which reflect cancer outcome. Previous studies reported the up regulation of claudin expression including CL-1 and CL-4 in colorectal cancer [15,16]; however, these studies did not demonstrate the histological localization of the claudins. In our results, CL-1 expression was significantly decreased at the invasive front (advanced margin) of colorectal cancer, while cancer tissues generally showed high-grade CL-1 expression, i.e., no significant decrease of CL-1 expression was detected in the central parts of colorectal cancer [17,18].

Several studies reported the decreased claudin expression correlated with aggressive behaviors of colorectal cancer [19], and supported our results. We speculated that the low-grade CL-1 expression at the invasive front plays a role in cancer invasion and metastasis, and that metastasis/recurrence is correlated with decreased CL-1 expression using digital quantitative evaluation. Previous reports described the altered expression of E-cad in colorectal cancer [20-24]; however, our results showed that the expression pattern of E-cad was not significantly correlated with clinicopathological factors. The discrepancy between the previous reports and our study may result from the different fixation/staining conditions or different evaluations (entire tumor or part of cancer tissues such as the invasive front). Based on the present study, we speculated that CL-1 at the invasive front may affect cancer invasion and metastasis more effectively than the expressions of CL-4 and E-cad. In conclusion, decreased CL-1 expression at the invasive front is thought to be an important prognosis prediction factor.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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